NOVEL SUPRAMOLECULAR ION SENSING SYSTEMS AND THEIR APPLICATION IN MOLECULAR LOGIC GATES

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Approval of the Graduate School of Natural and Applied Sciences

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ABSTRACT

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Recognition and sensing of ions is an important front in supramolecular organic chemistry. One remarkable extension of this kind of work is the application of selective switching processes to logic gate operations. In this study, we have designed selective metal ion chelators for zinc and cadmium ions based on dansylamide fluorophores and dipicolylamine chelators. The zinc complex of a previously reported difluoroboradiazaindacene-bipyridyl derivative was shown to respond anions by an increase in emission intensity. We also discovered a hitherto unknown reaction of difluoroboradiazaindacenes and showed that this reaction can be exploited in a very selective sensing of fluoride ions in acetone solutions. The remarkable chemistry of these boradiazaindacene dyes, especially the bipyridyl derivative, allowed us to propose the first example of a unimolecular "molecular subtractor". A single molecule can carry out substraction of binary inputs, when these inputs are fluoride anion and zinc cation.

Keywords; molecular recognition, logic gate, difluoroboradizaindacene

ÖZ

YENİ SÜPRAMOLEKÜLER İYON ALGILAYICI SİSTEMLER VE MOLEKÜLER MANTIK KAPILARINDAKİ UYGULAMALARI

COŞKUN, ALİ
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Tez Yöneticisi: Prof. Dr. Engin U. AKKAYA

Eylül 2003, 88 sayfa

İyonların tanınması ve algılanması süpramoleküler organik kimyanın önemli bir cephesidir. Bu gibi çalışmaların bir ilginç uzantısı da, seçici sinyal değişimlerinin mantık kapısı işlemlerine uygulanmasıdır. Bu çalışmada, dansilamid floroforu ve dipikolilamine şelatörü üzerine kurulu çinko ve kadmiyum seçiciliği gösteren metal şelatörleri sentezlemiş bulunuyoruz. Grubumuzda daha önce sentezlenen bir bipiridil-boradiazaindasen türevinin çinko kompleksinin anyonları emisyon artışı ile sinyallediğini gösterdik. Ayrıca, boradiazaidasenlerin bugüne kadar bilinmeyen bir reaksiyonunu ortaya çıkardık ve bu reaksiyonun aseton çözeltisi içinde son derece seçici olarak florür anyonunu sinyallediğini gösterdik. Boradiazaidasen boyarmaddelerinin ve özellikle bipiridil türevinin ilginç kimyası, bize tek moleküllü bir "moleküler çıkarma işlemcisi"ni önermemize imkan tanıdı. İnput'lar çinko katyonu ve florür anyonu olduğunda, tek bir molekül "binary" inputları birbirinden çıkarabilmektedir.

Anahtar kelimeler; moleküler algılayıcı, mantık kapısı, boradiazaindasen

To my family

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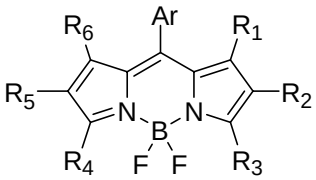
PET:	Photoinduced electron transfer
PCT:	Photoinduced charge transfer
LMCT:	Ligand to metal charge transfer
FE:	Fluorescence enhancement
FEF:	Fluorescence enhancement factor
FQF:	Fluorescence quenching factor
CHEF:	Chelation enhanced fluorescence
CHEQ:	Chelation enhanced quenching
TFA:	Trifluoroacetic acid
BODIPY:	Boradiazaindacene



R= H or any group

CHAPTER 1

INTRODUCTION

1.1. What is Supramolecular Chemistry?

The field of supramolecular chemistry has been defined as 'chemistry beyond the molecule' and involves investigating new molecular systems in which the most important feature is that the components held together reversibly by intermolecular forces, not by covalent bonds. Chemists working in this area can be thought of as architects combining individual covalently bonded molecular building blocks, designed to be held together by intermolecular forces, in order to create functional architectures.[1]

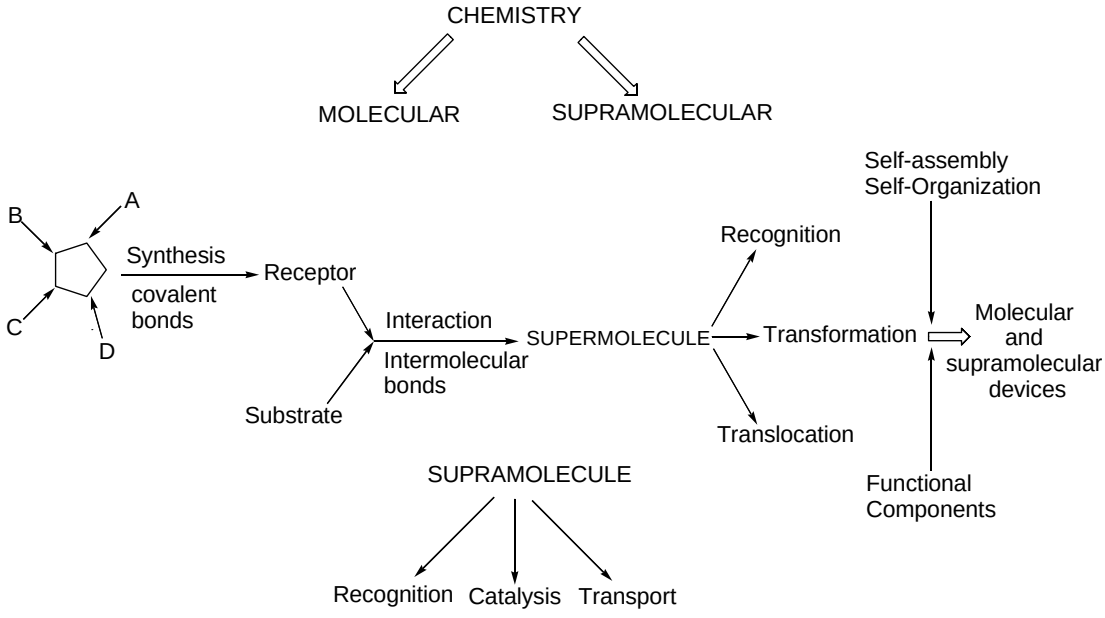


Figure 1. Comparison between the scope of molecular and supramolecular chemistry according to Lehn

Supramolecular chemistry is a multidisciplinary field, and therefore requires a grasp of a range of basic principles. This introduction describes a generalized apporach to supramolecular science and provides an indication of the wide ranging interests of chemists working in this area. Biological systems often provide inspiration, organic and inorganic chemistry are required for the synthesis of pre-designed supramolecular components, and physical chemistry is used to fully understand their properties. Finally, a degree of technical expertise can lead to functioning devices ready for application to the real world.

1.2. Molecular Recognition

1.2.1 Recognition, Information, Complementarity

Molecular recognition is defined by the energy and the information involved in the binding and selection of substrate(s) by a given receptor molecule; it may also involve a specific function. Mere binding is not recognition, although it is often taken such. One may say that recognition is binding with a purpose, like receptors are ligands with a purpose. It implies a pattern recognition process through a structurally well-defined set of intermolecular interactions. Binding of σ to ρ forms a complex or supermolecule characterized by its thermodynamic and kinetic stability and selectivity, i.e., by the amount of energy and of information brought into operation.

Molecular recognition thus implies the (molecular) storage and (supramolecular) read out of molecular information. These terms have become characteristic of the language of supramolecular chemistry. Although the notions of recognition and information were used in connection with biological systems, they effectively pervaded the realm of chemistry only at the beginning of the 1970's in connection with, and as generalization of, the studies on selective complexation of metal ions. Since then molecular recognition has become a major area of chemical research and a very frequently used term.[2]

Information may be stored in the architecture of the receptor, in its binding

sites and in the ligand layer surrounding bound σ; it is read out at the rate of formation and dissociation of the supermolecule. In addition to size and shape. A receptor is characterized by the dimensionality, the connectivity and the cyclic order of its structural graph. The binding sites are characterized by their electronic properties (charge, polarity, polarisability, van der Waals attraction and repulsion), their size, shape, number and arrangement in the receptor framework as well as their eventual reactivity that may allow the coupling of complexation with other processes (such as protonation, deprotonation, oxidation or reduction). The ligand layer acts thorugh its thickness, its lipo- or hydrophilicty and its overall polarity, being exo/endo-lipo/polarophilic. In addition, stability and selectivity depend on the medium and result from a subtle balance between solvation and complexation (i.e., ''solvation '' of σ by ρ). Finally, for charged complexes medium dependent cation-anion interactions influence markedly the binding stability and selectivity.

The information is a key notion of supramolecular chemistry, in fact the most fundamental and the general one, that constitutes the common thread running through the whole field. Indeed, in this respect supramolecular chemistry can be considered as a chemical information science or molecular ''informatics'' concerned with the molecular storage and the supramolecular reading and processing of the information via the structural and temporal features of molecules and supermolecules. [3,4]

Recognition implies geometrical and interactional complementarity between the associating partners, i.e., optimal information content of a receptor with respect to associating partners, i.e., optimal information content of receptor with respect to a given substrate. This amounts to generalized double complementarity principle extending over energetic features as well as over the geometrical ones represented by the ''lock and key '', steric fit concept of Emil Fischer. Emil Fischer described this idea in 1894, Figure 2 shows a stylized representation of the lock and key. The arrangement of binding sites in the host (lock) is complementary to the guest (key) both sterically and electronically.

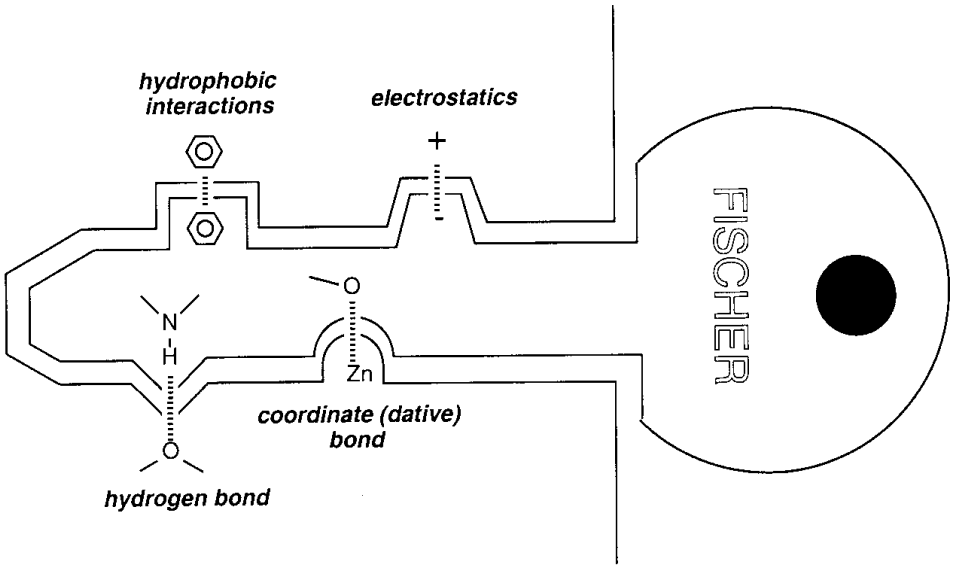


Figure 2. The lock and key principle: receptor sites in the host (lock) are complementary to the guest(key).

An example of the lock and the key principle in nature is provided by carboxypeptidase-A, an enzyme that selectively catalyses the hydrolysis of the C-terminal amino-acid residues of proteins.[5]

High recognition by a receptor molecule ρ consists in a large difference between the binding free energies of a given substrate σ and of the other substrates. It results in marked deviation from the statistical distribution. In order to achieve large differences in affinity several factors must be taken into account:

1) steric (shape and size) complementarity between ρ and σ

2) interactional complementarity, i.e. presence of complementary binding sites (electrostatic such as positive/negative, charge/dipole, dipole/dipole, hydrogen bond donor/acceptor, etc.) in the correct disposition on σ and ρ so as to achieve complementary electronic and nuclear distribution (electrostatistic, H-bonding and van der Waals) maps

3) large contact areas between ρ and σ so as to contain

4) multiple interaction sites, since non-covalent interactions are rather weak compared to covalent bonds.

5) Strong overall binding; although high stability does in principle not

necessarily imply high selectivity, this is usually the case; indeed, the differences in free energy of binding are likely to be larger when the binding is strong; high binding efficiency requires strong interaction; thus, in order to achieve efficient recognition, both high stability and high selectivity, strong binding of σ by ρ is required.

In addition, medium effects play an important role through the interaction of solvent molecules with σ and ρ as well as with each other; thus the to partners should present geometrically matched hydrophobic/hydrophobic or hydrophilic/hydrophilic domains.

1.2.2 Non-covalent interactions

The glue used by supramolecular chemists to hold molecules together is non-covalent, and there are a number of such interactions that can be utilized. They include;

a) electrostatics (ion-ion, ion-dipole and dipole-dipole);
b) hydrogen bonding ;
c) π-π stacking interactions;
d) dispersion and induction forces (van der Waals forces);
e) hydrophobic or solvatophobic effects.

The bond energy of a typical single covalent bond is around 350 $kJmol^{-1}$ rising up to 942 $kJmol^{-1}$ for the very stable triple bond in N_2. The strengths of many of the non-covalent interactions used by supramolecular chemists are generally much weaker ranging from 2 $kJmol^{-1}$ for dispersion force, through to 20 $kJmol^{-1}$ for a hydrogen bond to 250 kJ/mol for ion-ion interaction. The power of supramolecular chemistry lies in the combination of a number of weak interactions, allowing strong and selective recognition of specific guests to be achieved.

Electrostatic interactions (such as the ion-dipole interactions that operate in valinomycin) are based on the Coulombic attraction between opposite charges (Figure 3). Ion-ion interactions are non-directional, whilst for ion-dipole interactions the dipole must be suitably aligned for optimal binding efficiency. The high strength

of electrostatic interactions has made them a prized tool amongst supramolecular chemists for achieving strong binding. There are many receptors for cations and anions which employ electrostatic interactions to hold the guest in place.

Arrays of hydrogen bonds, such as those employed in biological systems (i.e. the DNA double helix), have been utilized in receptors designed to coordinate neutral organic species such as barbiturates, short chain alcohols and amides, and also anions.[6] The directional nature of hydrogen bonds, combined with the precision with which the individual components can be built into molecular systems has made them especially attractive to molecular designers. This has facilitated the construction of complex architectures.

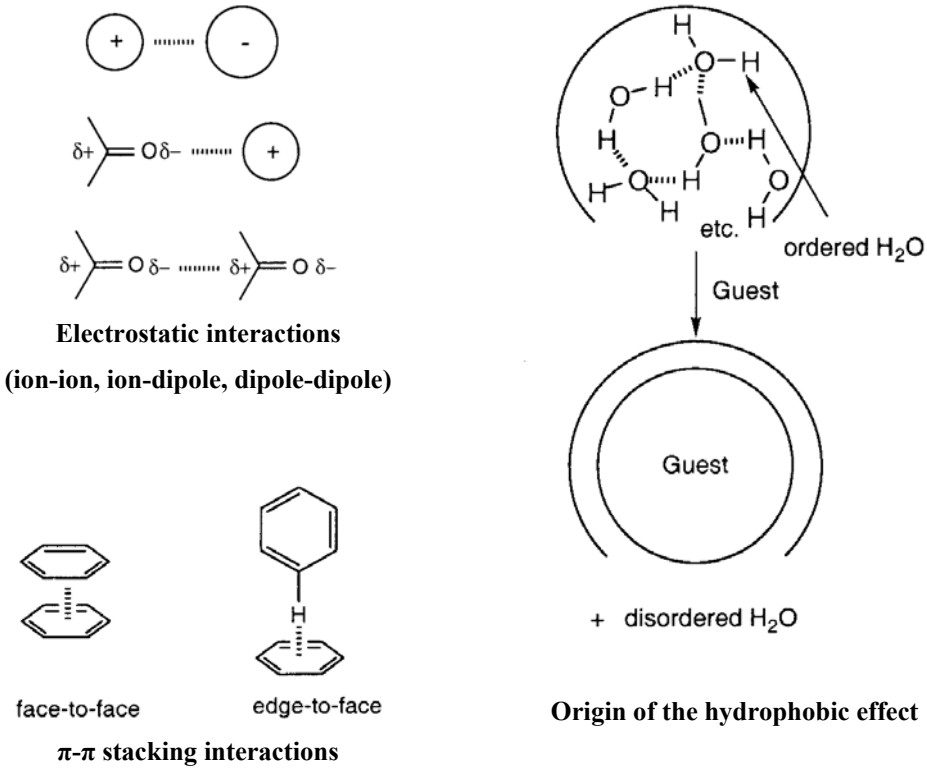


Figure 3. Some examples of Non-covalent interactions

π-π stacking forces occur between systems containing aromatic rings in Figure 3. Attractive interactions can occur in either a ‘’face-to-face’’ or ‘’edge to face’’ manner (for example benzene crystallizes in a ‘herring-bone’ arrangement maximising edge-to-face contacts). Current theories suggest this attractive force is

electrostatic in nature. Some very elegant receptors have been synthesized employing π-π interactions, including a receptor for benzoquinone.[7]

Dispersion forces (or induced dipole-induced dipole interactions) are attractive forces between molecules that occur when instantaneous dipoles in the electron clouds around each molecule interact favourably. These van der Waals forces are believed to provide additional enthalpic stabilisation to the coordination of a hydrophobic guest into a hydrophobic cavity. They are, however, of a very general nature and so it is difficult to design receptors specifically to take full advantage of them. One such system may be a self-assembled 'tennis ball' that can encapsulate xenon atoms

The hydrophobic effect (Figure 3) is the specific driving force for the association of apolar binding partners in aqueous solution. Water molecules around the apolar surfaces of a hydrophobic cavity arrange themselves to form a structured array. With guest complexation the water molecules are released and become disordered. This result in a favourable increase in entropy. In addition, there is believed to be an enthalpic component to the hydrophobic effect.Receptors containing hydrophobic interior cavities designed to encapsulate organic guest molecules in aqueous solution include the cyclophanes and cyclodextrins

Classical coordination chemistry (i.e. the coordination of metals by ligands donating two electrons to form a dative bond) although not strictly a non-covalent interaction is also widely used in supramolecular chemistry. The geometric requirements of metal ions, combined with the design of specific ligands has permitted the construction of complex and eye-catching molecular topologies catenanes and double and triple helices, and molecular grids.

Steric repulsion, this diminishes the strength of interactions as two molecules cannot occupy the same space. As may be expected from the lock and key analogy, however, it can play a very important role in determining the selectivity of a receptor species for a particular substrate and the stability of a specific complex

1.2.3 Design principles of a Supramolecular Host : chelate and macrocyclic effects

Molecular receptors are defined as organic structures held by covalent bonds that are able to bind selctively ionic or molecular substrates by means of various intermolecular interactions, leading to an assembly of two or more species, a supermolecule. The design of molecular receptors amounts to expressing in an organic molecule the principles of molecular recognition. Receptor chemistry,[8] the chemistry of artificial receptor molecules, represents a generalized coordination chemistry, not limited to transition-metal ions but extending to all types of substrates: cationic, anionic, or neutral species of organic, inorganic, or biological nature[5]. In order to obtain high recognition, the non-covalent forces described above must be taken into account in the design of the receptor. Design principles are therefore applied in order to achieve the desired intermolecular interaction, with a number of factors being used to increase the strength of the intended host-guest complex. In particular, chelating or macrocyclic ligands are frequently employed due to the high thermodynamic stability of their complexes.the chelate effect refers to the enhaced stability of a copmlex containing chelate rings as compared to a similar system containing fewer or no rings. This is most clearly illustrated by compairing two different ligands: ethylene diamine and ammonia .

The metal complex containing bidentate ethylene diamine is almost ten orders of magnitude more stable than that containing no chelating ligands. The reason for this lies in thermodynamic cosiderations. An increase in the overall binding constant (β) corresponds to more negative value of ΔG^o. This would result from either a more negative enthalpy or a more positive entropy on complexation.

$$[Ni(H2O)_6]^{2+}_{(aq)} + 6NH_{3(aq)} \rightleftharpoons [Ni(NH_3)_6]^{2+}_{(aq)} + 6H_2O_{(aq)} \quad \text{Log } \beta = 8.61$$

$$[Ni(H2O)_6]^{2+}_{(aq)} + 3en_{(aq)} \rightleftharpoons [Ni\ (en)_3]^{2+}_{(aq)} + 6H_2O_{(aq)} \quad \text{Log } \beta = 18.28$$

For amonia binding, six ligands replace six waters and so the number of independent species in solution remains the same. Ethylene diamine, however is

bidentate so three ligands displace six waters, increasing the number of independent species in the system and causing an increase in entropy, thus lowering ΔG^o.
Interestingly, from five membered chelate rings upwards, the chelate effect decreases in magnitude with increasing ring size. This can be explained by considering the configurational entrophy of the chelate chain. The longer the chain, the higher the configurational entrophy and so ring formation becomes increasingly improbable.

The macrocyclic effect is related to the chelate effect and refers to the increased thermodynamic stability of macrocyclc systems compared to their acyclic analogues. Macrocyclic hosts are less heavily solvated than their acyclic analogues and therefore less energy is required for desolvation (coordination is more entalpically favourable). Macrocyclic ligands are less flexible and consequently have less disorder to lose on complexation than their acyclic analogues(in other words, coordination is more entropically favourable because of the relative rigidity of the receptor).

The enhanced binding of guest species provided by chelating or macrocyclic hosts has been employed in the design of many receptors operating through a variety of intermolecular forces.[9]

1.3. Molecular Sensors

A receptor may be used as a sensor if it can report the presence of the guest by some physical means. Sensor should ideally be selective for a particular guest and not only report the presence of the guest molecule, but should also allow the chemist to monitor its concentration. This is important medically (for monitoring indicators of physical function) and enviromentally (monitoring pollutant levels). Two different strategies have been applied to sensor production.[10]

Firstly, the receptor can be used to create a modified material, for example an electrode. The receptor is incoorporated into a polymer electrode, and this modified electrode can then show a selective response to the presence of the ion for which the receptor is selective, allowing the quantitative determination of ion concentrations in solution.

Alternatively, the sensing function can actually be incorporated at a molecular level. This is achieved by combining a binding site and a reporter group in one molecule. The reporter group is choosen to have electrochemical or spectroscopic properties that are altered by proximate host-guest interaction. This electrochemical or spectroscopic output can therefore be used to quantitatively detect specific guests.

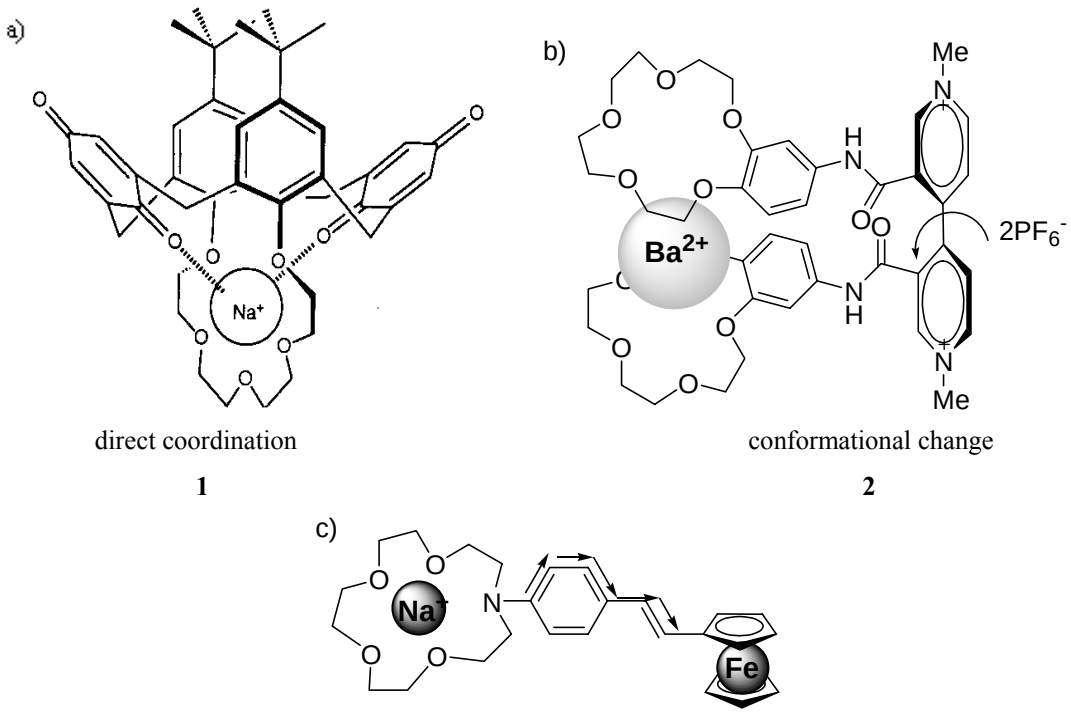
1.3.1. Electrochemical sensors

Electrochemical sensors can be created by attachment of redox active group to a receptor. For such a sensor to useful, the receptor should be selctive for guest of interest and the binding process must be coupled to the redox reaction; in other words the redox active centre must 'feel the presence' of the bound guest. Many redox-active groups have been incorporated into this type molecular sensor; e.g. ferrocene, quinone and bipyridinum (Figure 4). So far, the coupling has been realized through one or a combination of the following pathways

a) Direct cordinate bond formation between the redox centre and the complexed guest.[11]

b) Induced conformational perturbation of the redox centre(s) caused by guest complexation.[12]

c) Through-bond electrostatic communication; Electrochemical investigations reveal the binding of Na^+, K^+, and Mg^{2+} guest cations at the response crown ether coordinating sites results in shifts of the ferrocene oxidation wave to more positive potentials if a conjugates π-electron system links the heteroatoms of the ionophore to the redox center. The magnitude and type (one or two waves) of the anodic shift are related to the charge:radius ratio of the cationic guest, Mg^{2+} producing the largest value and K^+ the smallest.[13]



direct coordination

1

conformational change

2

through bond

3

Figure 4. Electrochemical recognition must be coupled to complexation for a redox sensor to work.

A change in the redox properties of the receptor can be detected by an electrochemical technique such as cyclic voltametry (CV). Changes in the cyclic voltamogram can therfore be used to sense the presence of this guest.

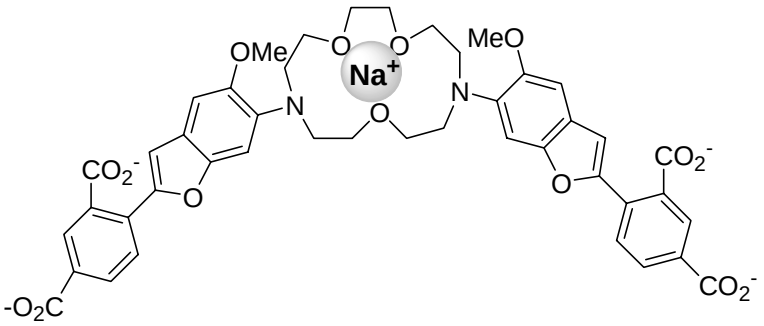
1.3.2. Optical sensors

The most common type of optical sensor is fluorescent, there is also colorimetric, [14] and electron-transfer (ET) path-selective sensors, [15] , the response in the last two exmaples are followed according to change in their absorbance. Fluorescence detection has three major advantages over other light-based investigation methods: high sensitivity, high speed, and safety. The point of safety refers to the fact that samples are not affected or destroyed in the process, and no hazardous by products are generated.

