FLUORESCENT SIGNALLING BY NOVEL CHEMOSENSORS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE MIDDLE EAST TECHNICAL UNIVERSITY

BY

GÜLAY DİLEK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
IN
THE DEPARTMENT OF CHEMISTRY

AUGUST 1999

Approval of the Graduate School of Natural and Applied Sciences

Prof. Dr. Tayfur Öztürk
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Erdal Bayramlı
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Assoc. Prof. Dr. Engin Umut Akkaya
Supervisor

Examining Committee Members

Prof. Dr. Haluk Hamamcı
Prof. Dr. Ali Gökmen
Assoc. Prof. Dr. Akgül Yeşilada
Assoc. Prof. Dr. Engin Umut Akkaya
Ass. Prof. Dr. Metin Zora

ABSTRACT

FLUORESCENT SIGNALLING BY NOVEL CHEMOSENSORS

Dilek, Gülay

M. S. Department of Chemistry

Supervisor:Assoc. Prof. Dr. Engin Umut Akkaya

August 1999, 102 pages

Design of fluorescent chemosensors with red to NIR emission is an active area of research. Fluorescent chemosensors synthesized until now, function at short wavelength region of the visible spectrum. This creates a significant background fluorescence and is highly damaging to the cell,

complicating the study of in vivo processes. Short wavelength excitation also necessitates the use of expensive quartz optical components. In spite of this recognized short-coming, there are very few examples of chemosensors that "signal" in red, in part because of the synthetic challenge involved in assembling this type of sensor molecules. Squaraines with their long wavelength excitation and emission proved to be highly amenable to modification yielding novel fluorescent chemosensor. The photochemical and photophysical properties of squaraines can be conveniently modified by substitutional changes.

In the first part of this study, a series of trimethylindolenine-derived squaraines with different alkyl groups on the indolenine nitrogen is synthesized. In the course of this study of squaraine based molecular signalling devices, the participation of groups attached to indolenine system and the squaryl oxygen in metal ion coordination is investigated.

The second part of this study involves the synthesis of novel Kemp's triacid phenanthridinediimide derivative which has selective response to zinc ions compared to cadmium ions.

Key words: Chemosensor, squaraine, molecular recognition, Kemp's triacid.

ÖZ

YENİ MOLEKÜLER ALGILAYICILAR İLE FLORASAN SİNYALLEME

Dilek, Gülay

Yüksek Lisans. Kimya Bölümü

Tez Yöneticisi: Doç. Dr. Engin Umut Akkaya

Ağustos 1999, 102 sayfa

Kırmızı ve yakın kızıl ötesi emisyonu olan floresan moleküler algılayıcıların tasarımı aktif bir araştırma alanıdır. Şu ana kadar geliştirilen floresan moleküler algılayıcılar genel olarak görünür bölge spekturumunun kısa dalga bölgesinde çalışırlar. Bu önemli bir arka plan floresanı yaratır ve

hücre içi süreçlerin çalışılmasını güçleştirerek hücreye oldukça zarar vermektedir. Kısa dalgaboyu uyarılması pahalı kuvars optik bileşenleri de gerektirir. Bu engelin bilinmesine rağmen hala kırmızı bölgede sinyali olan çok az sayıda moleküler algılayıcı örneği vardır. Bu da kısmen böyle bir algılayıcı molekülün oluşturulmasındaki sentetik zorluktur. Uzun dalgaboyu uyarılmaları ve emisyonları ile " squaraine" ler yeni floresan moleküler algılayıcıların geliştirilmesine imkan tanımışlardır. "Squaraine" lerin fotofiziksel ve fotokimyasal özellikleri yapısal sübstitüsyonlarla elverişli bir şekilde modife edilebilir.

Bu çalışmanın ilk kısmında "indolenine" azotunda farklı alkil grupları bulunan bir seri "trimethyl indolenine" türevli "squaraine" ler sentezlenmiştir. "Squaraine" tabanlı moleküler sinyalleme aygıtlarının bu çalışmasında "indolenine" sistemine iliştirilmiş grupların ve metal iyon koordinasyonunda "squaryl" oksijeninin katılımı araştırılmıştır.

Bu çalışmanın ikinci kısmı kadmiyum iyonuna göre çinko iyonuna daha seçici cevap veren yeni "Kemp's triacid phenanthridinediimide" türevini içermektedir.

Anahtar kelimeler: Moleküler algılayıcı, "squaraine", moleküler tanıma, "Kemp's triacid".

To my family

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my supervisor Assoc. Prof. Dr. Engin U. Akkaya for his guidance and support during the course of this research work.

I would like to thank all my friends in the biochemistry laboratory for their encourangement.

Special thanks go to my family and Makbule Dilek for their continuous support and help.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

CHAPTER 1

INTRODUCTION

1.1. From Molecular to Supramolecular Chemistry

For the selective complexation of metal ions, the design of chelating ligands has been an important goal of coordination and supramolecular chemistry for several decades (Krämer, 1998). Beyond molecular chemistry based on the covalent bond lies supramolecular chemistry based on molecular interactions – the association of two or more chemical entities as well as the intermolecular bond (Lehn, 1978).

Classically, the term synthesis has a meaning of the construction of molecular systems as a result of the sequential formation of covalent bonds (Fyfe, et. al, 1997). Molecular chemistry is the chemistry of the covalent bond and it is concerned with uncovering and mastering the rules that govern the structures, properties, and transformations of molecular species. Nonetheless, the chemistry of the covalent bond has now almost been stretched to its conceptual limits. Even the best present-day synthetic chemist can not hope to fabricate complicated nanosystems using only the currently available covalent bond (Whitesides, 1990). In order for the synthetic chemist to be able to build nanosystems another type of bond-

specifically, the intermolecular, noncovalent bond-must be learned to control. The synthesis of nanoscale composites by employing the noncovalent bond has led to the birth of a highly interdisciplinary field of chemical research – supramolecular chemistry – in recent years (Lehn, 1988). This branch of contemporary science is concerned with advancing structural complexity from inclusion complexes toward ordered oligo- and polymolecular entities which are held together using noncovalent, intermolecular bonds.

In the broad field of this science, the design and hence the use of chemosensors for ion and molecule recognition have developed at an extraordinary rate. This imaginative and creative area which involves the interface of different disciplines, e.g. organic and inorganic chemistry, physical chemistry, biology, medicine, environmental science, is not only fundamental in nature. It is also clear that progress is most rewarding for several new sensor applications deriving from the specific signal delivered by the analyte-probe interaction.

Indeed, if calcium sensing in real time for biological purposes is actually possible, because of the emergence of efficient fluorescent receptors, other elements can also be specifically detected, identified and finally titrated using tailored chemosensors (Czarnik, 1992). In addition, pollutants such as heavy metals or radionuclides are among the main targets since their detection and removal could be envisioned at very low concentrations with sensors displaying specific and strong complexing abilities. Besides, various species of biological interest including sugars and other micellaneous molecules such as oxygen and carbondioxide can be actually probed with optodes and similar devices.

Supramolecular chemistry may be defined as "chemistry beyond the molecule," bearing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces (Lehn, 1985).

Supramolecular chemistry – chemistry of systems (supermolecules) – is made up of molecular components in the same way as molecules are made up of atoms: adducts (Colquhoun, et. al, 1986), cage-type compounds (Belser et al, 1988; Lehn, 1988; Sargeson, 1979; Cram et al, 1988), catenones (Dietrich – Buchecker, et. al., 1984), chromoinophores, chundles (channel+bundle) (Lehn, 1988), electron donor-acceptor complexes, host-quest systems (Vögtle, 1981; Vögtle, 1982, Vögtle, 1984; Saenger, 1980; Breslow, 1982), inclusion compounds (Weber, 1987), intercalates (Barton, 1986), ion pairs (Vogler, et. al, 1990), molecular devices (Lehn, 1988), supermolecules (Lehn, 1988, Balzani, et. all, 1986).

Advances in biology and materials science will require the preparation of new supramolecular structures. The development of supramolecular chemistry requires the use of all resources of molecular chemistry combined with the designed manipulation of noncovalent interactions so as to form supramolecular entities, supermolecules. One may say that supermolecules are to molecules and the intermolecular bond what molecules are to atoms and the covalent bond (Lehn, 1988).

Molecular associations have been recognized and studied for a long time (Lehn, 1985) and the term "Übermolecüle", i.e., supermolecules, was introduced already in the mid-1930s to describe entities of higher organization resulting from the association of coordinatively saturated species (Wolf, 1949).

The partners of a supramolecular species have been named molecular *receptor* and *substrate* (Lehn, et. al, 1973), the substrate being usually the smaller component whose binding is being sought. This terminology conveys the relation to biological receptors and substrates for which Paul Ehrlich stated that molecules do not act if they are not bound.

Any general definition of supermolecule is necessarily arbitary and the word may have different meanings depending on the area to which it is

applied (Lehn, 1985; Balzani, 1987; Lehn, 1978; Ringsdorf, et. al, 1988). Conceptually, the feature that distinguishes a supermolecule from a "large molecule" is the possibility to split the supermolecule into individual molecular subunits (components) capable of a separate existence as they are or with minor modifications. The subunits are therefore characterized by a set of intrinsic properties that can be derived from a study of the isolated subunits or of suitable model compounds. However, the systems in large molecules would completely lose their chemical identity upon fragmentation. Many of the intrinsic properties of each component are expected to be maintained in the supramolecular structure with relatively minor changes that can be ascribed to the mutual perturbation between the subunits. However, the properties of a supermolecule will not generally be a superposition of those of the subunits. It is possible that processes involving two or more components take place in a supermolecule, such as

(i) intercomponent transfer processes (for example, electron or energy transfer) or

(ii) cooperative effects (for example, complexation at other species by two or more components).

These processes may cause the dissappearance of some intrinsic property of the components and/or the appearance of completely new properties, characteristic of the supermolecule.

The ultimate aim of supramolecular chemistry is to become the "science of informed matter" (Lehn, 1995), i.e., it seeks to create functioning, organized nanoscale devices which will be able to stockpile and process information (Whitesides, 1995), by analogy with the countless marvelous examples of machine-like systems which are present in nature.

Compared to the preparation of designed molecular structures, the preparation of designed supramolecular structures is a more difficult problem.

Whereas the atoms in a molecule are held together by strong covalent bonds, the molecules in a supramolecular structure are held together by numerous and relatively weak forces such as hydrogen bonding and van der Waals interactions. Also, other factors such as molecular shape and symmetry play a role in determining supramolecular structure.

In the same way as combination of atoms leads to molecules, combination of molecular components leads to supramolecular species, supermolecules. The current literature clearly shows that chemical research is rapidly moving from molecular to supramolecular species. There are at least four reasons for this trend:

1. the high degree of knowledge reached on molecular species;
2. the extraordinary progress made by synthetic methods;
3. the continuous search for new chemical functions;
4. the need to fill the gap which separates chemistry from biology.

The award of the 1987 Noble Prize in Chemistry to C.J. Pedersen, D.J. Cram, and J.M. Lehn "for their development and use of molecules with structure-specific interactions of high selectivity" has given new impetus to studies of supramolecular chemistry.

1.2. The Need for Nanochemistry

The scientific community has been fascinated and inspired by the creation of nanometer-scale (nanoscale) devices for more than quarter of a century (Philp, et. al, 1996). Enzymes and biogenic receptors are impressive examples of nanoscale devices. The development of nanoscale (10-500 °A) functional devices and growth of a nanotechnological capability will require

methods to prepare and characterize larger molecules or molecular aggregates than have been prepared (Webb, et. al, 1990). Although there are no fundamental factors impeding the development of nanoscale structures, it is realized that "engineering down" (Robinson, 1984) approaches, in other words a reduction in the size of structures generated by lithographic techniques below the present lower limit of roughly 1μm, may become impractial.

The electronics industry is keen to exploit the increased signal processing speeds and lower power consumption of very large scale integrated-nanoscale-electronic architectures. Another primary aim of the industry is the development of a three-dimensional array of switching elements which permits higher levels of integration. However, these goals conflict with the reduction in the size of silicon components. For semiconductor devices, scientists may come across with difficulties as a result of the failure of insulating carriers in the materials used.

Hence, the possibility of "engineering up" from a molecule to functioning electronic devices has become an increasingly attractive prospect (Kuhn, 1981). The requirements of molecular electronics demand that we not only provide molecular scale bistable devices (switches), but also the communication infrastructure (input/output) necessary to exchange information with the outside world (Philp, et. al, 1996). Moreover, the switches must be completely controllable, reversible, and readable at the molecular level. To be able to meet these challenges, the chemist must understand and apply the rules of molecular self-organization, self-assembly, and self-synthesis. Fortunately, biological systems display a diverse array of functional nanoscale structures.

Molecular interactions form the basis of the highly specific recognition, reaction, transport, regulation, etc. processes that occur in biology, such as substrate binding to a receptor protein, enzymatic reactions, assembling of

protein-protein complexes, immunological antigen-antibody association, intermolecular reading, translation and transcription of the genetic code, signal induction by neurotransmitters, and cellular recognition. The design of artificial, abiotic receptor molecules capable of displaying processes of highest efficiency and selectivity requires the correct manipulation of the energetic and stereochemical features of the noncovalent, intermolecular forces (electrostatic interactions, hydrogen bonding, van der Waals forces, etc.) within a defined molecular architecture. In doing so, the chemist may find inspiration in the ingenuity of biological events and encouragement in the demonstration that such high efficiencies, selectivites, and rates can be attained. However, chemistry is not limited to systems similar to those found in biology, but is free to create novel species and to invent processes.

Significant progress can be made in the development of nanoscience by transferring concepts found in biological world into the chemical arena. The science of supramolecular chemistry has started to bridge the gap between molecular and macromolecular structures. By utilizing interactions as diverse as aromatic $\pi - \pi$ stacking and metal-ligand coordination as the information source for assembly processes, in the last decade chemists have begun to use biological concepts such as self-assembly to construct nanoscale structures and superstructures with a variety of forms and functions (Philp, et. al, 1996). Therefore, it can be provided a flavor of how self-assembly operates in natural systems and can be harnessed in unnatural ones.

Binding of substrate to its receptor yields the supermolecule and involves a molecular recognition process. In order for the receptor to "recognize" a potential substrate and bind to it, the two species must complement each other both in size and shape (geometry) and binding sites (energy). This extends Emil Fischer's "lock and key" concept from steric fit to other, intermolecular properties. Therefore, receptor chemistry may be considered as the coordination chemistry and it extends the purpose of

designed organic complexing agents from the coordination of transition metal ions to the coordination of all kinds of substrates: cationic, anionic, and neutral species of an inorganic, organic, or biological nature. In addition to binding sites, the receptor may bear reactive sites that transform the bound substrate, which would make the receptor a molecular reagent or catalyst. If it is fitted with lipophilic groups that allow it to dissolve in a membrane, it may act as a molecular recognition, catalysis (transformation), and transport (translocation). Thus, molecular recognition, transformation, and translocation represent the basic functions of supramolecular species (Figure 1).

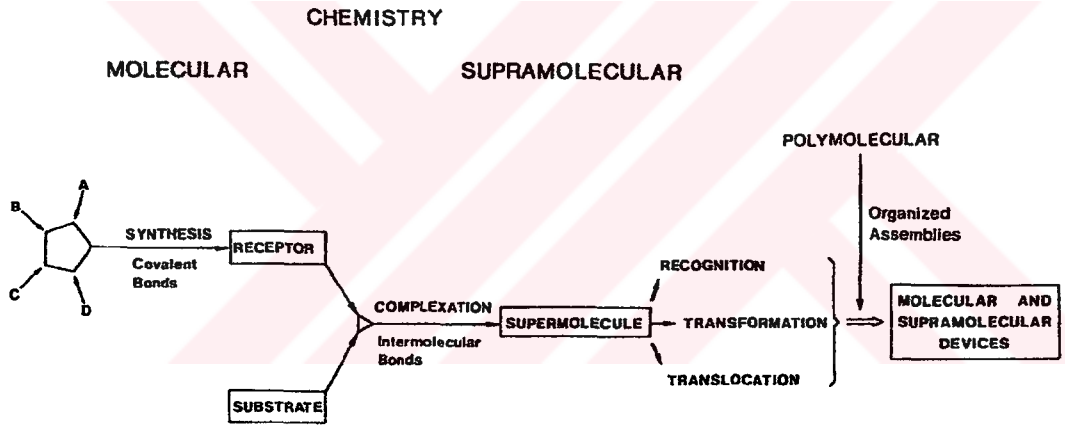


Figure 1. From molecular to supramolecular chemistry: molecules, supermolecules, molecular and supramolecular devices (Lehn, 1988).

In summary, chemistry can be likened to "language". The atoms are the "letters". The molecules are the "words". Assemblies of molecules make up the "sentences". The sets of assembled molecules of supermolecules are the "paragraphs". The ways in which the molecular assemblies and supramolecular arrays contain and express information are the "chapters". The manner in which this information is conveyed at both a molecular level is

the "book". Finally, chemistry has to tell a "story". The life sciences are composed of really wonderful chemical "stories". They have been written by nature using "ancient languages".

Chemists are just starting to write their own "stories". They know how to produce the "words". Now, they are learning how to write the "sentences". The "grammar" they will use will be dictated by the nature of the noncovalent bond. The "modern languages" are about to evolve. Materials science and life sciences will be a beneficiaries. As a discipline, chemistry will be enriched.

1.3. Molecular Recognition, Information, and Signals

"Molecular recognition" is an active area of chemical research that seeks to understand how to bind a "guest" molecule to a "host" molecule or material selectively and it has been defined a process involving *both binding* and *selection* of subtrate (s) by a given receptor molecule, as well as possibly a specific *function.* Molecular recognition thus is a question of *information storage* and *read out* at the supramolecular level (Lehn, 1988). It requires the design of receptors possessing steric and electronic features complementary to those of the substrate to be bound and a balance between rigidity and flexibility suitable for the function to be performed. Molecular recognition events may give rise to changes in electronic, ionic, optical and conformational properties and thus translate themselves into the generation of a signal.

Molecular recognition is one of the cornerstones of supramolecular chemistry because of its implications in many fields such as chemistry, biology, medicine (clinical biochemistry), environment, etc. In particular, selective detection of metal cations involved in biological processes (e.g., sodium, potassium, calcium, magnesium), in clinical diagnosis (e.g., lithium,

potassium, aluminum) or in pollution (e.g., lead, mercury, cadmium) has received considerable attention (Valeur, 1993).

There is at present a need in several areas for a rational approach toward ligand design for selective complexation of metal ions in solution. Such areas would be, for example, design of ligands as therapeutic reagents for the treatment of metal intoxication, design of antibiotics that owe their antibiotic action to specific metal complexation, design of complexes to act as imaging agents in the body, design of functional groups for chelating ion-exchange materials, and metal ion sequestering agents in detergents (Lauffer, 1987). At the same time, an understanding of the principles of selectivity would be invaluable in understanding the metal ion selectivity displayed by biological cation transport systems such as in the cell wall, metal ion binding proteins such as metallothionein, and how metal ions are distributed in the environment (Martell, 1989).

For the selective complexation of metal ions, one of the goal of supramolecular chemistry is the design of chelating ligands and chemical sensors for metal ions become accessible by combining recognition event with an easily quantiable signal event. The properties of an "ideal" sensor include high selectivity for one metal ion only, high sensitivity, fast and reversible (real-time) response, real-space response down to the micrometer level, and easy handling. Fluoresence signaling offers the advantage of high sensitivity, ease of automation, and it can be directly used for sensors with fiber optic systems. Therefore, fluorescence probing of the structure and dynamics of matter or living systems at a molecular or supramolecular level has been the object of numerous investigations in various fields such as polymers, solid surfaces, surfactant solutions, biological membranes, vesicles, proteins, nucleic acids, living cells, fluoroimmunochemistry, clinical diagnosis, etc. In fact, because of the sensitivity of fluorescent molecules to their microenvironment, information can be obtained on local physical and structural parameters (polarity, fluidity, order parameters, molecular mobility,

distances at a supramolecular level) as well as local chemical parameters (pH, ion concentration) (Czarnik, 1993). Such a local information is seldom accessible by other techniques.

The increasing interest of researchers for fluorescent chemosensors can be explained by the great improvement of the sensitivity and the spatial or temporal resolution of instruments, and by the development of a wide choice of commercially available probes for particular applications. The rapid analysis of trace metal cations for environmental and biomedical applications is particularly demanding since it requires the specific recognition of a particular element in the presence of numerous closely related species. In particular, sensors based on fluorescence measurements are desired due to their working detection limits typically below 0,1 μm analyte concentrations.

As an analytical tool, fluorescence offers unique and useful properties. The sensitivity of a fluorescence assay can be exquisite; in the extreme, single atoms can be visualized. With appropriate fluorophores, fluorescence emission can often be viewed at relatively long wavelengths that minimize background signals. Furthermore, there are many ways in which a fluorescence signal can be modulated, including intensity increase or decrease at a single wavelength, simultaneous intensity increase at one wavelength and decrease at another (ratiometry), fluorescence lifetime change, and fluorescence depolarization. The intensitometric methods suffer from certain limitations, as do all of above (Czarnik, 1993). However, the mode of signal transduction has found increasing utility in recent years in the construction of fluorescent chemosensors for producing real-time sensing of cationic, anionic, and neutral analyses. However, there is still a need for probes with improved specific response and minimum perturbation of the microenvironment, particularly in the field of ion recognition.

1.4. Fluorescence Spectrometry

Fluorescence Spectrometry is becoming increasingly popular in many branches of the chemical and biological sciences. It is used in studies of molecular structures and molecular interactions, in the localization of molecules (especially in biological systems), and in many types of trace analysis.

Fluorescence was first observed as long ago as 1565 and gets its name from the fact that the mineral fluorspar (calcium fluoride) was found to glow under UV radiation. Biologists have long been aware of the great sensitivity of fluorescence assay and were among the first to utilize it. The fluorescence of quinine, chlorophyll, and other plant materials was known to Stokes, who in the mid 19^{th} century first reported that fluorescence emission occurs at a longer wavelength than excitation. This wavelength shift, termed the Stokes shift, provides the primary conceptual basis for the enormous sensitivity of fluorimetric analyses. Stokes also outlined the relationship between the concentration of a fluorophore and observed fluorescence intensity, describing the quenching of fluorescence at high concentration and by the presence of foreign substances. Neither of these effects occurs in absorption spectroscopies, which led Stokes in 1852 to propose that fluorescence be used for the detection of organic subtances.

The transduction of information from the molecular world to ours can be achieved naturally by means of light signals arising from molecular species. Such fluorescence can arise against a dark background, the detection of fluorescent chemical species can approach the ultimate limit of a single molecule (Chen, et. al, 1994). Chemists and physicists have had over a century of experience in quenching fluorescence, which means that fluorescence signals can be easily switched "off" or "on" (de Silva, et. al, 1992). In fact, environmental factors can be responsible for fluorescence

switching which means that the fluorescence signals can directly relay information from the molecular sensor's environment. Such information can be highly resolved in space and time because of the use of molecules of <nm dimension and excited state lifetimes of ns-ms (Bissell, et. al, 1994). The use of light as an information carrier naturally allows multiplexing. Parallel operation with different colours is possible. Imaging is easily done with naked eye viewing. Moreover, picogram quantities of luminescent materials can frequently be studied. Owing to the sensitivity of fluorescent molecules to their microenvironment, information can be obtained on local physical and structural parameters as well as local chemical parameters as pH, ion concentration. Such local information is seldom accessible by other techniques. Light can travel through and exit from environments without an absolute requirement for a physical waveguide, electrochemical sensors rely on wires. This provides the simultaneous visualization of concentrations in all regions of a living cell.

Besides, dilute and concentrated solutions, suspensions and solid surfaces can all be readily studied, and combinations of fluorescence spectoscopy and chromatography are especially useful since fluorescence technique is selective, deriving in part from the two characteristic wavelengths (excitation, fluorescence).

Among these, the principle advantage of fluorescence signalling is its use in remote sensing applications with fiber optic techniques (optrodes), which permit "wireless" communication between the analyte and the detection element, making it particularly attractive for *in-situ* remote sensing applications. Moreover, because signals of many different wavelengths can propagate in either direction within a fiber optic, sensor arrays with multiple sensing capabilites may be constructed. Fiber optic sensors are not subject to electrical interferences and do not require a reference. These devices can be miniaturized.

Moreover, fluorescent chemosensors can be used at all levels of organization from whole organs to isolated tissues and can be incorporated into the intact functioning cells without destruction.

In-situ monitoring or *in-vivo* imaging of a binding event to a fluorescent chemical reagent, detection of a single molecule fluorescence, micro-sample analyses, remoteness of fiber-optic data aquision, on-site measurements, long-term multi-location continuous observations of chemical processes, etc. These are among the most attractive features of the new spectroscopic methodologies. Because of its high inherent sensitivity and selectivity, fluorescence spectroscopy is widely used in various fields of physics, chemistry, biology, and medicine. It is essential to develop fluorescence techniques for metal trace detection or for the recognition and measurement of metal components in various systems, especially in biological materials. However, among these techniques, those with improved specific response and minimum perturbation of the microenvironment will be the most useful ones.

