

MOLECULAR LOGIC IN THE ACTIVITY MODULATION OF
POTENTIAL PDT AGENTS & RATIONAL DESIGN OF SELECTIVE
CHLORIDE SENSORS

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DOCTOR OF PHILOSOPHY

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February, 2013

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ABSTRACT

MOLECULAR LOGIC GATES IN THE ACTIVITY MODULATION OF POTENTIAL PDT AGENTS & RATIONAL DESIGN OF SELECTIVE CHLORIDE SENSORS

Sündüs Erbaş-Çakmak

PhD in Materials Science and Nanotechnology

Supervisor: Prof. Dr. Engin U. Akkaya

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Considering the time arrow of science, things are getting smaller and smarter as the information grows immensely. More is known about the molecular and genetic basis of diseases, and therapies become more personalized accordingly. In parallel, organic devices are becoming progressively intelligent. Molecular logic gates increase the flexibility in functionality of the Boolean logic operations, and allow the creation of demanding complex functionalities for different purposes. The missing link between the design of any kind of complex logic operation and assignment of a real function is addressed by our work. A dual-activatable photosensitizer acting as an AND logic gate enables a more selective photodynamic therapy with the use of biologically relevant concentrations of acid and glutathione. Additionally, with a combinatorial therapy and imaging approach, a proof of principle theranostic device with DEMUX logic behaviour is developed to select between singlet oxygen generation and emission in response to an address input (acid) in organic solvent. In another project, relay of information between two independent logic gates embedded into a physically constraining microenvironment was successfully demonstrated to report the activity of a pH-activatable photosensitizer as an enhanced emission. Independently, a series of highly selective fluorescent chloride anion sensors based on BODIPY-triazolophane conjugate are introduced with a surprisingly large dynamic response range.

Keywords: Photodynamic therapy, logic gate, activatable photosensitizer, Cl^- sensor

ÖZET

POTANSİYEL FDT AJANLARININ MOLEKÜLER MANTIK KAPISIYLA AKTİVİTE KONTROLÜ & SEÇİCİ Klorür ANYONU ALGILAYICILARININ RASYONEL TASARIMI

Sündüs Erbaş-Çakmak

Doktora, Malzeme Bilimi ve Nanoteknoloji

Tez Yöneticisi: Prof. Dr. Engin U. Akkaya

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Bilimsel gelişimin izlediği yola bakıldığında artan bilgi birikiminin, fonksiyonel aygıtların küçülmesiyle orantılı olduğu görülür. Bunun yanı sıra, hastalıkların temelinde yatan moleküler ve genetik süreçler hakkında edinilen bilgi arttıkça uygulanan tedaviler daha vakaya özgü olma yolunda ilerlemektedir. Bu gelişmelere paralel olarak, organik malzemeler daha da akıllı hale gelmektedir. Moleküler mantık kapıları geleneksel Boole cebirine işlevsel esneklik kazandırmakta ve çeşitli amaçlar için ihtiyaç duyulan karmaşık işlemleri yapabilme olanağı sunmaktadır. Çalışmalarımızda, herhangi bir moleküler mantık kapısına atfedilen karmaşık işlemlerin uygulanabilir ve anlamlı bir yapıya sahip olacak şekilde iyileştirilmesi amaçlanmıştır. Bu bağlamda, biyolojik olarak anlamlı asit ve glutatyon derişimlerinde VE mantık kapısı olarak davranan aktifleştirilebilir bir fotodinamik terapi ajanı oluşturulmuştur. Bunun yanı sıra, tedavi ve görüntülemeyi birleştiren teranostik yaklaşımı kullanılarak, DEMUX mantık kapısı olarak davranan fotoduyarlaştırıcının, organik çözücünde singlet oksijen üretimi ve ışıması, asit adres girdisi ile kontrol edilmiştir. Bir diğer projede, sınırlı bir mikroalanda, birbirinden bağımsız mantık kapıları arasında bilgi iletişimi sağlanmış, pH ile aktifleşen fotodinamik ajanının aktivitesi, diğer bir mantık kapısı aracılığıyla emisyon artışı şeklinde takip edilebilmiştir. Bağımsız olarak, BODIPY ve triazolofen temelli, seçici bir seri floresan klorür anyonu algılayıcısı sentezlenmiş ve şaşırtıcı derecede geniş bir derişim aralığında bu anyonun algılandığı gözlenmiştir.

Anahtar kelimeler: Fotodinamik terapi, mantık kapısı, aktifleşebilir fotoduyarlaştırıcı, Cl^- algılayıcısı

Dedicated to my parents...

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LIST OF ABBREVIATIONS

BODIPY:	Boradiazaindacene
DEMUX:	Demultiplexer
DMF:	Dimethylformamide
DMSO:	Dimethylsulfoxide
DPBF:	1,3-Diphenylisobenzofuran
EET:	Electronic Energy Transfer
EtOH:	Ethanol
FL:	Fluorophore
FRET:	Förster-Type Energy Transfer
GSH:	Glutathione (reduced)
GSSG:	Glutathione (oxidized)
HOMO:	Highest Occupied Molecular Orbital
HRMS:	High Resolution Mass Spectrometry
ICT:	Intramolecular Charge Transfer
LUMO:	Lowest Unoccupied Molecular Orbital
MeOH:	Methanol
MUX:	Multiplexer
NMR:	Nuclear Magnetic Resonance
PDT:	photodynamic Therapy
PET:	Photoinduced Electron Transfer
PS:	Photosensitizer
TBACl:	Tetrabutylammonium Chloride
TFA:	Trifluoroacetic Acid
THF:	Tetrahydrofuran
TLC:	Thin Layer Chromatography

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CHAPTER 1

1. INTRODUCTION

This thesis presents four different projects related to use of chemical logic gates to allow mutually exclusive outcome of imaging and therapy in a unimolecular theranostic device (chapter 3), to develop dual-parameter activatable photodynamic therapy agent (chapter 4), to obtain functionally concatenated gate of bimolecular activatable photosensitizer embedded into a micelle with an activity reporting probe (chapter 6). In a separate context, this thesis also includes the synthesis and detailed photophysical analysis of BODIPY-based fluorescent sensors bearing triazolophane receptor for selective detection of chloride anion (chapter 5).

Chemical logic gates, first introduced by de Silva *et al.*¹ are primitive mimics of their silicon-based counterparts and can perform simple logic operations. The operation of these molecular devices is in response to a predetermined chemical or photonic input that enables a detectable output. Like digital electronics, thresholds are determined to structure the character of the output such as emission intensity. Since the device is molecular, the idea is promising considering the space limits of traditional Boolean computation. Indeed, certain semi-sophisticated processes have been conducted using chemical logic gates such as flip/flop memory devices² which are the basic element of random-access memory (RAM) in modern technology, key-pad-lock³ systems that produce an output only in the presence of certain order of inputs like in the case of a keypad of an ATM machine, and with the use of oligonucleotides, molecules are even designed to play games like Tic-Tac-Toe⁴. In this thesis, logic gates are used for different biomedical applications in different functional framework (chapters 3, 4, 6).

Conventional diagnosis techniques for life threatening diseases such as cancer may require painful or uncomfortable operations such as biopsy, radio imaging, magnetic resonance imaging etc. Yet, the dose and effectiveness of the therapeutic agents

cannot be adjusted in accord with the stage and advance of the malignancy in different regions of the tissue. A number of different studies associated with selective accumulation or activation of the drugs in tissue of interest were reported. Most of them depend on the disease specific markers, such as over-expressed receptors,^{5,6} symptomatic metabolites,⁷ enzymes,^{8,9} pH,¹⁰ reducing power¹¹ and oligonucleotide hybridization.¹² However, only a few have the imaging ability together with a remedy¹³ and none have reversible, interdependent, coupled therapeutic action with the diagnostic moieties.

Here in this thesis, a smart theranostic system is proposed in which the disease state is monitored and corresponding information is used *in situ* for therapeutic action. To create a theranostic agent, DEMUX logic gate design is used and a pH-induced switch in the direction of excitation energy transfer between two chromophores is combined with the concept of theranostics. Photodynamic singlet oxygen generation and fluorescence emission was only mutually observed and the switch between the two modes of operation is reversibly controlled by pH, acidic pH being a well-known tumor symptom.¹⁴ The idea presented in chapter 3, is a proof of principle of molecular theranostics, unifying diagnosis with therapeutic activity.

Logic gates are potential molecular devices for use as smart therapeutic agents. AND and OR gates with therapeutic activity is previously reported where the dual activity of both inputs are required for the therapy action in the former and activity of any of the two inputs are enough for the latter.^{15,16,17} To improve the biological relevancy of logic gate inputs, an extensive optimization process and pragmatical design is done to control the activity of the photosensitizer in second project presented in this thesis (chapter 4). Inputs are chosen as low pH and glutathione, two common parameters observed in most of the tumors. Compound is designed to show response to these parameters with the help of protonation induced spectral shift and reduction of the disulfide bond by glutathione to release energy acceptor moiety. Highly promising results are obtained such that compound displays a more singlet oxygen generation efficiency in the presence of both of these inputs.

The following step in molecular logic gate subject is to combine multiple simple logic gates to create a functional signal relay cascade. Attempts to integrate multiple gates are successfully performed in literature.^{18,19,20} However, functional assignment for such logic gates is still to be improved. In chapter 6, a research on the functional concatenation of simple logic gate is proposed where a pH-activatable photosensitizer acting as an AND gate is integrated, with the produced singlet oxygen, to a singlet oxygen reporter. The latter reporter act as either an AND gate with the use of light and singlet oxygen as inputs or INHIBIT gate with inputs singlet oxygen and glutathione. The controlled activity of the first photosensitizer is monitored with a concatenated manner by the second and a functional relay of the signal between two logic gates is successfully observed.

The era of molecular sensor is highly rich in binding motifs selective for certain analyte and there are a number of different anion and cation sensors. Among them, triazolophanes²¹ are also promising receptor for halides showing a size-dependent selectivity for chloride. Since it is not usual for a receptor to differentiate between halides with such selectivity, a fluorescent sensor with this binding module seems encouraging. With this motivation, in the research introduced in chapter 5, a BODIPY-based fluorescent chloride sensor is developed. With the two modes of response, binding to the receptor and basicity/redox property of the halide provide surprisingly large dynamic range of response. With the aid of control compound lacking receptor module, the contribution of other pathways involved in sensing is analyzed.

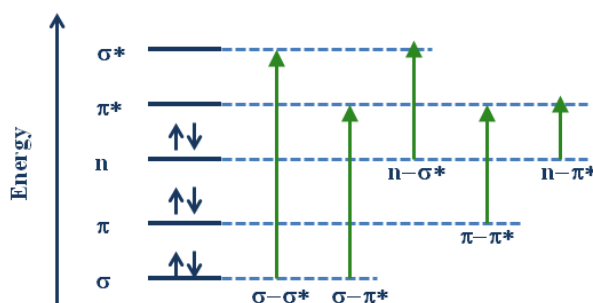
In summary, this thesis consists of functional improvement of molecular logic gates for biological applications such as development of a theranostic agent, dual-activatable photosensitizers and self-activity reporting activatable photosensitizers. In addition, the first fluorescent sensor developed with triazolophane-BODIPY conjugate with a highly promising binding behaviour in organic solvent is introduced.

CHAPTER 2

BACKGROUND INFORMATION

2.1 Photophysics of Light Absorption by an Organic Molecule

Excitation of an organic molecule by means of light involves transition of an electron from the orbital in the ground state to an unoccupied orbital with higher energy called excited state. Electronic transition within the molecule can take place between different molecular orbitals as far as the quantum selection rules are obeyed. The two major selection rules are the ones related to orbital symmetry and the spin multiplicity. Scheme 1 shows the possible transitions in an organic compound containing heteroatom in its structure. Transitions between the orbitals $n\text{-}\pi^*$ and $\pi\text{-}\pi^*$ require less energy. Transitions involving non-bonding orbitals usually result in a redistribution of electron density from heteroatom to the rest of the molecule and thus said to have a charge transfer character.²² The change in the dipole moment with respect to ground state as a result of charge transfer in certain molecules guides the design of chemosensors²³, logic gates²⁴ and some other smart organic materials.



Scheme 1. Relative energy levels of molecular orbitals in an organic molecule containing heteroatom and possible electronic transitions

The interaction of polarized light with the molecule during the absorption process is determined by the alignment of the transition moment of the molecule during the absorption process. Different transitions have different transition moment vectors on

an aromatic molecule and the parallel alignment of any of these moments with respect to polarization axis of the light increases the probability of the transition. Thus, any type of photon excitation would be expected to be related to the relative orientation of molecules and the polarization of light. Although this nature of transition is of great importance in confined molecular assemblies such as liquid crystals or membrane structures²⁵, most of the spectroscopic applications are in solution with randomly oriented molecules excited with non-polarized light.

Dependence of light absorption on the properties of absorbing species was first formulated by August Beer, Johann H. Lambert and Pierre Bouguer. Considering the intensity of incident light and the light that is transmitted, the law (Beer-Lambert law) states a correlation between the concentration, light path length and the absorption as shown below:

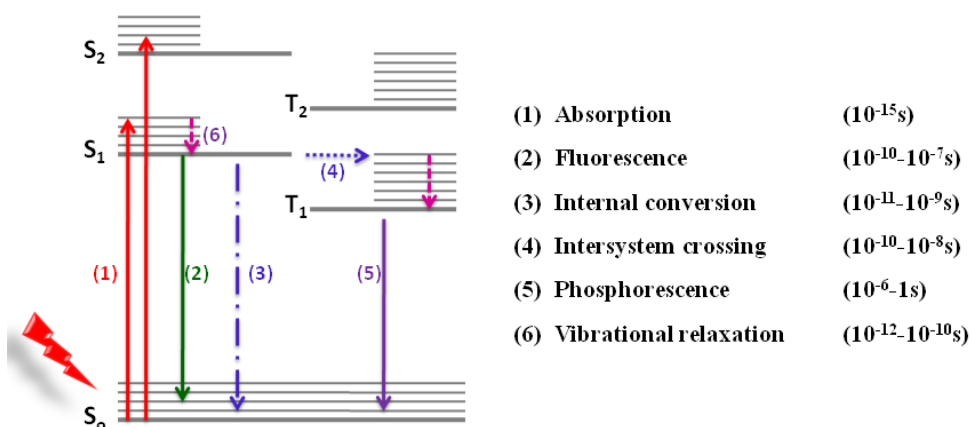
$$\log (I_0/I) = A_\lambda = \varepsilon(\lambda) C l \quad \text{Equation 1}$$

where I_0 and I represents the initial and transmitted light respectively, A_λ is the absorption of the molecule in a given wavelength (λ), ε is the molar absorption coefficient of the molecule in that wavelength, C is the concentration of the molecule and the l is the length of the absorbing medium through which light passes. Deviation from the ideal linear dependence appeared in higher concentrations either due to the aggregation of the absorbing molecules or emergence of new absorbing species such as excimers.²²

2.2 Providence of an Excited Molecule

Upon excitation by a photon, a molecule can go through a number of diverse physical pathways depending on the nature of its electronic, vibrational or rotational characteristics or the properties of media it is in. All of the possible well-defined pathways are illustrated in a Jablonski diagram²⁶ shown in Scheme 2. The initial essential process is the excitation and the transition would be to higher vibrational energy levels of the excited state (route 1). This process is exceedingly fast (10^{-15} s) and usually the reorientation of the nucleus during the process is not expected to

occur. Vibrational relaxation to the ground state vibrational level of excited electronic state (route 6) results in a subsequent lower energy emission profile which is called Stoke's shift.²² The extent of this spectral shift is very important for some applications including luminescent solar concentrators in which larger Stoke's shifts are demanded to decrease the self-absorption of the chromophore.²⁷ The lifetime of singlet excited state differs a lot but most of the chromophores have lifetimes around 0.1-100 ns.²² This lifetime depends on the relative fastness and the dominance of other deactivating pathways such as internal conversion (route 3) or intersystem crossing (route 4). Energy or electron/proton transfer routes to the molecules in close proximity, excimer or exciplex formation, interactions with the solvent or other molecules also change the excited state lifetime of a molecule.



Scheme 2. Possible photophysical processes taking place after the excitation by photon and their time scales, the Jablonski diagram

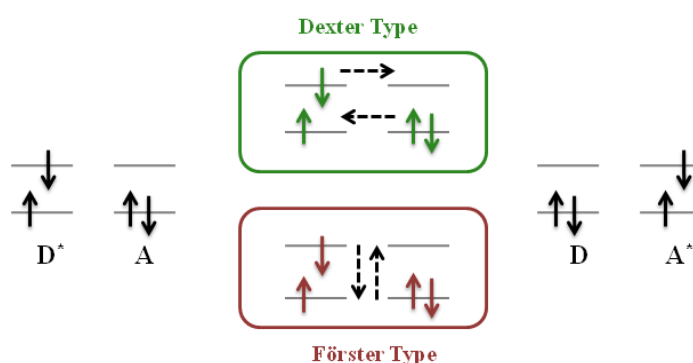
Amongst the deactivation pathways, perhaps the intersystem crossing is of great importance especially for some related applications such as photodynamic therapy. Selection rules forbids the transition between states of different multiplicity. However, presence of heavy atom creates a magnetic torque by the nucleus on the electron and mixes the spin states to some extent through spin-orbit coupling and allows such transitions.²² Molecules whose S_0 - S_1 transition is $n-\pi^*$ type, have more efficient intersystem crossing. Apart from heavy atoms, some molecules such as fullerene are shown to have this effect.²⁸ Identical molecules attached to one another in a perpendicular orientation and thus having close excited singlet states tend to

facilitate this transition as well.²⁹ The fate of a triplet state in an aerated solution is usually the quenching by molecular oxygen. Phosphorescence or delayed fluorescence is the emission from this state and usually can be observed at low temperatures in degassed solutions. Triplet-triplet annihilation is one important subsequent process of triplet state transition. In this phenomenon two molecules in triplet state collide and contribute their energy to the excitation of one of them or another molecule to singlet excited state. Since the energy given initially to excite the molecule is lower than the triplet-triplet annihilation induced excitation energy, this photophysical track is used as an energy upconverting method.³⁰ The intersystem crossing phenomenon, succeeding interactions with ground state oxygen and their importance in photodynamic therapy will be explained in details in following sections.

2.3 Energy and Electron Transfer Processes

An excited molecule can transfer its energy to another molecule in close vicinity or the electron transfer between two different species can take place. While the former requires spectral overlap between the emission of the donor and absorbance of the acceptor moiety, redox potentials and relative alignment of energy levels of the two species are important for the latter.

2.3.1 Electronic Energy Transfer



Scheme 3. Types of energy transfer

Energy transfer is the initial process in photosynthesis where perfectly arranged chromophores within a protein scaffold absorb and transfer energy through the

reaction center where series of redox reactions take place.³¹ Inspired by the beautiful example of nature, scientists develop energy transfer systems for a range of purposes such as for determining biochemical interactions³², for solar cell applications²⁷, for regulation of activity of photosensitiser⁸. Förster and Dexter extensively explained the nature of the process and formulate it.^{33,34} There are basically two different types of energy transfer mechanisms Förster type resonance energy transfer or electronic energy transfer (EET, FRET) and Dexter type energy or electron transfer as shown in Scheme 3.

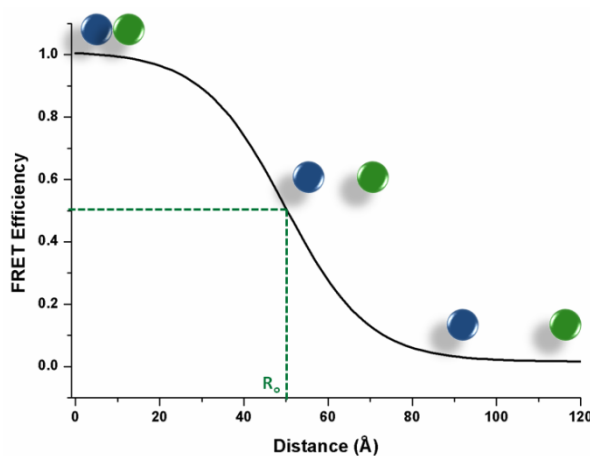


Figure 1. Distance dependency of FRET efficiency

Förster type energy transfer is essentially a dipole interaction between one excited and one ground state chromophore. For the process to take place two moieties should be close enough (usually less than 10 nm, Figure 1) and the spectral overlap between the donor emission and acceptor absorbance is required for efficient process. The dependency of distance and overlap integral is given in equation 2 and 3 below:

$$E_{\text{FRET}} = [1 + (R/R_0)^6]^{-1} \quad \text{Equation 2}$$

$$R_0^6 = [9 Q_0 (\ln 10) \kappa^2 J] / [128 \pi^5 n^4 N_A] \quad \text{Equation 3}$$

where, R_0 is Förster radius and defined as the distance between donor and acceptor moieties that enables 50% energy transfer efficiency. R is the separation between FRET modules, Q_0 is the fluorescence quantum yield of free FRET donor, κ is the

orientation factor of the dipole, J is the overlap integral, N_A is Avagadro's number, n is the refractive index of the medium. As it is clearly seen from the formula, increase in spectral overlap enhances FRET efficiency and the efficiency decreases dramatically depending on the 6th power of distance between donor and acceptor modules. This reliance is also evidently revealed in Figure 1, in essence no FRET is observed beyond 10 nm.

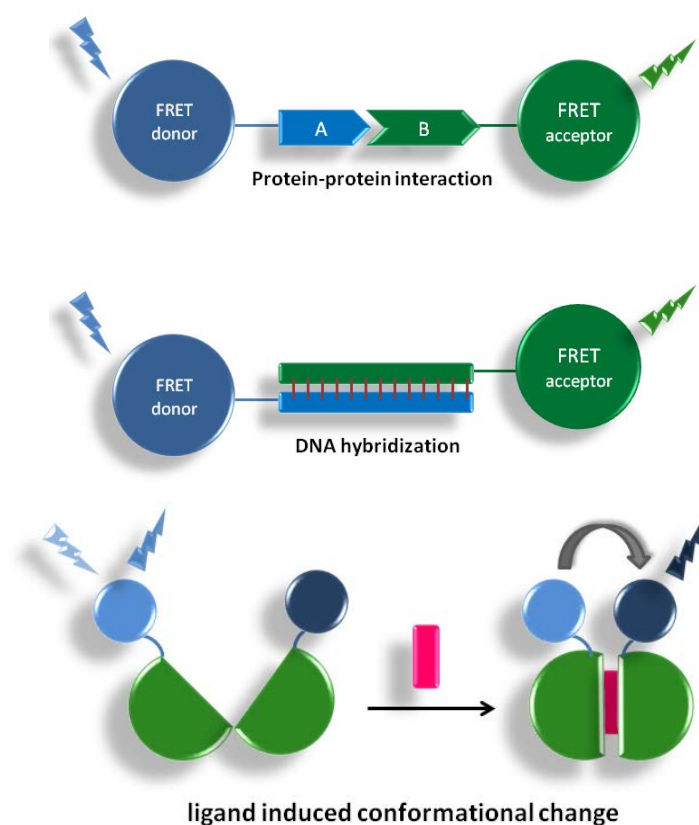


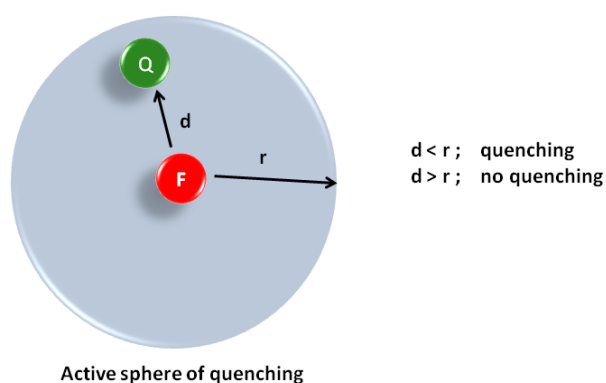
Figure 2. Use of FRET for biological applications

This property of EET is used widely to determine conformation of biological macromolecules such as proteins, DNA/RNA hybridization assays, to determine protein-protein, protein-DNA interactions, and as a biological metric system for differentially labeled regions or compartments, Figure 2.³² In order to determine the interaction partners of a protein in a downstream signaling pathway of a biochemical process or disease,³⁵ protein of interest is labeled and screened against tagged protein pool. The same analogy applies to DNA/DNA, DNA/RNA base pairing within or between molecular species to monitor the disease state, for diagnosis or gene

expression analysis etc. Since the conformational status of most of the biological molecules is closely related to their functionality, the information on their folding state is highly important. Stimulus or ligand induced change in the conformation in a receptor can also be scrutinized using the FRET method.³⁶

Dexter energy transfer requires a shorter distance between donor and acceptor due to the fact that an orbital overlap is required for the route.³⁴ Hence, the maximum distance required for the process is 1 nm which is 10 times less than FRET. In this process, electron exchange between the two modules is essential. This phenomenon is usually much faster and more efficient compared to FRET and usually occurs within conjugated systems.

2.3.2 Quenchers, Quenching Mechanism and Applications



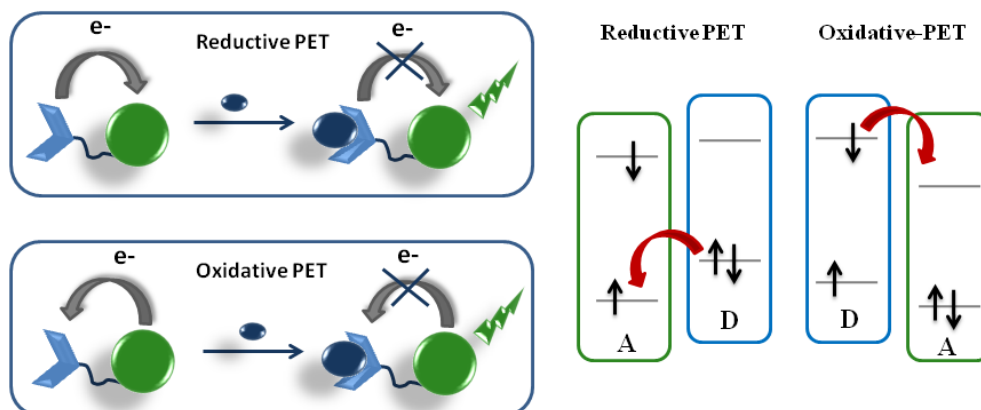
Scheme 4. The dependency of quenching on distance, F represents FRET donor and Q represents quencher.

For most applications, FRET is used between two fluorescent molecules and the change in the wavelength of emission is monitored as an output signal. However, sometimes, use of non-fluorescent molecules as an energy acceptor is required. In this case, fluorescence or activity of a molecule or sensitizer is controlled by a FRET to quencher in the vicinity which is usually called molecular beacon.⁸ A number of quenchers are present in literature and some are commercially available. The basic mechanism of quenching is described in Scheme 4, where the energy transfer to the beacon within the active sphere is followed by radiationless dissipation of energy. Most of the molecular beacons shows dynamic quenching where the absorption of

the species does not change but the excitation energy is lost through non-radiationless processes.²² In the other form of quenching called static quenching, physical interaction with the quencher is required to form a non-fluorescent complex. Since the time scale of energy transfer is much shorter than both fluorescence and triplet intersystem crossing lifetimes, presence of a beacon as an energy acceptor competes well with the fluorescence outcome or singlet oxygen generation process.^{8,22} Once the quencher diffuses out of the active sphere either followed by chemical dissociation or change in microenvironment, quenching efficiency disappears. The control of the dissociation or physical separation may depend on the activity of an enzyme that cleaves the linker between the two FRET modules or the change in the environment such as the polarity that physically partitions the modules apart.

Molecular beacons or quencher-based systems with fluorescent compounds are widely used as chemosensors. Among them, control of photodynamic activity is intriguing in view of the fact that it enables the creation of activatable photosensitizers which will be examined in following sections in detail.

2.3.3 Photoinduced Electron Transfer (PET)

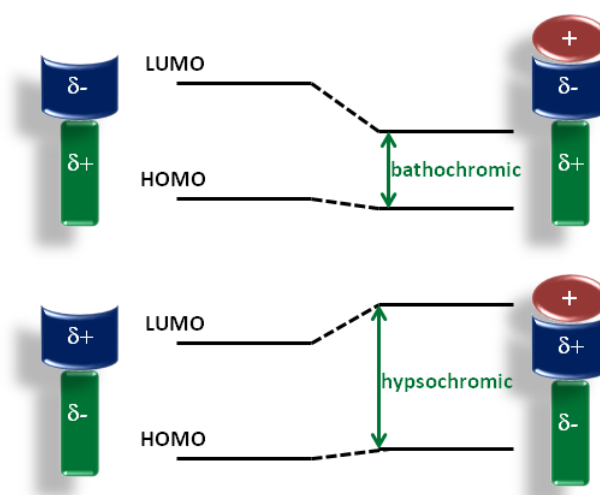


Scheme 5. Mechanism of photoinduced electron transfer and its use in chemosensor

Photoinduced electron transfer (PET) is an excited state phenomenon where an electron from the highest occupied molecular orbital (HOMO) of the nearby moiety is transferred to the HOMO of the fluorophore followed by the excitation of the

fluorophore, Scheme 5. In the case of oxidative PET (or reverse PET) electron excited to lowest unoccupied molecular orbital (LUMO) of the molecule is transferred to a closely spaced moiety having relatively lower LUMO level. For the two processes to take place, energy levels should be compatible to allow this redox processes to take place. Since both of these electron transfer processes competes with fluorescence the compound is not emissive. Any interaction that change the levels of either fluorophore or electron donor/acceptor part in such a way that the process is no more favourable, fluorescence quantum yield tends to increase. With appropriate design of the receptor or fluorophore, analyte responsive fluorescence control can be achieved and chemosensors can be constructed accordingly.²³

2.3.4 Photoinduced Intramolecular Charge Transfer (ICT)



Scheme 6. ICT process in cation sensing

Molecules possessing electron donating and/or electron withdrawing groups in their structure tend to show an increased dipole moment upon excitation.²² The redistribution of the charge is established by the reorganization with respect to solvent to attain thermodynamic equilibrium. These type of compounds show solvent and polarity dependent fluorescent properties and when rationally designed, interaction with the analyte can be used to control the ICT character of the molecule. A spectral shift in absorbance/emission upon binding to analyte also enables ratiometric sensing (Scheme 6). Depending on the electronic character of the

receptor and analyte, ligand can induce a hypsochromic or bathochromic spectral shift in electronic absorption and/or fluorescence spectra.

In Figure 3, two pH-responsive BODIPY fluorophores with a strong charge transfer property is shown. BODIPYs have electron-donating 4-(dimethylamino) phenyl and electron withdrawing pyridine groups in their structures. While protonation induces a hypsochromic shift in the first one, it results in a bathochromic shift in the second.³⁷ This distinct characteristic shifts in electronic absorptions of these two BODIPYs are used in the first project presented in this thesis to construct a molecular demultiplexer (DEMUX) logic operation.

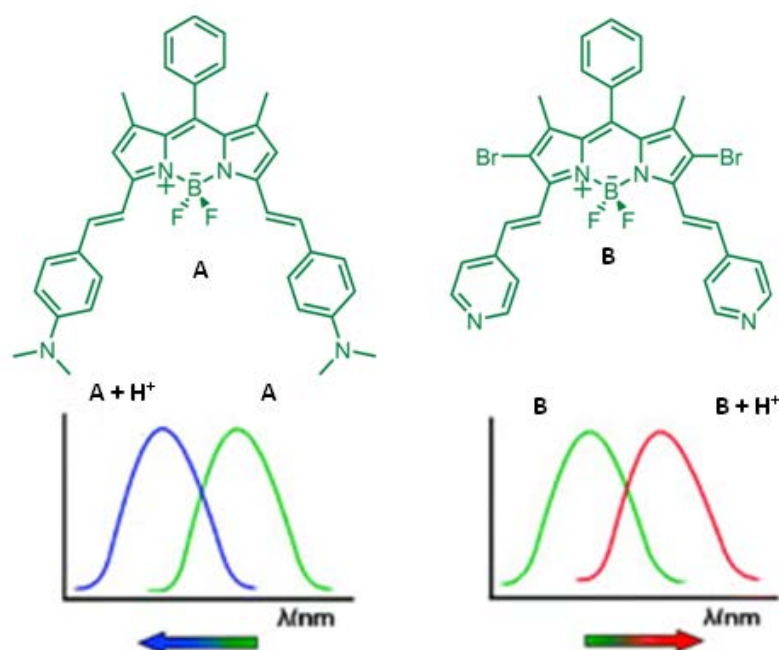


Figure 3. pH responsive BODIPY dyes with strong ICT character.

2.4 Fluorescent Chemosensors

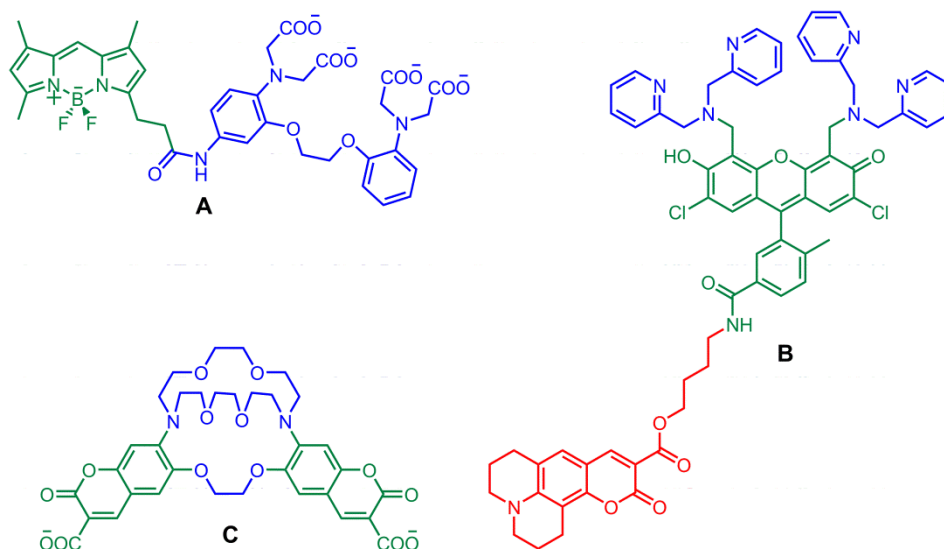
In many cases, detection of concentrations of certain analyte is highly important. To detect the toxic inorganic compounds in the environment, drinking water is critical. Determining the concentration fluctuations of inorganic ions and biomolecules in certain biological processes gives highly valuable scientific information and helps to understand the molecular biology of the process. The recognition of analytes also used for diagnostic purposes. Roles of ion transport across membrane in neuron excitation and muscle contraction, calcium release in fertilization, chloride

channelopathies in cystic fibrosis, dependence of ATP synthesis on proton gradient are all understood with the aid of molecular sensors.

When an appropriate binding pocket is attached to the structure of the fluorescent molecule reasonably, an interaction-induced observable change in fluorescence or electrochemical property can be obtained. The resultant response can be a change in intensity of emitted light (turn on/off sensor) or a shift in the emission wavelength (ratiometric). The mechanism of the first case is usually PET while it is ICT in the second. A great number of sensors have been developed and used for the detection of cations, anions, biomolecules.²³ Some of them are exemplified in following sections.

2.4.1 Cation sensors

Cations are important functional components of biology. They have important roles in neural activities, cell homeostasis, enzyme activity and a number of diseases are related to deficiencies in regulation of their concentration in the body. To detect these important analytes, a number of highly important chemosensors are developed. Some important examples with ICT, PET and FRET character are given in Scheme 7. BAPTA (1,2-bis (o-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid) motif in the receptor part of compound **A** (Scheme 7) is widely used in literature as Ca^{2+} binding module.³⁸ The BODIPY fluorophore is internalized into the cell in ester form, hydrolyzed within the cell and display increase in fluorescence intensity upon Ca^{2+} binding. Dipicolyl amine moiety is a Zn^{2+} chelator group and binding of this cation blocks the reductive photoinduced electron transfer from amine group to fluorescein in compound **B**.³⁹ Whence the PET is no more effective, fluorescein's green emission is observed. The coumarin attached through ester bond is hydrolyzed within the cell and this part is released consequently. The comparison of the blue emission of coumarin dye and green fluorescein emission enables real-time ratiometric sensing of the cation within the cell. This approach decreases the error associated with bleaching of the molecule or inhomogeneity in concentration of the fluorophore. Compound **C** is a K^+ sensor developed to determine extracellular concentration of this cation.⁴⁰



Scheme 7. Three of the Ca^{2+} (A), Zn^{2+} (B) and K^{+} (C) fluorescent chemosensors in literature

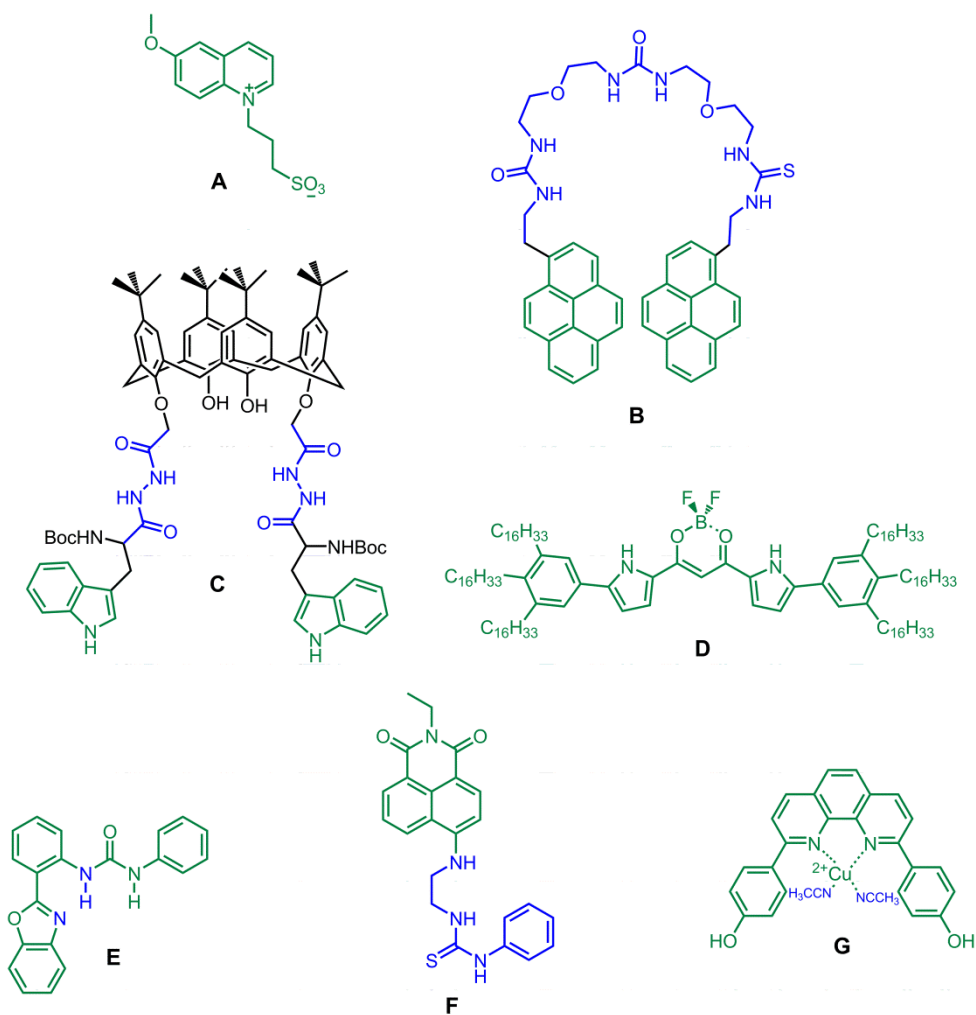
2.4.2 Anion Receptors and Sensors

Anions are essential components of biology as do cations. Phosphates are fundamental part of energy metabolism, they are used to modify proteins hence control their function through modulating the interaction with other biomolecules. They also form the backbone of DNA and RNA polymers. Iodide is important for the synthesis of thyroid hormone. Citrate is an essential intermediate in Krebs cycle. Chloride regulates cell volume and pH, has roles in bone degradation and is associated with the disease cystic fibrosis.²¹ And for a number of anions, the biological roles are still to be determined. For that reason it is important to develop sensors to detect their level in biological media.

Although selective detection of cation in aqueous media is improved a lot and most of them are in use, anion sensing is still challenging. The large radius of most anions requires tricky binding pockets. Most of the receptors developed in literature use either species with opposite charge to take advantage of electrostatic interaction, ion-dipole interaction or strong hydrogen bond donors such as urea or pyrrole to enable stable coordination.⁴¹ There are very few examples of fluorescent chloride sensors in literature and most of them are turn-off type. Some of them are given in Scheme 8. The halide response of 6-methoxy-N-(3-sulfopropyl) quinolinium (SPQ) is first analyzed by Wolfbeis and Urbano in 1983 and derivatives of this compound are

commercially available now for chloride sensing.⁴² The turn off response of this and similar dyes are explained by collisional quenching facilitated by reduction of the species.⁴³ Although the response is turn-off, the detection range of these dyes for halides is quite high and spans beyond 100 mM.⁴⁴

When two pyrene units come close enough the excited state interaction between the molecules produce a new lower energy fluorescence called excimer (excited dimer) emission.⁴⁵ This property of pyrene is used to detect fluoride, acetate, bisulfate and dihydrogen phosphate among others, (Scheme 8, compound **B**).⁴⁶ Upon binding of these anions to the thiourea groups the molecule folds to bring pyrene units closer in dimethylsulfoxide solution. The excimer emission of pyrene increases as a result. Although the selectivity for a certain anion is poor, this molecule provides a ratiometric sensing.



Scheme 8. Examples of fluorescent anion sensors

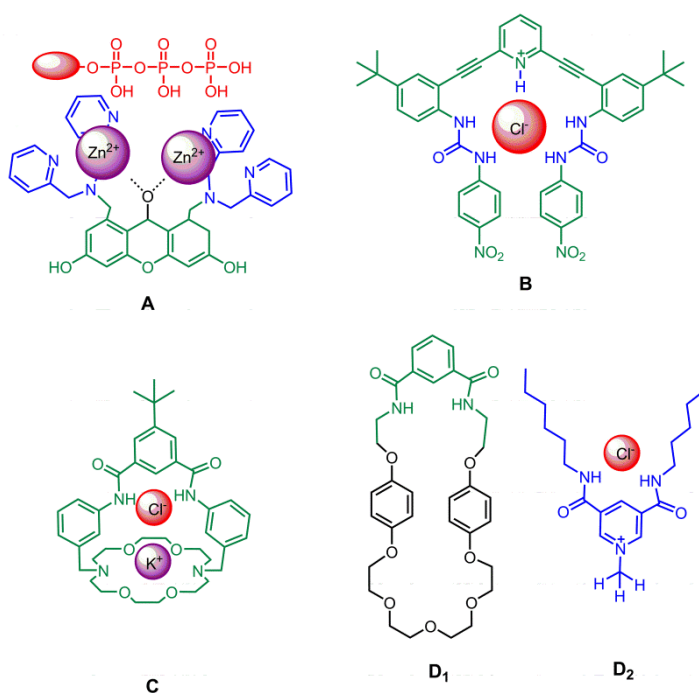
Compound **C** shown in Scheme 8, has chiral binding pocket and can discriminate between D-mandelate from the L-enantiomer. Quenching of the emission is more pronounced in the first one.⁴⁷ Compound **D** is a BF₂-bridged dipyrrolyldiketone and chloride anion interferes with the sol-gel transition temperature of this compound. Anion-induced decomposition of the gel structure at RT results in a bright orange fluorescence.⁴⁸ Compound **E** exhibits excited state intramolecular proton transfer (ESIPT) phenomenon which is transfer of proton from one group to another at the excited state due to change in acidity upon excitation.^{49,50} Fluoride abstracts the proton and prevents tautomer emission. The resultant emission is at 495 nm. Lower basicity of other anions can not manage to deprotonate the urea NH thus tautomer emission survives. Finally, emission of compound **F** is highly quenched upon addition of fluoride which is attributed to PET from deprotonated urea to naphthalimide fluorophore.⁵¹

In compound **G** in Scheme 8, authors take advantage of the ligand exchange of Cu (II) phenanthroline complex. Whence acetonitrile is coordinated to Cu (II), the emission is quenched. Change of the ligand with the chloride anion regenerates the emission at 402 nm.⁵²

2.4.2.1 Use of Co-ligand; Ion Pair Receptors

Sometimes, presence of other ions may facilitate the recognition event or transport of ions across lipid membrane.⁵³ The co-ligand is usually chosen to have opposite charge to benefit from electrostatic interactions. Scheme 9 depicts some important examples selected in literature. Since the strong Zn²⁺ binding of dipicolyl amines and tough phosphate - Zn²⁺ interaction are known, combination of both can be used to enable nucleoside polyphosphate⁵⁴ sensing. The fluorescence of xanthene is re-established upon binding induced aromatization with the aid of Zn²⁺ bound water. Johnson and Haley *et al.* developed urea and ethynylpyridine binding motif to enable halide binding. The molecule **B** is non-emissive in neutral and slightly emissive in protonated form (TFA is used as organic acid).⁵⁵ Chloride binding to protonated compound results in an enhanced emission in chloroform. Crystallographic data supports the binding and protonation.