Fluorescence is the phenomenon in which absorption of light of a given wavelength by a fluorescent molecule is followed by the emission of light at longer wavelengths. The distribution of wavelength-dependent intensity that causes fluorescence is known as the fluorescence excitation spectrum, and the distribution of wavelength-dependent intensity of emitted energy is known as the fluorescence emission spectrum.[16]

Sensitivity is an important issue because the fluorescence signal is proportional to the concentration of the substance being investigated. Sensitiviy of fluorescence arises from the differences between the excitation and emission wavelength. Relatively small changes in ion concentration in living cells can have significant physiological effects. Whereas absorbance measurements for colorimetric sensors can reliably determine concentrations only as low as several tenths of a micromolar, fluorescence techniques can accurately measure concentrations one million times smaller -- pico- and even femtomolar. Using fluorescence, one can monitor very rapid changes in concentrations. Due to this advantages fluorescent sensors are especially attractive as they give a meaningful physical output which is easy to measure even at very low concentrations. They are, therefore, very sensitive and suitable for use in biological systems. But the biggest disadvantage of this fluorescent sensors, they mainly work in organic media, and in the aqueous media they dont show any respose. Recently, there is a strong demand in this area to synythesize water soluble derivatives of fluorescent sensors. Due to these limitations the number of commercial fluorescent sensors on the market is still reletively small.

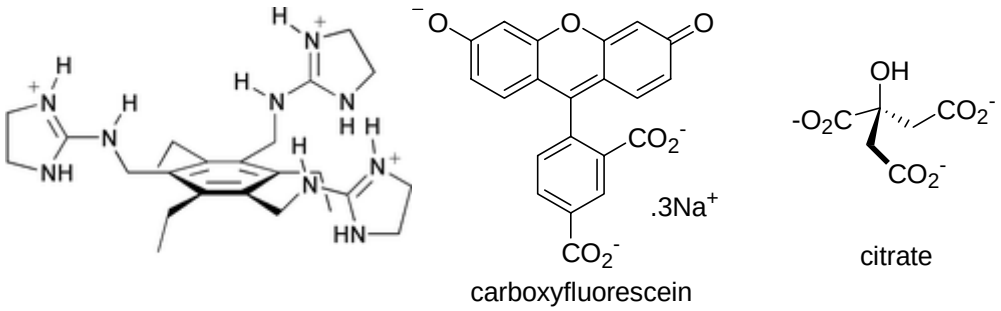
Sensor **4** in Figure 5 is commercially used to monitor physolocigal levels of sodium ions. The crown ether binds the guest cation, the charge on which alters the electric field experienced by the fluorophore. This changes the wavelengths and intensities of fluorescence (absorbtion/emission) and allows the concentration of sodium ions to be determined.[17]



4

Figure 5. Fluorescent probe for Na^+

Chemists are now developing sensors for more structurally demanding guests in competitive solvents; a challenging goal. An excellent example is provided by a sensor for citrate anions developed in research from the group of Eric Ansyln in figure 6 ,[18]. Tridentate guanidinium based receptor shows a high affinity and selectivity for **7 (**tricarboxylate citrate) anion. Neither of these two components are fluorescent, and in order to convert the receptor into a sensor, a clever strategy was utilized. A mixture of **5** and **6** (carboxyfluorescein) was made. Susbstrate which is fluorescent binds to the receptor, but quite weakly. When tricarboxylate citrate is added to the mixture it displaces the substrate. The fluorescent properties of substrate changes considerably on its release from the comples, and in this way sensory response to the addition of citrate is obtained. The sensor detected the concentration of citrate anions even in the presence of common anionic contaminants present in beverages, such as ascorbate and phosphate.



5 **6** **7**

Figure 6. Selective receptor for citrate anions binds weakly and allows the system to be used as a fluorescent sensor.

1.4. Fluorescence signalling phenomena

The most widely used mode of fluorescence modulation is the decrease or increase of fluorescence intensity at a single emission wavelength upon analyte binding. For halide ions and molecular oxygen, non-chelative quencing is usually the method in analysis. With intrinsic fluorescent chemosensors, where donor atoms of the ligand is a part of the fluorophore, the comploexation of metal ion results in either fluorescence enhancement (chelation-enhanced fluorescence,CHEF) or decrease in fluorescence (chelation-enhanced quenching, CHEQ). The cations, which usually display CHEF are non-redox active, closed shell cations; e.g., Zn^{2+}, Cd^{2+}, Al^{3+}. CHEQ is usually demonstrated when an intrinsic chemonsensor is complexed with a suitable quenching ion;e.g., Cu^{2+}, Hg^{2+},Ni^{2+}. One of the mostly used phenomena in supramolecular recognition by chemosensors is PET (Photoinduced Electron Transfer), which is discussed in the following section. Another event, which is also in our anion sensor, is the formation of equilibrium between boron and fluoride anion, which affects both absorbance and emission of the fluorophore.[19]

1.4.1. Photoinduced electron transfer (PET)

In photoinduced electron transfer, absorption of light by a molecule causes an electron to jump to another molecule or component of a composite system. Once the electron has jumped, a molecular radical ion pair is formed or in an organic/semiconductor nanostructure, an electron-hole pair is created. These ions or electron hole pairs have a finite lifetime after which they recombine (mostly geminate recombination) through radiative or nonradiative channels.

This type of system consists of a fluorophore linked to a donor atom (usually an amino nitrogen). Upon excitation of fluorophore, an electron transfer occurs from the HOMO of the donor to the low-lying HOMO of the acceptor, fluorophore. Thus fluorescence doesn't take place. When cation binds to the recognition moiety where the donor atom is present, the energy of the HOMO of the ion receptor is lowered so

that the photoinduced electron transder can't happen from HOMO of the donor to the fluorophore. This is displayed as enhancement in fluorescence.[20]

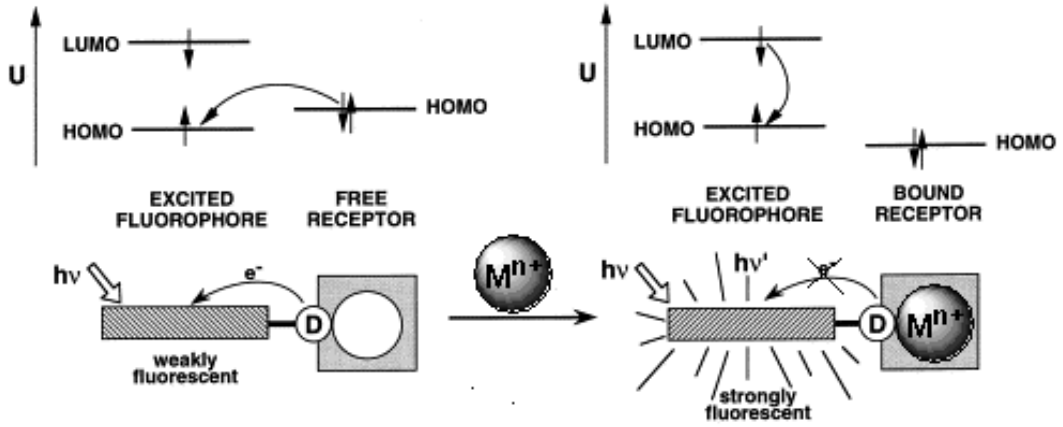
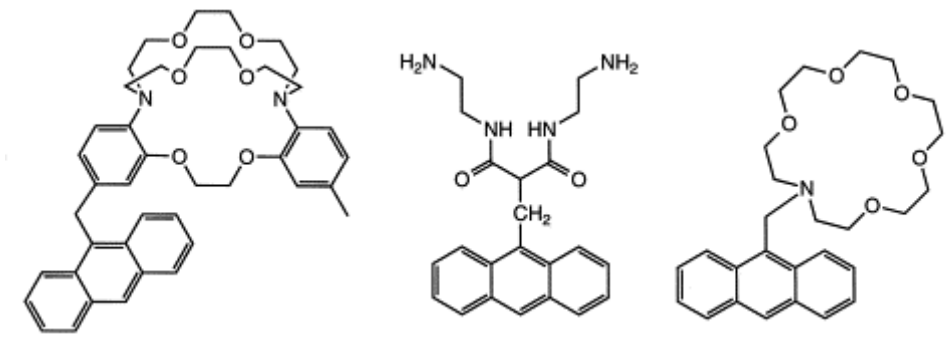


Figure 7. PET mechanism

Many of the fluorescent chemosensors work with this principle. Selectivity for ions is achieved by the correct choice of recognition moiety for the desired ion. A classical example is compound **10**, [21] . The recognition moiety is not necessary to be a crown ether. Cryptands like **8** [22], podands **9** [23], chelating [24], calixarene type receptors can also serve as ion binding sites in Figure.8



8 **9** **10**

Figure 8. Some examples of PET based fluorescent chemosensors

The presented basic scheme is not only PET mechanism. With transition metals, electron transfer may occur from fluorescent chemosensor to the coordinated metal ion or vice versa, [25]. Also in some instances, this result in quencing of the fluorescence by non-radiative energy-transfer according to Dexter mechanism. PET may sometimes occur from acceptor to donor. Then it is called oxidative PET.

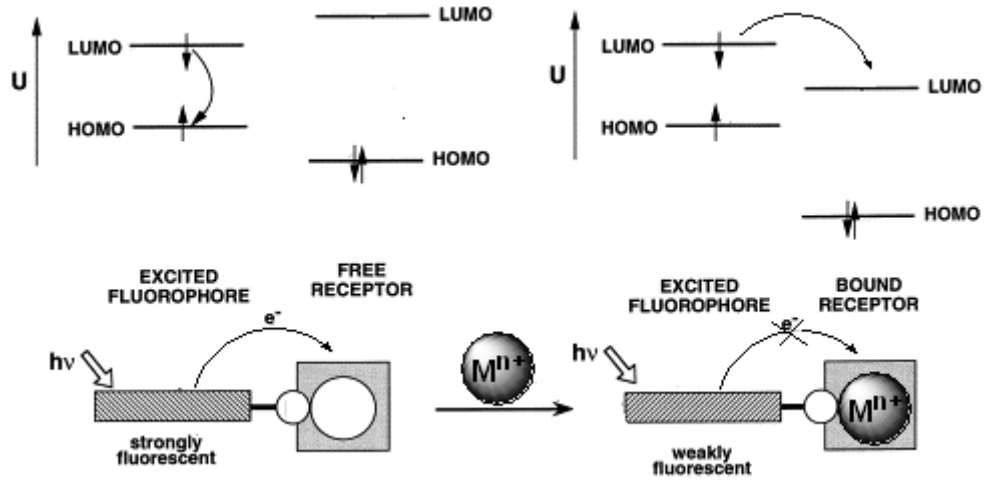


Figure 9. Oxidative PET mechanism

In some instances, after the prevention of PET by metal binding, excitation energy is transferred from the fluorophore, through ligand to another bound cation like Eu^{3+} or Tb^{3+}. This transfer is seen as the disapperance of emission signal from the fluorescent cations. An example for oxidative PET, we can cite a recent work from our laboratory, [26]. BODIPY dyes (borondipyrromethene dyes) typically have very high quantum yield (near to 1). They are also photostable and insensitive to the pH chnages; with this properties they are potential sensors for both cation and anion sensing. In that particular study (Figure 10), without zinc ion fluorophore has a bright-green fluorescence and with the addition of zinc fluorescence is quenched via oxidative PET mechanism.

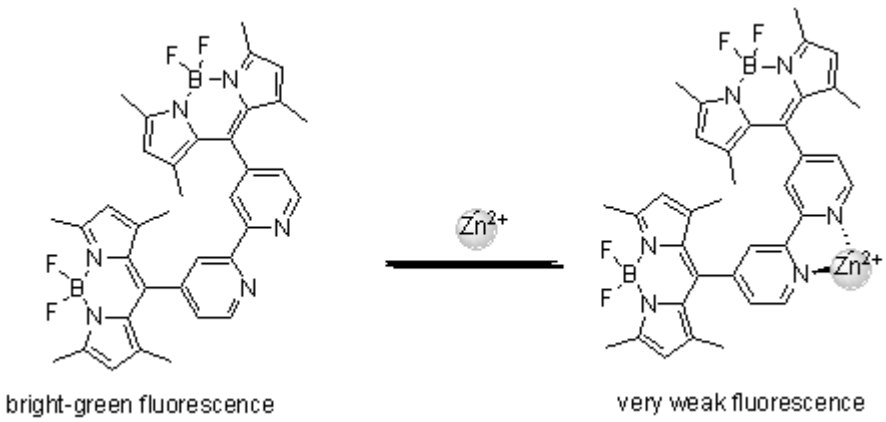


Figure 10. Complexation of zinc with bis-bipyridyl BODIPY fluorophore by oxidative PET mechanism

1.4.2. Photoinduced charge transfer(PCT)

When a fluorophore contains an electron-donating group (often an amino group) conjugated to an electron-withdrawing group, it undergoes intramolecular charge transfer from the donor to the acceptor during excitation by light. The consequent change in dipole moment results in a Stokes shift that depends on the microenvironment of the fluorophore. It can thus be anticipated that cations in close interaction with the donor or the acceptor moiety will change the photophysical properties of the fluorophore because the complexed cation affects the efficiency of intramolecular charge transfer [27,28].

When a group (like an amino group) playing 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, a blue shift of the absorption spectrum is expected together with a decrease of the extinction coefficient. Conversely, a cation interacting with the acceptor group enhances the electron-withdrawing character of this group; the absorption spectrum is thus red-shifted and the molar absorption coefficient is increased. The fluorescence spectra are in principle shifted in the same direction as those of the absorption spectra. In addition to these shifts, changes in quantum yields and

lifetimes are often observed. All these photophysical effects are obviously dependent on the charge and the size of the cation, and selectivity of these effects are expected.

Let us consider only the case where the dipole moment in the excited state is larger than that in the ground state. Then, when the cation interacts with the donor group, the excited state is more strongly destabilized by the cation than the ground state, and a blue shift of the absorption and emission spectra is expected (however the fluorescence spectrum undergoes only a slight blue shift in most cases; this important observation will be discussed below). Conversely, when the cation interacts with the acceptor group, the excited state is more stabilized by the cation than the ground state, and this leads to a red shift of the absorption and emission spectra in Figure 11.

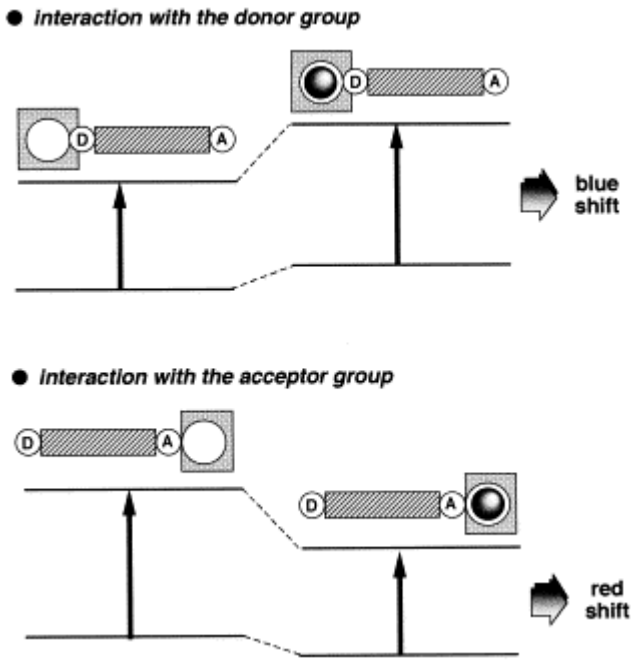


Figure 11. Spectral displacements of PCT sensors resulting from interaction of a bound cation with an electron-donating or electron-withdrawing group.

Many fluoroionophores have been designated according to the following principle; the cation receptor is an azacrown containing a nitrogen atom which is conjugated to an electron withdrawing group.

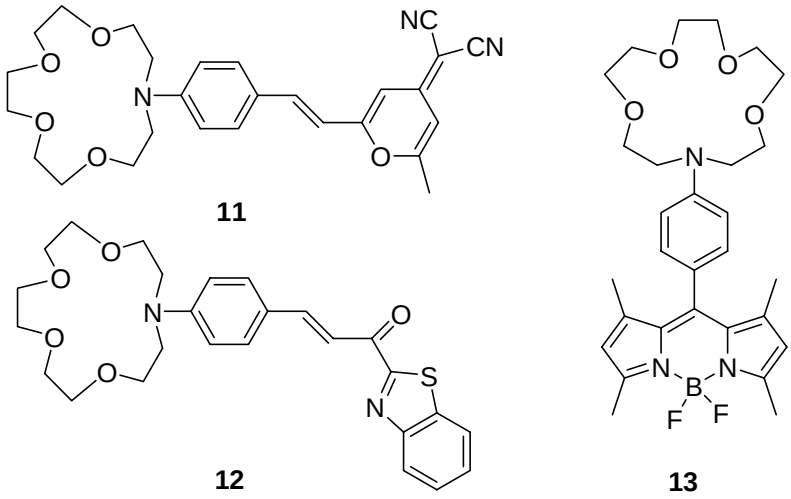


Figure 12. Crown-containing PCT sensors in which the bound cation interacts with the donor group.

In compounds **11** and **12** ,[29,30], the blue shift of the abosrbtion spectrum is much larger than that of the emissionspectrum on cation binding and small shift occurs in the fluorescence spectrum, the PCT reduces the electron density on the nitrogen atom of the crown, and this nitrogen atom becomes a noncoordinating atom of the crown because it is positively polarized. The fluorescence spectrum is thus only slightly affected because most of the fluorescence is emitted from species in which the interaction between the cation and the fluorophore doesnt exist any more or is much weaker. Generally , the changes in fluorescence intensity upon cation binding is not very large in these PCT molecular sensors (factors of ca. two to five) as compared to PET sensors. A remarkable exception is offered by **13** in Figure 12, [31], whose electron-withdrawing group is boron-dipyrromethene: the fluorescence enhancement factor varies from 90 for Li^{+} to 2250 for Mg^{2+}.

1.5. Cation Recognition

Fluorescent senors for pH measurements and metal ion recognition are widely used because they offer distinct advantages in terms of senstivity, selectivity, response time, local observation. Various fields are concerned by such sensors: biology, medicine(clinical biochemistry), enviroment, etc. Considerable effort being made in the development of fiber-optic chemical sensors because of their potential

for use in clinical and enviiromental applications,[32,33]. Detecting cations is great interest to many scientists, including chemists, biologists, clinical biochemsits and enviromentalists. Sodium, potassium, magnesium, calcium, zinc are involved in biological processes such as transmission of nerve impulses, muscle contraction, regulation of cell activity, etc. Moreover, various metal ions belong to metalloenzymes. In medicine, it is important to control the serum levels of lithium in patients under treatment of manic depression, and potassium in the case of high blood pressure. Regarding aluminum, its toxicity has long been recognized and there is controversy about its possible implications in Alzheimers disease. In chemical oceanography, it has been demostrated that some nutrients required for the survival of microorganisms in sea water contain zinc, iron, mangase as enzyme cofactors. Finally, its well known that mercury ,lead and cadmium are toxic for organisms, and early detection in the enviroment is important. Among the numerous analytical methods that are available for the detection of cations, flame photometry, atomic absorbtion spectrometry, ion sensitive electrodes, electron microprobe analysis, neutron activation analysis, etc., are expensive, often require samples large size and don't allow continuous monitoring. In contrast, the methods based on fluorescent sensors offer distinct advantages in terms of sensitivity, selectivity, response time. Moreover , remote sensing is possible by using optical fibers with a molecular sensor immobilized at the tip. Therefore, considerable efforts are being made to develop selective fluorescent sensors for cation detection.

Such fluorescent sensors consists of a fluorophore linked to an ionophore and is thus called a fluoroionophore in Figure 13, the criteria for good sensors are stability, metal selectivity, metal affinity, signal transduction, fluorescent signalling, kinetically rapid sensitization, ease of delivery to target systems and availability. In the desing of such sensors, [34], attention should be paid to both recognition and signalling moieties. The signalling moiety acts as a signal transducer, i.e.it converts the information (recognition event) into an optical signal expressed as the changes in the photophysical characteristics of the fluorophore. These changes are due to the perturbation (by the bound cation) of photoinduced processes such as electron transfer, charge transfer, energy transfer or dissapperance etc.

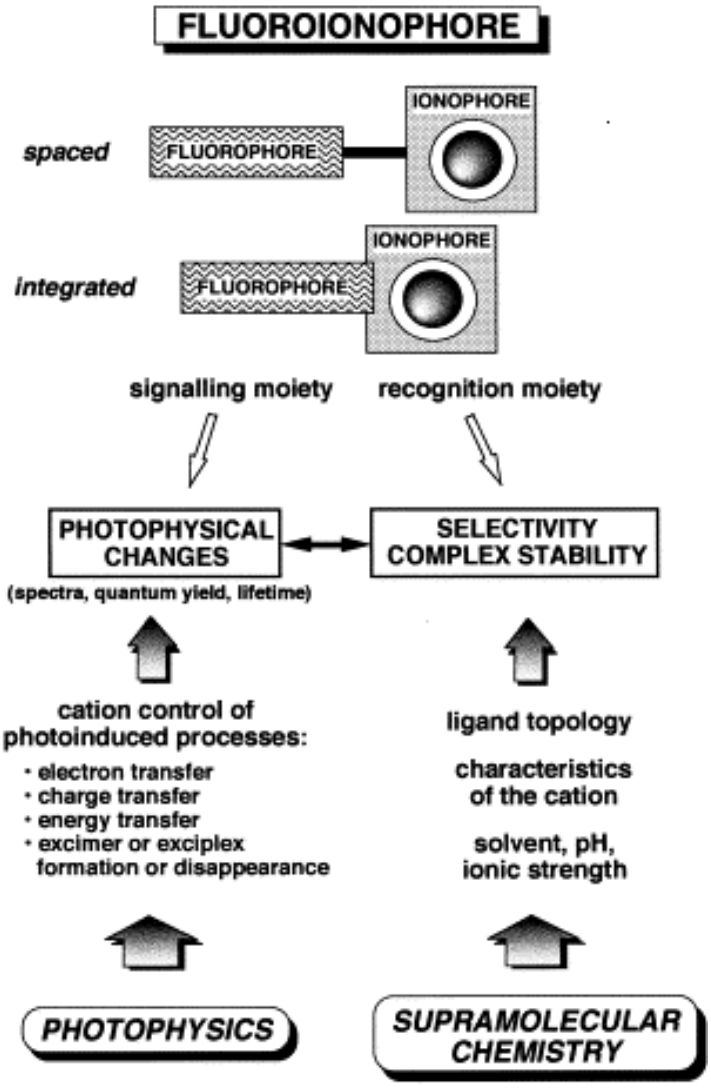


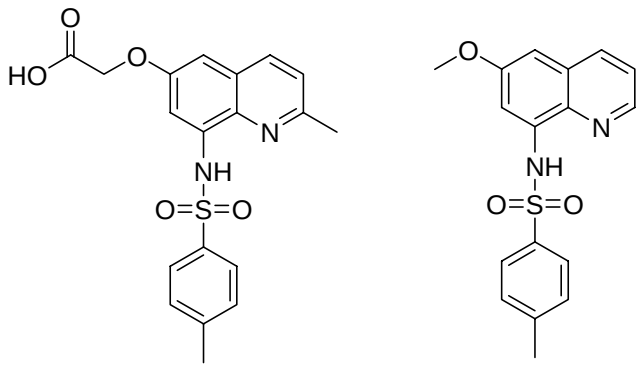
Figure 13. Main aspects of fluorescent molecular sensors for cation recognition

As indicated above cations has mas physiological effecsts on living cells, the effets of the many of these cations were explored. Alhough Zn^{2+} has many important cellular roles, little is known about the cellular recognition of Zn^{2+} in comparison with other cations such as Ca^{2+}, Na^{+}, K^{+}, etc. Zinc (Zn^{2+}) is the second most abundant heavy metal ion after iron, and it is an essential component of many protein scaffolds**.** Chelatable (Zn^{2+}) is released from nerve terminals by excitatory signals and binds to the N-methyl-D-aspartate receptor, changing its function, [35]. Zn^{2+} also suppresses apoptosis, and iduces the formation of β-amyloid, [36], which is thought to be related to the etiology of Alzheimer's disease. Zinc also plays a role

in following disesaes Crohn's disease, Diabetes, Growth of children, Abnormal outcomes of pregnancy, Diarrhea, Pneumonia.

Therefore, several chemical tools for measuring Zn^{2+} in living cells have recently been developed to clarify its physiological its physological significance, There are two types of fluorescent sensor molecules for Zn^{2+}, one based on a quinoline structure excitable with UV light (TSQ **15** [37], Zinquin **14** [38]) in Figure.14 and the other based on fluorescein (Zinpyr-1) and (ZnAF-1) [39] in Figure.15 excitable with visible light [40,41].

Zinquin ethyl ester and TSQ are analogues of each other. Zinquin ethyl ester is thus useful to detect the intracellular zinc ions. It forms a complex with a zinc ion with two nitrogen atoms in the structure. Generally most of the zinc sensors in literature senses the cadmium ion and makes fluorescent complex with the cadmium ion, however cadmium ions are not contained in normal living cells, The water solubility of zinquin ethyl ester and TSQ are poor.



Zinquin **14** TSQ **15**

Figure 14. Quinoline based Zn^{2+} sensors

A cell-permeable Zn^{2+} sensor molecule basedon fluorescein (Zinpyr-1) and ZnAF-1 was reported recently. Zinpyr-1 fluoresces strongly upon addition of Zn^{2+} to cells. However, it has the disadvantages that the basal fluorescence is high (quantum yield, 0.39) and is pH-sensitive with a p*K*a of 8.3. Thus, the fluorescence can be changed by intracellular pH changes under physiological conditions, and such pH

changes are observed in many cells exposed to certain biological sitmuli. ZnAF-1 is improved derivative of Zinpyr-1, in ZnAF-1 initially fluorescence is nearly Zero due to the PET mecahanism and with the addition of Zinc it fluoresces so it can be used

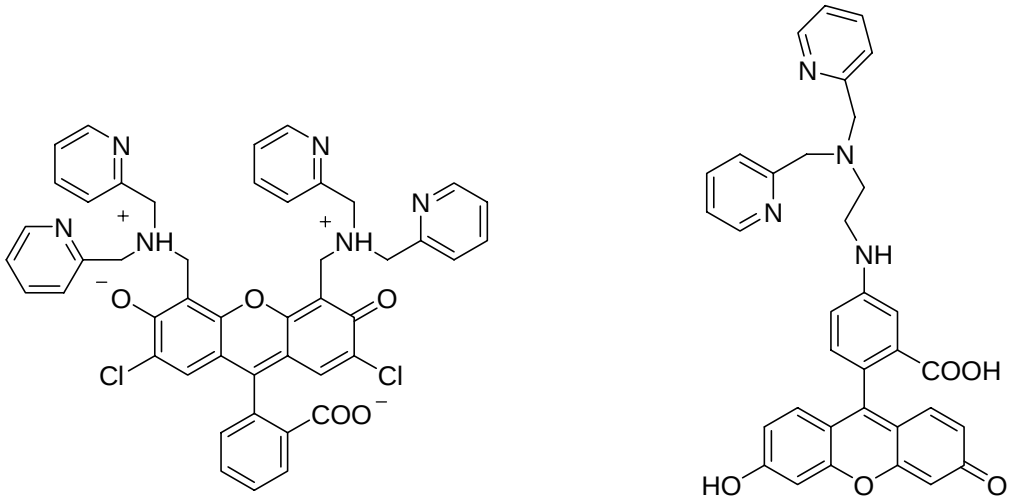


Figure 15. Fluorescein based Zn^{2+} sensors

for cellular activitives, and fluorescent of this compound is not much sensitive to physiological pH changes. The biggest disadvantage of ZnAF-1 is its multi-step synthesis. The following compound is synthesized by Kimura et.al and it shows selectivity for zinc at nanomolar concentration. Preliminary experiments of compound **18** in Figure 16 showed low toxicity against several cell lines and good cell permeability [42].