The essence of fluorescence spectrometry is that a molecular sample, illuminated by light from an external source, emits fluorescence at a different wavelength, generally larger than that of the exciting light. The fluorescence phenomenon may be explained as follows (Guilbault, 1990): the absorption of a quantum of light by a molecule results in the promotion of an electron from the molecule's ground electronic state where the total electron spin is zero and the state is called as "singlet" (S_0) to one of several vibrational levels in the excited electronic state where the total electron spin is still zero. In solution, the excited state molecule relaxes to the lowest vibrational level of the lowest electronic state (S_1) rapidly. Therefore, the energy stored in the excited state may be released in several ways. The electron may return to the electronic ground state with only the release of heat, which is also called "radiationless relaxation". After relaxing thermally to the lowest vibrational energy level of the S_1 state, the electron may return to the S_0 state with light

emission. This process is called *fluorescence*. If the molecule is sufficiently long-lived in the S_1 state, it may cross into a lower energy triplet state, which is donated by T_1 and T_1 has nonzero total electron spin and exhibits magnetic properties. Relaxation from the T_1 state to the S_0 state can also occur with light emission in solids (phosphorescence), by chemical reaction or by the release of energy (radiationless transition).

Loss of energy by vibrational relaxation and internal conversion involving excited state is generally much more rapid than the fluorescence and phosphorescence transitions, which normally originate from the ground vibrational states of S_1 and T_1, respectively. This energy loss ensures that the wavelength of maximum fluorescence is langer than the wavelength of maximum absorption (solvent relaxation effects also contribute to this result): further, since the T_1 level is of lower energy than S_1, the phosphorescence maximum is at a longer wavelength than the fluorescence maximum.

Fluorescence signalling permits the monitoring of both excitation and emission wavelengths. The emission signal can be observed in the form of intensity, intensity ratio or lifetime measurements.

Signal transduction is the mechanism by which an interaction of sensor with analyte yields a measurable form of energy (Czarnik, 1992). A sensor is a device that interacts with matter or energy and it yields a measurable signal response. A chemical sensor is a micro- or macroscopic device that interacts reversibly with a chemical analyte with signal transduction. However, a chemosensor is an abiotic device, molecule-sized or larger, that signals interactions with analytes reversibly and in real-time. On the other hand, a biological sensor is a micro- or macroscopic device that interacts reversibly with a biological analyte with signal transduction. A biosensor can be described as a biotic device that signals the presence of matter or energy.

In order to detect ions, fluorescent sensors are designed which can bind ions and after binding they undergo photophysical changes.

Among these sensory systems, properly designed fluorescent chemosensors are the best techniques for the recognition of ions inside the cells. Since chemosensors using well-defined thermodynamic equilibria to measure analytes in the cells inherently measure the free ion concentrations or activities that are usually biologically relevant and they are more interpretable than measurements of the total concentration (bound plus free) of the analyte in dead tissue (Tsien, 1983). The analytical techniques such as flame photometry, atomic absorption spectrophotometry, neutron activation analysis, radioisotope equilibration, electron-beam microscope analysis are the destructive methods for measuring total concentrations and usually they are unable to give reliable information about the more relevant and dynamic free ion concentrations. Moreover, optical techniques are generally fast, relatively non-invasive, sensitive and easily adapted to high spatial resolution via microscopic imaging and also fluorescence is one of the best modes of optical read out.

1.5. Fluorescent Chemosensors for Transition-Metal Ions

During the last decade, a research area of fascinating interest in supramolecular chemistry has counted on the conception and the study of molecular devices endowed with both photoactive and recognition properties (Lehn, 1990). The creative imagination of chemists has led to the generation of supermolecules and materials which present outstanding potential as artificial photosynthetic systems or recognition-directed entities for molecular electronics or signal processing.

In that connection, fluorescent chemosensors occupy a central place (Czarnik, 1992). Compounds incorporating a binding site, a fluorophore, and a mechanism for communication between the two are called fluorescent chemosensors. A fluorescent sensor is essentially a two component compound, in which a light-emitting group is covalently linked to a receptor specific for a particular ion. They are specifially designed to exhibit selective complexation affinity toward a given species and upon guest binding they undergo profound photophysical changes (extinction coefficient, fluorescence intensity, shifts in absorption and emission spectra) (Figure 2)

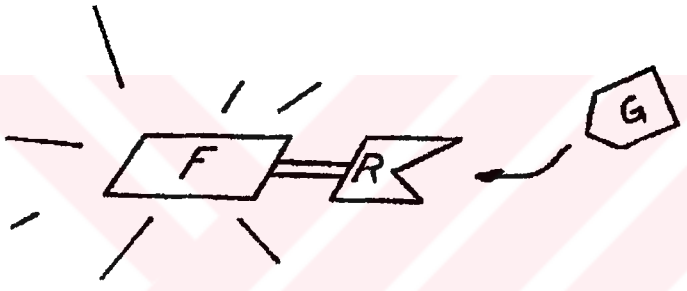


Figure 2. Schematic representation of a photoresponsive receptor system; G= guest species, F= fluorophore group, R= recognition site.

Sensor efficiency requires that the ion-receptor interaction modifies the fluorescence of the light-emitting unit. Changes of fluorescence intensity of two orders of magnitude and more are often observed, in other words the fluorescence can be switched on and off.

The basic requirements for the design of an artificial photoresponsive supramolecular system are as follows:

(a) a component of the supramolecular system must be able to absorb light;

(b) as a consequence of light excitation, the choromophoric component (or another component) must undergo a structural change;

(c) such a molecular structural change must cause a "functional" change (that is a change in some properties relevant to a function) in another component or in the whole supramolecular structure.

Due to the inherent sensitivity of fluorescence, the photoresponsive molecular probes that satisfy these characteristics are of considerable interest for the detection of trace analytes in solution. Actually, the case of metal cations has attracted much of the attention of the chemists in the field. However, there is also a real demand for the development of fluorescent chemosensors for the recognition of various other kinds of substrates, such as inorganic anions, molecular ions, neutral molecules, and radical species, and this area has not yet been so extensively investigated.

Because the selective binding of metal ions from water is considerably easier than that of anions or neutrals, the development of fluorescent chemosensors for metal ions occured early. Goppelsröder reported in 1867 that morin forms a strongly fluorescent chelate with Al(III). Fluorimetric methods for the determination of many other metal ions, with vastly differing degrees for ion discrimination, have been described in the anteceding century (Czarnik, 1994). Until only recently, these sensing molecules were invariably heterocycles bearing chelating arrays of donor atoms, which are classified as *intrinsic* chemosensors. Intrinsic chemosensors are the ones in which the mechanism for signal transduction involves interaction of the analyte with a ligand which is the part of the fluorophore π-system.

During the past 25 years, scientists have studied on the *de novo* design of abiotic receptors, in an endeavor progressively termed host-guest chemistry, biomimetic chemistry, molecular recognition, and supramolecular chemistry. An ability to design receptors from scratch complements and the ability to generate such receptors from biotic sources has proven useful in

the mimicking enzyme catalysis and in transport phenomena. The application of supramolecular receptors to fluorescence sensing was first described by Sausa in 1977. When naphthalene is outfitted with a crown ether binding site, the association of alkali earth metals is signalled by changes in the naphthalene fluorescence; because all of the donor atoms remain in the insulated from the fluorophore π - system. Such compounds are classifed as *conjugate* chemosensors. In conjugated chemosensors the mechanism for signal transduction involves interaction of the analyte with a ligand electronically-insulated from the fluorophore π- system.

About ten years ago, the first examples of fluoroionophores emerged as prototypes of a new class of fluorescent probes particularly well suited for the detection of alkali- and alkaline-earth metal ions (Löhr et al, 1985). These compounds are based on crown-ethers and cryptands as complexing units (ionophores) and aromatic hydrocarbons or organic dyes as fluorophores, the two components being chemically connected in such a manner that an internal transduction mechanism can effect the conversion of a chemical information into an optical signal at the molecular level.

Because of the importance of s-block metal ions in biology, fluoroionophore research area has been very productive and still remains of current interest. Some systems already found practical applications, such as Tsien's calcium chemosensors based on EDTA-type chelating structures that are being widely used by biologists in order to assess and image Ca^{2+} concentration jumps in living cells. Moreover, in 1992, a fluorescent chemosensor was synthesized for monitoring potassium *in-vivo* continuously in blood during open-heart surgery and accross biological membranes (Masilamani, et. al, 1992).

In contrast to the case of s-block cations, the number of fluorescent chemosensors for transition- and heavy-metal ions detection remains very small. Transition metals are required for life processes as they participate in

many biological reactions in plants, microorganisms, animals, and human beings. Especially, first row metals, such as manganese, iron, cobalt, nickel, copper, and zinc, play a central role in biological, environmental, and marine media, although neutral natural concentrations of these bioactive trace metals are extremely low, typically within the nano- to picomolar range. These metals can also be pollutants derived from diverse sources and thus may exhibit more or less high toxicity, depending on the nature of the element. Besides, it is now well established that it is the chemical form of a metal rather than its total concentration which determines its bioavailability and toxicity in natural media. Therefore, chemical speciation of transition metals is receiving considerable attention in biology, ecotoxicology, geology, and oceanography. For this reason, fluorescent chemosensors might represent versatile analytical tools to allow not only the determination, but also the speciation, of bioactive trace metals present at extremely low concentrations in complex natural media.

Actually, transition-metal ions have been known to effectively quench fluorescence for quite a long time. Indeed, 3d-block metal ions and many of their chelates with organic ligands possess low-lying energy levels and exhibit redox activity, so that they may be very efficient in promoting energy or electron transfer processes.

Among those two-component supramolecules that were specifically designed to complex transition-metal ions, most were shown to exhibit the expected metal induced quenching of the ligand-appended chromophore fluorescence, thus operating according to a chelation-enhanced quenching (CHEQ) mechanism (Fages, 1992) (Figure 3).

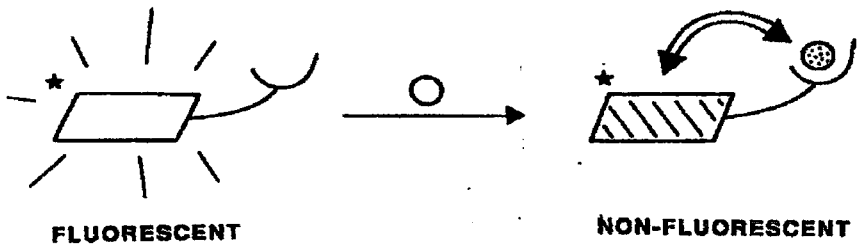


Figure 3. Transition-metal ion-induced ON-OFF switching of fluorescence in a two compenent molecular system. Quenching occurs *via* electron-transfer and/or energy-transfer mechanisms, due to the interaction between the complexed metal and the excited chromophore.

The measurement of fluorescence quenching as a general method for the determination of inorganic quenchers is known not to be very specific for it may be subject to the interferences from other quenching species also present in solution, such as dissolved dioxygen, anions, etc. This may contribute to poor motivation to chemists to synthesize fluorescent receptors based on metal-induced quenching of fluorescence and to use them as optical sensors but such molecules may become attractive and high-performance sensors provided that the following critical characteristics are fulfilled (Fages, 1992):

1. The fluorescent probe must exhibit an intense fluorescence emission in the uncomplexed state and present a selective affinity for the metal ion under investigation,

2. The structure of the complex must flavor the generation of metal-chromophore interactions, so that complexation is followed by the full extinction of chromophore fluorescence.

1.6. Requirements for the Design of Fluorescent Chemosensors

The design of any fluorescent chemosensor requires knowledge of three topics (Czarnik, 1993):

1. how can one bind a specie with selectivity,
2. how can one generate signals from such binding events that are easy to measure,
3. What mechanisms for binding and signal transduction intersect.

With regard to selective binding , the classes of compounds known as crown ethers and their nitrogen analogues have found extensive application. Especially for metal ions which are highly regulated in biological systems or those implicated in such regulatory mechanisms, there have been a number of proposals as potential chemosensors. As the field matures, characteristics for a desirable chemosensor have become apparent.

Recognition of ions requires special care in the design of fluorescent probes because attention should be paid to both recognition and signalling moieties. Recognition is responsible for selectivity and efficiency of binding, which is relevant to the field of supramolecular chemistry and signalling converts the information into an optical signal which should be as selective as possible of the species to be probed. Therefore, selectivity must be viewed in terms of both selectivity of binding and selectivity of photophysical effects.

In order to make use of the advantages of chemosensors, the binding domain of the fluorescent chemosensor must have sufficient selectivity for the analyte of interest as compared to others present (Minta, 1989). Moreover, the analyte must be capable of dissociating from the binding

domain in real-time and the fluorescence should be as intense as possible. It should be characterizable by a product of extinction coefficient and fluorescence quantum yield exceeding 10^3-10^4 M^{-1} cm^{-1}. That is, the chemosensor should have good quantum yield in aqueous solutions and high extinction coefficient to ensure that small concentrations of the chemosensor is sufficient for a good signal and also to avoid any buffering of ion fluctuations.

Furthermore, the medium in which recognition takes place is of major importance: parameters such as nature of the solvent (polarity, hydrogen-bonding ability, protic or aprotic character), pH, ionic strength, etc. play a role since they can affect not only the efficiency and selectivity of binding, but also the photophysical characteristics of the fluorophore (for example, protonation may compete with cation binding). In many cases and for biological samples, aqueous solutions are mostly considered and water soluble probes are desirable, but in some analytical applications (e.g., based on extraction) the probe can be in an organic phase.

Binding of the chemosensor to cellular constituents and membranes can be minimized or controlled. Usually, enough charged polar groups such as carboxylates should be added to the chemosensor to render it water-soluble and impermeant through membranes, so that once introduced into cells it does not rapidly leak out again. Unless the intention is to stick to membranes nonspecifically, large domains of unrelieved hydrophobicity should be avoided. The polar groups should be maskable by nonpolar protecting groups which are removable by cytoplasmic enzymes, so that large population of cells can be loaded with the indicator by incubating them with the polar membrane-permeant derivative (Minta, 1989).

If a chemosensor does not shift wavelength but only gets brighter or dimmer upon binding of the analyte, then changes in the cell shape, lamp brightness, indicator content or detection efficiency appears as changes in

analyte concentration. However, such cases are largely excluded by ratioing. Measurements of excited-state lifetime can give similar cancellation (Lakowicz, 1992).

Toxicity (intrinsic and photodynamic) should be minimized.

Photostability in the presence of dissolved oxygen is also highly desirable. In other words, it should have good photo- and chemical stability. For intracellular analytes, the chemosensor should be more or less irreversibly placed into the cell preferably by passive diffusion (incubation with the chemosensor).

Besides, excitation and emission wavelengths should be at the red end of the visible spectrum. Excitation wavelengths should exceed 340 nm, because shorter wavelengths require expensive quartz rather than glass microscope optics and are strongly absorbed by nucleic acids and aromatic aminoacids. Also, emission wavelengths should exceed 500 nm to reduce overlap with tissue autofluorescence, which peaks near 460 nm due to reduced pyridine nucleotides. The use of fluorescence assays can be hindered by autofluorescence from the samples, which almost invariably limits the ultimate sensitivity and autoflorescence and absorbance of biological materials decrease with increasing wavelength (particularly above 600 nm).

Among these, the requirement for long wavelength excitation and emission stands out as the principal requirement for a successful chemosensor, including "ratiometric" chemosensors that is either the absorption or emission spectrum should show a shift that would enable ratioing of the signals of the bound and free chemosensors. Binding of the analyte should cause a large wavelength shift in the excitation and emission spectrum or both (Tsien, 1986; Bright, 1989). Such ratioing is the instrumental analog of color vision and it is important in canceling out the

irrelevancies as dye concentration, cell thickness, and wavelength-independent variations in illumination intensity and detection efficiency. This is needed for low background signals in any biological application, as the endogenous fluorophores typically fluorescence brightly when excited at short wavelengths, and also UV or blue light is highly damaging to the cell complicating the study of *in vivo* processes. Long wavelength chemosensors are more compatible with fiber optics and lifetime-based applications. Rugged and inexpensive laser diodes can be used for excitation. In addition, short wavelength excitation also necessitates the use of expensive quartz optical components. Therefore, there is a great impetus for the design of a "long-wavelength" chemosensor and a red fluorescing chemosensor would bring many opportunities in many practical applications.

1.7. Intrinsic and Conjugate Chemosensors

In the design of fluorescent chemosensor, there are two approaches that one could choose. First one is conjugate chemosensors, which are also known as photoinduced electron transfer (PET) sensors, CHEF (chelation enhanced fluorescence) sensors, etc. The design is simple; one has to find a nitrogenous-ligand or chelator which is sufficiently selective for the analyte of interest and then covalently bind this to a fluorophore. For the sensor to be a pure "conjugate" chemosensor, the ligand-chelator should not be conjugated; it should have noninteracting π- systems. In this case, there is just one fluorescent species, the issue is whether that fluorescence is quenched or not. When the amine-nitrogens of the ligand/chelator is "free" they will quench the fluorescence in solution *via* photoinduced electron transfer, sometimes up to 1000-fold. However, when the amine electrons are involved in coordinate interactions with metal ions, or hydrogen bonding with the analyte, quenching does not occur and the original fluorescence of the parent

fluorophore is restored. Therefore, the analyte is signalled by an increase in the fluorescence intensity without any spectral shift. Although spectral shifts can not be obtained with this class of chemosensors, some advantages of conjugate probes are also known. Firstly, synthesis can be very straight-forward. Secondly, the binding constants and the selectivity ratios are mostly unchanged in the chemosensor compared to the free ligand/chelator. Because the ligand/chelator is not perturbed by the fluorophore attachment. Moreover, in connection with the fluorescence lifetime techniques, concentration dependent artifacts can be overcome.

In intrinsic chemosensors, the ligand/chelator is clearly a part of the fluorophore π- system. Therefore, when the analyte interacts with the binding region a different absorbing species is formed, with different quantum yield and sometimes with different emission maximum. So the ratio of emission intensities on excitation at two different wavelengths can be the parameter to relate the ion concentrations to the fluorescence signal. Concentration dependent artifacts are avoided completely, but being in conjugation to the fluorophore, the binding strength and the selectivity depend on the electronic structure of the fluorophore very much. In most cases, the ligand is the donor (D) part of the Donor-Acceptor (D-A) molecules, so the binding affinity may be significantly decreased compared to the free ligand. The other limiting factor has been the synthetic challenge of assembling an intrinsic chemosensor. However, there are good examples of both classes of chemosensors in the literature.

1.7.1. Recognition Based on Cation of Photoinduced Electron Transfer in Nonconjugated Donor-Acceptor Systems

This type of probe, often called fluorescent photoinduced electron transfer (PET) sensors, has been studied extensively. Photoinduced electron transfer (PET) is the process which exploits the light driven redox reactions for signalling purposes for proton and metal ions. Fluorescent PET sensors can be formalized as "fluorophore-spacer-receptor" systems (Figure 4). The two moieties are separated spatially from each other by an inert, σ-bonded spacer. Therefore, there is a long range interaction between the receptor and fluorophore for their communication.

Figure 4. Schematic representation of a fluorescent PET chemosensor.

When the fluorophore is photoexcited, this strong long-range interaction develops in the form of an electron transfer from the ion-free receptor to the fluorophore, however; in their ground state, these subunits show some features of supramolecular assembly with weak interactions between their π- and n-electron systems.

Figure 5 illustrates how a cation can control the photoinduced electron transfer in a fluoroionophore in which the cation receptor is an electron donor (e.g., amino group) and the fluorophore (e.g., anthrancene) plays a role of an acceptor. On excitation of the fluorophore, an electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied

molecular orbital (LUMO), which enables photoinduced electron transfer from the HOMO of the donor (belonging to the free cation receptor) to that of the fluorophore, causing fluorescence quenching of the latter. On the other hand, when cation binds to the receptor, the redox potential of the donor is increased so that the HOMO of the receptor becomes lower in energy than that of the fluorophore. Therefore, photoinduced electron transfer is not possible any more and fluorescence quenching is suppressed. That is, upon cation binding fluorescence intensity is increased (Valeur, 1994b).

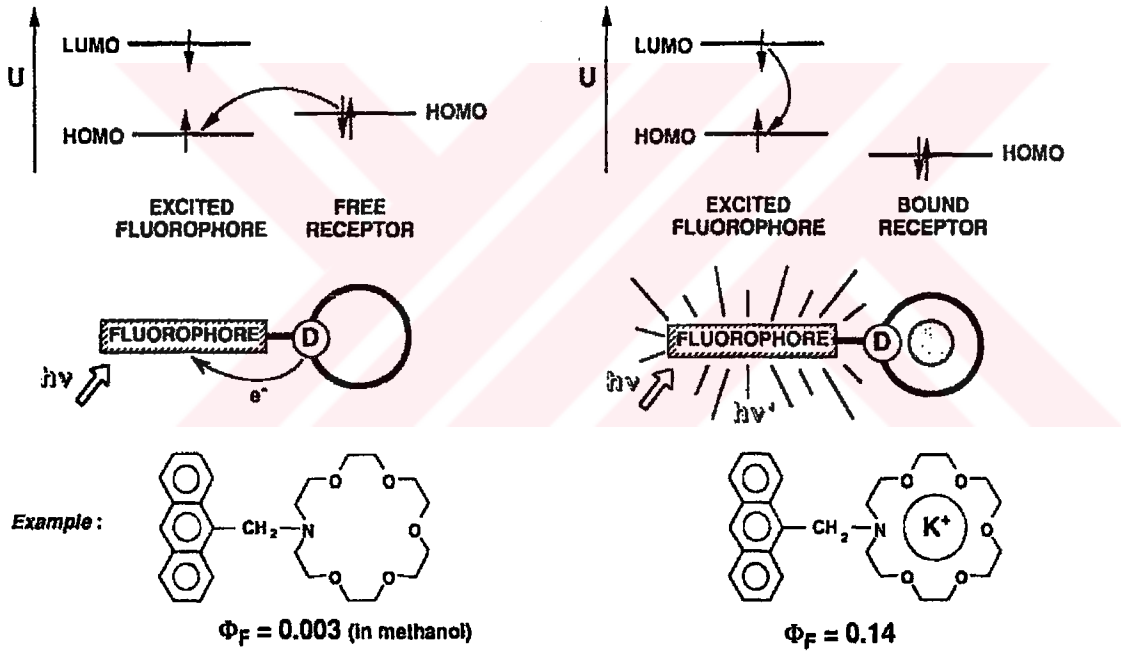


Figure 5. Principle of cation recognition based on cation control of photoinduced electron transfer in nonconjugated donor-acceptor systems (Valeur, 1994), (Example from de Silva, et. al, 1986).

It has long been discovered that the fluorescence of aromatic hydrocarbons is quenched by aliphatic or aromatic amines because of PET from the latter to the former.

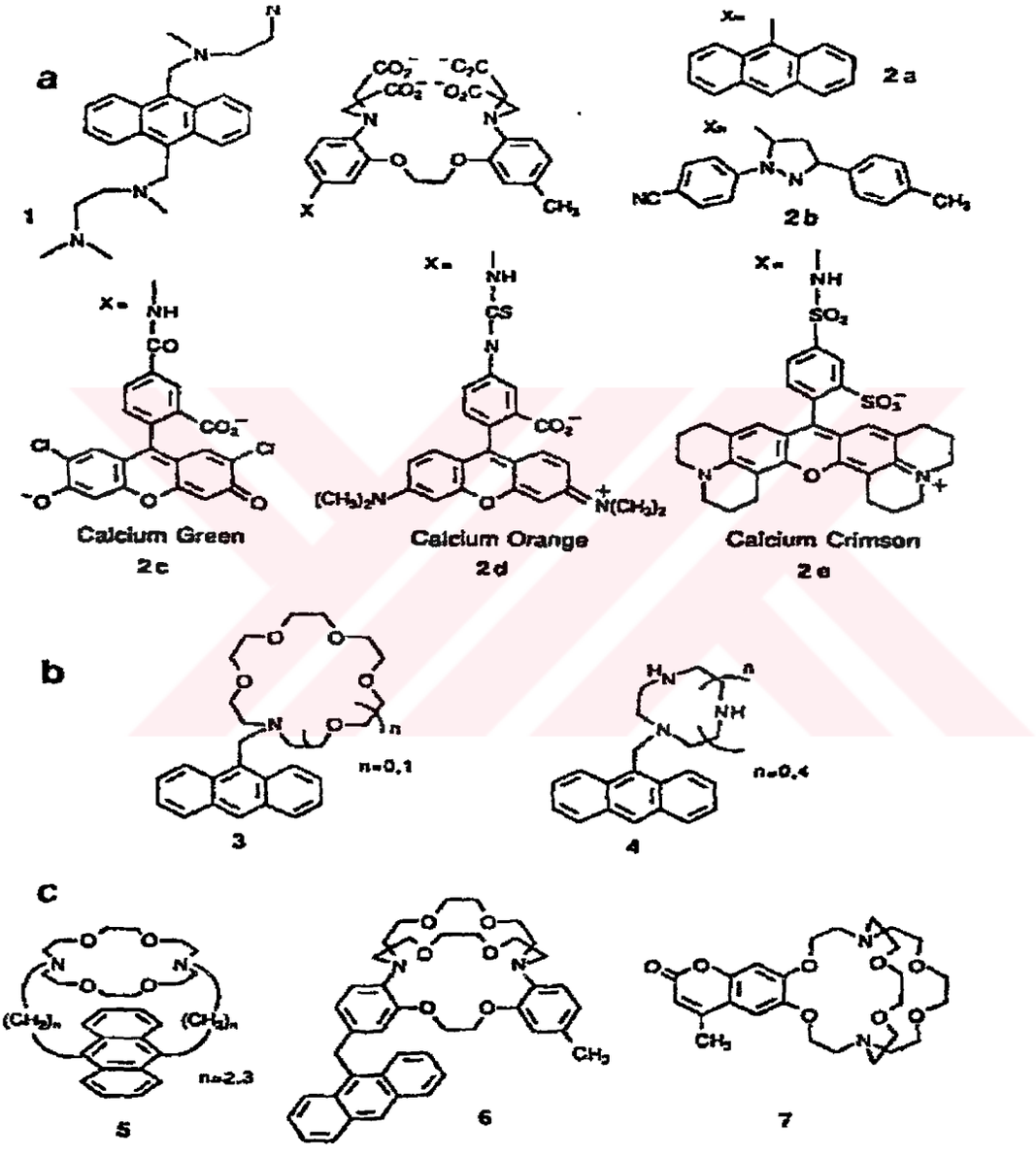


Figure 6. Examples of fluoroionophores based on cation control of photoinduced electron transfer. (a) Chelators; (b) coronands; (c) cryptands. (Valeur, 1994b).