Compound **C** in Scheme 9 shows an enhanced binding of chloride in the presence of either sodium or potassium cation bound to crown ether moiety. Also the binding of these cations to a chloride-bound receptor is facilitated compared to free receptor.⁵⁶ This cooperative binding enables co-transportation of both ions across lipid membrane.⁵⁷ Lastly, in compound **D**, pyridinium in the chloride receptor of one unit interacts with crown ether moiety in the second to construct a more confined binding cavity for the anion.^{58,59} A pseudorotaxane is formed between the two units with the aid of chloride and pyridinium ions.



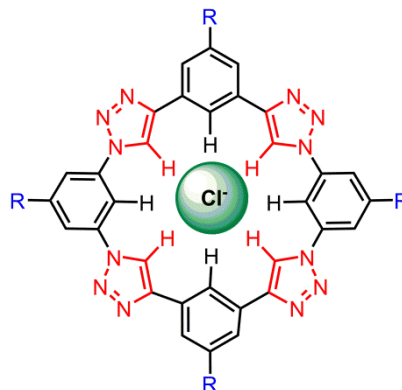
Scheme 9. Ion pair receptors

In the fluorescent chloride sensor project presented in this thesis, proton is used as a co-ligand within the chloride binding pocket with the intension of measuring chloride anion level in acidic media.

2.4.2.2 Triazolophanes as Chloride Receptor

Triazolophanes and related foldamer-like structures, novel binding form for halides, selectively for chloride are discovered independently by three different research groups (Scheme 10).^{60,61,62} Highly preorganized cavity of the receptor use the

unconventional hydrogen bonding between carbon-bound hydrogen and halide. The dipole (4.7D) on the triazole moiety polarizes the CH groups making them good hydrogen-donors. Overall dipole orientation of the molecule decreases the electron density within the cavity hence increasing the anion binding further.



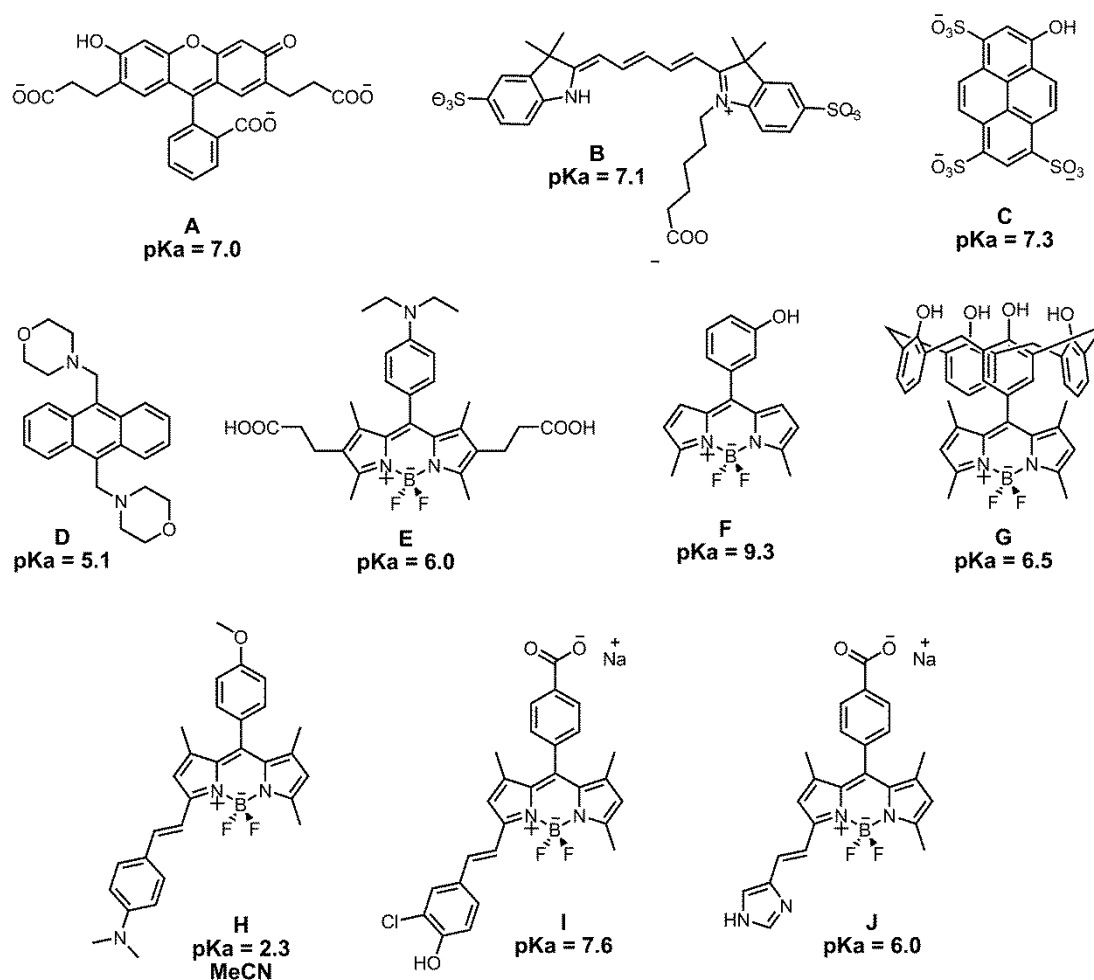
Scheme 10. Structure of triazolophane

The halide selectivity of triazolophane results from size of the cavity to which these anions perfectly fit. The size of the space is estimated to be 0.38 nm. The diameter of chloride and bromide anions are 0.36 and 0.39 nm respectively.²¹ Fluoride is too small and iodide is too large for this cavity. Among bromide and chloride, the stronger binding of chloride (10^7 M^{-1} in CH_2Cl_2) is attributed to its Lewis basicity.⁶³ In the fluorescent chloride sensor project documented in this thesis, triazolophane is used as chloride receptor to benefit from its size selectivity.

2.4.3 pH Indicators

Since this thesis includes a pH-activatable photosensitizer, pH responsive fluorophores are briefly reviewed in this section. In view of the fact that the pH inside and outside the cell, within organelles, in tissues is highly regulated and the change in pH during certain biochemical or physiological processes strictly determines the functionality, it is important to build up reliable sensors. The pH of the exterior of the cell is almost neutral (around 7.4) while within the intracellular organelles it drops down to 4.5 (lysosomes).⁶⁴ Some of the pH sensors with different pH response in literature are given in Scheme 11. Fluorescein derivative (compound A) has a pKa value of 7.0 and suitable for cytosolic pH measurements which usually

varies between 6.8 and 7.4.⁶⁵ Once the dye is internalized by the cell, leakage is fairly slow and the dye retains within the cytoplasm. However, the main drawback of this dye is its relatively fast photobleaching.⁶⁶ Compound B is a cyanine-based near IR pH indicator with a pKa value suitable for cytoplasmic pH measurement. The dye absorbs and emits at 645 and 665 nm respectively.⁶⁷ 8-hydroxypyrene-1,3,6-trisulphonic acid or pyranine (compound C) is a water soluble non-toxic dye with a unit fluorescence quantum yield with a pKa value of 7.3.^{68,69} The absorbance of the compound shifts from 405 to 465 nm upon deprotonation of the hydroxyl group. Cellular uptake of the compound is maintained by using sulfonic acid protection. With the aid of esterases, deprotection takes place within the cell and dye retains inside. For sensing acidic organelles, anthracene-based compound D is suitable with a pKa value of 5.1. In acidic organelles such as in lysosome, protonation of the amine group blocks the PET and an increase in emission intensity is observed.⁷⁰



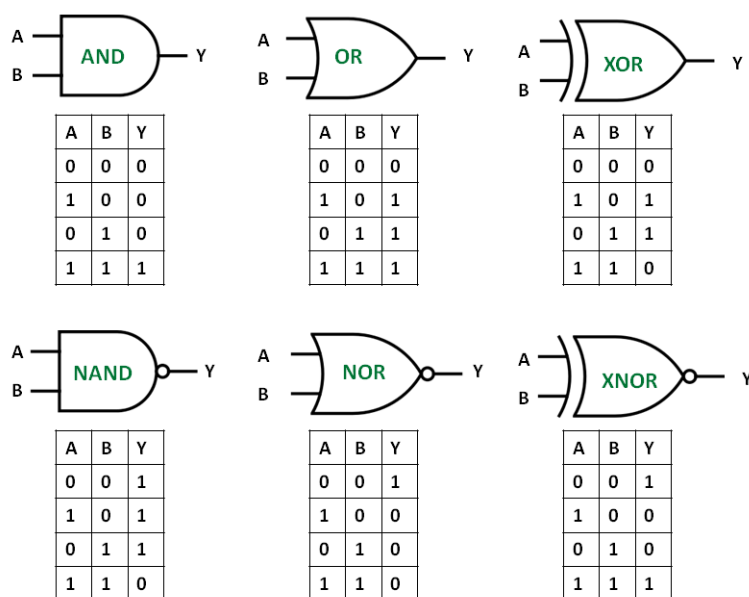
Scheme 11. Fluorescent pH sensors

Versatility of BODIPY dyes allows different functionalization. This helps the development of different pH indicators with different pH response range. Among them, a few are given in Scheme 11. Compound **E** is non-emissive in neutral solution while PET is hindered upon protonation of amine and emission is reinstated. Imaging of the viable cancer cells are accomplished with this dye.⁷¹ For alkaline solutions compound **F** with pKa 9.3 is developed.⁷² Calixarene-BODIPY conjugate compound **G** is developed by Akkaya *et al.* with a pKa value of 6.5 which is suitable for most of the biological applications.⁷³ Among styryl BODIPY dyes, dimethylaminophenyl (pKa = 2.3 in acetonitrile, compound **H**)⁷⁴, 4-hydroxy-3-chlorophenyl (pKa = 7.6, compound **I**),⁷⁵ and imidazole (pKa = 6.0, compound **J**)⁷⁶ derivatives are reported. Compound **H** shows a hypsochromic shift upon protonation and the absorbance and emission is solvent dependent due to strong ICT character of the dye. Compound **I** display a bathochromic shift upon deprotonation and quantum yield decreases a lot, protonation results in a small hypsochromic shift in emission of compound **J**.

2.5 Logic Gates

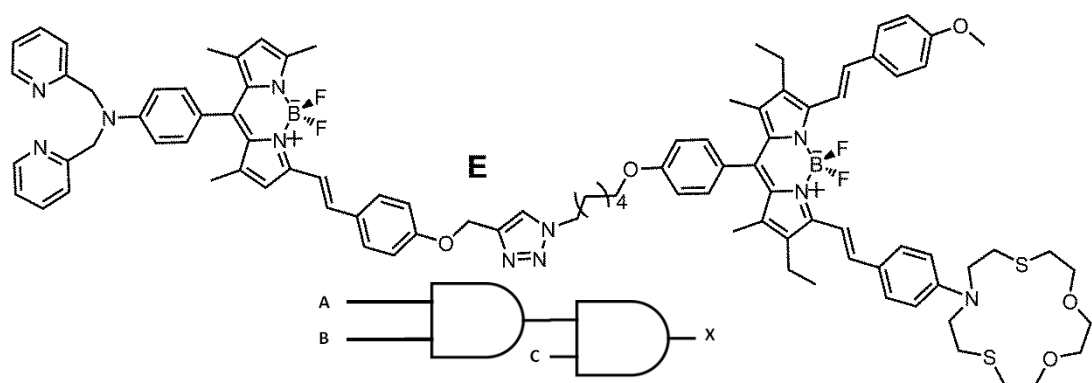
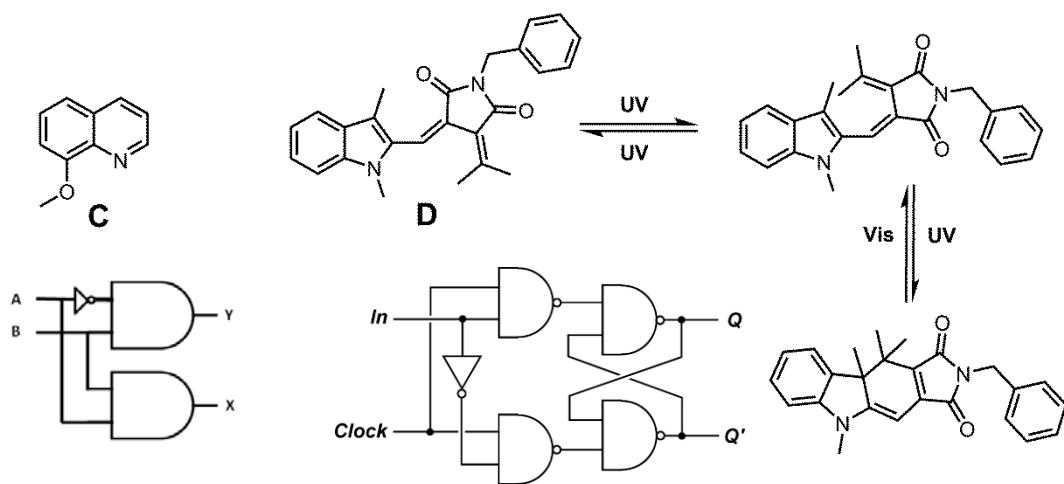
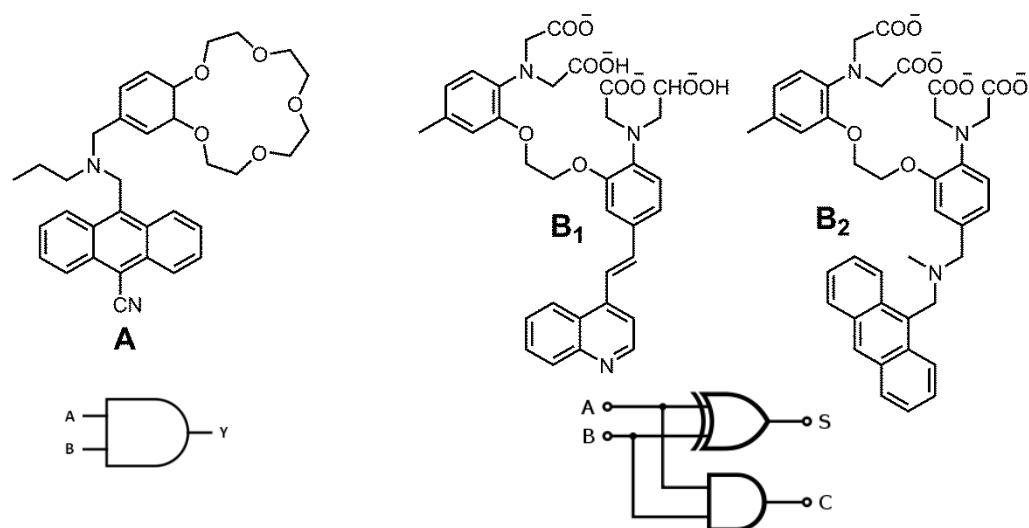
Physical operations that satisfy Boolean logic algebra with a suitable, readable input and output set are defined as logic gate. The computer science in the last century use the simplest logic operations, integrate them to build highly complex computational skills and electronic devices. In digital electronics in general, the input or output data are taken as discrete bands and a threshold level is defined to prevent noise and other unwanted small fluctuations.

This analogy is first used in chemical counterparts in 1993 by de Silva.¹ This is the first logic gate that works as an AND operation with chemical inputs and spectral outputs. Anthracene emission is restored only when effective PET from amine and crown ether moieties is blocked by protonation and sodium binding respectively. The truth table for AND and other basic logic operations are given in Scheme 12. From the first introduction of first chemical logic gate, a number of simple and more complex integrated logic operations have been developed. Some of the examples with quiet complex logic behaviour are shown in Scheme 13 together with the pioneering work of de Silva (compound **A**).



Scheme 12. Basic logic gates, schematic representations and truth tables

The first molecular calculator was developed by de Silva *et al.* and composed of two different molecules performing AND and XOR logic operations separately (compound B).⁷⁷ Molecules use same inputs Ca^{2+} and H^+ but AND gate is constructed by hindering of the PET pathway by the inputs while in XOR gate these inputs results in spectral shift of the anthracene transmittance in opposite direction. The output of the former logic is used as ‘carry’ for the addition algebra. Credi *et al.* used 8-methoxyquinoline as both a demultiplexer and multiplexer logic gate.⁷⁸ A multiplexer (MUX) is a data-selector device that selects one of the multiple inputs to an output. A demultiplexer (DEMUX) on the other hand, does the opposite and takes one input and selects between different outputs. The selection in both logic gates is done in the presence of another input called control or address input. Protonation of compound C shifts the absorbance and emission peak of the compound. Two wavelengths of excitation, each exciting protonated and neutral compound selectively are taken as MUX input and relative acid concentration as control input. Output is 474 nm emissions. Switch between the two excitation energy is chosen by the acid to obtain the output as emission. In the case of DEMUX logic gate, isobestic absorbance point (262 nm) is taken as input and acid enables a selection between the two emission states, protonated and neutral. Thus, with the use of a very simple compound a rather complex logic operation is obtained.

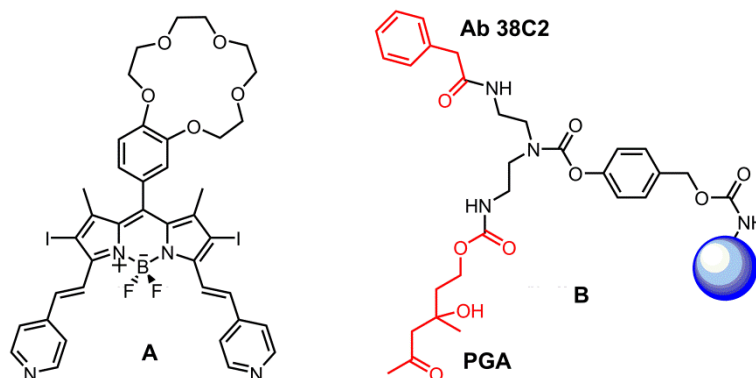


Scheme 13. Molecular logic gate examples

Photochromic compounds are widely used in logic gate era and make all-photonic systems possible.⁷⁹ In this type of information processing, the character of input and output is essentially light thus there is no input-output inhomogeneity unlike in the case of most chemical logic gates. Light induced reversible change in the shape and spectroscopic properties of the molecules provides manufacture of advance molecular logic operations. Andreasson and Pischel *et al.* developed the first molecular D-Flip Flop logic gate using fulgimide (compound **D**).² D-Flip Flop consists of a clock input that determines the output-input relation and builds a memory device. If this input is 1, the output takes the value of input. If not, then the previous output survives. Fulgimide is emissive in the closed form (640 nm) which is quantitatively generated upon 4 min UV-irradiation from E-form. E-form of the compound absorbs maximally at 374 nm while closed form absorbs at 523 nm. Two light source of 532 nm and 1064 nm were used as input, the former being the clock and emission at 644 nm is followed as output. If none of the inputs are present, the output remains the same due to lack of photochromism in the absence of light. When the clock is 1 but the other input is 0, then nonfluorescent E form dominates. As a result output always reads 0 (same as input). When both inputs are present (both lights are on), third harmonic generating crystals converts them to 355 nm light which has enough energy for the formation of emissive closed form. Thus, the input (value 1) is reproduced by the output (value 1). When only 1064 nm input is present, for the same reason, previous output reads as the next output. Hence, the past of the device has a role in its future. Taking advantage of photochromic character of the compounds and with rational combination of optical inputs, scientists are able to develop a highly complex logic operation.

Finally, Akkaya *et al.* showed that two logic gates can be concatenated to form a more complex integrated logic device.⁸⁰ Zn^{2+} and H^{2+} sensitive chromophores are clicked together to form an energy transfer donor-acceptor dimer (compound **E**). Zn^{2+} and light is used as an input to construct an AND gate for the first part of the molecule. Presence of Zn^{2+} blocks the PET process and enables emission. In the covalently bound molecules, the energy is an output through a FRET for the next molecule. Upon binding of Hg^{2+} to FRET acceptor, hypsochromic shift in absorption from 680 nm to 640 nm increases the spectral overlap and emission intensity at 660

nm increases. This approach of integrating multiple logic gates opens a path for developing complex functionalities out of chemical logic devices like their silicon counterparts.



Scheme 14. Logic gates for smart drug design

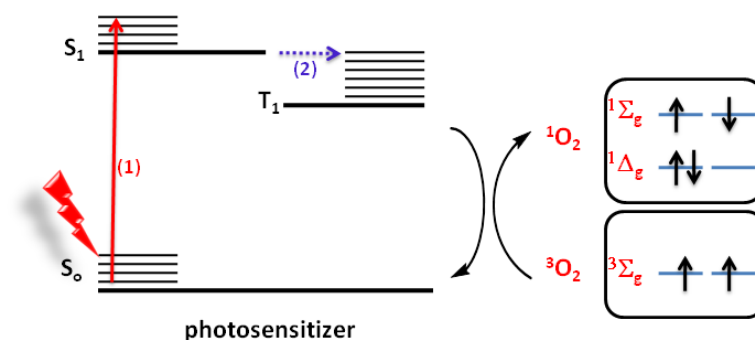
Although most of the molecular logic gates are introduced without significant functionality but a mimick of digital logic gates, some have important functional meaning. Upon them, two therapeutic approaches are exemplified in Scheme 14. In compound **A**, developed by Akkaya *et al.* is a BODIPY photosensitizer that is actively generates toxic $^1\text{O}_2$ in organic solvent only in the presence of tumor related inputs acid and Na^+ .¹⁵ Basically, binding of Na^+ to the crown ether blocks PET and protonation shifts the absorbance to excitation wavelength. Hence an AND logic is generated. Compound **B** is introduced by Shabat *et al.* and mutual enzymatic activity of two different biomolecules (catalytic antibody Ab38C2 and penicillin G amidase PGA) at two different sites generates an amine nucleophile which attacks the carbamate and liberates anti-cancer drug doxorubicin.¹⁷ Thus, an OR gate is built for smart drug release. Improvements of such kind would be a hope for intelligent drug design and release strategies.

2.6 Photodynamic Therapy

Photodynamic therapy (PDT) is a method clinically used for the remedy of certain cancers such as ocular melanoma, basal cell carcinoma, pancreas, lung, genitourinary cancers and diseases such as papillomas, rheumatoid arthritis and age-related macular degeneration.⁸¹ It basically requires generation of toxic singlet oxygen upon

excitation of a photosensitizer (PS) by light of appropriate energy. In the vicinity, reaction with the biomolecules and irreversible oxidative damage induces cell death. Short lifetime in aqueous solutions (< 40 ns) and short diffusion distance (< 2 nm) in accordance, makes the therapy non-invasive and promising.⁸²

2.6.1 Photophysics and Biochemistry of Photodynamic Therapy



Scheme 15. Jablonski diagram of $^1\text{O}_2$ generation

The photophysical pathways involved in $^1\text{O}_2$ generation is given in Scheme 15. Following the excitation of the molecule to singlet excited state, among other pathways transition to triplet state (intersystem crossing) is required. This is enhanced in the presence of heavy atoms or exciton coupling. Perpendicular orientation of bichromophoric molecules with respect to one another also helps this transition.²⁹ This step is required to enable a normally spin forbidden process, transition of ground state triplet oxygen to singlet excited state. Molecular oxygen indeed has two excited singlet states with quite different energies and orbital occupancies. Higher energy singlet state has shorter lifetime and relaxes to the lower singlet state. Phosphorescence from the longer-lived excited state can be followed at 1270 nm with an IR detector.

The singlet oxygen reacts readily with biomolecules within the cell. If the primary reactant is the excited PS, hydroxyl radicals, superoxide anion, hydrogen peroxide are formed afterwards; the reaction is defined as type I.⁸³ The generated radicalic compounds react further with other biomolecules or molecular oxygen to form $^1\text{O}_2$. In type II reaction, $^1\text{O}_2$ generated upon sensitization is the primary reactant. PDT response involves both types of reactions.⁸⁴ Oxidative damage given by $^1\text{O}_2$ is

basically lipid peroxidation, oxidation of thiol and amine groups, genotoxic damage such as DNA strand breaks and cross-linking which leads to mutation at the end.⁸⁵ Lipid peroxidations by $^1\text{O}_2$ influence the lipid fluidity and membrane leakage. Oxidation of the thiol and amines on amino acids, thus proteins, interfere with their proper folding hence functionality. One intriguing result of thiol oxidation is believed to be the conversion of mitochondrial membrane into a leaky, more permeable form allowing the passage of mitochondrial constituents which persuade cell death.⁸⁶ Each oxidative reaction of $^1\text{O}_2$ generates reactive products which amplify the initial effect leading to cell death decision. Thymine groups on DNA are the main targets of hydroxyl radicals produced by this cascade.⁸⁷ Since the proper functioning of the repair enzymes are eradicated, mutations accumulate to trigger apoptosis.⁸⁸

Biological response of PDT is related to oxidative damage and all lead to cell death (apoptosis or necrosis depending on the localization of the PS), shut down of tumor microvasculature and stimulation of the immune system.⁸⁹ The last response is quite unique to PDT and enables the development of new concepts such as cancer vaccine. Singlet oxygen mediated damage to membrane proteins and lipids disintegrate the membrane and phospholipids are liberated. Subsequent reactions of these lipid elements produce immune stimulatory molecules including leukotrienes.⁹⁰ In addition PDT-induced overexpression of HSP70 chaperone proteins attract neutrophils to initiate an immune response.⁹¹ The immune stimulating property of the therapy is used to develop a potential cancer vaccine. Promising regression of the tumor was observed upon administration of previously PDT-applied cell lysates to model animal mice.⁹²

2.6.2 Requirements for a Photosensitizer

Since a photosensitizer is prepared with the aim of a biomedical application, it should meet the criteria of biocompatibility, acceptable water solubility or stable conjugation with an appropriate carrier moiety such as micelle or polymers and finally absence of dark toxicity. Besides, the main requirement for photodynamic therapy is efficient singlet oxygen generation. Since this process requires the spin-

forbidden excitation of ground state triplet molecular oxygen to singlet excited state the molecule has to be modified to enhance this transition. Heavy atoms are known to facilitate this transition by the exertion of a magnetic torque by the nucleus of the heavy atom to spin momentum of the electron so-called spin-orbit coupling (SO coupling).

The heavy atom effect on tetraarylazadipyrrromethenes is studied extensively and results indicate that bromo-photosensitizer produce thousand-fold more singlet oxygen than heavy-atom free one.⁹³

Since the activity in photodynamic therapy is initiated by light irradiation, photosensitizer is expected to be stable against bleaching. Toxic effect of the PS should be only exerted under light irradiation, namely the compound should not have any dark-toxicity.

Finally, the energy of light used is also important for efficient PDT action. Since the organic compounds absorb characteristically at certain wavelengths, the energy of light source is chosen in view of that. However, due to the presence of absorbing species in the tissue such as melanin, flavins, and haemoglobins, the penetration depth of light through the body is highly limited to NIR-region specifically between 620-850 nm.^{94,95} This range is called therapeutic window of the body and photosensitizers should be chosen or functionalized to be efficiently excited with light of this wavelength.

Apart from playing with the absorption properties of the photosensitizer, there are other ways to overcome this problem. Among them, use of upconverting systems⁹⁶ or chemiluminescent coupled photosensitizations⁹⁷ worth attention. The former requires an upconverting system that activates a photosensitizer with shorter wavelength absorption by a lower energy light. Upconversion is enabled by a nonlinear anti-Stokes phenomenon created by the lanthanide lattice of the inorganic nanoparticles. In the latter case, energy of activation is provided by a chemiluminescence of a nearby oxalate ester.

2.6.3 **Activatable Photosensitizers**

Activation of photodynamic therapy agent only in the presence of light and oxygen makes the method already non-invasive without any side-effect reported. In addition, due to leaky vasculature of the tumor tissue, photosensitizers tend to accumulate selectively in the tumor tissue. This phenomenon is called enhanced permeation and retention (EPR) and is a kind of passive targeting. Even though the accumulation is more prominent in tumor tissue, nearby healthy cells are still affected from this therapy and further selectivity is required.

With the wish of more selective therapy, in combination with the EPR-dependent targeting, it is important to develop photosensitizers to be activated only in the presence of certain disease parameters. The deactivation of the PS in the absence of the disease microenvironment can be through a number of different mechanisms. The simplest form of controlling PDT action spatially is by means of targeting chemical/biochemical groups. With the improved knowledge in molecular biology and genetics of diseases, the genes and gene products associated with a syndrome can be determined. For tumor tissue, overexpression of surface receptors and transporters are well-established. Among them, folate receptor and glucose transporters are important targets. Hence, photosensitizers with 2-deoxyglucose and/or folic acid in their structures tend to accumulate selectively in tumor site.⁹⁸

Alongside the control on tissue accumulation, activity of the photosensitizer can be controlled as well. A PS can be controlled at any time of its photophysical journey depending on the character of the quenching. PS can be quenched before triplet state transition by means of singlet-singlet energy transfer or the quenching can occur through the self-quenching of the PS itself. Photoinduced electron transfer at excited state may be a way to deactivate the photosensitizer. After ISC, singlet oxygen scavengers can be used to decrease photodynamic action. Some important examples from literature involving activatable photosensitizers with different mechanisms of action are given in Figures 4-7 in following sections.

2.6.3.1 FRET-based Quenching of the PS

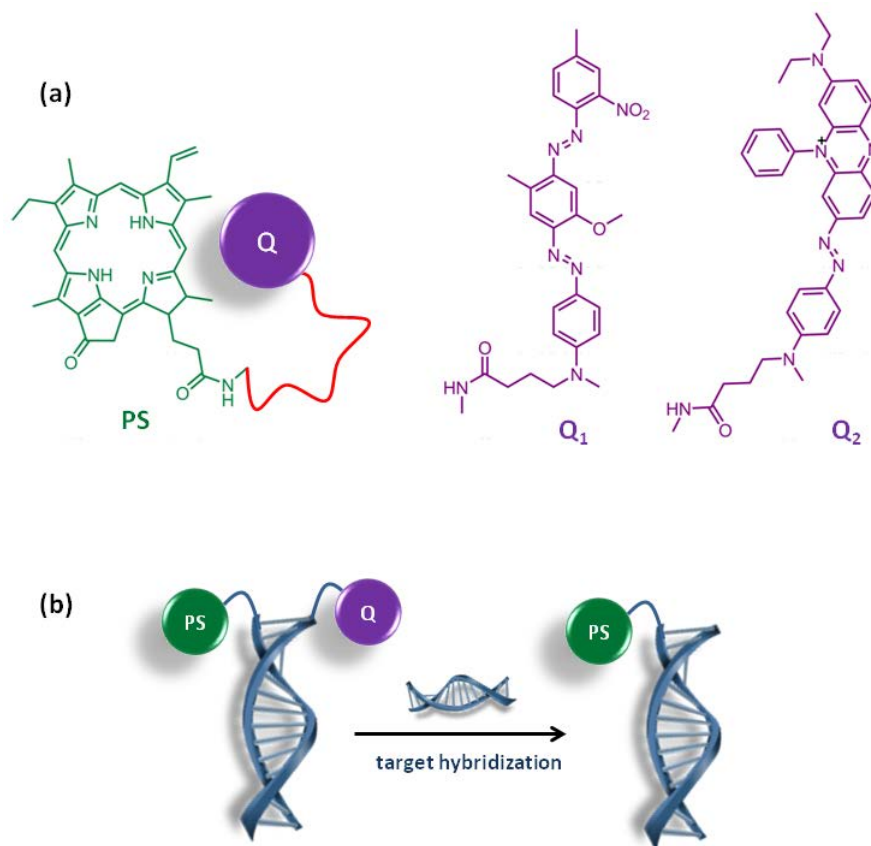


Figure 4. Energy transfer based quenching of $^1\text{O}_2$ production

Excited photosensitizers can transfer its energy to an adjacent molecule with appropriate spectral properties. If the requirements for a dynamic quenching phenomenon process are met such as spectral overlap between emission of energy donor and absorption of acceptor; and short-enough separation between the two interacting species (essentially < 10 nm); then, FRET efficiency do increases. Cleavage or dissociation of the quencher from the PS and diffusional drifting away from the active zone of quenching leads to activation of the PS. FRET acceptor are usually chosen as non-emissive and the energy is dissipated as heat at the end. There are a number of different nonemissive quenchers with different absorption properties in literature and some are commercially available. In Figure 4, examples involving commercially available black-hole quenchers BHQ-1 (Q₁) and BHQ-2 (Q₂) are shown. Pyropheophorbide-a is conjugated to this quenchers through lysine linker and the quenching behaviour of these quenchers with different linker length and spectral

overlap are analyzed.⁹⁹ Consistent with the FRET theory, shorter separation and greater spectral overlap facilitate the quenching process. The BHQ-3 is observed to be a better quencher. In a separate work, the linker is selected as a Matrix metalloproteinase 7 (MMP7) target.⁸ This protease is known to be overexpressed in tumor tissue and has important roles in extracellular matrix remodelling. Tumor metastasis is known to be associated with MMPs. It has been shown *in vitro* and in mouse animal models that the cleavage of the peptide linker with the enzyme releases the PS and activates it. The enzyme-dependent activity is proved also with the use of enzyme inhibitor. Similar construct with a linker which is substrate of tumor associated fibroblast activation protein is also reported with promising *in vivo* activity.¹⁰⁰

Enzymes are not the only activating disease parameters. Nucleic acids are also targeted for this purpose using the specific hybridization properties. Hybridization of the DNA strands tagged with PS and quenchers through base-pairing quenches the PS (Figure 4).¹⁰¹ Displacement of the strand with the target oligonucleotide liberates the PS and activity is restored

2.6.3.2 Deactivation Through Self-Quenching

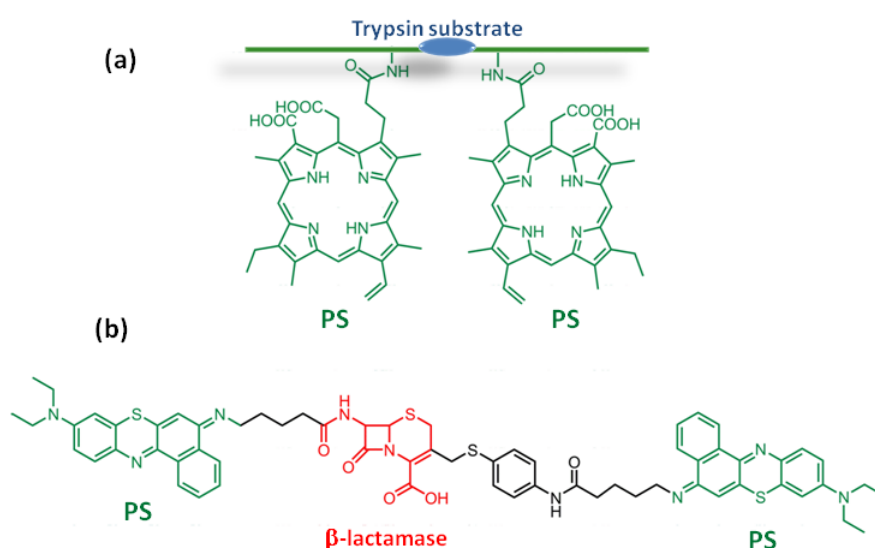


Figure 5. Deactivation of the PS through self-quenching

Ground state interactions between planar chromophores those are stacked in an aggregate usually produce a non-emissive or quenched product. This behaviour is usually followed with a change in electronic absorption spectra and called self-quenching. This phenomenon is also used in literature for controlled PS deactivation. Figure 5 shows two PSs quenched by this mechanism. Chorin-e₆ photosensitizer attached to one another via lysine linker on a polymer backbone displays decreased ¹O₂ generation efficiency due to high density within the polyethylene matrix. However, once the linker is cleaved by the metalloprotease cysteine cathepsin, the PS is activated.¹⁰² Likewise, self-quenching of two 5-ethylamino-9-diethylaminobenzo(a)phenothiazinium photosensitizers is eradicated by the activity of β-lactamase.¹⁰³ This enzyme is responsible for the antibiotic resistance (ampicillin) and antibiotic resistant strain of *Staphylococcus aureus* becomes susceptible to PDT action and can be destroyed by the activation of the PS. Enzyme cleaves β-lactam ring to release the PS.

2.6.3.3 Singlet Oxygen Quencher

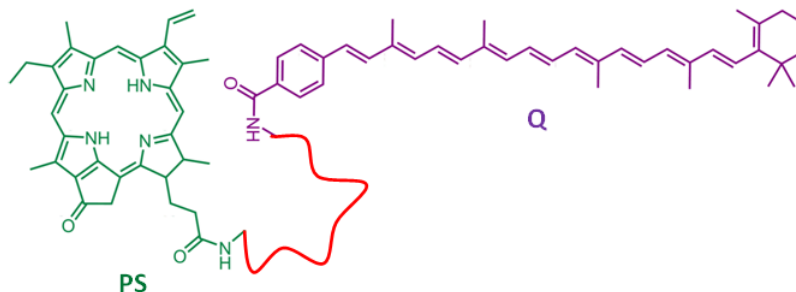


Figure 6. Quenching of PS through ¹O₂ scavanger

Photodynamic action can be controlled after the generation of ¹O₂. This requires singlet oxygen quenchers. There are a number of different ¹O₂ quenchers that either act physically (azide, amines)¹⁰⁴¹⁰⁵ through charge transfer or show their effects through chemical reaction (thiols, ascorbate).¹⁰⁶ Carotenoid, a physical ¹O₂ quencher is covalently attached to the pyropheophorbide-a photosensitizer through a caspase-3 susceptible peptide linker and display a more efficient toxic effect upon cleavage by this apoptotic enzyme (Figure 6).¹⁰⁷

2.6.3.4 Deactivation Through Electron Transfer

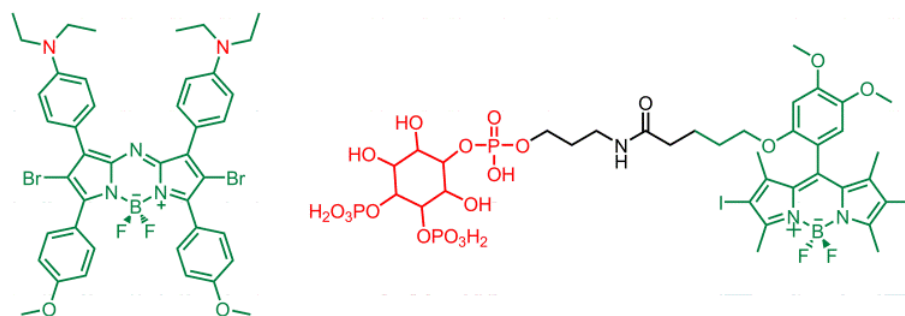


Figure 7. PS deactivation by means of PET

Photoinduced electron transfer is a widely-used fluorescence quenching or PDT deactivation method. With the mechanism described in previous sections, electron rich compounds adjacent to fluorophore or PS reduce the excited molecule and generate non-emissive fluorophore, inactive PS. Depending on the relative orientation of HOMO and LUMO levels of the electron donor and acceptor moieties direction of electron transfer may change. Block of PET pathway by means of disease associated molecules or malignant environment is used for PS activation. In Figure 7, the first aza-BODIPY is inactive due to PET from amine groups. Once the amine is protonated ($pK_a = 6.6$), PET is no more effective since the HOMO of the electron donor is stabilized with respect to HOMO of the PS. This enables improved $^1O_2$ generation, more than 10-folds.¹⁰⁸

The second BODIPY dye conjugated to inositol 1,4,5-trisphosphate, a ligand of the receptor of the same name (Figure 7).¹⁰⁹ As extensively described by Marcus's theory, the PET process is highly dependent on the polarity and character of the solvent/microenvironment.¹¹⁰ Authors designed a PET pathway between the IP_3 to BODIPY dye in polar solvent. However, once the electron acceptor moiety is buried within the cavity of the receptor, the non-polar environment with lower dielectric constant affect the PET process by stabilizing the S_1 state of the PS and the photosensitizer is activated. This innovative work is proposed for the selective light-induced inactivation of the receptor by means of PDT.

As described in previous sections, disease parameter-responsive PET and ICT coupled systems are designed to control the PS activity with AND logic construct.^{15,16,17}

2.7 Tumor Biology and Disease Symptoms

Effective, targeted therapy of any kind requires knowledge about the genetic profile and molecular biology of the disease. This knowledge is inevitable when cancer is in concern. Although a perfect medication of this disease is still not available, highly improved molecular knowledge is accumulated on this subject.

Cancer is defined as unnatural cell proliferation that produces an uncontrollable growth and division of the cells and usually transformed cells invade other regions of the body through metastasis. The demography of this disease is frightening since the numbers indicate that the 13% of all human deaths are a result of this disease.¹¹¹ There are many different types of cancers and the genetic and molecular basis or outcomes of each differ. However, there are certain molecular signs quite common in all tumor types. Considering the altered expression profile of a gene or the activity of a protein, the main contributors are the ones that control cell division. Ras protein is one of such proteins and has role in signal transduction of cell proliferation. A single mutation results in an overactive protein that produces a proliferative signal constitutively. Some chromosomal defects such as gene amplification, chromosomal translocations do also contribute the development of this disease. Although the genetic change that leads to improved cell division is supposed to be the reason, other genetic cooperative mutations are usually required or occur as a result of genetic instability. These are either genetic defects in tumor suppressor genes (such as *rb*, *p53*) that normally function to suppress the expression of cancer-promoting genes or DNA repair enzymes that correct the mismatch defects. The lack of control over cell division by the suppressor genes leads or aids tumor development. Likewise, lack of proper activity of repair enzymes provokes accumulation of mutations and contributes to the disease development. Together with the molecular and genetic background explained above, lack of normal apoptotic activity, telomere

instability and many other factors all together or as a combination of some will result in the progress of the disease.¹¹²

Beside this molecular data, particularly important contributors/symptoms are introduced in this section; low tumor pH and glutathione level. In the research which will be presented in the following sections uses these informations about tumor molecular biology.

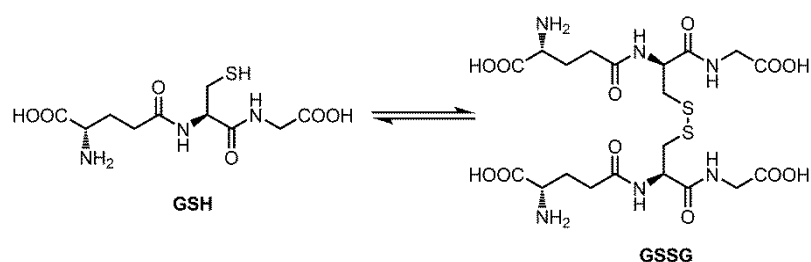
2.7.1 Walburg Effect and Low Tumor pH

The energy metabolism of cancer cells are usually depends on the glycolysis and lactic acid production as described first by Nobel laureate Otto Heinrich Warburg.¹¹³ In healthy cells, the oxidation process continues within the mitochondria to produce more energy. The reduced activity of pyruvate kinase enzyme in cancer cells prevents further oxidation of pyruvate and also reduces the production of reactive oxygen species. The latter activity decreases oxidative stress hence helps cell survival and proliferation. The lack of proper oxygen supply (hypoxia) to the tumor site and mitochondrial defects are hypothesized to be the cause of such high glycolysis rate. Cancer cells even still keep high glycolysis rate in the presence of oxygen. The main outcome of this metabolic shift is the change in the pH of the tumor microenvironment as a result of hypoxia, lactic acid accumulation and change in the ion transport character across the plasma membrane.¹¹⁴ The extracellular pH is reported to drop down to 6^{115,116} for some cancer types but for most tumors pH values between 6.5-7.0 are reported.¹¹⁷

2.7.2 Redox Status of Tumor and Glutathione

One of the other widespread tumor metabolic hallmark is increased level of intracellular reduced glutathione (GSH). Glutathione is an electron donor composed of tripeptide of cysteine, glycine and glutamate amino acids (Scheme 16) and is an important element of cellular redox homeostasis together with cysteine, nicotinamide dinucleotide (NADH, NADPH), thioredoxin, peroxiredoxin, flavin adenine dinucleotide (FAD) and oxidized forms of these compounds. Glutathione is

synthesized by successive activity of two different enzymes γ -glutamylcysteine synthetase and glutathione synthetase. The molecule is in balance with its oxidized disulfide form (GSSG) and it is regenerated from this form by glutathione reductase enzyme. The oxidized glutathione produced within the cell is drained out of the cell and degradation of this compound occurs in extracellular medium. This balance is a determinant of protein activity and the progress of cell cycle. Reducing ability of GSH enables the maintenance of thiol proteins and regulates the signal transduction. In addition of its role as reducing agent, GSH also have active role in DNA synthesis and regulation of immune response.



Scheme 16. Chemical structures of reduced (GSH) and oxidized (GSSG) glutathione.