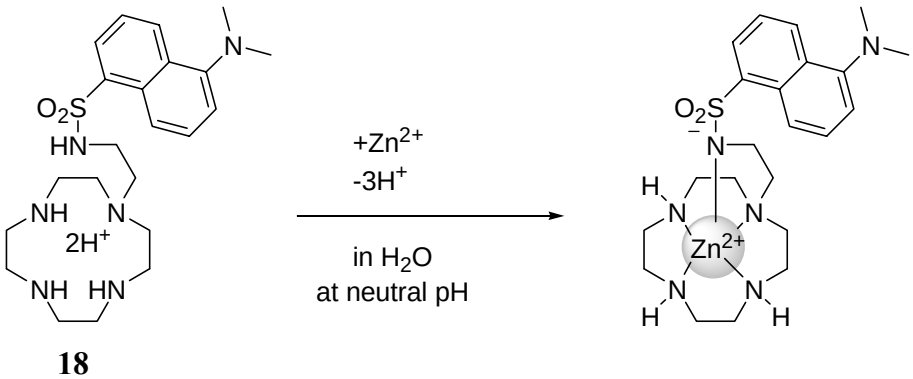
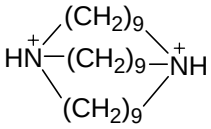


Figure 16. Dansyl-based (dansylamidoethylcyclen) Zn^{2+} sensor

1.6. Anion Recognition

Anion recognition chemistry has its roots in work conducted in the late 1960s around the same time that Pedersen reported the synthesis and coordination chemistry of crown ethers and Lehn published the first accounts of cation coordination chemistry by cryptands. In the 1970s, the coordination chemistry of group 1 and 2 metal and ammonium cations attracted most interest and consequently cation recognition is now a well-developed and mature area of supramolecular chemistry. Compared with the cation receptors, anion receptors were developed much later.[43]. In 1968 the first synthetic receptor for inorganic anions was reported (size selective binding of Cl^- anions was described [44] with diprotonated 1,11-diazabicyclo-[9.9.9]nonacosane) **19**. The field started to develop in 1976 when Graf and Lehn reported [45] that protonated cryptate **20** encapsulates F^-, Br^- and Cl^- anions. Since then several other anion receptors have been developed.



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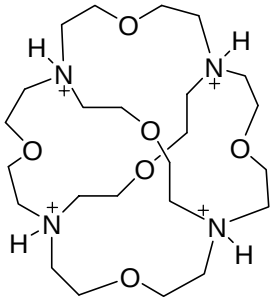


20

Figure 17. First examples of anion sensors

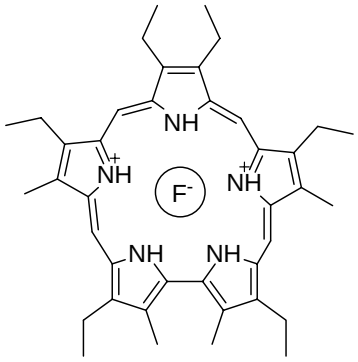
There are a number of reasons for this sudden growth in this new area of coordination chemistry. Anions are found biological systems and in biological processes. They carry genetic information (DNA is a polyanion) and the majority of enzyme substrates and co-factors are anionic. Anions also play roles in the areas of medicine and catalysis, pollutant anions have been linked to eutrophication (increasing of biomass) of rivers (from the over use of phosphate-containing fertilizers) and metabolites of nitrate. The production of technetate during the reprocessing of nuclear fuel (and its subsequent discharge into the seas and oceans) is also a matter of environmental concern. The design of anion receptors is

particularly challenging. There are a number of reasons for this. Anions are larger than isoelectronic cations and therefore have a lower charge to radius ratio. This means that electrostatic binding interactions are less effective than they would be for the smaller cation. Additionally anions may be sensitive to pH values (becoming protonated at low pH and so loosing their negative charge), thus receptors must function within the pH window of their target anion. Anionic species have a wide range of geometries and therefore a higher degree of design may be required to make receptors complementary to their anionic guest. Solvent effects also play a crucial role in controlling anion binding strength and selectivity. Electrostatic interactions generally dominate in anion solvation, and hydroxylic solvents in particular can form strong hydrogen bonds with anions. A potential anion receptor must therefore effectively compete with the solvent environment in which the anion-recognition event takes place. For example, a neutral receptor that binds anions solely through ion-dipole interactions may only complex anions in aprotic organic solvents, whereas a charged receptor has the capacity to bind highly solvated (hydrated) anions in protic solvent media. It is no coincidence that biological anion receptor systems are optimized to operate in a very specific range of environments where the source of selectivity for the biological anion is the difference in free energy lost on dehydrating the anion and that gained by the interaction of the anion with the binding site.[46]

For the sensing of anions in the past few years a wide range of anion sensors are synthesized, and they show varying degrees of affinity(and selectivity) towards anions such as F^-, Cl^-, $H_2PO_4^-$, and carboxylates. These are sapphyrins **21**, calixpyrroles **22, 23**, polyamines, guanidium, and we recently synthesized a BODIPY derivative for anion sensing [47]. Moreover, anions like phosphate, arsenate, technetate, etc., are important pollutants and selective recognition and/or signalling of these species is of prime importance.

Pyrrolic NH groups can be combined with electrostatic interactions to produce receptors that have an extremely high affinity for anions. Early work by Sessler et al. demonstrated that sapphyrins [48] are capable of coordinating to anions. The core of the sapphyrin macrocycle **21** may be doubly protonated to form a receptor with a positive charge and an array of five NH hydrogen-bonding groups.

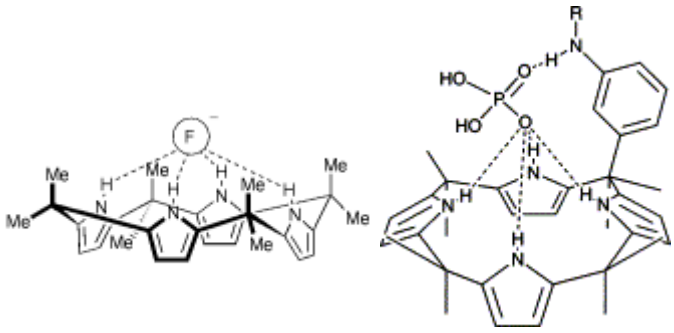
Solution-phase experiments indicated that fluoride ions bind over 10^3 times more strongly to diprotonated sapphyrin than either bromide or chloride ions.



21

Figure 18. Fluoride complex of sapphyrin

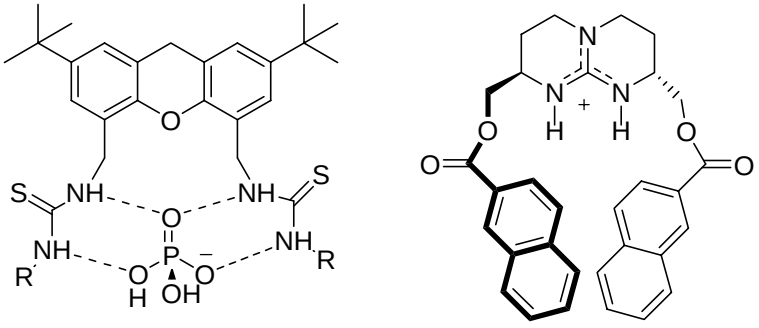
In 1996, Sessler and co-workers reported that calix[4]pyrroles (*meso*-octaalkylporphyrinogens), macrocycles first synthesized in the nineteenth century by Baeyer,also coordinate to anions, [49]. *meso*-Octamethylcalix[4]pyrrole **22** in Figure.19 was shown to form complexes with fluoride, chloride, and dihydrogen phosphate with stability constants of 17 200, 350, and 100 M^{-1} respectively . The conformation of the macrocycle in the solid state changes dramatically upon anion complexation. The free calixpyrrole adopts a 1,3-*alternate* conformation wherein adjacent rings are oriented in opposite directions. However the crystal structure of the chloride complex of **22** reveals that the macrocycle forms a *cone* conformation with the four pyrrole NH groups forming hydrogen bonds to the bound chloride ion



22 **23**

Figure 19. F^- and $H_2PO_4^-$ selective calixpyrrole derivatives

The increased affinity for phosphates is obtained for **23** [50], `two-point' interaction between the receptor and the bound anion provides a mode of binding which is not possible with the smaller anions like chloride, fluoride etc. R group in **23** could be fluorescein, dansyl or any fluorescent label, by this way with the binding of anions to this calix-pyrrole moieties a fluorescence response is obtained.



24 **25**

Figure 20. Bidentate anion sensors

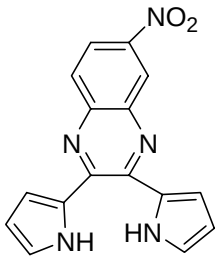
Urea and thiourea are particularly good hydrogen-bond donors and are excellent receptors for Y-shaped anions such as carboxylate through the formation of two hydrogen bonds. The very simple urea-based receptor **25** shows increasingly stable complexes with more highly charged and more basic bidentate anions .[51]

As has already been mentioned in the introduction, care must be taken with protonated polyammonium receptors so that the environment is sufficiently acidic for them to remain protonated whilst not too acidic to protonate any anionic guest. Guanidine is readily protonated to form the guanidinium ion **24** in Figure.20, which is stabilized by resonance and charge delocalization. With a pK_a of 13.6, the guanidinium cation is approximately three orders of magnitude more stable than a protonated secondary amine (pK_a≈10.5). Guanidinium therefore remains protonated up to high pH values, and is ideal for extending the pH range over which anion receptors operate. Schmidtchen and co-workers incorporated the guanidinium group into a bicyclic ring to form **24**. These receptors possess hydrogen-bonding arrays similar to those present in ureas. This has led to extensive use of guanidinium-based receptors for binding complementary carboxylate or phosphate guests.

Among the range of biologically important anions, fluoride is of particular

interest due to its established role in preventing dental caries. Fluoride anion is also being explored extensively as a treatment for osteoporosis, and, on a less salubrious level, can lead to fluorosis, a type of fluoride toxicity that generally manifests itself clinically in terms of increasing bone density. This diversity of function, both beneficial and otherwise, makes the problem of fluoride anion detection one of considerable current interest. While traditional methods of fluoride anion analysis such as ion selective electrodes like LaF_3 and 19F NMR spectroscopy remain important, electrodes and the methods for determining F^- concentrations are sensitive and selective, but under certain circumtances direct visualisation of intracellular F^- would be of great advantage of especially analytical biochemists. So the fluorescence signalling of fluoride anion remains as an important target, there is a few easy-to-use signalling agents which can recognize fluoride anion in solution and signal its presence via easy-to-detect optical signature. Sapphyrins **21** and calixpyrroles **22** are potential fluoride sensors, especially Sessler et.al [52] , calixpyrroles are not fluorescent, their working principle is the same as tridentate guanidinium **5**, firstly addition weakly binding fluorescent molecule at this instance fluorescence is quenched and with the addition of anion such as fluoride, chloride and phospahate , the fluorescent molecule will be released and the change in the emission intensity will be observed.

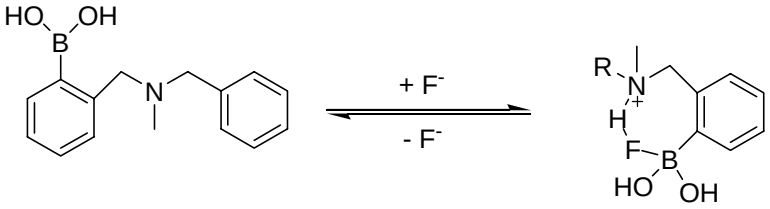
Sessler and co-workers reported that 2,3-dipyrrol-2′-ylquinoxalines such as **26** provide a simple, unexplored class of anion receptors that allow for the detection of fluoride ions in dichloromethane and DMSO under both visual (that is, naked eye) and fluorescence emission conditions,[53]. In fact, **26** undergoes a clear yellow to purple color change on addition of fluoride ions that is not observed on addition of other anions. The observed color changes also take place in DMSO, but reversed upon addition of water. This is presumably because water competes with the pyrrolic NH hydrogen bond donating sites for fluoride ions. Compound **26** in Figure.21 shows a remarkable selectivity for the fluoride ion (K_a(F)/K_a(Cl)>1800; K_a(F)/K_a($H_2PO_4^-$)>1400).



26

Figure 21. Dipyrrolylquinoxalines based Fluoride sensor

Another example for potential fluoride sensors are Boronic acid based sensors, fluoride has interesting interaction with Boron, and Cooper et.al, [54], showed that in the presence of fluoride -OH groups of Boronic acid is replaced by Fluoride.



27

Figure 22. Fluoride equilibrium of Boronic acid based fluoride sensor

With this equilibrium **27** shows exclusive selectivity for F^- and similar selectivity was observed by Shinkai and coworkers with ferrocene boronic acid, [55]

1.7. Molecular Switches

Logic gate is an elementary building block of a digital circuit. Most logic gates have two inputs and one output. At any given moment, every terminal is in one of the two binary conditions *low* (0) or *high* (1), represented by different voltage levels. The logic state of a terminal can, and generally does, change often, as the circuit processes data.

Developments in supramolecular chemistry and nanotechnology shows great interest in the construction of simple electronic or photonic driven systems and

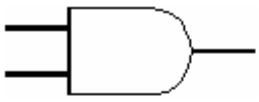
network that function as molecular level devices which are working by logic gates.[56]

As indicated at the beginning the binary logic of computing is based on bits that can be written and read as 0 or 1. This is achiavable in molecules as in many ways, but the most common are based on switching the optical properties of the molecule. Putting photons into molecules (absorbtion) and the collection of light that comes out of them (luminescence) are experimentally trivial processes that dont require physical linkages to be manufactured between components are in direct contrast to the problems associated with attracting a molecular wire to an electron source and measuring what comes out the other end. The so-called 'connection problem' is dramatically reduced when photons are used instead of electrons.

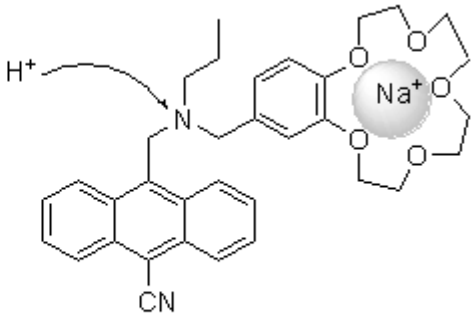
Fluorescent chemosensing is useful in biomedical research and it has been very recently developed into chemical logics, [57]. In the chemical logic system, the binding of a guest molecule to a host compound corresponds to the logic input and the resulting physical property change such as absorbtion and/or fluorescence spectra corresponds to the logic output.

There are seven basic logic gates: AND, OR, XOR, NOT, NAND, NOR, and XNOR. And all of this logic gates were achieved by molecules.

The *AND gate* is so named because, if 0 is called "false" and 1 is called "true," the gate acts in the same way as the logical "and" operator. The following illustration and table show the circuit symbol and logic combinations for an AND gate. (In the symbol, the input terminals are at left and the output terminal is at right.) The output is "true" when both inputs are "true." Otherwise, the output is "false". The first case of a molecule scale logic gate designed of primary importance was the AND gate. This molecule **28** displayed the property that two possible PET channels from the receptors needed to be suppressed if a strong fluorescence output was to be obtained. This was arranged by providing the two guest species that these two receptors were selective. The amine unit required H^+ ($input_1$) whereas the benzocrown ether moiety required Na^+ ($input_2$). This satisfied AND logic.[58]



Input 1	Input 2	Output
0	0	0
0	1	0
1	0	0
1	1	1

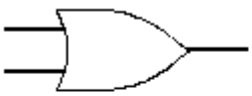


28

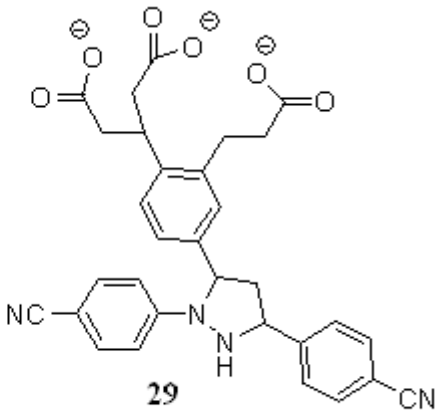
AND Gate

Figure 23. Digital and Molecular representation of AND logic gate

The *OR gate* gets its name from the fact that it behaves after the fashion of the logical inclusive "or." The output is "true" if either or both of the inputs are "true." If both inputs are "false," then the output is "false." The OR gate requires a set of nonselective receptors gives a positive optical response upon cation binding. The less selective the receptor, the operationally better OR action. The first intentionally designed logic OR gate in which Ca^{2+} and Mg^{2+} produce essentially identical fluorescence enhancements **29**.[59]



Input 1	Input 2	Output
0	0	0
0	1	1
1	0	1
1	1	1

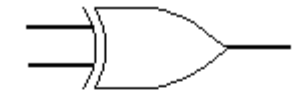


29

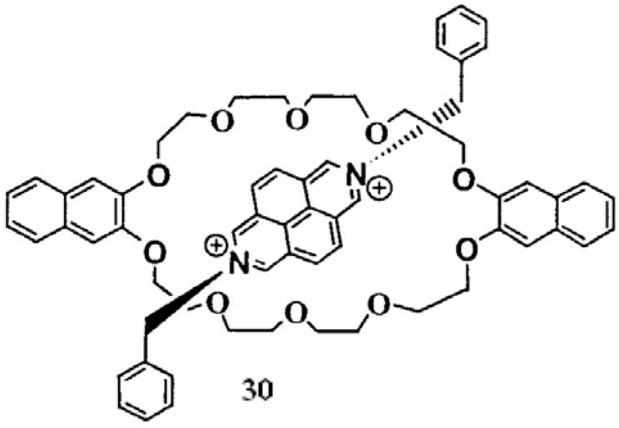
OR Gate

Figure 24. Digital and Molecular representation of OR logic gate.

The *XOR* (*exclusive-OR*) *gate* acts in the same way as the logical "either/or." The output is "true" if either, but not both, of the inputs are "true." The output is "false" if both inputs are "false" or if both inputs are "true." Another way of looking at this circuit is to observe that the output is 1 if the inputs are different, but 0 if the inputs are the same. XOR is the one of the hardest logic gate to proceed chemically. In today's processors, addition is performed with an AND gate which gives the carry digit and an XOR gate is actually a comparator because it can establish whether the two inputs have the same value. The pesudorotaxane **30** in Figure 25 results from the self-assembly of the electron-accepting 2,7-dibenzyldiazapyrenium dication with the crown ether which contains two 2,3-dioxynaphthalene units. Because of the electron donor/acceptor interaction, a low energy CT excited state is formed which is responsible for (i) the presence of a weak and broad absorbtion band (ii) the dissappearance of the strong fluorescence exhibited by two separated components.[60]



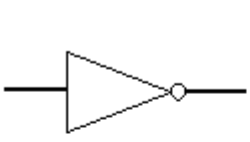
Input 1	Input 2	Output
0	0	0
0	1	1
1	0	1
1	1	0



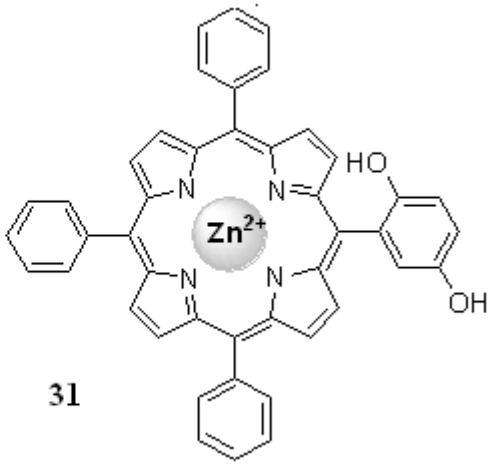
XOR Gate

Figure 25. Digital and Molecular representation of XOR logic gate.

A logical *inverter*, sometimes called a *NOT gate* to differentiate it from other types of electronic inverter devices, has only one input. It reverses the logic state. For **31** a bright green fluorescence is observed only when the guest is absent from the receptor site of porphyrin unit in Figure 26.[61]



Input	Output
1	0
0	1

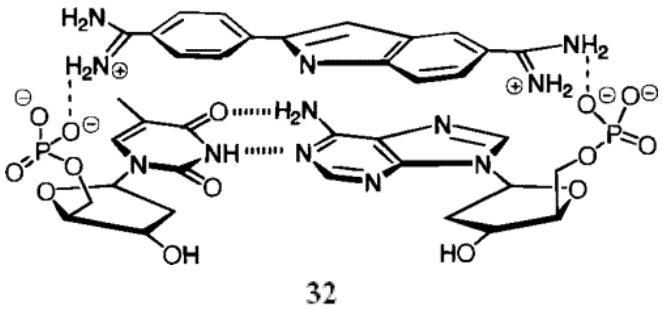


31

Figure 26. Digital and Molecular representation of NOT logic gate.

The *NAND gate* operates as an AND gate followed by a NOT gate. It acts in the manner of the logical operation "and" followed by negation. The output is "false" if both inputs are "true." Otherwise, the output is "true." A DNA-binding dye, 4',6-diamidino-2-phenylindole (DAPI) signals AT base pairing with a shift in the fluorescence emission spectrum. The signaling follows Watson-Crick base-pairing rules, and both dAMP and dTMP are required for the largest spectral shift. Thus, the dye with its two phosphate receptor sites functions as a molecular NAND gate accepting nucleotides as inputs. this DAPI-based system is the first report of a gate design in which hydrogen bonding interactions have been utilized.[62]

Input 1	Input 2	Output
0	0	1
0	1	1
1	0	1
1	1	0

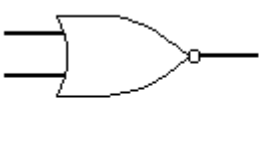


32

NAND Logic gate

Figure 27. Digital and Molecular representation of NAND logic gate

The *NOR gate* is a combination OR gate followed by an inverter. Its output is "true" if both inputs are "false." Otherwise, the output is "false. Fluorescence from **33** is switched 'OFF' by Zn^{2+}. Similar action of H^{+} can also be seen.[63]



Input 1	Input 2	Output
0	0	1
0	1	0
1	0	0
1	1	0

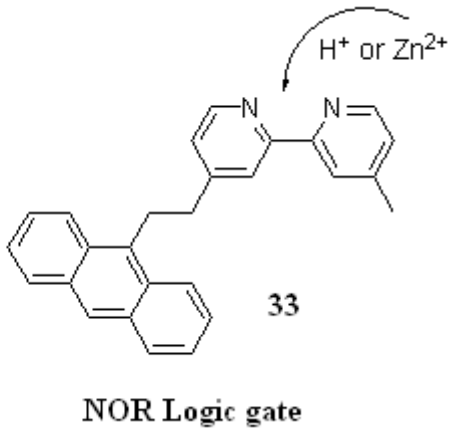
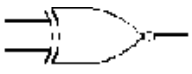


Figure 28. Digital and Molecular representation of NOR logic gate

The *XNOR (exclusive-NOR) gate* is a combination XOR gate followed by an inverter. Its output is "true" if the inputs are the same, and "false" if the inputs are different. The complex in Figure.29 which forms with the combination of **34** and **35** displays a charge transfer absorbtion band, equivalent to an output 1.[64]



Input 1	Input 2	Output
0	0	1
0	1	0
1	0	0
1	1	1

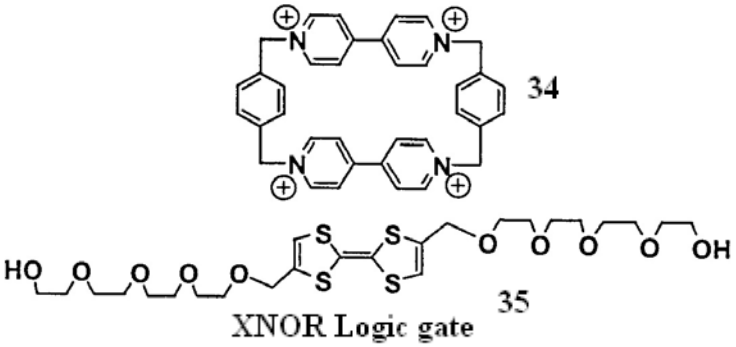


Figure 29. Digital and Molecular representation of XNOR logic gate

CHAPTER 2

EXPERIMENTAL

2.1. Instrumentation

1H and 13C NMR spectra were recorded on a Bruker Instruments Avance Series-Spectrospin DPX-400 Ultra shield (400 MHz) High Performance digital FT-NMR spectrometer (METU, NMR Laboratory). All chemical shifts are referenced to residual signals previously refenced to TMS and splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), p (pentet), dt (doublet of triplet) and br (broad).

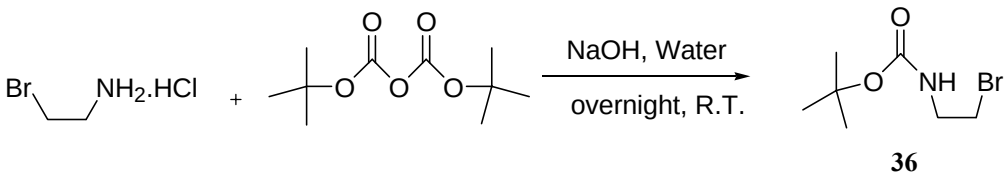
Electronic absorbtion spectra were recorded on a Shimadzu UV-1601 spectrophotometer. A Perkin-Elmer LS 50 B luminescence spectrometer was used for recording the fluorescence emission spectra. All instrumental parameters were controlled by Fluorescence Data Manager Software (FLDM). Measurements were conducted at 25^{o}C using a 1x0.5 cm rectengular quartz cuvette.

ESI-MS analysis were recorded on a Agilent 1100 MSD spectrometer (TUBITAK-ATAL analysis laboratory)

Chemicals and solvents were purchased from Aldrich and used without further purification. Column chromatography of all the products were performed using Merck Silica Gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM) pretreated with eluent. Reactions were monitored by thin layer chromatography using Merck Silica Gel 60 Kiesegel F_{254} TLC Aluminum Sheets 20x20 cm.

2.2. Synthesis of (2-bromoethyl)-carbamic acid tert-butyl ester (36)

2-Bromoethylamine hydrochloride (5.00 g, 24,4 mmol) was added to a cooled solution of sodium hydroxide (3,886 g, 97 mmol) in 70 mL water. Di-tert-butyl dicarbonate (Boc_2O) (5,761 g, 26,4 mmol, 1,1 equiv.) was carefully added over a period of 30 min and the reaction mixture was stirred overnight at room temperature (r.t.). The product was extracted with ethyl acetate (2x50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to obtain the protected amine N-Boc-2-bromoethylamine as a pale yellow oil.(5,2 g, 93%). ^1H NMR ($CDCl_3$) :δ (ppm) 1.26 (s, 9H, $C(CH_3)_3$), 3.27 (t, 2H, *J*=5.2, -CH_2NH-), 3.34 (t, 2H, *J*=5.2, -CH_2Br), 4.88 (br, 1H, -NH-). Without further purification **36** was used through next step.