Many examples related with PET chemosensors are present in literature and various systems have been designed in which anthracene is linked, *via* a spacer, to an ionophore containing a nitrogen atom. Figure 6 gives some examples: chelator **1** (Czarnik, 1988), **2a** and **2b** (Prosanna de Silva, 1990), and **4** (Akkaya, 1990), cryptands **5** (Lehn, 1985; Fages, 1989) and **6** (Fages, et. al, 1989), **2c, 2d** and **2e** (Lakowicz, et. al., 1992), coronands **3** (de Silva, 1990). Anthracene fluorescence, which is efficiently quenched by the lone pair of the nitrogen atom, is recovered on binding of a cation which suppresses electron transfer. In the other fluoroionophores, i.e., **2b, 2c, 2d, 2e,** and the coumaro-cryptand **7** (Kurtz, et. al, 1990) fluorescence intensity is increased on cation binding. Note that in **2c, 2d** and **2e** the spacer is –CO-NH - . Rigid and multiple spacers can increase the weak coupling between the fluorophore and receptor moieties usually. The CH_2 spacer is the advantageous because of the fact that it allows PET rates. Since there is a small distance of separation between the fluorophore and receptor. Moreover, it is almost convenient for synthesis *via* benzylic functionalities.

Very large enhancement of fluorescence intensity can be observed. For instance, the fluorescence of a solution of **1** in acetonitrile is multiplied by a factor of 1000 on addition of $ZnCl_2$ (Czarnik, 1988).

It should be noted that the quantum yield is affected by cation binding but the absence of the emission or excitation spectra precludes the possibility of intesity-ratio measurements at two wavelengths.

For PET chemosensor, the analyte is signalled only by an increase in the fluorescence intensity without any spectral shift. That spectral shift cannot be obtained in this class of fluorescent chemosensors is a major drawback. However, it is known that fluorescent PET chemosensors have some advantages. First of all synthesis can be very straight-forward. Secondly, the binding constants and selectivity ratios are mostly unchanged in the fluorescent chemosensor compared to the free receptor because receptor is

not perturbed by the fluorophore attachment. Besides, concentration dependent artifacts can be overcome by the help of the fluorescence life-time techniques.

1.7.2. Recognition Based on Cation Control of Photoinduced Charge Transfer in Conjugated Donor-Acceptor Systems

Many fluorophores contain an electron-donating group (often an amino group) conjugated to an electron-withdrawing group (e.g. squaric acid) so that they undergo intramolecular charge transfer from the donor to the acceptor on excitation by light. The consequent change in dipole moment results in a Stokes shift that depends on the microenvironment of the fluorophore; polarity probes have been designed on this basis. 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.

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 extinction 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 is expected.

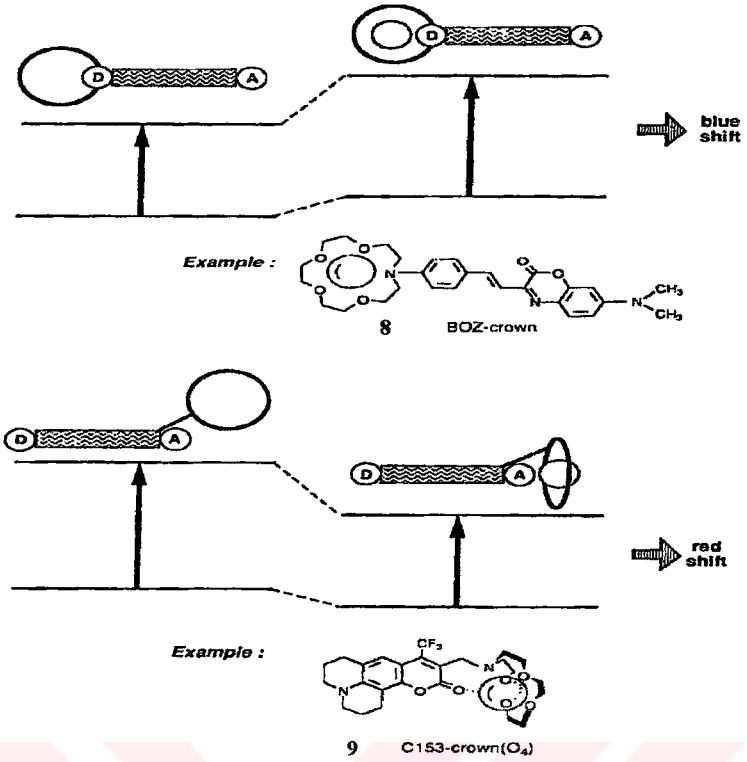


Figure 7. Spectral displacement of fluoroinophores based on cation control of photoinduced charge transfer in conjugated donor-acceptor systems. (Valeur, 1994b).

The photophysical changes on cation binding can also be described in terms of charge dipole interaction keeping in mind that the dipole moment in the excited state is larger than that in the ground state. 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. 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 (Figure 7).

In Figure 8, fluoroionophores **10** (Valeur, 1988; Valeur, 1990), **11** (Valeur, 1989), **12-14** (Retting, 1993), **15** (Verheoven, et. al, 1989; Verhoeven, et. al 1990) contain the same macrocycle (monoaza-15-crown-5) as the ionophore; the nitrogen of the crown plays a role of an electron donor with respect to both fluorophore and cation. These compounds undergo a more or less drastic blue shift of their absorption spectrum upon cation binding, the alkaline-earth metal ions inducing much larger effects than alkali metal ions, as expected for the double charge of the former. The largest blue shift of the fluorescence spectrum observed for calcium ion in compounds **10-15** are explained by the fact that it fits well into the cavity of monoaza-15-crown-5.

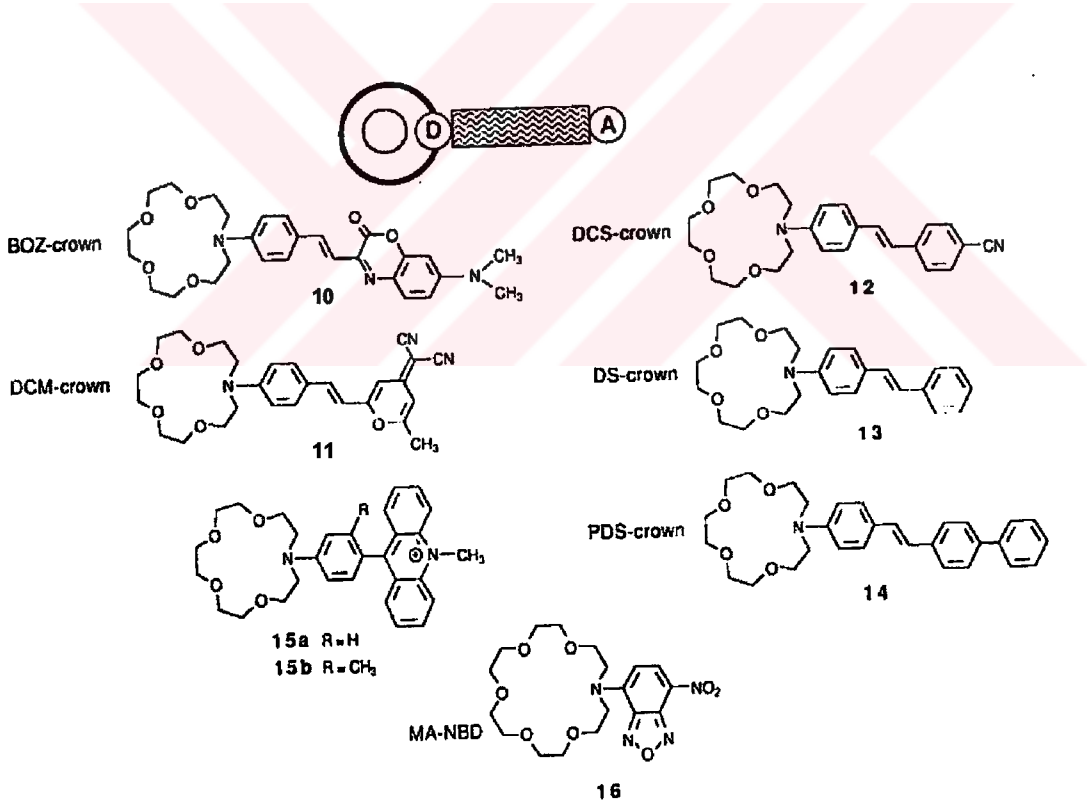


Figure 8. Examples of donor-acceptor fluoroionophores in which electron-donating character of the donor (nitrogen atom of the crown) is cation-controlled.

The interaction of the analyte with the binding region forms a different absorbing species with different quantum yields since the receptor is the part of the fluorophore π- system. Therefore, the ratio of emission intensities on excitation can be the parameter to relate the ion concentrations to the signal. In principal, this avoids concentration dependent artifacts completely. However, binding strength and selectivities depend on the electronic structure of the fluorophore because of being in conjugation to the fluorophore. The other limiting factor is the synthetic challenge of assembling a conjugated donor-acceptor system. However, there is a number of good examples found in literature.

1.8. Basic Ideas for Future Developments

A large number of sensitive fluorescent probes are now available for the measurement of pH and the concentration of ions.

Each indicator has to be designed according to the ion probed in its particular environment and the technique used, so that the possibility of preparation of a universal molecular system is unlikely. The indicators have to fulfill some minimum requirements. Obviously, the binding properties of the ionophore have to be matched with the target ion to be monitored in its environment. But also the electronic interaction of the ionophore with the fluorophore in the ground or the excited states has to be significantly altered by the presence of the ion.

Some of the research directions under current consideration contain the following ideas:

1. With conjugated D-A systems which increase their dipole moment during the electronic excitation, the charge transfer (CT) contribution leads to a long-wavelength CT transition in absorption which can be suppressed with an ion on the donor side or increased when it is on the acceptor side. With

respect to the fluorescence, most of the known results show only a very slight short-wavelength shift of the spectra when complexed probes are excited, which possess strong D-A interactions. This shift can be increased with less polar suitable probes because then the coordination of the cation is partially or totally maintained in the excited state. For cases where the ratio method with one excitation wavelength is compulsory, e.g., with emission ratio imaging, the design of suitable probe could thus be achieved.

2. Nevertheless, the D-A systems where the D group is part of the ionophore possess the inconvenience of leading, in the presence of cations, to a fluorescence at short wavelength where the free probe has its charge transfer absorption band. To overcome this difficulty D-D conjugated systems with one D substituent part of the ionophoric group have been designed, which in the presence of cations lead to a $D^{\delta+}$ - $D^{\delta-}$ - M^{+} system with a long-wavelength emission.

For this type of fluoroinophores, the photochemical processes can be accelerated and can lead to very fast and reversible photochemical ion release or ion takeup.

3. Virtually all PET probes devised until now function in the same way in that they disfavor ET on complexation because the complexing site is an integral part of the donor system. A principally different behaviour with PET on complexation is expected if the complexing site is part of the acceptor system.

4. As a possible source for new long-wavelength dye systems, biradicaloid dyes possessing energetically close-lying frontier molecular orbitals could be used. An example of such dye systems already in extensive use in imaging is squaric acid derivatives.

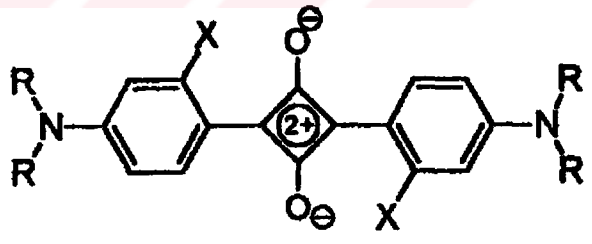
1.9. Squaraines as Long Wavelength Fluorophores

Design of fluorescent or chromogenic chemosensors for the selective detection of low concentrations of ions is an active area of research. With a few exceptions, most of the studies have been concerned with nonaqueous solutions of ions. Moreover, most of the chromophores hitherto reported suffer from the requirement of short wavelength excitation. For detection of metal ions in naturally occuring systems, particularly for intracellular applications,near-infrared absorbing water soluble chemosensors would be highly desirable. Squaraines form a class of dyes possessing intense absorption in the visible and NIR region. The strong fluorescence emission and the photosensitivity in the red-IR region which makes the squaraine dyes potentially useful as fluoroionophores and as fluorophores in biological applications have also been explored. Most of the reported studies however have dealt with the 1,3-disubstituted squaraines. Although a number of such dyes possess strong absorption at wavelengths greater than 600 nm, those possessing absorption in the 780-840 nm region are limited. Recently, the synthesis and characterization of a new class cationic squaraine dyes were reported. These dyes which are easily solubilized in water behave as chromoinophores for the detection of trace quantities of transition – and rare-earth-metal ions in aqueous media. These dyes also exhibit a relatively high efficiency for fluorescence emission and are suitable as IR sensitizers for extending the photoresponse of large band-gap semiconductors.

Rhodamine and BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indicane) (Trademark of Molecular Probes, Inc.) derivatives and cyanines are known to fluorescence in the long wavelength region of the visible spectrum, they all leave something to be desired. Rhodamines and BODIPY derivatives are for most considerations not red enough and also highly light-sensitive Cyanines are an alternative but, they suffer low quantum yields in aqueous solutions and photobleach rapidly. On the other hand, 3, 4-Dihydroxycyclobut–3-ene-

1,2-dione, commonly known as squaric acid have been the subject of many recent investigations both because of their unusual electronic structures as well as their properties as semiconductive and photogeneration materials in electrophotographic photoreceptors. They are used for photosensitization in imaging processes, in solar energy systems, in optical data storage, in fluorescent labels, and in nonlinear optics; but their very promising spectral properties like long wavelength absorption and emission (620-670 nm) with very high molar extinction coefficients (~10^5 cm^{-1}, M^{-1}), high quantum yields, intense fluorescence emission with small Stokes shifts have not been exploited so far in relation to chemosensor design. This may be partly due to their solubility characteristics, most are sparingly soluble in all solvents except CH_2Cl_2 and $CHCl_3$ and tend to form various aggregate structures in other solvents. A list of spectral properties of representative squaraines can be found in Table 1.

TABLE 1. Spectral Properties of Selected Squaraines



substituent	λ_{max} *(absorption) (nm)*	λ_{max} *(emission) (nm)*	ε *(cm^{-1} M^{-1})*	*Quantum Yield in Dichloromethane*
R=CH_3, X=H	627.6	646	309,000	0.65
R=C_2H_5, X=H	634.1	650	324,000	0.69
R=C_3H_7, X=H	638.8	651	339,000	0.70
R=C_4H_9, X=H	640.0	664	339,000	0.70
R=$C_{18}H_{37}$, X=H	641.8	664.5	331,000	0.74
R=CH_3, X=CH_3	643.5	669.5	263,000	0.023
R=C_2H_5, X=CH_3	651.0	671.5	309,000	0.036
R=C_4H_9, X=CH_3	657.1	675	295,000	0.054
R=CH_3, X=OCH_3	631.8	661	251,000	0.042
R=C_2H_5, X=OCH_3	638.8	665	302,000	0.049
R=C_4H_9, X=OCH_3	643.5	664	324,000	0.26
R=CH_3, X=OH	635.9	660	331,000	0.86
R=C_2H_5, X=OH	641.1	661.5	372,000	0.86
R=C_4H_9, X=OH	648.2	665	363,000	0.83

As the numbers in Table 1 indicate that this class of compounds have superior qualities, especially considering high quantum yields and high extinction coefficient. The only hurdle is the appropriate derivatization to introduce analyte-sensitive functions and improve solubility in water. With appropriate modifications on the amine function, solubility and aggregation behavior can be modulated to a great extent.

Squaraines are 1,3-derivatives of squaric acid **17** in which the substituents are amines or highly electron-donating aromatic or heterocyclic groups. Since the discovery of these compounds by Treibs and Jacob in 1965, their electronic structure has been the subject of considerable discussion. Treibs and Jacob depicted the structure of bis-2-pyrrylsquaraines by **18** because the properties of **18** were similar to the properties of the trimethine cyanine dyes. At a later time Sprenger and Zeigenbein described the electronic structures as either **21** or a hybrid of **21** and **22.** Canonical form **21** is particularly attractive because it corresponds to a Hückel aromatic system (N=0).

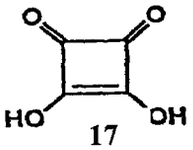
More recently the consensus has shifted back to the original Treibs and Jacob proposal, i.e., that a dipolar cyanine structure (e.g., **19**) and not a cyclobutenediylium structure best represents the electronic configuration of these compounds. The evidence for this conclusion rests on several observations (Kazmaier, et. al., 1990).

1. The visible spectra of squaraines show close similarity to cyanine dyes

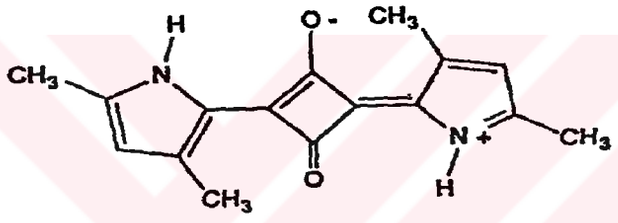
2. The solubility of squaraines, like cyanines, is very low in most organic solvents consistent with a dipolar structure.

3. Squaraines, like cyanines, readily undergo addition with nucleophilic reagents.

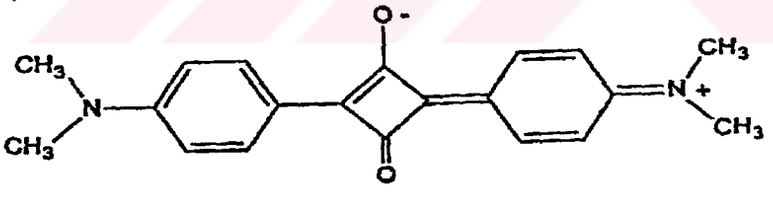
4. More recently,semiempirical molecular orbital (MNDO) studies on **19** have shown a distinct polyene character to squaraine chromophore. This quinoid character is enhanced when explicit solute/solvent interactions are taken into account.



17



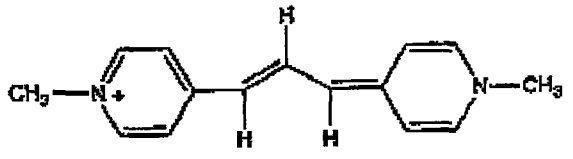
18



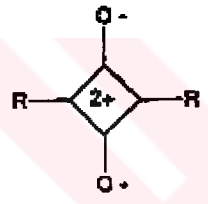
19

Both experimental and theoretical studies on a related series of aminosquaraine compounds (**23** and **24**) indicated that these compounds experience considerable C-N π- bonding between the central four-membered ring and the amino substitutents, supporting the MNDO evidence presented for aryl squaraines.

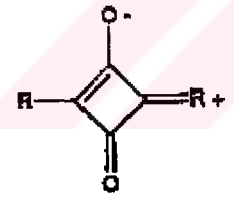
The conclusion that canonical form **22** best approximates the electronic structure of squaraines leads to a number of implications for the NMR of these compounds. If the central ring has the charge distribution of **22** rather than **21,** then one would expect this ring to behave more like an α, β-unsaturated ketone than an aromatic system.



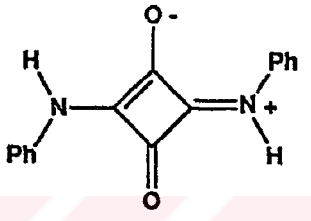
20



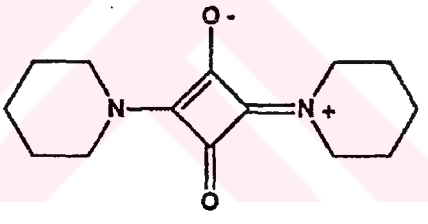
21



22



23



24

Secondly, canonical representation **22** indicates that the positive charge is delocalized to the pendent aromatic groups and that these groups would have a pseudo-quinoid structure.

Squaraines are obtained by treating 1 equivalent of squaric acid with 2 equivalent of a N,N-dialkylanilines at reflux temperature using a mixture of toluene and n-butanol as the solvent. No catalysis is required and water formed in the reaction is removed azeotropically in a Dean-Stark apparatus.

Most symmetrical (same dialkylaminophenyl group on both sides of the squaryl-moiety) squaraines are synthesized in this way with high yields. Asymmetrical squaraines can also be synthesized either by a mixed reaction or by multi-step sequence of reactions involving a cycloaddition. The general synthesis of squaraines is shown in Figure 9.

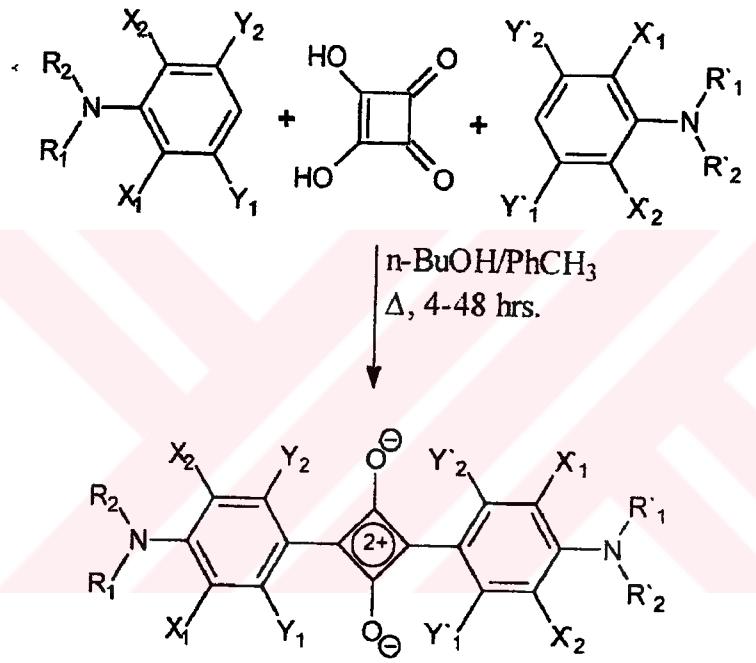


Figure 9. General synthesis of squaraines

In most synthesis, the yield is in excess of 70%.

For squaraine dyes, squaric acid (C_4O_2 unit) moiety is the electron acceptor and the nitrogen atom of the dialkylaniline moiety is the electron donor part. The absorption and emission spectra of the squaraine dye has been suggested to arise from charge transfer transitions. Although the charge transfer is primarily confined to the central C_4O_2 unit (from oxygen to

the four-membred ring), there is a minor contribution from the dialkylaniline moiety, also. These transitions are highly sensitive to substitutional changes as well as the nature of the solvent medium.

Ionophores that are covalently linked to chromophores exhibit intramolecular charge transfer (ICT) transitions. Excitation of such molecules causes a redistribution of their charge densities, which can significantly affect the metal ion binding ability of the ionophoric unit. Alternatively, the ICT transition can be severely affected by complexation with metal ions, leading to significant changes in the absorption and emission properties of these molecules. Such molecules can therefore be utilized for the selective and quantitative detection of biologically important metal ions such as Li^+, Na^+, K^+, and Ca^{2+}. For intracellular applications it is desirable to design fluoro- or chromoinophores that are water soluble and absorb strongly in NIR region. Additionally, if the chromophoric unit is redox active, such molecules can be utilized for transportation of ions across membranes as well as in the design of ion sensitive electrodes. Squaraine dyes possess the ability to undergo reversible redox processes. Squaraine type chemosensors where the ionophore covalently linked to redox active residues are very important since these are potential sensitive probes for metal ions and for transport of metal ions across membranes.

Squaraine dyes have important applications in imaging science and in the sensitization of wide band gap semiconductor materials. The symmetric D-A-D (donor-acceptor-donor) arrangement of symmetrical squaraine dyes causes an interesting effect on the formation of intramolecular charge transfer states. As a result, the photophysical properties of these dyes are highly sensitive to substituent groups and the solvent medium. For example, the fluorescence yield of hydroxy derivative of squaraine dye can be greatly enhanced by microencaging within a polymer matrix or β-cyclodextrin (Kamat, et. al., 1996).

In the solid state, due to the extensive intermolecular charge-transfer interactions, the absorption becomes very broad. It covers most of the visible region and extends to the NIR where the solid-state diode lasers emit (Law, 1993). These optical characteristics have made squaraines very attractive for a number of industrial applications, e.g., xeropraphic photoreceptors, organic solar cells and optical recording.