Under normal conditions, the interior of the cell is more reducing due to higher level of GSH compared to GSSG. Concentration of GSH in blood is about 2-4 μM ¹¹⁸ whereas the intracellular concentrations are thousand folds higher.¹¹⁹ The extracellular space of air-way fluid of the lung also has a GSH concentration as high as 200-400 μM .¹²⁰ These values differ during cancer progression. Change in the redox balance of the cell interferes with cell proliferation and metastasis.¹²¹ In order for the cell to proliferate the reactive oxygen species should be lowered. This is maintained by the high level of NADPH and GSH. Both forms of reducing species are elevated in tumor tissue by the activity of cancer promoting biochemical changes. MYC, a cancer promoting transcription factor, increases glutamine uptake, a precursor in GSH synthesis pathway.¹²² Although, elevated cellular GSH helps the cells to develop resistance to chemotherapy, one can take advantage of this. Indeed, thiol exchange reaction with GSH or the reducing power of this compound is used a lot in literature for drug release purposes.¹²³

CHAPTER 3

Proof of Principle for a Molecular 1:2 Demultiplexer to Function as an Autonomously Switching Theranostic Device¹²⁴

This work is partially described in the following publication

Sundus Erbas-Cakmak, O. Altan Bozdemir, Yusuf Cakmak, Engin U. Akkaya

Chem. Sci., **2013**, 4, 858-862

3.1. Objectives

Although simple logic behaviour of the molecular logic devices have been examined a lot, most of them are not ascribed a meaningful functionality. To build a logic gate with a defined application is challenging though. Here, guided by the digital design concepts, a two-module molecular Demultiplexer (DEMUX) is synthesized where the output is switched between emission at near IR, and cytotoxic singlet oxygen, with light at 625 nm as the input (I), and acid as the control (c). In the neutral form, the compound fluoresces brightly under excitation at 625 nm, however, acid addition moves the absorption bands of the two modules in opposite directions, resulting in an effective reversal of excitation energy transfer direction, with a concomitant upsurge of singlet oxygen generation and decrease in emission intensity.

3.2 Introduction

Ever since the seminal de Silva article was published in 1993,¹ chemical logic gate research is in a process of rapid progress and evolution. Now closing in on its second decade, chemical equivalents of many two input logic gates were reported,^{125,126} along with more complex digital systems featuring higher levels of virtual integration.^{127,128,129,130,131,132,133,134,135,136,137} Finding tangible applications for these molecular constructs with digital capabilities has been in the forefront of research in recent years.^{138,139} Progress in this field was essentially based on the premise that

molecular logic gates, while getting their inspiration from their silicon based counterparts in digital electronics, the limitations and potential opportunities do not necessarily show a one-to-one correspondence. Often stated limitation of “input-output heterogeneity” can be molded into a distinct advantage, since the outputs can be molecular in nature which may interact with other, and more complex molecular and supramolecular phenomena such as metabolism, thus opening a new path to information processing therapeutic agents.^{140,141,142,143} In addition, certain design flexibilities available in molecular logic gates, have no counterparts in semiconductor based analogues. Functional/ virtual integration of logic gates and possibility of superposed logic gates are just two examples. These two uniquely molecular features are exploited in a typical design of molecular multiplexers^{144,78} (MUX) and demultiplexers^{145,78} (DEMUX). In digital signal processing, a multiplexer is a combinatorial circuit which selects from a number of inputs and directs it to an output. Demultiplexer is a complementary device, taking a single input signal and switching it between multiple output signals. Both can be considered as controlled switches.^{146,147}

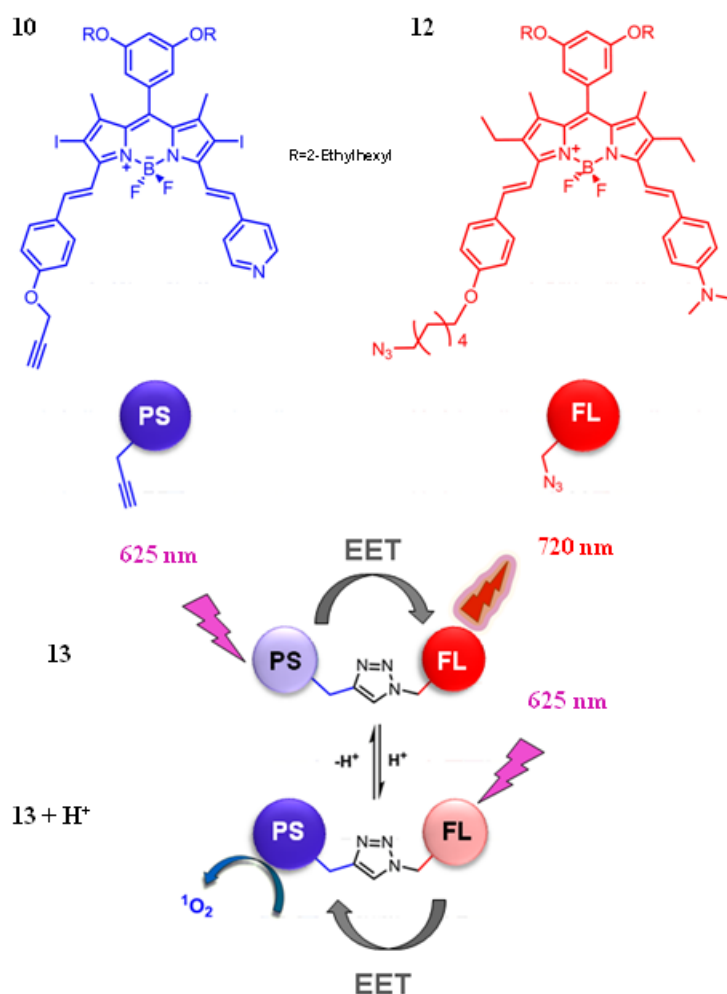
Previously, it has been shown¹⁵ that even a simple AND logic gate within the context of photodynamic therapy can bring in highly precious surplus value of enhanced selectivity. Photodynamic therapy is a non-invasive methodology of treatment of various cancers and non-cancerous indications.¹⁴⁸ This methodology is based on the excitation of a particular photosensitizer within the tumor tissue, generating singlet oxygen and other reactive oxygen species (ROS), and thus, regiospecifically destroying the targeted tumor. In recent years, combined diagnosis and therapy functions received particular attention, with a new buzz word to accompany this heightened interest: theranostics. Diagnosis component obviously include, but not limited to imaging of the region of interest. As for all therapeutic methodologies, such multifunctional delivery agents would be useful for photodynamic therapy as well.

It occurred that a molecular DEMUX device, which can switch between two outputs such as light and cytotoxic singlet oxygen can be very useful in a photodynamic theranostics context. Such a molecular device when directed to the tumor region,

may fluoresce brightly in the surrounding healthy tissue, and inside the tumor, appear dark while generating singlet oxygen on excitation. The switch can be made by the acidity difference between the tumors and the healthy tissue without any need to change the wavelength of excitation. It is widely known that most tumors are considerably acidic compared to neighbouring healthy tissues as a result of Warburg effect.¹¹³ If the design is made properly, the emission can be at the red or near IR region of the spectrum making imaging more valuable.

3.3 Design of the Molecular Demultiplexer

With these goals in mind, a proof-of-principle DEMUX logic is designed, which can operate in organic solvents, incorporating switchable through space energy transfer coupled to mutually exclusive generation of red/near IR emission or singlet oxygen generation depending on the acidity of the medium. Here, the light at 625 nm (supplied by a LED array) is the input (I), and the acid (Trifluoroacetic acid, TFA) is the control (or address input). The target molecule is composed of two distyryl-Bodipy modules clicked together by Huisgen cycloaddition (Scheme 17). Previously examined distyryl-Bodipys³⁷ taught that pyridylethenyl and dimethylaminophenyl ethenyl substituted Bodipys respond by opposite spectral shifts on protonation (blue shift with dimethylaminophenyl, and red shift with pyridyl) as a result of different ICT characteristics. When excited at a fixed wavelength, it is reasonable to expect a switch in the direction of EET (Figure 8, 9). Furthermore, one of the Bodipy modules (compound **12**, **FL** module) is designed to be fluorescent, and especially more so on protonation. The other one (compound **10**, the **PS** module) carries heavy atoms (two iodine atoms at 2,6-positions) facilitating intersystem crossing to yield a higher rate of ¹O₂ generation. It is clear that the switch is not going to be absolute, i.e., both modules will be excited at varying degrees, nevertheless an inspection of Figure 8 reveals that at the optimal wavelength (635 nm), **PS** to **FL** absorptivity ratio changes from 2 to 0.5, altering the position of preferential initial excitation.



Scheme 17. Modular assembly of the molecular DEMUX device with two different outputs depending on the acidity of the medium.

3.4 Results and Discussion

The operation of the DEMUX can be broken down into superposed action of two modules clicked together. Compound **13** shows a broad band and it is difficult to assess spectral changes on the addition of control input TFA (Figure 8b). But, when the two components are separately studied as independent modules, the operation of compound **13** can be better understood. When no acid is added, maximum absorbance of the isolated PS module (**10**) is at 647 nm and that of FL module (**12**) is at 681 nm, however, when TFA is added, the absorption peak of **10** shifts to 671 nm, whereas that of **12** shifts to 647 nm (Table 1, Figure 8a). An opposite shift is

also observed in emission spectra of modules **10** and **12** (Figure 9b). While the difference seems to be small, adding the auxochromic nature of the protonation of **12**, it should not be surprising to see that the compound **13** works in accordance with the design expectations. For compound **13**, when there is no added acid ($c = 0$), on excitation at 625 nm, an intense emission at 715 nm from the **FL** module is observed in accordance with the operation of DEMUX device corresponding to input $h\nu_{625} = 1$, $c = 0$; therefore output is switched to output 1, which is $h\nu_{715}$. The faster rate of excited state energy transfer process with respect to intersystem crossing was used⁹⁹ to quench the singlet oxygen generation near neutral pH and yield near IR emission instead (*vide infra*).

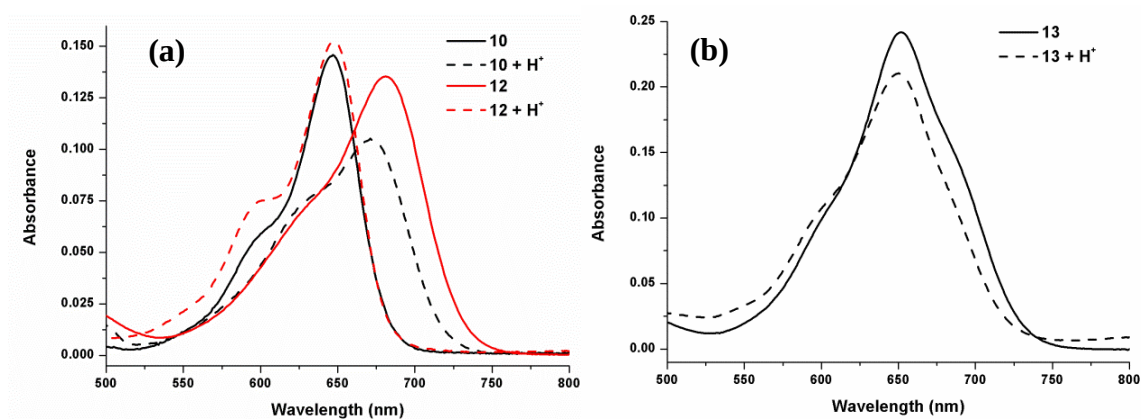


Figure 8. Absorbance spectra of equal concentrations (2 μM) of the photosensitizer (PS, **10**) and the fluorophore (FL, **12**) modules in the presence (dashed curves) and absence (solid curves) of added TFA in chloroform (a), absorbance spectra of compound **13** (2 μM) in chloroform in the absence (solid) and presence (dashed) of TFA.

Excitation energy transfer (EET) from the **PS** to **FL** in DEMUX compound **13** is confirmed by the decrease in the singlet oxygen generation capacity of the **PS** module (Figure 10, 11, Table 2), even though it actively generates $^1\text{O}_2$ when that module alone was excited separately, both under acidic and neutral conditions (Figure 10, 11). On acid addition, ($c = 1$) and excitation at the fixed wavelength of 625 nm, simultaneous ICT shifts in **PS** and **FL** change the direction of EET and cytotoxic singlet oxygen is then produced, while the emission of **FL** is quenched

(Figure 12). Energy transfer to **PS** module in compound **13** is also confirmed by the decrease in the excited state lifetime of protonated compound **13** (0.03 ns, $\chi^2 = 1.43$) compared to protonated donor **FL** module (3.62 ns, $\chi^2 = 1.21$) in CHCl_3 , indicating a very high energy transfer efficiency (99%).

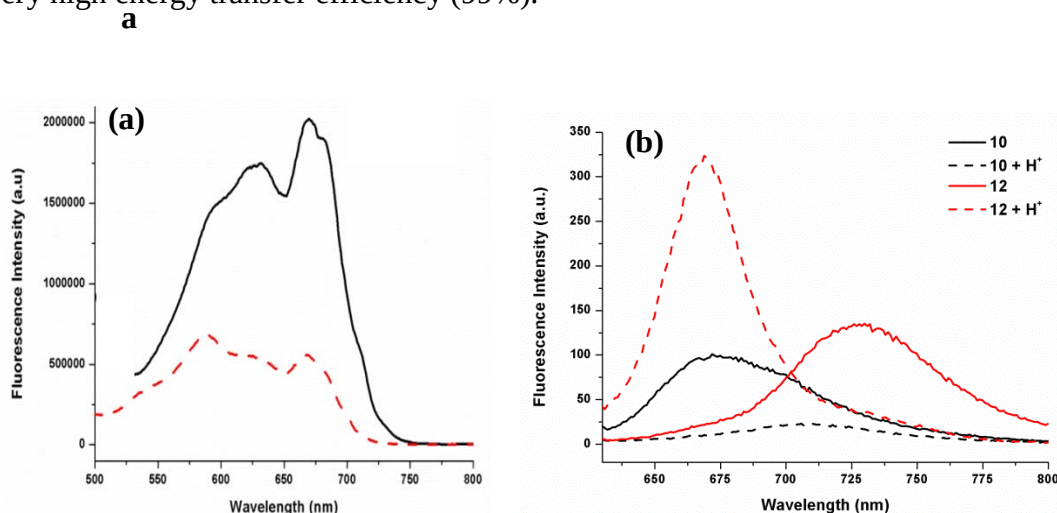


Figure 9. Excitation spectra (a) of 2 μM of molecular demultiplexer (compound **13**) in the absence and presence of acid in chloroform. Overlapping peaks result in the observed broad absorption peak. Emission was collected at 715 nm and at 700 nm for compound **13** and acid-added compound **13** respectively. Emission spectra of module compounds **10** and **12** (2 μM) in acidic and neutral media in chloroform (b).

Table 1. Photophysical characterization of compounds **10**, **12** and **13** in chloroform.

Compound	λ_{abs} [nm]	ϵ [$\text{M}^{-1}\text{cm}^{-1}$]	λ_{F} [nm]	$\phi_{\text{F}}^{[a]}$ [$\lambda_{\text{exc}}(\text{nm})$]	$\tau_{\text{F}}^{[b]}$ [ns]
11	647	73000	665	0.22	1.71
11 + H^+	671	53000	694	0.06	0.33
2	681	68000	729	0.16	3.37
12 + H^+	647	76000	665	0.41	3.62
13	652	121000	715	0.42	4.05
13 + H^+	650	105000	700	0.02	0.03

^a Quantum yields were calculated using Sulforhodamine 101 (excitation at 550 nm, in EtOH) as reference. All other compounds were dissolved in chloroform. ^b All measurements are taken in CHCl_3 using 650 nm (for compounds **10** and **12**) and 667 nm (for compound **13**) NanoLEDs.

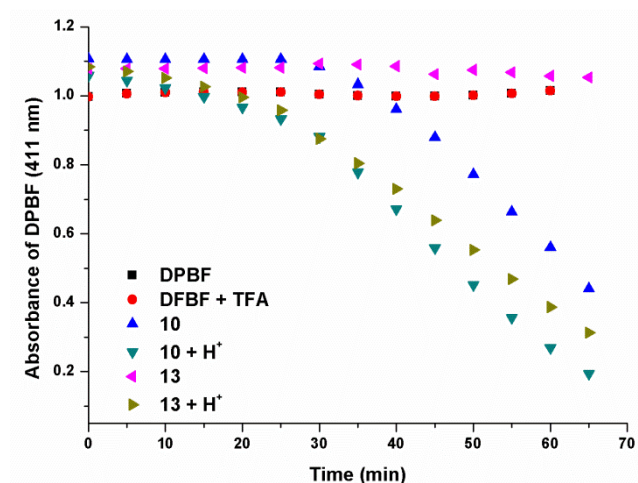


Figure 10. Singlet oxygen mediated photobleaching of DPBF with molecular demultiplexer, (a.k.a, DEMUX device, compound **13**, purple), compound **13** in acidic media (light green), **PS** (blue), **PS** in acidic media (green), DPBF alone (black), DPBF alone in acidic media (red), followed by decrease in its absorbance at 411 nm.

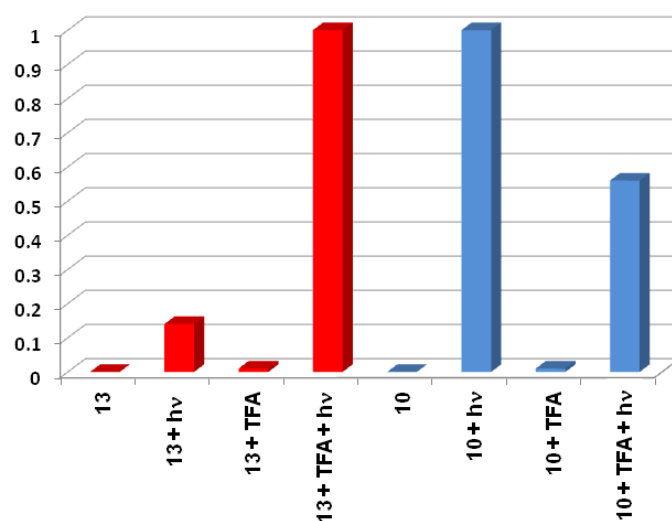


Figure 11. Relative singlet oxygen generation efficiency of photosensitizer (compound **10**, blue) alone or molecular demultiplexer (compound **13**, red) in the presence or absence of light and/or TFA.

Three negative and two positive control experiments support the mutually exclusive character of near IR fluorescence emission and singlet oxygen generation (Table 2). In positive controls, singlet oxygen generation activity of 20 nM compound **10** (**PS** module, same concentration as the demultiplexer **13**) was studied and found to be active in sensitization, both in the presence or absence of trifluoroacetic acid (Figure 10, 11, Table 2). This shows that the decrease in the singlet oxygen generation rate is mostly due to the presence of fluorophore module as an energy sink. The relative singlet oxygen generation efficiencies were calculated by normalizing the rate of initial decrease in the DPBF degradation/bleaching. In **10** (**PS**) alone case (positive control-1), the efficiency seems to decrease to 0.56 compared to compound **13** under acidic conditions.

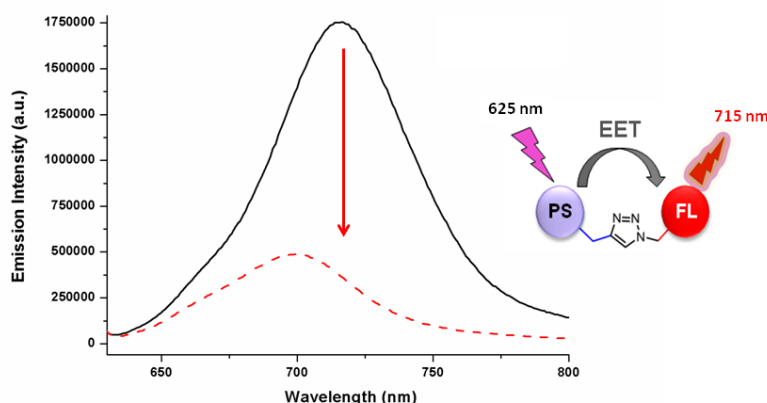


Figure 12. Emission spectra of the DEMUX device **13**, under neutral (black, solid) and acidic (red, dashed) conditions excited at 625 nm in chloroform.

This is probably due to the larger absorptivity of the protonated **FL** unit which leads to excitation of the **PS** in **13** by EET. **PS** under acidic conditions exhibits a decrease in the rate of singlet oxygen generation which is due to decrease in molar absorptivity at the wavelength of excitation (625 nm) as a result of bathochromic shift on protonation. In addition, by using two negative controls, the amount of degradation of the trap molecule (diphenylisobenzofuran, DPBF) in the absence of photosensitizer under neutral and acidic conditions (Table 2) is determined. The third negative control was carried out to determine the extent of singlet oxygen generation of compound **13** under neutral conditions, i.e., in the absence of added acid. In this

experiment, due to effective reversed EET from the **PS** to **FL**, no singlet oxygen generation is observed as expected (Table 2).

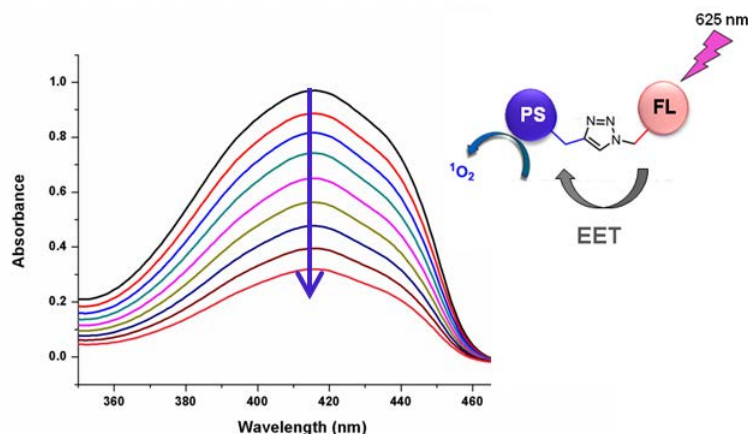


Figure 13. Singlet oxygen mediated photobleaching of DPBF (50 μM) in the presence of 20 nM compound **13** and TFA (20 μl /3mL) in chloroform.

Control experiments with trap alone were performed to ensure the reliability of the data. In acidic conditions, reciprocal shifts in the absorbance of the two modules of the compound **13** leads to a change in the direction of electronic energy transfer. The **12 (FL)** becomes the donor, and excitation energy is transferred to **10 (PS)**, which becomes activated and generates cytotoxic singlet oxygen effectively at concentrations as low as 20 nM (Figure 13, Table 3). Response of compound **13** and all other control experiments under neutral and acidic conditions in dark and under red LED irradiation at 625 nm is shown in Table 3.

The overall working principle of the compound presented here as a molecular DEMUX device is as follows; in the absence of acid, there is an intense emission near IR at around 715 nm (Figure 12, 14). When the acid is added, the fluorescence of the compound **13** is completely quenched due to EET to non-emissive **PS** module. This decrease in emission is linked to the efficient generation of singlet oxygen. With pH-driven change in the EET direction, **PS** module becomes active and generates therapeutic output, singlet oxygen. This action is dependent on change in EET direction, thus, with a similar demultiplexer based on this proof of principle, but with

additional practical considerations in mind (solubility, pK_a values of the acid sensitive groups), sensitization to produce singlet oxygen would be only in the acidic medium (as found in most tumors) and the boundaries of the tumor region can be imaged by the change in fluorescence intensity (which would appear as darker regions, as opposed to red/near IR fluorescence from the surrounding healthy tissues).

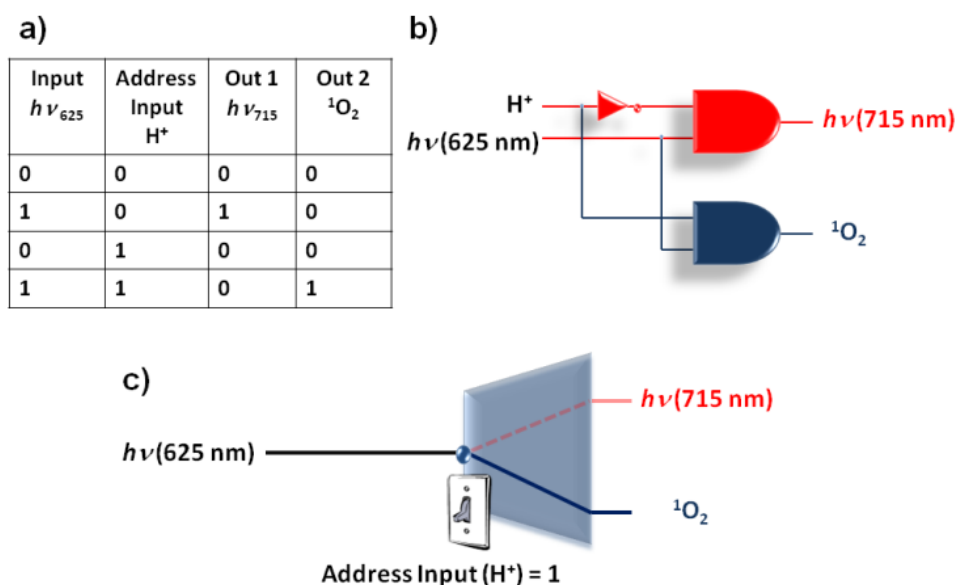


Figure 14. Truth table (a), circuit diagram (b) and schematic switching operation (c) of the 1:2 molecular DEMUX logic device.

For the singlet oxygen generation experiments, the compounds in each solutions were 20 nM and the trap was 50 μ M. Measurements were done in the dark for the first 25 min. Samples were then irradiated with 625 nm LED starting at $t=25$ minutes, and measurements were repeated in 5 minute intervals.

Table 2. Relative rates of singlet oxygen generation in positive and negative control experiments.

Experiment Type		DEMUX	Positive 1	Positive 2	Negative 1	Negative 2	Negative 3	Negative 4
Contents	DPBF ^a	50 μ M	50 μ M	50 μ M	50 μ M	50 μ M	50 μ M	50 μ M
	TFA ^b	20 μ l/3ml	-	20 μ l/3ml	-	-	20 μ l/3ml	20 μ l/3ml
	10 ^c	-	20 nM	20 nM	-	-	-	-
	13 ^d	20 nM	-	-	20 nM	-	-	20 nM
Irradiation (625 nm)		Light	Light	Light	Dark / Light	Dark / Light	Dark/ Light	Dark
Relative Rate of ¹ O ₂ Generation		1	1	0.56	~0/0	~0	~0	0.14

^a DPBF: 1,3-diphenylisobenzofuran, ^b TFA: Trifluoroacetic acid, ^c PS: photosensitizer, compound 10, ^d Molecular Demultiplexer

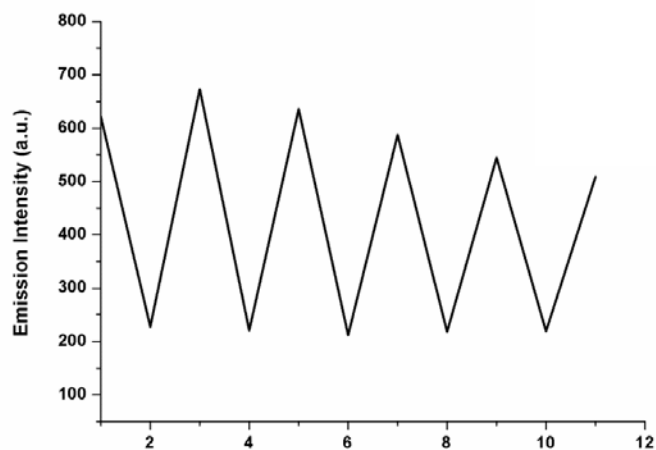


Figure 15. Reversibility of fluorescence of compound 13 in the presence of acid and base in chloroform followed at 715 nm. The first measurement was done in neutral conditions, for each following measurements TFA and piperidine were added in 50 μ l portions, times of addition were given as numbers following the name of reagents.

The demultiplexer device described in this work acts reversibly and retains most of its fluorescence intensity even after 5 cycles of pH oscillations (Figure 15).

Table 3. Experimental data for the generation of photonic and chemical outputs in response to the input acted by the address switch.

Compound	Irradiation (625 nm)	H ⁺ (Address Input)	Relative Emission Intensity (715 nm)	Relative ¹ O ₂ Generation Efficiency ^a
13	0	0	0	0
13	1	0	1	0.14
13	0	1 ^b	0	< 0.01
13	1	1 ^b	0.21	1.0
10	0	0	-	0
10	1	0	-	1
10	0	1	-	< 0.01
10	1	1	-	0.56

^a ^aRelative singlet oxygen generation was calculated by setting the rate of DPBF degradation at input=1, address=1 case to be 1. ^b 20 μ L TFA was added to 3 mL of each sample. Details of calculations were given in Supporting Information.

The importance of multifunctional drugs and delivery systems are well appreciated, and in this work, we demonstrated that the two critical functions, i.e., diagnostic and therapeutic actions can be in principle, implemented using a single molecular agent. We expect this proof of principle model of a demultiplexer to pave the way to multifunctional molecular systems, which would interactively deliver the therapeutic agent, while offering diagnostic imaging possibilities. The work described here suggest that even at a relatively rudimentary stage, information processing molecules can produce unprecedented capabilities, such as autonomous decision making to switch between diagnostic and therapeutic modes. And this is made possible by a unimolecular superposed logic operation with no semiconductor counterpart. The future of molecular logic gate research looks very bright.

3.5 Experimental Details

3.5.1 General

All chemicals and solvents purchased from Sigma-Aldrich were used without further purification. Spectra of ^1H NMR and ^{13}C NMR were recorded using a Bruker DPX-400 in CDCl_3 with TMS as internal reference. Splitting in the spectra are shown as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), and br (broad).

Absorption spectrometry was performed using a Varian spectrophotometer. Steady state fluorescence measurements were conducted using a Varian Eclipse spectrofluorometer. Column chromatography of all products was performed using Merck Silica Gel 60 (particle size: 0.040–0.063 mm, 230–400 mesh ASTM). Reactions were monitored by thin layer chromatography using fluorescent coated aluminum sheets. Solvents used for spectroscopy experiments were spectrophotometric grade. Fluorescence life time measurements were determined on a HORIBA Jobin Yvon fluorolog, FL-1057. HORIBA Scientific NanoLEDs at 650 and 667 nm (pulse width < 250 ps) was used. The instrument response function was measured with an aqueous Ludox solution. The decays were analyzed with a multi-exponential fitting function by iterative reconvolution and chi-square minimization. Mass spectra were recorded on Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS.

3.5.2 Determination of FRET Efficiencies

Energy transfer efficiencies between donor and acceptor moieties are determined using the fluorescence lifetimes of modules and molecular demultiplexer. The following formula is used for this purpose:

$$E = 1 - \tau_{\text{DA}}/\tau_{\text{D}} \quad \text{Equation 4}$$

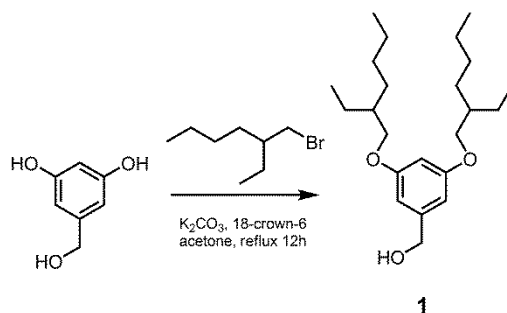
where τ_D and τ_{DA} refer to excited state decay time (lifetime) of protonated donor (protonated compound **12**) alone and donor as a part of molecular demultiplexer (protonated compound **13**) respectively. Both measurements were done in the presence of trifluoroacetic acid (TFA) and absorbance values are all below 0.1 to avoid inner filter effect.

3.5.3 $^1O_2$ Generation Experiments

For $^1O_2$ measurements, bleaching of singlet oxygen trap molecule 1,3-diphenylisobenzofuran (DPBF) was used. The corresponding reaction with $^1O_2$ is given in scheme. 625 nm emitting LED is used as a light source with 10 cm separation from the sample cuvette. Solution containing DPBF (50 μ M), compound **13** (20 nM) and TFA (20 μ l/3ml) is prepared in $CHCl_3$. Two negative control solutions are prepared with DPBF alone (50 μ M) (with TFA and without TFA) and DPBF (50 μ M) with compound **13** (20 nM) (without TFA). As positive control, a solution with DPBF (50 μ M) and photosensitizer (compound **10**) (20 nM) is prepared. Solutions are aerated for 5 minutes prior to each experiment. Then, absorption spectra are recorded in 5 minutes intervals while the solutions are either kept under dark (first 15 min) or irradiated with 625 nm light.

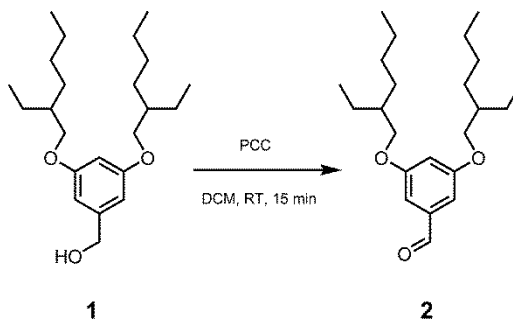
The decrease in absorbance of DPBF at 411 nm is used to calculate relative singlet oxygen generation efficiencies. Slope of the graph of absorbance vs time are calculated and normalized with respect to photosensitizer or protonated compound **13**. The experimental artifact resulting from decomposition of DPBF by trifluoroacetic acid is corrected by subtracting the slope of this control graph from TFA containing solutions.

3.5.4 Synthesis



Synthesis of Compound 1: 3, 5-dihydroxybenzylalcohol (2 g, 14.3 mmol) and 2-ethylhexyl bromide (5.8 ml, 28.56 mmol) were dissolved in 50 ml acetone. K_2CO_3 (16 g, 112 mmol) and catalytic amount of 18-crown-6 were added. The reaction mixture was refluxed for 12h. Then, acetone was evaporated in vacuum evaporator; the crude product was extracted with water and chloroform. Organic layer was collected, dried with Na_2SO_4 and evaporated in vacuo. The product was obtained as viscous yellow liquid (13.73 mmol, 4.98 g, 96%).

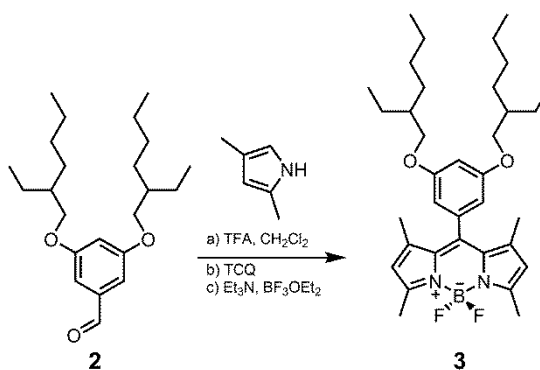
^1H NMR ($CDCl_3$, 400 MHz, δ ppm) 6.54 (d, 2H, J = 2.2 Hz; ArH), 6.41 (t, 1H, J = 2.2 Hz; ArH), 4.64 (s, 2H, CH_2O) 3.85 (dd, 4H, J = 3.64 Hz; OCH_2), 3.50 (1H, CH_2OH), 1.76 (m, 2H; OCH_2CH), 1.3-1.6 (m, 16H; CH_2), 0.9-1.0 (m, 12H; CH_3).



Synthesis of Compound 2: 3, 5-di(2-ethylhexyl)benzylalcohol, compound 1 (4.2 g, 11.6 mmol) and pyridinium chlorochromate (5 g, 20 mmol) were dissolved in 50 ml dichloromethane. The reaction mixture was stirred at room temperature for 30 minutes. Then, the crude product was filtered from celite, filtrate was collected, vacuum evaporated. The product was purified by silica gel column chromatography using $CHCl_3$ as mobile phase. Fraction containing compound 2 was collected then the solvent was removed under reduced pressure (3.91 g, 10.8 mmol, 93%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 9.92 (s, 1H), 7.02 (d, 2H, $J = 2.16$ Hz; ArH), 6.73 (t, 1H, $J = 2.00$ Hz; ArH), 3.90 (dd, 4H, $J = 3.64$ Hz; OCH_2), 1.76 (m, 2H; OCH_2CH), 1.3-1.6 (m, 16H; CH_2), 0.9-1.0 (m, 12H; CH_3)

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 191.5, 160.9, 138.4, 107.8, 107.3, 70.6, 39.3, 30.5, 29.0, 23.8, 23.0, 14.0, 11.0.

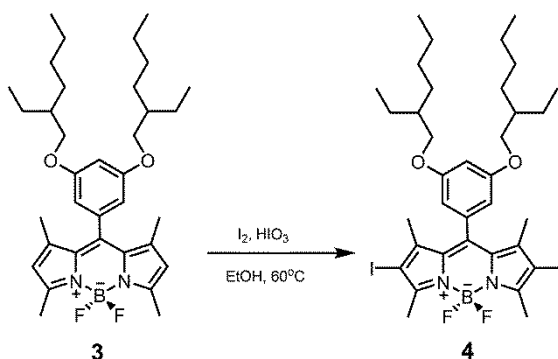


Synthesis of Compound 3: CH_2Cl_2 (300 ml) was purged with Ar for 30 min. 3,5-di(2-ethylhexyl)benzaldehyde, compound **2** (1.76 g, 4.85 mmol) and 2,4-dimethylpyrrole (1 ml, 9.71 mmol) were added. The color of the solution turned into red after the addition of 3 drops of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12h. Then, tetrachloro-1,4-benzoquinone (1.192 g, 9.7 mmol) was added and the reaction mixture was stirred at room temperature for 45 min. Then triethyl amine (6 ml) and boron trifluoride diethyl etherate (6 ml) were added sequentially. After stirring at room temperature for 30 min, it was extracted with water. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl_3 /Hexane (50:50/v:v) as mobile phase. Fraction containing compound **3** was collected then the solvent was removed under reduced pressure (673.5 mg, 1.16 mmol, 24%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 6.58 (t, 1H, $J = 2.2$ Hz; ArH), 6.45 (d, 2H, $J = 2.2$ Hz; ArH), 6.01 (s, 2H; ArH), 3.85 (dd, 4H, $J = 3.64$ Hz; OCH_2), 2.60 (s, 6H; ArCH_3), 1.75 (m, 2H; OCH_2CH), 1.60 (s, 6H; ArCH_3), 1.3-1.6 (m, 16H; CH_2), 0.9-1.0 (m, 12H; CH_3)

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 161.4, 155.4, 143.2, 136.4, 121.0, 113.2, 110.0, 106.3, 102.3, 71.0, 39.3, 30.5, 29.1, 23.8, 23.0, 14.6, 14.2, 14.1, 11.1.

HRMS (TOF-ESI): m/z calcd for $C_{35}H_{51}BF_2N_2O_2$: 579.3933 $[M-H]^-$; found: 579.4002 $[M-H]^-$, $\Delta = 11.8$ ppm.

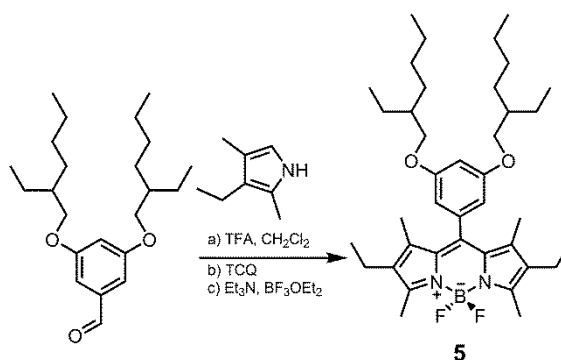


Synthesis of Compound 4: Compound **3** (300 mg, 0.516 mmol) and I_2 (328 mg, 1.29 mmol) were dissolved in ethanol (150 ml). Iodic acid, HIO_3 (181.6 mg, 1.32 mmol) was dissolved in a few drops of water and added into previous solution. The reaction mixture was stirred at $60^\circ C$ for a few hours until all reactant was consumed. After the reaction was completed, saturated sodium thiosulfate solution was added (50 ml) and it was stirred at room temperature for additional 30 min. Following that, it was extracted with $CHCl_3$ and water. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$ /Hexane (50:50, v/v). Fraction containing compound **4** was collected, the solvent was removed under reduced pressure (280 mg, 0.34 mmol, 66%).

^1H NMR ($CDCl_3$, 400 MHz, δ ppm) 6.60 (t, 1H, $J = 2.2$ Hz; ArH), 6.40 (d, 2H, $J = 2.2$ Hz; ArH), 3.81 (dd, 4H, $J = 3.94$ Hz; OCH_2), 2.68 (s, 6H; Ar CH_3), 1.73 (m, 2H; OCH_2CH), 1.60 (s, 6H; Ar CH_3), 1.2-1.5 (m, 16H; CH_2), 0.91 (m, 12H; CH_3)

^{13}C NMR ($CDCl_3$, 400 MHz, δ ppm) 55.161.7, 156.7, 145.4, 141.5, 136.0, 131.0, 106.0, 102.6, 85.5, 71.1, 39.3, 30.5, 29.1, 23.9, 23.0, 16.9, 16.0, 14.1, 11.1.

HRMS (TOF-ESI): m/z calcd for $C_{35}H_{49}BF_2I_2N_2O_2$: 831.1866 $[M-H]^-$; found: 831.1951 $[M-H]^-$, $\Delta = 10.0$ ppm.

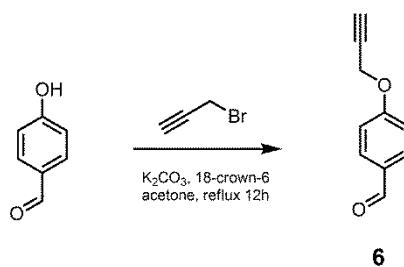


Synthesis of Compound 5: CH₂Cl₂ (300 ml) was purged with Ar for 30 min. 3, 5-di(2-ethylhexyl)benzaldehyde, compound **2** (1.3 g, 3.57 mmol) and 2,4-dimethyl-5-ethyl pyrrole (0.96 ml, 7.14 mmol) were added. The color of the solution turned into red after the addition of 3 drops of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12h. Then, tetrachloro-1,4-benzoquinone (878 mg, 3.57 mmol) was added and the reaction mixture was stirred at room temperature for 45 min. Then triethyl amine (7 ml) and boron trifluoride diethyl etherate (7 ml) were added sequentially. After stirring at room temperature for 30 min, it was extracted with water. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃ as mobile phase. Fraction containing compound **5** was collected then the solvent was removed under reduced pressure (394 mg, 0.62 mmol, 17%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 6.58 (t, 1H, *J* = 2.3 Hz; ArH), 6.45 (d, 2H, *J* = 2.3 Hz; ArH), 3.82 (dd, 4H, *J* = 3.7 Hz; OCH₂), 2.55 (s, 6H; ArCH₃), 2.33 (q, 4H, *J* = 11.3 Hz; ArCH₂CH₃), 1.73(m, 2H; OCH₂CH), 1.49 (s, 6H; ArCH₃), 1.2-1.6(m, 16H; CH₂), 1.02(t, 6H, *J* = 16.0 Hz; ArCH₂CH₃), 0.8-1.0 (m, 12H; CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 161.4, 153.6, 140.3, 138.4, 137.2, 132.6, 130.5, 106.6, 102.2, 71.0, 39.3, 30.5, 29.1, 23.9, 23.1, 17.1, 14.6, 14.1, 12.5, 11.5, 11.1.

HRMS (TOF-ESI): *m/z* calcd for C₃₉H₅₉BF₂N₂O₂: 635.4559 [M-H]⁺; found: 635.4643 [M-H]⁺, Δ = 13.0 ppm.

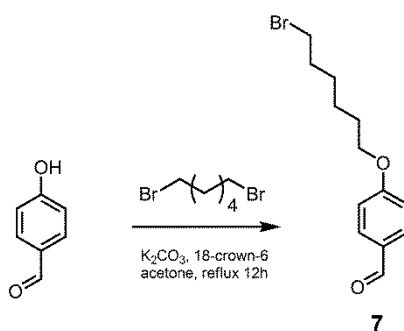


Synthesis of Compound 6: 4-hydroxybenzaldehyde (2.45 g, 20 mmol) and propargyl bromide (2.5 g, 30 mmol) were dissolved in 100 ml acetonitrile. K_2CO_3 (5.5 g, mmol) and a few crystals of benzo-18-crown-6 were added. The reaction was refluxed until all 4-hydroxybenzaldehyde was consumed. The solvent was evaporated in vacuo, extracted with water and $CHCl_3$. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$ /Hexane (50:50, v/v). Fraction containing compound **6** was collected then the solvent was removed under reduced pressure (2.85 g, 17.8 mmol, 89%).

^1H NMR ($CDCl_3$, 400 MHz, δ ppm) 9.92 (s, 1H), 7.87 (d, $J=8.92$ Hz, 2H, ArH), 7.11 (d, $J=8.72$ Hz, 2H, ArH), 4.80 (d, $J=2.44$ Hz, OCH_2), 2.58 (t, $J=2.48$ Hz, 1H, OCH_2CCH).

^{13}C NMR ($CDCl_3$, 400 MHz, δ ppm) 190.8, 162.4, 131.9, 130.0, 115.2, 77.5, 76.4, 55.9.

HRMS-ESI: calculated for $M+Na$ 183.0422, found 183.0422, $\Delta=0$ ppm.