36

Figure 30. Boc protection of compound **36**

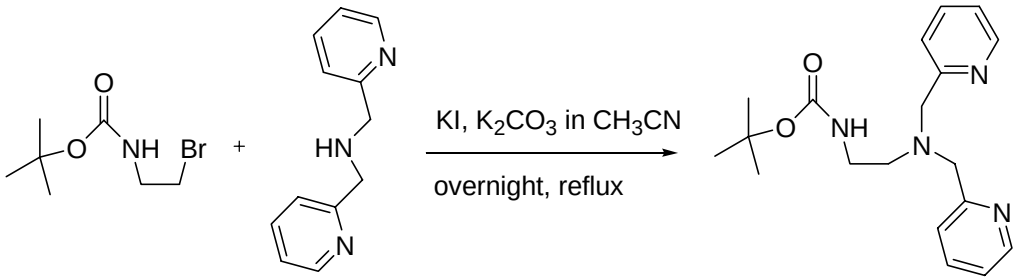
2.3. Synthesis of [2-(Bis-pyridin-2-ylmethyl-amino)-ethyl]-carbamic acid tert-butyl ester (37)

A suspension of **36** (1.688 g, 7.54 mmol), 2,2'-dipicolylamine (1.5143 g, 7.60 mmol), KI (2.1581 g, 13 mmol) and K_2CO_3 (1.797 g, 13 mmol) in 150 ml Acetonitrile was refluxed overnight. Acetonitrile was removed by evaporation, and the residue was diluted with 100 ml 2N sodium carbonate and extracted 2 times with dichloromethane. The organic phase was washed with brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was chromatographed on silica gel and eluted with chloroform-methanol 9:1 (v/v) to yield 1.172 g **37**. Yield 46%. Modified procedure adapted from [65]

^{1}H NMR ($CDCl_3$) :δ (ppm) 1.14 (s, 9H), 2.40 (t, 2H, *J*=5.7), 2.92 (t, 2H), 3.55 (s, 4H), 5.78 (br, 1H), 7.07 (m, 2H), 7.34 (d, 2H, *J*= 7.7), 7.55 (dt, 2H, *J*=7.7), 8.46 (d, 2H, *J*=4.1). (Figure 62)

^{13}C NMR ($CDCl_3$) :δ (ppm) 28.36, 38.78, 53.89, 60.27, 78.58, 122.43, 123.28, 136.81, 149.23, 156.19, 159.55. (Figure 63)

ESI-MS (m/z); 343.20 [M+H], calc. 343.21[M+H]. (Figure 73)



36 **37**

Figure 31. Reaction scheme for the formation of **37**

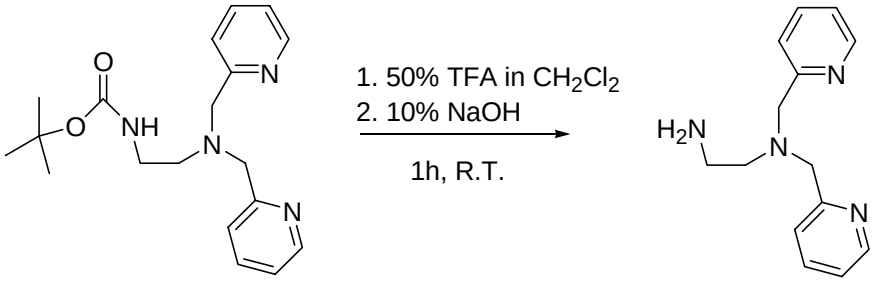
2.4. Synthesis of 2-(Bis-pyridin-2-ylmethyl-amino)-ethylamine (38)

The boc-protected picolyamine derivative **37** (1.5 g, 4.38 mmol) was deprotected with a 50 % TFA-solution in CH_2Cl_2 for 1h. Then the excess TFA and CH_2Cl_2 was evaporated and 10% NaOH solution (100 mL) was added to the reaction mixture. Aqueous solution was extracted three times with 50 ml portions of CH_2Cl_2 and dried over Na_2SO_4 and evaporated to dryness to afford 1.069 mg of **38**. Yield 92%.

^{1}H NMR ($CDCl_3$) :δ (ppm) 1.95 (br, 2H), 2.56 (t, 2H, *J*= 5.9), 2.69 (t, 2H, *J*= 5.9), 3.74 (s, 4H), 7.05 (m, 2H), 7.40 (d, 2H, *J*= 7.5), 7.56 (dt, 2H, *J*= 7.5), 8.44 (d, 2H, *J*= 4.5). (Figure 64)

^{13}C NMR ($CDCl_3$) :δ (ppm) 39.88, 57.54, 60.77, 122.01, 123.14, 136.39, 149.23, 159.88. (Figure 65)

ESI-MS (m/z); 243.10 [M+H], calc. 243.15 [M+H]. (Figure 73)



36 **38**

Figure 32. Deprotection of Boc-protected picolylamine derivative

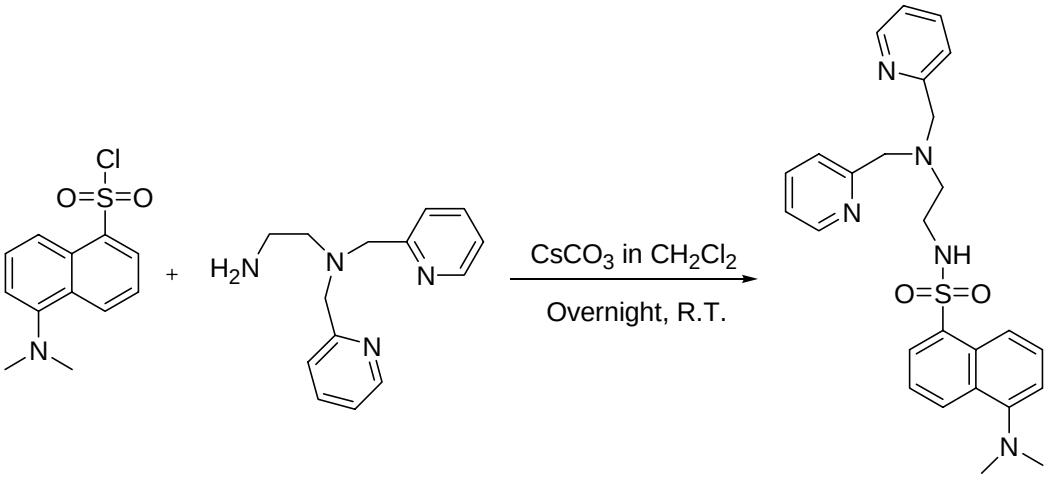
2.5. Synthesis of 5-Dimethylamino-naphthalene-1-sulfonic acid [2-(bis-pyridin-2-ylmethyl-amino)-ethyl]-amide (40)

A mixture of dansylchloride **39** (122 mg, 0.45mmol), **38** (130 mg, 0,54 mmol) and $CsCO_3$ (176 mg, 0,54 mmol) in 10 ml CH_2Cl_2 was stirred overnight at R.T. The reaction mixture was filtered through celite. The residue dried over Na_2SO_4 and evaporated to dryness. The crude product was chromatographed on silica gel and eluted with chloroform-methanol 85:15 (v/v) to afford 163 mg of **40.** Yield 76 %.

^{1}H NMR ($CDCl_3$) :δ (ppm) 2.50 (t, 2H, *J*=5.5), 2.62 (s, 6H), 2.77 (m, 2H), 3.45 (s, 4H), 6.99-7.06 (m, 4H), 7.31-7.47 (m, 4H), 8.03 (br, 1H), 8.17 (dd, 1H, *J*=7.4), 8.41 (t, 2H, J= 7.4), 8.50 (d, 2H, J= 4.1). (Figure 66)

^{13}C NMR ($CDCl_3$) :δ (ppm) 41.87, 45.78, 59.55, 115.33, 119.97, 122.50, 123.28, 123.57, 128.13, 129.62, 130.23, 130.25, 130.29, 135.87, 136.91, 149.39, 152.12, 159.18. (Figure 67)

ESI-MS (m/z); 476.20 [M+H], calc. 476.20 [M+H]. (Figure 74)



39 **38** **40**

Figure 33. Reaction scheme for compound **40**

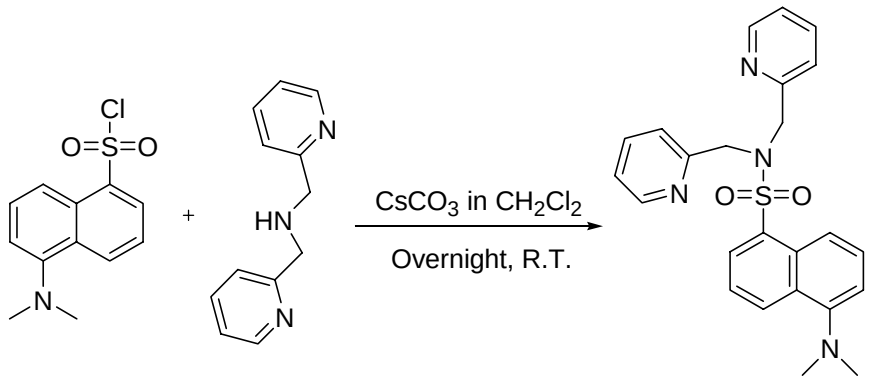
2.5. Synthesis of 5-Dimethylamino-naphthalene-1-sulfonic acid bis-pyridin-2-ylmethyl-amide (41)

A mixture of dansylchloride **39** (337 mg, 1.25mmol), 2,2'-dipicolylamine (300 mg, 1,50 mmol) and $CsCO_3$ (489 mg, 1,50 mmol) in 15 mL CH_2Cl_2 was stirred overnight at rt. The reaction mixture was then filtered through celite. The residue dried over Na_2SO_4 and evaporated to dryness. The crude product was chromatographed on silica gel and eluted with chloroform-methanol 97:3 (v/v) to afford 340 mg of **41.** Yield 63%.

^{1}H NMR ($CDCl_3$) :δ (ppm) 2.99 (s, 6H), 4.85 (s, 4H), 7.13 (t, 2H, *J*= 5.0), 7.28 (t, 3H, *J*= 11), 7.52-7.57 (m, 3H), 7.63 (t, 1H, *J*= 8.4), 8.35 (d, 1H, *J*= 7.2), 8.46 (d, 2H, *J*= 5.0), 8.52 (d, 1H, *J*= 8.4), 8.59 (d, 1H, J= 8.4). (Figure 68)

^{13}C NMR ($CDCl_3$) :δ (ppm) 45.83, 53.66, 115.57, 119.95, 122.63, 122.81, 123.52, 128.56, 130.16, 130.37, 130.54, 130.77, 135.68, 136.76, 149.41, 152.12, 156.65. (Figure 69)

ESI-MS (m/z); 433.10 [M+H], calc. 433.16 [M+H]. (Figure 74)



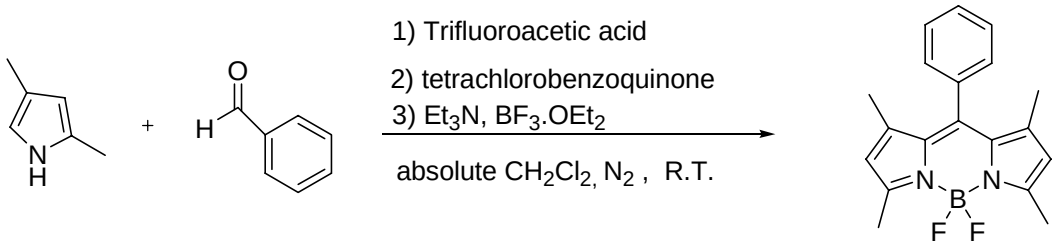
39 **41**

Figure 34. Reaction scheme for compound **41**

2.6. Synthesis of 1,3,5,7-Tetramethyl-8-phenyl-difluorobordiaza-s-indacene (42)

2,4-dimethylpyrrole (220 mg, 2.32 mmol) and benzaldehyde (130 mg, 1.16 mmol) were dissolved in 250 mL absolute CH_2Cl_2 (N_2 was bubled through CH_2Cl_2 for 30 min) under nitrogen atmosphere. One drop of TFA was added and the solution stirred at r.t. until TLC-control (silica: CH_2Cl_2) showed the complete consumption (nearly 2h) of the aldehyde. At this point, a solution of tetrachlorobenzoquinone (285 mg, 1,16 mmol)in 100 mL absolute CH_2Cl_2 was added, stirring was continued for15 min followed by the addition of 3 mL of Et_3N and 3 mL of $BF_3.Et_2O$. After stirring for 30 min the reaction mixture was washed with water, dried over Na_2SO_4 and evaporated to dryness. the residue was chromatographed on silica gel and eluted with CH_2Cl_2 to afford 162 mg **42** as orange needles. Yield 44 %.[66]

^{1}H NMR ($CDCl_3$) :δ (ppm) 1.37 (s, 6H), 2.56 (s, 6H), 5.98 (s, 2H), 7.26-7.30 (m, 2H), 7.47-7.50 (m, 3H). (Figure 71)



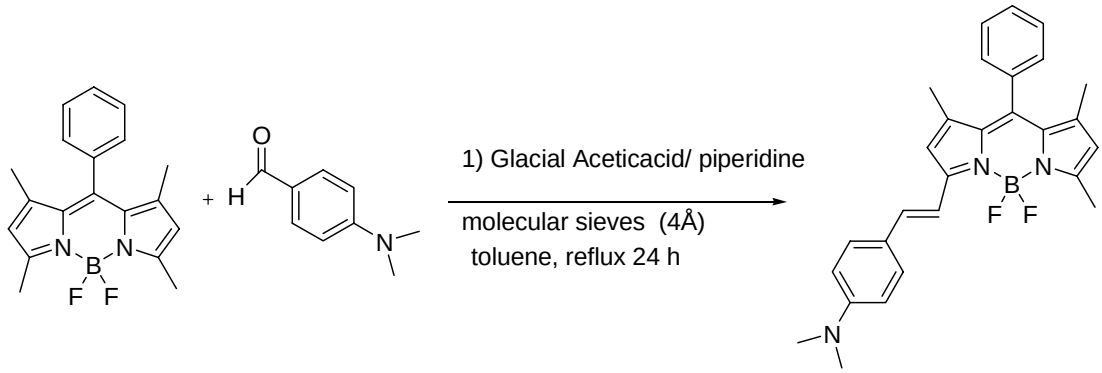
42

Figure 35. Synthesis of BODIPY derivative **42**

2.7. Functionalization of boron-dipyrromethene dye (44)

Boron-dipyrromethene dye **42** (50 mg, 0.154 mmol) and 4-dimethylaminobenzaldehyde **43** (24mg, 0.155 mmol) were refluxed overnight in a mixture of toluene (6 ml), glacial acetic acid (0.115 mL) and piperidine (0.140 mL) together with small amount of molecular sieves(4Å). After cooling to room temperature the mixture was placed on the top of silica column and eluted with CH_2Cl_2/hexane (1/1). The blue fraction was collected and recrystallized from $CHCl_3$/Hexane (1/1.5) to afford 22.4 mg as purple needles. Yield 32%. The procedure adapted from [67]

^{1}H NMR ($CDCl_3$) :δ (ppm) 1.39 (s, 3H), 1.44 (s, 3H), 2.60 (s, 3H), 3.06 (s, 6H), 5.96 (s, 1H), 6.59 (s, 1H), 6.69 (d, 2H, *J*= 8.6), 7.19 (d, 2H), 7.28-7.33 (m, 2H), 7.45-7.55 (m, 6H). (Figure 70)



42 **43** **44**

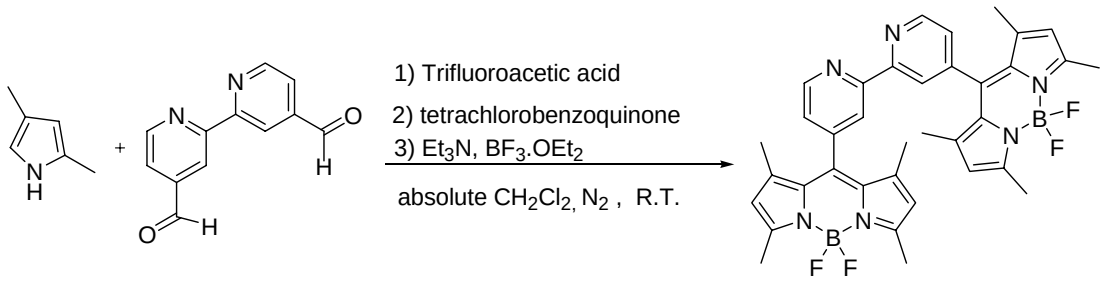
Figure 36. Synthesis of functionalized BODIPY dye **44**

2.8. Synthesis of Bis-boradiazaindacenyl-bipyridine BODIPY (46)

2,4-dimethylpyrrole (204 mg, 1.38 mmol) and bipyridinedicarboxyaldehyde (0.35 mmol) **45** were dissolved in 200 mL absolute CH_2Cl_2 (N_2 was bubled through CH_2Cl_2 for 30 min) under nitrogen atmosphere. One drop of TFA was added and the

solution stirred at r.t. until TLC-control (silica: CH_2Cl_2) showed the complete consumption (nearly 3h) of the aldehyde. At this point, a solution of tetrachlorobenzoquinone (340 mg, 1.38 mmol)in 50 mL absolute CH_2Cl_2 was added, stirring was continued for15 min followed by the addition of 3 mL of Et_3N and 3 mL of $BF_3.Et_2O$. After stirring for 30 min the reaction mixture was washed three times with water, dried over Na_2SO_4 and evaporated to dryness. the residue was chromatographed on silica gel and eluted with CH_2Cl_2 to afford **46**. It was the third band, orange in color, having a bright green fluorescence. Yield 20 %. [66]

^{1}H NMR ($CDCl_3$): δ (ppm) 1.37 (s, 12H), 2.50 (s, 12H), 5.92 (s, 4H), 7.28 (d, 2H, *J*=4.9), 8.48 (s, 2H), 8.70 (d, 2H, *J*=4.9). (Figure 72)



45 **46**

Figure 37. Synthesis of Bis-boradiazaindacenyl-bipyridine BODIPY **46**

CHAPTER 3

RESULT AND DISCUSSION

In the first part of study, we synthesized Bis-boradiazaindacenylBODIPY **46** and with the addition of $Zn(ClO_4)_2$ in acetonitrile fluorescence of this compound is quenched due to oxidative PET mechanism. In an earlier work in our lab was proposed as zinc ion sensor [26], the fluorescence emission intensity of the 1:1 Zn(II) complex of doubly boradiazaindacene (BODIPY) substituted bipyridyl ligand **46** is highly sensitive to anion coordination to the metal center. Oxidative PET, which is responsible for the quenching of the fluorescence in the complex is effectively inhibited by anion coordination, leading to a 25-fold enhancement of the emission intensity, at different concentrations of anions.

In second part, we investigated that exquisite selectivity was shown for the F^- ion by BODIPY unit and we synthesized different BODIPY derivatives **42, 44** for this purpose. We proposed that this sensing occurs due to the deboronylation equilibrium between fluoride anion and the BODIPY unit.

In third part logic gate behaviour of compound **46** was presented, here the inputs are Fluoride ion and Zinc ion, As a result of this studies we obtained a substraction process, this is the first example of this by using BODIPY unit. The key issue here the exquisite selectivity of BODIPY unit for Fluoride anion and the queching of fluorescence of **46** with the addition of Zinc ion.

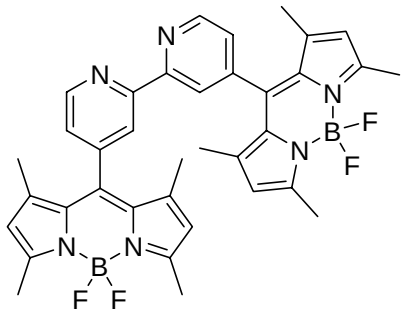
In the last part; we synthesized two different compounds **40** and **41** which contain a chelator Picololyl amine unit and its derivative **38**. As a fluorophore dansyl unit **39** was used in this structures. The zinc selectivity of this compound was

obtained at less than μM concentration of zinc ion in aqueous media. Also in this study we showed the effect of –NH group in sulfonamide unit for zinc selectivity with the comparison of **40** and **41,** compound **41** doesnt contain this unit.

3.1. Novel fluorescent chemosensor (46) for anions via modulation of oxidative PET, [47]

Effective signaling of anions remains to be a challenging target of chemosensor designs. Anions in general, play important roles in biological processes and anomalies in regulation and intake of certain anions are implicated in disease states. Moreover, anions like phosphate, arsenate, technetate, etc., are important pollutants and selective recognition and/or signaling of these species is of prime importance. Fluorescence signaling is preferable considering a multitude of practical issues, including sensitivity and inherent selectivity.

As a part of our own research program in exploring photophysical phenomena for fluorescent chemosensor applications [68], a synthesis of bis-BODIPY(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene)-substituted bipyridyl ligand was reported by our group(**46**).[26]

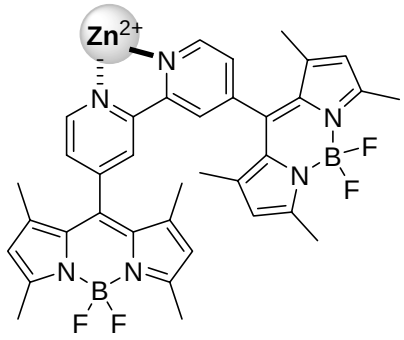


46

Figure 38. Structure of Bis-boradiazaindacenyl-bipyridine BODIPY **46**

The ligand has a very high quantum yield in organic solvents (in acetonitrile, Φ_f= 0.39), but metal chelation quenches the fluorescence. One of the most effective quenchers of the fluorescence of compound **46** is Zn(II), thus eliminating the

possibility of a simple paramagnetic/HTM related quenching. In fact, we proposed that the quenching was due to oxidative photoinduced electron transfer (PET); from the excited BODIPY units to the metal complexed bipyridyl group.



47

Figure 39. Zinc complex of compound **46**

Considering the magnitude and effectiveness of this quenching, we explored processes which could restore the BODIPY emission, thereby signaling the causative analyte. There have been some recent examples of target analytes, [69], chelating to a metal complex and thus signaling the analyte anion concentration. However, the observed effects are only incremental changes in emission intensity. We reasoned that total restoration of the BODIPY emission should be possible with simple chelating anions like phosphate and acetate.

To explore the anion response of the metal complex, we have prepared a solution of the complex in acetonitile. To a $2.3x10^{-7}$ M solution of the ligand, we added 0.1 mM Zn(II) in the form of perchlorate salt. Given the stoichiometry and association constant with Zn(II), we estimate that more than 99% of the ligand is metal-bound. The metal complex was then titrated with tetrabutylammonium salts, F^-,Cl^-, Br^-, SO_4^{2-}, HPO_4^{2-} and acetate. For this purpose 10 mM anion solutions were prepared in acetonitrile, it can be clearly seen from figure 40 at 0.25 mM anion concentration, Among these anions fluoride, phosphate and acetate displayed strongest binding effects. Depending upon the binding ability of anions they shows differences in emission spectrum

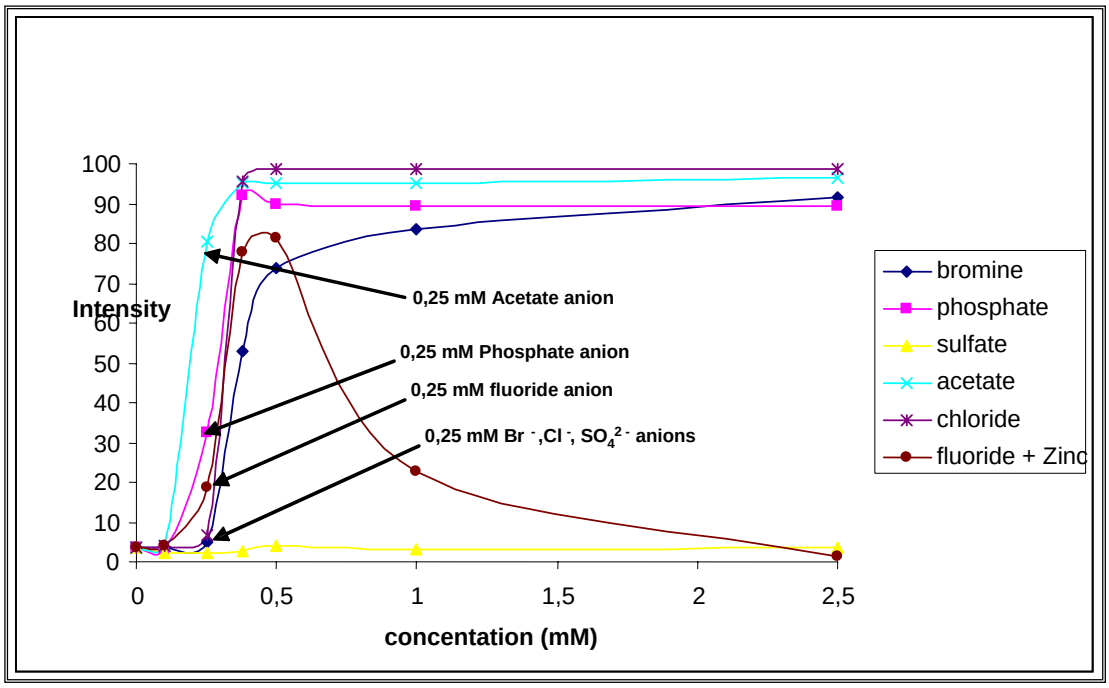


Figure 40. The change in the intensity of Zinc-BODIPY complex with different anions, excited at 480 nm

Apparent dissociation constants (K_d) were determined by plotting the emission values against the concentration of anions and fitted to the following equation, $F=(F_o+F_{max}[A]/K_d)/(1+[A]/K_d)$, where F_o is the initial F value for the zinc complex in the absence of anions, F_{max} is the maximum F value and [A] is the anion concentration. The dissociation constants (K_d) determined this way are as follows: acetate 0.21 mM; phosphate 0.25 mM; fluoride 0.24 mM; chloride 0.31 mM and bromide 0.40 mM in figure 40.

The reversible chelation of the anions to the metal center is electrostatic in nature with very little coordination complex character. This is of course desirable for the signaling of analytes in dynamic systems. As expected, neither the zinc complexation nor the added anion causes a change in the absorption spectrum. The effect of phosphate binding on the emission spectrum is shown in figure 41 . There is a remarkable and to best of our knowledge, unprecedented 25-fold enhancement of the emission intensity at 518 nm on phosphate binding. This really one of the most important outcome of our reseach, because most of the anion sensors in

literature show, mostly quenching of fluorescence or slight enhancement of emission intensity upon anion binding.