Squaraine dyes are intriguing compounds both due to their unusual electronic structures as well as to their properties as semiconductors and photogeneration materials in electrophotography. Most applications using squaraine derivatives employ them as solids, usually microcrystalline, in which there is clear indication that the squaraine chromophores are aggregated. Several studies have shown that squaraines can exist as different kinds of aggregates, with at least two prominent limiting forms commonly occurring. In a recent study, the type of aggregation for a number of squaraines has been shown to depend on the structure of the chromophores in mixed-solvent experiments. Studies of crystalline solids show, for example, that bis(4-methoxyphenyl)squaraine exists in a solid as a "card pack" array in which the planar squaraines are stacked vertically (Farnum, 1974). This structure exhibits a spectrum strongly blue-shifted compared to that of the monomer in dilute solution. In contrast, bis[2-methyl-4-(dimethylamino)phenyl]squaraine forms crystals in which the squaraine chomophores are in a "slipped stack" arrangement; in this case the crystals show a prominent transition strongly red-shifted compared to the solution monomer (Wingard, 1982).

The behaviour of other solid squaraines usually shows one of these characteristic spectroscopic features – blue-shifted corresponding to an "H" aggregate or red-shifted characteristic of a "J" aggregate, respectively. Since the classical exciton treatments of Kasha and Hochstrasser and Kuhn and co-workers predict shifts similar to those observed in the crystals for molecular arrangements in aggregates, it is reasonable to assure that many

squaraines aggregates exhibiting similar absorption spectra in microcrystals, films, or other media may show similar intermolecular arrangements. Interestingly, it is found that several surfactant of amphiphilic squaraines form stable monolayer films at the air-water interface that can be transferred to optically rigid supports. Most of the freshly prepared Langmuir-Blodgett (LB) films of these squaraines show blue-shifted or "H" aggregates which would be anticipated if the molecules are arranged in the film in a simple "card-pack" array. However, in a number of cases it is found that heating of these films results in their conversion to a red-shifted aggregate, a process which may be reversed by heating the product films in a humid atmosphere. While the absorption of the aggregate may be blue-shifted or red-shifted relative to the solution monomer absorption, very little is known regarding the structural detail of these squaraine aggregates and the driving forces that control aggregation. The aggregational behaviour can be rationalized in terms of an optimization between hydrophobic (between hydrocarbon chains) and charge transfer interactions (between squaraine chromophores) during the aggregation process.

Strong hydrophobic interaction is the driving force for the formation of blue-shifted aggregate where the hydrophobic interaction forces a closer interaction between the hydrocarbon chains. However, in the J-aggregate, the transition dipole moments are expected to be tilted, so charge-transfer interaction between the anilino group and the four-membered ring occurs. This charge-transfer interaction is assumed to be the driving force for the formation of the J-aggregate.

The molecular arrangement of the red-shifted aggregate is not well-defined as the blue-shifted aggregate, but it is believed that some sort of intermolecular charge-transfer interaction occurs in a J-aggregate. It may due to the partial tilting of the transition dipole moment along the packing direction or due to the tilting of the transition dipole moment along the pack axis but in two opposite directions (Kasha, 1965). Because of the increased polarity in

the solvent mixture, intermolecular charge-transfer interaction appear to be dominant to lead to the formation of J-aggregate. One type of an aggregate can rearrange to another if the solvent polarity or temperature of the medium is changed.

There are already successful examples of squaraine-based chemosensors in the literature, and such modified squaraines were shown to signal pH, alkaline, and earth-alkaline metals in various solvent systems. When the range of selectivities that could be achieved by the judicious choice of the crown ethers is considered, azacrown-appended squaraines (squaraine fluoroionophores) are targets of prime importance.

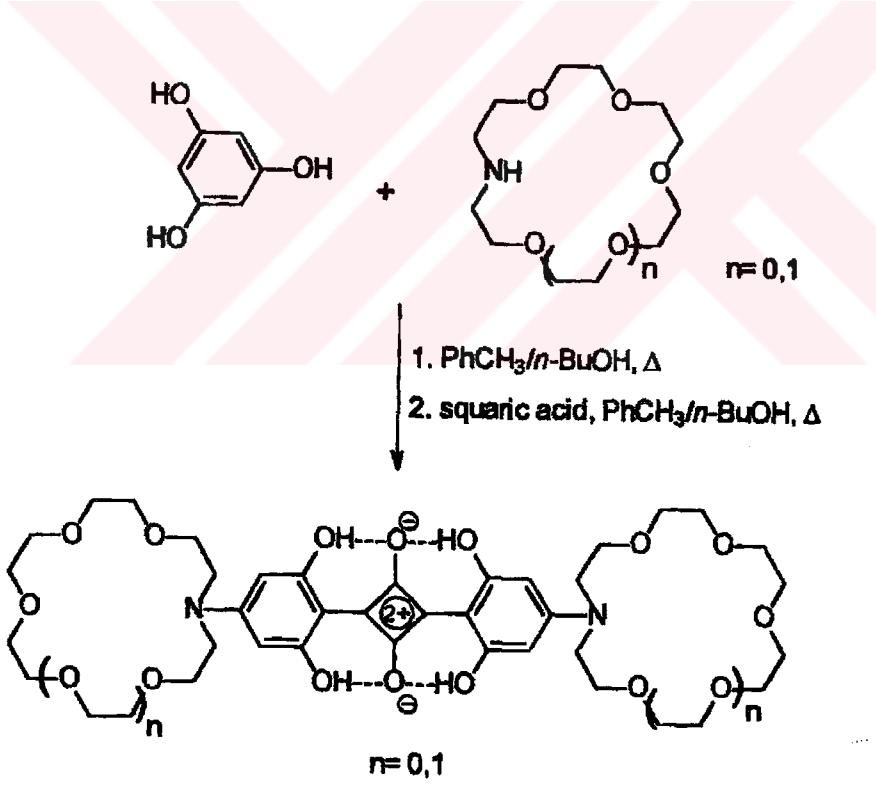


Figure 10. An example to azacrown-appended squaraine. (Akkaya, 1997)

Crown ethers are macrocyclic polyethers that can play the role of multidentate ligands towards a variety of metal ions and organic cations. Since the formation constant of the resulting complex depends on the fitting of the cavity to the size of the metal ion, crown ethers can be designed to be selective. Crown ethers are flexible, so that their conformation can be easily changed if they are covalently bound to components that undergo structural changes. Therefore, the coordinating ability of supramolecular systems that contain both crown ethers and photoisomerizable components may be controlled by light.

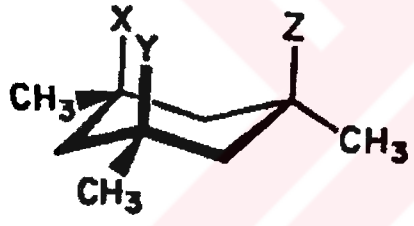
The ion-binding ability of crown ether components in supramolecular systems can also be controlled by other means such as acid-base processes, redox processes, coordination of heavy-metal ions in remote sites, and temperature. An excellent review on redox-responsive macrocyclic receptor molecules containing transition-metal redox centers has been published recently.

1.10. The Design of a Kemp's Triacid Diimide Based Selective Fluorescent Chemosensor

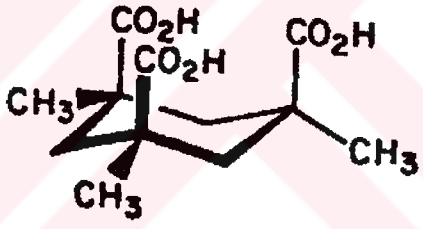
The selective chelation of divalent metal cations is important in studying the action of natural ionophores where biologically active metals must be selectively transported across membranes. For example, biological decontamination requires isolation of poisonous metals while leaving essential metals untouched. Quantitative and qualitative analysis for trace metals is another area for selective ionophores.

Roughly in the last 15 years, research groups have designed and synthesized ionophores targeted for alkaline earth and transition metals with the common feature of two carboxyl groups. The theoretically neutral complexes allowed efficient and selective transport ability.

Proximity effects can result in dramatic changes in functional group behaviour which have provided many speculative models for enzymatic catalysis. Most such models have involved pairs of proximate functional groups, and therefore the scientists were attracted to the general cyclohexane structure **25** which positions three functional groups at an ~0,25-nm separation.



25



26

As shown in Figure 11, Kemp's triacid (1,3,5- trimethylcyclohexane – 1,3,5 – tricarboxylic acid) can be prepared in three steps in an overall yield of 22% by an oxidative degradation of 3,5,7-trimethyladamantan-1-ol **(27)**. Fragmentation of an initially formed hypobromite parallels related fragmentations reported by Black for 1-adamantanol and forms a cyclohexanone which undergoes α bromination under the reaction conditions to form **28.** This substance can be oxidized in 64% yield to **29** by means of potassium permanganate in water-pyridine. Conversion of the resulting

lactone-acid **29** to **26** can then be effected by a catalytic amount of ruthenium dioxide in the presence of periodic acid.

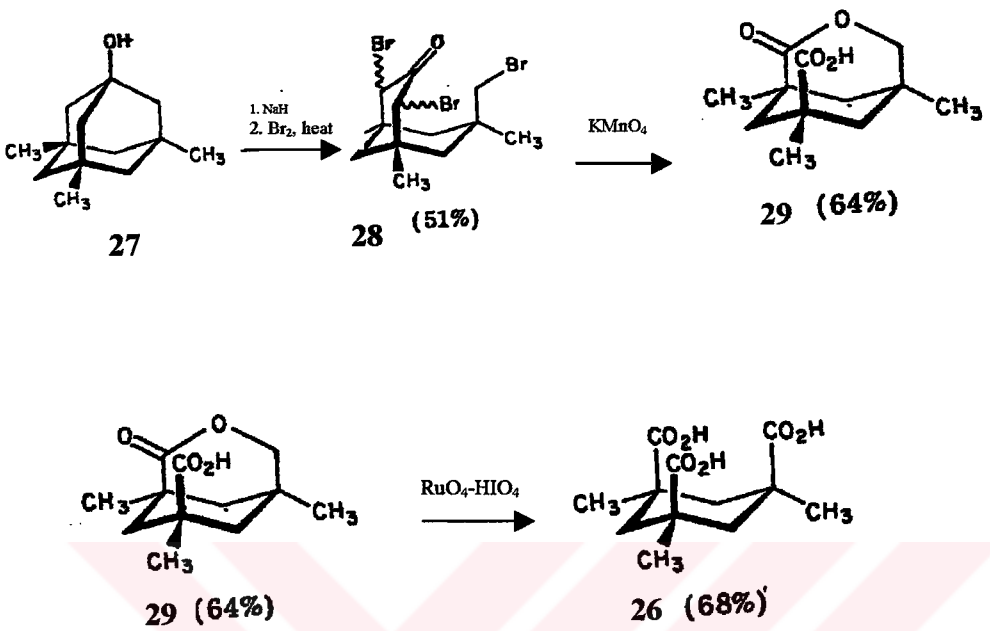
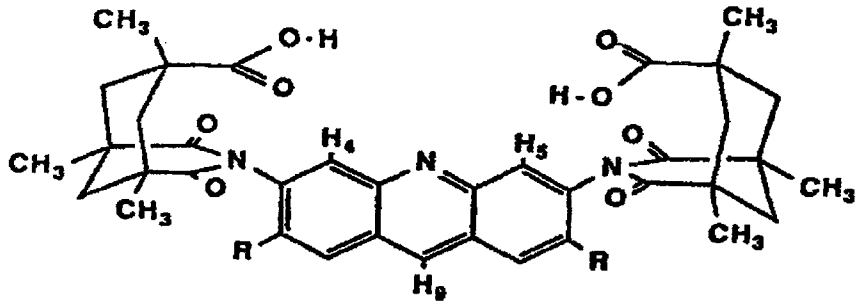


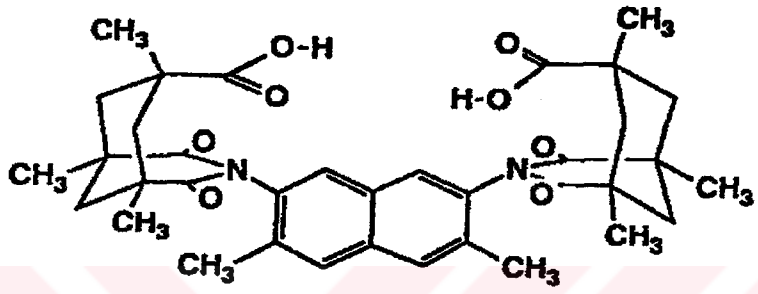
Figure 11. Synthesis of Kemp's triacid

Rebek and co-workers introduced the model receptors as a chelating agents derived from 1,3,5-trimethylcyclohexane–1,3,5-tricarboxyclic acid (Kemp's triacid) derivatives **30**,**31** and they gave evidence of their unique binding capacities.

In these structures two carboxyl groups converge on a molecular cleft in a manner similar to the convergence of the carboxyl functions in enzymes such as lysozyme and the aspartic proteinases. This feature accounts for the unusual acid dissociation constants of the diacids and is the crucial element in their ability to recognize smaller molecules of complementary size, shape, and functionality.



30 a R = H
30 b R = CH_3



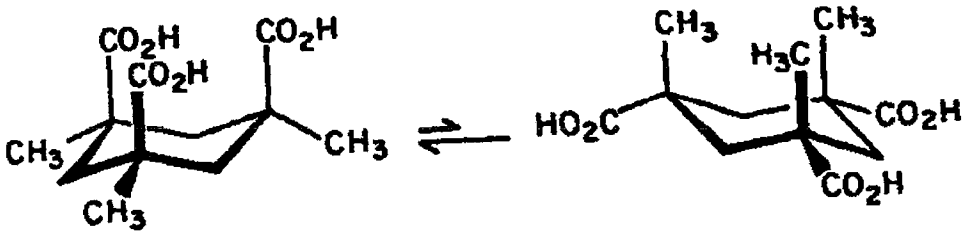
31

The aromatic spacer groups in **30** and **31** prevent the formation of intramolecular hydrogen bonds between the opposing carboxyl groups, yet these functions are ideally positioned for intermolecular hydrogen bonding (Rebek, 1987).

1.10.1. Probable Structure for Kemp's Triacid and Its Trianion

Of the two chair conformations available to **26, 32** in which the three carboxyl groups are oriented triaxially, is likely to be more stable. Although A values alone are likely to provide only a crude approximation in cases involving 1,3-diaxial interactions, it is noteworthy that methyl shows a significantly larger A value than carboxyl. More cogently, it is evident from

space-filling models that relatively flat carboxyl groups can pack in a triaxial array with less crowding than three methyl functions, and intramolecular hydrogen bonding is likely to enhance this bias.



26 **32**

On the other hand, the polyanions derived from **26** might be expected to show the reverse conformational preference. Successive ionization of the carboxylic acid groups of **26** forms species which must be destabilized by electrostatic repulsion in the chair conformation with triaxial carboxylates. Either the dianion or the trianion of **26** might therefore assume the triequatorial conformation as its more stable orientation. It is interesting to note that a rapid conformational change which affects the binding constant at each of several identical reactive sites is the defining feature of the concerned model for allosteric proteins.

A candidate for such a property is the very large difference in chemical shift (δ 1.5-1.7) which is seen for the resonances of the nonequivalent methylene hydrogens of **26** and its triester (Table 2). This difference is largely attributable to the anisotropy of the carbonyl groups of **26** and its derivatives. It is only consistent with a preferred conformation for **26** in which the two methylene hydrogens experience, on the average, a very different orientation with respect to the neighbouring acyl functions.

TABLE 2. ^{1}H Chemical Shift Values

		Chemical shift, δ		
entry	structure (solvent)[a]	CH_3C	CH_2	Δ_{CH_2} [b]
1a	26	1.23	ca. 1.2, [c] 2.63	
b	26(CD_3OD)	1.25	1.20, 2.70	1.50
c	26, monosodium salt	1.20	1.13, 2.54	1.41
d	26, disodium salt	1.20	1.15, 2.47	1.32
e	26, trisodium salt	1.30	1.50, 2.10	0.60
2a	26, trimethyl ester (CD_3OD)	1.20	1.10, 2.70	1.60
b	26, thrimethyl ester ($CDCl_3$)	1.20	1.00, 2.70	1.70

[a] Unless specified, the solvent is 1:1 CD_3OD-D_2O

[b] Δ_{CH_2} = difference in chemical shift values for axial and equatorial methylene protons in parts per million. [c] Resonance masked by absorption due to methyl groups.

A symmetrical, unhindered, aliphatic tricarboxylic acid with no inductive interactions between its acidic groups is expected to exhibit three pK_a values centered at about 4.7 and separated by a statistical factor of log 3= 0.5.

An acid with neighbouring ionizable groups is expected to show a much larger separation between pK_a values. An example is phthalic acid, for which the pK_a's for the first and second dissociations are separated by 2.5. Although part of this difference is probably due to stabilization of the monoanion through intramolecular hydrogen bonding, the larger part can be attributed to destabilizing charge repulsion in the dianion.

As seen from Table 3, the first two pK_a values of **26** are separated by 2.5 pK_a units. As expected to a triacid and its monoanion with proximate carboxylate functions as in **32.** Were this triaxial orientation shared with the trianion of **26,** one would expect at least as large a separation between the second and third pK_a values, resulting form the destabilizing effect of additional charge density. The smaller separation that is observed is only in

accord with a conformational change that separates the three negative charges of the trianion.

TABLE 3. pK_a Values at 25°C

	H_2O		CH_3OH-H_2O (1:1 v/v)	
Substance	pK_a	ΔpK_a [a]	pK_a	ΔpK_a [a]
26	3.3		4.7	
	5.85		7.6	
		1.5		1.2
	7.3		8.8	
29			6.3	

Even though the trianion of **26** is less basic than one would expect if it assumed the triaxial chair conformation it still may hold the record for the most basic example of a carboxylic acid anion. Even the hexaanion of benzene- 1,2,3,4,5,6-hexacarboxylic acid is a weaker base ($pK_{a\text{-}29}$ = 6.76).

1.11. Aim of the Work

In this study, our aim is to apply a cation controlled PET process in the detection of Zn^{2+} ions at very low concentrations by the synthesis of a series of novel squaraine fluorophores, Kemp's triacid dimide derivative and also azacrown derivative of squaric acid for the detection of K^+ ions.

We have synthesized a series of trimethylindolenine-derived squaraines with different alkyl groups on the indolenine nitrogen. The side chains (DMAE, DMAP, TMEDA, 18-azacrown-6) carry potential donor atoms for metal ion coordination. Squaryl oxygen also participate in the chelation of

Zn (II) ions, leading to a variety of fluorescence and absorption signals. Our aim is to observe the effect of the side chain of indolenine on absorbance and fluorescence emission intensity.

We have also included a squaraine derivative possessing an azacrown-ether function capable of complexing with K^+ ion which have recently been studied in a different context. The interest came from the realization that dyes with multiple fluorescence are candidates of choice for developing ion-sensitive fluorescence probes. Moreover, as the squaraine dyes studied here absorb in the long-wavelength region of the visible spectrum, they possess potential interest as laserdiode-excitable fluorescence probe.

Moreover, a new class of chelating agent derived from 1,3,5-trimethyl cyclohexane-1,3,5-tricarboxylic acid (Kemp's triacid) and 3,8-diamino-6-phenylphenanthridine was synthesized. We attempted to produce this derivative which had a potential of providing a rigid NNN coordinating environment towards Zn^{2+} ion and Cd^{2+} ion. Based on literature evidence some selectivity was to be expected.

CHAPTER 2

EXPERIMENTAL

In this study, the compounds were characterized by Nuclear Magnetic Resonance (^{1}H, ^{13}C) Spectrum.

^{1}H and ^{13}C-Nuclear Magnetic Resonance Spectra were carried out with Bruker Gmbh DPX-400, 400 MHz High Performance Digital FT-NMR Spectrometer (METU NMR Laboratory) by using DMSO-d_6 as a solvent and TMS as an internal reference. Spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), m (multiplet).

Absorbance values were measured in Schimadzu UV-2100 Spectrophotometer.

Fluorescence values were measured in Perkin Elmer LS 50 B Luminescence Spectrometer.

All chemicals were purchased from Aldrich Chemical Company, unless otherwise stated.

Column chromatography was performed using Merck Silica Gel 60 (particle size : 0.040-0.063 mm, 230-400 mesh ASTM).

2.1. Synthesis of Phenylhydrazine (33)

A stirred slurry of 0.20 mole of aniline **(32)** (18.63 gr) in concentrated hydrochloric acid (80 mL) was treated dropwise at 0°C or below with sodium nitrite (0.20 mole, 13.8 gr) in cold water (70 mL). The mixture was stirred for 0.5 hour at about 0°C, filtered (if necessary), and in a few cases added to 100 gr of chopped ice before proceeding with the reduction. Dropwise addition (stirring) at about 0°C of stannous chloride dihydrate (0.6 mole, 113.77gr) in cold concentrated hydrochloric acid (140 mL) then produced a slurry of the tin double salt of the hydrazine (or in a few cases of the hydrazine hydrochloride). This slurry was refrigerated overnight, filtered and the precipitate was washed with saturated sodium chloride (about 400 mL) followed by 2:1 (200:100 mL) petroleum ether-ether. The filtered solid was treated with excess concentrated sodium hydroxide and the hydrazine was extracted into ether (5x100 mL). The ether extract was washed with water (1x100 mL) and dried with sodium sulfate, decolorized (if necessary). Hydrochloric acid (80 mL) was added dropwise to obtain a white precipitate (the hydrochloride of phenylhydrazine, **33**). The precipitated product was collected by filtration and dried *in vacuo*. The synthesis is shown in Figure 12. The yield was 8.84 gr (31%).

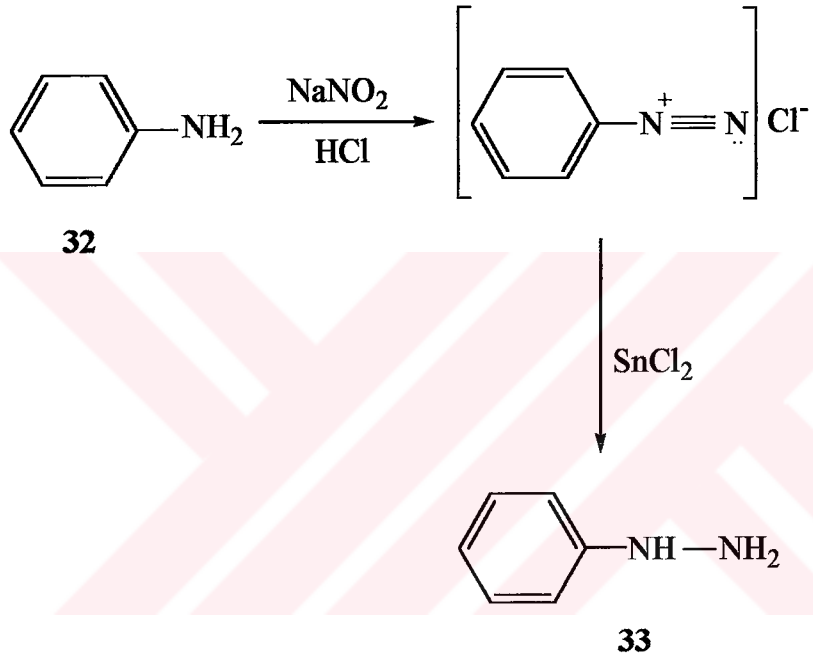


Figure 12. Synthesis of phenylhydrazine **(33)**.

2.2. Synthesis of 2,3,3-Trimethylindolenine (35)

A mixture containing the hydrochloride of phenylhydrazine **(33)** (30mmol, 4.335gr), methylisopropyl ketone (3-methyl-2-butanone) (42mmol, 3.7gr) and glacial acetic acid (30mL) was stirred for 30 minutes at room temperature. A spectrum taken on a sample withdrawn from the mixture at this time showed a strong absorption at 275 nm of the phenylhydrazone **(34)** and the 232 nm maximum of the phenylhydrazine **(33)** was absent. The mixture was then stirred and heated at reflux for 1 hour. By that time the maximum at 275 nm had disappeared and been replaced by the 260 nm maximum of **35.** After that point saturated K_2CO_3 in water (30 mL) was added to make the solution basic and the mixture was extracted with dichloromethane (3x25 mL). Removal of the solvent under reduced pressure and a rotary evaporator left a residue. The yellow-brown tint was dried *in vacuo.* The synthesis is shown in Figure 13. The isolated product was in 61% yield (3.95gr). mp.155-165°.