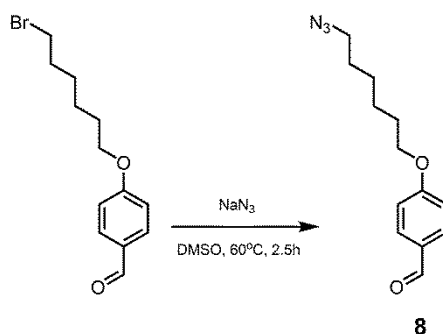
Synthesis of Compound 7: 4-hydroxybenzaldehyde (5 g, 41 mmol) and 1,6-dibromohexane (2 g, 82 mmol) were dissolved in acetonitrile (150 ml). K_2CO_3 (17.2 g, 123 mmol) and catalytic amount of benzo-18-crown-6 were added. The reaction mixture was refluxed for 12h. Then, acetonitrile was evaporated in vacuo and

extracted with water and chloroform. Organic layer was dried with Na₂SO₄ and evaporated in vacuo. The product was purified by silica gel column chromatography using CHCl₃/Hexane (75:25, v/v). Fraction containing compound **7** was collected then the solvent was removed under reduced pressure (13.4 mmol, 3.82 g, 32%)

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.7 (s, 1H), 7.68 (d, *J*= 8.64 Hz, 2H, ArH), 6.82 (d, *J*= 8.76 Hz, 2H, ArH), 3.88 (t, *J*= 6.4 Hz, 2H, OCH₂), 3.4 (t, *J*= 6.68 Hz, 2H, CH₂Br), 1.65 (m, 4H, CH₂CH₂Br), 1.35 (m, 4H, OCH₂CH₂).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 190.6, 164.1, 131.9, 129.7, 114.7, 68.1, 44.9, 32.4, 28.8, 26.5, 25.2.

HRMS (TOF-ESI): *m/z* calcd for C₁₃H₁₇BrO₂: 285.04902 [M+H]⁺; found: 285.05413 [M+H]⁺, Δ = 17.9 ppm.

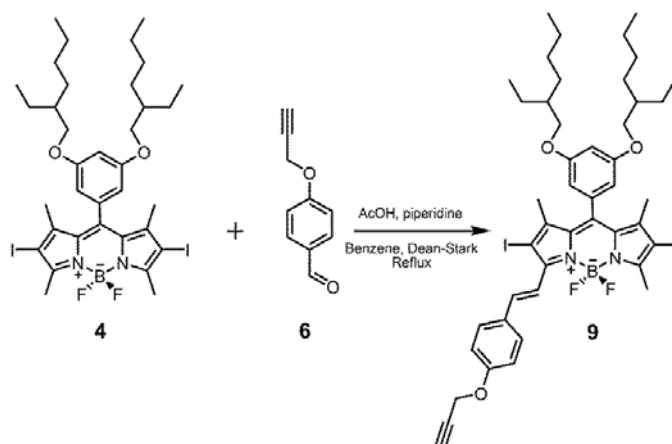


Synthesis of Compound 8: 4-(6-bromohexyloxy)benzaldehyde, compound **7** (1.5 g, 5.3 mmol) was dissolved in 25 ml DMSO. Sodium azide (1.37 g, 21.2 mmol) was added and the reaction mixture was stirred at 60°C for 2.5 hours. Then, it was extracted with water and CHCl₃ a few times and organic layer was collected, dried with Na₂SO₄ and evaporated under reduced pressure. No further purification was required.

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.86 (s, 1H), 7.82 (d, *J*= 8.80 Hz, 2H, ArH), 7.00 (d, *J*=8.76 Hz, 2H, ArH), 4.05 (t, *J*=6.40 Hz, 2H, OCH₂), 3.28 (t, *J*=6.80 Hz, 2H, N₃CH₂), 1.75-1.85 (m, 2H, N₃CH₂CH₂), 1.55-1.65 (m, 2H, OCH₂CH₂), 1.4-1.55 (m, 4H, CH₂).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 190.8, 164.1, 132.0, 129.8, 114.7, 68.1, 51.3, 28.9, 28.7, 26.4, 25.6.

HRMS (TOF-ESI): m/z calcd for $C_{13}H_{17}N_3O_2$: 248.13990 $[M+H]^+$; found: 248.14573 $[M+H]^+$, $\Delta = 23.5$ ppm.

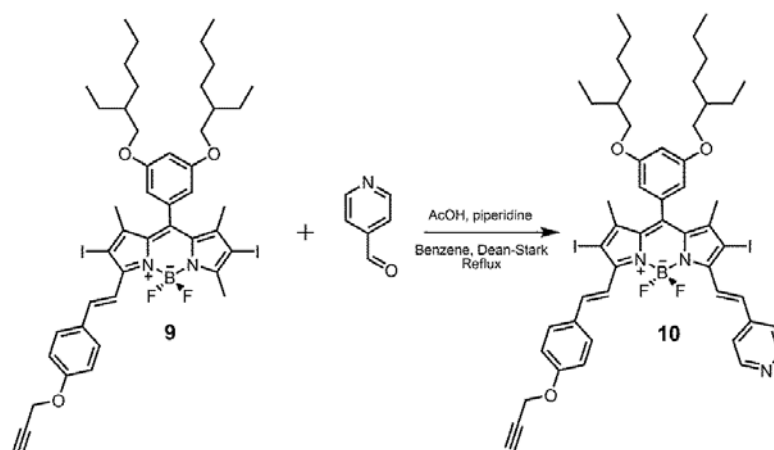


Synthesis of Compound 9: Compound **4** (200 mg, 0.24 mmol) and compound **6** (31 mg, 0.19 mmol) were dissolved in benzene (40 ml). Piperidine (0.3 ml) and acetic acid (0.3 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with $CHCl_3$ and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$ /Hexane (50:50, v/v). Fraction containing compound **9** was collected then the solvent was removed under reduced pressure (73 mg, 0.074 mmol, 39%).

^1H NMR ($CDCl_3$, 400 MHz, δ ppm) 8.11 (d, 1H, $J = 16.6$ Hz; CH), 7.61 (d, 2H, $J = 8.8$ Hz; $ArCH_3$), 7.55 (d, 1H, $J = 16.8$ Hz; CH), 7.02 (d, 2H, $J = 8.8$ Hz; ArH), 6.60 (t, 1H, $J = 2.2$ Hz; ArH), 6.41 (d, 2H, $J = 2.2$ Hz; ArH), 4.71 (d, 2H, $J = 2.4$ Hz; OCH_2), 3.85 (dd, 4H, $J = 3.8$ Hz; OCH_2), 2.70 (s, 3H; $ArCH_3$), 2.55 (t, 1H, $J = 2.4$ Hz; CH), 1.75 (m, 2H; OCH_2CH), 1.65 (s, 3H; $ArCH_3$), 1.60 (s, 3H; $ArCH_3$), 1.2-1.5 (m, 16H; CH_2), 0.8-1.0 (m, 12H; CH_3).

^{13}C NMR ($CDCl_3$, 400 MHz, δ ppm) 161.7, 158.5, 156.9, 150.4, 146.1, 145.1, 140.2, 138.6, 136.3, 132.0, 131.7, 130.4, 129.1, 117.1, 115.3, 106.3, 102.6, 106.3, 102.6, 86.1, 82.1, 78.3, 75.8, 71.1, 55.9, 39.3, 30.5, 29.1, 23.8, 23.0, 17.3, 16.9, 16.2, 14.1, 11.1.

HRMS (TOF-ESI): m/z calcd for $C_{45}H_{49}BF_2I_2N_2O_2$: 973.2285 $[M-H]^-$; found: 973.2347 $[M-H]^-$, $\Delta = 6.4$ ppm.

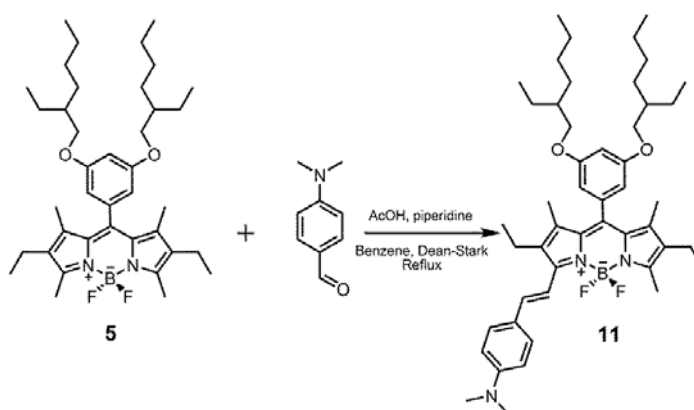


Synthesis of Compound 10, PS: Compound **9** (75 mg, 0.075 mmol) and 4-pyridinecarboxaldehyde (17 mg, 0.15 mmol) were dissolved in benzene (40 ml). Piperidine (0.3 ml) and acetic acid (0.3 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CHCl_3 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ (95:5, v/v). Fraction containing **10 (PS)** was collected then the solvent was removed under reduced pressure (35 mg, 0.033 mmol, 44%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.67(d, 2H, $J = 5.8$ Hz; ArH), 8.23(d, 1H, $J = 16.8$ Hz; CH), 8.01(d, 1H, $J = 16.8$ Hz; CH), 7.86(d, 1H, $J = 16.7$ Hz; CH), 7.65(d, 2H, $J = 8.8$ Hz; ArH), 7.62(d, 1H, $J = 16.9$ Hz; CH), 7.52(d, 2H, $J = 6.0$ Hz; ArH), 7.06(d, 2H, $J = 8.8$ Hz; ArH), 6.61(t, 1H, $J = 2.2$ Hz; ArH), 6.42(d, 2H, $J = 2.2$ Hz; ArH), 4.79(d, 2H, $J = 2.4$ Hz; CH_2), 3.75(dd, 4H, $J = 3.8$ Hz; CH_2), 2.48(t, 1H, $J = 2.3$ Hz; CH), 1.65(m, 2H; OCH_2CH), 1.58(s, 3H; ArCH_3), 1.53(s, 3H; ArCH_3), 1.2-1.5(m, 16H; CH_2), 0.7-0.9(m, 12H; CH_3)

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 161.8, 158.9, 152.7, 150.3, 148.1, 147.5, 144.9, 144.3, 140.7, 139.9, 136.3, 134.8, 130.1, 129.5, 123.3, 121.5, 116.9, 115.4, 106.4, 102.7, 78.2, 75.9, 71.1, 55.9, 39.3, 30.5, 29.7, 29.1, 23.9, 23.0, 17.7, 17.1, 14.1, 11.1.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{51}\text{H}_{58}\text{BF}_2\text{I}_2\text{N}_3\text{O}_3$: 1062.2550 $[\text{M}-\text{H}]^-$; found: 1062.2625 $[\text{M}-\text{H}]^-$, $\Delta = 7.1$ ppm.

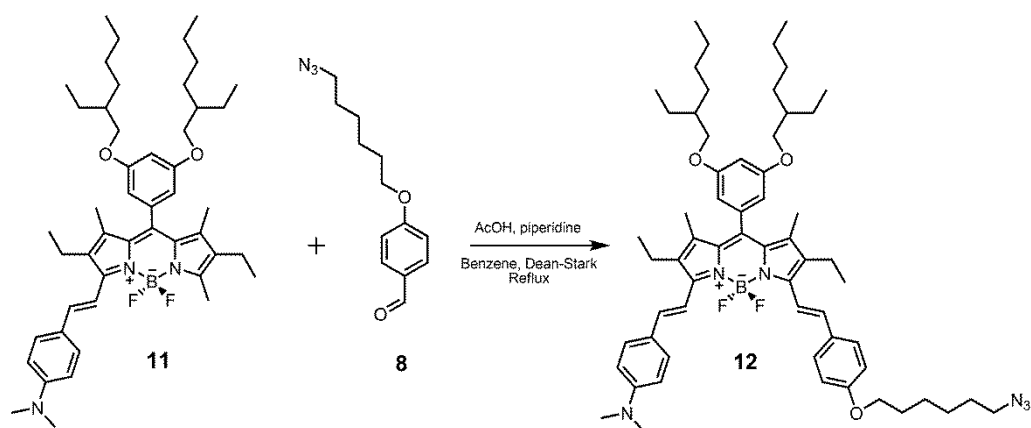


Synthesis of Compound 11: Compound **5** (200 mg, 0.314 mmol) and 4-dimethylaminobenzaldehyde (37.5 mg, 0.25 mmol) were dissolved in benzene (40 ml). Piperidine (0.3 ml) and acetic acid (0.3 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CHCl_3 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl_3 /Hexane (75:25, v/v). Fraction containing compound **11** was collected then the solvent was removed under reduced pressure (0.039 mmol, 30 mg, 16%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.60(d, 1H, $J = 16.6$ Hz; CH), 7.54(d, 2H, $J = 8.8$ Hz; ArH), 7.22(d, 1H, $J = 16.7$ Hz; CH), 6.73(d, 2H, $J = 8.6$ Hz; ArH), 6.57(t, 1H, $J = 2.2$ Hz; ArH), 6.48(d, 2H, $J = 2.2$ Hz; ArH), 3.85(dd, 4H, $J = 3.6$ Hz; OCH_2), 3.03(s, 6H; $\text{N}(\text{CH}_3)_2$), 2.64(q, 2H, $J = 11.1$ Hz; ArCH_2), 2.59(s, 3H; ArCH_3), 2.36(q, 2H, $J = 11.3$ Hz; ArCH_2), 1.74(m, 2H; OCH_2CH), 1.52(s, 3H; ArCH_3), 1.49(s, 3H; ArCH_3), 1.2-1.5(m, 16H; CH_2), 1.19(t, 3H, $J = 6.0$ Hz; ArCH_2CH_3), 1.03(t, 3H, $J = 6.0$ Hz; ArCH_2CH_3), 0.9-1.0(m, 12H; CH_3)

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 161.5, 150.9, 150.8, 139.0, 138.5, 137.5, 136.2, 133.0, 132.7, 131.8, 131.1, 131.0, 128.7, 115.8, 115.7, 112.2, 107.0, 102.2, 71.0, 40.4, 39.3, 30.5, 29.1, 23.8, 23.0, 18.5, 17.2, 14.7, 14.1, 14.0, 12.6, 11.5, 11.3, 11.1

HRMS (TOF-ESI): m/z calcd for $\text{C}_{61}\text{H}_{83}\text{BF}_2\text{N}_6\text{O}_3$: 767.5373 [M]; found: 767.5446 [M+H], $\Delta = 9.5$ ppm.

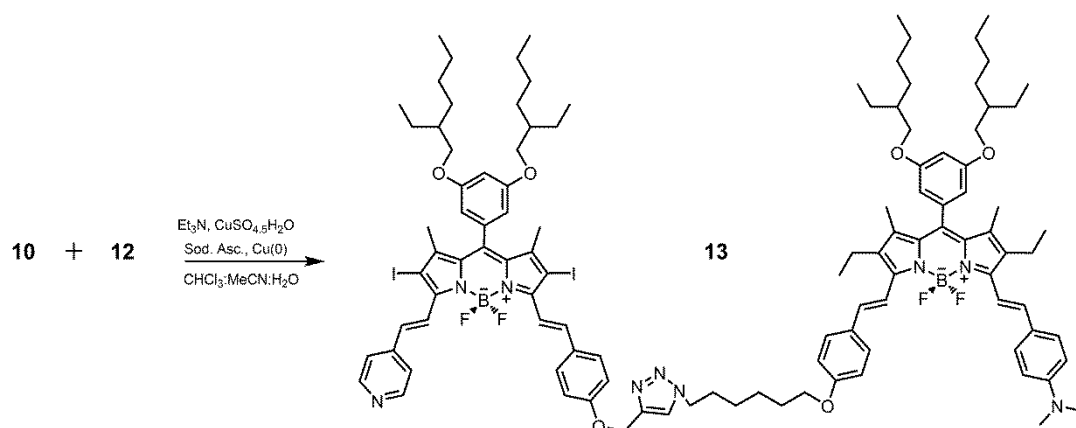


Synthesis of Compound 12, FL: Compound **11** (30 mg, 0.039 mmol) and compound **8** (11.6 mg, 0.047 mmol) were dissolved in benzene (40 ml). Piperidine (0.2 ml) and acetic acid (0.2 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CHCl_3 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using $\text{CHCl}_3/\text{Hexane}$ (75:25, v/v). Fraction containing compound **12** (**FL**) was collected then the solvent was removed under reduced pressure (0.022 mmol, 21.5 mg, 56 %).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.70 (d, 1H, $J = 16.6$ Hz; CH), 7.65(d, 1H, $J = 17.0$ Hz; CH). 7.56(m, 4H; ArH), 7.27(d, 1H, $J = 18.0$ Hz; CH), 7.18(d, 1H, $J = 16.6$ Hz; CH), 6.94(d, 2H, $J = 8.4$ Hz; ArH), 6.75(d, 2H, $J = 8.2$ Hz; ArH), 6.58(s, 1H; ArH), 6.49(s, 2H; ArH), 4.03(t, 2H, $J = 6.4$ Hz; OCH_2CH_2), 3.85(d, 4H, $J = 5.8$ Hz; OCH_2CH), 3.31(t, 2H, $J = 6.8$ Hz; CH_2N_3), 3.07(s, 6H; $\text{N}(\text{CH}_3)_2$), 2.65(m, 4H; ArCH_2CH_3), 1.85(m, 2H; OCH_2CH_2), 1.2-1.9(m, 24H; CH_2), 1.51(s, 6H; ArCH_3), 1.20(t, 6H, $J = 6.5$ Hz; ArCH_2CH_3), 0.95(m, 12H; CH_3)

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 161.4, 159.4, 151.8, 150.9, 149.1, 139.0, 137.7, 137.1, 136.7, 134.4, 133.7, 133.0, 132.3, 131.7, 130.6, 128.9, 128.7, 125.9, 124.6, 118.4, 115.8, 114.7, 112.2, 107.2, 102.2, 71.1, 67.9, 51.4, 40.4, 39.4, 30.5, 29.2, 29.1, 28.8, 26.5, 25.7, 23.8, 23.0, 18.5, 14.1, 14.1.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{61}\text{H}_{83}\text{BF}_2\text{N}_6\text{O}_3$: 996.65878 [M]; found: 996.67410 [M], $\Delta = 15.4$ ppm.



Synthesis of Compound 13, the Molecular Demultiplexer: **10 (PS)** (22 mg, 21 μ mol) and **12 (FL)** (16 mg, 16 μ mol) were dissolved in CHCl_3 /acetonitrile mixture (10 ml: 10 ml). Triethylamine (20 μ l) was added. Saturated aqueous solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1 ml) and Sodium ascorbate (1 ml) were prepared in separate vials. Then, 0.15 ml of each solution were added to reaction mixture. A small piece of Cu (0) wire was added. The mixture was stirred overnight at room temperature. Then, it was extracted using CHCl_3 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl_3 /MetOH (96:4, v/v). Fraction containing **13** was collected then the solvent was removed under reduced pressure (10 μ mol, 21 mg, 63%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.67(d, 2H, $J = 6.1$ Hz; ArH), 8.23(d, 1H, $J = 16.4$ Hz; CH), 8.00(d, 1H, $J = 16.8$ Hz; CH), 7.85(d, 1H, $J = 16.8$ Hz; CH), 7.69(d, 1H, $J = 17.2$ Hz; CH) 7.8-7.4(m, 11H; CH, ArH), 7.25(d, 1H, $J = 16.6$ Hz; CH), 7.17(d, 1H, $J = 17.1$ Hz; CH), 7.08(d, 2H, $J = 8.8$ Hz; ArH), 6.92(d, 2H, $J = 8.8$ Hz; ArH), 6.73(d, 2H, $J = 8.9$ Hz; ArH), 6.61(d, 2H, $J = 2.2$ Hz; ArH), 6.57(d, 2H, $J = 2.2$ Hz; ArH), 6.47(d, 2H, $J = 2.2$ Hz; ArH), 6.42(d, 2H, $J = 2.2$ Hz; ArH), 5.30(s, 2H; OCH_2), 4.42(t, 2H, $J = 7.2$ Hz; NCH_2), 4.02(t, 2H, $J = 6.3$ Hz; OCH_2CH_2), 3.85(m, 8H; OCH_2CH), 3.03(s, 6H; $\text{N}(\text{CH}_3)_2$), 2.63(m, 4H; ArCH_2CH_2), 2.00(m, 2H; NCH_2CH_2), 1.82(m, 2H; OCH_2CH_2), 1.8-1.7(m, 4H; CH_2), 1.70(s, 3H; ArCH_3), 1.68(s, 3H; ArCH_3), 1.52(s, 6H; ArCH_3), 1.5-1.2(m, 32H; CH_2), 1.16(m, 6H; ArCH_2CH_3), 1.0-0.8 (m, 24H; CH_3)

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 161.8, 161.3, 159.7, 159.4, 150.9, 150.2, 143.8, 140.8, 137.7, 136.8, 136.2, 134.8, 134.3, 133.7, 133.0, 130.6, 129.8, 128.9, 128.7, 125.8, 122.6, 121.5, 118.4, 116.7, 115.8, 115.2, 114.8, 112.1, 107.2, 106.3, 102.7, 102.2, 71.1, 67.7, 62.2, 50.4, 40.3, 39.4, 30.5, 30.2, 29.7, 29.1, 26.3, 25.6, 23.8, 23.0, 18.5, 17.6, 17.2, 14.1, 11.3, 11.1, 8.6.

CHAPTER 4

Control of PDT Action with an Implemented AND Logic Operation; Glutathione and pH Dual Activatable Photosensitizer

Sundus Erbas-Cakmak, Fatma Pir, Engin U. Akkaya

4.1 Objectives

Molecular logic gates are primitive mimics of their electronic counterparts and are a promising research area. Even a number of two input logic operations and a number of more advanced operations such as key-pad-lock³, flip-flop logics² are constructed; their use in place of traditional computational methodology is still not realistic. On the contrary, use of such logic operations in other research areas such as in biology, using wet chemistry is highly promising since biochemical processes has this intrinsic property as well. In the research presented in this part, an AND logic gate is designed with a photosensitizer to control its activity in a biologically meaning media. Two inputs glutathione (GSH) and acidity, associated with cancer are used to control the selective activation of photosensitizer under real disease conditions.

4.2 Introduction

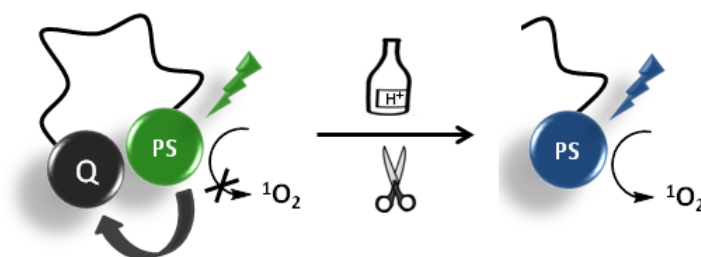
Use of molecular logic gates for diagnosis¹⁴⁹ and therapeutic^{16,17} purposes is introduced into the literature by a number of different groups. Among the examples, control of singlet oxygen generation through an AND logic gate is remarkable.¹⁵ In this work, photodynamic activity depends on the sodium dependent abolishing of PET process and protonation induced spectral shift in absorbance. Although choice of inputs are realistic in this proof-of-principle study, the response range is not meaningful since neither the sodium concentration is that high nor the pH is that low

in tumor tissue. In another proof-of concept work, the DEMUX¹²⁴ logic gate is implemented on a photosensitizer and fluorophore and the presence of acid enables the switch between emission to photodynamic activity under irradiation which is interpreted as imaging and therapy. Still, the inputs are beyond the range of biological importance and for realistic arguments, the inputs should be chosen rationally or the sensitivity of the receptor to those inputs should be optimized accordingly.

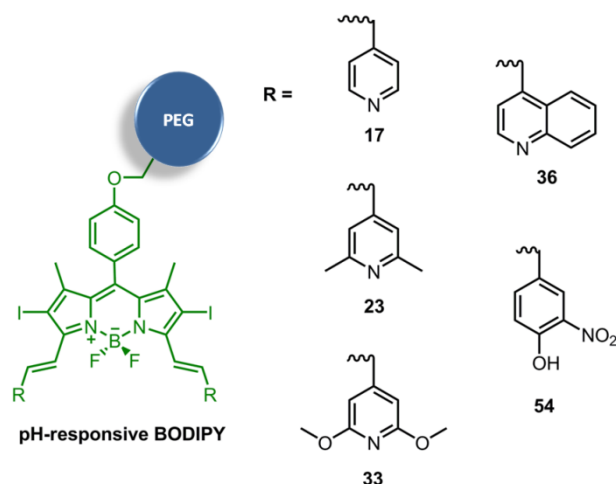
Considering this, the project aims to optimize the input responsiveness of the logic gate to develop a more realistic activatable photosensitizer with a potential use as photodynamic therapeutic agent.

4.3 Design of the AND Logic Gate

Molecular AND logic construct of photosensitizer is designed taking the two of the cancer related input into consideration; high glutathione concentration and low pH. Upon these inputs, pH sensitivity is attained by an ICT character on the BODIPY by means of pH responsive functional groups. GSH responsiveness is maintained by building an electronic energy transfer system with the use of a lower energy BODIPY chromophore to quench the photosensitizer before the generation of toxic ¹O₂. Scheme 18 shows representative activation of the photosensitizer.

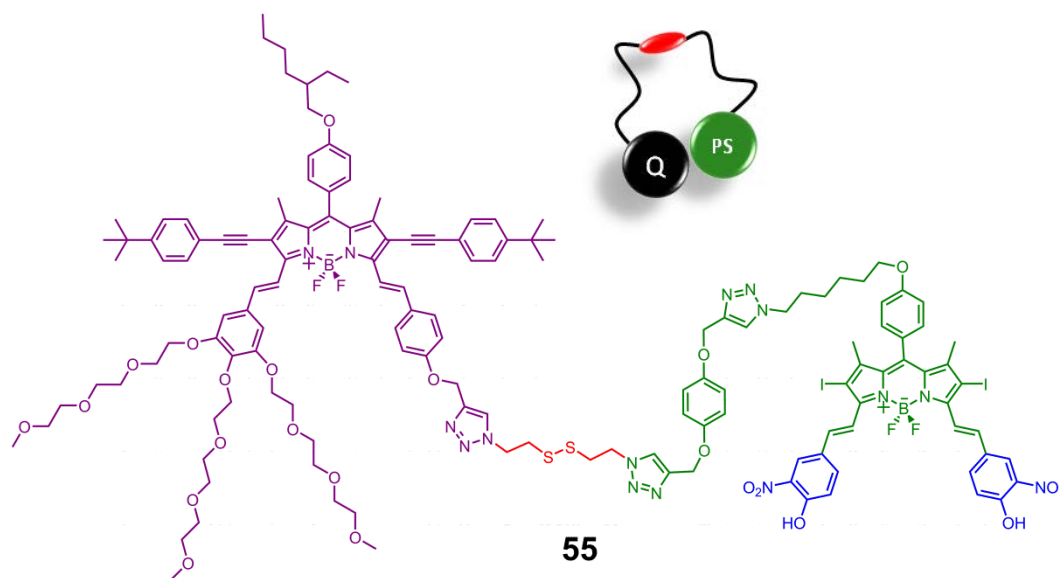


Scheme 18. Representative illustration of cleavage of the disulfide bond and protonation of the photosensitizer by glutathione and acid respectively.



Scheme 19. Structures of compounds bearing different pH-sensitive groups

A spectral shift with a huge extinction coefficient change upon protonation is required for the photosensitizer to be activated only in the acidic conditions. Besides, the pKa of the compound should be in biologically relevant range which is above 6.5 for most tumors.¹¹⁷ Most of the pH responsive groups in literature have a pKa value below 6 which makes them unsuitable for therapeutic purposes unless the highly acidic intracellular organelles such as lysosome are targeted. To optimize the pKa value, a number of different water soluble compounds have been synthesized. The semi-schematic representations of these compounds are given in Scheme 19, the complete chemical structures and their synthesis can be found in experimental part of this chapter. General approach for pKa optimization in the project is to incorporate electron donating or withdrawing groups into the structure to attain desired sensitivity and spectral properties.



Scheme 20. Chemical structure of AND logic construct of photosensitizer with GSH and pH responsive moieties shown in red and blue respectively.

With the intention of GSH responsiveness, a near-IR absorbing energy acceptor BODIPY dye with an appropriate spectral character for EET is attached to the photosensitizer through a bioreducible disulfide linker. For reasonable reaction conditions the quenching module and the photosensitizer are attached to one another through disulfide bridge using Huisgen 1,3-dipolar cycloaddition, namely ‘click’ reaction. The BODIPY dye used as an energy sink is prepared by the Sonogashira coupling at 2,6-positions together with Knoevenagel condensation to increase the conjugation further.

The structure of the final AND logic construct is given in Scheme 20 where each functional moiety is color-coded. The quencher moiety is shown in purple, the reducible disulfide bridge in red, the photosensitizer in green and finally the pH responsive phenolic moiety in blue. To increase the solubility of the compound throughout the reaction pathways, triethyleneglycol and 2-ethylhexyl moieties are attached to the structure.

4.4 Experimental Results and Discussion

4.4.1 pH Optimization

With the pH of tumor tissue in mind, water soluble BODIPYs with pH responsive groups with an ICT character have been synthesized and the pKa values are determined in aqueous solutions. As a start, compound **17** a distyryl-BODIPY with 4-pyridinecarboxaldehyde was synthesized and the pKa value is determined to be 3.42 with a protonation-induced bathochromic shift from 594 nm to 615 nm (Figure 16). Since the pH of biological media is not suitable for the protonation of the PS, a strategy to increase the pKa has been adapted. This strategy is basically to attach electron donating groups into the structure of the pH responsive moiety to enhance its basicity and as a consequence to raise the pKa. As electron donating group methyl and methoxy set have been chosen and 2,6-modified pyridinecarboxaldehyde were synthesized bearing these groups.

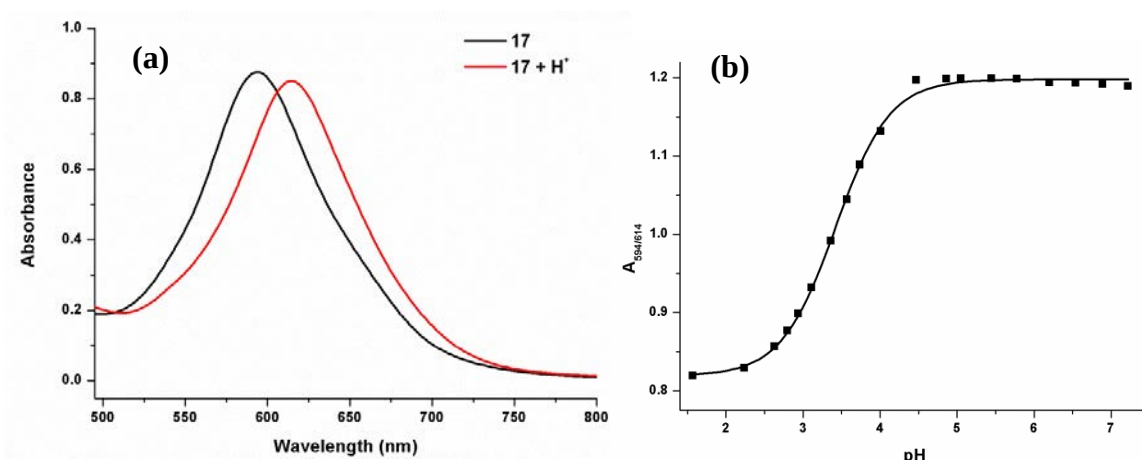


Figure 16. Electronic absorption spectra of protonated and neutral compound **17** in water (a) and the pH titration curve of the same compound (b).

Upon these candidates, compound **23** with 2,6-methylpyridine in its structure is able to show the desired spectral shift in absorbance, namely, protonation induces a large hypsochromic shift in absorbance from 723 nm to 601 nm (Figure 17). However, the pKa of the compound is experimentally determined to be 4.21 and the compound is pH-responsive essentially in the range 3.5 to 5. Although, consistent with the design

strategy an increase in pKa is observed, the value it reaches is not still truly biologically significant.

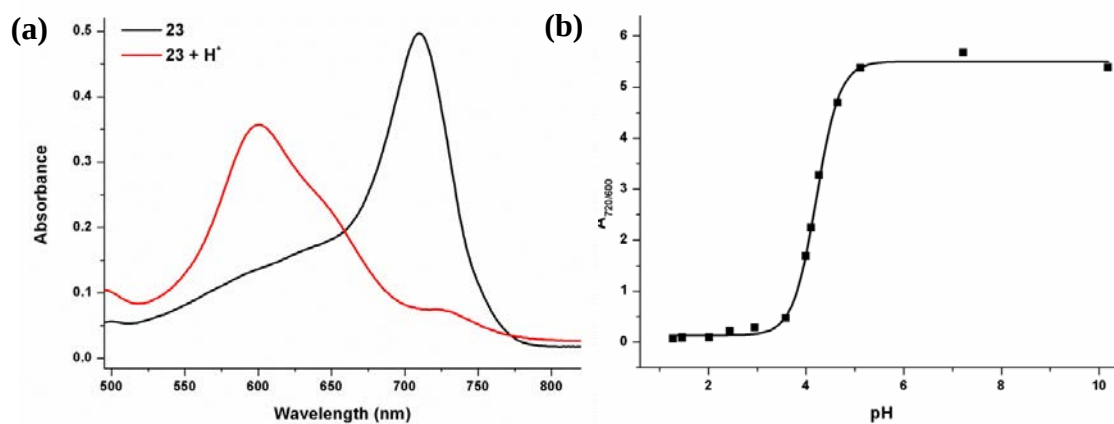


Figure 17. Electronic absorption spectra of protonated and neutral compound **23** in water (a) and the pH titration curve of the same compound (b).

The PS with 2,6-dimethoxypyridine, compound **33** fails to display a observable spectral shift upon protonation below pH 8 and the pKa can not be determined using UV-Vis absorbance since the compound decomposes rapidly at higher pH solutions. (Figure 18). The compound absorbs maximally at 626 nm.

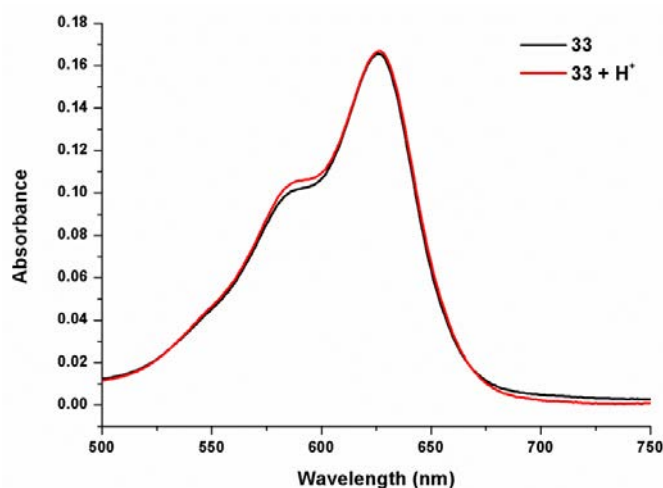


Figure 18. Electronic absorption spectra of protonated and neutral compound **33** in 40% THF in water.

Since the quinoline has a pKa of 4.85¹⁵⁰ and the value for the related compound 4-methylquinoline (lepidine) is 5.67¹⁵¹, the BODIPY construct of this nominee worths trying. For this reason, compound **36** is synthesized. In spite of the applicable spectral shift in absorbance upon protonation, the compound fails to display a significant pKa (Figure 19). The pKa is calculated to be 2.62 and the absorbance maxima reallocates from 636 nm to 660 nm upon protonation in 40% THF in water. Thus the compound has not met the criteria of the desired PS.

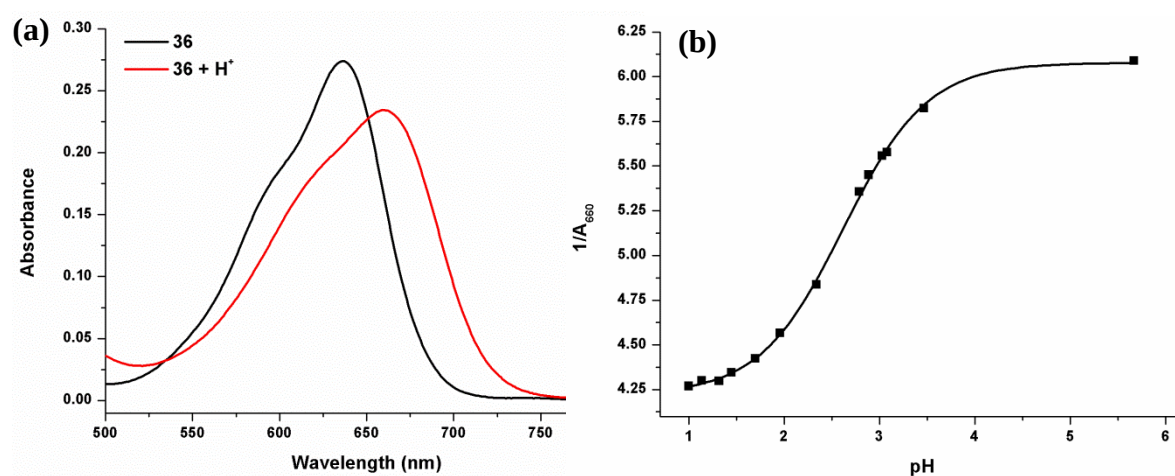


Figure 19. Electronic absorption spectra of protonated and neutral compound **36** in water (a) and the pH titration curve of the same compound (b).

In literature, monostyryl BODIPY with a 3-chloro-4-hydroxyphenyl moiety was reported to have a pKa of 7.6.⁷⁵ As a final attempt, a new version of this compound with an improved electron withdrawing group is designed with an expectation of decrease in pKa. Compound **38** and **54** with a nitro group in place of chloro is synthesized in view of that. The pH response of compound **38** is investigated within a micelle in aqueous solution since this compound and the final AND logic gate construct is not soluble in water. Fortunately and with the exertion of rational design, the compound is determined to have a pKa assessment of 6.92 in cremophor EL micelle with a tremendous spectral change in absorbance from 649 nm to 730 nm on account of deprotonation (Figure 20).

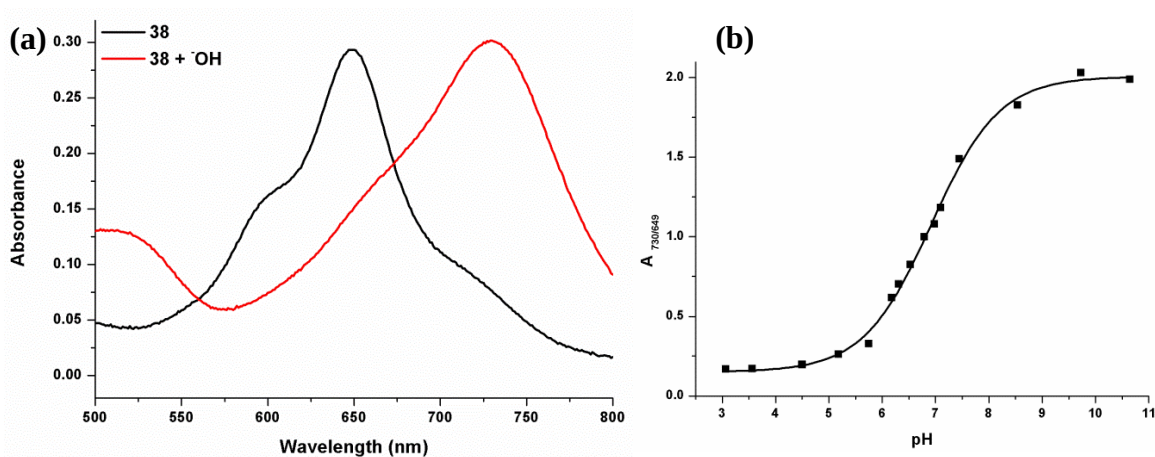


Figure 20. Electronic absorption spectra of deprotonated and neutral compound **38** in micelle in water (a) and the pH titration curve of the same compound (b).

To understand if the pH response is consistent in non-micellar system, a water soluble version (compound **54**) is pH-titrated in 40% THF in water and the pKa is determined to be 6.62 (Figure 21). 0.3 unit difference may result from the fact that, relatively more hydrophobic microenvironment within the micelle may encumber the deprotonation process since the charged species can not solvated easily and the process is less favourable accordingly. The absorption spectrum of deprotonated compound is essentially the same as in micelle. The pKa values and pH dependent spectral shifts in absorption of all compounds are given in Table 4.

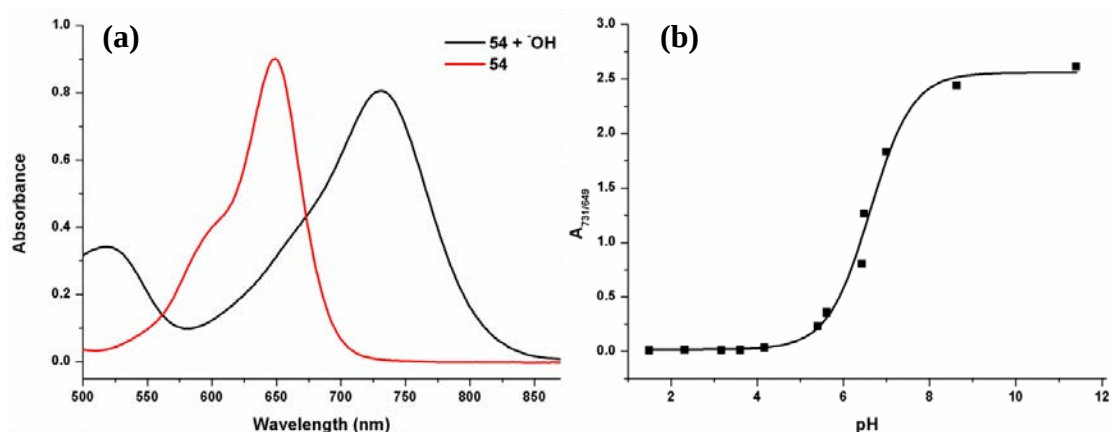


Figure 21. Electronic absorption spectra of deprotonated and neutral compound **54** in 40% THF in water (a), and the pH titration curve of the same compound (b).

Table 4. Absorbance values of compounds in water

Compound	$\lambda_{\max 1}^a$	$\lambda_{\max 2}^b$	pKa
17	615	594	3.42 ^c
23	601	723	4.21 ^c
33	626	626	- ^d
36	660	636	2.62 ^e
38	649	730	6.92 ^f
54	649	731	6.62 ^c

^a the value for fully protonated compound, ^b value for fully deprotonated compound, ^c the measurement was done in water, ^d the value can not be calculated accurately since the compound decomposes beyond pH 8 in 40% THF in water, ^e the measurement was done in 40% THF in water, ^f the measurement was done in water using the micellar form of the compound.

With the results obtained from pKa optimization study as described above, pH dependent component of the molecular AND logic gate is built with 4-hydroxy-3-nitrophenyl moiety.

4.4.2 Photophysical Characterization

UV-Vis absorption and emission spectra of deprotonated and neutral forms of compounds **38**, **51** and **55** in THF are given in Figure 22, Table 5. Energy transfer to a chromophore in the vicinity is expected to compete singlet oxygen generation by the photosensitizer. For that, the emission of the donor (photosensitizer) has to have spectral overlap with the absorption of the energy acceptor (quencher) in close proximity. As shown in Figure 23, this criterion is met in the molecule. Absorption of the quencher part, as represented by compound **51** overlaps well with the emission of photosensitizer part (compound **38**). In deprotonated forms, the spectral compatibility is less pronounced, however since in this form the compound is not truly excited at the chosen wavelength (625 nm LED is used for photodynamic measurements), and is already quenched by other deactivation pathways (as observed by a dramatic decrease in emission) this incoherence is not an immense drawback.

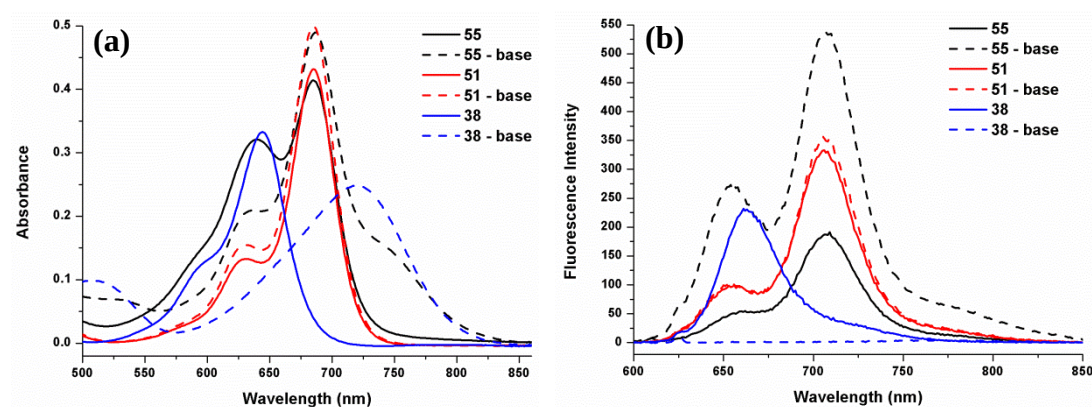


Figure 22. Electronic absorption (a) and fluorescence (b) spectra of 7.5 μ M of neutral (solid) and deprotonated (dash) compounds **55** (black), **51** (red) and **38** (blue) in THF. Piperidine is used to deprotonate the compounds and the excitation is done at 625 for fluorescence measurements.