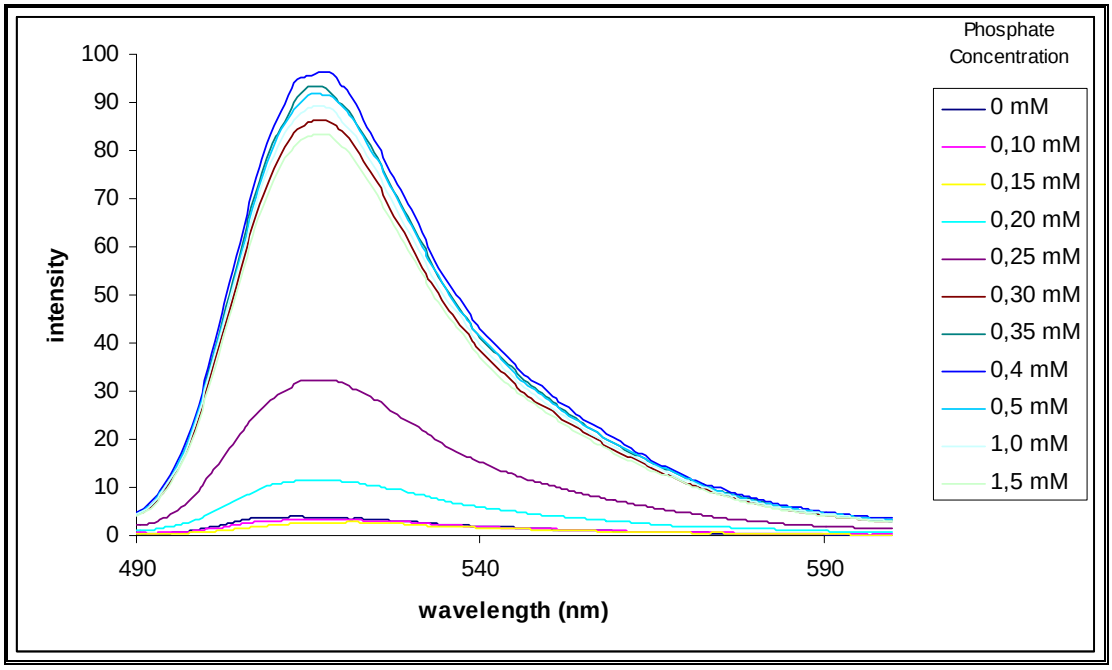


Figure 41. Change in the emission spectrum of Zinc-BODIPY complex with the addition of phosphate anion excited at 480 nm

In this novel anion sensing system the enhancement of emission is truly spectacular. BODIPY-Zn(II) complex solution of no visually discernable fluorescence emission becomes bright green fluorescent on the addition of less than a millimolar of phosphate. Proposed equilibrium for the binding of phosphate anion is given in figure 42.

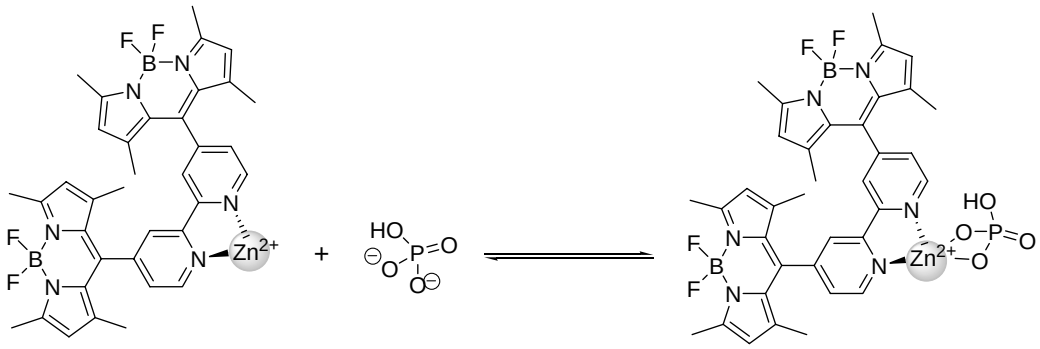


Figure 42. Equilibrium between BODIPY-Zinc complex and phosphate anion

Unlike many other anion sensing systems where only a few fold change in emission intensity is the norm, here, even in relatively polar acetonitrile solution a remarkable change is observed. Quantum yield varies between less than 0.002 to 0.39. The origin of this enhancement is the inhibition of oxidative PET. The added anions are not strong chelators that could remove the complexed Zn(II), but through simple electrostatic interactions they can, at least partially, neutralize the charge on the metal center. Thus, otherwise favorable oxidative PET process becomes thermodynamically unfavorable, hence the full emission intensity of the boradiazaindacene fluorophore is restored. We believe by appropriate selection of the chelator units (i.e.; stronger metal binding ligands) tethered to the fluorophore, this approach can be extended to polar and more competitive media like water. The fluorescent enhancement factor (FEF) is comparable for all anions at saturation, except for sulfate and fluoride. Sulfate, in agreement with earlier findings, [66], does not induce any change in the emission intensity. In figure 40 we also expect for fluoride to reach saturation restore the full emission intensity of boradiazaindacene fluorophore. Fluoride, surprisingly causes a decrease in the emission intensity. Then the absorbance spectrum of **46** was taken, at different

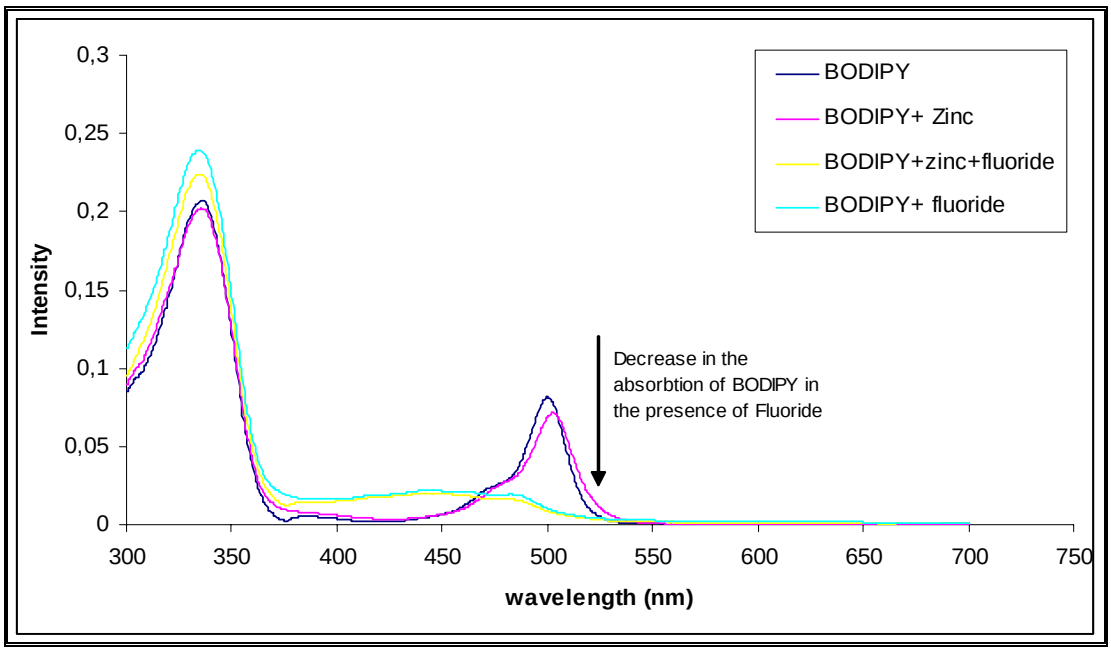


Figure 43. Absorbance spectrum of BODIPY **46**

conditions in figure 43. As expected there is no difference in the absorbance spectrum of BODIPY and Zinc-BODIPY complex, but with the addition of fluoride both in the presence and the absence of zinc ion the absorbance of boradiazaindacene fluorophore is decreased, new and broad absorbance band is formed. Also the in figure 44 change in the intensity of BODIPY unit can be seen both in the presence and in the absence of zinc ion. In the presence of zinc, firstly interaction of fluoride anion with the metal center, as a result of this, our fluorophore

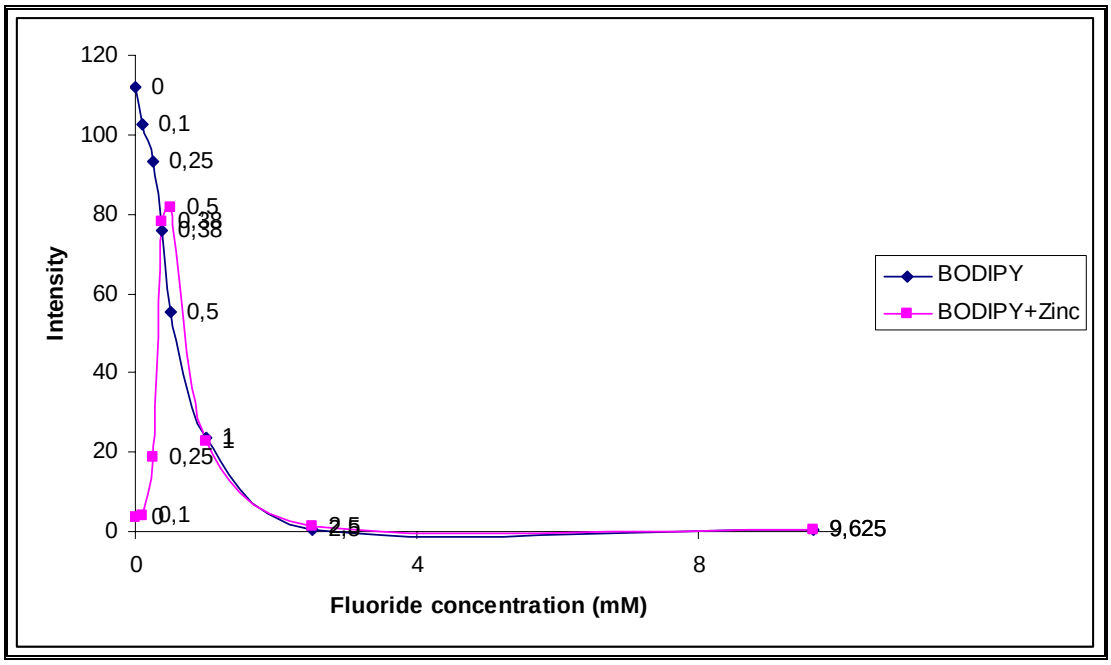


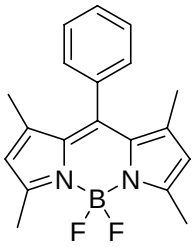
Figure 44. Emission spectrum of Both BODIPY and Zinc-BODIPY complex with increasing concentration of Fluoride anion.

partially restored the emission intensity. When it reaches to the saturation point it can be clearly seen from the graph that the emission intensity of Fluorophore is at the baseline. In the absence of zinc ion, there is no metal center for anion coordination, and we can clearly see the effect of fluoride anion, it directly decreases the emission of **46**. When we combine our results both from the absorbance and the emission data, the result is that there has to be a structural change in the BODIPY

unit, and the only possible place for this change is the boron linkage in the body of BODIPY. With this outcome of this project, we decided to use BODIPY unit as a fluoride sensor. For this purpose we synthesized, different BODIPY derivatives. The results will be explained in the second part in detail.

3.2. Deboronylation Equilibrium of Extended Conjugation Difluorobora-*s*-diazaindacene Dyes: A Fluoride Chemosensor with an Exquisite Selectivity

As we discovered in the first part for compound **46**, fluoride binding to the zinc(II) complex of this compound had an unexpected emission response which was not shared by other anions, the emission intensity at long wavelengths decreased. To perform further investigations on this unexpected result, the parent BODIPY derivatives were synthesized according to known literature procedure, and we made improvements in the synthesis of BODIPY derivatives; in the first step of the BODIPY synthesis, the CH_2Cl_2 should be saturated with argon after that the pyrrole, aldehyde and TFA added to this argon saturated CH_2Cl_2 solution. At this step, condensation reaction occurs between aldehyde and the pyrrole, TFA was used in catalytic amount just to catalyze the reaction. Excess TFA can cause oxidation reactions. After nearly 2h, a solution of p-chloroanil in CH_2Cl_2 was added, the p-chloroanil is a reducing agent and it oxidizes the condensation product, after this step firstly 3 mL of Et_3N and then 3 mL of $BF_3.OEt_2$ is added. With the addition of Et_3N, the TFA in the media is neutralized, after the additon of $BF_3.OEt_2$, the bright green fluorescence was observed, at this stage nucleophilic attack occurs to the electron deficient boron centre from two nitrogens in the pyrrole units. In this study, we showed that there is an equilibrium between the boradiazaindacenes and the ring opened deboronylation product. This equilibrium shows us the BODIPY unit is a potential fluoride selective sensor. To observe this selectivity over fluoride, we first synthesized the following simple BODIPY derivative **42**. In the presence of millimolar fluoride result in abosorbtion and emission spectra distinctly different than a typical



42

Figure 45. Structure of Simple BODIPY derivative **42**

boradiazaindacene. In figure 46, the emission spectrum of **42** is shown and with increasing fluoride concentration, the emission of **42** decreases even at 0.25 mM fluoride concentration.

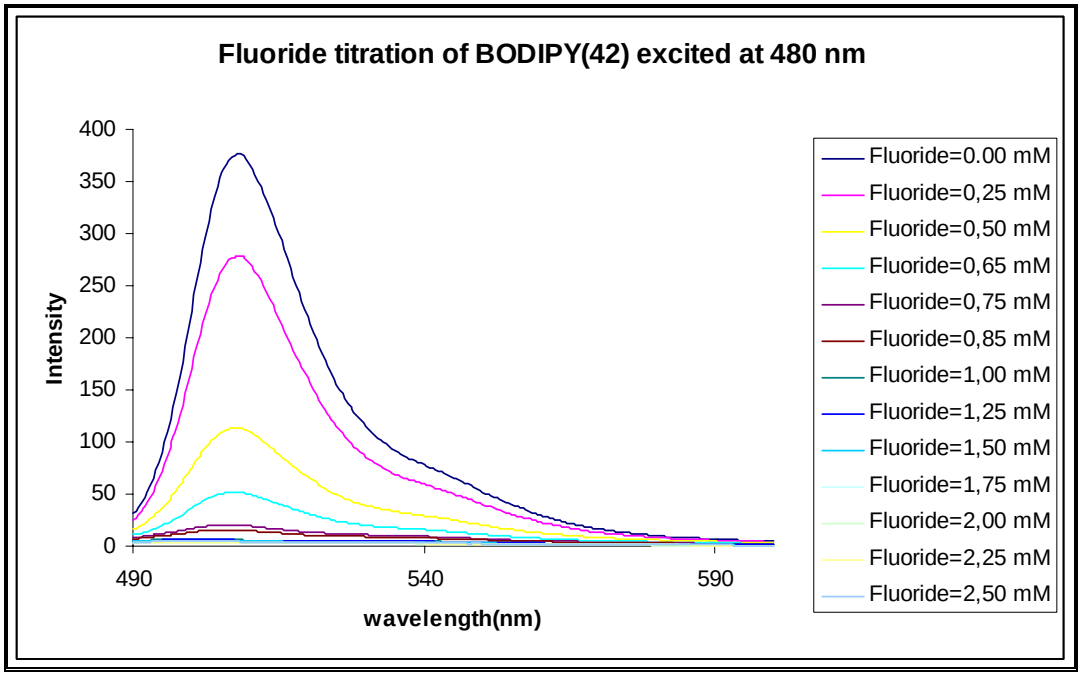


Figure 46. Emission spectrum of BODIPY(42) ligand conc: $2{,}3 \times 10^{-7}$, and the titration of **42** with fluoride anion, excitation was at 480 nm.

As expected other anions do not show any change in the emission spectrum of **42,** in figure 47, The fluoride selectivity of BODIPY can clearly be seen.

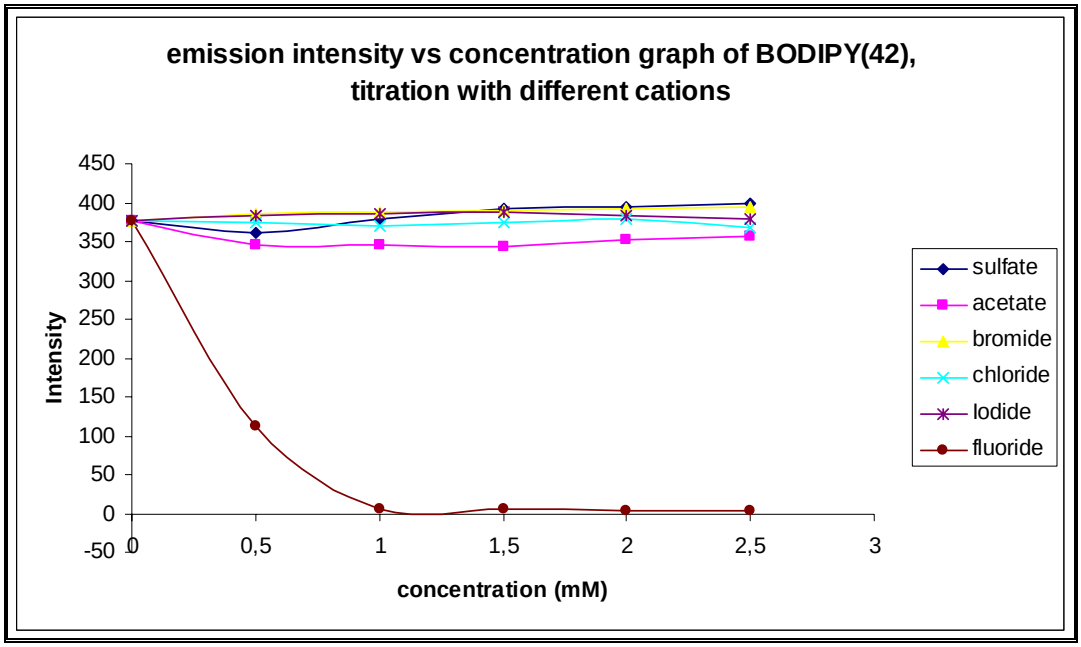


Figure 47. Emission response of **42** various anions.

As we mentioned earlier, there is a deboronylation equilibrium between fluoride and boron in the structure of BODIPY, if somehow we could get back the fluoride anion from the solution, then it would be possible for us to prove the reversibility of this reaction. But, the mere fact that less than maximal concentrations yield lesser changes, prove that reaction is not stoichiometric.

During the fluorescence measurements we realized that after the addition of fluoride (nearly 15 min later) the blue fluorescence observed, with higher quantum yield. That is another important aspect of the project, although we are working with relatively polar solvents and in this media, we still obtained exquisite selectivity for fluoride. When we excite at 410 nm, a distinct emission corresponding to ring opening product is observed. We see the real effect of fluoride at 510 nm, the emission of **42** decreases and at 450 and 480 nm two new emission maxima appears.

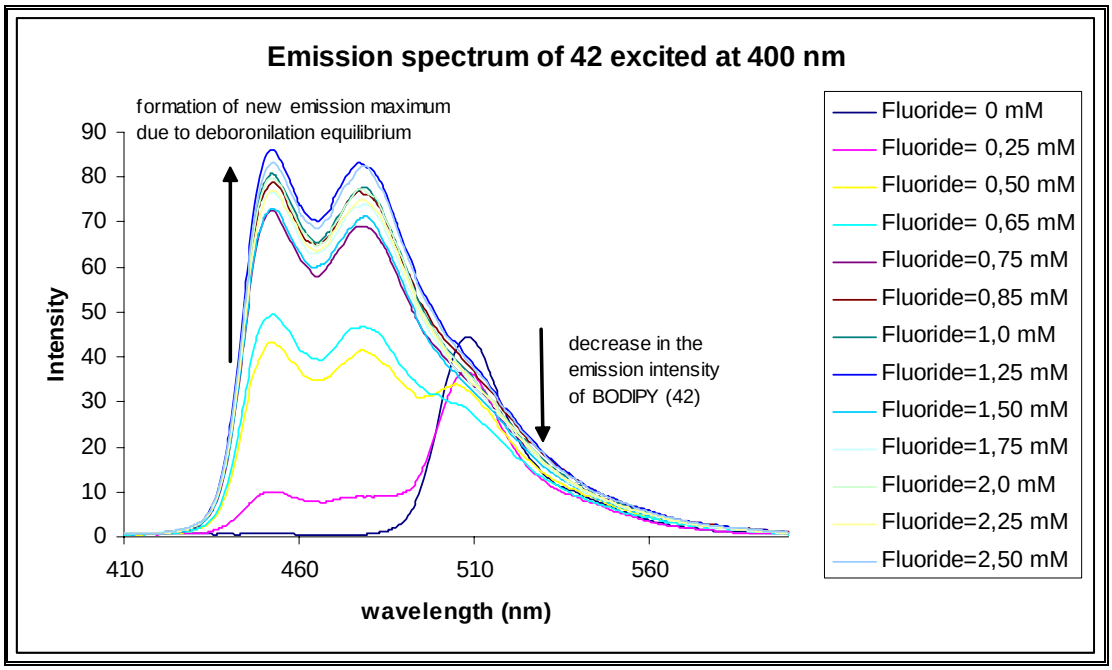
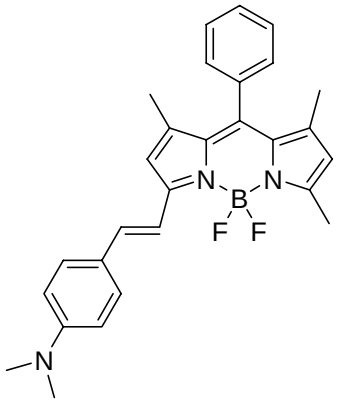


Figure 48. Emission spectrum of BODIPY(42) ligand conc: $2{,}3\times10^{-7}$, and the titration of **42** with Fluoride anion(excited at 400 nm).

After 0.85 mM fluoride concentration the change in the emission intensity reachs to the saturation, after this point the addition of fluoride anion causes no change in the emission spectrum of BODIPY(42).

In most of the optical and fluorescent chemosensors for anions was reported. Higher charge to size ratio of fluoride in many cases is responsible for larger signals obtained for fluoride. An exclusive selectivity is rare, ratiometric sensing is rarer still. In order to investigate the possibility of extending the chemosensing into the red end of the visible spectrum, we studied the fluoride response of a recently reported extended conjugation boradiazaindacene derivative **44**. [67]



44

Figure 49. Structure of BODIPY **44**

The absorption spectrum of **44** is not sensitive to solvent polarity, but emission shifts to longer wavelengths solvatochromically. In nonpolar media it has higher quantum yield, and with the increasing polarity quantum yield is increases. Broadening of the emission peak together with decreasing quantum yield is consistent with the charge transfer (CT) character of the emissive state. In acetone, we determined that the absoption peaks at 599 nm (ε = 81,000 M^{-1} cm^{-1}), emisson peaks at 686 nm. Quantum yield in acetone is 0.15.

Absorption spectrum is obtained in the presence of various anions at 2.5 mM as tetrabutylammonium salts. Only fluoride showed any change. Blue color of the solution faded to yield a practically colorless solution in figure 50. The effect of fluoride can be seen on this BODIPY derivative.

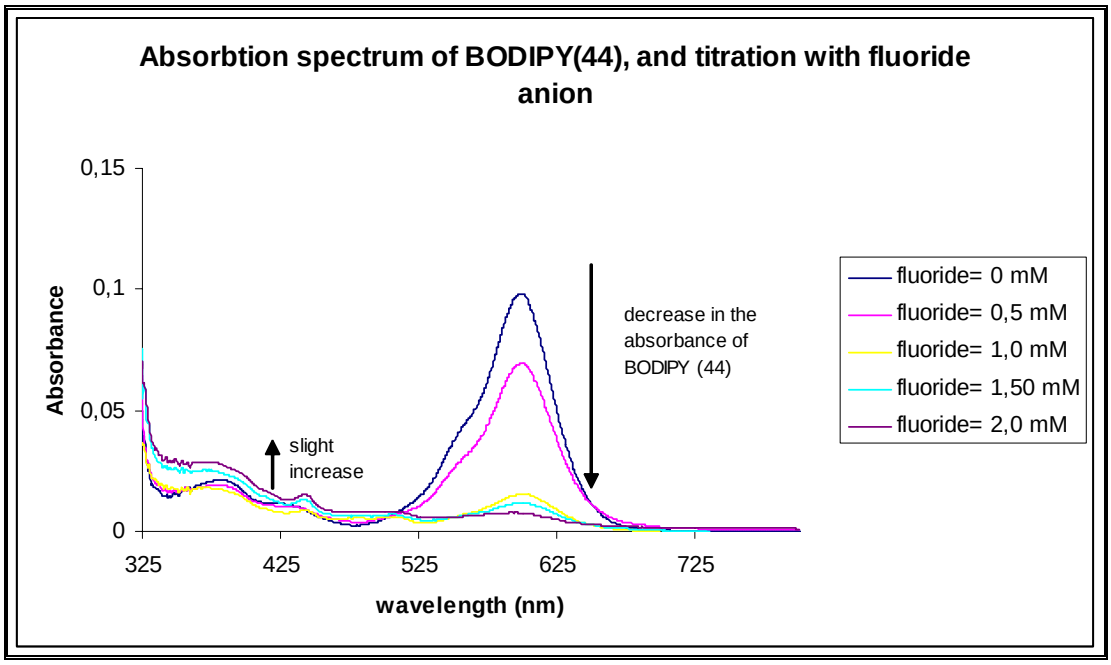


Figure 50. Absorbance spectrum of BODIPY (44), as a function of increasing fluoride concentration.

Also the following absorbance for BODIPY(44) picture taken at ambient light conditions with different anions. It shows the selectivity of Boradiazaindacene derivatives for fluoride anion.

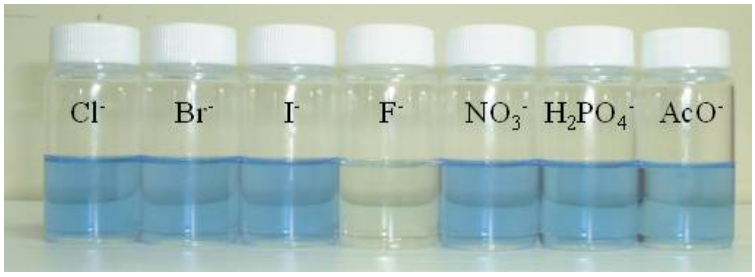


Figure 51. The color change under ambient light on the addition of 2.5 mM anions in the form of tetrabutylammonium salts in acetone.

The emission spectrum showed even more spectacular changes: Titration with increasing concentrations of fluoride clearly showed the disapperance of the long wavelength peak with a concomitant growth of two peaks at 452 and 482 nm. Again, the effect was only observed in the case of fluoride and there is no change in

the emission spectrum when high concentrations of Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, $H_2PO_4^-$, were added in figure 52. To test whether the effect was due to or initiated by a simple acid base interaction, we added Et_3N to the acetone solution, no change either in absorption or emission spectrum was observed. This clearly shows us that this is not a simple acid-base reaction.

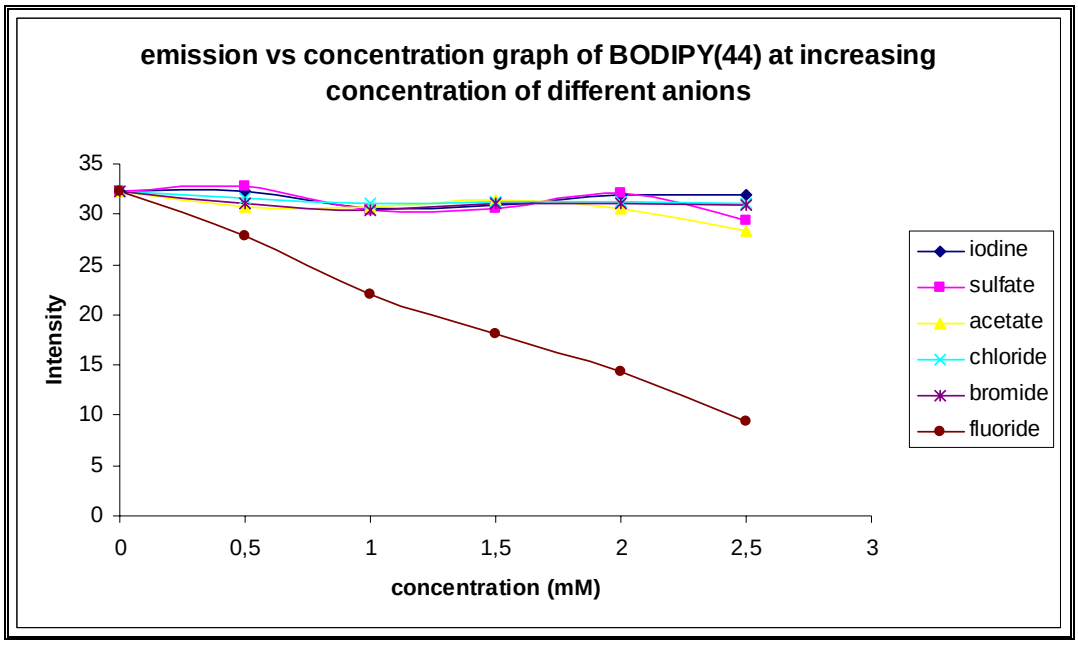


Figure 52. Emission vs concentration graph of BODIPY (44) ligand concentration; $2{,}3x10^{-7}$ M at increasing anion concentrations.