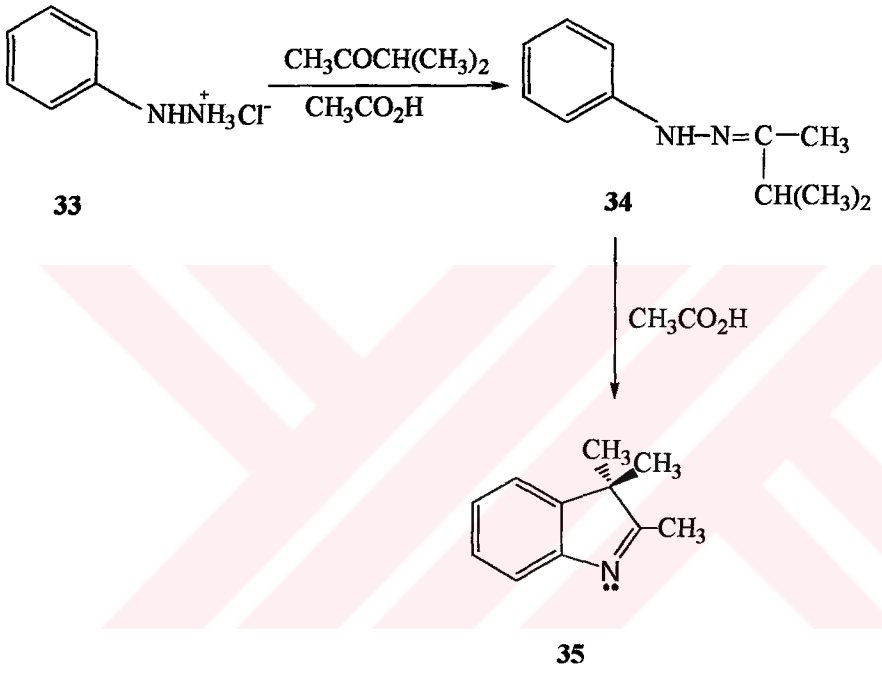
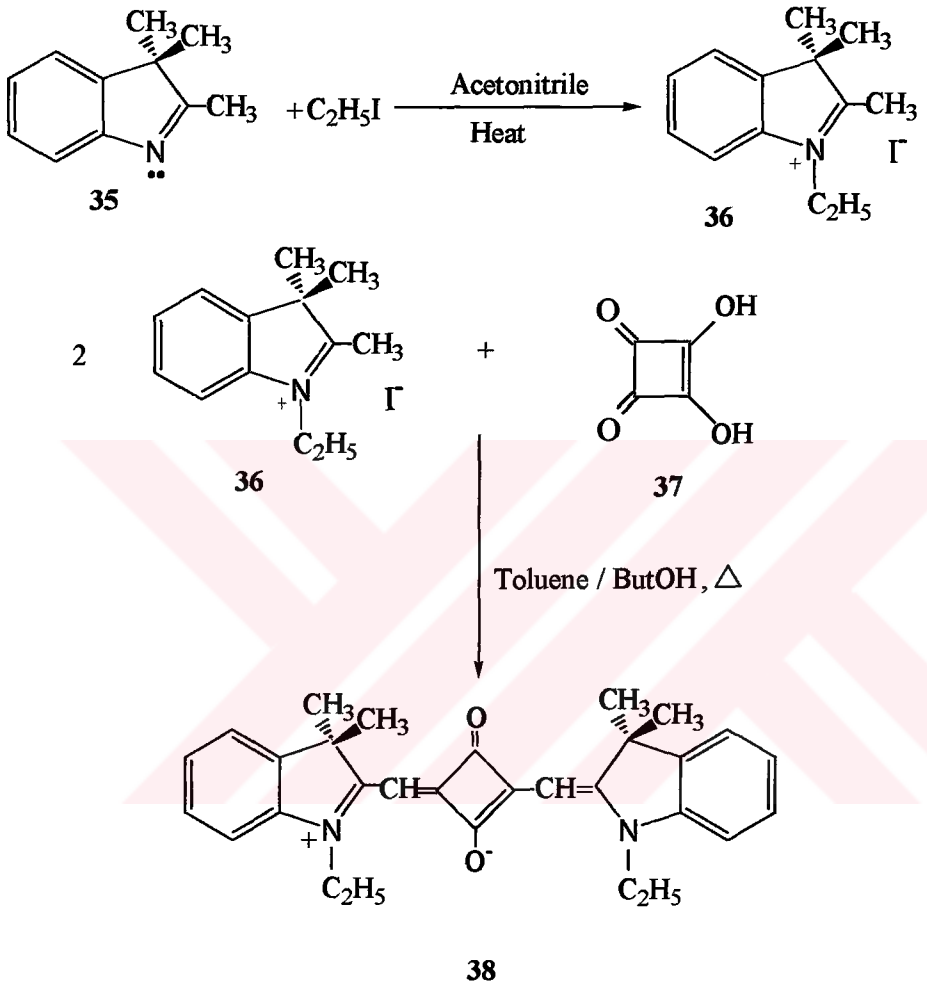


Figure 13. Synthesis of 2,3,3-Trimethylindolenine **(35)**.

2.3. Synthesis of Ethylsquaraine 38

2,3,3-Trimethyl indolenine **(35)** (1mmole, 0.16gr) and ethyliodide (1mmole, 0.156 gr) were heated to reflux in a solvent containing acetonitrile (10 mL) at 120°C. Reaction mixture was cooled down to room temperature and solvent was evaporated and the solid was triturated with petroleum ether. The resulting dark pink solid **36** was dried *in vacuo*. Without any isolation attempt, the solid was reacted with 3,4-dihydroxy-3-cyclobutene-1,2-dione **(37)** (0.5 mmole, 0.057 gr) in a solvent mixture containing toluene (13.3 mL) and n-butanol (6.6mL) by refluxing for 17 hours in the Dean-Stark apparatus accompanied by azeotropic distillation of water. Reaction mixture was cooled down to room temperature. After the evaporation of the solvent, the resulting dark-green solid **38** was dried *in vacuo*. The synthesis is shown in Figure 14. The yield was 0.172 gr (76%). ^{1}H NMR (DMSO-d_6) δ 1.33 (t, 6H), 1.72 (s,12H), 4.01 (m, 4H), 5.91 (s, 2H), 6.92 (d, 2H), 7.08 (t, 2H), 7.20-7.32 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 11.933, 13.382, 18.876, 26.912, 38.220, 49.224, 72.225, 86.169, 109.056, 122.249, 123.617, 127.725, 141.901, 142.170, 169.566, 179.496, 182.257.



35 36 36 37 38

Figure 14. Synthesis of ethylsquaraine **38**

2.4. Synthesis of Dimethylaminoethylsquaraine 41

2,3,3-Trimethylindolenine **(35)** (1 mmole, 0.16 gr) and 2-dimethyl-aminoethylchloride **(39)** (1 mmole, 0.144 gr) were heated to reflux in a solvent containing acetonitrile (10 mL) at 120°C. Reaction mixture was cooled down to room temperature and solvent was evaporated. The solid was triturated with petroleum ether and the resulting N-1-(N'-dimethylaminoethyl)-2,3,3-trimethylindolenine chloride **(40)** was dried *in vacuo*. Without any isolation attempt, the solid was reacted with 3,4-dihydroxy-3-cyclobutene-1,2-dione **(37)** (0.5 mmole, 0.057 gr) in a solvent mixture containing toluene (13.3 mL) and n-butanol (6.6 mL) by refluxing for 17 hours in the Dean Stark apparatus, accompanied by azeotropic distillation of water. Reaction mixture was cooled down to room temperature. After the evaporation of the solvent, the resulting dark-green solid **41** was dried *in vacuo*. The synthesis is shown in Figure 15. The yield was 0.202 gr (75%). ^{1}H NMR (DMSO-d_6) δ 1.51 (s, 12H), 2.93 (s,12H), 3.41-3.98 (m, 8H), 5.64 (s, 2H), 7.21 (t, 2H), 7.27-7.50 (m, 4H), 7.58 (d, 2H). Although many squaraines have been prepared in laboratories, most of these compounds are too insoluble to permit the accumulation of solution ^{13}C NMR spectra. Some compounds initially appear soluble enough but crystallize out of solution after several hours before spectrum accumulation is complete. For these reasons ^{13}C NMR spectra were not obtained.

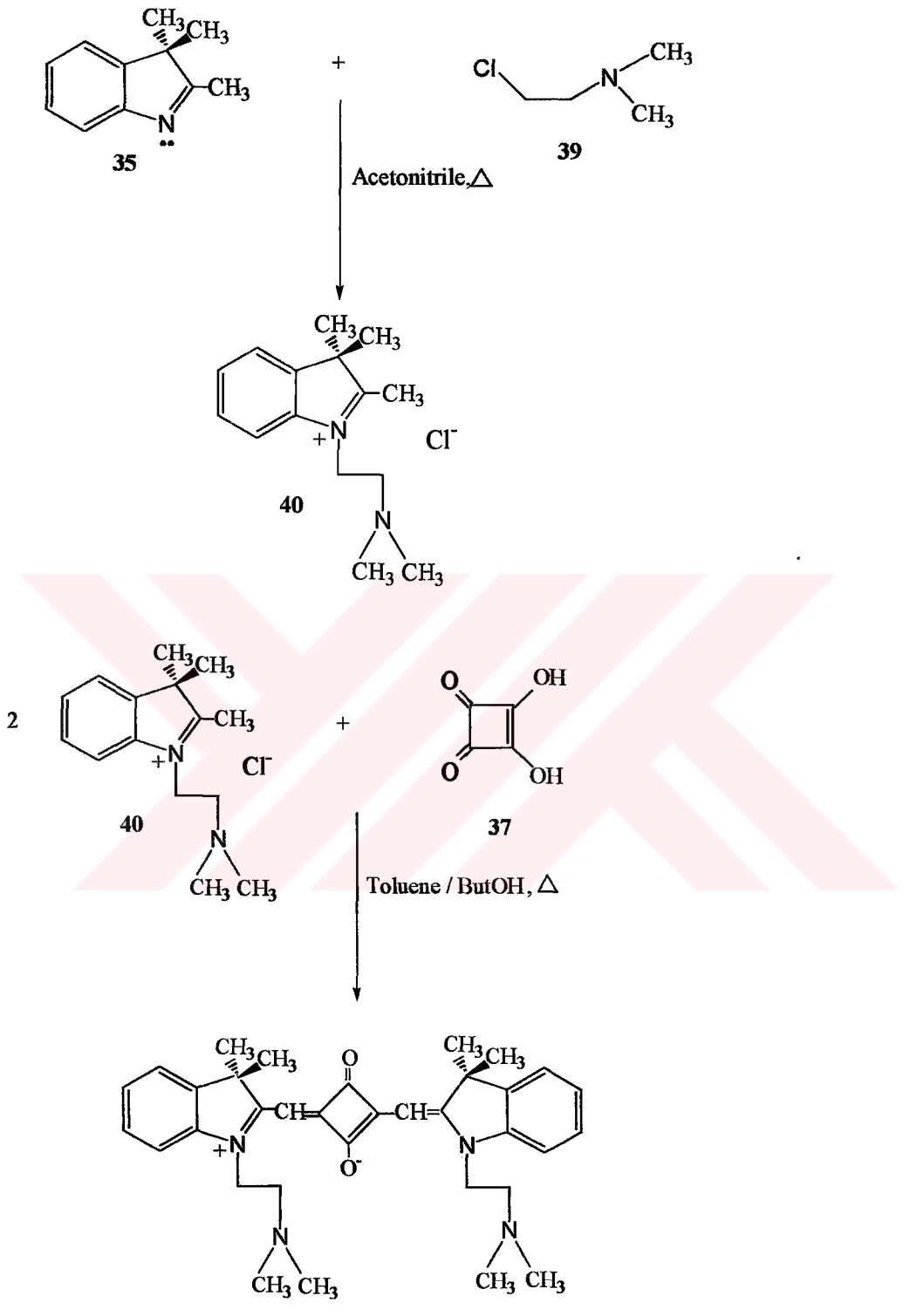


Figure 15. Synthesis of dimethylaminoethylsquaraine **41**

2.5. Synthesis of Dimethylaminopropylsquaraine 44

2,3,3-Trimethylindolenine **(35)** (1 mmole, 0.16 gr) and 3-dimethyl-aminopropylchloride **(42)** (1 mmole, 0.158 gr) were heated to reflux in acetonitrile (10 mL) at 120°C. Reaction mixture was cooled down to room temperature and solvent was evaporated. The mixture was triturated with petroleum ether and the resulting N-1-(N'-dimethylaminopropyl)-2,3,3-trimethylindolenine chloride **(43)** was dried *in vacuo.* Without any isolation attempt, the solid was reacted with 3,4-dihydroxy-3-cyclobtene-1,2-dione **(37)** (0.5 mmole, 0.057 gr) in a solvent mixture containing toluene (13.3 mL) and n-butanol (6.6 mL) by refluxing for 17 hours in the Dean-Stark apparatus, accompanied by azeotropic distillation of water. Reaction mixture was cooled down to room temperature. After the evaporation of the solvent, the resulting dark green solid **44** was dried *in vacuo.* The synthesis is shown in Figure 16. The yield was 0.223 gr (79%). ^{1}H NMR (DMSO-d_6) δ 1.49 (s, 12H), 3.48-3.78 (m,24H), 5.98 (s, 2H), 7.20 (t, 2H), 7.27-7.43 (m, 4H), 7.58 (d, 2H). The solubility of squaraine **44** is very low in organic solvents. The low solubility of dimethylaminopropylsquaraine **44** did not permit ^{13}C NMR measurements in solution.

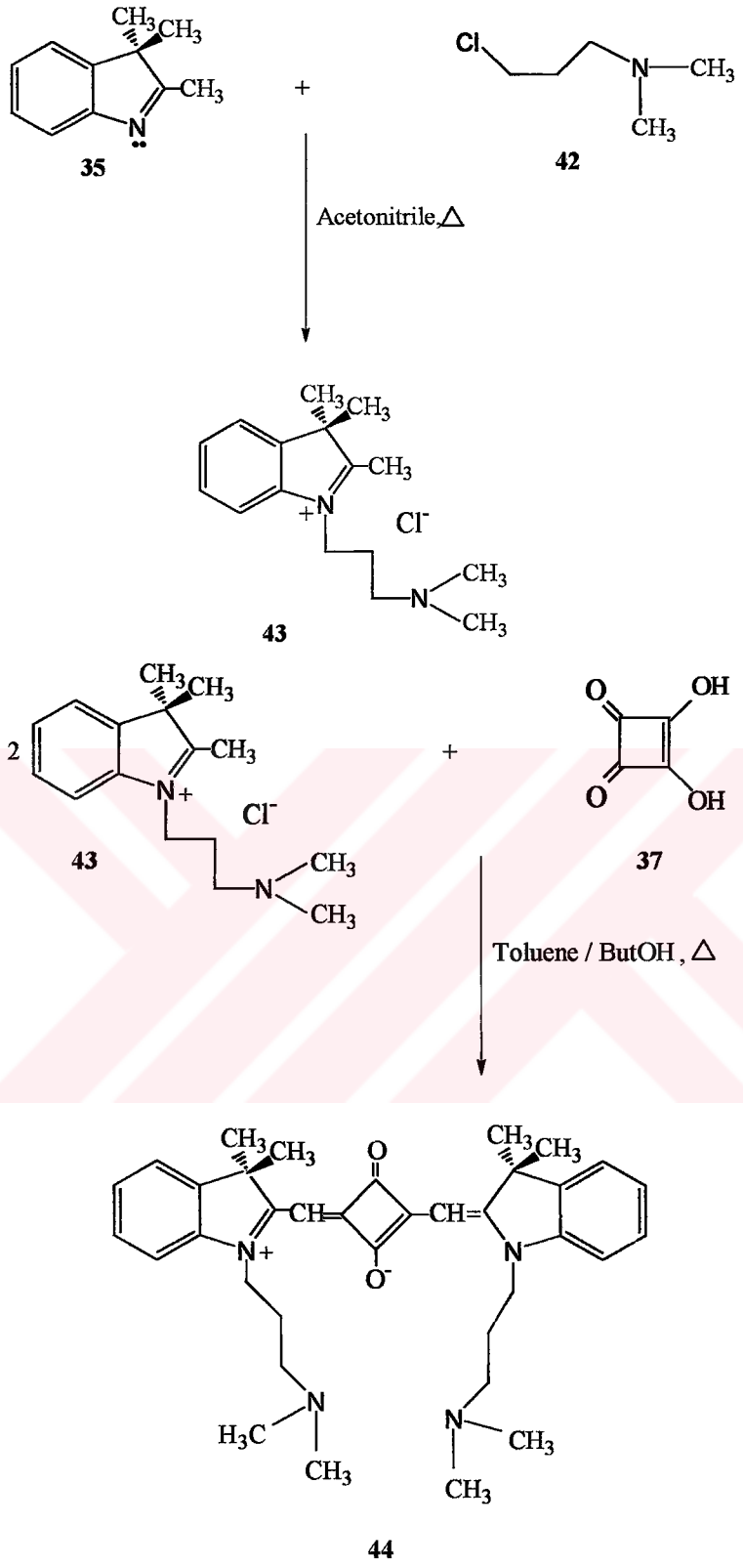


Figure 16. Synthesis of dimethylaminopropylsquaraine **44**

2.6. Synthesis of Trimethylethylenediaminesquaraine 48

N-(N',N'-Dimethylaminoethyl)-N-methylaminoethanol **(45)** (6.84 mmol, 1 gr) is converted to the corresponding halide **46** by refluxing it in $SOCl_2$ (10 mL) for 5 hours. Reaction mixture was cooled down to room temperature and the solvent was evaporated. The solid was triturated with diethyl ether (30 mL). The resulting white powder **46** was dried *in vacuo.* The yield was 1.35 gr (83%). 2,3,3-Trimethylindolenine **(35)** (1 mmole, 0.16 gr) and N-(N',N'-dimethylaminoethyl)-N-methylaminoethylchloride **(46)** (1 mmole, 0.238 gr) were heated to reflux in acetonitrile (10 mL) at 120°C. Reaction mixture was cooled down to room temperature and solvent was evaporated. The solid obtained was triturated with petroleum ether and the resulting N-1-(N'-N'-dimethylaminoethyl)-2,3,3-trimethylindolenine **(47)** was dried *in vacuo* (Figure 17). Without any isolation attempt, the solid was reacted with 3,4-dihydoxy-3-cyclobutene-1,2-dione **(37)** (0.5 mmole, 0.057 gr) in a solvent mixture containing toluene (13.3 mL) and n-butanol (6.6 mL) by refluxing for 17 hours in the Dean-Stark apparatus, accompanied by azeotropic distillation of water. Reaction mixture was cooled down to room temperature. After the evaporation of the solvent, the resulting dark green solid **48** was dried *in vacuo.* The synthesis is shown in Figure 18. The yield was 0.277 gr (85%). ^{1}H NMR (DMSO-d_6) δ 1.53 (s, 12H), 3.32 (br. s,12H), 3.35-3.85 (m, 22H), 5.62 (s, 2H), 7.18 (t, 2H), 7.25-7.39 (m, 4H), 7.54 (d, 2H). The solubility of squaraine **48** is very low in organic solvents. The low solubility of trimethylethylenediaminesquaraine **48** did not permit ^{13}C NMR measurements in solution.

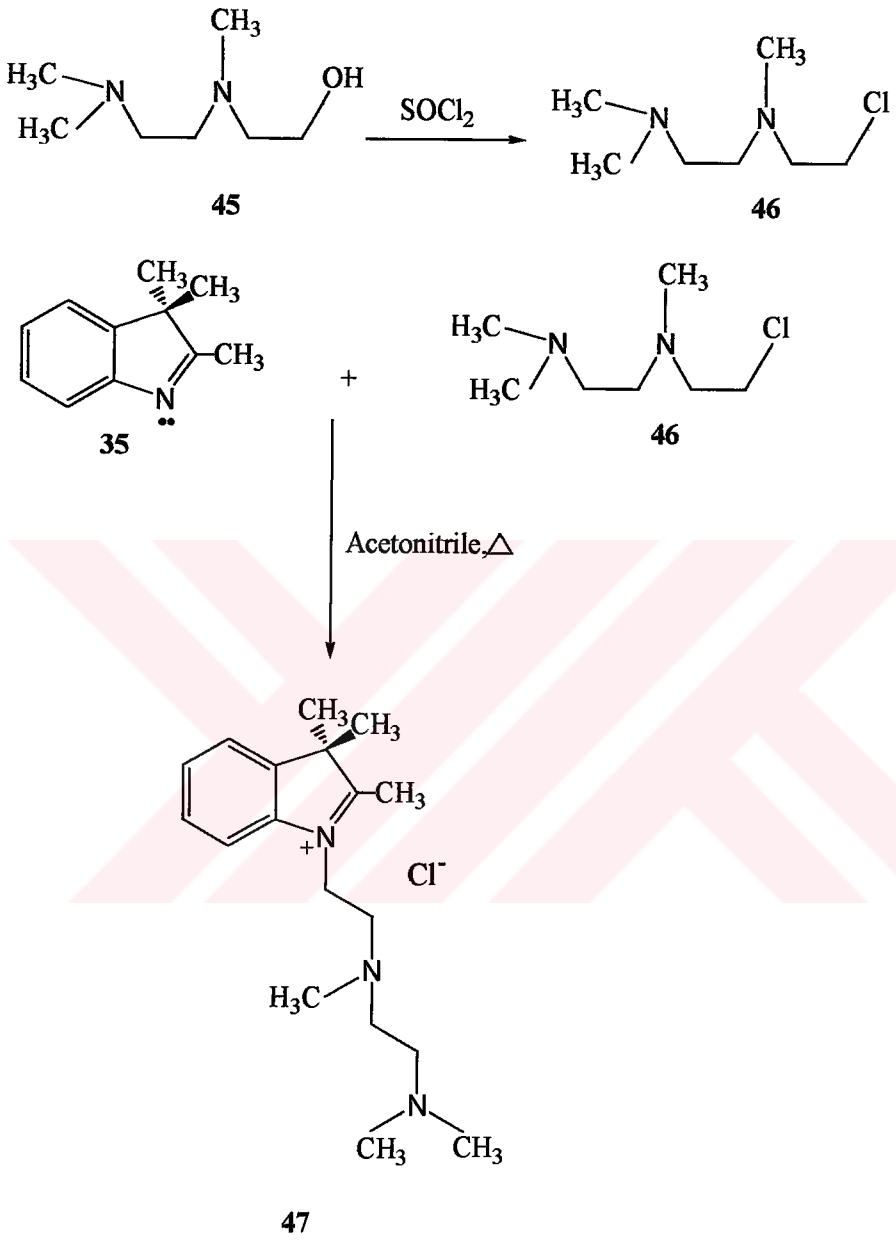


Figure 17. Synthesis of N-1-(N'-N'-dimethylaminoethyl)-2,3,3-trimethylindolenine **(47)**

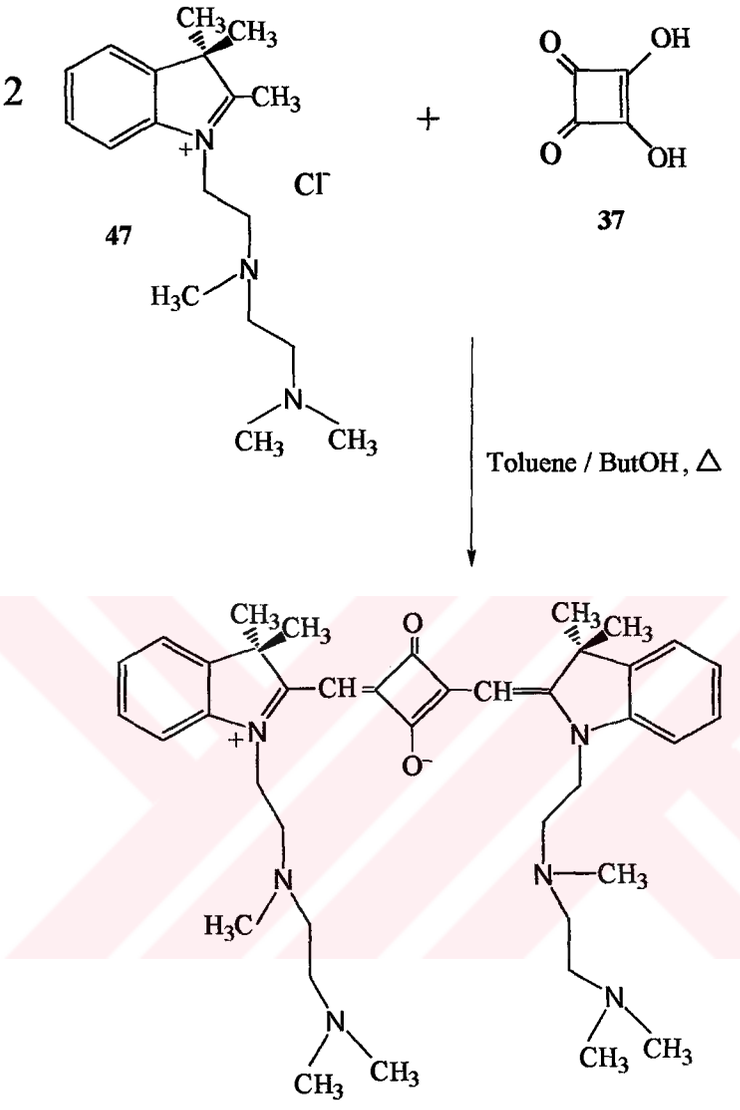
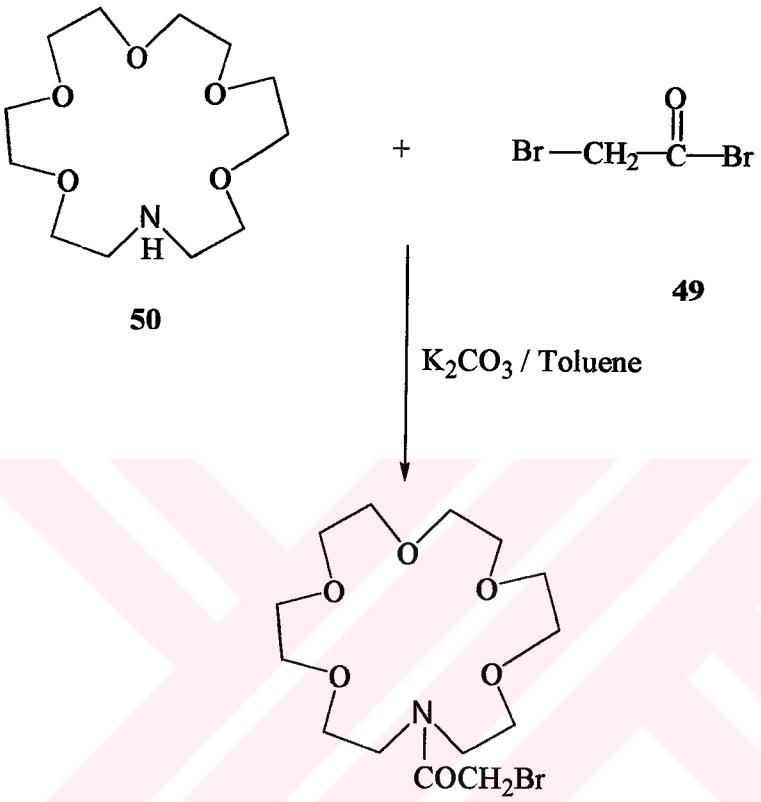


Figure 18. Synthesis of trimethylethylenediaminesquaraine **48**

2.7. Synthesis of an Azacrownsquaraine Conjugate 53

A solution of bromoacetylbromide **(49)** (1.12 mmol, 0.226 gr) in 4.9 mL of toluene was added dropwise to a stirred mixture of 1-Aza-18-crown-6 **(50)** (1.12 mmol, 0.295 gr) and powdered K_2CO_3 (2.24 mmol, 0.310 gr) in 14.7 mL of toluene. After it was stirred for 16 hours, the mixture was filtered and the filtrate was evaporated to an oil. Yield was 0.33 gr (98%) (Figure 19).