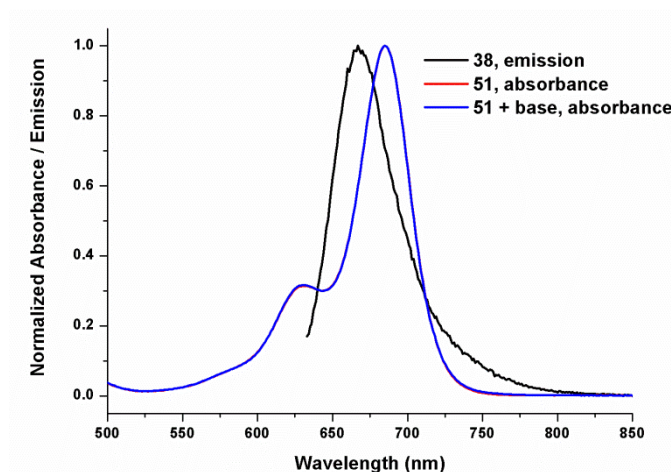


Figure 23. Normalized electronic absorption spectra of compound **51** in neutral (red) and basic (blue) conditions, and emission spectra of compounds **38** in THF. Piperidine is used as base and the compound **38** is excited at 625 nm for fluorescence measurements.

Table 5. Photophysical characterization of compounds **38**, **51** and **55** in THF.

Compound	Absorption		Emission		ϵ ($M^{-1}cm^{-1}$)		ϕ_F	
	λ_{max1}^a	λ_{max2}^b	λ_{max1}^a	λ_{max2}^b	λ_{max1}^a	λ_{max2}^b	λ_{max1}^a	λ_{max2}^b
38	645	720	667	-	40000	30000	0.13	-
51	685	685	707	-	57000	63000	0.76	-
55	640	687	654	707	42000	70000	0.24	0.15
	685		707		56000			

^a the value for fully protonated compound, ^b value for fully deprotonated compound.

The UV-Vis absorption of micellar compound **55** in water is given in Figure 24a. Two peaks corresponding to two absorbing modules converge to give an almost single one at higher wavelength upon deprotonation. For equal concentrations of compounds **55** and **38**, emission spectra shows a decrease in the emission of PS part compared to free compound **38**, Figure 24b, which indicates energy transfer. Since the deprotonated form of free PS, compound **38** is non-emissive, the same analysis can not be performed for this form. The energy transfer is not proved clearly with excitation spectra, no observable change is detected which may be due to low emission intensity of each module and close absorption properties.

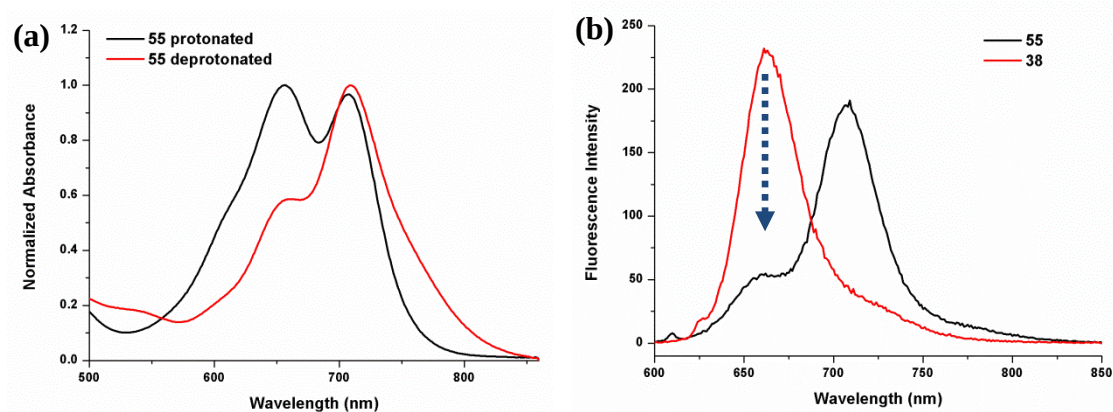


Figure 24. Electronic absorption spectra of neutral (black) and deprotonated (red) forms of micellar compound **55** in aqueous solution (a), comparison of fluorescence spectra of neutral compounds **38** and **55** in micelle in water (excited at 625 nm).

4.4.3 Analysis of Reduction by Glutathione

The cleavage of the disulfide bond is analyzed by incubating the micellar compound **55** for 12 hour at room temperature and comparing it with the GSH-free compound **55** both through spectroscopic analysis and High Resolution Mass Spectra (HRMS). As shown in Figures 25 and 26, the compound is successfully cleaved by GSH and thiol form of the free photosensitizer and disulfide form of quencher are detected by HRMS.

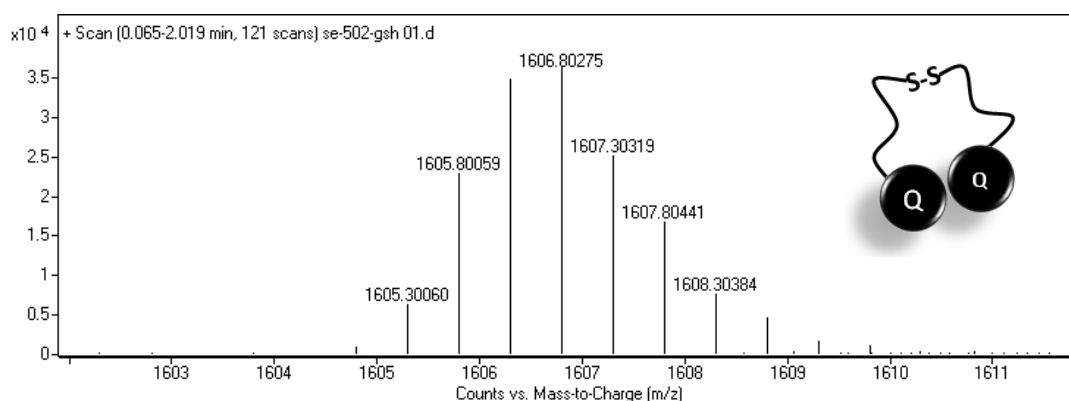


Figure 25. The cleavage of quencher from the photosensitizer as analyzed by HRMS, ($\Delta = 8.8$ ppm) after incubation with GSH for 12 h.

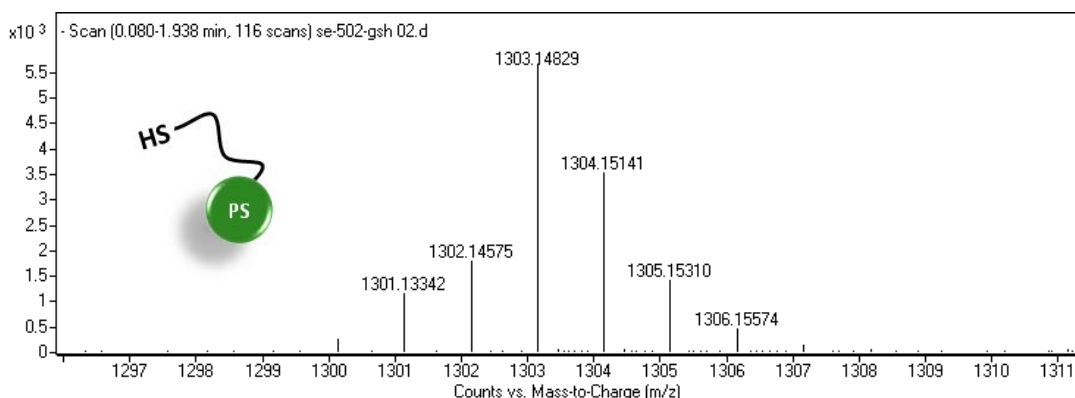


Figure 26. The cleavage of quencher from the photosensitizer as analyzed by HRMS, ($\Delta = 4.65$ ppm) after incubation with GSH for 12 h.

After resolving the reduction of the disulfide linker by GSH through HRMS analysis, a spectral examination is also performed to understand energy transfer. Since the EET efficiency is expected to decrease upon release of the energy donor part, the emission of this part is predicted to increase. Unsurprisingly, an increase in emission of the photosensitizer part is clearly observed in fluorescence spectra, Figure 27a, which indicates that the energy transfer is less effective in the free form. However, the excitation spectra do not show a clear distinction between GSH containing and GSH-free samples (Figure 27b).

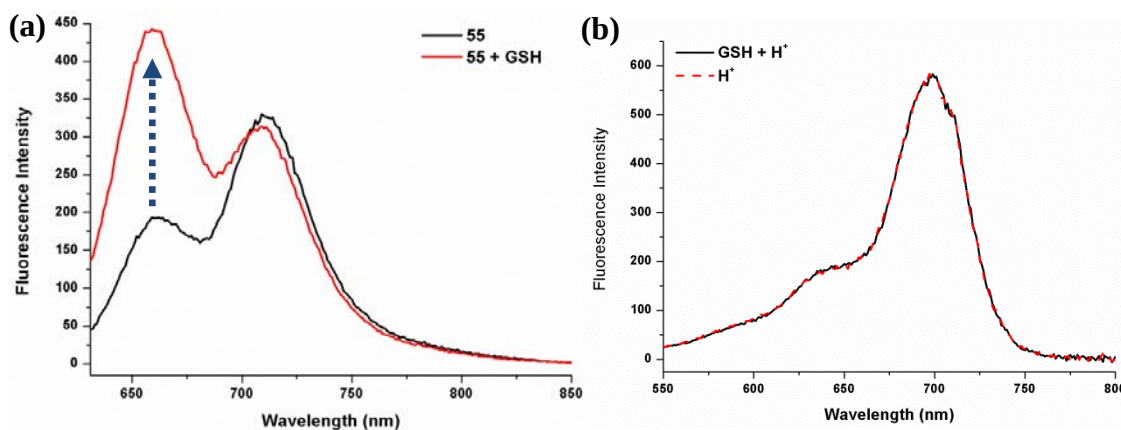


Figure 27. Emission spectra of compound **55** in the absence (black) and the presence of GSH (red) in micelle, in water. The spectrum is taken by excitation at 625 nm. The compound **55** is incubated with 2.5 equivalents of GSH over 12 h in water (a). Excitation spectra of micellar compound **55** in the presence (black, solid) and absence (red, dashed) of GSH in acidic aqueous solution (b).

4.4.4 Photodynamic Activity Measurements and AND Logic Gate

In order the compound to behave as an AND logic operation it needs to show more efficient photodynamic activity in the presence of two inputs, GSH and acid. The photodynamic activities are measured with the micellar forms of the compounds **38** and **55** in water and pH was adjusted to deprotonate the compounds in certain experiments. Compound **38** is used for the GSH-cleaved situation since the presence of excess GSH interferes with the singlet oxygen generation experiments.

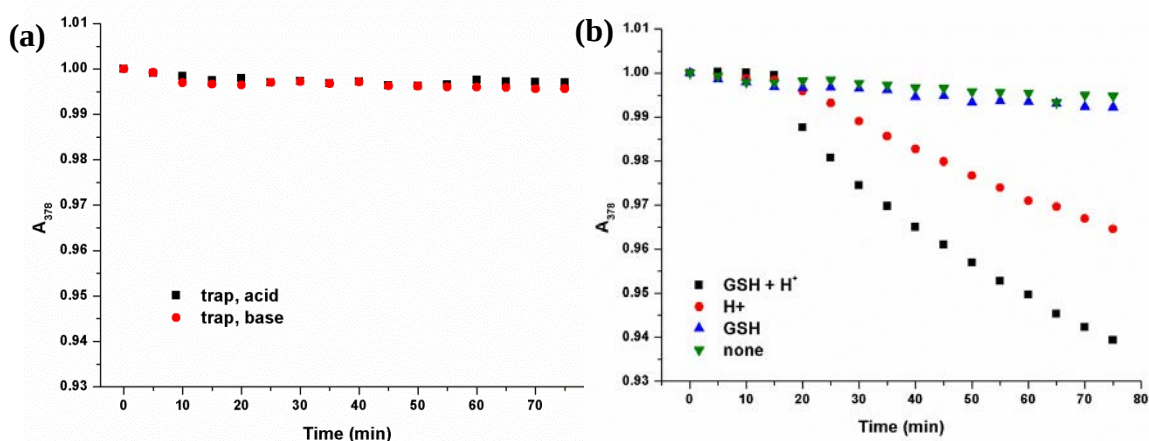


Figure 28. Control experiment with the solution containing trap molecule only in acidic (black) and basic (red) aqueous conditions (a) Comparison of $^1\text{O}_2$ generation of micellar forms of molecular AND logic construct (7.5 μM) in the presence of different combinations of inputs as followed by the decrease in $^1\text{O}_2$ trap absorbance at 378 nm in water (b). In the first 15 min, the samples are in dark whereas they are irradiated with a 625 nm LED from that time on.

$^1\text{O}_2$ generation experiment was done with the use of a trap molecule. The reaction of this molecule with $^1\text{O}_2$ is described in experimental section. Absorbance decrease at 378 nm is followed as a measure of $^1\text{O}_2$ production. First, to show that the trap molecule does not decompose in the absence of photosensitizer, control experiments under dark and 625 nm irradiation are performed in similar conditions. Result in Figure 28a, display that trap is stable under experimental conditions. On the other hand, the photosensitizer free from quencher shows a greater extent of $^1\text{O}_2$ in the presence of slightly acidic media, Figure 28b. Although compound **55** produces singlet oxygen to some extent in the absence of GSH, still this is less efficient

compared to free photosensitizer. The results are depicted as relative $^1\text{O}_2$ generation efficiency in Figure 29 as measured by percent decrease of trap absorption at 378 nm in each experimental condition. The threshold value of $^1\text{O}_2$ generation efficiency for AND logic gate is determined to be 4 and none of the input combinations but one manage to reach this value. Thus, the PS actively produces $^1\text{O}_2$ only in the presence of both inputs acid and GSH, as expected.

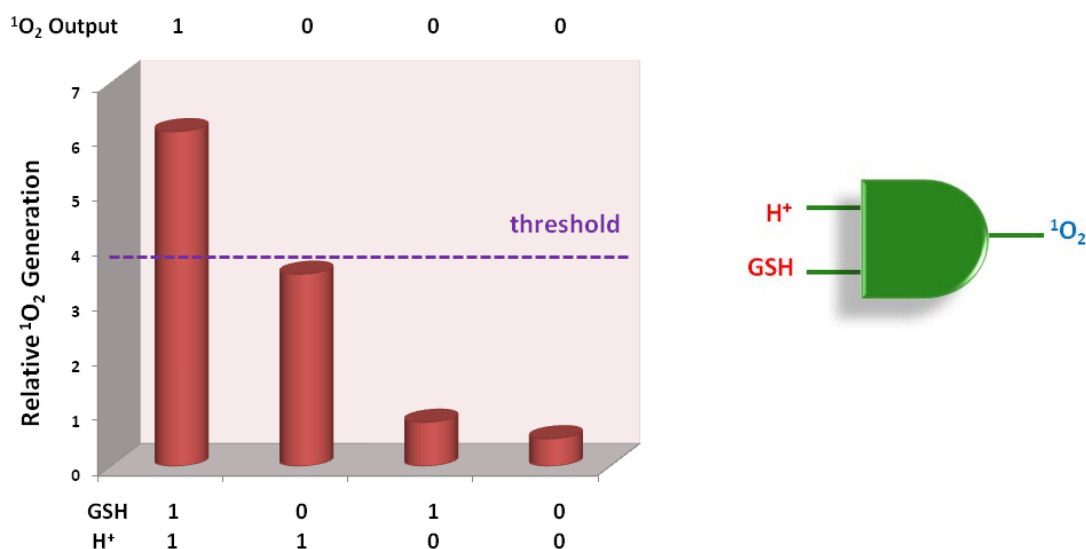


Figure 29. Comparison of relative $^1\text{O}_2$ generation as measured by the percent decrease in absorbance of trap molecule and the threshold level determined for AND logic.

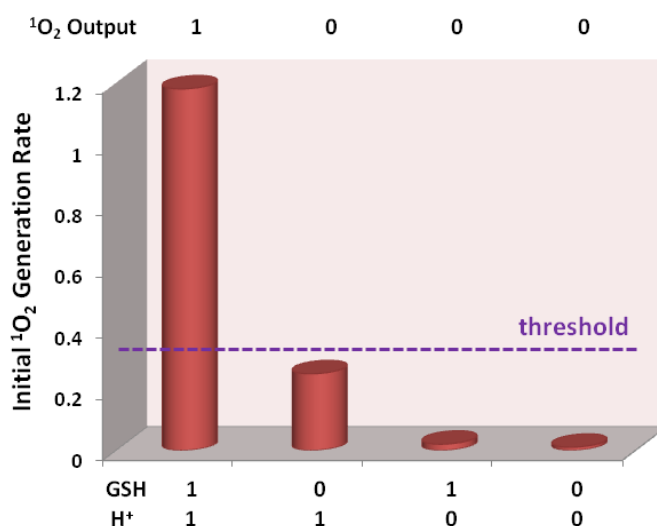


Figure 30. Comparison of initial $^1\text{O}_2$ generation rate as measured by the percent decrease in absorbance of trap molecule within 5 min of light irradiation.

Looking at the initial rates of $^1\text{O}_2$ production, GSH and H^+ both input case shows a distinct rate compared to other combinations of inputs (Figure 30). Since the $^1\text{O}_2$ generation is dependent on the dissolved O_2 concentration, the PDT action slows down in time. In real tissue, the O_2 supply is expected to be constant within the time scale of experiment. Thus, initial rates may be more valuable to compare the relative singlet oxygen generation efficiency.

4.5 Experimental Details

4.5.1 pKa Determination of Compounds 17, 23, 33, 36, 38 and 54

Aqueous solutions of each compound were prepared and were titrated with aliquots of acid (HCl) and base (NaOH) solutions. Each time, pH of the solution was measured with the aid of a pH meter and the spectra are recorded. Since there are two different absorbing species, one protonated/deprotonated and one neutral, two different peak absorbance wavelengths are observed. Plotting pH versus the ratio of absorbance at these two wavelengths and subsequent non-linear curve fit in *Origin* software gives the experimental pKa values in water. For some compounds **33**, **36** and **54** 40% THF was used to increase solubility.

Since compound **38** is the true module of the target compound (compound **55**) and is not soluble in water, the pKa measurements were performed after the formation of micelle.

4.5.2 Preparation of Micelle

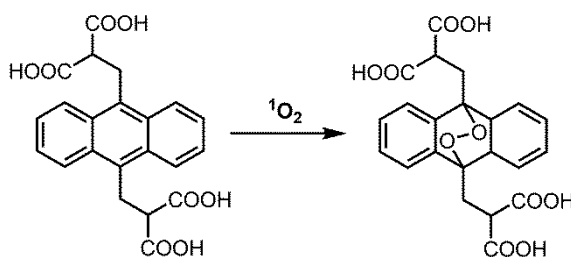
Micelles of compounds **38** were prepared with Cremophor EL using the procedure in literature.¹⁵² 50 mg Cremophor EL and compound **38** (6 mg, 5 μmol) or compound **55** (14 mg, 5 μmol) were dissolved in 330 ml freshly distilled tetrahydrofuran. The solution was sonicated for 30 min, while the sonication water bath was kept below 35 $^{\circ}\text{C}$. Then, THF was evaporated under reduced pressure and the remaining compounds were dissolved in water (5 ml). The suspension was filtered through 0.45 μm PTFE filter. For each measurements micelles were prepared freshly. Concentrations of

solutions of the compounds in micelles were predicted using their extinction coefficients in THF.

GSH (2.5 equivalent) was added to the solution of compound 55 before the preparation of the micelle and the sample was incubated at RT for 12 h, before HRMS and spectroscopic analysis were performed.

4.5.3 $^1\text{O}_2$ Generation Experiments

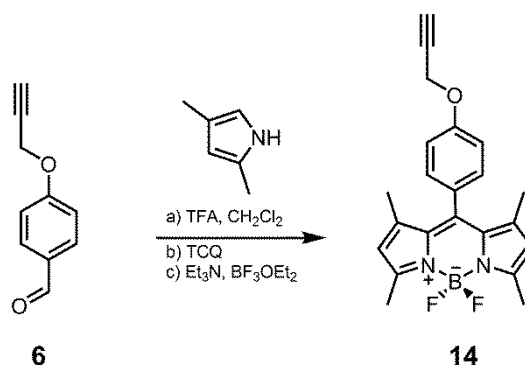
$^1\text{O}_2$ dependent degradation of water soluble trap, 2,2'-(anthracene-9,10-diylbis(methylene)dimalonic acid was used to measure photodynamic activity since the absorption of this compound decreases indicating the generation of $^1\text{O}_2$.¹⁵³ Corresponding reaction is given in Scheme 21.



Scheme 21. Reaction of trap molecule with $^1\text{O}_2$.

Since the water solubility of the anthracene-based trap is poor in water, samples were sonicated for 15 min to obtain clear solutions. Measurements were performed using 625 nm LED and samples were irradiated with the light source from a 5 cm distance. All samples were aerated for 5 min prior to experiments. After incubation under dark for 15 min, light was irradiated for 60 min and UV-Vis spectra were recorded at each 5 min intervals. Relative singlet oxygen efficiency was calculated by the percent decrease in trap absorbance at 378 nm within 60 min light irradiation period.

4.5.4 Synthesis

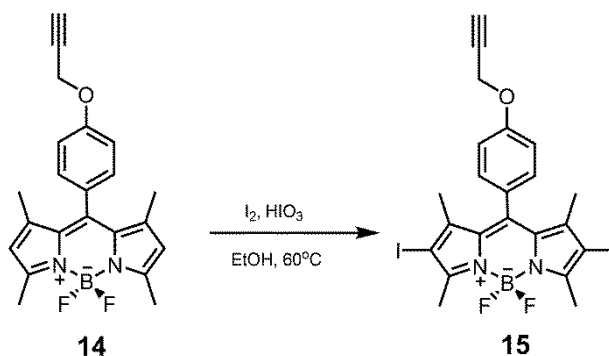


Synthesis of Compound 14¹⁵⁴: CH₂Cl₂ (300 ml) was purged with Ar for 30 min. 4-(prop-2-yn-1-yloxy) benzaldehyde **6** (1 g, 6.2 mmol) and 2,4-dimethyl pyrrole (1.2 g, 12.6 mmol) were added. The color of the solution turned into red after the addition of 3 drops of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12h. Then, tetrachloro-1,4-benzoquinone (1.52 g, 6.2 mmol) was added and the reaction mixture was stirred at room temperature for 45 min. Then triethyl amine (6 ml) and boron trifluoride diethyl etherate (6 ml) were added sequentially. After stirring at room temperature for 30 min, it was extracted with water. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃. Fraction containing compound **14** was collected then the solvent was removed under reduced pressure (260 mg, 0.68 mmol, 11%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 1.43 (s, 6H), 2.56-2.58 (m, 6H+1H), 4.77 (d, J =2.4 Hz, 2H), 5.99 (s, 2H), 7.10 (d, J =8.7 Hz), 7.21 (d, J =8.7 Hz, 2H).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 14.5, 14.6, 56.0, 75.9, 78.0, 115.6, 121.2, 128.0, 129.2, 131.8, 141.5, 143.1, 155.4, 158.1.

HRMS-ESI: calculated for M+Na 401.1617, found 401.1644, Δ = 6.7 ppm.

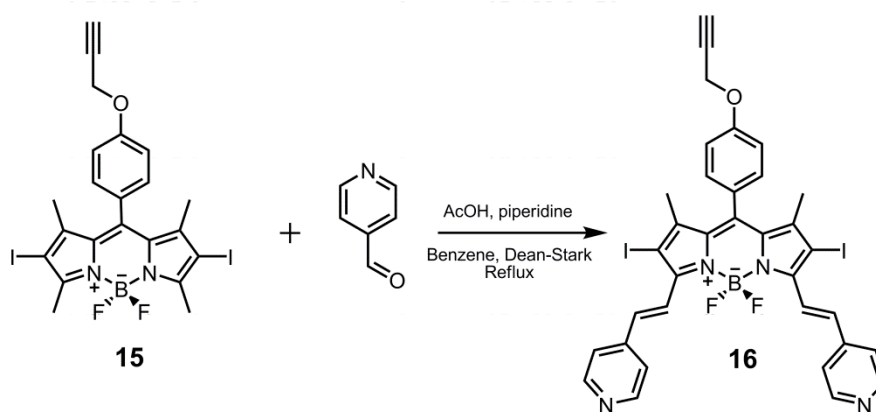


Synthesis of Compound 15: 1,3,5,7-Tetramethyl-8-(4-Propargyloxyphenyl)-4,4-difloroboradiaza-s-indacene **14** (150 mg, 0.39 mmol) and I_2 (249 mg, 0.99 mmol) were dissolved in ethanol (100 ml). Iodic acid, HIO_3 (138 mg, 0.78 mmol) was dissolved in a few drops of water and added into previous solution. The reaction mixture was stirred at 60°C for a few hours until all reactant was consumed. Then, saturated sodium thiosulfate solution was added (50 ml) and it was stirred at room temperature for additional 30 min. Then, it was extracted with CHCl_3 and water. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $\text{CHCl}_3/\text{Hexane}$ (50/50, v/v). Fraction containing compound **15** was collected then the solvent was removed under reduced pressure (218 mg, 0.35 mmol, 90 %).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 1.36 (s, 6H), 2.49 (s, 1H), 2.56 (s, 6H), 4.70 (s, 2H), 7.04 (d, $J=8.8$ Hz, 2H), 7.09 (d, $J=8.8$ Hz, 2H).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 16.0, 17.1, 56.1, 76.1, 85.8, 100.1, 116.0, 127.8, 129.1, 131.6, 141.4, 146.0, 156.7, 158.6.

HRMS-ESI: calculated for $\text{M}+\text{Na}$ 652.9550, found 652.9515, $\Delta=5.4$ ppm.

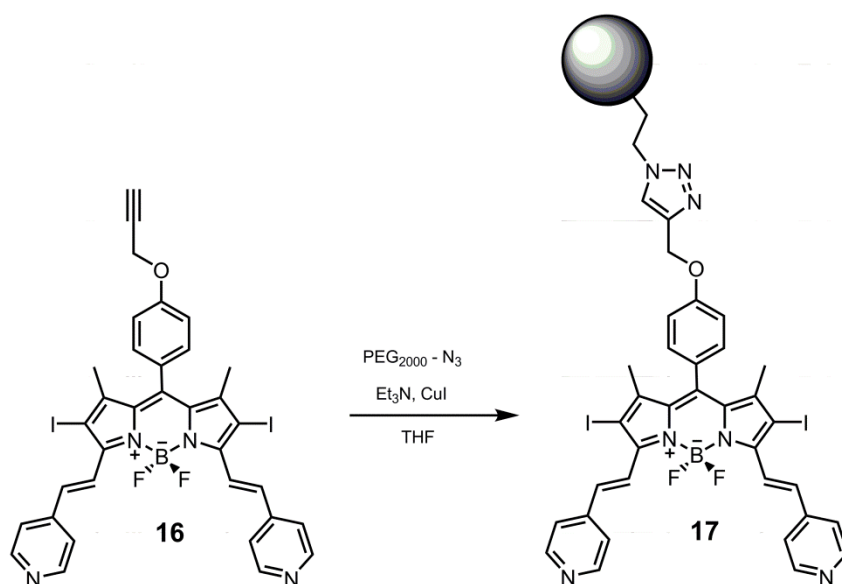


Synthesis of Compound 16: Compound **15** (120 mg, 0.19 mmol) and 4-pyridinecarboxaldehyde (51 mg, 0.48 mmol) were dissolved in benzene (40 ml). Piperidine (0.4 ml) and acetic acid (0.4 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CHCl₃ and water. Organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃/MeOH (95:5, v/v). Fraction containing compound **16** was collected then the solvent was removed under reduced pressure (0.09 mmol, 75 mg, 49%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 8.69 (d, 2H, J = 6.0 Hz; CH), 8.08 (d, 2H, J = 16.7 Hz; ArH), 7.84 (d, 2H, J = 16.7 Hz; CH), 7.50 (d, 4H, J = 6.0 Hz; ArH), 7.22 (d, 2H, J = 8.4 Hz; ArH), 7.18 (d, 2H, J = 8.4 Hz; ArH), 4.81 (d, 2H, J = 2.4 Hz; OCH₂), 2.60 (t, 1H, J = 2.4 Hz; OCH₂CH), 1.50 (s, 6H; ArCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 158.8, 150.3, 149.9, 147.1, 143.7, 141.4, 136.6, 133.9, 129.3, 127.5, 122.8, 121.5, 116.2, 83.7, 77.7, 76.2, 56.1, 17.8.

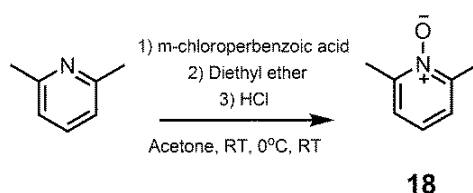
HRMS (TOF-ESI): m/z calcd for C₃₄H₂₅BF₂I₂N₄O: 809.0252 [M+H]⁺; found: 809.0290 [M+H]⁺, Δ = 4.70 ppm.



Synthesis of Compound 17: Compound **16** (50 mg, 62 μ mol) and azide functionalized polyethylene glycol monomethylether (2000MW, 124 mg, 62 μ mol)

were dissolved in tetrahydrofuran (2 ml). Triethylamine (430 μ l) and CuI (24 mg, 0.126 mmol) were added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, it was extracted with CHCl_3 and brine. The organic layer was collected and dried over Na_2SO_4 , followed by evaporation of the solvent under reduced pressure. The product was purified by silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ (95:5, v/v) as mobile phase. Fraction containing compound **17** was collected then the solvent was removed under reduced pressure (34 μ mol, 100 mg, 54%).

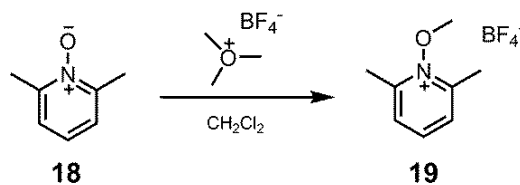
^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.62 (4H; CH), 8.04 (d, 2H, $J = 16.72$ Hz; CH), 7.80 (d, 2H, $J = 16.80$ Hz; CH), 7.51 (d, 2H, $J = 5.52$ Hz; ArH), 7.19 (2H + 2H; ArH), 5.25 (2H; OCH_2), 4.58 (t, 2H, $J = 5.08$ Hz; NCH_2), 3.90-3.30 (PEG, $\text{OCH}_2\text{CH}_2\text{O}$), 1.50 (s, 6H; ArCH_3).



Synthesis of Compound 18¹⁵⁵: 2,6-lutidine (5 g, 47 mmol) was dissolved in 60 ml acetone. m-chloroperbenzoic acid (13 g, 75 mmol) was dissolved in 60 ml acetone and was added to previous mixture dropwise during the course of 10 minutes. The reaction mixture was stirred for 90 min. at room temperature. Then, it was cooled using an ice bath for 30 min. Following this, 20 ml of ice cold diethyl ether was added and HCl gas was bubbled through the reaction for 10 min. The solid produced as a result of bubbling was filtered, washed two times with ether. Then, salt was dissolved in 20 ml of water; pH was adjusted to be above 10 using NaHCO_3 . Finally, the solution was extracted with CHCl_3 , solvent was evaporated to yield liquid colorless compound **18** (40 mmol, 4.9 g, 85%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.18 (d, 2H, $J = 7.24$ Hz; ArH), 7.00 (t, 1H, $J = 7.56$ Hz; ArH), 2.45 (s, 6H; ArCH_3).

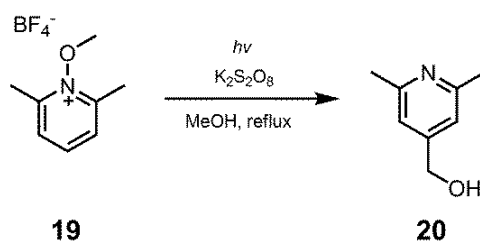
^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 148.7, 124.4, 123.8, 18.1.



Synthesis of Compound 19: Compound **18** (6 g, 32 mmol) was dissolved in 100 ml dichloromethane. Equal amount of trimethyloxonium tetrafluoroborate (32 mmol, 7.05 g) was added and the reaction mixture was stirred for 5h at RT. Solvent was vacuum evaporated to yield a white solid (quantitative, used without further purification).

^1H NMR (D_2O , 400 MHz, δ ppm) 8.18 (t, 1H, $J = 7.92$ Hz; ArH), 7.75 (d, 2H, $J = 7.96$ Hz; ArH), 4.22 (s, 3H; OCH_3), 2.81 (s, 6H; ArCH_3).

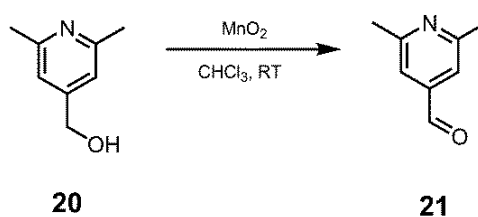
^{13}C NMR (D_2O , 400 MHz, δ ppm) 153.6, 143.8, 128.0, 66.6, 16.7.



Synthesis of Compound 20: Compound **19** (5.19 g, 23.1 mmol) was dissolved in 65 ml MeOH. Potassium peroxodisulfate (1.53 g, 5.65 mmol) was dissolved in 6 ml H_2O and was added to previous reaction mixture. The solution was refluxed for 30 min while it was irradiated with light. Following this, more of potassium peroxodisulfate (3.06 g, 11.30 mmol) was added and the reaction was refluxed for additional 30 min. The excess $\text{K}_2\text{S}_2\text{O}_8$ was filtered off and the solvent was vacuum evaporated to yield brown oil. The product was further purified by column chromatography using CHCl_3 :MeOH (90/10; v/v) as mobile phase (yellow oil, 0.86 g, 6.3 mmol, 27%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.48 (s, 2H; ArH), 4.91 (s, 1H; CH_2OH), 2.60 (s, 6H; ArCH_3).

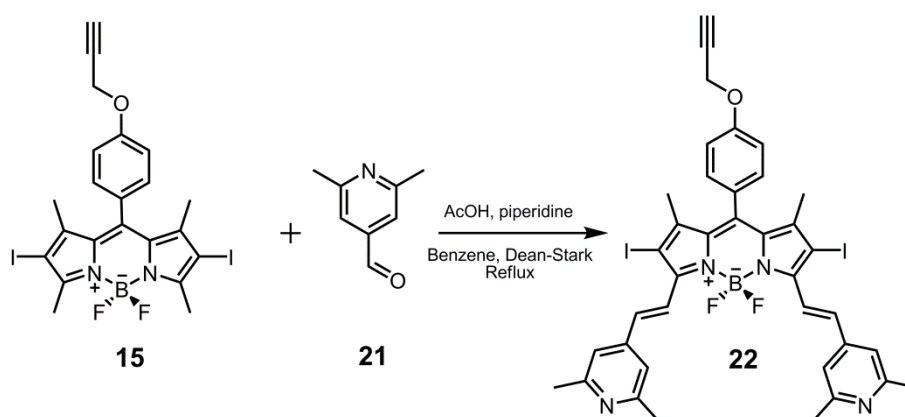
^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 161.6, 152.5, 121.1, 61.5, 18.5.



Synthesis of Compound 21¹⁵⁶: Compound **20** (864 mg, 6.3 mmol) was dissolved in 2.5 ml CHCl₃ and 1 ml methanol. The solution was heated to 35°C to dissolve the compound. Then, 1.1 equivalents of MnO₂ (0.61 g, 7 mmol) was added at RT. After stirring 2h at RT, additional amount of MnO₂ (0.51 g) was added. After 2h, the solid precipitates were removed by filtering over celite. The solvent was removed by vacuum evaporation. Then the product was purified further by precipitation of the impurities in CHCl₃. (white solid, 101 mg, 0.75 mmol, 12%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.93 (s, 1H), 7.30 (s, 2H; ArH), 2.53 (s, 6H; ArCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 192.1, 159.5, 124.1, 119.0, 24.3.



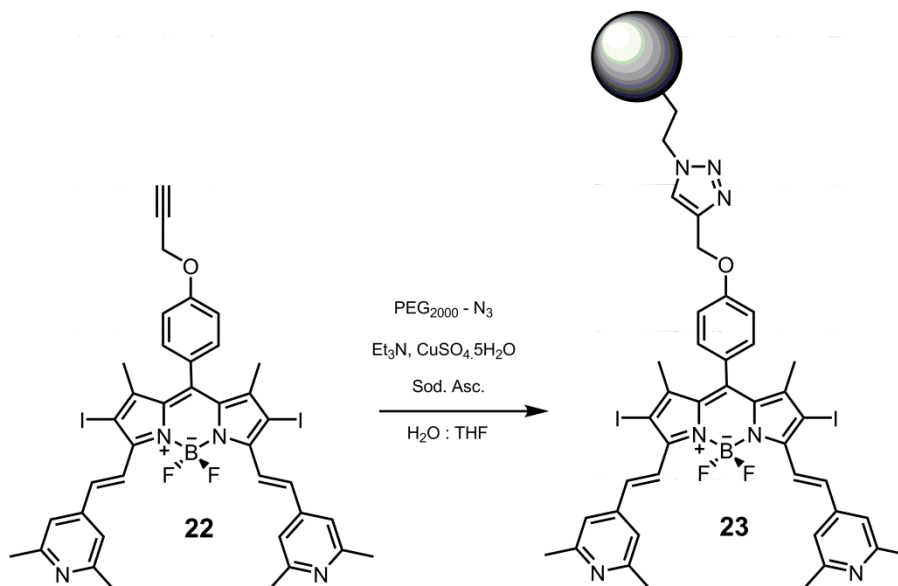
Synthesis of Compound 22: Compound **15** (93 mg, 0.15 mmol) and 2,6-dimethyl-4-pyridinecarboxaldehyde (60 mg, 0.44 mmol) were dissolved in benzene (15 ml). Piperidine (0.4 ml) and acetic acid (0.4 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CHCl₃ and water. Organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃/MeOH (95:5, v/v) as

mobile phase. Fraction containing compound **22** was collected, then the solvent was removed under reduced pressure (0.07 mmol, 61 mg, 47%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.00 (d, 2H, $J = 16.73$ Hz; CH), 7.78 (d, 2H, $J = 16.69$ Hz; CH), 7.21 (d, 2H, $J = 8.80$ Hz; ArH), 7.18 (d,s, 2H + 1H, $J = 8.91$ Hz; ArH), 4.81 (d, 2H, $J = 2.36$ Hz; OCH_2), 2.6 (s, 12H, ArCH_3), 2.5 (1H, CH), 1.52 (s, 6H, ArCH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 158.3, 150.1, 147.0, 144.4, 141.1, 137.2, 133.8, 129.3, 127.5, 122.3, 118.8, 118.4, 116.2, 105.9, 77.8, 76.2, 56.1, 24.1, 17.7.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{38}\text{H}_{34}\text{BF}_2\text{I}_2\text{N}_4\text{O}^+$: 865.0878 $[\text{M}+\text{H}]^+$, found: 865.07406 $[\text{M}+\text{H}]^+$, $\Delta = 1.59$ ppm.

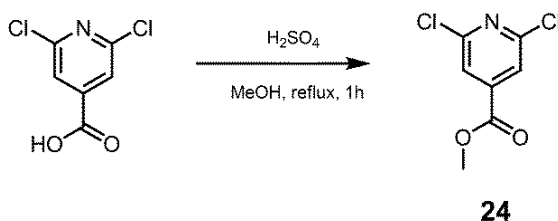


Synthesis of Compound 23: Compound **22** (42 mg, 49 μmol) and azide functionalized polyethylene glycol monomethylether (2000MW, 84 mg, 62 μmol) were dissolved in tetrahydrofuran (2 ml) and water (0.1 ml). Triethylamine (50 μl) was added and the reaction was stirred for 5 min. Then, CuSO₄·5H₂O (4 mg, 29 μmol) and sodium ascorbate (6 mg, 29 μmol) were added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, it was extracted with CHCl₃ and brine. The organic layer was collected and dried over Na₂SO₄, followed by evaporation of the solvent under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃/MeOH (92:8, v/v) as

mobile phase. Fraction containing compound **23** was collected then the solvent was removed under reduced pressure (6 μ mol, 18 mg, 12%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.00 (d, 2H, $J = 16.73$ Hz; ArH), 7.96 (s, 1H; ArH), 7.81 (d, 2H, $J = 16.76$ Hz; ArH), 7.25-7.15 (m, 2H + 2H + 4H; ArH), 5.29 (s, 2H; OCH_2), 4.12 (t, 2H, $J = 4.72$ Hz; NCH_2), 2.60 (s, 12H; ArCH_3), 1.52 (s, 6H; ArCH_3).

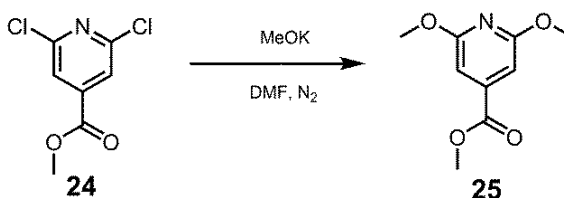
HRMS (TOF-ESI): Distribution around 2800 with separation of 44 corresponding to ethylene glycole unit.



Synthesis of Compound 24¹⁵⁷: 2,6-dichloronicotinic acid (500 mg, 2.6 mmol) was dissolved in 5.2 ml MeOH. 78 μ l concentrated H_2SO_4 was added. The reaction mixture was refluxed for 1h. Then, the reaction was cooled to RT and was quenched with NaHCO_3 . It was extracted with CHCl_3 and water. The organic layer was collected and dried over Na_2SO_4 , followed by evaporation of the solvent under reduced pressure. The product was purified by silica gel column chromatography using CHCl_3 as mobile phase. Fraction containing compound **24** was collected then the solvent was removed under reduced pressure (white solid, 2.4 mmol, 490 mg, 92%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.84 (s, 2H; ArH), 4.01 (s, 3H; OCH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 163.2, 151.5, 142.4, 122.6, 53.3.

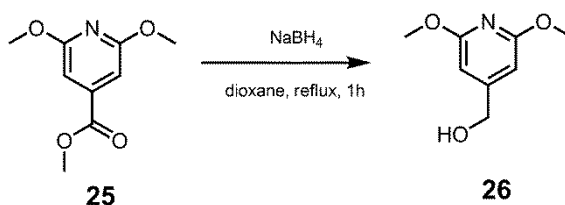


Synthesis of Compound 25: Compound **24** (250 mg, 1.21 mmol) was dissolved in 10 ml of anhydrous dimethylformamide. Ar was purged in the solution for 15 min. Potassium methoxide (255 mg, 3.64 mmol, 864 μ l) was added to the reaction mixture

and it was refluxed for 12h. Then, the reaction mixture was neutralized with HCl solution. It was extracted with CHCl_3 and water. The organic layer was collected and dried over Na_2SO_4 , followed by evaporation of the solvent under reduced pressure. The product was purified by silica gel column chromatography using CHCl_3 /Hexanes (3/2; v/v) as mobile phase. Fraction containing compound **25** was collected then the solvent was removed under reduced pressure (white solid, 0.51 mmol, 100 mg, 42%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 6.87 (s, 2H; ArH), 3.96 (s, 6H; ArOCH_3), 3.92 (s, 3H; OCH_3).

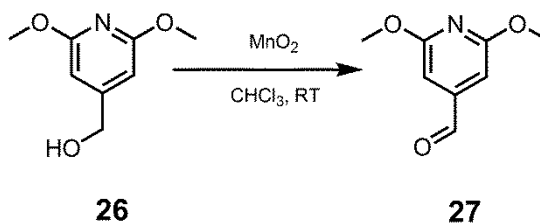
^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 165.6, 163.8, 142.7, 101.2, 53.9, 52.5.



Synthesis of Compound 26: Compound **25** (800 mg, 4.06 mmol) and 2 equivalents of NaBH_4 (309 mg, 8.12 mmol) were dissolved in 10 ml dioxane. It was refluxed for 1h. Then, the reaction was cooled to RT and quenched with ice cold water. It was extracted with CH_2Cl_2 . The organic layer was collected and dried over Na_2SO_4 , followed by evaporation of the solvent under reduced pressure (686 mg, quantitative).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 6.30 (s, 2H, ArH), 4.62 (s, 2H; ArCH_2), 3.91 (s, 6H; ArOCH_3), 1.93 (b, 1H; ArCH_2OH).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 163.5, 155.6, 98.3, 63.8, 53.6.



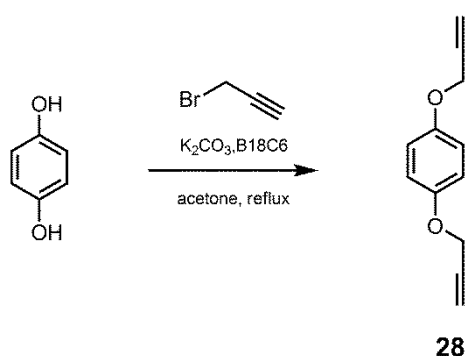
Synthesis of Compound 27: Compound **26** (172 mg, 1.02 mmol) was dissolved in 6 ml of CHCl_3 . 1.1 equivalents of MnO_2 (98 mg, 1.12 mmol) was added, and the

reaction mixture was stirred at RT for 12 h. After completion of the reaction as followed by thin layer chromatography, the reaction mixture was filtered over celite to get rid of MnO₂ by products. Solvent was removed under reduced pressure to yield compound **27** (yellow solid, 118 mg, 68%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.94 (s, 1H), 6.73 (s, 2H; ArH), 4.00 (s, 6H; ArCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 191.2, 164.3, 147.5, 100.7, 54.0.