There are recent reports of boron-based anion sensors,[70] in these examples fluoride attacks the sp^2 hybridized boron and converts it to sp^3 hybridization state, thereby disrupting the p-π conjugation in the fluorophore and generating a signal. In a more elaborate design,[71] trianthrylboron moiety is coupled to a porphyrin unit, thus establishing an energy transfer pathway which is modulated similarly by fluoride binding. In the present case however, boron is already sp^3 hybridized in **44**, and only possibility is a nucleophilic displacement, breaking a B-N bond and forming a B-F bond.

Figure 53. Fluorescence emission response of compound 1 on the addition of 2.5 mM anions in the form of tetrabutylammonium salts in acetone. Excitation is at 360 nm using a hand-held UV lamp.

This ring opening deboronylation equilibrium is established only in the presence of fluoride, in part due to smaller size and larger charge density of this anion.

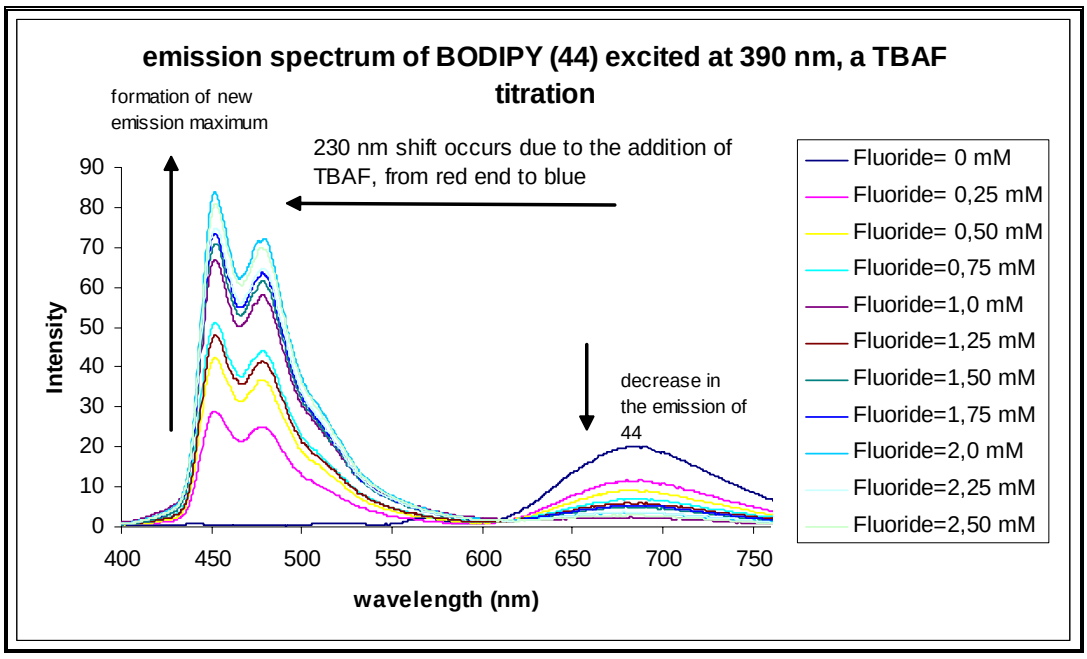


Figure 54. TBAF titration of **44** in acetone. The emission spectra were obtained by excitation at 390 nm. Slit widths were 5 nm.

In figure 54 due to the deboronylation equilibrium 230 nm shift occurs. The equilibrium constant is calculated based on the decrease in absorbance at 600 nm after 15 minutes of equilibration in the presence of varying amounts of fluoride, $K_a = 2x10^{-3}\ M^{-1}$. Longer incubation periods at high acetate (> 2.5 mM) concentrations also showed smaller but detectable changes. There is no example in literature which

can show a exquisite selectivity towards fluoride or any anion which can cause 230 nm spectral shift in the emission spectrum together with a change in the absorbance. The bond enthalpy of B-F is higher than bond enthalpy of B-N. Stronger affinity of fluorine towards boron is apparent when single bond enthalpies are compared (B-F 757 kJ/mol vs. B-N 389 kJ/mol). This ring opening deboronylation equilibrium is established only in the presence of fluoride, yielding a very selective fluorescent dual-channel chemosensor with a 230 nm shift in the maxima of the emission peak; practically one end of the visible spectrum to the other end. As a bonus naked eye detection of fluoride also possible in figure 51. Proposed deboronylation equilibrium is shown in figure 55.

The most important outcome of this project, all BODIPY derivatives can be used as fluoride sensor due to the enourmous selectivity of the BODIPY unit for fluoride anion, the change in the absorbance and the emission spectrum shows us this interaction between, fluoride and boron occurs through deboronylation equilibrium, another important indication for this, at lower concentrations like 0.10, 0.25 we didn't observe the distinct color change in the absorbtion. At higher concentrations of fluoride anion deboronylation equilibrium is forced to the right side and as a result, a distinct change in both absorbance and equilibrium occurs.

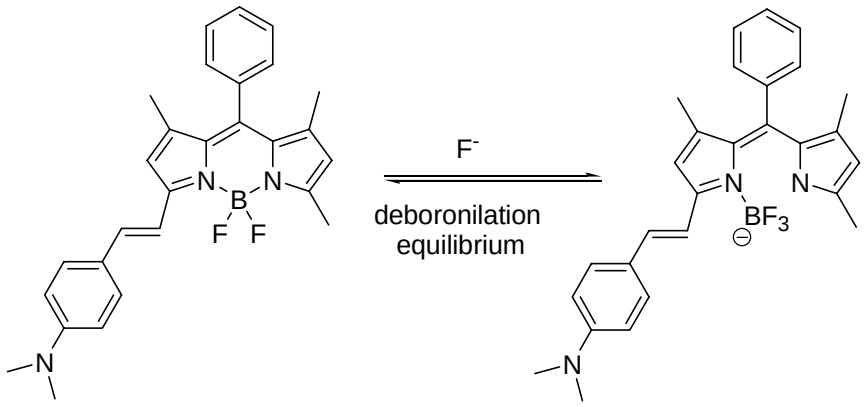


Figure 55. Proposed equilibrium between cyclic and the ring-opened forms of **44**

3.3. The first example of molecular half substractor BODIPY(46)

A half-subtractor is a combinatorial curciut that subtracts two bits and produces their difference. It also has an output to specify if a 1 has been borrowed. Let's designate the minuend bit by x and subtrahend bit by y. To perform x-y, we have to check the relative magnitudes of x and y. If $x \geq y$, we have three possibilities: 0 – 0 = 0, 1 – 0 = 1, and 1 – 1 =0. The result is called the diffrence bit. If $x < y$, we have 0 –1, and it is necessary to borrow a 1 from the next higher stage. The 1 borrowed from the next higher stage adds 2 to the minuend bit, just as in the decimal system, a borrow adds 10 to a minuend digit. With the minuend equal to 2, the diffrence becomes 2 – 1 = 1. The half-subtractor needs two outputs. One output generates the diffrence and will be designated by the symbol D. The second output, designated B for borrow, generates the binary signal that informs the next stage that a 1 has been borrowed. The truth table for the input-output relationships of a half-subtractor can now be derived as follows:

Table 1. The truth table for the input-output relationship of a half-substractor.

X (1st input)	Y (2nd input)	B (borrow)	D (difference)
0	0	0	0
0	1	1	1
1	0	0	1
1	1	0	0

While analyzing the emission data that we obtained from bis-boradiazaindacenyl-bipyridine derivative, we realized that by focussing on the emissions at two different wavelengths (blue and green emissions), we can obtain two logic gate operations, when fluoride and zinc(II) are used as chemical inputs. At long wavelength, when both inputs are 0; that is no addition, the output signal is high (1). When fluoride is added, it decreases (0), when zinc(II) alone is added, it is again low (0), but when both inputs are added, there is a criticial concentration of fluoride where the emission is high (mM). This output is actually just the reverse of what is needed in the diffrence bit (EQUIVALENCE), but when it is read in negative

logic, it becomes an XOR gate. At short wavelength however, since only when fluoride alone causes a spectral shift, we directly obtain the INHIBITION logic gate behavior.

If one shows the truth table one more time with the chemical inputs, it would look like this:

Table 2. The truth table for the input-output relationship of a half-substractor BODIPY (46)

Zn(II) input 1	**F^- input 2**	**Borrow output (intensity at 450 nm)**	**Difference output (positive logic, intensity at 515 nm)**	**Difference output (negative logic, intensity at 515 nm)**
0	0	0	1	0
0	1	1	0	1
1	0	0	0	1
1	1	0	1	0

The reason why the gate response is such can be explained in this way: Normally, with what we have discoved about the deboranylation, one should expect low fluorescence output at long wavelength (515 nm) whenever fluoride is present. But, when together with Zn(II) situation is different, because fluoride has a higher affinity for the chelated Zn(II) center, thus in fact contrary to expectations increase the emission intensity of the boradiazaindacene. Of course, as the fluoride concentration increases, deboranylation takes over, decreasing the long wavelength emission and increasing the short wavelength emission.

Thus, this single molecule is apperently capable of doing arithmetics. Earlier A. P. de Silva had published a system where there are two molecules acting as two separate logic gates and doing binary addition. Ours is the first system, which can

signal the subtraction of chemical inputs, and in addition, it is a unimolecular system.

Target regions for Zn(II) and fluoride ions are shown below.

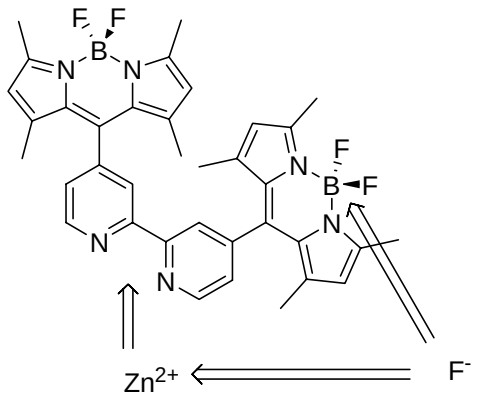
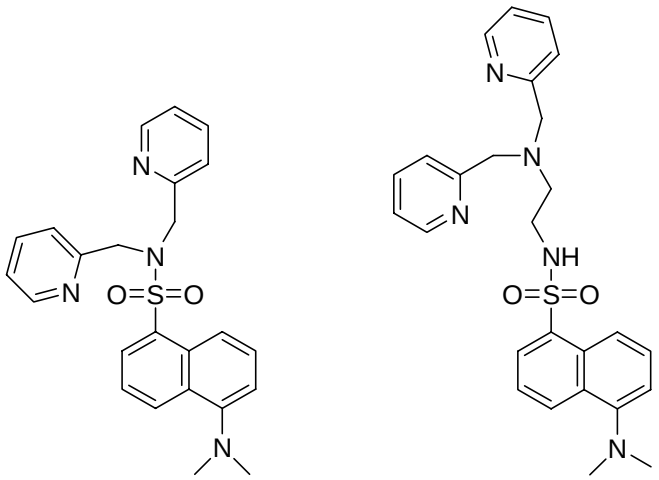


Figure 56. Target regions of BODIPY(46) for Zn(II) and fluoride ions

3.4. Novel dansyl-modified Zinc(II) chelators as fluorescent chemosensors.

The synthesis of these zinc(II) chelators is straight forward, 2,2'-picolylamine unit is known in literature as a strong chelator for zinc (II) but it needs an additional donor atom for stronger binding. In this study two derivatives of this chelator were synthesized and we used dansyl moiety as a fluorophore unit, because it is known in literature that one of the most outstanding zinc(II) properties is a strong affinity to aromatic sulfonamides, there is a formation of strong bonds between zinc(II) and the deprotonated sulfonamide N^- anions at physiological pH [72]. To show this difference in affinity two different derivatives of picolyl amine chelator was synthesized.



41 **40**

Figure 57. Structures of dansyl modified zinc(II) Chelators

In compound **41** there is no sulfonamide proton, at physological pH, it is not possible to obtain N^- unit, also in compound **41** picolylamine is directly connected to the dansyl fluorophore so, the flexibility of this chelator is limited.

In the synthesis of compound **41,** dansylchloride and 2,2'-picolylamine were dissolved in CH_2Cl_2, to make the solution basic $CsCO_3$ was used, after nearly 2h (TLC) showed formation of expected product, once the reaction was completed the

solution was filtered through celite and after evaporation of solvent, it was purified via column chromatography (silica gel, chloroform:methanol, 97/3), ^{1}H-NMR, ^{13}C-NMR and ESI-MS results shows the formation of pure product. (Figure 69, 70, 74)

In ESI-MS result in all cases [M+H] peak was observed, molecular ion peak expected for [M+H] should be at 433.16 and the peak was at 433.10

In the case of compound **40,** there is a sulfonamide proton which can be deprotonated at physiological pH, so in this case it is possible to connect zinc(II), and it is also possible to see the effect of N^- unit for the binding of zinc(II), by comparring zinc(II) abilities of compound **40** and **41**.

In the synthesis of **40** a multi-step synthesis was carried out. First , HCl salt of bromoethyl amine is protected by using Boc group in the prescence of NaOH, the yield of this reaction is quantitative, and without further purification, its direcly used through next step,

Then boc-protected bromo-ethyl amine is reacted with 2,2'-picolylamine in the presence of KI and K_2CO_3 as a base. Solvent was acetonitrile. At this stage reaction mixture was refluxed overnight, maximum yield obtained for this reaction was 46%. In the purification of compound **41,** there was no problem but for compound **40,** due to the extra amine group the polarity of this compound increased. For the purification of this compound 15%(MeOH-$CHCl_3$) mixture was used and following silica-gel chromatography, preparative TLC was used for further purification of this compound. This Boc-protected unit **37** well characterized by ^{1}H-NMR, ^{13}C-NMR and ESI-MS results. (Figure 62, 63, 73)

In ESI-MS result in all cases [M+H] peak was observed, molecular ion peak expected for [M+H] should be at 343.21 and the peak was at 343.20 and these results clearly indicate the formation of this product.

At this stage compound **37** has to be purified because in the next step, purification is very difficult due to the high polarity of the obtained zinc(II) chelator unit. For the synthesis of compound **38**, **37**was deprotected according to well-known procedure, the compound **37** was dissolved in absolute CH_2Cl_2 then equal amount of TFA was added (by volume), after 1h TLC shows the complete deprotection of

compound **37** after the that solvent was evaporated through rotary evaporator and neutralized by using 10% NaOH solution, after work up pure product was obtained and compound **38** was well characterized by ^{1}H-NMR, ^{13}C-NMR and LC-MS results prove the identity of these compounds. (Figure 64, 65, 73 respectively)

In ESI-MS result in all cases [M+H] peak was observed, molecular ion peak expected for [M+H] should be at 243.15 and the peak was at 243.10

At the last step compound **38** and dansylchloride were dissolved in CH_2Cl_2. As a base again $CsCO_3$ was used, after the reaction complete, the suspension filtered through celite and after evaporation of solvent crude product was purified via column chromatography (silica gel chloroform:methanol-85:15(v/v/)). Due to the presence of additional amine group obtained product was highly polar so for further purification, preparative TLC was used. Compound **40** well-characterized by ^{1}H-NMR, ^{13}C-NMR and ESI-MS data. (Figure 66, 67, 74 respectively)

In ESI-MS result in all cases [M+H] peak was observed, molecular ion peak expected for [M+H] should be at 476.20 and the peak was at 476.20

The following equilibrium shows us the zinc-complex of **40,** as it can be seen sulfonamide nitrogen plays important role for the complexation of Zinc(II).

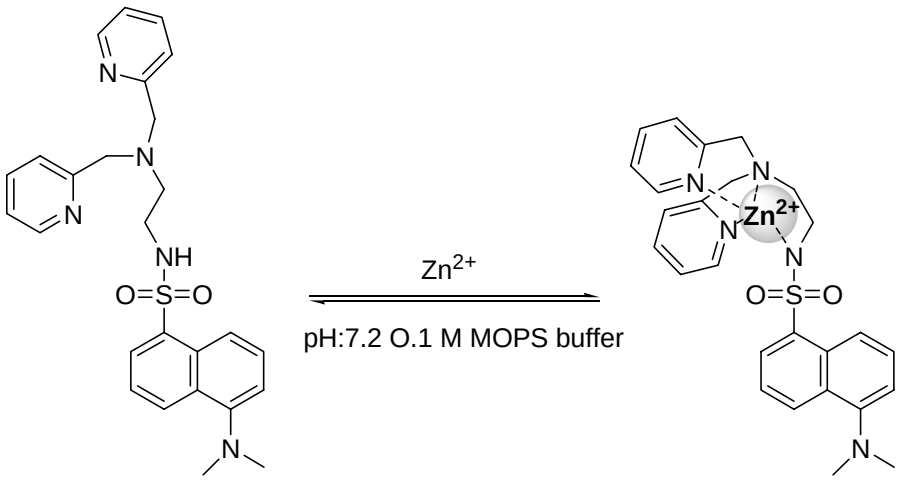


Figure 58. The zinc complex of compound **40**

To study the effect of various metal ions on the fluorescence of dansyl

modified sensors, series of stock solutions were prepared, ligand concentration in each measurement in solution was 10 μM. In our first experiments, we tried to prepare metal stock solutions at pH 7.2, 0.1 M MOPS buffer, metal stock solutions were prepared from their corresponding salts ; $Zn(ClO_4)_2.6H_2O$, $Cd(ClO_4)_2.6H_2O$, $Cu(ClO_4)_2.6H_2O$, $Co(ClO_4)_2.6H_2O$, $Mg(ClO_4)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Pb(NO_3)_2$. In the first instance, all metal stock solutions were prepared in MOPS buffer, solubility for Pb^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+} salts were limited. For this reason considering solubility, we prepared our stock solutions in DMSO. 1, 2 , 4, 8, 10 ,16 mM metal stock solutions were prepared for all cations. The total volume of the solutions during our measurements were 4 ml, from each of the metal stock solutions, 100 μl was taken and diluted to 4000 μl and in solution series of cations in different concentration was obtained, these are 25, 50, 100, 200, 400 μM. Then 1 mM solutions of compound **40** and compound **41** in acetonitrile were prepared. Due to solubility problem we prepared our stock solutions for dansyl modified ligands in acetonitrile, and acetonitrile was miscible with water, 1 mM solutions for each in acetonitrile was prepared and in solution 40 μl is diluted to 4000 μl, so the ligand concentration obtained in solution was 10 μM. The rest of solutions were completed by pH 7.2 , 0.1 M MOPS buffer. Also in this final solutions we observed precipitation of Pb^{2+} as $Pb(OH)_2$ at 200 and 400 μM metal concentration,

In figure 59 effect of various metal ions on the fluorescence of **40** and **41** is presented. For compound **40**, As expected Cu^{2+}, Co^{2+}, Ni^{2+} quenches the fluorescence, because they are open shell transition metal elements. With copper ion extremely stable four coordinated complex forms. In the case of Mg^{2+} only 4% quenching occurs that is due to low binding ability of magnesium with comparison to the other cations that causes nearly no change, for Pb^{2+}, it behaves like Mg^{2+} only 4% enhancement occurs, we can call for $Mg^{2+}$and Pb^{2+} causes no change in the emission of **40**.

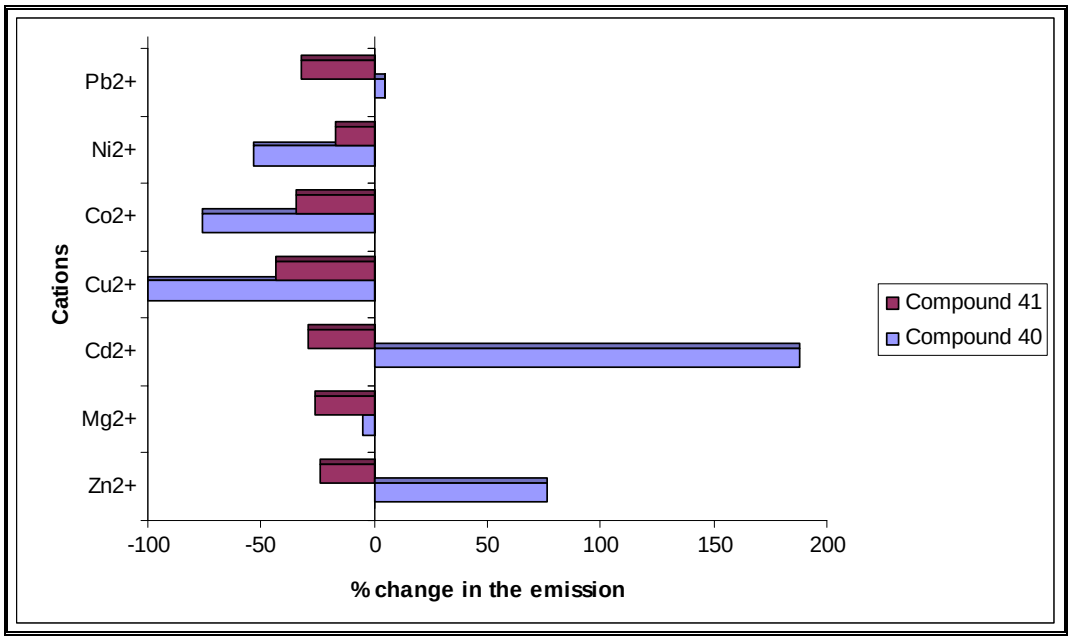


Figure 59. Effect of various metal Ions on the fluorescence of dansyl modified sensors,ligand conc.: 10 μM, metal concentration: 25 μM, in pH:7.2, 0.1 M MOPS buffer, percent change in the emission was obtained for different cations.

The largest effects obtained for zinc(II) and cadmium(II), as indicated in literature zinc and cadmium shows similar behaviours in all zinc sensors which are synthesized up to now, but in our case **40** shows higher selectivity for cadmium(II). It doesn't cause any problems. It is to be used for intracellular sensing of zinc(II), and only trace amount of Cadmium is present in cell due to its high toxicity.. Compound **40** causes % 80 enhancement in the emission, and for cadmium(II) %188 enhancement in the emission occurs. From this results, it can be concluded that Compound **40** can be used for both sensing of zinc(II) and cadmium(II). As mentioned above for intracellular sensing only zinc selectivity of this compound important, because there is a trace amount of cadmium in the cell. Also sensing of cadmium(II) is prime important in the environment due to its high toxicity. With compound **40,** %188 change observed and much higher than that of the change for zinc(II). By this way compound **40** can be used for both sensing of cadmium(II) and zinc(II).

A small blue shift was observed for both. The enhancement for zinc(II) and

cadmium(II) occurs due to photo induced charge transfer mechanism which was explained in introduction part in detail, the excited state is more strongly destabilized by the cation than the ground state, and a blue shift of the absorption and emission spectra is expected. Initially internal electron transfer occurs from electon donating amino group to the dansyl moiety. With the binding of zinc (as shown in figure 58) this electron transfer terminated, the amino group coordinated to zinc and that causes the slight blue shift. Initially, yellowish fluorescence was observed and with the addition of zinc the fluorescence emission turns to the bright green, and also quantum yield for compound **40** increases upon the binding of zinc(II). In the case of **41**, in all cases quenching of fluorescence occurs. That is due to the presence of sulfonamide proton, at physiological pH, its deprotonated and N^- forms, that causes the binding of zinc(II) but in the case of **41** there is no dansyl amide proton which can be deprotonated. By this we clearly show the effect of sulfonamide group on the binding of zinc(II). In the case of **41** the mechanism goes probably through PET mechanism and upon the complexation of electron transfer from pyridine unit to dansyl unit is cancelled and quenching of fluorescence was observed. Compound **40** has higher flexibility that **41** due to the ethylene bridge and also has higher binding ability for zinc(II) due to the presence of N^- unit in sulfonamide group.

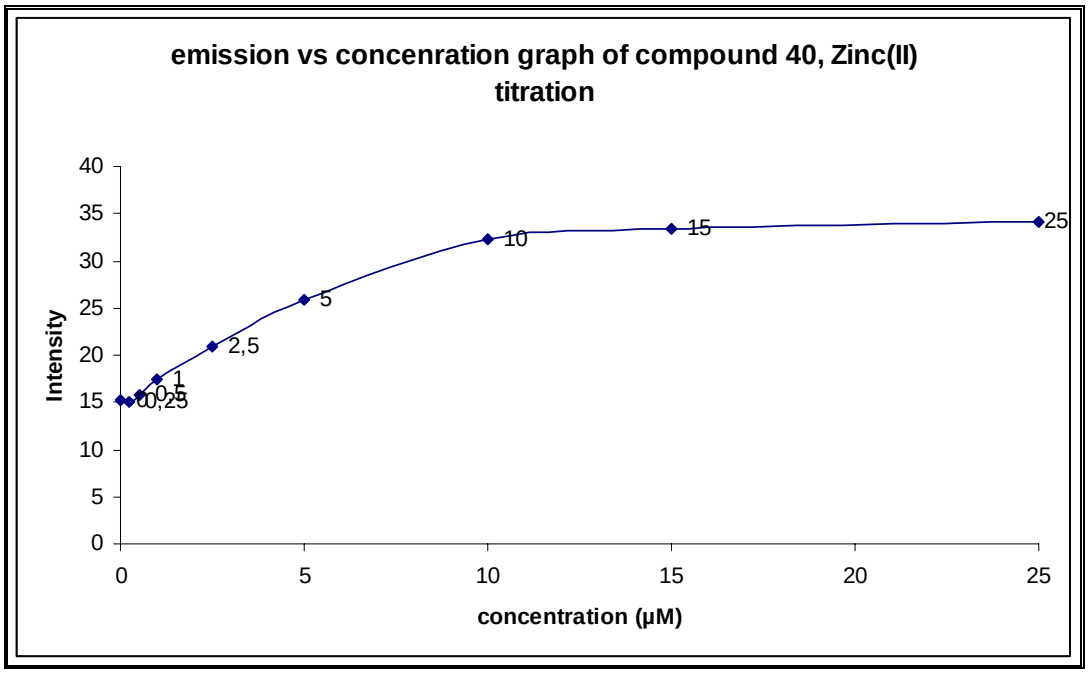


Figure 60. Fluorescence emission response of **40** (10 µM) to increasing levels of Zinc(II) at 25 °C and pH 7.2 (0.1 M MOPS buffer). The emission maximum is at 527 nm, excitation was at 322 nm

The response increases from 1 µM to 10 µM. Until it reaches a 1:1 [L]/[zinc(II)] ratio, indicating that the increase in fluorescence is due to the ZnL complex formation, and, moreover for ZnL is stable. The zinc(II)-dependent flurescence was unaffected by the presence of 2.5 or more excess amount of Zinc(II) ion. The earlier examples of zinc(II) sensor's (TSQ, Zinquin) complexation neither strong nor stoichiometric, in our case we obtained binding of Zinc in stoichiometric amount.