The resulting bromoacetyl-1-aza-[18]-crown-6 **(51)** (0.65 mmol, 0.225 gr) and 2,3,3-trimethylindolenine **(35)** (0.65 mmol, 0.103 gr) were heated to reflux in 10 mL acetonitrile for 5 hours at 120°C. Reaction mixture was cooled down to room temperature and solvent was evaporated. The resulting solid was triturated with petroleum ether and the resulting dark pink solid was dried *in vacuo* (Figure 20). Without any isolation attempt, the solid **52** was reacted with 3,4-Dihydroxy-3-cyclobutene-1,2-dione **(35)** (0.325 mmole, 0.037 gr) in a solvent mixture containing toluene (13.3 mL) and n-butanol (6.6 mL) by refluxing for 17 hrs in the Dean-Stark apparatus, accompanied by azeotropic distillation of water. Reaction mixture was cooled down to room temperature. After the evaporation of the solvent, the resulting dark-green solid **53** was dried *in vacuo.* The synthesis is shown in Figure 21. The yield was 0.234 gr (72%) : mp>250°C. ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 12H), 4.01-4.90 (m,24H), 5.15 (s, 4H), 5.60 (s, 2H), 6.95 (d, 2H), 7.05-7.30 (m, 4H), 7.52 (d, 2H). The solubility of squaraine **53** is very low in organic solvents. The low solubility of azacrownsquaraine conjugate **53** did not permit ^{13}C NMR measurements in solution.



51

Figure 19. Synthesis of bromoacetyl-1-aza-[18]-crown-6 **(51)**

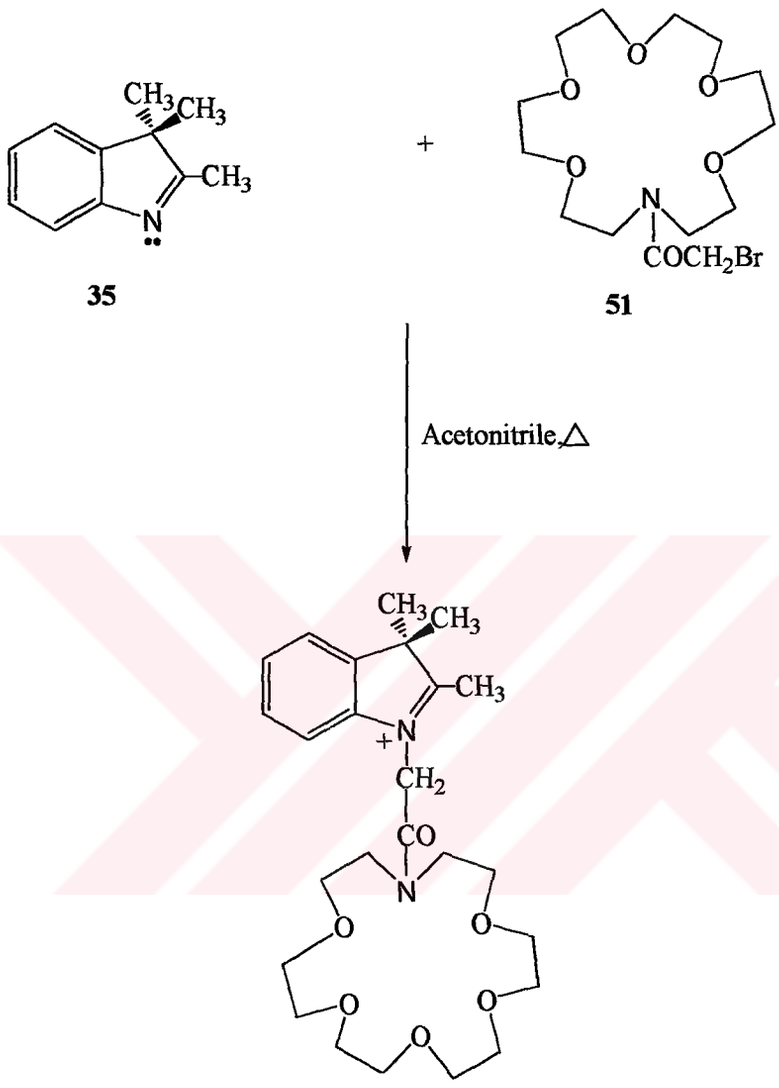
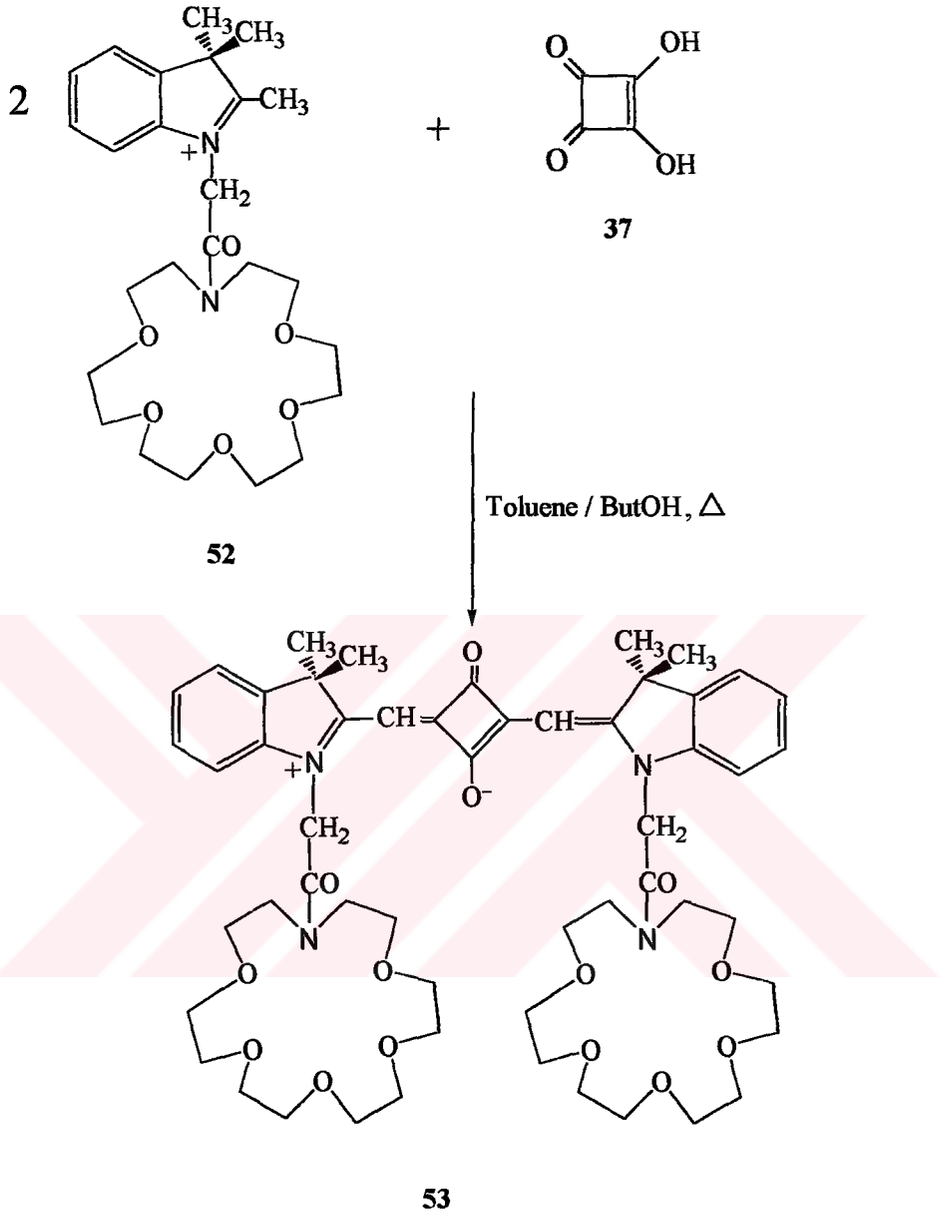


Figure 20. Synthesis of an azacrownindolenine conjugate **52**



53

Figure 21. Synthesis of an azacrownsquaraine conjugate **53**

2.8. Synthesis of Kemp's Triacid Phenanthridinediimide Derivative 56

A mixture of 269 mg of 3,8-diamino-6-phenylphenanthridine **(55)** (0.94 mmole) and 487 mg of the triacid **54** were ground together and then heated in a loosely stoppered flask for 5 h in a sand bath at ~240°C. The mixture fused but did not liquefy. The cooled reaction mixture was dissolved in 50 mL of CH_2Cl_2, filtered through Celite, and then evaporated. The reaction gave a mixtrue of mono(substituted) Kemp's triacid phenanthridine-diimide and bis(substituted) one . The separation of mono and bis-(substituted) Kemp's triacid diimide derivatives was accomplished by short column chromatography on silica gel. Chloroform/methanol (9:1) as the eluting solvent eluted the mono(substituted) Kemp's triacid diimide and unreacted 3,8-diamino-6-phenylphenanthridine first and then yielded the bis-(substituted) Kemp's triacid diimide. The yield was 219 mg (32%) (Figure 22). ^{1}H NMR (DMSO-d_6) δ 1.25-1.75 (m, 30H), 7.59-7.70 (m, 4H), 7.80-7.90 (m, 2H), 7.92-8.08 (m, 2H), 8.10-8.16 (br. m, 1H), 8.97 (d, 1H), 9.06 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 25.56, 25.58, 30.65, 30.78, 39.07, 38.28, 39.49, 39.70, 39.91, 40.12, 40.24, 40.26, 40.33, 41.30, 41.33, 42.12, 42.43, 43.46, 43.62, 122.18, 122.62, 123.02, 124.22, 128.04, 128.34, 128.94, 129.50, 129.81, 131.95, 132.09, 135.63, 137.39, 139.07, 143.45, 160.16, 176.15, 176.22, 177.47, 177.61.

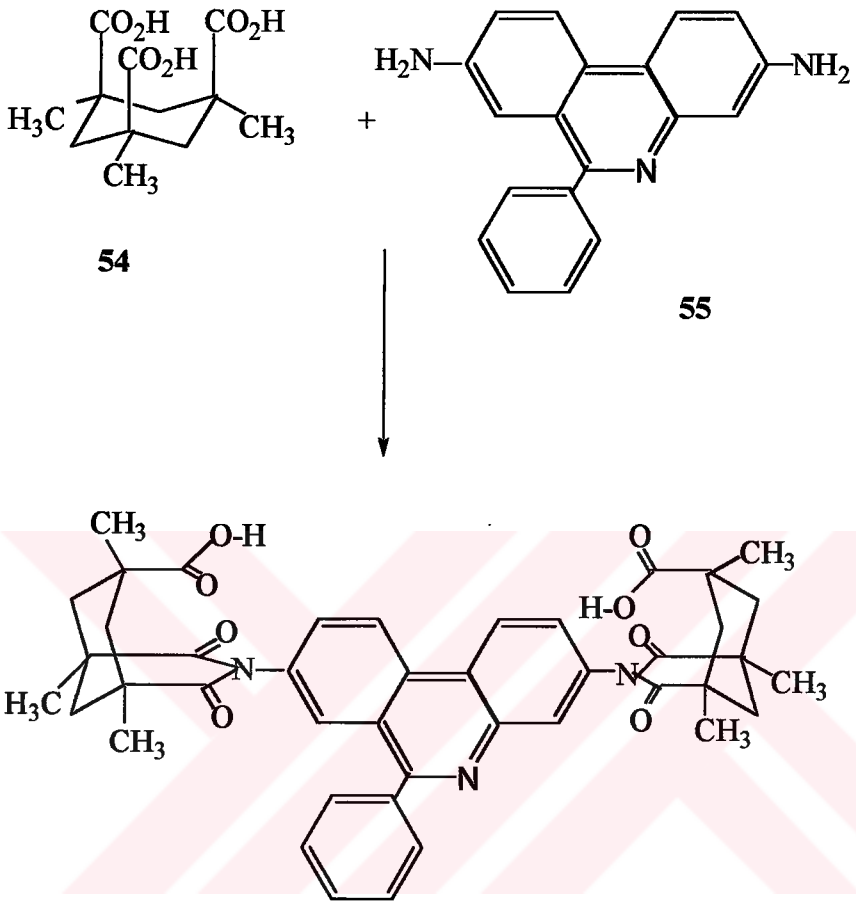


Figure 22. Synthesis of Kemp's triacid phenanthridinediimide derivative **56**

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Spectral Properties of Trimethylindolenine-derived Squaraines

We have synthesized a series of trimethylindolenine-derived squaraines with different alkyl groups on the indolenine nitrogen. The side chains carry potential donor atoms for metal ion coordination. Squaryl oxygens also participate in the chelation Zn (II) ions, leading to a variety of absorbance and fluorescence signals.

In the course of our study of squaraine based molecular signalling devices, we have investigated the participation of groups attached to indolenine system and the squaryl oxygen in metal ion coordination. Substituents in the trimethylindolenine ring have profound effects on the maximum wavelength of absorption and emission. Squaraines have very promising spectral properties for molecular sensors, including high quantum yield, long wavelength excitation and emission. The squaraines as expected had sharp absorption peaks around 635-645 nm.

It is known that squaraine dyes possess a sharp and intense absorption in the visible region in solution. In the solid state, due to the crystal packing and strong charge-transfer interactions, the absorption shows a considerable bathocromic shift to near-infared (NIR) region. These optical

absorption characteristics have made squaraines suitable materials for several technological applications such as xerography, optical data storage, and solar energy conversion.

As a monomer in solution, these compounds absorb strongly at 620-670 nm with very high molar extinction coefficients and exhibit intense fluorescence emissions with small Stokes shifts. In the microcrystalline solid state, due to the strong intermolecular D-A interactions, the solid state absorption is very broad, from 550 to 900 nm, and is red-shifted from the monomer absorption (Law, 1995). The solid state absorption at 450-500 nm is relatively weak, and this low absorbance has hindered the use of squaraine-based photoreceptors in copiers, where a flat response across the visible region (450-650 nm) is desirable (Law, 1995). In an attempt to improve the spectral property, trimethylindolenine derived-squaraines were designed and synthesized. Expectedly, the introduction of a less powerful electron-donating group reduces the charge-transfer interaction, producing a spectral blue-shift in the monomer absorption. The blue-shift has translated into an enhanced absorptivity between 500 and 550 nm. Recent xerographic data indicate that the spectral sensitivity improved from 450 to 650 nm (Law, 1992). The absorption maxima (λ_{max}) of the symmetrical squaraines vary from 500 to 550 nm, depending on the substituent in the trimethylindolenine ring. Their absorption coefficients are of the order of 2.5×10^5 cm^{-1} M^{-1} and they are organic photoconductors that are photosensitive in the near IR where solid-state laser emit (780 nm). Our studies utilize squaraines in solution, so solution properties are more relevant for us. But, squaraines also have high aggregation tendencies, therefore one has to be careful in the interpretation of squaraine spectra.

3.1.1. Spectral Properties of Trimethylethylenediaminesquaraine 48

Squaraines with their long wavelength excitation and emission proved to be highly amenable to modification yielding novel fluorescent chemosensors. The squaraine derivative **48** was designed for the detection of Zn^{2+} ions. This novel fluorescent compound can be excited at long wavelength which is very important for the living organisms. Since short wavelength excitation creates a significant background fluorescence (autofluorescence) and is highly damaging to the cell, complicating the study of *in vivo* process. Short wavelength excitation also necessitates the use of expensive quartz optical components. In addition, laser-diode excitable chemosensors offer a number of advantages like potential for miniaturization, compatibility with fiber-optics applications, transdermal sensing, etc. So, there is a great impetus for the design of "long-wavelength" chemosensors.

The chemosensor is designed as an intrinsic chemosensor, i.e.; the ligand is an integral part of fluorophore π-system. It is well established that fluorescence signal to be obtained using such probes would be stronger or carry more information.

In the study of squaraine based molecular devices, we have synthesized trimethylethylenediamine squaraine **48** in order to investigate the participation of groups attached to the indolenine system and the squaryl oxygen in metal ion coordination. As a control, R= CH_2 CH_3 squaraine **38** was also synthesized. This squaraine showed no response to Zn (II) at any concentration up to 0.2 M, either in absorption or emission spectrum (Figure 24), demonstrating that the squaryl oxygens alone are not sufficient to interact with Zn (II). The trimethylethylenediamine squaraine **48** on the other

hand, has a very interesting response both in the absorption spectrum and more importantly in the emission spectrum in a solvent system of 50% acetonitrile-50%n-butanol. At low concentrations of Zn (II) the emission spectrum shifts to longer wavelength (Figure 26). When excited at 600 nm, the emission maximum at 660 nm shifts to 665 nm with an increase in intensity up to 1μM Zn (II), but further increase in the ion concentration decreases the emission intensity and by 100 mM Zn (II) concentration, the emission is shifted to 650 nm. When followed at 625 nm, the fluorescence emission intensity first decreases 3-fold at low concentrations and increases 2-fold at high concentrations compared to the free squaraine ligand. Thus, the molecule offers three Zn (II) addressable states, providing the first example of a molecular ternary logic: state 0, 0 Zn (II) medium fluorescence, state 1, 5 μM high fluorescence, state 2, 50 mM, low fluorescence (Figure 23).

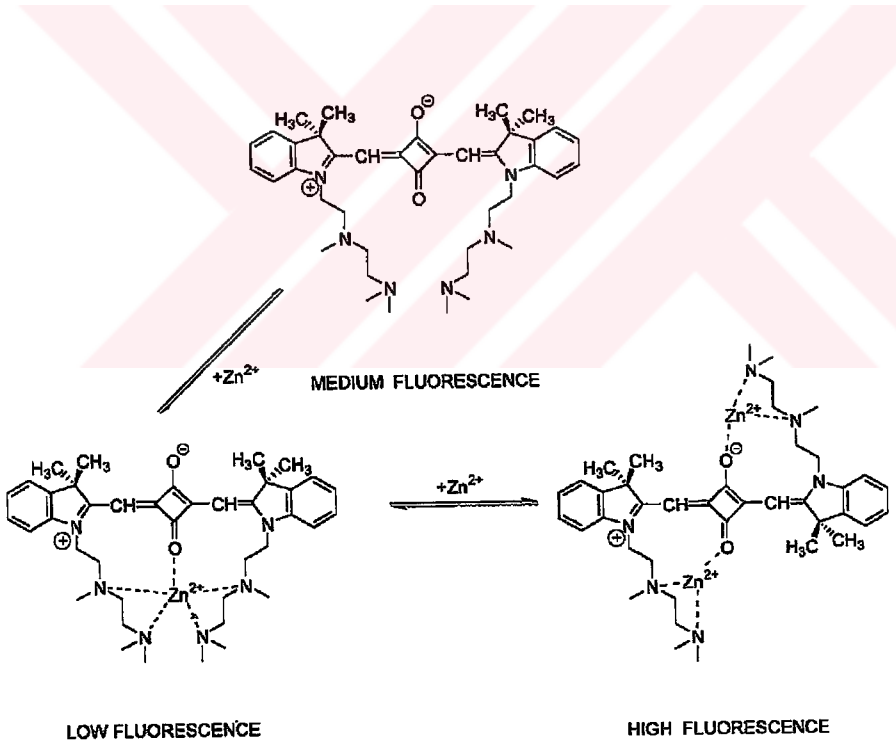


Figure23. Two different binding modes leading to three levels of fluorescence signal.

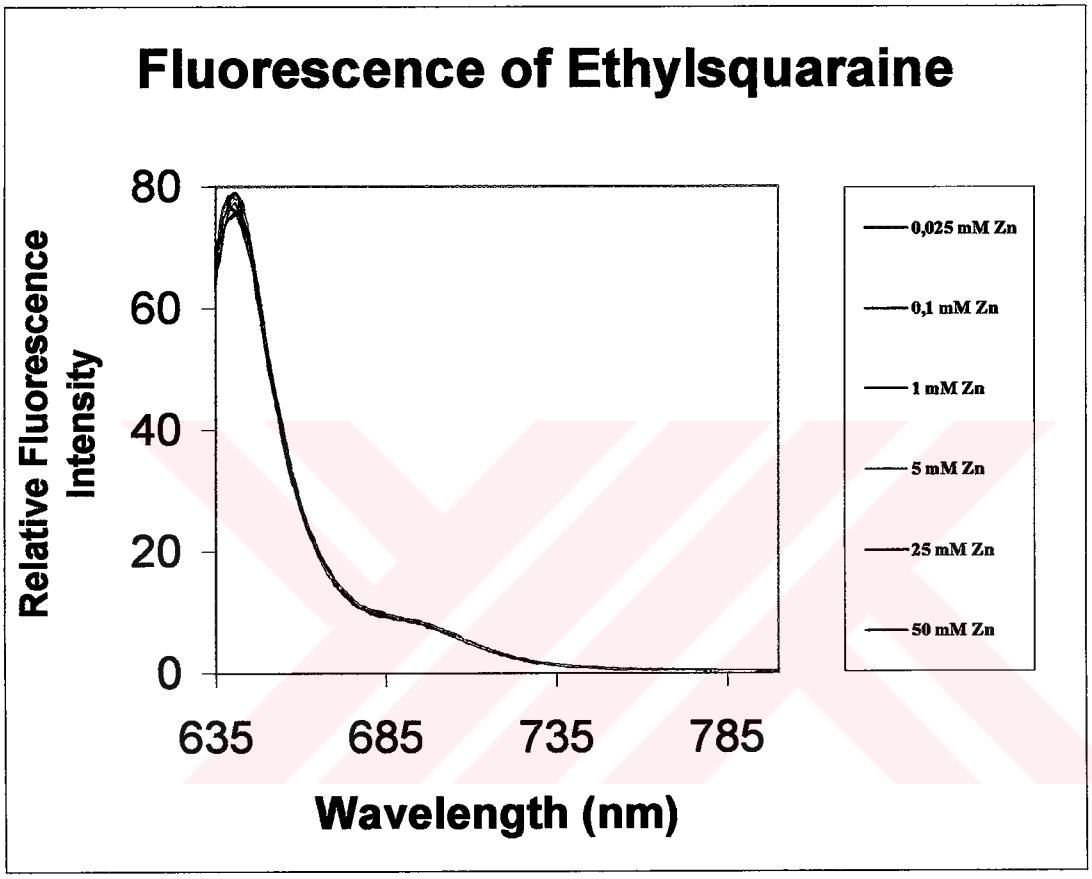


Figure 24. Emission spectrum of 0.4 μM ethylsquaraine **38** in acetonitrile as a function of increasing Zn^{2+} concentrations (λ_{exc} = 625 nm).