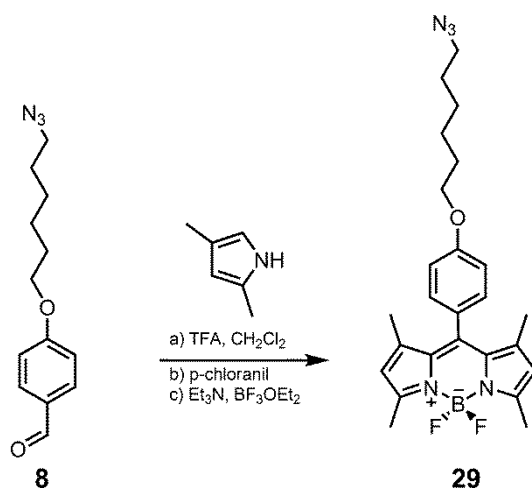
HRMS (TOF-ESI): m/z calcd for C₈H₁₀NO₃⁺: 168.0655 [M+H]⁺, found:168.06143 [M+H]⁺, Δ = 2.42 ppm.



Synthesis of Compound 28: Hydroquinone (2 g, 18.2 mmol) was dissolved in 30 ml acetone. 5 equivalents of K₂CO₃ (12.6 g, 91 mmol) was added and the reaction mixture was refluxed for 30 min. Then, 3 equivalents of propargyl bromide (6.48 g, 54.6 mmol) was added dropwise. The reaction mixture was refluxed for additional 12 h. Then, it was cooled to RT. Following the extraction with CHCl₃, the organic layer was collected and dried over Na₂SO₄, followed by evaporation of the solvent under reduced pressure. Crude product was crystallized in hexanes to yield compound **28** (white solid, quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 6.94 (s, 2H; ArH), 4.67 (d, 4H, J = 2.44 Hz; ArOCH₂), 2.53 (t, 2H, J = 2.44 Hz; CCH).

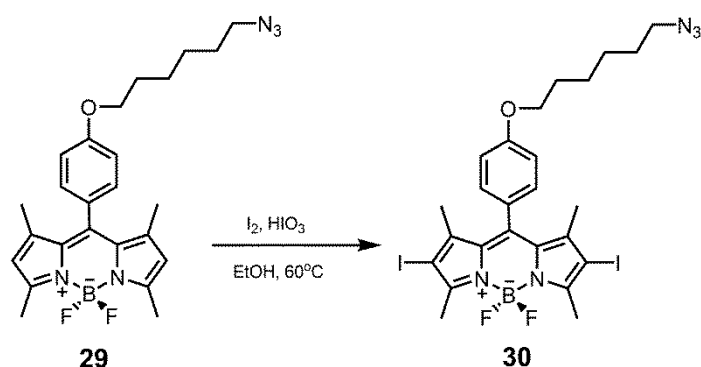
¹³C NMR (CDCl₃, 400 MHz, δ ppm) 152.4, 116.0, 78.8, 75.4, 56.5.



Synthesis of Compound 29: CH₂Cl₂ (300 ml) was purged with Ar for 30 min. Compound **8** (1.1 g, 4.45 mmol) and 2,4-dimethyl pyrrole (0.96 ml, 9.4 mmol) were added. The color of the solution turned into red after the addition of 3 drops of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12h. Then, tetrachloro-1,4-benzoquinone (1.09 g, 4.45 mmol) was added and the reaction mixture was stirred at room temperature for 45 min. Then triethyl amine (5 ml) and boron trifluoride diethyl etherate (5 ml) were added sequentially. After stirring at room temperature for 30 min, it was extracted with water. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃. Fraction containing compound **29** was collected then the solvent was removed under reduced pressure (400 mg, 0.86 mmol, 19%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.20 (d, 2H, J = 8.60 Hz; ArH), 7.01 (d, 2H, J = 8.64 Hz; ArH), 6.00 (s, 2H; ArH), 4.05 (t, 2H, J = 6.44 Hz; OCH₂), 3.31 (t, 2H, J = 6.80 Hz; NCH₂), 2.58 (s, 6H; ArCH₃), 1.86 (m, 2H; CH₂), 1.70 (m, 2H; CH₂), 1.55 (m, 4H; CH₂), 1.45 (s, 6H; ArCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 159.6, 155.2, 143.2, 141.9, 131.9, 129.2, 126.9, 121.1, 115.1, 67.9, 51.4, 29.1, 28.8, 26.6, 25.7, 14.6, 14.5.

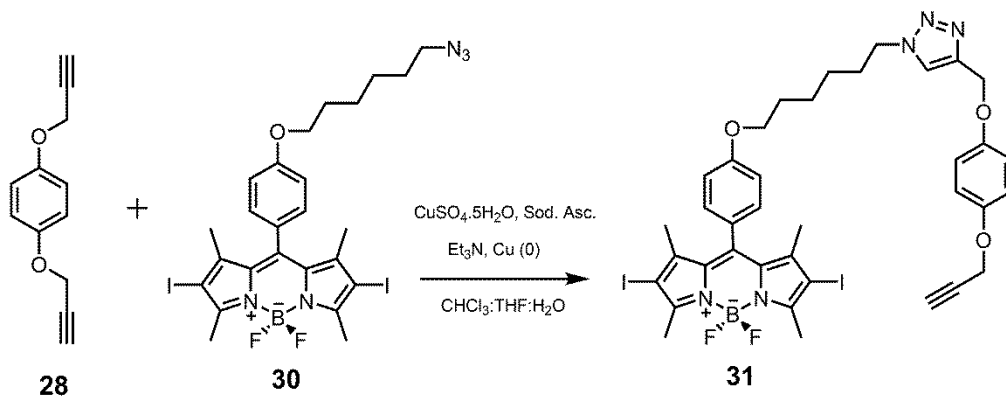


Synthesis of Compound 30: Compound **29** (270 mg, 0.58 mmol) and I₂ (368 mg, 1.45 mmol) were dissolved in ethanol (100 ml). Iodic acid, HIO₃ (204 mg, 1.16 mmol) was dissolved in a few drops of water and added into previous solution. The reaction mixture was stirred at 60°C for a few hours until all reactant was consumed. Then, saturated sodium thiosulfate solution was added (50 ml) and it was stirred at room temperature for additional 30 min. Then, it was extracted with CHCl₃ and water. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure (415 mg, quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.22 (d, 2H, J = 8.68 Hz; ArH), 7.02 (d, 2H, J = 8.68 Hz; ArH), 4.04 (t, 2H, J = 6.36 Hz; OCH₂), 3.30 (t, 2H, J = 6.80 Hz; NCH₂), 2.63 (s, 6H; ArCH₃), 1.85 (m, 2H; CH₂), 1.70 (m, 2H; CH₂), 1.55 (m, 4H; CH₂), 1.45 (s, 6H; ArCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 160.1, 156.6, 145.5, 141.6, 131.8, 129.1, 126.6, 115.5, 85.8, 68.0, 51.4, 29.1, 28.8, 26.6, 25.7, 17.2, 16.0.

HRMS (TOF-ESI): m/z calcd for C₂₅H₂₇BF₂I₂N₅O⁺ 716.03716 [M-H]⁺, found: 716.06297 [M-H]⁺, Δ = 36.0 ppm.

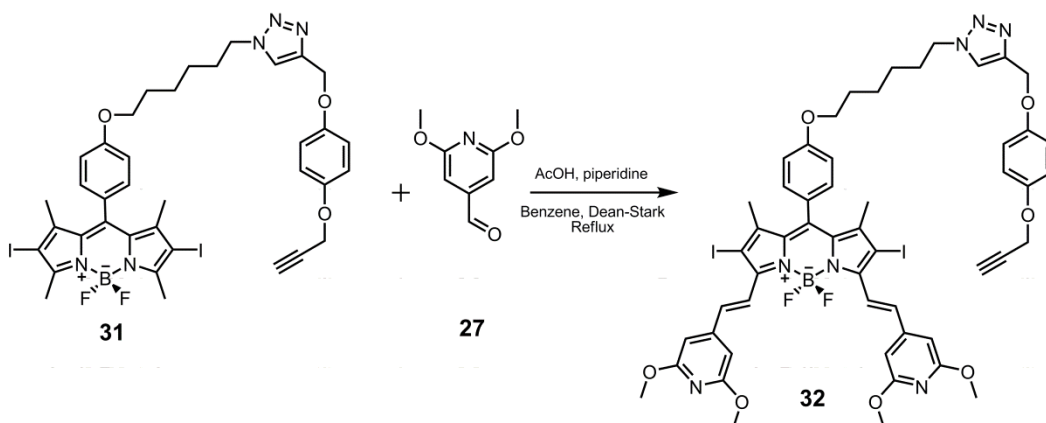


Synthesis of Compound 31: Compound **28** (519 mg, 2.8 mmol) and compound **30** (200 mg, 0.28 mmol) were dissolved in CHCl_3 (3 ml) and THF (3 ml). Triethylamine (200 μl) was added and the reaction was stirred for 5 min. Then, saturated solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (200 μl) and sodium ascorbate (200 μl) were added. Catalytic amount of Cu (0) was added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, it was extracted with CHCl_3 and organic layer was evaporated under reduced pressure. It was purified by silica gel column chromatography using CHCl_3 as mobile phase. Fraction containing compound **31** was collected then the solvent was removed under reduced pressure (0.27 mmol, 241 mg, 96%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.60 (s, 1H; ArH), 7.21 (d, 2H, $J = 8.12$ Hz; ArH), 7.02 (d, 2H, $J = 8.17$ Hz; ArH), 6.93 (s, 4H; ArH), 5.16 (s, 2H; OCH_2), 4.62 (d, 2H, $J = 1.52$ Hz; OCH_2), 4.48 (t, 2H, $J = 7.12$ Hz; NCH_2), 4.01 (t, 2H, $J = 6.28$ Hz; OCH_2), 2.63 (s, 6H; ArCH_3), 2.02 (t, 1H, $J = 1.40$ Hz; CH), 1.96 (m, 2H, CH_2), 1.82 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 1.44 (s, m, 6H + 2H, $\text{ArCH}_3 + \text{CH}_2$).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 160.0, 156.5, 153.1, 152.1, 145.4, 144.4, 141.7, 131.7, 129.1, 126.6, 122.5, 116.1, 115.8, 115.4, 78.8, 75.4, 67.9, 62.7, 56.5, 50.3, 30.2, 29.0, 26.3, 25.6, 17.2, 16.0.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{37}\text{H}_{38}\text{BF}_2\text{I}_2\text{N}_5\text{NaO}_3^+$: 926.1017 $[\text{M}+\text{Na}]^+$, found: 926.08227 $[\text{M}+\text{Na}]^+$, $\Delta = 20.98$ ppm.



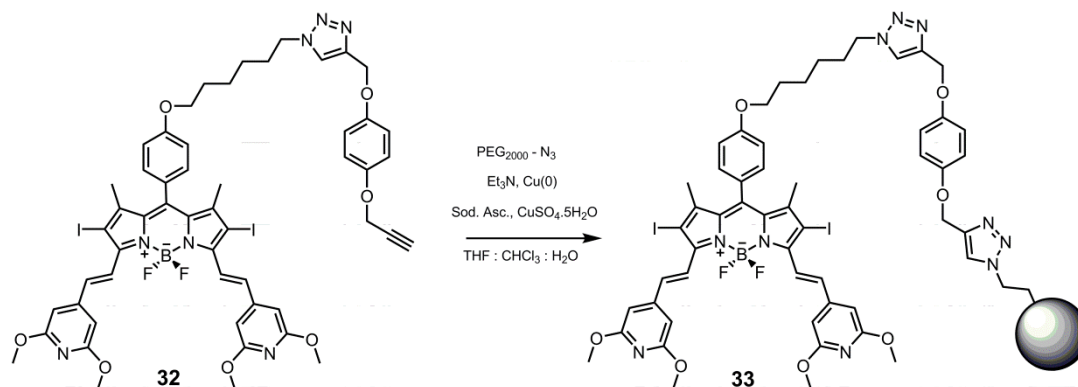
Synthesis of Compound 32: Compound **31** (125 mg, 0.14 mmol) and 2,6-dimethoxy-4-pyridinecarboxaldehyde (92 mg, 0.56 mmol) were dissolved in benzene (30 ml). Piperidine (0.3 ml) and acetic acid (0.3 ml) were added. The reaction mixture was

refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CHCl_3 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl_3 as mobile phase. Fraction containing compound **32** was collected, then the solvent was removed under reduced pressure (62 μmol , 75 mg, 44%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.94 (d, 2H, $J = 16.68$ Hz; CH), 7.72 (d, 2H, $J = 16.64$ Hz; CH), 7.59 (s, 1H; ArH), 7.16 (d, 2H, $J = 8.57$ Hz; ArH), 7.04 (d, 2H, $J = 8.64$ Hz; ArH), 6.93 (s, 4H; ArH), 6.57 (s, 4H; ArH), 5.20 (s, 2H, OCH_2), 4.65 (d, 2H, $J = 2.44$ Hz; OCH_2), 4.41 (t, 2H, t, $J = 7.12$ Hz; NCH_2), 4.04 (t, 2H, $J = 6.32$ Hz; OCH_2), 3.98 (s, 12H, OCH_3), 2.52 (t, 1H, $J = 2.42$ Hz; CH), 2.01 (m, 2H, CH_2), 1.85 (m, 2H, CH_2), 1.62 (s, 6H; ArCH_3), 1.48 (m, 4H; CH_2).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 163.8, 160.21, 153.1, 152.2, 150.0, 149.0, 146.7, 144.5, 137.2, 133.8, 129.3, 128.3, 126.6, 122.4, 122.3, 116.1, 115.8, 115.5, 99.3, 78.8, 75.4, 67.9, 62.8, 56.5, 53.7, 50.3, 30.2, 29.0, 26.3, 25.6, 17.8.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{53}\text{H}_{52}\text{BF}_2\text{I}_2\text{N}_7\text{NaO}_7^+$: 1224.1971 $[\text{M}+\text{Na}]^+$, found: 1224.1762 $[\text{M}+\text{Na}]^+$, $\Delta = 17.07$ ppm.

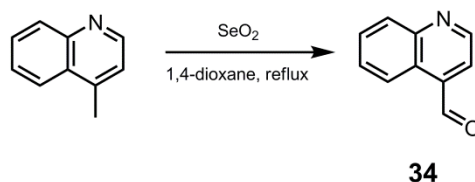


Synthesis of Compound 33: Compound **32** (17 mg, 14 μmol) and azide functionalized polyethylene glycol monomethylether (2000MW, 31 mg, 16 μmol) were dissolved in CHCl_3 (1 ml) and THF (1 ml). Triethylamine (50 μl) was added and the reaction was stirred for 5 min. Then, saturated solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (150 μl) and sodium ascorbate (150 μl) were added. Catalytic amount of Cu (0) was added. The reaction mixture was stirred for 12 h at room temperature. After the

reaction was completed, the crude product was applied to silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ (90:10, v/v) as mobile phase. Fraction containing compound **33** was collected then the solvent was removed under reduced pressure (8 μmol , 26 mg, 58%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.96 (d, 2H, $J = 16.72$ Hz; CH), 7.83 (s, 1H; ArH), 7.73 (d, 2H, $J = 16.64$ Hz; CH), 7.62 (s, 1H; ArH), 7.17 (d, 2H, $J = 8.76$ Hz; ArH), 7.05 (d, 2H, $J = 8.28$ Hz; ArH), 6.93 (s, 4H; ArH), 6.54 (s, 4H; ArH), 5.19 (s, 2H; OCH_2), 5.17 (s, 2H; OCH_2), 4.58 (t, 2H, $J = 5.00$ Hz; NCH_2), 4.41 (t, 2H, $J = 7.64$ Hz; NCH_2), 4.10-3.30 (PEG), 2.01 (m, 2H, CH_2), 1.85 (m, 2H, CH_2), 1.62 (s, 6H; ArCH_3), 1.48 (m, 4H; CH_2).

HRMS (TOF-ESI): Distribution around 3000 with separation of 44 corresponding to ethylene glycole unit.

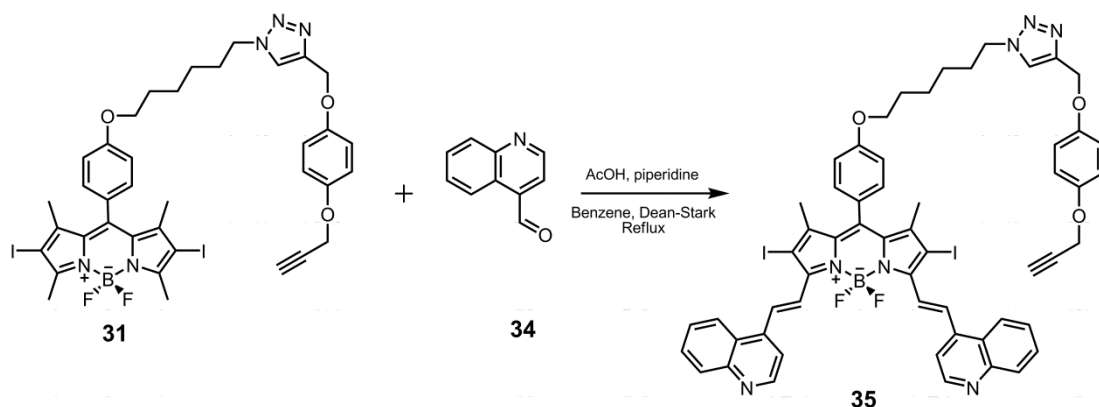


Synthesis of Compound 34¹⁵⁸: 4-methylquinone (1.32 ml, 10 mmol) was dissolved in 1,4-dioxane (12 ml). Selenium dioxide (1.12 g, 10.12 mmol) was added to the reaction mixture and it was refluxed for 8h. Then, solvent was removed under reduced pressure and the crude product was applied to silica gel column chromatography using ethyl acetate as mobile phase. Fraction containing compound **34** was collected, then the solvent was removed under reduced pressure (946 mg, 6 mmol, 60%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 10.50 (s, 1H), 9.19 (d, 1H, $J = 4.24$ Hz; ArH), 9.00 (dt, 1H, $J_1 = 0.64$ Hz, $J_2 = 7.03$ Hz; ArH), 8.21 (dt, 1H, $J_1 = 0.36$ Hz, $J_2 = 7.24$ Hz; ArH), 7.70-7.85 (m, 3H; ArCH).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 192.8, 150.4, 149.3, 136.7, 130.2, 130.0, 129.4, 125.8, 124.4, 123.8.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{10}\text{H}_8\text{NO}^+$: 158.0600 $[\text{M}+\text{Na}]^+$, found: 158.05593 $[\text{M}+\text{Na}]^+$, $\Delta = 25.75$ ppm.

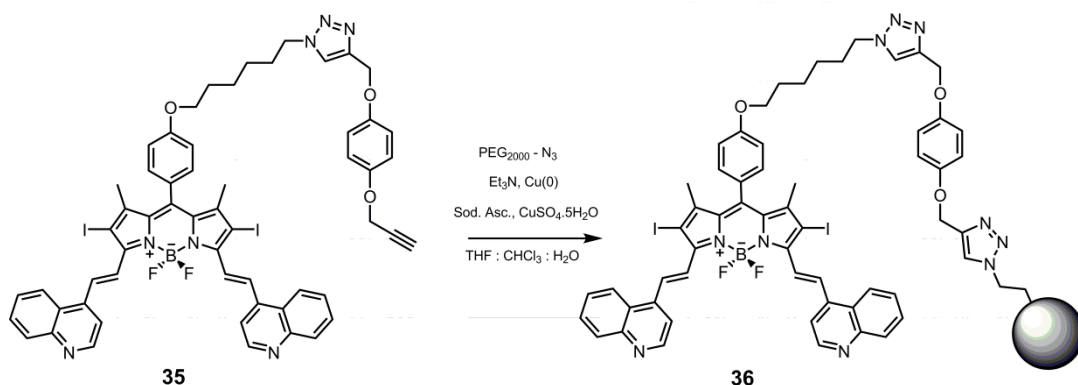


Synthesis of Compound 35: Compound **31** (91 mg, 0.1 mmol) and quinoline-4-carboxaldehyde, compound **34** (40 mg, 0.25 mmol) were dissolved in benzene (25 ml). Piperidine (0.3 ml) and acetic acid (0.3 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CHCl_3 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc as mobile phase. Fraction containing compound **35** was collected, then the solvent was removed under reduced pressure (84 μmol , 99 mg, 84%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 9.01 (d, 2H, $J = 4.64$ Hz; ArH), 8.96 (d, 2H, $J = 16.56$ Hz; CH), 8.32 (d, 2H, $J = 8.40$ Hz; ArH), 8.18 (d, 2H, $J = 8.36$ Hz; ArH), 7.94 (d, 2H, $J = 16.53$ Hz; CH), 7.81 (d, 2H, $J = 4.60$ Hz; ArH), 7.77 (t, 2H, 7.48 Hz; ArH), 7.62 (t, 2H, $j = 7.76$ Hz; ArH), 7.22 (d, 2H, $J = 8.41$ Hz, ArH), 7.09 (d, 2H, $J = 8.44$ Hz; ArH), 6.95 (s, 4H; ArH), 5.20 (s, 2H; OCH_2), 4.64 (d, 2H, $J = 2.04$ Hz; OCH_2), 4.43 (t, 2H, $J = 7.12$ Hz; NCH_2), 4.05 (t, 2H, $J = 6.17$ Hz; OCH_2), 2.52 (t, 1H, $J = 1.84$ Hz; CH), 2.02 (m, 2H; CH_2), 1.88 (m, 2H, CH_2), 1.60 (m, 8H, $\text{ArCH}_3 + \text{CH}_2$).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 163.8, 160.2, 153.1, 152.2, 150.0, 149.0, 146.7, 144.5, 137.2, 133.8, 129.3, 128.3, 126.6, 122.4, 122.3, 116.1, 115.8, 115.5, 99.3, 78.8, 75.4, 67.9, 62.8, 56.5, 53.7, 50.3, 30.2, 29.0, 26.3, 25.6, 17.8.

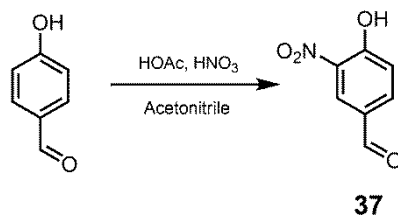
HRMS (TOF-ESI): m/z calcd for $\text{C}_{57}\text{H}_{48}\text{BF}_2\text{I}_2\text{N}_7\text{NaO}_3^+$: 1204.1861 $[\text{M}+\text{Na}]^+$, found: 1204.1620 $[\text{M}+\text{Na}]^+$, $\Delta = 20.01$ ppm.



Synthesis of Compound 36: Compound **35** (20 mg, 17 μmol) and azide functionalized polyethylene glycol monomethylether (2000MW, 37 mg, 19 μmol) were dissolved in CHCl_3 (2 ml) and THF (2 ml). Triethylamine (150 μl) was added and the reaction was stirred for 5 min. Then, saturated solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (250 μl) and sodium ascorbate (250 μl) were added. Catalytic amount of Cu (0) was added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, the crude product was applied to octadecyl functionalized silica gel column chromatography using CHCl_3 as mobile phase. The mobile phase was changed to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (80:20; v/v) after the starting compound was eluted from the column. Fraction containing compound **36** was collected then the solvent was removed under reduced pressure (13 μmol , 40 mg, 76%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 9.02-8.9 (m, 2H + 2H; ArH), 8.31 (d, 2H, $J = 8.17$ Hz; ArH), 8.17 (d, 2H, $J = 8.16$ Hz; ArH), 7.93 (d, 2H, $J = 16.85$ Hz; ArH), 7.85-7.70 (m, 2H + 2H + 1H; ArH), 7.60 (s + d, 1H + 2H; $J = 8.24$ Hz; ArH), 7.20 (d, 2H, $J = 8.16$ Hz; ArH), 7.09 (d, 2H, $J = 8.37$ Hz; ArH), 6.92 (s, 4H, ArH), 5.16 (s, 2H; OCH_2), 5.13 (s, 2H; OCH_2), 4.54 (t, 2H, $J = 4.76$ Hz; NCH_2), 4.40 (t, 2H, $J = 6.92$ Hz; NCH_2), 3.90-3.30 (PEG), 2.00 (m, 2H; CH_2), 1.85 (m, 2H; CH_2), 1.40 (m, 4H; CH_2), 1.30 (s, 6H; ArCH_3).

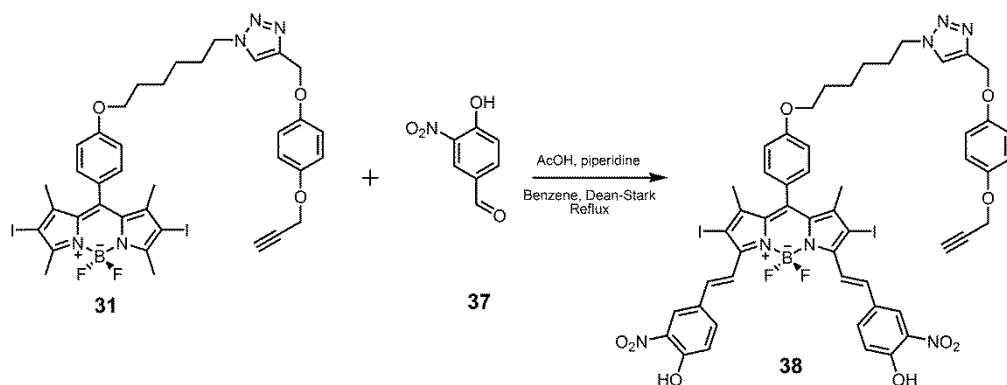
HRMS (TOF-ESI): Distribution around 3000 with separation of 44 corresponding to ethylene glycole unit.



Synthesis of Compound 37¹⁵⁹: 4-hydroxybenzaldehyde (1.22 g, 10 mmol) was dissolved in 20 ml acetonitrile. Acetic acid (10 ml) and nitric acid (0.75 ml) were added and the reaction was refluxed for 3h. Then, it was cooled to RT and extracted with EtOAc and water. Organic layer was collected and dried with Na₂SO₄, solvent was evaporated under reduced pressure. (9.1 mmol, 1.52 g, 91%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 11.05 (s, 1H; ArOH), 9.98 (s, 1H), 8.68 (s, 1H, ArH), 8.17 (d, 1H, J = 8.61 Hz; ArH), 7.34 (d, 1H, J = 8.68 Hz; ArH).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 188.7, 159.3, 136.4, 128.6, 126.2, 121.3, 115.7.

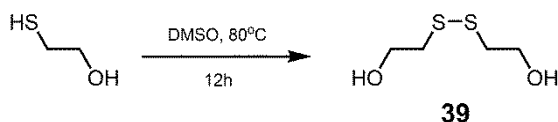


Synthesis of Compound 38: Compound **31** (200 mg, 0.22 mmol) and 4-hydroxy-3-nitrobenzaldehyde, compound **37** (110 mg, 0.66 mmol) were dissolved in benzene (20 ml). Piperidine (0.3 ml) and acetic acid (0.3 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CH₂Cl₂ and water. Organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc as mobile phase. After the impurities are eluted from the column, the mobile phase was changed to CH₂Cl₂/MeOH/AcOH (90/5/5; v/v). Fraction containing compound **38** was collected, then the solvent was removed under reduced pressure (0.15 mmol, 180 mg, 68%).

^1H NMR (**38** + AcOH: CDCl_3 , 400 MHz, δ ppm) 8.79 (s, 2H; ArH), 8.19 (d, 2H, J = 16.81 Hz; ArH), 7.97 (d, 2H, J = 8.80 Hz; ArH), 7.60 (d + s, 2H + 1H, J = 16.65 Hz; ArH), 7.23 (d, 2H, J = 8.80 Hz; ArH), 7.19 (d, 2H, J = 7.44 Hz; ArH), 7.05 (d, 2H, J = 8.24 Hz; ArH), 6.93 (s, 4H; ArH), 5.20 (s, 2H; OCH_2), 4.64 (s, 2H; OCH_2), 4.41 (t, 2H, J = 7.12 Hz; NCH_2), 4.05 (t, 2H, J = 5.84 Hz; OCH_2), 2.52 (s, 1H; CH), 1.85 (m, 2H, CH_2), 1.60-1.40 (m, 12H, CH_2 + ArCH_3).

^{13}C NMR can not be recorded due to poor solubility.

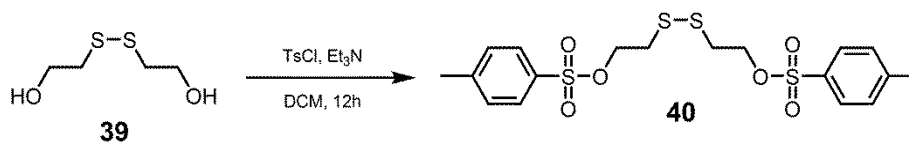
HRMS (TOF-ESI): m/z calcd for $\text{C}_{51}\text{H}_{43}\text{BF}_2\text{I}_2\text{N}_7\text{O}_9$. 1200.1278 $[\text{M-H}]^-$, found: 1200.13078 $[\text{M-H}]^-$, Δ = 2.48 ppm.



Synthesis of Compound 39¹⁶⁰: mercaptoethanol (5 g, 64 mmol) was dissolved in DMSO (20 ml). The reaction mixture was stirred at 80°C for 12h. Then, it was cooled to RT and was extracted with brine and EtOAc. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using Hexanes/EtOAc 75/25; v/v) as mobile phase. Fraction containing compound **39** was collected, then the solvent was removed under reduced pressure (29 mmol, 4.5 g, 86%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 3.90 (t, 4H, J = 5.84 Hz; OCH_2), 7.30 (t, 4H, J = 5.88 Hz; SCH_2).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 60.4, 41.3.



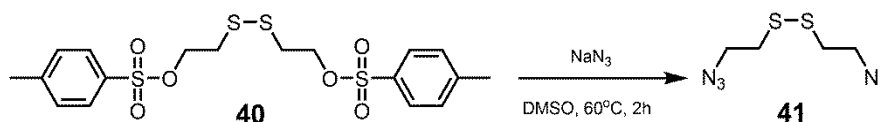
Synthesis of Compound 40¹⁶⁰: 2-hydroxyethyl disulfide, compound **39** (1 g, 6.5 mmol) was dissolved in 20 ml CH_2Cl_2 and 2 ml Et_3N . In a dropper, p-toluene sulfonyl chloride (1.44 g, 13 mmol) was dissolved in CH_2Cl_2 10 ml and was added to the previous solution dropwise while the reaction mixture was being cooled with ice bath. It was stirred for 12h. After the extraction with water, organic layer was

collected and dried with Na₂SO₄, evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃ as mobile phase. Fraction containing compound **40** was collected, then the solvent was removed under reduced pressure (white solid, 6.5 mmol, 3 g, quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.83 (d, 4H, J = 8.04 Hz; ArH), 7.38 (d, 4H, J = 7.93 Hz; ArH), 4.21 (t, 4H, J = 6.61 Hz; OCH₂), 2.85 (t, 4H, J = 6.53 Hz; SCH₂), 2.48 (s, 6H, ArCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 145.2, 130.0, 128.0, 67.5, 36.9, 21.7.

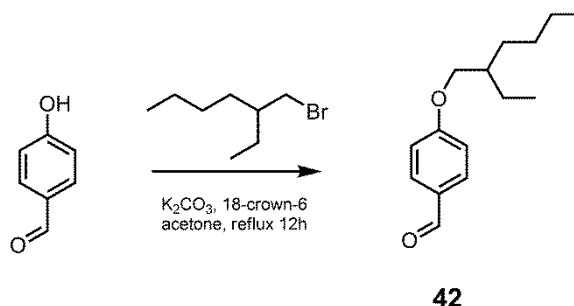
HRMS (TOF-ESI): m/z calcd for C₁₈H₂₂NaO₆S₄⁺ 485.0191 [M+Na]⁺, found: 485.0104 [M+Na]⁺, Δ = 17.94 ppm.



Synthesis of Compound 41: Compound **40** (1.2 g, 2.6 mmol) was dissolved in 10 ml DMSO and sodium azide (12 mmol, 780 mg) was added to the reaction mixture. It was stirred 2h at 60 oC. After cooling to RT, it was extracted with EtOAc. Organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. (yellow oil, 2.47 mmol, 0.5 g, 95%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 3.63 (t, 4H, J = 6.76 Hz; NCH₂), 2.89 (t, 4H, J = 6.76 Hz; SCH₂).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 49.9, 37.6.



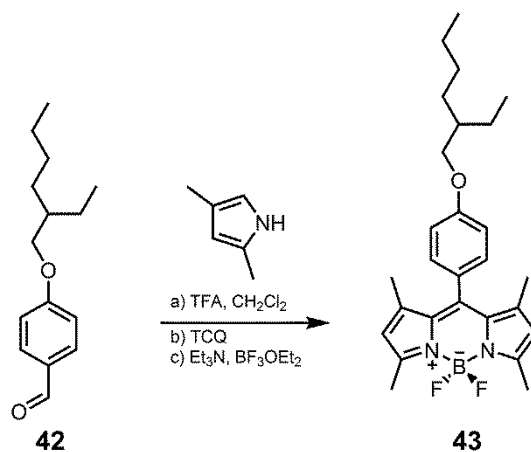
Synthesis of Compound 42: 4-hydroxybenzaldehyde (4 g, 33 mmol), 2-ethylhexyl bromide (6.7 ml, 36 mmol) and catalytic amount of benzo-18-crown-6 were dissolved in 60 ml acetonitrile. K₂CO₃ (13.6 g, 98 mmol) was added and the reaction mixture was refluxed for 12 h. The solvent was evaporated under reduced pressure

and the crude product was extracted with CH₂Cl₂. Organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. (yellow oil, 7.7 g, quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.88 (s, 1H), 7.83 (d, 2H, J = 8.24 Hz; ArH), 7.01 (d, 2H, J = 8.33 Hz; ArH), 3.94 (d, 2H, J = 5.64 Hz; OCH₂), 1.77 (m, 1H; CH), 1.45 (m, 4H; CH₂), 1.32 (m, 4H; CH₂), 0.93 (m, 6H; CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 190.7, 164.5, 131.9, 129.7, 114.8, 70.9, 39.3, 30.4, 29.0, 23.8, 23.0, 14.0, 11.1.

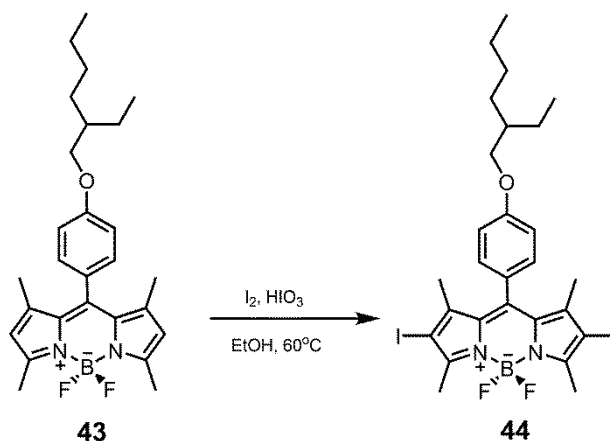
HRMS (TOF-ESI): m/z calcd for C₁₅H₂₃O₂⁺ 235.16926 [M+H]⁺, found: 235.17327 [M+H]⁺, Δ = 17.1 ppm.



Synthesis of Compound 43: CH₂Cl₂ (400 ml) was purged with Ar for 30 min. Compound **42** (2.4 g, 10.24 mmol) and 2,4-dimethyl pyrrole (2.3 ml, 22.53 mmol) were added. The color of the solution turned into red after the addition of 3 drops of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12 h. Then, tetrachloro-1,4-benzoquinone (2.52 g, 10.24 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. Then triethyl amine (9 ml) and boron trifluoride diethyl etherate (9 ml) were added sequentially. After stirring at room temperature for 1 h, it was extracted with water. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃ and then EtOAc/Hexanes (20/80; v/v) as mobile phase. Fraction containing compound **43** was collected then the solvent was removed under reduced pressure (810 mg, 1.8 mmol, 18%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.17 (d, 2H, $J = 8.76$ Hz; ArH), 7.01 (d, 2H, $J = 8.80$ Hz; ArH), 5.99 (s, 2H; ArH), 3.92 (d, 2H, $J = 5.93$ Hz, OCH_2), 2.55 (s, 6H, ArCH₃), 1.79 (m, 1H, CH), 1.60-1.40 (m, s, 4H + 6H; CH₂ + ArCH₃), 1.37 (m, 4H; CH₂), 0.96 (m, 6H, CH₃).

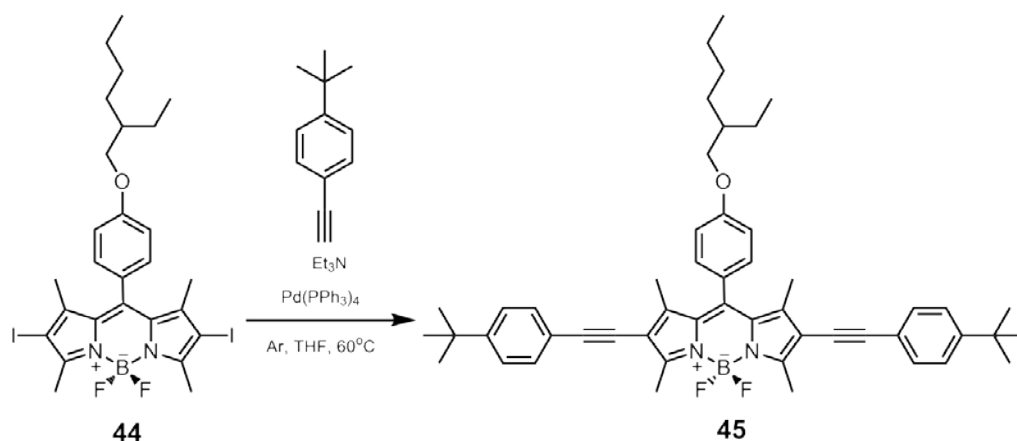
^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 160.0, 155.2, 143.2, 142.1, 131.9, 129.1, 126.8, 121.1, 114.8, 70.9, 39.4, 30.6, 29.1, 23.9, 23.0, 14.6, 14.1, 11.2.



Synthesis of Compound 44: Compound **43** (330 mg, 0.73 mmol) and I₂ (389 mg, 1.53 mmol) were dissolved in ethanol (200 ml). Iodic acid, HIO₃ (256 mg, 1.46 mmol) was dissolved in a few drops of water and added into previous solution. The reaction mixture was stirred at 60°C for 1 h until all reagent was consumed. Then, saturated sodium thiosulfate solution was added (50 ml) and it was stirred at room temperature for additional 30 min. Then, it was extracted with CHCl₃ and water. Organic layer was dried with Na₂SO₄ and compound **44** was obtained by evaporation of the solvent under reduced pressure (514 mg, quantitative).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.12 (d, 2H, $J = 8.68$ Hz; ArH), 7.03 (d, 2H, $J = 8.72$ Hz; ArH), 3.92 (d, 2H, $J = 5.88$ Hz, OCH_2), 2.64 (s, 6H, ArCH₃), 1.79 (m, 1H, CH), 1.60-1.40 (m, s, 4H + 6H; CH₂ + ArCH₃), 1.36 (m, 4H; CH₂), 0.96 (m, 6H, CH₃).

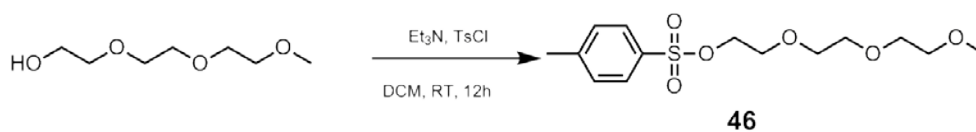
^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 160.2, 156.7, 145.8, 142.1, 132.1, 129.8, 126.8, 115.8, 85.9, 71.2, 39.6, 31.0, 24.3, 17.6, 16.3, 14.3, 11.7.



Synthesis of Compound 45: Compound **44** (100 mg, 0.14 mmol) was dissolved in tetrahydrofuran (30 ml) and triethylamine (5 ml). Argon was purged for 30 min. Then, 15% mole equivalent of Pd(PPh₃)₄ (24 mg, 21 μmol) was added. 4-(Tert-butyl)phenylacetylene (88 μl, 0.49 mmol) was added via syringe and the reaction mixture was stirred 12 h at 60°C. After it was cooled to RT, it was extracted with CH₂Cl₂ and brine. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/Hexanes (10/90; v/v) as mobile phase. Fraction containing compound **45** was collected then the solvent was removed under reduced pressure (92 mg, 0.12 mmol, 86%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.43 (d, 4H, J = 8.16 Hz; ArH), 7.37 (d, 4H, j = 8.48 Hz; ArH), 7.18 (d, 2H, J = 8.52 Hz; ArH), 7.06 (d, 2H, J = 8.61 Hz; ArH), 3.95 (d, 2H, J = 5.76 Hz, OCH₂), 2.73 (s, 6H, ArCH₃), 1.80 (m, 1H, CH), 1.60-1.30 (m, 4H + 6H + 18H; CH₂ + ArCH₃ + CCH₃), 0.96 (m, 6H, CH₃).

HRMS (TOF-ESI): m/z calcd for $C_{51}H_{60}BF_2N_2O^+$ 765.4761 $[M+H]^+$, found: 765.4540 $[M+H]^+$, $\Delta = 28.87$ ppm.

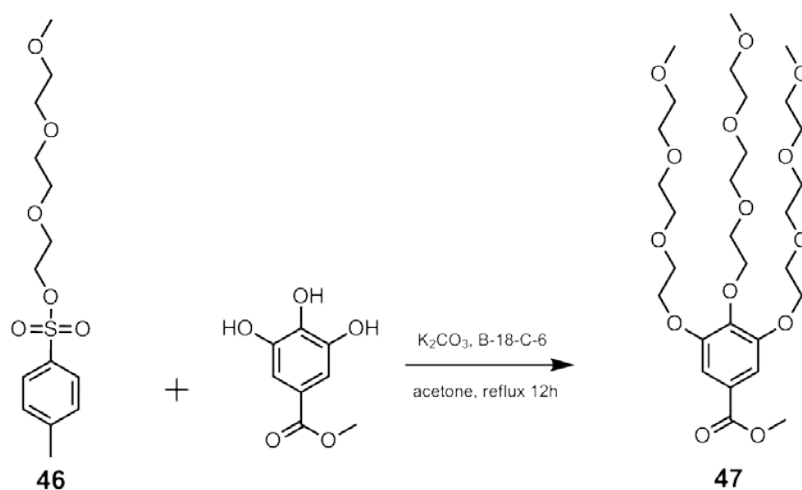


Synthesis of Compound 46: triethyleneglycol monomethyl ether (10 g, 61 mmol) was dissolved in 100 ml CH_2Cl_2 and 13 ml Et_3N . In a dropper, p-toluene sulfonyl chloride (12 g, 63 mmol) was dissolved in CH_2Cl_2 (20 ml) and was added to the previous solution dropwise while the reaction mixture was being cooled with ice bath. It was stirred for 12 h. After the extraction with water, organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. (yellow oil, 55 mmol, 17.5 g, 90%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.78 (d, 2H; $J = 8.24$ Hz; ArH), 7.32 (d, 2H, $J = 8.04$ Hz; ArH) 4.15 (t, 2H; $J = 4.77$ Hz; OCH_2), 3.67 (m, 4H; OCH_2), 3.60 (m, 4H; OCH_2), 3.51 (m, 2H; OCH_2). 3.34 (s, 3H; OCH_3), 2.42 (s, 3H; ArCH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 144.8, 133.0, 129.8, 127.9, 71.9, 70.7, 70.5, 70.5, 69.3, 68.6, 59.0, 21.6.

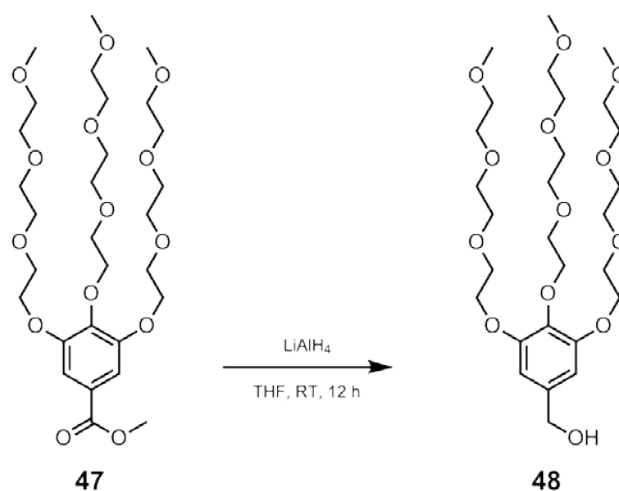
HRMS (TOF-ESI): m/z calcd for $\text{C}_{14}\text{H}_{22}\text{NaO}_6\text{S}^+$ 341.1029 $[\text{M}+\text{Na}]^+$, found: 341.10639 $[\text{M}+\text{Na}]^+$, $\Delta = 10.23$ ppm.