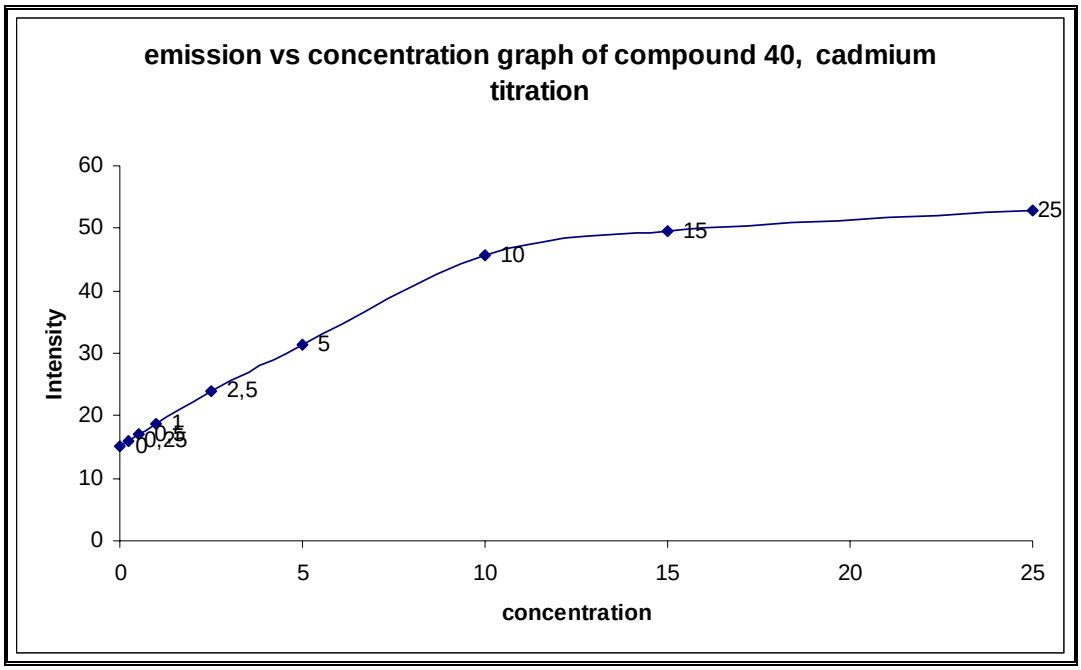


Figure 61. Fluorescence emission response of **40** (10 μM) to increasing levels of cadmium(II) at 25 °C and pH 7.2 (0.1 M MOPS buffer). The emission maximum is at 527 nm, Excitation was at 322 nm

The response increases from 1 μM to 10 μM. Until it reaches a 1:1 [L]/[cadmium(II)] ratio, indicating that the increase in fluorescence is stoichiometric, totally due to the CdL complex formation, and, moreover for CdL is stable. The Cadmium(II)-dependent flurescence was unaffected by the presence of 2.5 or more excess amount of Cadmium(II) ion. In the earlier examples, all Zinc sensors also shows the same effect for cadmium. In our case our dansyl modified sensor shows %110 more affinity towards cadmium(II), and the binding is stoichiometric for cadmium(II).

The one of the most outstanding property of our ligand it causes nearly 80 percent enhancement for zinc in the emission and selectivity over various cations and %188 enhancement for cadmium. Although cadmium(II) causes larger emission changes than zinc(II), in biological media, cadmium(II) concentration is practically nill. Therefore in such media, this chemosensor can be used as a zinc(II)-selective probe. On the other hand in various environments cadmium(II) at higher concentrations so in environmental monitoring, this property of the chemosensor would make it a cadmium(II)-selective probe.

CHAPTER 4

CONCLUSION

In four diffent parts of this M.Sc. thesis study, we obtained significant results.

In the first part of the study, we showed that anion binding generates a very large fluorescence signal when a zinc complex of a well-designed boradiazaindacene ligand is used as a chemosensor. The magnitude of the signal change with phosphate is unprecedented, and while carrying out a routine survey of anions, we discovered that fluoride seems to cause an unexpected change in the emission spectrum. On follow-up, we found that fluoride-mediated ring opening is quite a general mode of reactivity for difluoroboradiazaindacenes. We decided to extend the sensing action to the red end of the visible spectrum, and thus synthesized a recently reported red-fluorescing dye of the same class. As expected, this compound showed a spectacular change in the presence of fluoride.

On further analysis of the data obtained from the bipyridyl derivative, we realized that if one switches to negative logic at one of the wavelengths, this compound seems to capable of doing binary subtraction, using the inputs of fluoride and zinc ions.

In the final part, we synthesized two different zinc chelators with dansylamide unit as a fluorophore. The results demonstrated the importance of dansylamide N-H in both zinc binding and perhaps in efficient signal generation.

We believe our results in this area will be helpful for a better understanding analyte-chemosensor interactions and their applications as logic gates and future information processors based on chemical systems.