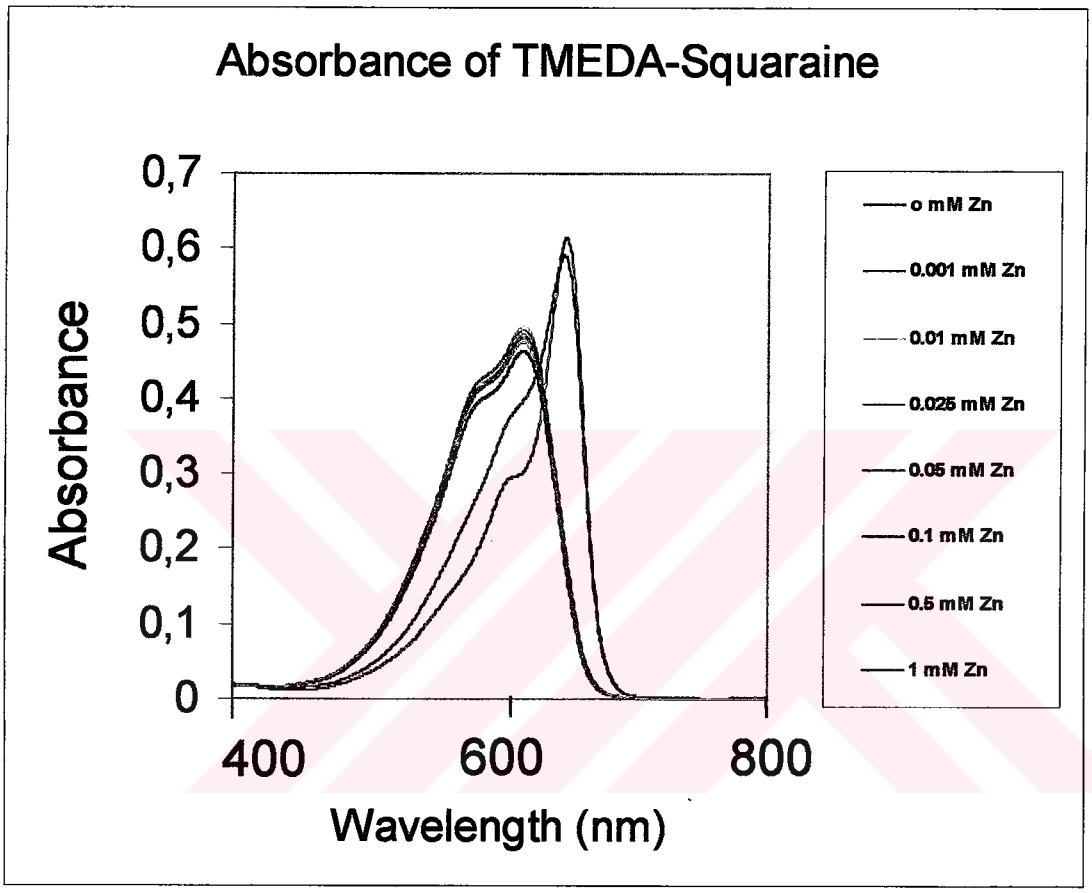


Figure 25. Absorption spectrum of 2.5 μM trimethylethylenediamine squaraine **48** in acetonitrile as a function of increasing Zn^{2+} concentrations.

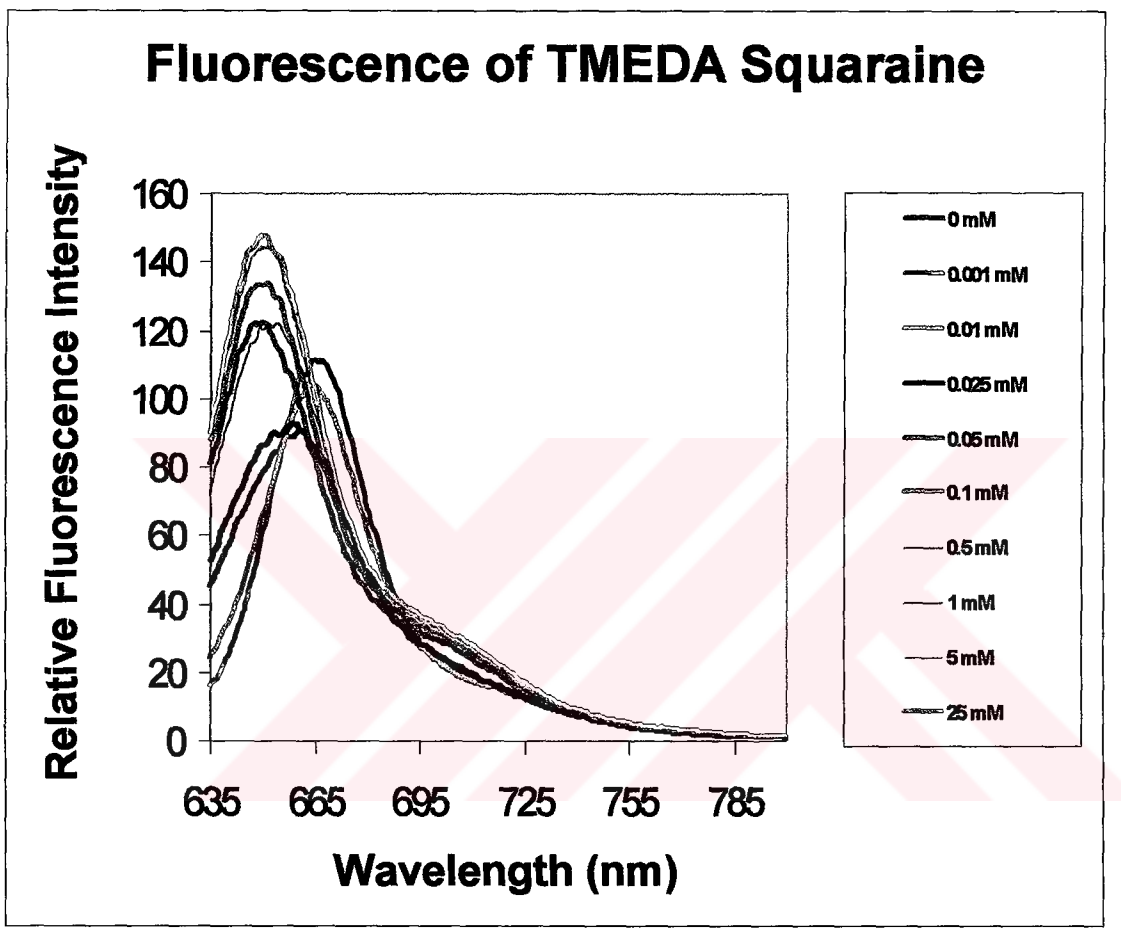


Figure 26. Emission spectrum of 1 μM trimethylethylenediamine squaraine **48** in acetonitrile as a function of increasing Zn^{2+} concentrations (λ_{exc} = 625 nm)

3.1.2. Spectral Properties of Dimethylaminoethylsquaraine 41

The photophysical and photochemical properties of squaraines can be conveniently modified by substitutional changes or by changing the nature of the medium (Das, 1996). The symmetric D-A-D (donor-acceptor-donor) arrangement at symmetrical squaraine dyes causes an interesting effect on the formation of intramolecular charge transfer states. As a result, the photophysical properties of these dyes are highly sensitive to substituent groups and the solvent medium.

The absorbance spectrum of dimethylaminoethylsquaraine shows an interesting and varied response to an increase in zinc concentrations. The spectrum is dominated by aggregate peaks. The intermolecular charge transfer interactions between the donor-acceptor groups are proposed to be the driving force behind the aggregation process. In fact, in the solvent mixture that we used, at $[Zn^{+2}]=0$, we have two peaks, one of which is clearly due to a blue-shifted aggregate structure, relative proportions change as the $[Zn^{+2}]$ concentrations increase. Also, new blue shifted (at 450 nm) and red shifted (at 750 nm) aggregate peaks appear in the spectra. In short, we concluded that the absorbance spectra are far too complicated changes for any ion-sensing application (Figure 25). Fluorescence spectra also do not display a clear trend (Figure 26).

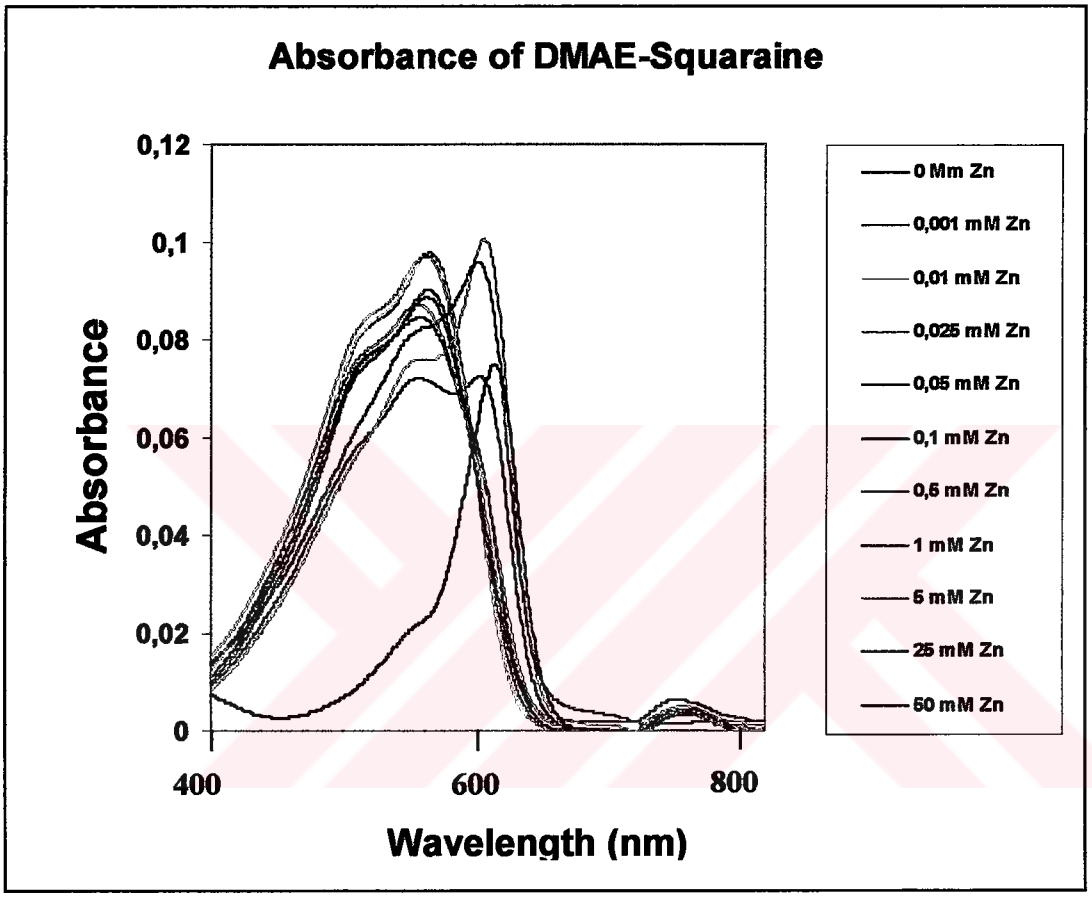


Figure 27. Absorption spectrum of 0.4 μM dimethylaminoethyl-squaraine **41** in acetonitrile as a function of increasing Zn^{2+} concentrations.

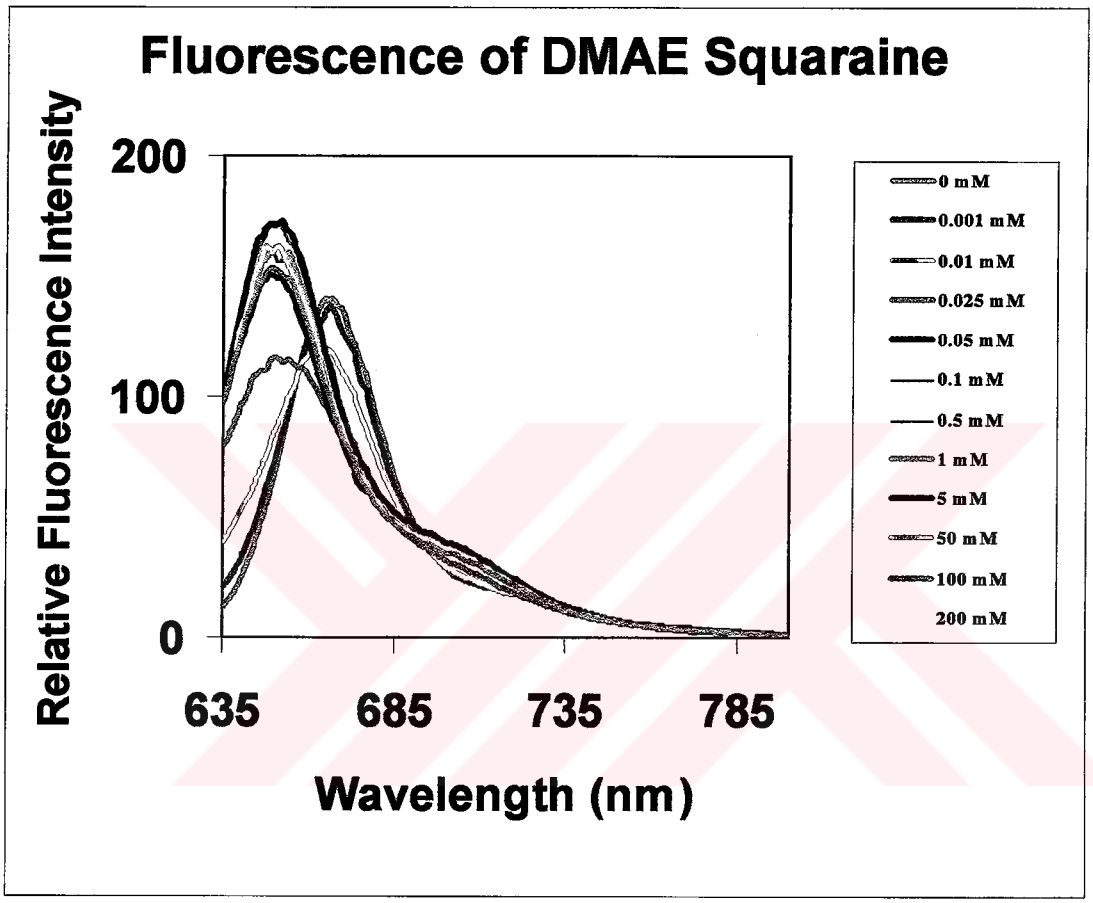


Figure 28. Emission spectrum of 0,4 μM dimethylaminoethylsquaraine **41** in acetonitrile as a function of increasing Zn^{2+} concentrations (λ_{exc} = 625 nm)

3.1.3. Spectral Properties of Dimethylaminopropylsquaraine 44

In the dimethylaminopropyl derivative, a bidirectional response was observed. The absorbance increases until a [Zn^{+2}] concentration of 0.05 mM (λ_{max} = 655 nm). At 0.5 mM concentration an abrupt decrease took place, indicating that probably a switching to 1:2 stoichiometry. Apparently, this mode of complexation causes a decrease in the planarity of the molecule leading to decreased extinction coefficient. Higher concentrations of zinc may cause a formation of complexes of other stoichiometries (1:3). This seems to restore planarity to the molecule with an increase in absorptivity (Figure 30).

The intense absorption at 655 nm suggests that the compound can be ideally excited with a laser diode. This would make this compound one of the few fluorescent chemosensors for Zn (II) that can be excited with such long wavelength sources.

It is very likely that this chemosensor will open a new path for the development of better chemosensors based on squaraine system, and would offer considerable advantages by enabling laser diode excitation leading to the development of compact solid-state devices for celluler ion-flux studies and real-time imaging (Akkaya, 1997). In the case of fluorescence spectra, changing stoichiometry of the interaction leads to an abrupt change in absorption spectra. However, there is a uniform increase in the fluorescence emission intensity (λ_{max} = 660 nm) in the solution of dimethylaminopropyl-squaraine conjugate (Figure 31). This clearly shows that 1:3 complex must have higher quantum yield.

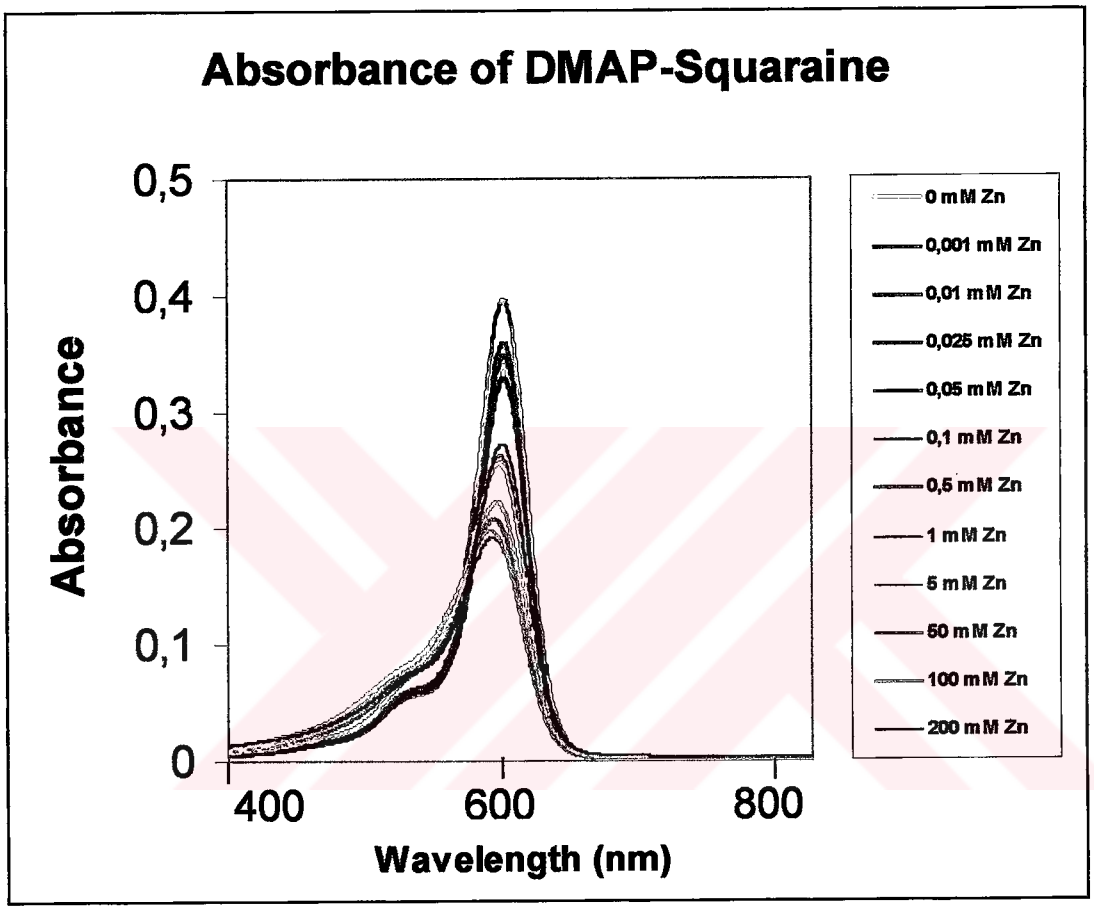


Figure 29. Absorption spectrum of 1.6 μM dimethylaminopropy-l squaraine **44** in acetonitrile as a function of increasing Zn^{2+} concentrations.

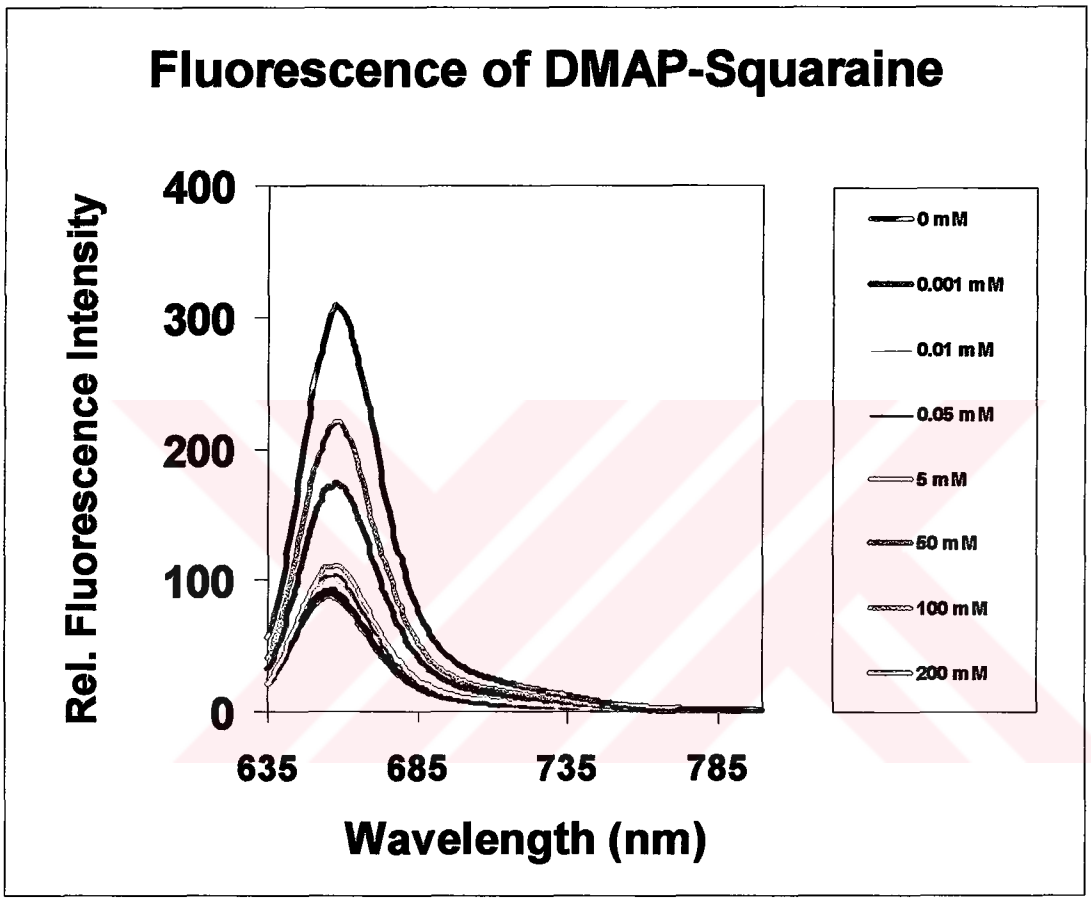


Figure 30. Emission spectrum of 0.4 μM dimethylaminoprohyl-squaraine **44** in acetonitrile as a function of increasing Zn^{2+} concentrations (λ_{exc} = 625 nm)

3.1.4. Spectral Properties of Azacrownsquaraine Conjugate 53

We have synthesized a new class of squaraine dyes that contain an ionophoric moiety. Ionophores that are covalently linked to chromophores exhibit intramoleculer charge transfer (ICT) transitions. Excitation of such molecules causes a redistribution of their charge densities, which can significantly affect the metal ion binding ability of the ionophoric unit. Alternatively, the ICT transition can be severely affected by complexation with metal ions, leading to significant changes in the absorption and emission properties of these molecules. Such molecules can therefore be utilized for the selective and quantitative detection of biologically important metal ions such as K^+. For intracellular applications it is desirable to design fluoro- or chomoionophores that are water soluble and absorb strongly in the near infrared region. Additionally, if the chromophoric unit is redox active, such molecules can be utilized for transportation of ions across membranes as well as in the design of ion sensitive electrodes.

Here, we report the metal ion binding properties of an crown ether derivative of squaraine dye.

The crown-ether-linked squaraine dye has very strong absorption band centered arround 625-630 nm.

As in the case of zinc sensitive squaraines, changing stoichiometry of the interaction between the fluorophore and K^+ leads to an abrupt change in absorption spectra (Figure 32). However, there is a uniform increase in the fluorescence emission intensity in the solutions of the azacrownsquaraine conjugate (λ_{max} = 650 nm) (Figure 33). This clearly shows that 1:2 complex has a higher quantum yield. The change in intensity is not spectacular, but it is clear that other squaraine derivatives based on this idea is likely to yield NIR emitting fluorophores.

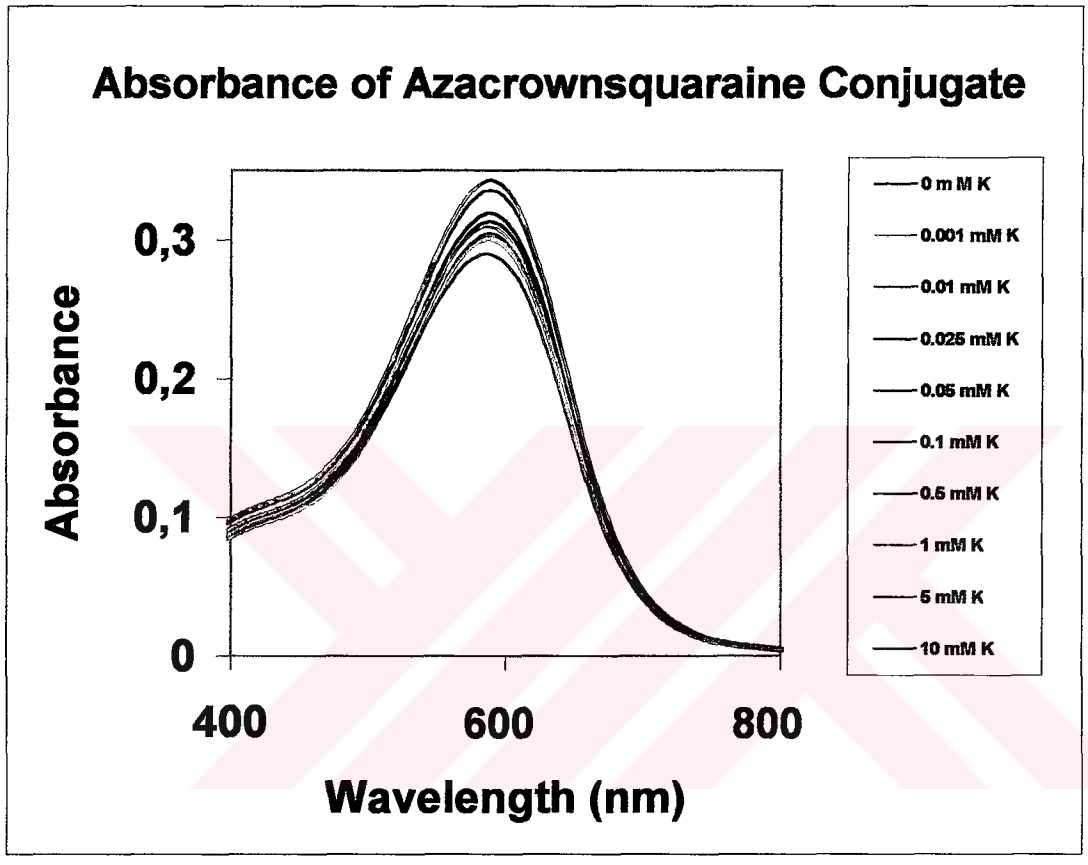


Figure 31. Absorption spectrum of 1.6 μM azacrownsquaraine co **53** in acetonitrile as a function of increasing K^+ concentrations.