Synthesis of Compound 47: methyl-3,4,5-trihydroxybenzoate (2.75 g, 15 mmol), compound **46** (15g, 47 mmol) and catalytic amount of benzo-18-crown-6 were dissolved in 60 ml acetone. K_2CO_3 (8.3 g, 60 mmol) were added and the reaction mixture was refluxed for 18 h. Then, solvent was removed under reduced pressure

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.30 (s, 2H; ArH), 4.22 (m, 2H + 4H; OCH₂), 3.88 (m, 4H + 3H; OCH₃ + OCH₂), 3.80 (m, 2H; OCH₂), 3.74 (m, 6H; OCH₂). 3.65 (m, 12H; OCH₂), 3.56 (m, 6H; OCH₂), 3.38 (s, 9H; OCH₃).

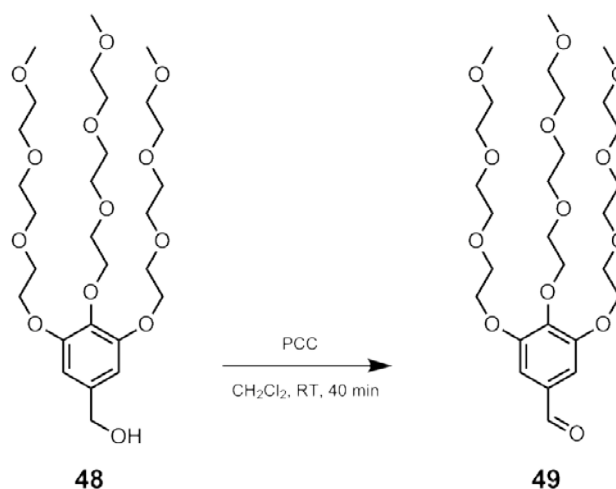
¹³C NMR (CDCl₃, 400 MHz, δ ppm) 166.5, 152.3, 142.6, 124.9, 109.0, 72.4, 71.9, 70.8, 70.7, 70.6, 70.5, 69.6, 68.8, 52.1.



¹H NMR (CDCl₃, 400 MHz, δ ppm) 6.63 (s, 2H; ArH), 4.58 (s, 2H; OCH₂), 4.15 (m, 2H + 4H; OCH₂), 3.84 (t, 4H, J = 5.29 Hz; OCH₂), 3.79 (t, 2H, J = 5.44 Hz; OCH₂),

3.73 (m, 6H; OCH₂). 3.65 (m, 12H; OCH₂), 3.54 (m, 6H; OCH₂), 3.38 (s, 9H; OCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 152.7, 137.8, 136.7, 106.6, 72.3, 71.9, 70.8, 70.7, 70.5, 69.8, 68.9, 65.2, 59.0.

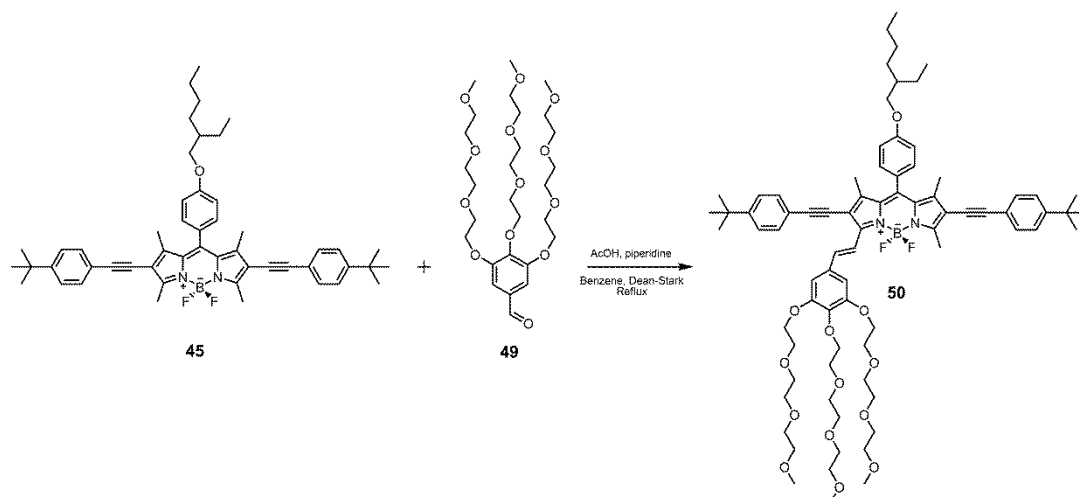


Synthesis of Compound 49: Compound **48** (2.4 g, 4 mmol) was dissolved in CH₂Cl₂ (25 ml). Pyridinium chlorochromate (2.15 g, 10 mmol) was added to the reaction mixture and it was stirred for 40 min at RT. Then, it was directly applied to silica column chromatography using EtOAc/MeOH (95/5; v/v) as mobile phase. Fraction containing compound **49** was collected then the solvent was removed under reduced pressure (colorless oil, 2.37 g, quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.82 (s, 1H), 7.14 (s, 2H; ArH), 4.21 (m, 6H; OCH₂), 3.89 (m, 4H; OCH₂), 3.82 (m, 2H; OCH₂), 3.80-3.50 (m, 24H; OCH₂), 3.38 (s, 9H; OCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 191.0, 153.0, 144.1, 131.6, 109.0, 72.5, 71.9, 70.8, 70.7, 70.6, 70.5, 69.6, 68.9, 59.0.

HRMS (TOF-ESI): m/z calcd for C₂₈H₄₈NaO₁₃⁺ 615.2987 [M+Na]⁺, found: 615.28633 [M+Na]⁺, Δ = 20.10 ppm.

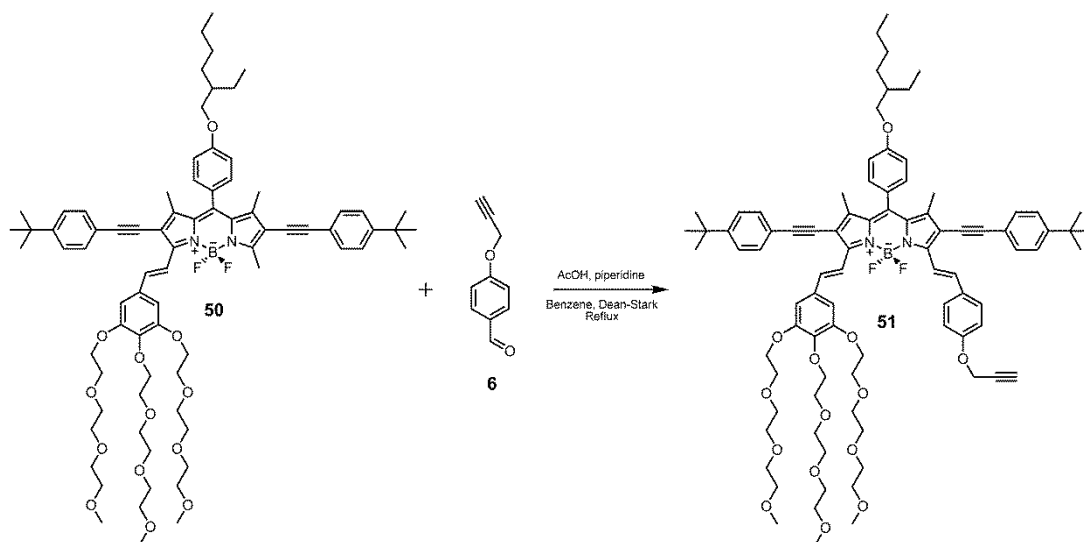


Synthesis of Compound 50: Compound **45** (150 mg, 0.2 mmol) and compound **49** (100 mg, 0.17 mmol) were dissolved in benzene (45 ml). Piperidine (0.3 ml) and acetic acid (0.3 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CH_2Cl_2 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/MeOH (85/15; v/v) as mobile phase. Fraction containing compound **50** was collected, then the solvent was removed under reduced pressure (41 μmol , 55 mg, 21%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.34 (d, 1H, $J = 16.24$ Hz; ArH), 7.62 (d, 1H, $J = 16.21$ Hz; ArH), 7.50-7.30 (m, 8H; ArH), 7.21 (d, 2H, $J = 8.60$ Hz; ArH), 7.06 (d, 2H, $J = 8.65$ Hz; ArH), 6.87 (s, 2H; ArH), 4.25 (m, 6H; OCH_2), 3.95 (d, 2H, $J = 4.56$ Hz; OCH_2), 3.90 (t, 4H, $J = 5.37$ Hz; OCH_2), 3.85 (t, 2H, $J = 4.40$ Hz; OCH_2), 3.80-3.50 (m, 24H; OCH_2), 3.38 (s, 9H; OCH_3), 2.77 (s, 3H; ArCH_3), 1.8 (m, 1H; CH), 1.65 (s, 3H; ArCH_3), 1.63 (s, 3H; ArCH_3), 1.60-1.40 (m, 6H; CH_2), 1.40-1.20 (m, 18H + 2H; $\text{CH}_3 + \text{CH}_2$), 0.95 (m, 3H + 3H; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 160.3, 152.9, 151.7, 151.5, 141.4, 140.0, 138.8, 132.5, 131.1, 130.8, 129.3, 126.4, 125.5, 125.4, 120.5, 120.4, 115.4, 107.5, 98.1, 96.7, 83.3, 81.0, 72.5, 72.0, 71.9, 71.0, 70.9, 70.7, 70.6, 69.8, 69.0, 59.0, 39.4, 34.8, 31.2, 30.6, 29.2, 23.9, 23.0, 14.1, 13.7, 13.4, 11.2.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{79}\text{H}_{105}\text{BF}_2\text{N}_2\text{NaO}_{13}^+$ 1361.7570 $[\text{M}+\text{Na}]^+$, found: 1361.7296 $[\text{M}+\text{Na}]^+$, $\Delta = 20.12$ ppm.

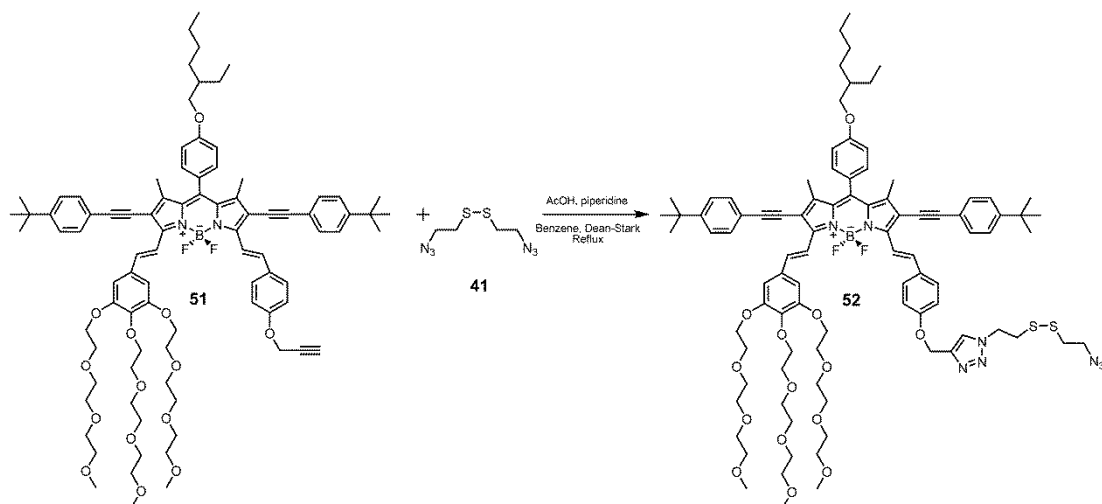


Synthesis of Compound 51: Compound **50** (45 mg, 30 μmol) and compound **6** (9 mg, 56 μmol) were dissolved in benzene (25 ml). Piperidine (0.2 ml) and acetic acid (0.2 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, it was extracted with CH_2Cl_2 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/MeOH (90/10; v/v) as mobile phase. Fraction containing compound **51** was collected, then the solvent was removed under reduced pressure (27 μmol , 40 mg, 90%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.49 (d, 1H, $J = 16.24$ Hz; ArH), 8.34 (d, 1H, $J = 16.16$ Hz; ArH), 7.74 (d, 1H, $J = 16.60$ Hz; ArH), 7.65 (s + d + d, 1H + 2H + 2H; ArH), 7.50-7.30 (m, 8H; ArH), 7.21 (d, 2H, $J = 8.09$ Hz; ArH), 7.10-7.00 (m, 2H + 2H; ArH), 6.90 (s, 2H; ArH), 4.77 (2H; OCH_2), 4.25 (m, 6H; OCH_2), 4.00- 3.50 (m, 32H; OCH_2), 3.40 (s, 3H; OCH_3), 3.37 (s, 6H; OCH_3), 2.60 (1H; CH), 1.80-1.20 (m, 23H; $\text{CH}_3 + \text{CH}_2$), 0.98 (m, 3H + 3H; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 160.3, 158.6, 152.9, 151.7, 145.3, 140.2, 139.7, 138.8, 132.7, 130.8, 130.7, 129.6, 129.2, 126.6, 125.6, 125.5, 120.6, 120.5, 115.3, 107.9, 98.4, 98.2, 83.4, 83.1, 77.4, 77.1, 76.7, 75.9, 72.5, 72.0, 71.9, 69.9, 69.2, 59.0, 59.0, 55.9, 39.4, 34.8, 31.2, 30.6, 29.2, 23.9, 23.1, 14.1, 11.2.

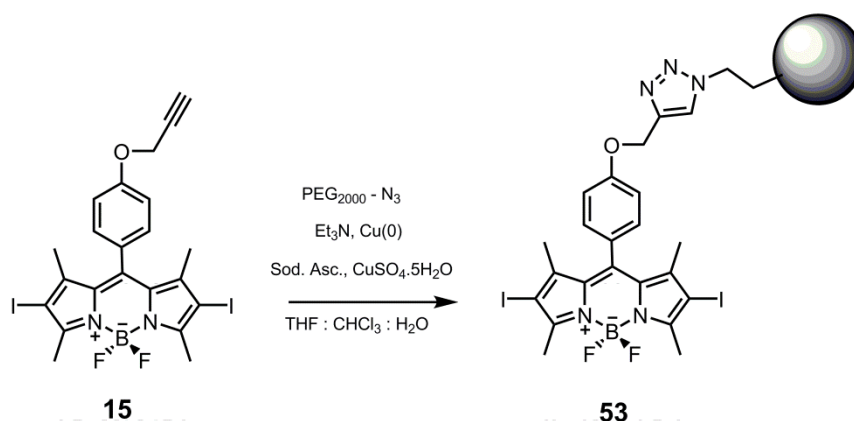
HRMS (TOF-ESI): m/z calcd for $\text{C}_{89}\text{H}_{111}\text{BF}_2\text{N}_2\text{NaO}_{14}^+$ 1503.7989 $[\text{M}+\text{Na}]^+$, found: 1503.7684 $[\text{M}+\text{Na}]^+$, $\Delta = 20.28$ ppm.



Synthesis of Compound 52: Compound **51** (45 mg, 30 μ mol) and compound **41** (62 mg, 300 μ mol) were dissolved in CH_2Cl_2 (6 ml) and MeOH (3 ml). Saturated solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (100 μ l) and sodium ascorbate (100 μ l) were added. Catalytic amount of Cu (0) was added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, it was extracted with CH_2Cl_2 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/MeOH (85/15; v/v) as mobile phase. Fraction containing compound **52** was collected, then the solvent was removed under reduced pressure (18 μ mol, 30 mg, 60%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.48 (d, 1H, $J = 16.32$ Hz; ArH), 8.34 (d, 1H, $J = 16.28$ Hz; ArH), 7.75-7.60 (d + s + d + d, 2H + 1H + 1H + 2H; ArH), 7.50-7.30 (m, 8H; ArH), 7.21 (d, 2H, $J = 8.33$ Hz; ArH), 7.08 (d, 2H, $J = 3.49$ Hz; ArH), 7.05 (d, 2H, $J = 3.40$ Hz; ArH), 6.91 (s, 2H; ArH), 5.31 (s, 2H; OCH_2), 4.73 (t, 2H, $J = 6.64$ Hz; NCH_2), 4.25 (m, 6H; OCH_2), 4.00- 3.50 (m, 32H; OCH_2), 3.40 (s, 3H; OCH_3), 3.37 (s, 6H; OCH_3), 3.22 (t, 2H, $J = 6.64$ Hz; NCH_2), 2.89 (m, 2H + 2H; SCH_2), 1.80-1.20 (m, 23H; CH_3 + CH_2), 0.98 (m, 3H + 3H; CH_3).

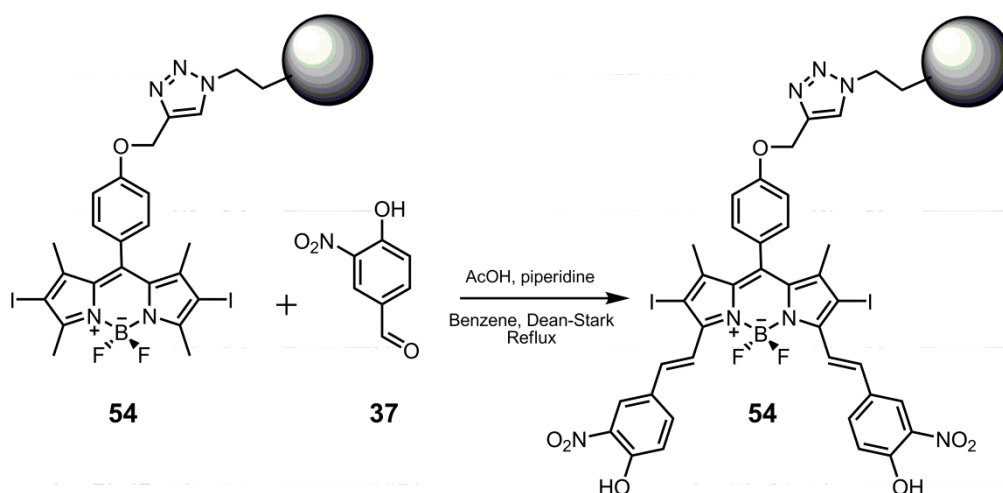
^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 160.3, 159.3, 153.0, 151.8, 145.3, 144.4, 140.1, 139.6, 138.9, 133.4, 132.7, 132.2, 131.9, 130.8, 130.4, 129.6, 128.6, 126.7, 125.5, 123.6, 120.6, 118.4, 117.1, 116.1, 115.3, 114.0, 107.9, 107.2, 98.4, 98.2, 83.3, 72.5, 71.9, 70.9, 70.8, 70.7, 70.5, 70.4, 69.8, 69.2, 68.9, 62.1, 59.0, 49.9, 48.9, 39.5, 37.7, 34.9, 31.3, 30.6, 29.2, 23.9, 23.1, 14.1, 13.5.



Synthesis of Compound 53: Compound **15** (127 mg, 0.2 mmol) and azide functionalized polyethylene glycol monomethylether (2000MW, 800 mg, 0.4 mmol) were dissolved in tetrahydrofuran (2 ml). Triethylamine (1.4 ml) and CuI (77 mg, 0.4 mmol) were added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, the crude product was purified by silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ (93:7, v/v) as mobile phase. Fraction containing compound **53** was collected then the solvent was removed under reduced pressure (red oil, 0.11 mmol, 300 mg, 55%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.93 (s, 1H, ArH), 7.12 (b, 2H + 2H), 5.24 (s, 2H; OCH_2), 4.58 (t, $J = 4.84$ Hz; NCH_2), 3.90-3.40 (PEG), 3.35 (s, 3H; OCH_3), 2.60 (s, 6H; ArCH_3), 1.40 (s, 6H; ArCH_3).

HRMS (TOF-ESI): Distribution around 2500 with separation of 44 corresponding to ethylene glycole unit.

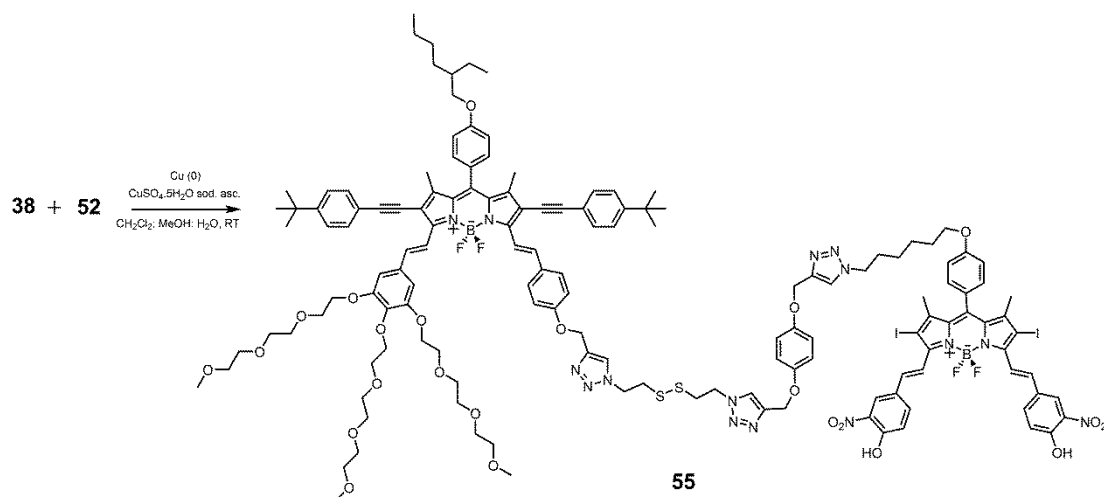


Synthesis of Compound 54: Compound **54** (120 mg, ~45 μmol) and compound **37** (23 mg, 135 μmol) were dissolved in benzene (25 ml). Piperidine (0.2 ml) and acetic acid (0.2 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all aldehyde was consumed. After the reaction was completed, the crude product was purified by silica gel column chromatography using $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (85/15; v/v) as mobile phase. Fraction containing compound **54** was collected, then the solvent was removed under reduced pressure (30 μmol , 90 mg, 66%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.24 (s, 2H; ArH), 8.06 (d, 2H, $J = 16.72$ Hz; ArH), 7.93 (m, 2H + 2H; ArH), 7.56 (d, 2H, $J = 16.69$ Hz; ArH), 7.20 (m, 2H + 2H + 2H; ArH), 5.28 (s, 2H, OCH_2), 4.60 (t, 2H, $J = 4.36$ Hz; NCH_3), 3.90-3.40 (PEG), 3.35 (s, 3H; OCH_3), 1.50 (s, 6H; ArCH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 159.6, 155.8, 149.8, 146.7, 143.2, 140.4, 136.2, 135.3, 133.7, 129.5, 127.2, 124.5, 124.4, 120.7, 119.3, 115.9, 94.3, 83.6, 72.0, 70.6, 69.6, 62.2, 59.0, 50.7, 50.4, 17.7.

HRMS (TOF-ESI): Distribution around 2800 with separation of 44 corresponding to ethylene glycole unit.



Synthesis of Compound 55: Compound **52** (40 mg, 24 μmol) and compound **38** (45 mg, 37 μmol) were dissolved in CH_2Cl_2 (6 ml) and MeOH (3 ml). Saturated solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (100 μl) and sodium ascorbate (100 μl) were added. Catalytic amount of Cu (0) was added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, it was extracted with CH_2Cl_2

and water. Organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/MeOH (85/15; v/v) as mobile phase. Fraction containing compound **55** was collected, then the solvent was removed under reduced pressure (12 μmol, 35 mg, 50%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 8.48 (d, 1H, J = 15.92 Hz; ArH), 8.32 (d, 1H, J = 15.84 Hz; ArH), 8.29 (s, 2H; ArH), 8.09 (d, J = 16.52 Hz, 2H; ArH), 7.95 (d, J = 8.04 Hz, 2H; ArH), 7.8-7.5 (m, 10H; ArH), 7.50-7.30 (m, 10H; ArH), 7.25-7.10 (m, 6H; ArH), 7.10-7.0 (m, 5H; ArH), 6.90 (m, 6H; ArH), 5.30 (s, 2H, OCH₂), 5.15 (s, 4H, OCH₂), 4.65 (s, 4H, NCH₂), 4.40 (m, 2H, OCH₂), 4.2 (m, 6H; OCH₂), 4.10-3.50 (m, 36H, OCH₂), 3.30 (s + s, 6H + 3H; OCH₃), 3.15 (m, 4H; SCH₂), 2.05 (m, 18H; CH₃), 1.8 (m, 4H), 1.60-1.0 (21H), 0.95 (m, 6H).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 167.8, 160.3, 160.2, 159.2, 158.6, 155.6, 152.8, 151.7, 149.8, 146.6, 138.8, 136.2, 135.4, 133.7, 132.7, 130.8, 130.3, 129.6, 129.5, 129.4, 129.3, 126.6, 125.6, 125.5, 124.4, 123.7, 120.7, 120.4, 119.4, 115.9, 115.8, 115.5, 115.3, 107.8, 72.4, 71.9, 70.9, 70.8, 70.6, 70.5, 69.8, 69.1, 59.0, 58.9, 48.9, 40.6, 39.4, 31.2, 30.6, 30.2, 29.2, 26.3, 25.6, 23.9, 23.0, 17.8, 14.1, 13.5, 11.2.

HRMS (TOF-ESI): m/z calcd for C₁₄₄H₁₆₂B₂F₄I₂N₁₅O₂₃S₂⁻ 2884.9 [M-H]⁻, found: 2884.9 [M-H]⁻.

CHAPTER 5

Turn-on BODIPY-based Fluorescent Sensor with a Large Dynamic Range for Measuring Chloride Level in Acidic Media

Sundus Erbas-Cakmak, Kevin P. McDonald, Amar H. Flood

5.1. Objective

There are only a few fluorescent chloride anion sensors in literature and most of them lack anion selectivity. Among anion binding motifs, novel triazolophane structures worth attention since they display strong and size-selective interaction with chloride anion. Here, a turn-on chloride sensor was created by combining a BODIPY fluorophore with a triazolophane from which was discovered a large dynamic range. The sensor employed protonated pyridine (pyrH^+) to quench BODIPY emission by photoinduced electron transfer (PET) to the pyrH^+ cation. Selective chloride binding into the cavity bearing the pyrH^+ destabilized the PET acceptor orbital allowing the BODIPY emission reinstated. Chloride binding properties in acidic and neutral organic solvents were investigated and the macrocycle was found to be responsive to chloride concentrations across the range of 0.2 μM to 80 mM in acidic dichloromethane with an increase in quantum yield from 0.06 (no chloride) to 0.73 (90 mM chloride). Apart from a simple 1:1 binding to triazolophane cavity, a second binding motif was observed which suggests possible contributions of anion induced deprotonation and/or charge transfer interactions between anion and electron deficient pyridinium moiety. By introducing this bright sensor into the era of anion detection, potential applications and improvements are discussed.

5.2. Introduction

Cations and anions are essential elements of human beings and other living organisms both for the normal functioning of biochemical processes and the survival of the organism itself. The main contribution of rare earth metals are their unique coordination properties that built up a reaction microenvironment within the active cavity of enzymes. Mg^{2+} , Fe^{2+} , Mn^{2+} would be the most common examples of such coordination dependent activity. Besides controlling the activity of enzymes, in some cases, binding of a cation to a protein would change the functionality or signaling event succeeded by this assembly. Calcium binding proteins are of great examples for such systems. Some cations assist the stability of a structure such as G-quadruplex structures in telomeres to control the cell cycle etc. The role of Na^+ and K^+ are well known in electrophysiological events such as neuron activation/deactivation and cognitive functionalities. Hence, determining real-time concentrations and consequent roles of inorganic compounds in physiological processes is challenging and without any doubt this challenge would give clues for a molecular level understanding of a number of diseases¹⁶¹ and biochemical processes¹⁶². Upon the detection methods, fluorescence is accepted to be the easiest and one of the most accurate technique.¹⁶³ Basically, in this technique, a fluorophore is responsive to the analyte of interest and either a change in the fluorescence intensity or a spectral shift is required for the sensing. For biological applications, the wavelengths of excitation (λ_{exc}) and detection (λ_{F}), biocompatibility and the reliability of ensuring data are important. Here, as a start in dichloromethane, we address some of these requirements and designed and synthesized a fluorescent chloride sensor based on a BODIPY dye¹⁶⁴ and a triazolophane receptor^{165,166,167} that is excited by light in visible region of the spectrum and the dynamic response spans a large concentration range.

Although chloride is one of the most abundant anions in the body (0.13 M in blood serum), its physiological roles in many different biological processes, such as the development of cystic fibrosis¹⁶⁸, cell volume and pH regulation¹⁶⁹, and in a number of different biological processes¹⁷⁰ and diseases are yet to be determined precisely. The level of this anion changes in cellular organelles and the exact level in acidic

organelles such as lysosomes are not well-known yet. For this reason, our attempt in this research is to develop a sensor that initiates an effort to detect chloride at acidic media.

Considering chloride sensors in literature, one of the early demonstrations by Illsley and Verkman employed 6-methoxy-N-(3-sulfopropyl) quinolinium (SPQ) as a chloride sensor.⁴²⁻⁴⁴ Although it can detect across relevant concentrations among other competing anions (not quenched by bicarbonate, phosphate, sulfate), it requires excitation and detection in the UV region involving energetic light, known to be hazardous, increase mutagenicity and give oxidative photodamage to cells. Quantum dots employing thiourea as the binding motif was used as a turn-off chloride sensor ($\lambda_{\text{exc}} = 425$, $\lambda_{\text{F}} = 620$ nm) to measure chloride level in epithelial cells.¹⁷¹ Lucigenin, being the most widely-used one for applications, is a turn-off sensor with an excitation wavelength of 430 nm.¹⁷² These are “turn-off” sensors, where the fluorescence intensity is quenched by the analyte. Consequently, there is usually no way to eliminate spurious quenching by solvents, other molecules in the media or photobleaching of the fluorophore. Turn-on sensors are also being developed. For example, urea-functionalized phenanthrolines, but they require UV excitation and the dynamic range is limited to the submillimolar region (less than 1 mM). The presence of other anions also interferes with their fluorescence output by quenching the fluorophore, which decreases the reliability of the data obtained from live media.¹⁷³ Genetically-encoded fused proteins (clomeleon) were used as ratiometric sensors to monitor chloride concentrations during neuronal development in rat cells.¹⁷⁴ A ratiometric chloride sensor was developed using a pyrene-modified calixarene ($\lambda_{\text{exc}} = 340$ nm) by Diamond *et al.*¹⁷⁵ Therein, excimer emission was abolished upon chloride binding and the monomer emission increases in response. However, detection is limited to micromolar concentrations (50 - 150 μM), which are very low compared to physiological values.

The concept of using a co-analyte, either to increase the binding strength of the analyte or modulate sensing, is another common strategy.⁵³ For instance, dipicolyl amine receptors are coordinated to Zn^{2+} for the detection of phosphate derivatives.⁵⁴ Recently, this approach was extended to gene silencing by taking advantage of the

interaction between the picolyl-bound Zn^{2+} and the phosphate groups of small interfering RNA (siRNA).¹⁷⁶ In the case of chloride sensing, a solvent molecule, dimethylsulfoxide (DMSO) present in the active site of a tetra-urea porphyrin chemosensor was shown to increase selectivity in favour of Cl^- .¹⁷⁷ Zhang *et al.* used a phenanthroline- Cu^{2+} complex to sense chloride selectively in organic solvents.⁵² Complexation with Cu^{2+} quenches the complex's emission at 402 nm and subsequent Cl^- binding restores it selectively. Severin *et al.* used a pyranine chromophore whose association with rhodium(III) results in a non-emissive dye.¹⁷⁸ However, the emission intensity at 510 nm increases more than three folds upon addition of Cl^- up to 30 mM. Mechanically interlocked squaraine rotaxanes were shown to be responsive to Cl^- both in solution and in a reverse-phase silica gel plate.¹⁷⁹ Within this rotaxane format, the tetralactam macrocycle which normally stabilizes the excited triplet state, chloride binding shifts the relative location of the macrocycle, in favor, the emission intensity and singlet excited state lifetime increases ($\lambda_{\text{exc}} = 365$). Recently, “turn-on” and “turn-off” responses resulting from different ethynylpyridine derivatives was examined by Johnson and Haley *et al.* in organic solvents.⁵⁵ In these works, hydrochloric acid but not trifluoroacetic acid (TFA) was shown to increase emission intensity ($\phi_{\text{F}} = 0.02$) on account of chloride binding that accompanied protonation. While a lot of approaches have been examined, there still remains a need to explore other sensors. Here, we take advantage of the chloride selectivity of triazolophanes.⁶⁰

5.3. Design of the Fluorescent Chloride Sensor

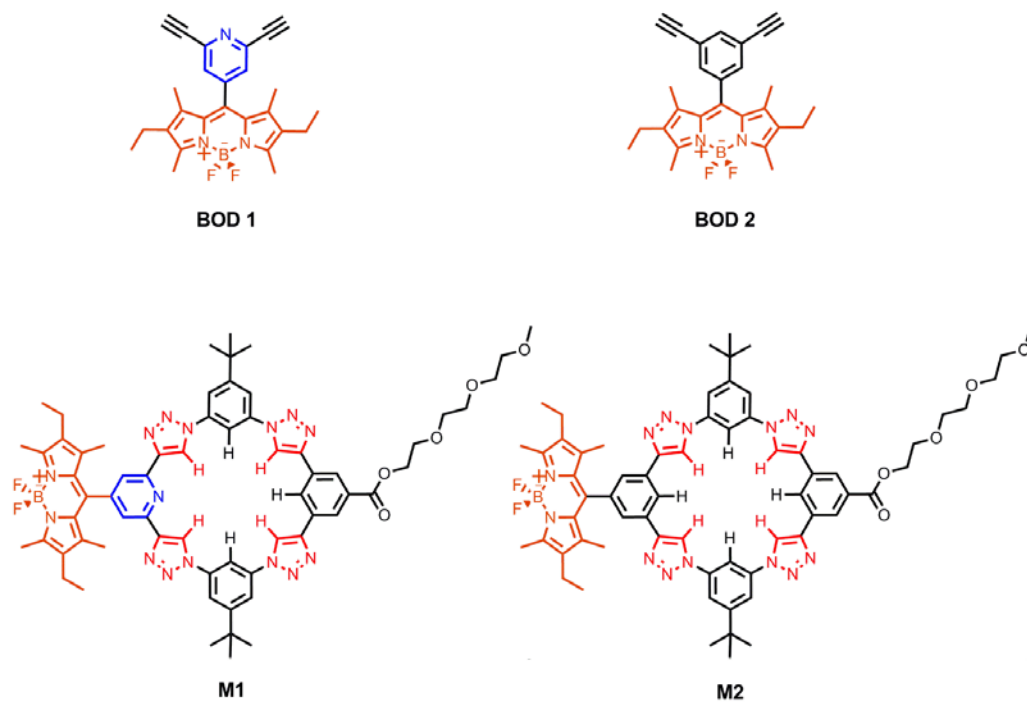
In this work, we describe a turn-on sensor for chloride concentrations in organic media as a pre-cursor to test the feasibility of appending a BODIPY dye to a chloride-selective triazolophane. We employ a proton as a “co-ligand” and consequently aim to develop a chloride sensor that works under commensurate conditions such as those of low pH in organic solvent. The structures of macrocycles and their BODIPY precursors are given in Scheme 22.

With acidic media in mind, we employed a BODIPY dye substituted in the *meso* position with a pyridine that is incorporated as one of the four aryl rings of a

triazolophane. Charge transfer quenching of the protonated and methyl-quaternized forms of similarly pyridine-substituted BODIPY dyes have been extensively studied by Ziessel and others.^{180,181,182} The triazolophane scaffold, which provides size-selective binding for chloride, has not yet been investigated for the purpose of fluorometric anion sensing. The molecular design (Scheme 22) was envisioned to allow sensing of chloride using a turn on mechanism. Briefly, the co-ligand, H^+ , will generate a pyridinium cation that will cause quenching of the BODIPY's emission by PET to the lowest unoccupied molecular orbital (LUMO) of pyridine. Addition of Cl^- will destabilize the LUMO of the pyridinium moiety, shutting off PET and turning the BODIPY emission back on. Therefore, as a long term goal, the use of the H^+ allows us to target acidic organelles (*trans*-Golgi network, lysosomes, late endosome, exosomes, secretory granules) with lower pH, thought to have Cl^- concentrations less than plasma concentrations of 130 mM.

As a starting point, we examine the sensitivity towards H^+ and Cl^- in organic solvents where we find a clear turn-on response exhibited in acidic dichloromethane and discovered an unexpectedly wide dynamic range (0.2 μM - 80 mM).

The macrocycle **M1** was designed as principle fluorescent sensor for chloride with protonatable pyridine moiety which constitutes a size-selective chloride binding cavity together with other triazole-linked aryl groups. **M2** was synthesized to examine the chloride binding properties of pyridine free macrocycles. Besides, two BODIPY precursors **BOD1** and **BOD2** that lack a chloride binding triazolophane binding motif are synthesized for control purposes.



Scheme 22. Structures of BODIPY building blocks and the derived triazolophane macrocycles

5.4. Results and Discussion

5.4.1. Photophysical Characterization

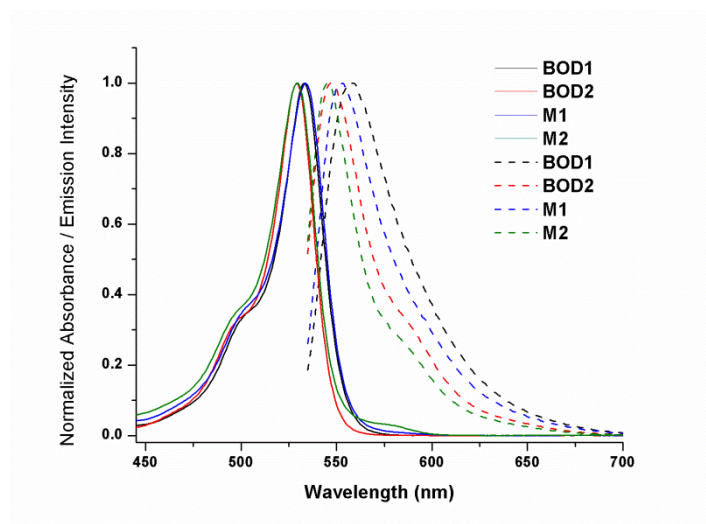


Figure 31. Normalized absorption and emission spectra of BODIPY building blocks and macrocycles in CH_2Cl_2 .

Both **BOD1** and **M1** display BODIPY-based absorption in the visible region at 532 nm (Figure 31, Table 6) whereas **BOD2** and **M2** show absorption maxima are at 529 nm. BODIPY band positions do not change upon formation of the macrocycle since the bulky phenyl at the meso-position of the BODIPY do not contribute to the conjugation a lot. As is the case with most BODIPY dyes, there is a small Stoke's shift in the emission maxima (Table 6). The smaller Stoke's shift displayed by **M1** and **M2** indicates that macrocyclic structure is less stabilizing towards the BODIPY excited states. Table 1 shows photophysical characterizations of the compounds. A decrease in quantum yield of **M1** from 0.66 to 0.06 upon protonation is observed which is restored back to 0.73 by the addition of 90 mM tetrabutylammonium chloride.

Table 6. Photophysical properties of BODIPY building blocks and macrocycles

Compound	Φ_F^a	$\epsilon(M^{-1}cm^{-1})^d$	λ_{max} (abs, emm)
BOD 1	0.62	58000	532, 558
BOD 2	0.83	59000	529, 547
M1	0.66	36000	533, 552
M1•H⁺	0.06	24000	541, 554
M1•H⁺•Cl⁻^b	0.73	36000	532, 553
M2	0.59	39000	529, 545
BOD-Q-1	0.01	44000	542, -
BOD-Q-1. Cl⁻^e	0.02	61000	539, 632
BOD-Q-2	0.01	19000	538, -
BOD-Q-2 Cl⁻^f	0.54	39000	530, 542

^a see electronic supporting information for details of calculations ^b in the presence of 90 mM TBACl ^d extinction coefficient at maximum absorption and emission wavelengths. ^e in the presence of 60 mM TBACl, ^f in the presence of 8 mM TBACl.

5.4.2. Acid-Responsive Behavior

The compounds analyzed in this work are not designed to work under biologically appropriate solvents and acid concentration. Rather, as a start, we try to develop a system that helps us understand the behavior of such fluorophore-triazolophane conjugate in a similar context in organic media. In order to understand the acid

response of macrocycle **M1**, trifluoroacetic acid (TFA) was titrated into CH_2Cl_2 solutions.

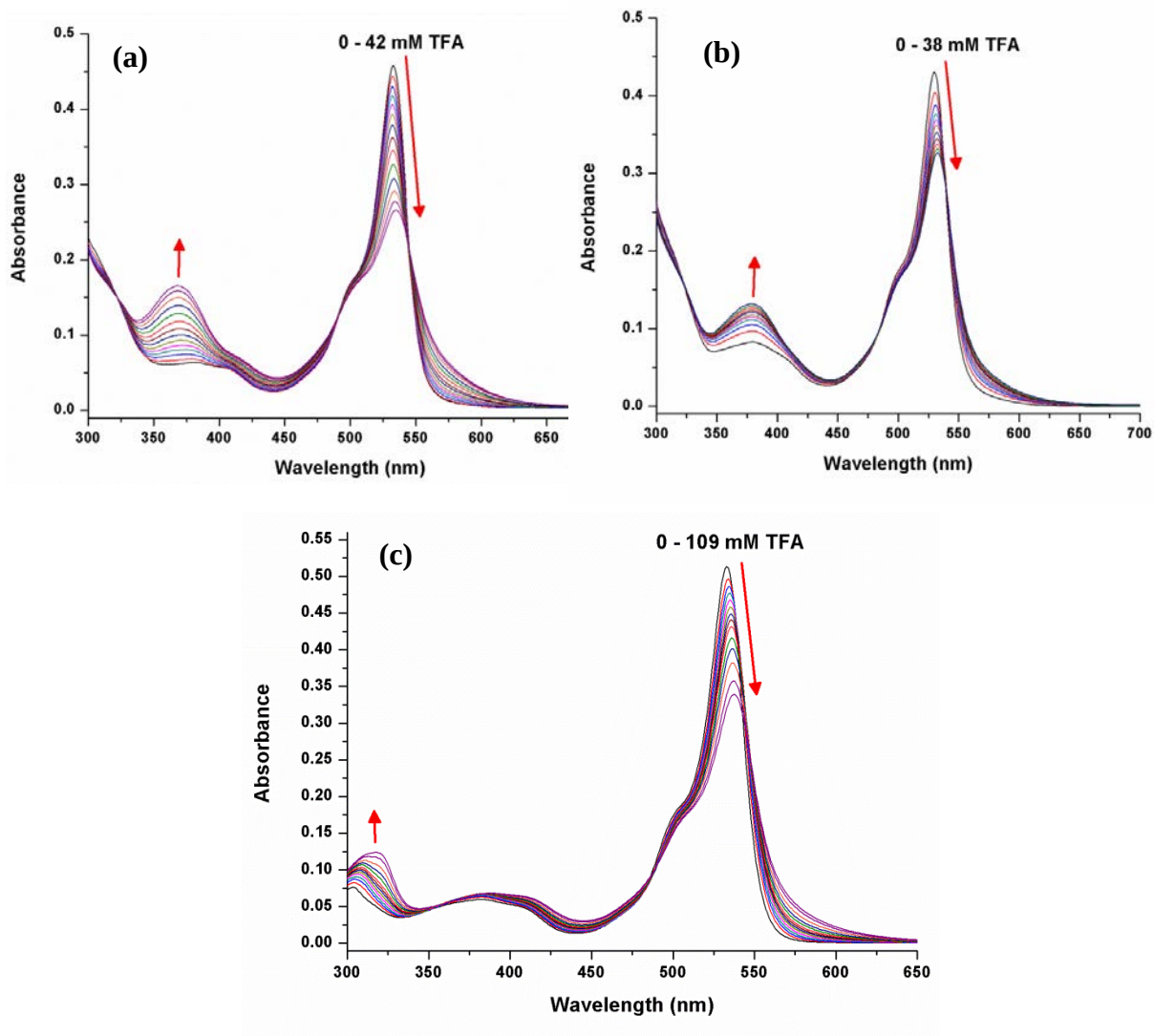


Figure 32. UV-Vis absorption spectra of **M1** (a), chloride-saturated **M1** (b) and **BOD1** (c) titrated with TFA in CH_2Cl_2 . (a) **M1** (10 μM) was titrated with 2.6 M TFA using 2 μl aliquots until there are no further spectral changes. (b) **M1** (10 μM , previously saturated with 2.5 equivalents of TBACl) was titrated with TFA until there are no further spectral changes. (c) **BOD1** (9 μM) was titrated with 1.3 M TFA using 2 μl aliquots.

The electronic absorption spectrum (Figure 32a) shows a bathochromic shift of the BODIPY transition from 532 to 537 nm accompanied by peak broadening.

Additionally a new peak grows in at 369 nm, which is attributed to the π - π^* transition of BODIPY on the basis of the emergence of a similar peak with **BOD1** at slightly shorter wavelengths and based on the assignments in literature (Figure 32a-c).¹⁸³ The same titration was performed with **M1**•Cl[−] where the macrocycle was previously saturated with chloride added as the tetrabutylammonium salt (TBACl). Similarly, a bathochromic shift from 529 to 534 nm was observed together with the new peak growing in at 375 nm (Figure 32b).