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APPENDIX A

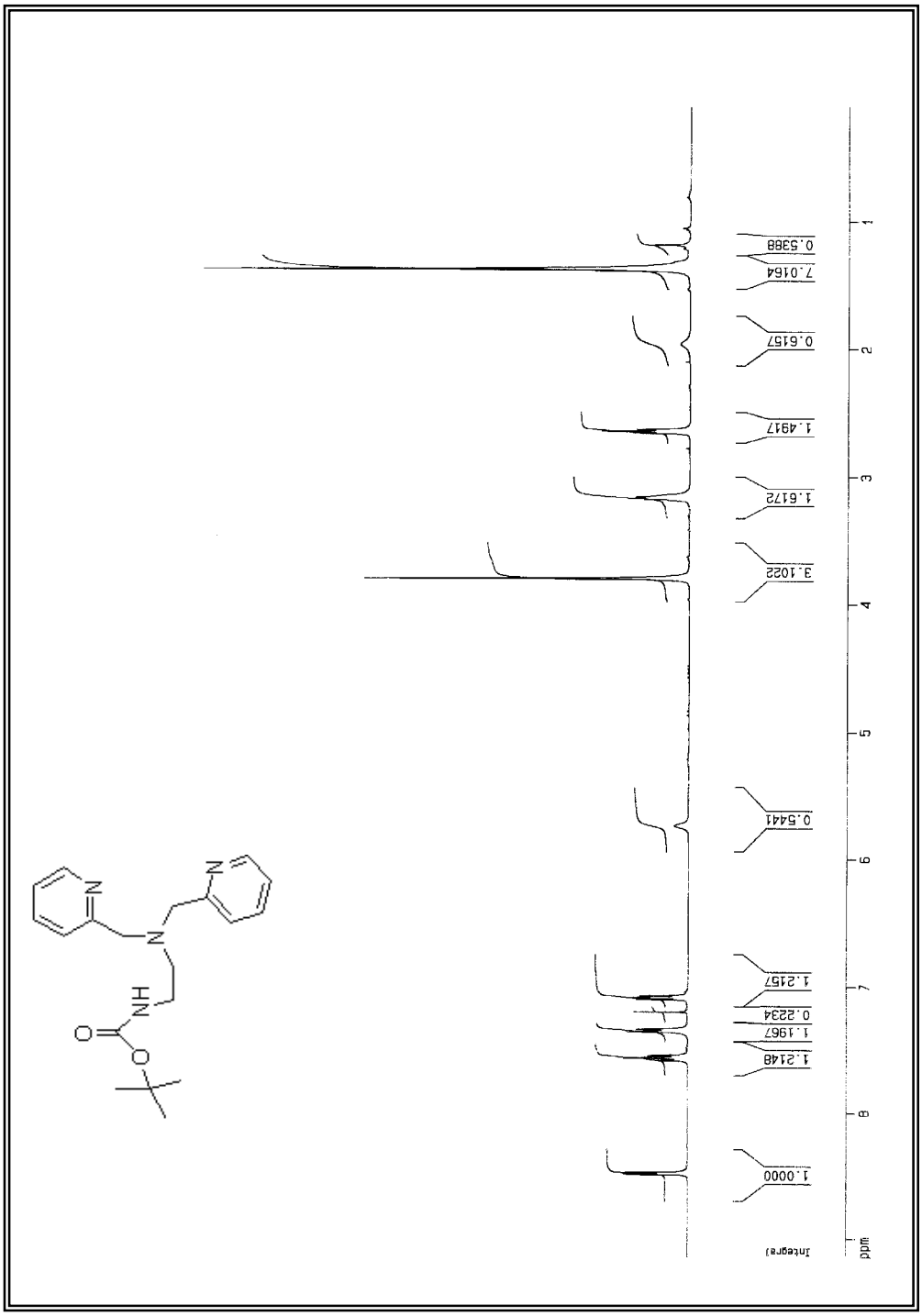

Figure 62. 1H NMR spectrum of compound 37

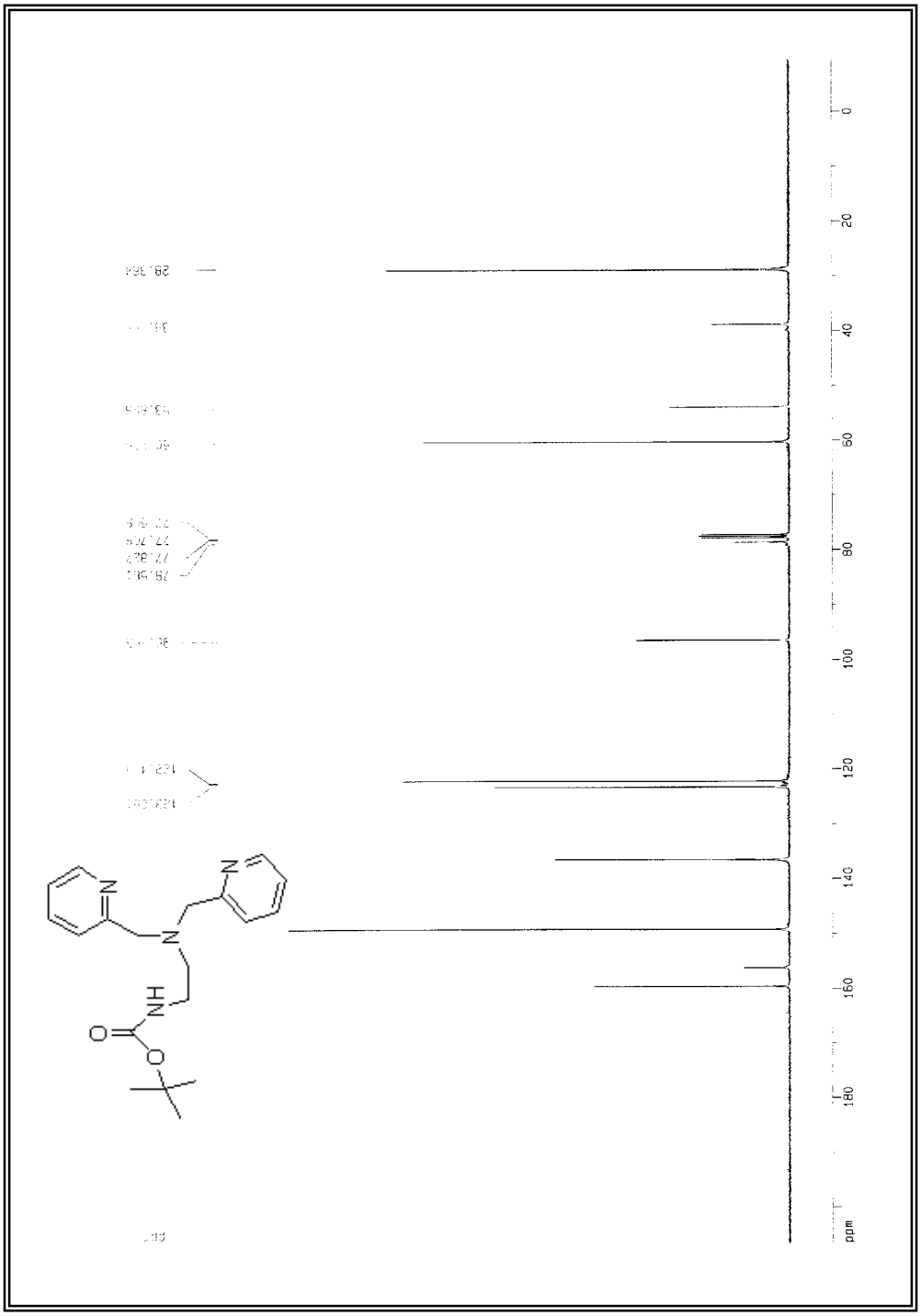

Figure 63. 13C NMR spectrum of compound 37

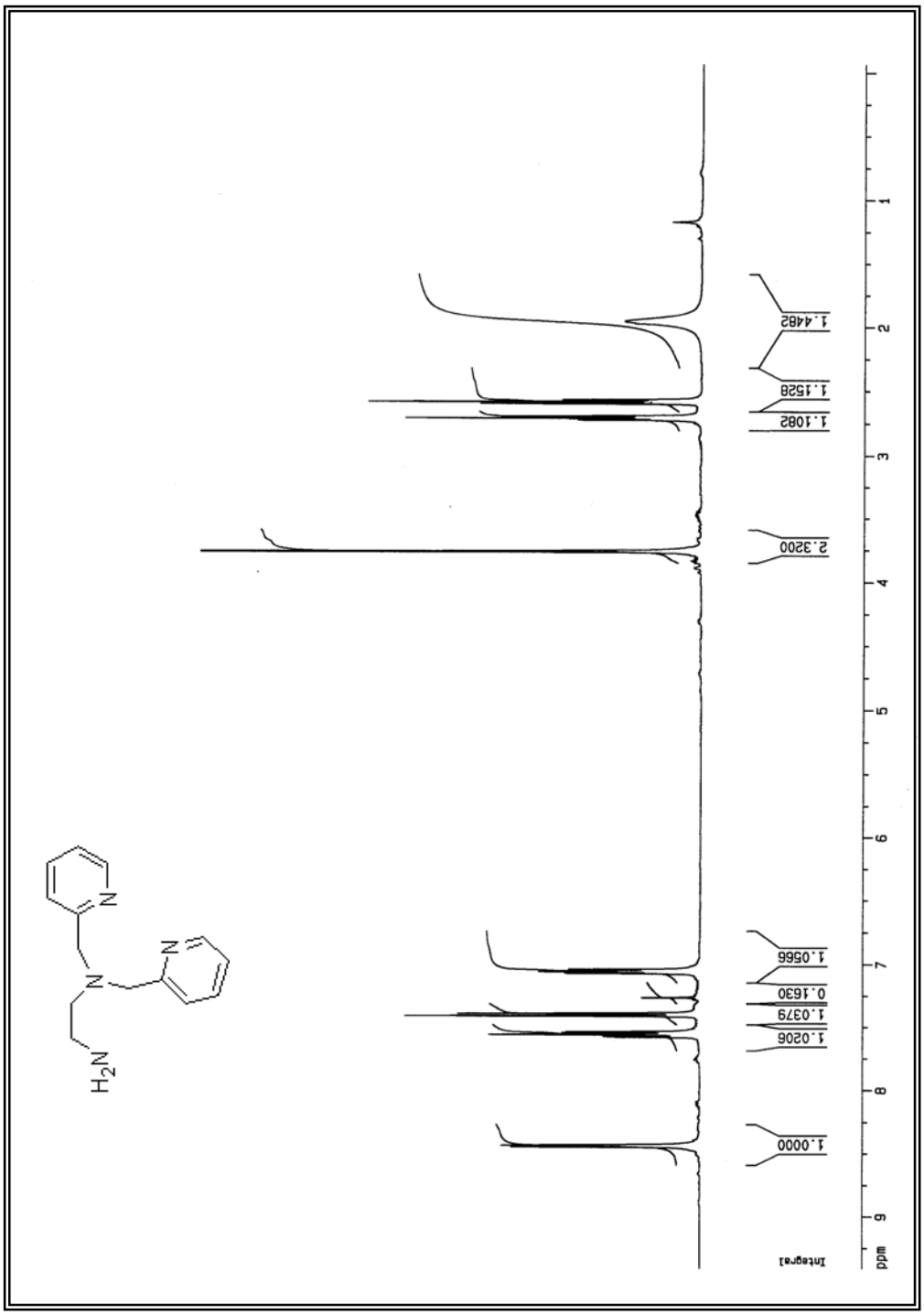

Figure 64. 1H NMR spectrum of compound 38

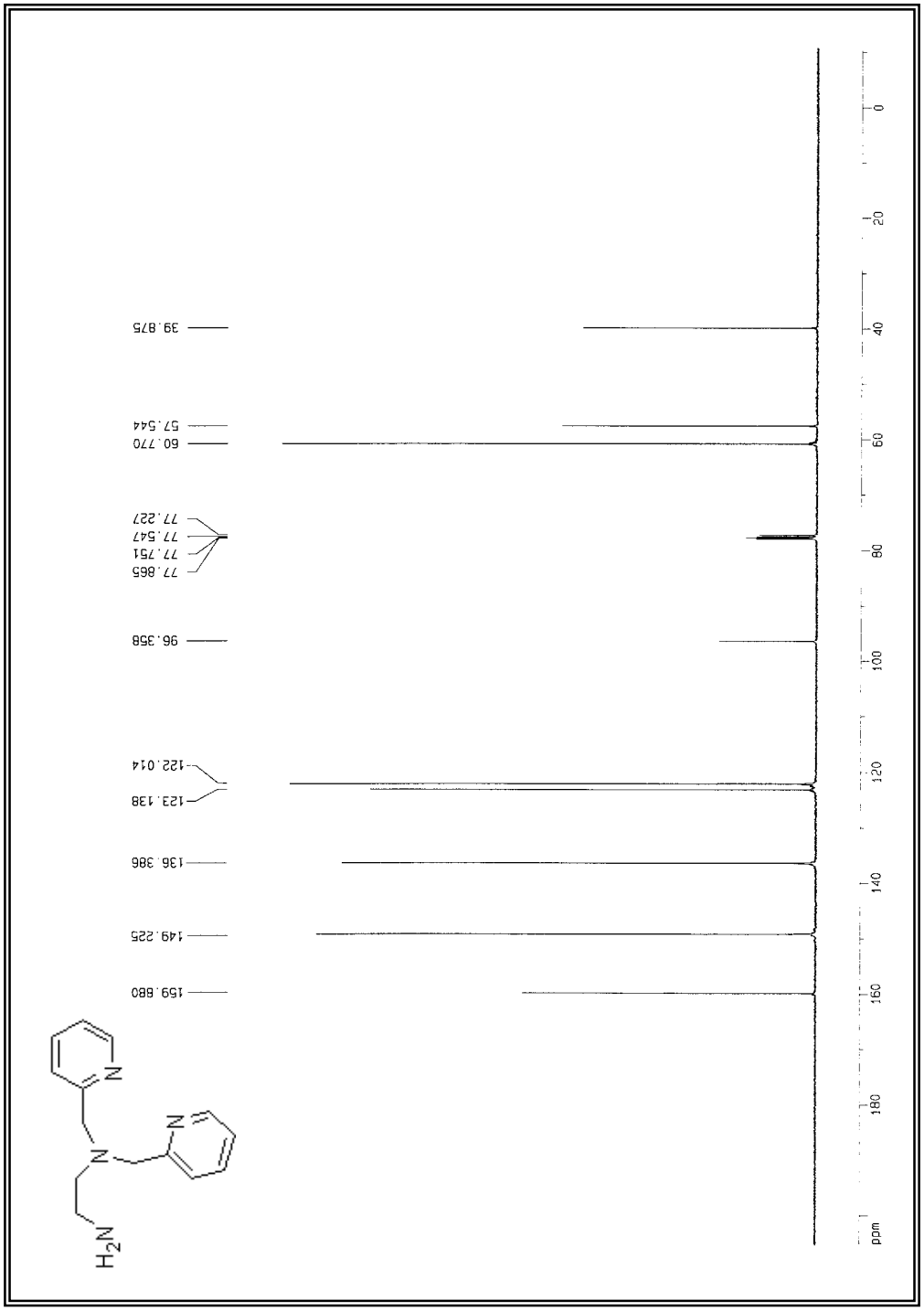

Figure 65. 13C NMR spectrum of compound 38

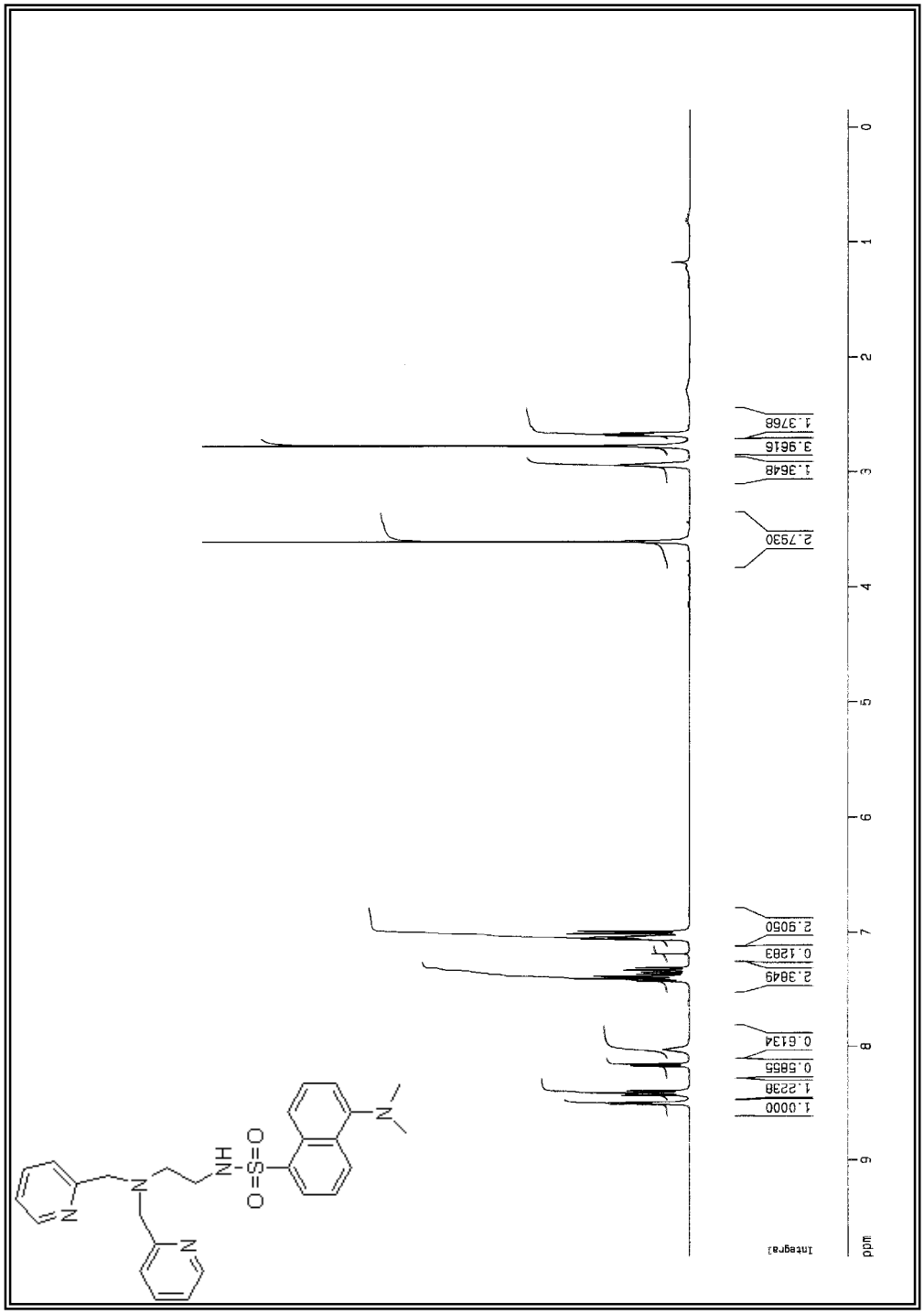

Figure 66. 1H NMR spectrum of compound 40

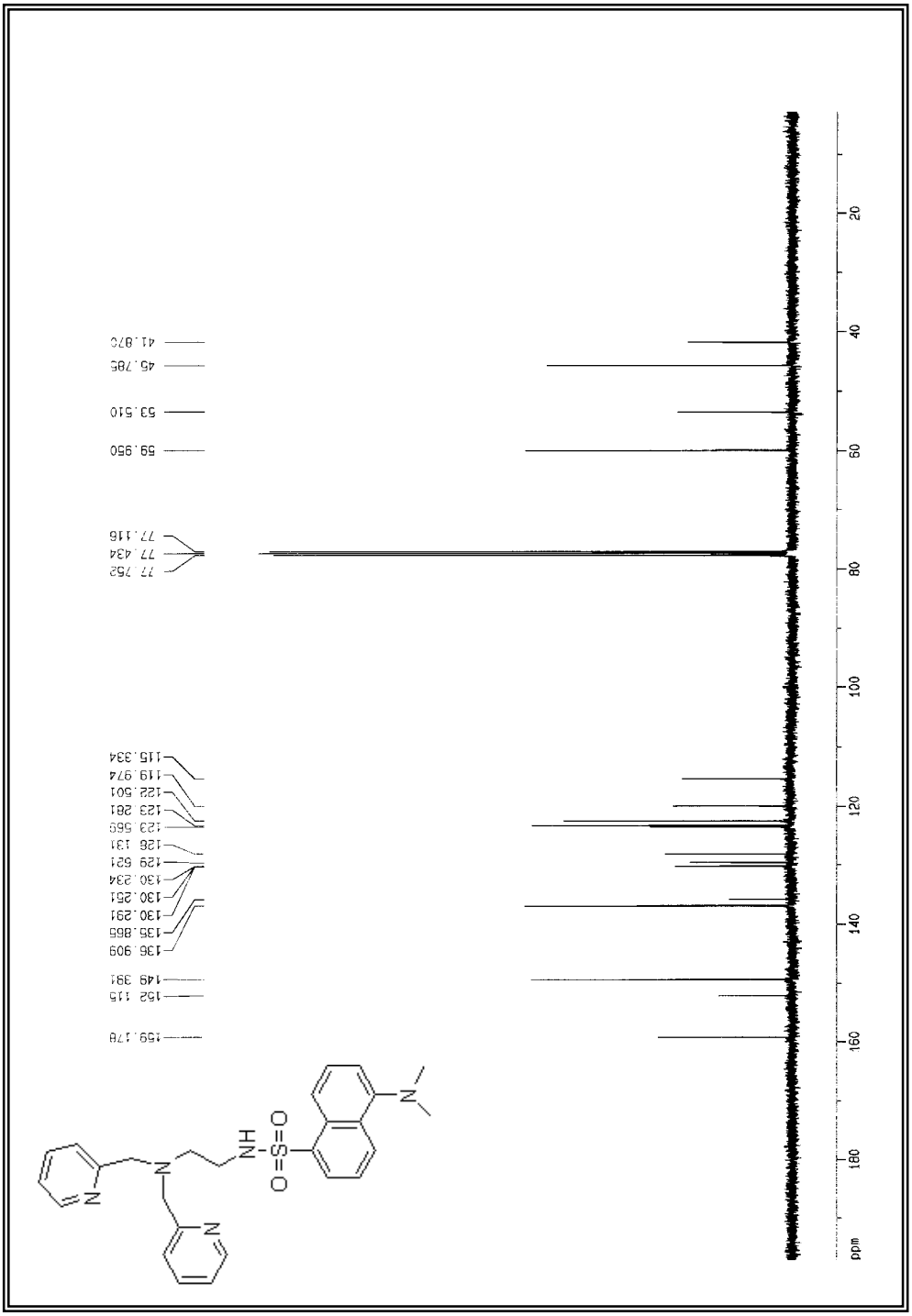

Figure 67. 13C NMR spectrum of compound 40

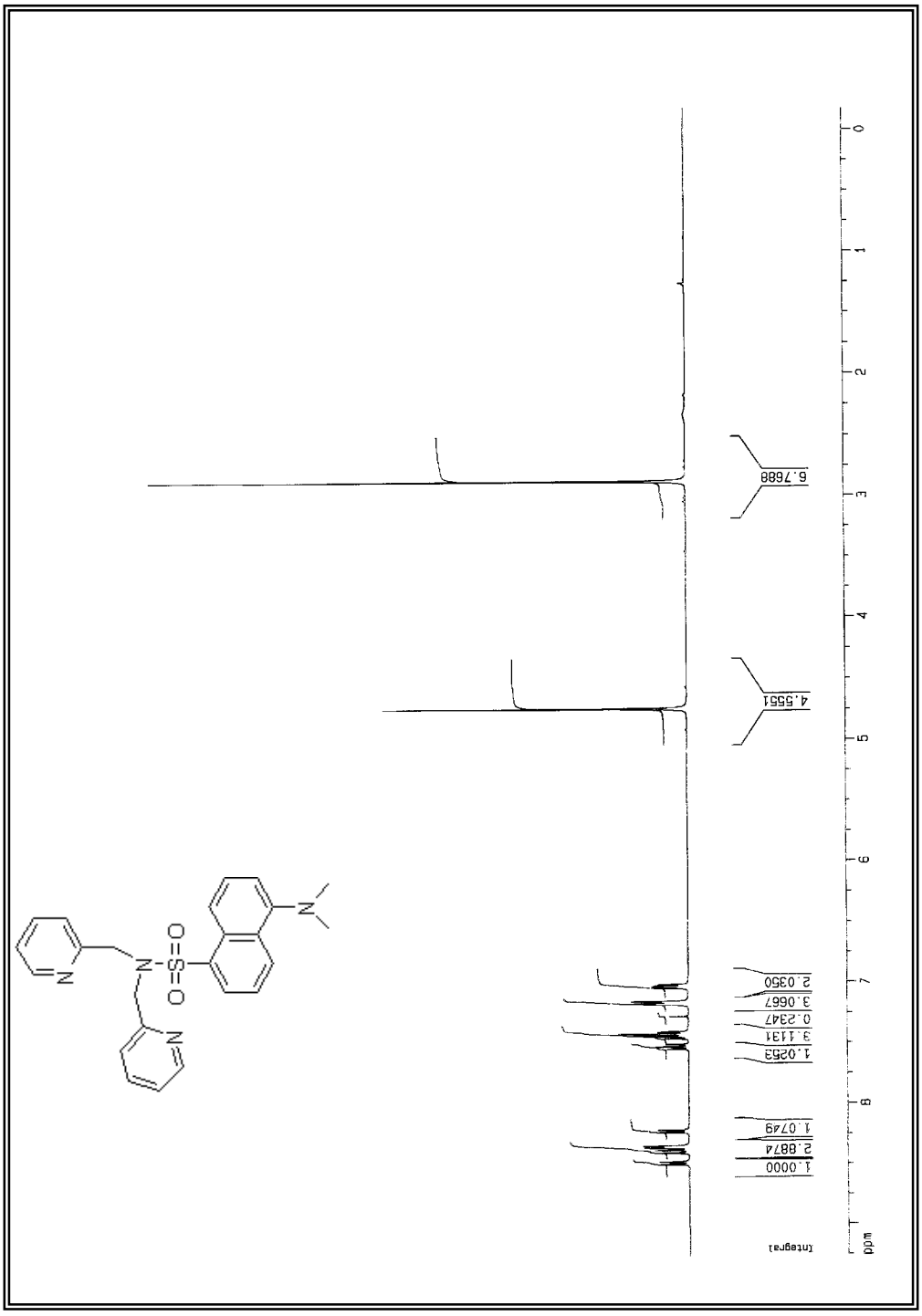

Figure 68. 1H NMR spectrum of compound 41

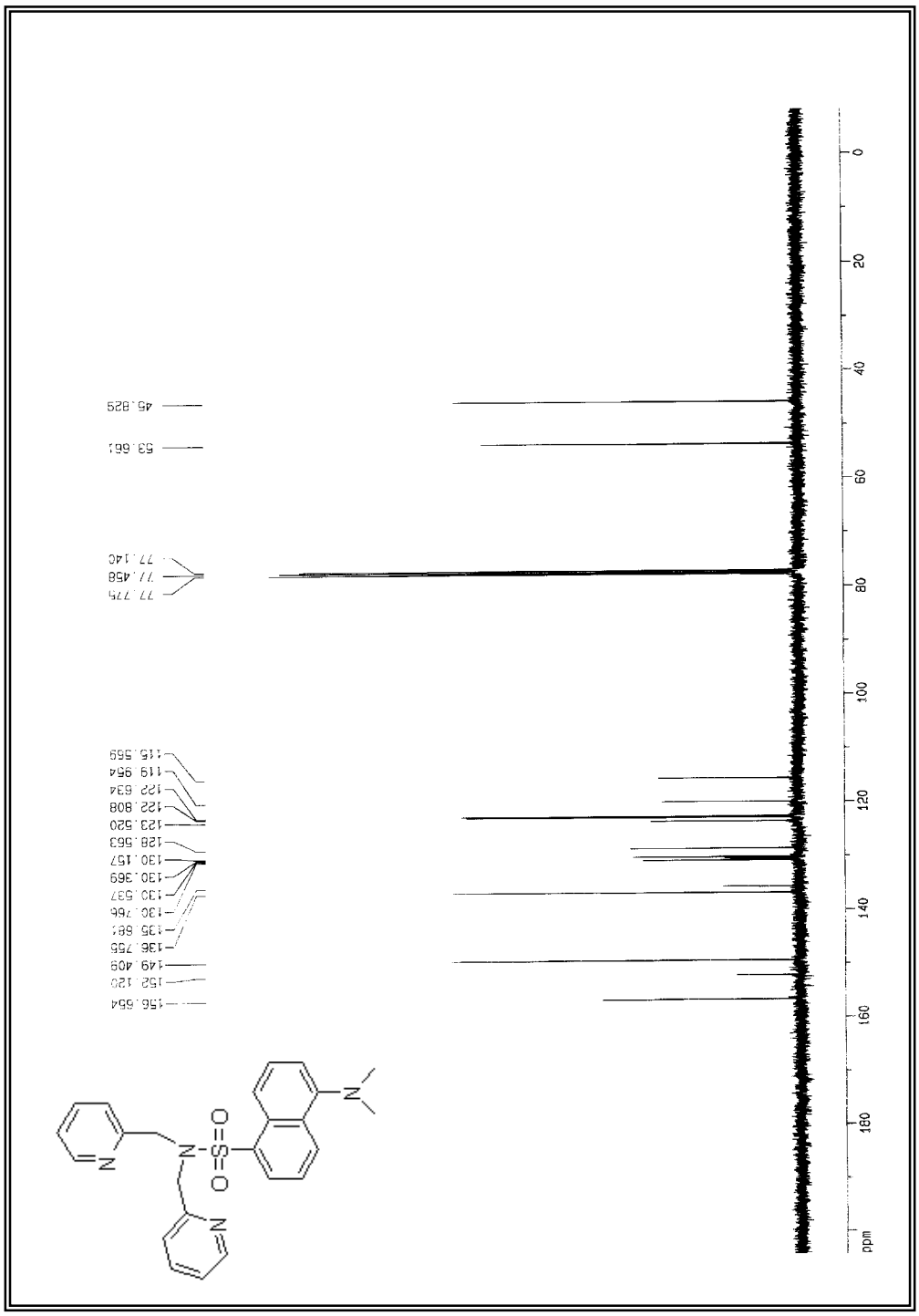

Figure 69. 13C NMR spectrum of compound 41

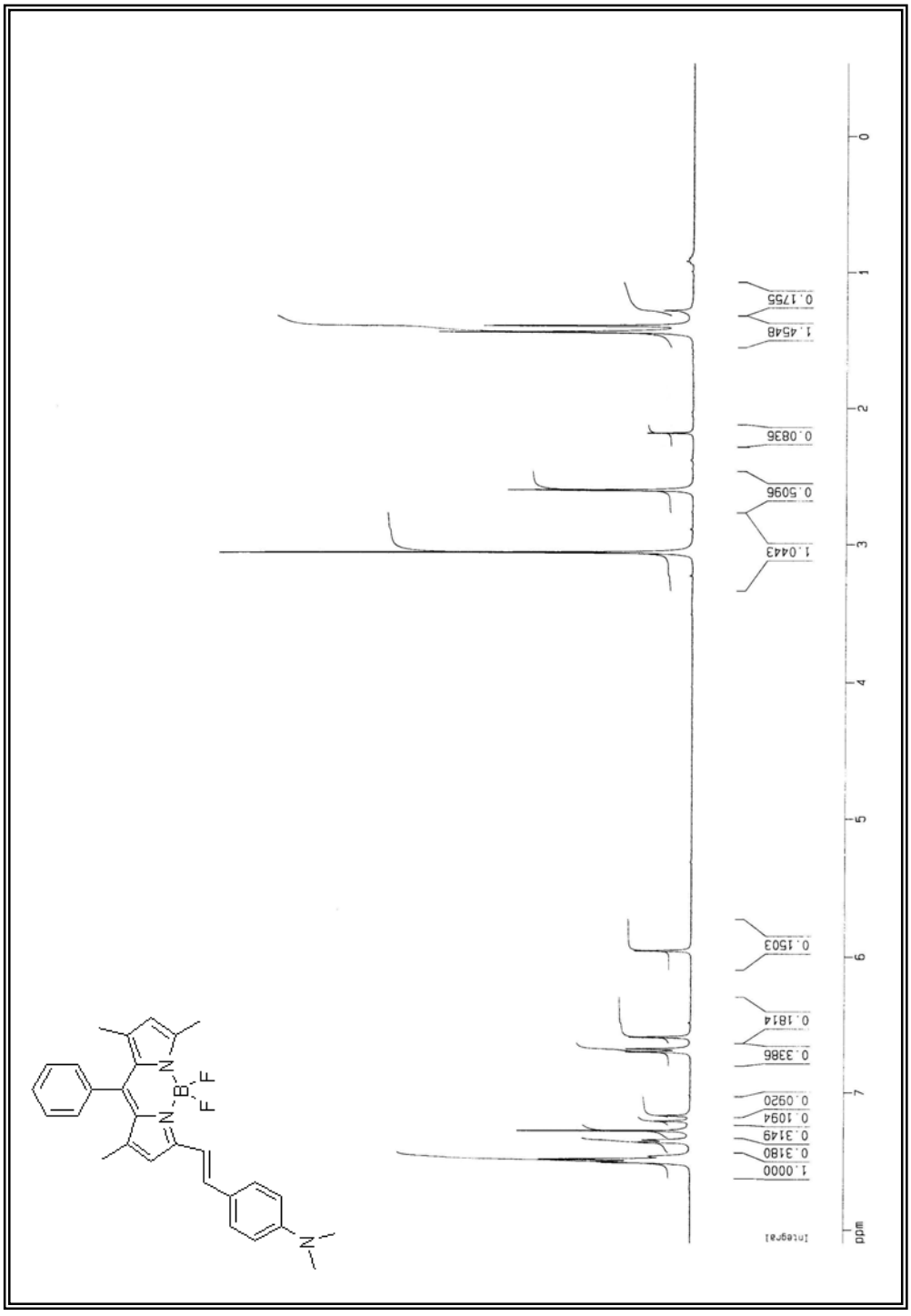

Figure 70. 1H NMR spectrum of compound 44

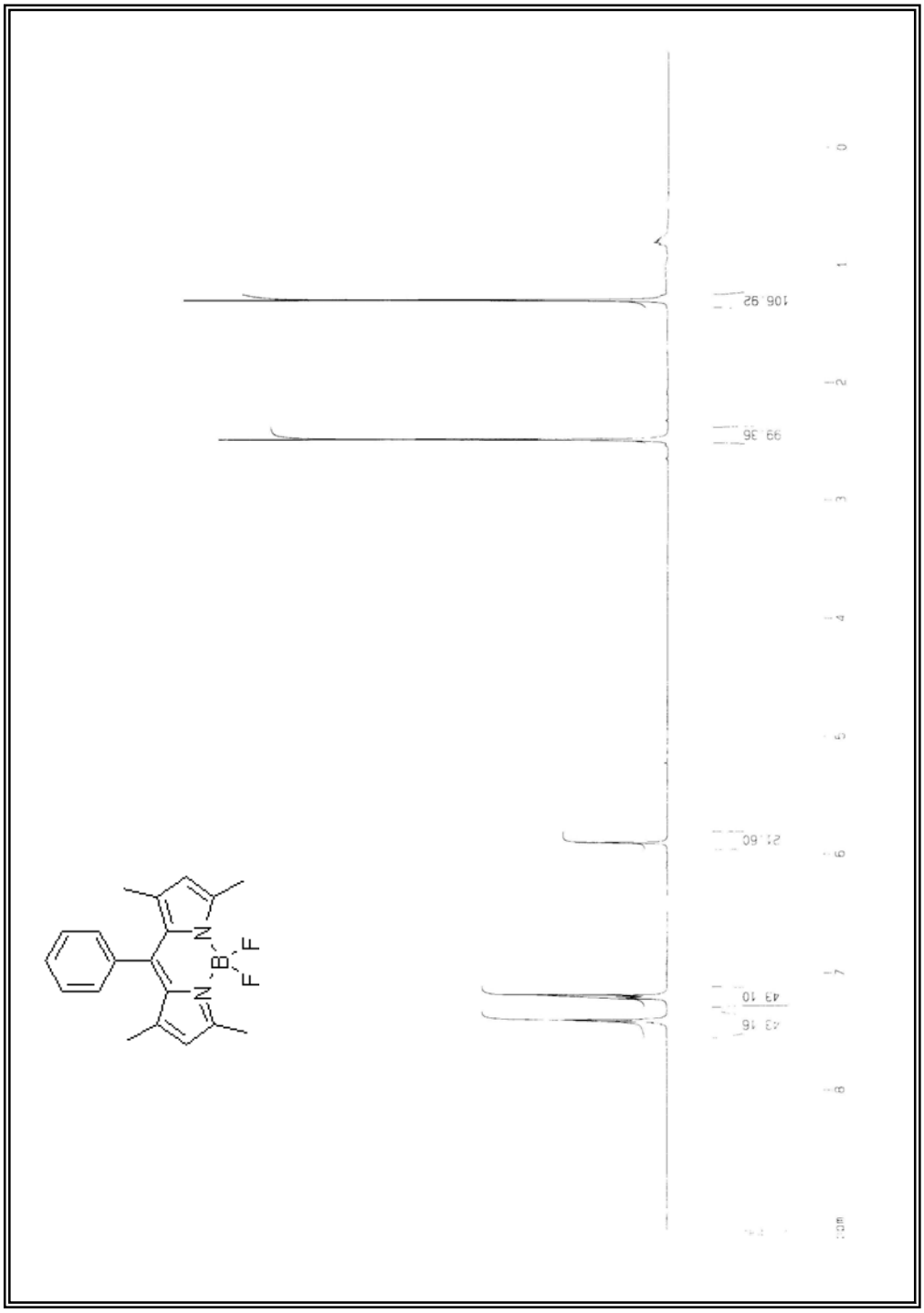

Figure 71. 1H NMR spectrum of compound 42

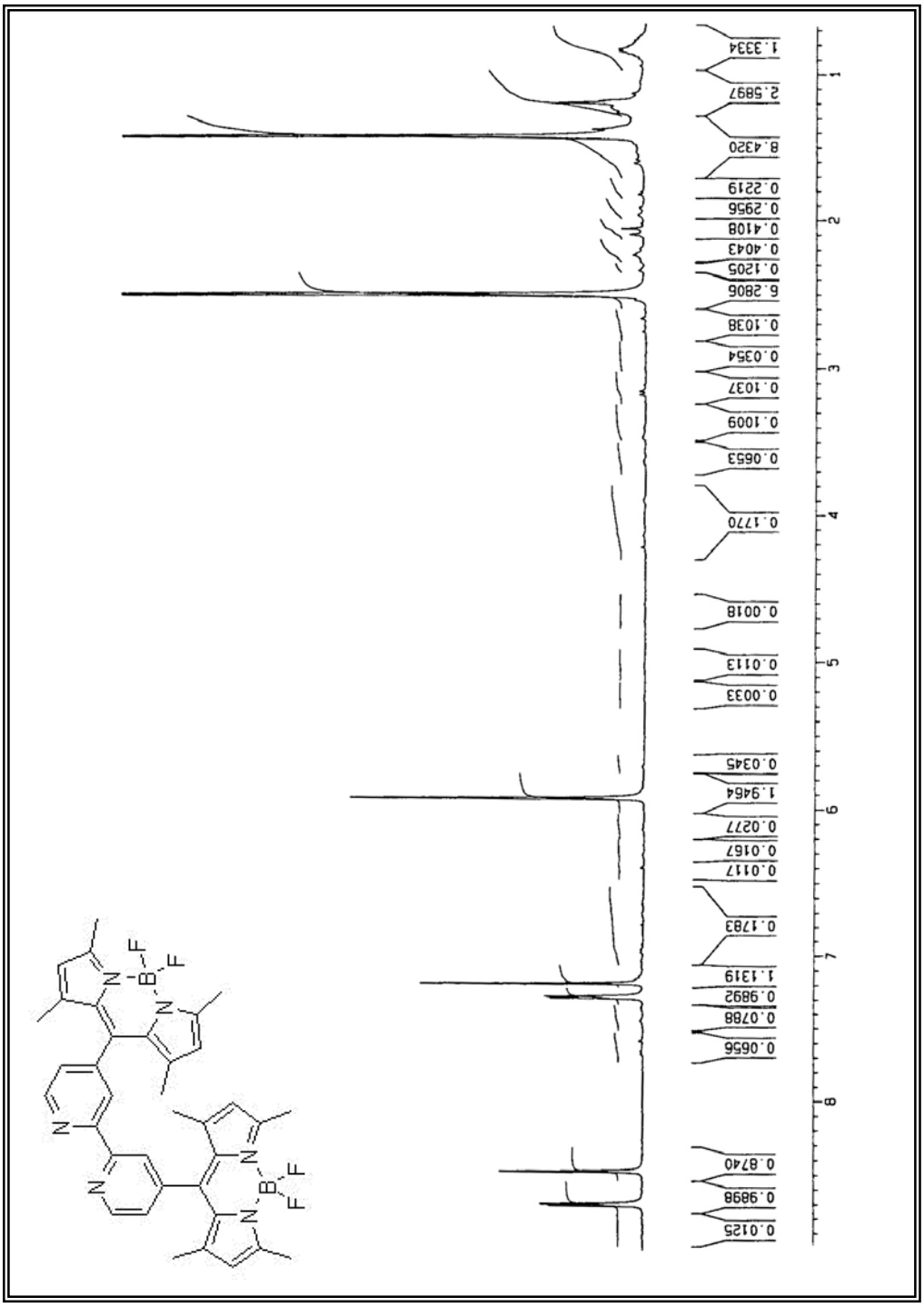

Figure 72. 1H NMR spectrum of compound 46

APPENDIX B

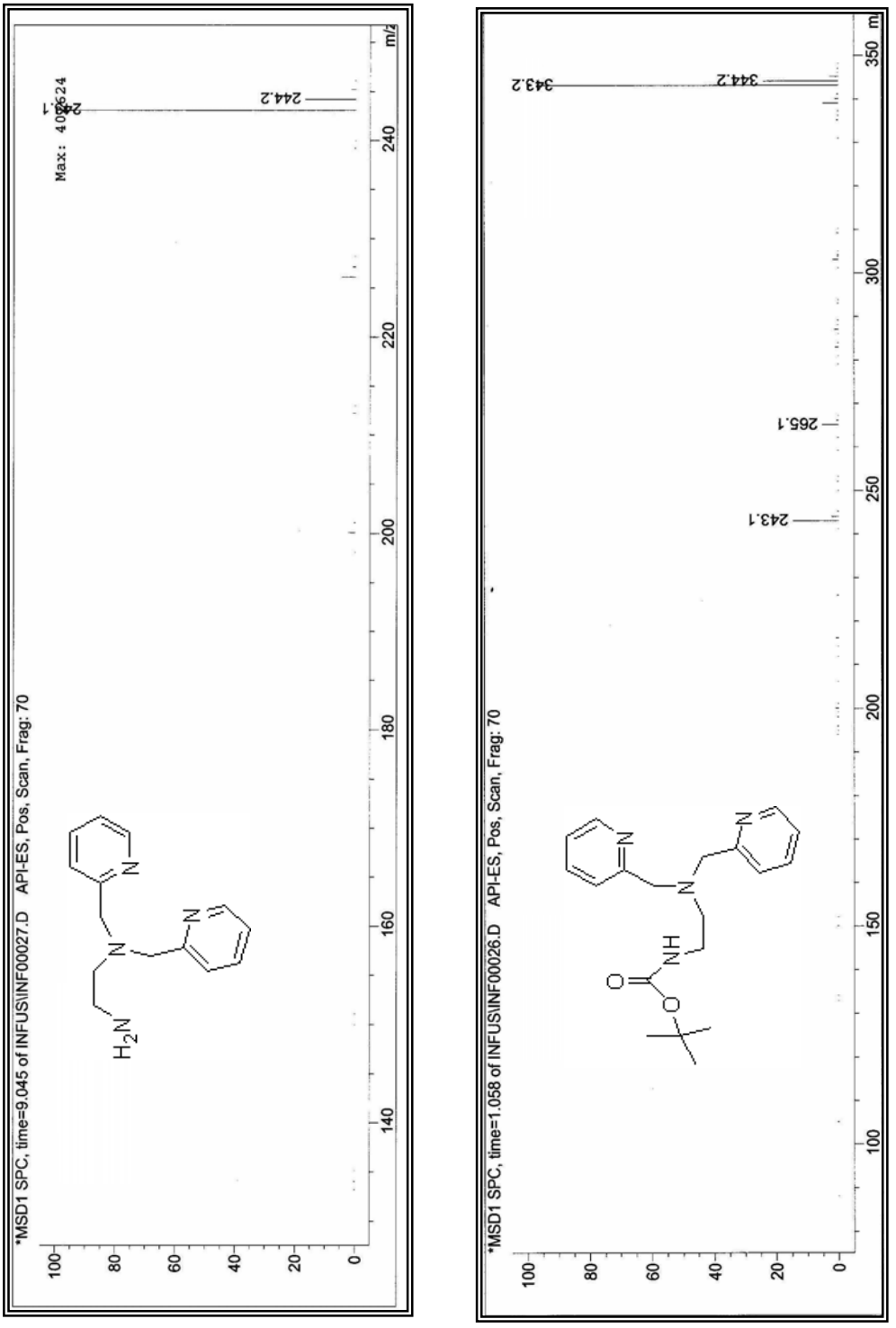

Figure 73. ESI-MS spectrums of compound 37 [M+H]; 343.2 and compound 38 [M+H]; 243.1 respectively

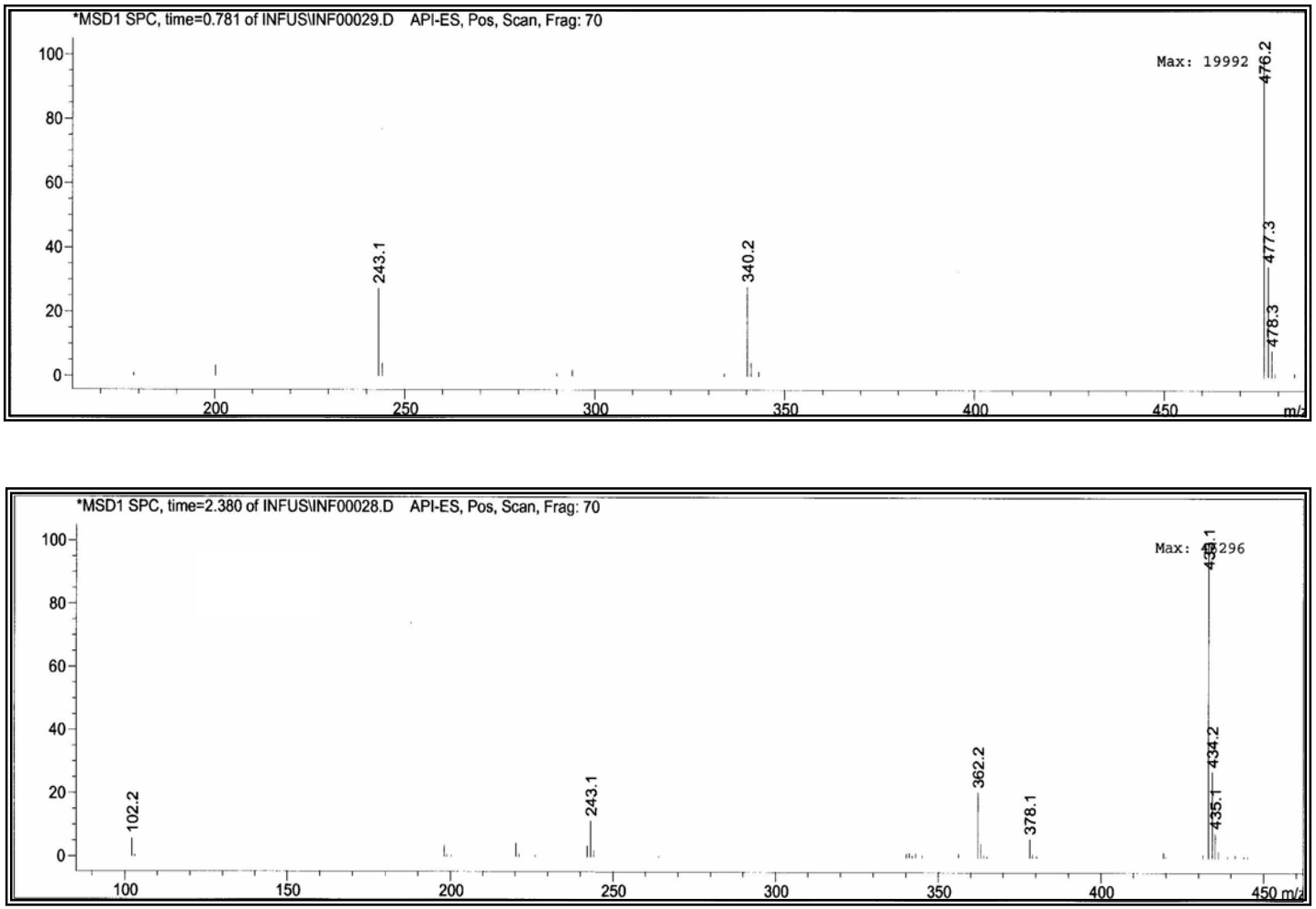

Figure 74. ESI-MS spectrums of compound 40 [M+H]; 476.2 and compound 41 [M+H]; 433.1 respectively