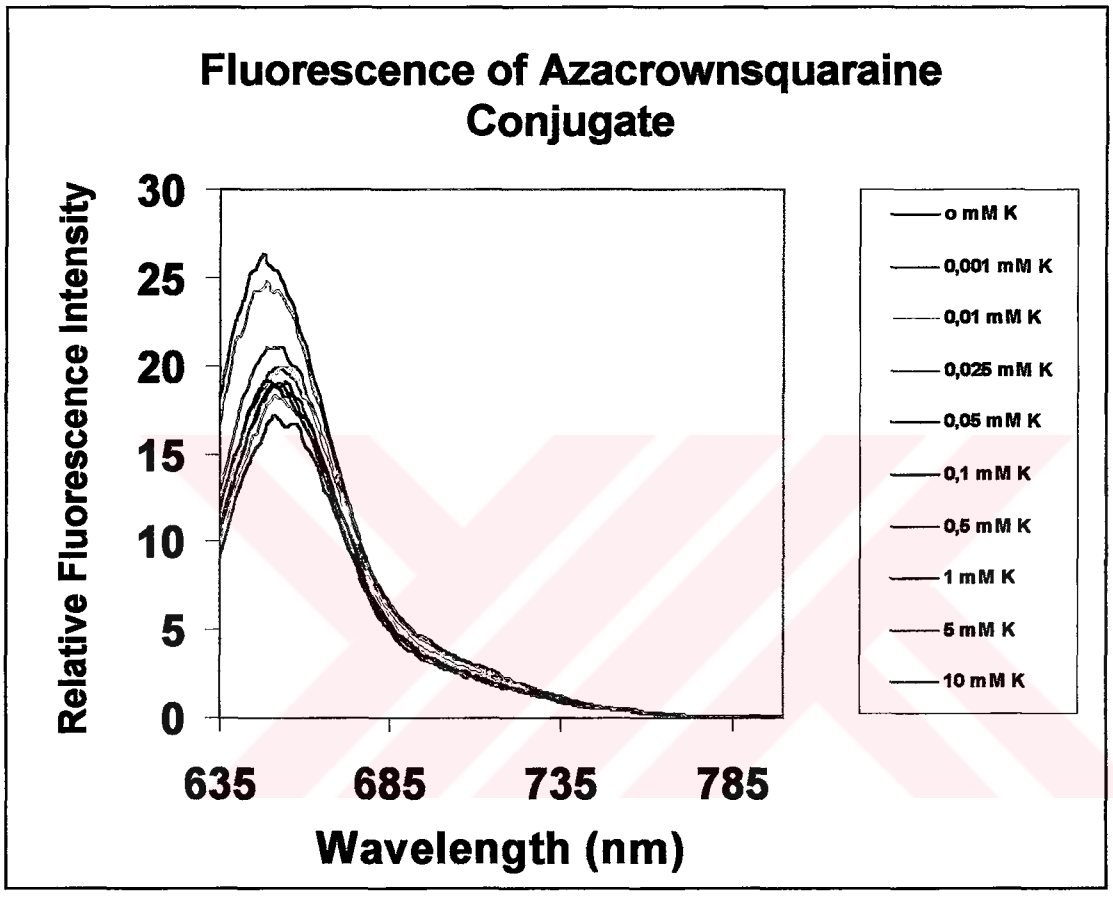


Figure 32. Emission spectrum of 1 μM azacrownsquaraine conjugate **53** in acetonitrile as a function of increasing K^+ concentrations (λ_{exc} = 625 nm)

3.2. Spectral Properties of Kemp's Triacid Phenanthridinediimide Derivative 56

We have designed and synthesized a new class of chelating agents derived from 1,3,5-trimethylcyclohexane-1,3,5-tricarboxylic acid (Kemp's triacid). It is known that Kemp's triacid imides are useful carriers for the transport of various metal ions. Amongst these carriers, the aromatic Kemp's triacid diimide **56** has an especially high transport ability and efficiency for Zn (II).

The novel Kemp's triacid diimide **56** was prepared as a chromogenic reagent for Zn^{2+}. The mechanism of the color change was demonstrated as being due to the promotion of the complex 1:1 (and/or 2:2) chromophore:metal ratio after the addition of transition- or heavy-metal ion to the chromophoric solution. The complexation causes proton transfer from the carboxyl group to the azo group, changing the color of the original chromophore **56.**

The emission spectra were measured using Cd $(ClO_4)_2$ and Zn $(ClO_4)_2$ in acetonitrile solution. Upon the addition of Zn^{2+} from 0.0 to 10^{-1} mM the emission band (λ_{max} = 490nm) intensity increased.

As it is seen in both Figure 34 and Figure 35, as the concentrations of Zn $(ClO_4)_2$ and Cd $(ClO_4)_2$ increase, fluorescence also increases, however, there was no change in the absorption spectra.

Kemp's triacid diimide derivative has a λ_{max} of 445 nm in chloroform. The compound is also highly fluorescent in chloroform. This chemosensor successfully signals Zn (II) and Cd (II) ions at micro to milimolar concentrations. Zn^{2+} and Cd^{2+} lead to a small blue shift in the emission spectrum. The absorption peak does not change as the metal ions are added, so there is no signal in the absorption spectrometry. But the emission

intensity at 445 nm increases about 6-fold in the presence of 10 mM Zn (II) in acetonitrile. A smaller concentration of cadmium perchlorate solution could be prepared, but at equal concentrations (1mM) zinc causes a 2-fold longer fluorescence signal. The binding cavity that is formed by the imide-carbonyls and carboxylic acid group apperently prefers Zn^{2+}, the harder metal ion, compared to Cd^{2+}. Further improvements are likely to improve this selectivity.

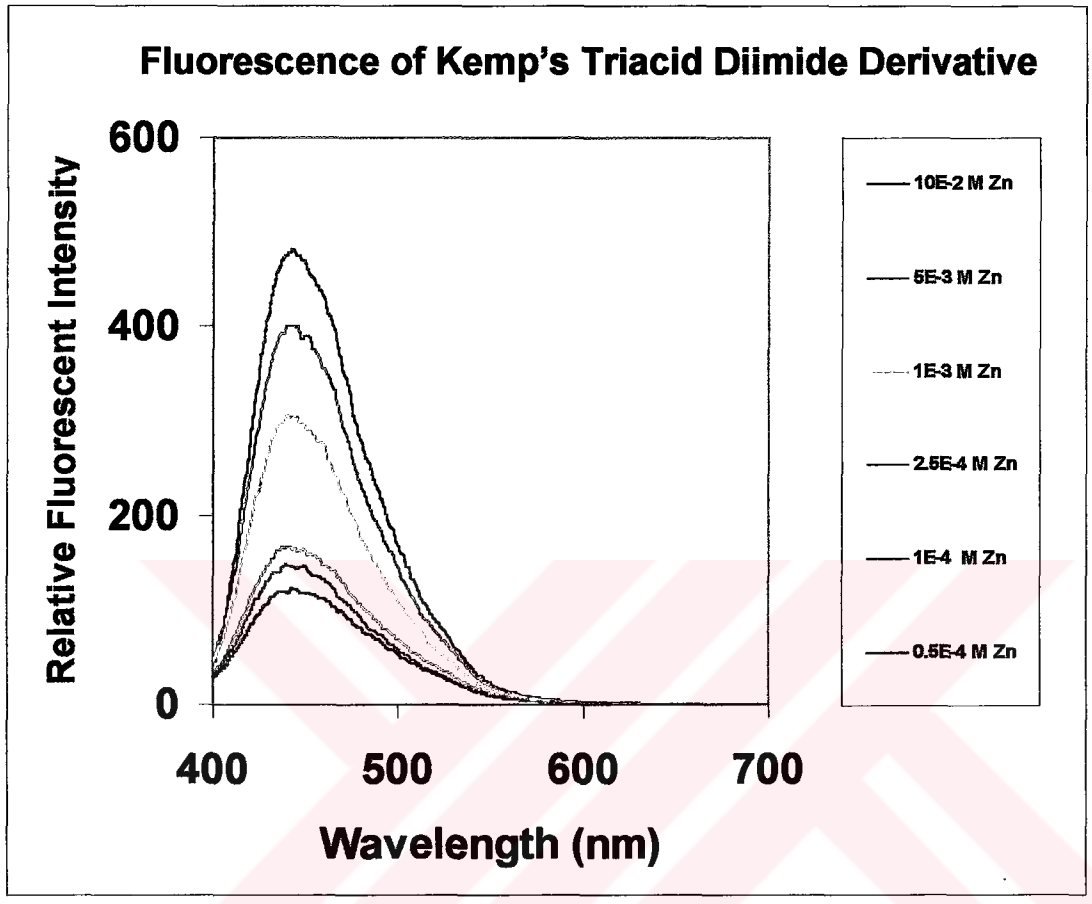


Figure 33. Emission spectrum of 0,4 μM Kemp's triacid phenanthridinediimide derivative **56** in acetonitrile as a function of increasing Zn^{2+} concentrations. (λ_{exc} = 390 nm)

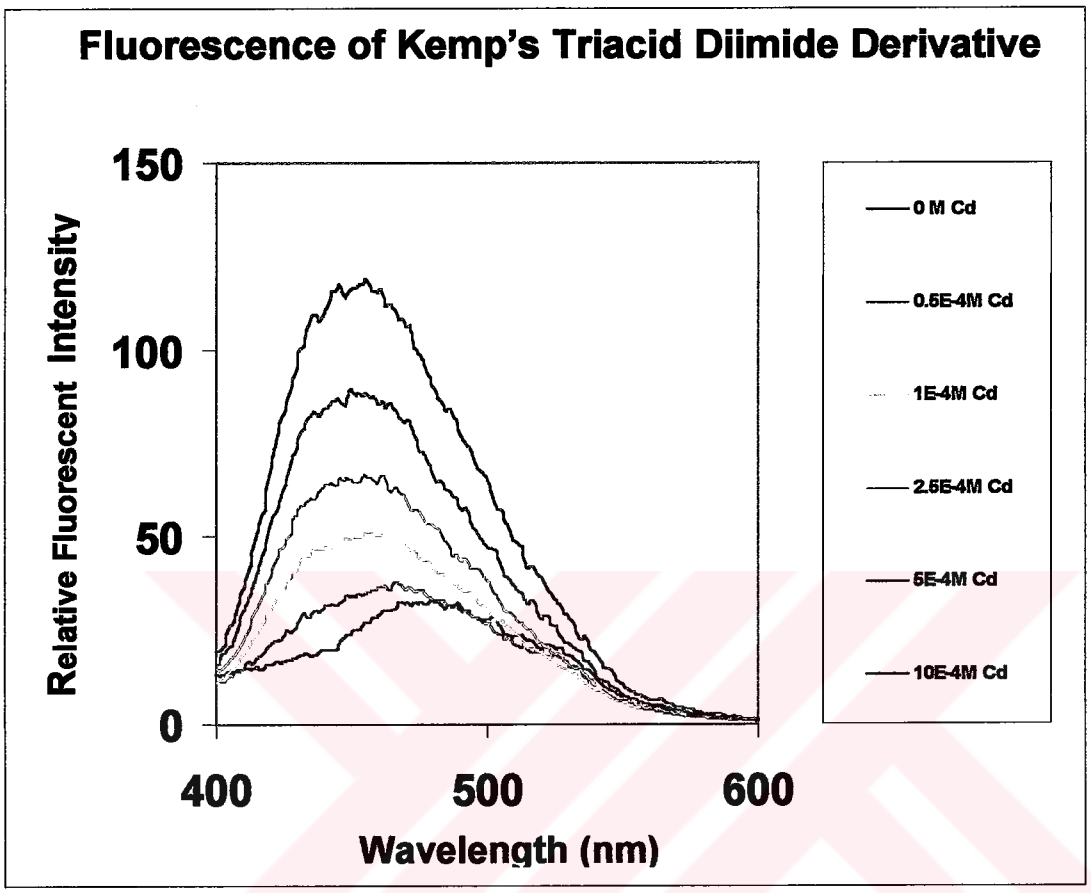


Figure 34. Emission spectrum of 0.4 μM Kemp's triacid phenanthridinediimide derivative **56** in acetonitrile as a function of increasing Cd^{2+} concentrations. (λ_{exc} = 390 nm)

CHAPTER 4

CONCLUSION

In this study a series of novel fluorescent chemosensors based on squaraine has been synthesized and spectrally characterized.

One of the most important accomplishments of this study was the long wavelength excitation and emission which proved to be highly amenable to modification yielding a novel fluorescent chemosensor. These novel fluorescent compounds work at long wavelengths which is very important for the living organisms. Moreover, they possess potential interest as laser-diode-excitable fluorescence probes. In conclusion, we have observed that the indolenine derived squaraine dyes can act as red to NIR emitting absorbing fluorophores for detecting low amounts of zinc ions in acetonitrile solution.

In the second part of the study a new class of squaraine dye that contain an ionophoric moiety was synthesized. As expected, this novel squaraine selectively signals potassium ions in acetonitrile solution although the change in the emission intensity is less than optimal.

In the last part of our study we synthesized a novel Kemp's triacid phenantridinediimide derivative which has selective response to zinc ions compared to cadmium ions. Among Zn (II) and Cd (II) ions, Zn^{2+} caused the greater change in the intensity of fluorescence emission. The mechanism of fluorescence enhancement could be the inhibition of photoinduced electron transfer from the carboxylate ions to phenanthridine moiety. Further

improvements are likely to yield a more selective agents for the detection of Zn^{2+} and other transition metal ions.

In further studies in this area considering the fact that two donor atom containing side chains complicate the spectra asymmetric squaraines with one side chain can be synthesized. In addition, instead of squaric acid croconic acid can be used to obtain near infrared absorbing dyes (croconium dyes). The practical potential for such molecular sensors would be greater.

REFERENCES

Akkaya, E. U.; Huston, M. E.; Czarnik, A.W. *J. Am. Chem. Soc.* **1990,** 112, 3590-3593.

Akkaya, E. U.; Oğuz, U. *Tetrahedron Letters* **1997,** Vol.38, 25, 4509-4512

Balzani, V., Sabbatini, N.; Scandola, F. *Chem. Rev.* **1986,** 86, 319

Balzani, *V. Supramolecular photochemistry* **1987,** 1

Barton, J. K. *Science* **1986,** 233, 727

Belser, P.; De Cola L.; von Zelewsky, A. *J. Chem. Soc., Chem. Commun.* **1988,** 1057

Bissell, R. A.; Bryan, A. J.; de Silva, A. P.; McCoy, C. P. *J. Chem. Soc., Chem. Commun* **1994,** 405

Breslow, R. *Science* **1982,** 218, 532

Bright, G. R.; Fisher, G. W.; Rogowska, J.; Taylor, D. L. *Fluorescence Microscopy of Living Cells in Culture PartB, Methods in Cell Biology Vol. 30,* Ed. Taylor, D. L.; Wang, Y.L. pp. 157-192, Academic Press, San Diego, 1989.

Chen, D. Y.; Adelhelm, K.; Cheng, X. L.; Dovichi, N. J. *Analyst* **1994,** 119, 349

Colquhoun, H. M.; Stoddart, J. F.; Williams, D. J., *Angew. Chem., Int. Ed. Engl.* **1986,** 25, 487

Cram, D. J.; Karbach, S.; Kim, Y. H.; Baczynskyj, L.; Marti, K.; Sampson, R.M.; Kalleymeyn, G. W. *J. Am. Chem. Soc.* **1988,** 110, 2554

Czarnik, A. W.; Huston, M. E.; Haider, K. W. *J. Am. Chem. Soc.* **1988,** 110, 4460-4462

Czarnik, A. W. *Fluorescent Chemosensors for Ion and Molecule Recognition,* Ed. Czarnik, A. W. ACS Symposium Series 538, pp2, American Chemical Society: Washington, D.C., 1992

Czarnik, A. W. *J. Org. Chem,* **1993,** 58, 5797-5801

Czarnik, A. W. *Acc. Chem. Res.* **1994,** 27, 302-308

Das, S. M. V.; Thomas, K. G.; Thomas, K. J.; George, M. V.; Liv, D.; Kamat, P. V.; *J. Chem. Soc., Faraday Trans.* **1996,** 92, 24, 4913-4916

de Silva, S. A.; Prasanna de Silva, A. *J. Chem. Soc., Chem. Commun.* **1986,** 1709.

de Silva, A. P.; Bissell, R.A.; Gunoratne, H.Q. *Chem. Soc. Rev.* **1992,** 187

Dietrich-Buchecker, C. O.; Sauvage, J. P.; Kern, J. M. *J. Am. Chem. Soc.* **1984,** 106, 3043

Fages, F. *J. Am. Chem. Soc.* **1989,** 111, 8672-8680

Fages, F.; Desvergne, J. P.; Bavas-Laurent, H. *J. Am. Chem. Soc.* **1989,** 96-102

Fages, F.; Desvergne, J. P.; Bovas-Laurent, H.; Marsau, P.; Lehn, J. M.; Kotzyba-Hibert, F.; Albrecht-Gary, A. M.; Al-Joubbeh, M. *J. Am. Chem. Soc.* **1989,** 111, 8672-8680

Fages, F.; Desvergne, J. P.; Bovas-Laurent, H.; Marsau, P. *Pure Appl. Chem.* **1992,** 69, 1231-1238

Farnum, D. G.; Neuman, M. A.; Suggs, W. T. Jr. *J. Cryst. Mol. Struct.* **1974,** 4,199

Fyfe, C. T. M.; Stoddart J.F. *Accounts of Chemical Research* **1997,** 30,10, 393-401

Goppelsröder, F. *J. Praxt. Chem.* **1867,** 101, 408

Guilbault, G. G *"Practical Fluorescence",* Marcel Dekker, Inc.: New York, 1990

Kamat, P. V.; Sauve, G.; Thomas, K. G.; Thomas, K. J.; Das, S.; George, M. V. *J. Phys. Chem.* **1996,** 100, 2117-2124.

Kasha, M.; Rawls, H. R.; El-Bayoumi, M. *Pure Appl. Chem.* **1965,** 11,371

Kazmaier, P. M.; Hamer, G. K.; Burt, R. A. *Can. J. Chem.* **1990,** 68, 530.

Krämer, R. *Angew. Chem., Int. Ed. Engl.* **1988,** 37,6,772-773

Kuhn, H. *Angew. Chem., Int. Ed. Engl.* **1981,** 20, 500

Kuhn, H.; Waser, J. *Angew. Chem.* **1981,** 93, 495

Kurtz, I.; Golchini, K; Malkovic-Basic, M.; Gharib, S. A.; Masilamani, D.; Lucas, M.E. *Am. J. Phys.* **1990,** 258, F438

Lacowicz, J. R.; Szmacinski, H.; Nowaczyk, K.; Johnson, M. L. *Cell Calcium,* **1992,** 13, 131

Lakowicz, J. R.; Szmacinski, H.; Nowaczyk, K.; Johnson, M. L. *J. Fluorescence* **1992,** 2,47

Lauffer, R. B. *Chem. Rev.* **1987,** 87, 901

Law, K. Y.; Bailey, F. C. *J. Org. Chem.* **1992,** 57, 3278-3286

Law, K. Y.; Bailey, F. C. *Can. J. Chem.* **1993,** 71, 494

Law, K. Y. *J. Phys. Chem.* **1995,** 99, 9818-9824

Lehn, J. M.; Simon, J.; Wagner, J. *Angew. Chem.* **1973,** 85, 621, 622

Lehn, J. M.; Simon, J.; Wagner, J. *Angew. Chem., Int. Ed. Engl.* **1973,** 12, 578, 579

Lehn, J. M. *Pure Appl. Chem.* **1978,** 50,871

Lehn, J. M. *Science,* **1985,** 227, 849.

Lehn, J. M.; Konopelski, J. P.; Kotzyba-Hibert, F.; Desvergne, J. P.; Fages, F.; Costellan, A.; Bavas-Laurent, H. *J. Chem. Soc., Chem. Commun.* **1985,** 433

Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1988,** 27, 89-112

Lehn, J. M., Angew. Chem., Int. Ed. Engl. **1990,** 29, 1304-1319

Lehn, J. M. *Supramolecular Chemistry;* VCH: Weinheim, Germany **1995**

Löhr, H. G.; Vögtle, F. *Acc. Chem. Res.* 1985, 18, 65-72

Martell, A. E. *Chem. Rev.* **1989,** 89, 1875-1914

Masilamani, D., Lucas, M. E. Fluorescent chemosensors for monitoring potassium in blood and accross biological membranes, in A. W. Czarnik (ed.), *ACS Symposium Series 538,* pp. 162-182, American Chemical Society: Washington, D. C., 1992.

Minta, A.; Tsien, R. Y. *J. Biol. Chem.* **1989,** 264, 19449

Philp, D.; Stoddart, J. F. *Angew. Chem., Int. Ed. Engl.* **1996,** 35, 1154-1196

Prasanna de Silva, A.; Nimal Gunarotne, H. Q. *J. Chem. Soc., Chem. Commun.* **1990,** 186

Rebek, J., Jr.; Nemeth, D.; Ballester, P.; Lin, F. T. *J. Am. Chem. Sec.* **1987,** 109, 3474-3475

Rettig, W.; Letard, J. F.; Lapouyade, R. *Pure Appl. Chem.* **1993,** 65, 1705-1712

Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988,** 27,113

Robinson, A. L. *Science* **1984,** 223, 267

Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980,** 19, 344

Sargeson, A. M., *Chem. Brit.* **1979,** 15, 23

Stokes, G. G. *Philos. Trans. R. Soc. London, A* **1852,** 142, 463

Tsien, R. Y.; Poenie, M. *Trends Biochem. Sci.* **1986,** 11, 450

Tsien, R. Y. Fluorescent and photochemical probes of dynamic biochemical signals inside living cells, in A. W. Czarnik (ed.), *ACS Symposium Series 538,* pp. 130-146, American Chemical Society: Washington, D. C., 1992.

Tsien, R. Y. *Annu. Rev. Biophys. Bioeng.* **1993,** 12, 94

Valeur, B.; Fery-Forgues, S.; Le Bris, M. T.; Guette, J. P. *J. Chem. Soc., Chem. Commun.* **1988,** 5,384-385

Valeur, B.; Fery-Forgues, S.; Le Bris, M. T.; Guette, J. P. *J. Phys. Chem.* **1988,** 92, 6233-6237

Valeur, B.; Bourson, J. *J. Phys. Chem.* **1989,** 93,3871.

Valeur, B.; Fery-Forgues, S.; Bourson, J.; Dallery, L. *New J. Chem.* **1990,** 14,617-623

Valeur, B. *Molecular Luminescence Spectroscopy, Methods and Applications Part 3,* Ed. Schulman, S. G. pp. 25-84, John Wiley & Sons, New York, 1993

Valeur, B. "Fluorescent Probe Design for Ion Recognition" in *Topics in Fluorescence Spectroscopy,* Vol.4, *Probe Design and Chemical Sensing,* Lakowicz, J. R. (Ed.), Plenum Press: New York, 1994a, 22

Valeur, B. "Fluorescent Probe Design for Ion Recognition" in *Topics in Fluorescence Spectroscopy, Vol. 4, Probe Design and Chemical Sensing,* Lakowicz, J. R. (Ed.), Plenum Press: New York, 1994b, 25.

Verhoeven, J. W.; Ariese, F.; Jonker, S. A. *Red. Trav. Chim. Pays-Bas* **1989,** 108,109-115

Verhoeven, J. W.; Jonker, S. A., Van Dijk, S. I; Goubitz, K.; Reiss, C. A.; Schuddeboom, W. *Mol. Cryst. Liq. Cryst.* **1990,** 183, 273-282

Vogler, A; Kunkely, H. *Topics Curr. Chem.* **1990,** 158

Vögtle, F. *Host-quest complex chemistry I. Topics Curr. Chem.* **1981,** 98

Vögtle, F. *Host-quest complex chemistry II. Topics Curr.* Chem. **1982,** 101

Vögtle, F.; Weber, E. *Host-quest complex chemistry III. Topics Curr. Chem.* **1984,** 121

Webb, T. H.; Wilcox, C. S. *J. Org. Chem.* **1990,** 55, 363-365

Weber, E. *Molecular inclusion and molecular recognition-clathrates I. Topics Curr. Chem.* **1987,** 140

Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1990,** 29, 1209-1218.

Whitesides, G. M. *Sci. Am.* **1995,** 273, 3, 114-117

Wingard, R. E. *IEEE Ind. Appl.* **1982,** 1251.

Wolf, K. L.; Frahm, H.; Harms, Z. *Phys. Chem. Abt. B* **1937,** 36,17