Binding constants were determined by analyzing UV-Vis titration spectrums on *Sivvu* software (Table 7).¹⁸⁴ The dissociation constants of trifluoroacetic acid, tetrabutylammonium chloride, tetrabutylammonium trifluoroacetate were also included in analysis. Further, to eliminate the effect of trifluoroacetate binding to cavity, the binding constant of this anion to **M1** was determined by titration with the tetrabutylammonium salt of this anion. *Sivvu* analysis for binding constants determination shows that the basicity, as reflected in the log β of **M1** increases from 4.24 to 5.41 in the presence of chloride. When **M1** is initially titrated with acid, the protonation is less effective. However, when the macrocycle is first saturated with chloride, protonation seems to be favored more (ΔG becomes −30.84 kJ/mol).

Table 7. Binding constants and ΔG values of macrocycles for chloride or proton^a

Compound	Log β^a	ΔG , (kJ/mol) ^a
M1 .Cl [−]	6.31	−35.99 ± 0.2
(M1 .H ⁺). Cl ^{−b}	7.48	−42.68 ± 1.6
(M1 .Cl [−]). H ^{+c}	5.41	−30.84 ± 0.1
M1 .TFA [−]	5.39	−30.72 ± 0.4
M1 .H ^{+d}	4.24	−24.18
M1 .TFAH	2.51	−14.34 ± 0.03
M2 . Cl [−]	7.51	−42.86 ± 0.4

^a see experimental part for details of calculations, ^b the data was obtained by titrating protonated **M1** with TBACl. ^c the data was obtained by titrating chloride saturated **M1** with TFA, ^d the ΔG value for this species is calculated from the **M1**. Cl[−] and (**M1**.Cl[−]). H⁺ equilibria.

This type of behavior, namely an increase in the observed pK_a in the presence of halides, was previously reported in bacteriorhodopsin.¹⁸⁵ In our case, this change is attributed to a modification in the pyridine microenvironment upon chloride binding. Highly electropositive cavity of the triazolophane obstructs a further positive charge inside. A similar phenomenon was extensively studied by Moore *et al.* on differently substituted ethynylpyridine oligomers where it was consistently shown that electron deficient environments destabilizes the pyridinium cation.⁵⁵ In our case, chloride binding reduces the electropositivity character of the cavity and aids protonation.

Fluorescence quenching profiles of BODIPY dyes in CH_2Cl_2 are given in Figure 33. Both **BOD1** and **M1** quenches almost entirely by the addition of 30 mM TFA. **BOD2**, which lacks a pyridine moiety in its structure, shows no acid response as expected. A small decrease (approximately 30%) in the emission of **M2** upon TFA addition was also observed. This behavior may be attributed to triazolophane protonation. Although this hypothesis cannot be proved by MASS Spect analysis, the protonated triazolophanes may provide an alternative but less effective route for PET process. The UV-Vis absorbance spectrum of this compound also shows a bathochromic shift upon TFA titration (Figure 34). Trifluoroacetate binding to triazolophane and triazole protonation may give rise to such a spectral change. On the other hand, almost no change was observed in the emission of **BOD2** upon TFA titration which confirms that the BODIPY core is not modified by TFA. In order to ensure that the compound had not decomposed after acid titration, chemical reversibility was also investigated using triethylamine (Et_3N) as base. The UV-Vis absorbance spectrum was restored back to its original non-protonated form (Figure 37).

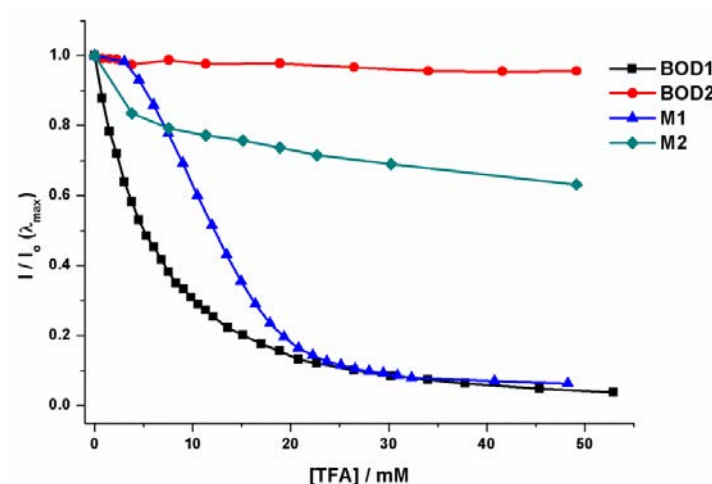


Figure 33. Quenching profiles of BODIPY building blocks and macrocycles. Stern-Volmer plots of 2 μM of **BOD1** (black), **BOD2** (red) and **M1** (blue) and **M2** (green) with TFA in CH_2Cl_2 followed by maximum emission wavelengths.

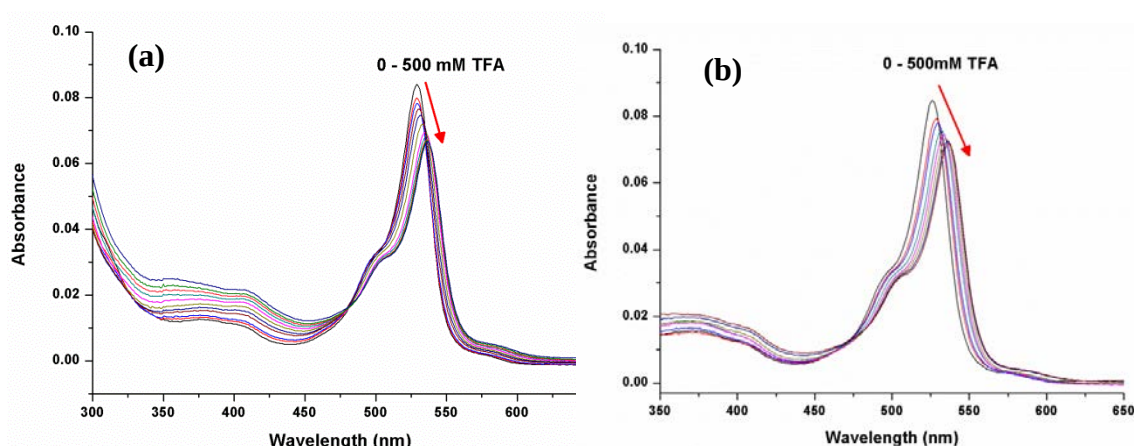


Figure 34. Titration of **M2** (2 μM) with TFA before (a) and after (b) saturation with 4 equivalents of TBACl in CH_2Cl_2 .

5.4.3. Chloride Binding

A small hypsochromic shift was observed in the BODIPY absorption upon chloride binding to both macrocycles, from 532 to 529 nm in **M1** and from 529 to 526 nm in **M2** (Figure 35, 42). Consistent with prior studies on chloride binding to triazolophanes⁹⁻¹², the region of the spectrum at 250 nm saw an increase in intensity.

Chloride binding constants of each macrocycles was estimated in the presence and absence of acid by fitting UV-Vis titrations using *Sivvu* software. For each analysis ion-pairing interactions were also included. The binding constants for chloride anion were determined to be $2.04 \times 10^6 \text{ M}^{-1}$ for **M1** and $3.03 \times 10^7 \text{ M}^{-1}$ for **M1** in the presence of acid. When the macrocycle is protonated, the positive charge inside the cavity increases the binding strength considerably due to an additional electrostatic benefit to the binding process. For **M2**, the binding constant was determined to be $3.26 \times 10^7 \text{ M}^{-1}$. The chloride binding constants and ΔG values for corresponding ions are given in Table 7.

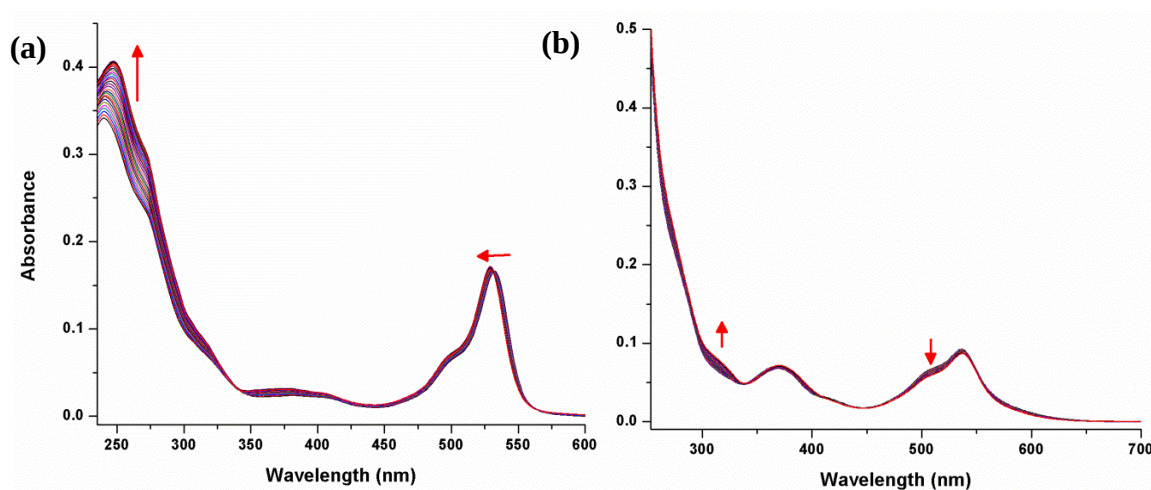


Figure 35. Titration of **M1** (4 μM) with TBACl (0.6 mM) before (a) and after protonation (b) in CH_2Cl_2 .

Initial ^1H NMR spectra of macrocycles **M1** and **M2** are broad and in both cases, there are three different triazole protons. ^1H NMR spectra with variable concentrations (1 - 10 mM) shows no significant change in peak positions or resolution (Figure 36a). Also, no spectral change was observed on UV-Vis during serial dilution from 346 mM **M1** to 2.7 μM (Figure 36b). This result suggests that the three triazoles resonances initially observed in **M1** is not due to aggregation but instead a structural asymmetry. Geometry optimization (HF/3-214^{*}) shows that the planarity of the triazolophane is distorted someway to break the symmetry of the triazoles closest to the western BODIPY side of the macrocycle. This distortion was removed upon Cl^- binding as confirmed by NMR titration (Figure 38) and geometry

optimization of the complex (HF/3-214). This structural distortion is also seen in the ^1H NMR spectra and the calculated spectra of **M2**. The ^1H NMR titration of **M1** with TBACl displays an overall downfield shift with the most profound shifts observed for the triazoles and the east phenylene protons (proton with label c) within the macrocycle (Figure 38). A Job's plot was generated using the chemical shift in proton c which indicates 1:1 binding of Cl^- to **M1**.

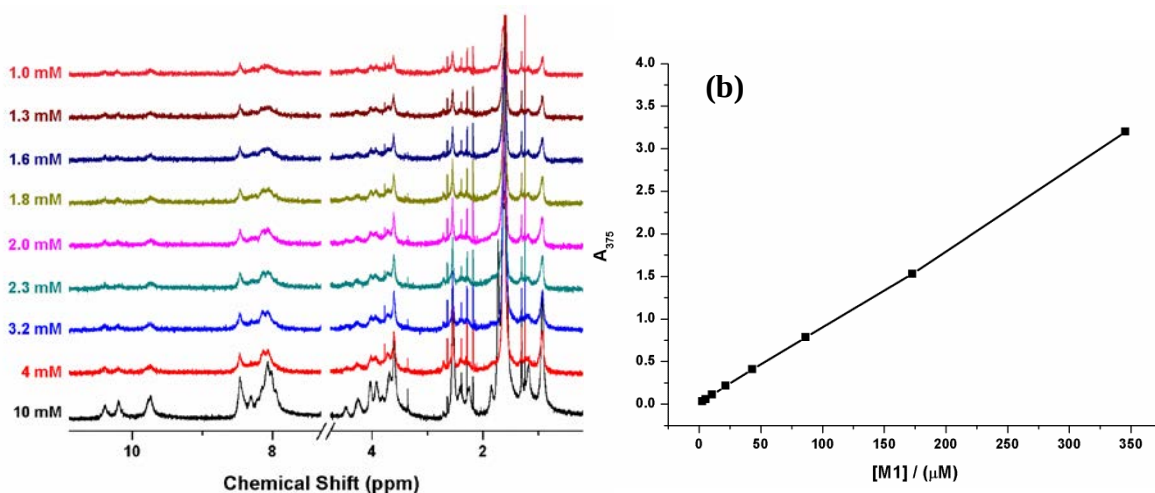


Figure 36. Concentration dependence of the ^1H NMR spectrum of **M1** (500 MHz, 298 K, CD_2Cl_2) (a), concentration dependence of the UV-Vis Absorbance spectrum of **M1** (345.6 – 2.7 μM , CH_2Cl_2) as followed by the absorbance change at 375.

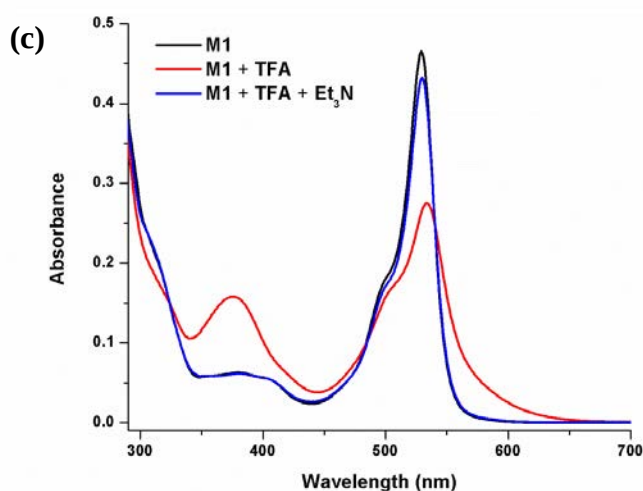


Figure 37. Characterization of the reversibility of the acid response of protonated **M1** (10 μM , CH_2Cl_2) by addition of Et_3N .

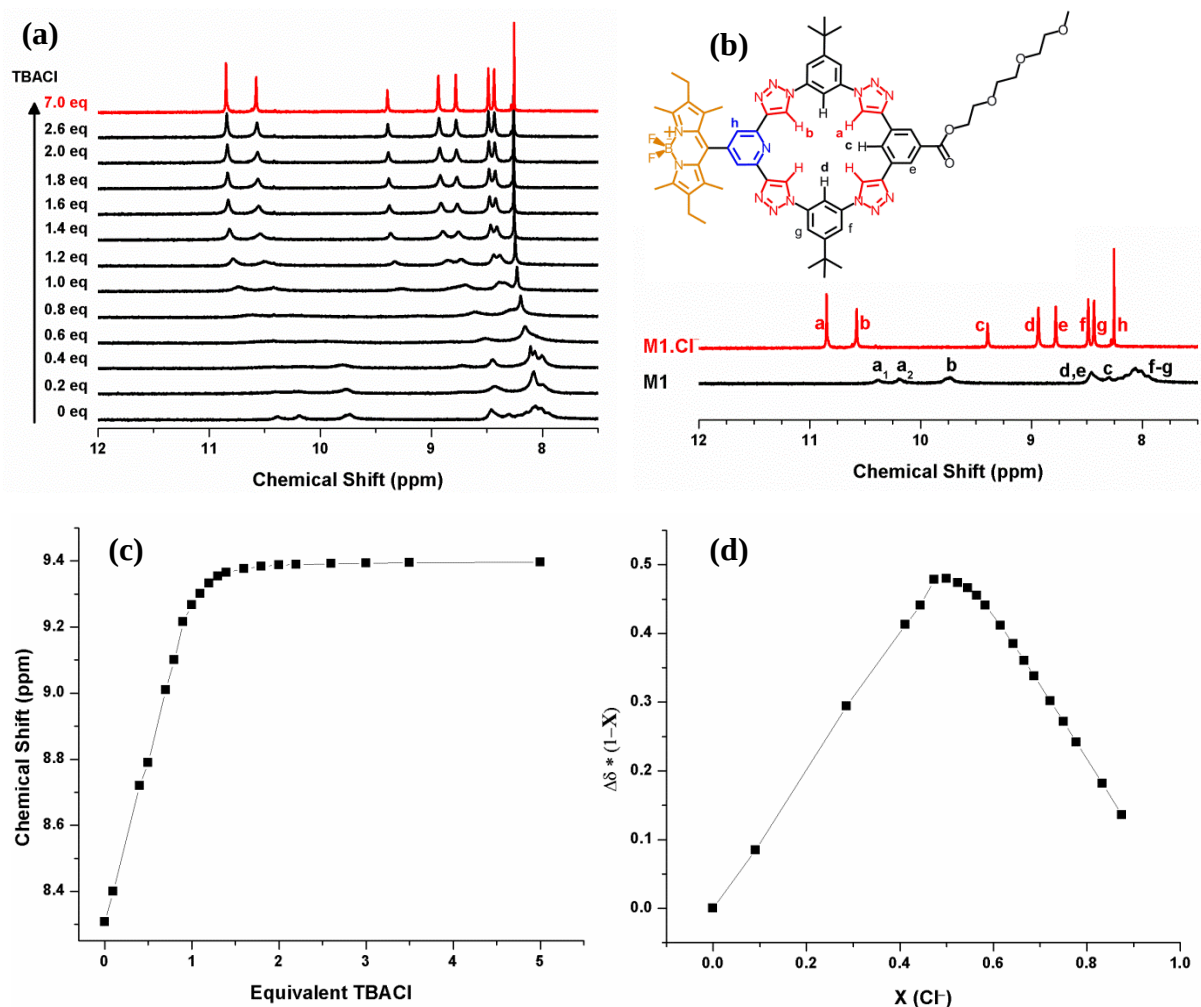


Figure 38. A) ^1H NMR titration of **M1** (10 mM) with TBACl (0.2 M) in CD_2Cl_2 . B) labeled chemical shifts, C) chemical shift in proton labeled as c within the macrocycle through TBACl titration D) Job's plot for TBACl binding using shifts in proton c.

5.4.4. Turn-on Sensing and Binding Equilibria

The overall reaction pathways between species accessible with protonation and Cl^- complexation, their associated emission properties and their proposed orbital diagrams are shown in Figure 39. Initially, there is an intense emission from the BODIPY fluorophore ($\phi_F = 0.66$). Along path 1, protonation is followed by the enhanced Cl^- binding. Upon protonation, oxidative PET from excited BODIPY to

the pyridinium is believed to account for the quenching with the quantum yield decreasing to 0.06. Upon Cl^- binding (Path 1b),

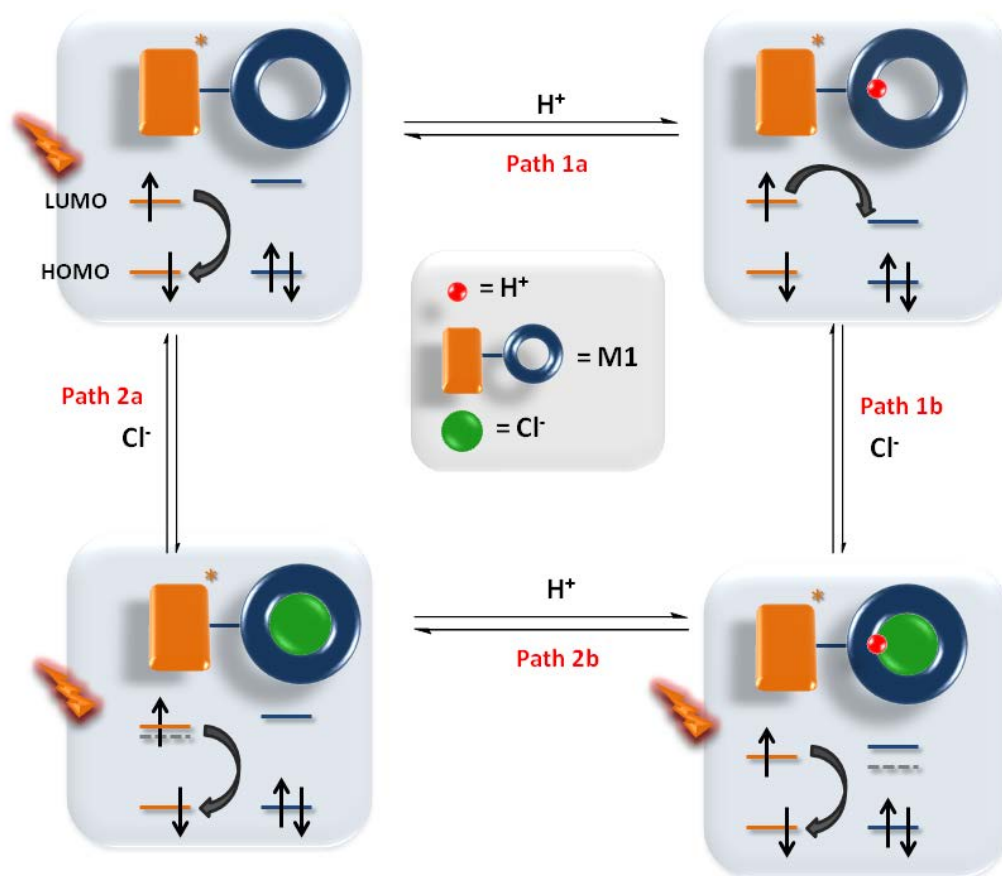


Figure 39. Schematic representation of binding equilibria for **M1** and the mechanism of action for turning on and off BODIPY emission. Path 1 represents sequential addition of acid then chloride whereas the order is reversed along path 2.

electrostatic destabilization of the pyridinium-localized LUMO competes PET and emission is restored to increasing levels depending on the Cl^- concentration such that, at Cl^- concentrations of 90 mM, the emission becomes even more intense than non-protonated **M1** (Table 6). In the second pathway, Cl^- binding is followed by facilitated protonation. The principle reactions taking place along each path include those shown in Figure 39 as well those for dissociation of the TFA into the

trifluoroacetate anion and a proton, and, the disassociation of the TBACl salt into chloride and the TBA^+ cation.

The acidity of TFA was estimated by fitting the UV-Vis absorbance data obtained by diluting TFA in CH_2Cl_2 and the ΔG value for TBACl ion pairing in CH_2Cl_2 was taken as is from literature.⁴² The constant for ion pairing of tetrabutylammonium cation and trifluoroacetate anion was determined by titrating TFA with TBACl. To determine the binding affinity of trifluoroacetate, a counterion involved during protonation process, titration of **M1** with tetrabutylammonium salt of this anion and subsequent *Sivvu* analysis were performed.

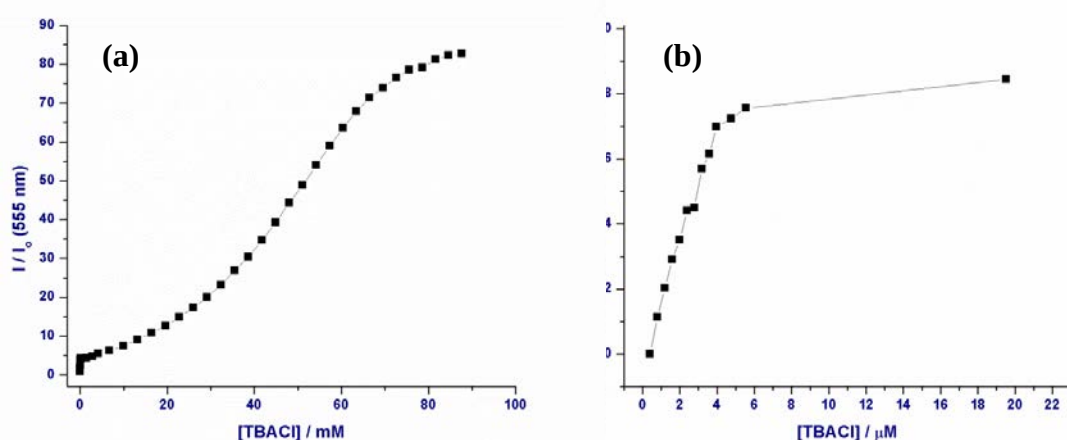


Figure 40. Restoration of emission of **M1** (2 μM) by TBACl in CH_2Cl_2 . (a) TBACl (0.6 mM, 10 mM, 0.1 M, 0.5 M, 2 M) was added to the **M1** (previously quenched by 120 mM TFA). (b) micromolar region of plot in A.

Upon protonation of **M1** (555 nm) emission is decreased by 97% and the quantum yield dropped from 0.66 to 0.06 upon addition of 120 mM TFA. PET processes usually require a spacer motif between the electron donor and acceptor moieties. In the previously reported pyridine BODIPYs, the two electronic systems are separated not in space but instead by the orientation, namely, pyridine at the *meso*-position of BODIPY is not coplanar with the BODIPY core. So, the two systems can behave as pseudo-independent electronic systems. In our case, the comparably bulky triazolophane orients almost perpendicular to BODIPY core on account of sterics. By analogy, intramolecular electron transfer between the covalently linked but electronically separated systems becomes possible.

When the protonated receptor, **M1.H**⁺ was titrated with TBACl (Figure 40), a gradual increase in emission intensity was observed during addition of more than 80 mM TBACl. The quantum yield of **M1.H**⁺ increased from 0.06 to 0.72 with increasing concentrations of chloride. The final quantum yield was even higher than the non-protonated macrocycle. Photophysical properties of the BODIPY building blocks and macrocycles are given in Table 6. The shape of the binding curve is interesting with two different sections, one below 4 μ M (Figure 40b) and one over 6 (Figure 40a). The overall dynamic range starts from sub-micromolar concentrations to above 80 mM.

To help understand the origin of the two-step chloride response in **M1** (Figure 40), **BOD1** was titrated first with H⁺ (Figure 41a) and followed with TBACl (Figure 41b). The emission was restored upon addition of 80 mM TBACl but the dynamic range of reaction starts at milimolar concentrations of chloride. Since this molecule lacks triazolophane binding pocket, the effect of chloride is unexpected! Halide induced quenching, rather than enhancing of quinolinium dyes are widely known in the literature and the reason for this quenching is explained by either halide induced intersystem crossing or by the collisional reduction of fluorophores. Structural similarity between the quinolinium dyes and the pyridinium moiety in **M1** and **BOD1** suggest a similar mechanism behind chloride induced turn on of the emission. This time however, the quaternized pyridine provides a PET route itself. Therefore, reduction of the pyridinium prevents PET and enables emission to be reinstated.

Since **BOD1** does not show a change in the presence of micromolar concentrations of chloride, two-step turn-on of the emission from **M1.H**⁺ can be explained as follows: The first step of the curve is associated with the saturation of the triazolophane's binding pocket. Thus, incorporating the triazolophane into the sensor's structure enables detection of chloride at micromolar concentrations. **BOD1** lacks this binding pocket and consequently, it lacks the sensitivity towards the μ M range and its detection curve shows one response that matches with the second of **M1.H**⁺. In the second step, the proposed collisional reduction by halide emerges and it requires higher chloride levels, namely milimolar concentrations. The behavior of

BOD1.H⁺ and **M1.H⁺** resemble each other at this range, suggesting a similar mechanism acting on both of them.

To see if the dynamic/collisional process is nonspecific, tetrabutylammonium salts of other halides (F⁻, Br⁻, I⁻), acetate (AcO⁻) and hydrogen sulfate (HSO₄⁻) were titrated (Figure 41). Addition of I⁻ did not alter the quenched profile but the color of the solution turned to gold. An increase in the emission, in the presence of HSO₄⁻ was observed beyond 110 mM. Likewise, addition of more than 80 mM of Br⁻, led to an increases in the emission intensity. The most obvious increase in emission was seen in samples titrated with acetate and fluoride anions, where the response is even

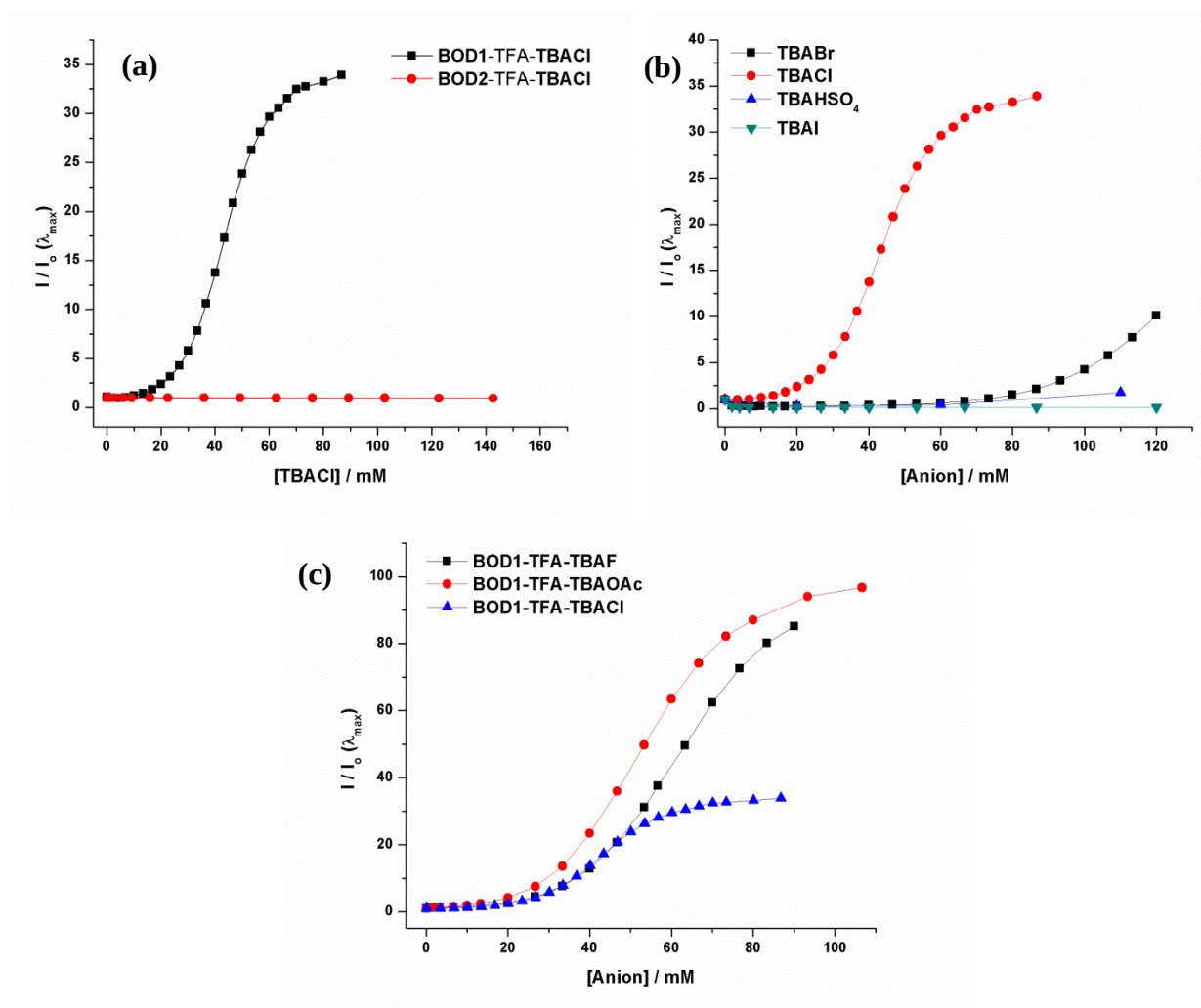


Figure 41. (a) Stern-Volmer plots of 2 μ M solutions of **BOD1** (black) and **BOD2** (red) with TBACl; (b) Fluorescence titration of **BOD1** (2 μ M) with TBACl (red), TBABr (black), TBAI (green) and TBAHSO₄ (blue); (c) Fluorescence titration of **BOD1** (2 μ M) with TBACl (blue), TBAF (red), TBAOAc (black) in CH₂Cl₂.

higher than the one observed for chloride. If the proposed charge transfer mechanism is true, then the magnitude of the effect and the selectivity should depend on the reduction potentials or basicities of anions. For fluoride and acetate, their higher basicity could deprotonate the pyridinium, which would contribute to the increase in emission intensity or it may even be the basis of the process. If the effect of F^- and AcO^- is due to their basicity, then, similarities between the absorbance spectra of neutral **BOD1** and **M1**, and the acidic solutions of the same compounds in the presence of excess F^- or AcO^- , can be explained solely by anion-induced deprotonation.

The tetraphenylene triazolophane in **M2** is not expected to show an acid response since the only possible protonation sites are triazoles and they are known to have very low pK_a values (1.17 for free triazoles).¹⁸⁶ However, when **M2** was titrated with TFA there was an obvious response in both the UV-Vis (Figure 34) and fluorescence spectra (Figure 33). There is a bathochromic shift in the absorbance spectra of **M2** both in the absence and presence of TBACl, (from 526 to 537 nm and from 529 to 537 nm, respectively).

Fluorescence titrations of **M2** with TFA also show an interesting result; it exhibits quenching upon acid titration accompanied by a bathochromic shift from 545 to 553 nm. However, the quenching is not as effective as **M1** (Figure 33). More acid (550 mM) is required compared to the amount required for **M1** (30 mM) to quench 80% of the initial emission in the case of **M2**. Knowing that BODIPY building-block does not get quenched with acid (Figure 33), this behavior can be attributed to protonation of the triazole nitrogens and generation of an additional route for the PET quenching process. During the period of the titration where the macrocycle **M2.H⁺** is saturated, there is an increase in emission intensity up to around 5 μ M TBACl, then, there is an decrease in emission intensity at 30 μ M chloride (Figure 44). This decrease may be explained by considering the protonation of the triazole facilitated in the presence of chloride within the macrocycle. Since there is incomplete quenching in the beginning, the presence of chloride may complete the process. However, unlike **M1**, the potential protonation sites face outwards, and thus they should be affected less by

the presence of chloride. Both quenching and chloride response of **M2** seems to be less effective than **M1**.

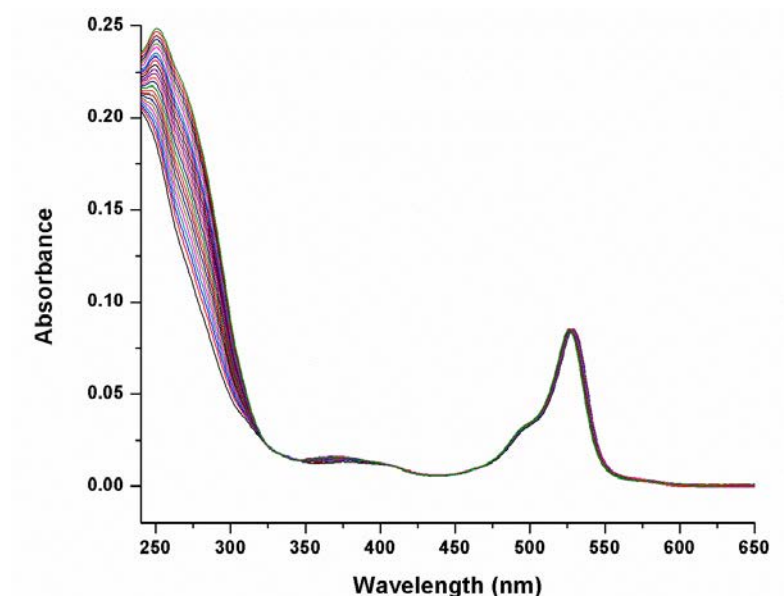


Figure 42. Titration of **M2** (2 μM , CH_2Cl_2) with TBACl up to 4 equivalents.

In an attempt to understand the ground state behavior of the BODIPY fluorophore in the presence of high concentrations of Cl^- , UV-Vis absorbance spectra of **BOD1**, **M1** and **M2** were recorded under various conditions (Figure 43). Each sample was subjected to different concentrations of TFA that are sufficient to saturate the compounds based on the knowledge obtained from the fluorescence experiments. These values are 25 μl (109 μM) for **BOD1** and **M1**, and, 120 μl (522 mM) for **M2**. For **M2** and neutral **M1** no significant change in absorbance was observed after the initial saturation of the macrocycle with TBACl ($\sim 3 \mu\text{M}$, Figure 43b, c). This suggests that after binding of chloride to the cavity of the macrocycle, the BODIPY core is not affected by excess TBACl in **M2** and neutral **M1**. However, **BOD1.H⁺** and **M1.H⁺** show a dramatic change in their spectra accompanying the addition of large amounts of TBACl. Considering the band structure of the BODIPY's core absorbance at 532 nm, the final spectra of **BOD1.H⁺** and **M1.H⁺** look as if the compounds are no longer protonated after excess TBACl addition (Figure 45). Apart from the spectral change in the visible region, the UV region shows the most obvious increase. A sharp peak grows in around 360 nm in both macrocycles and **BOD1**

during the addition of excess TBACl. The intensity of this new peak is not high in neutral **M1** relative to BODIPY core absorbance at 532 nm. Titrating TFA with excess TBACl explains the origin of these growing peaks since a similar increase in the spectrum was obtained thus attributed to ion pairing of tetrabutylammonium cation and trifluoroacetate anion.

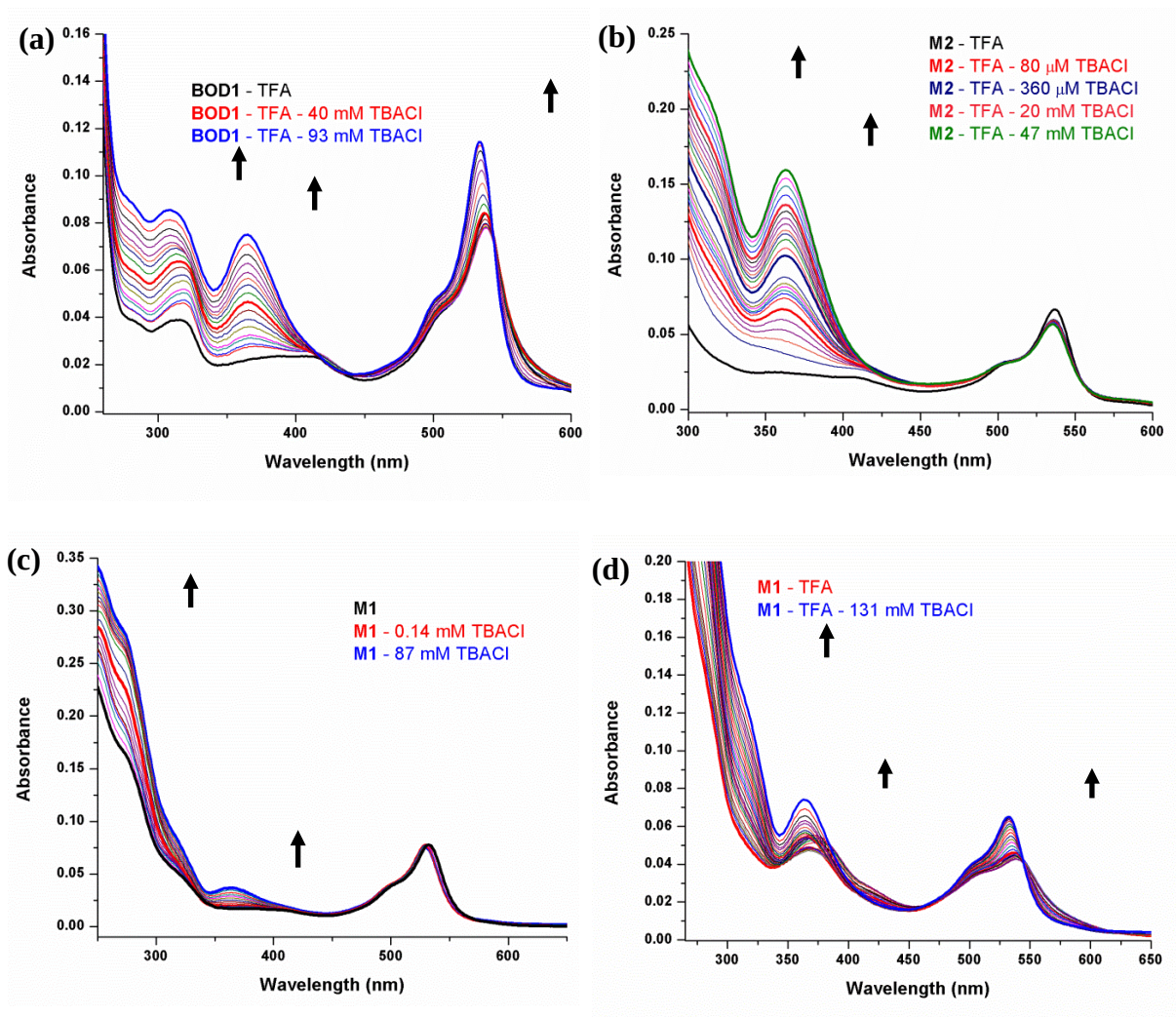


Figure 43. UV-Vis Spectra of 2 μ M solutions of **BOD1**, **M1** and **M2** in the presence of excess TBACl in CH_2Cl_2 . Except for the titration of **M1** in (c), TFA was added to the samples prior to TBACl titrations (25, 25 and 120 μ l to **BOD1**, **M1** and **M2** respectively). D) Absorbance change at 367 nm upon TBACl titration of **BOD1** and **M1**.

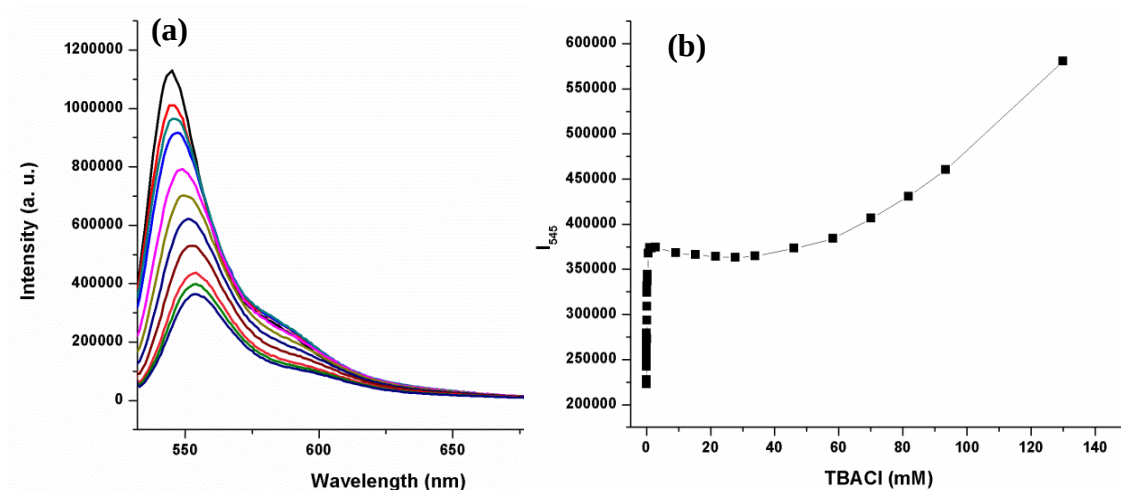


Figure 44. Acid and chloride fluorescence response of **M2** (2 μM, CH₂Cl₂). A) Fluorescence quenching and bathochromic shift upon TFA addition up to approximately 250000 equivalents. B) Titration of protonated **M2** (**M2**·H⁺) with TBACl (0.6 mM, 10 mM, 0.5 M, 2 M) as followed by intensity change at emission wavelength of 545 nm.

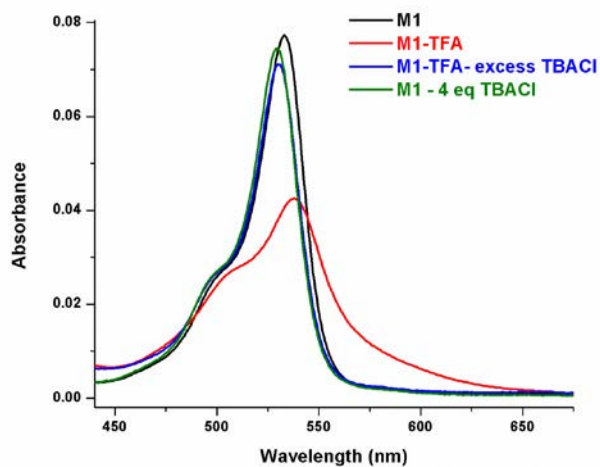
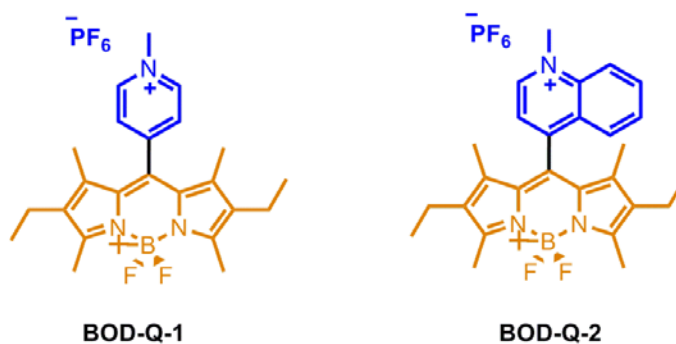


Figure 45. UV-Vis absorbance spectra of **M1** (black), protonated **M1** (red), protonated **M1** titrated with excess TBACl (blue) and **M1** titrated with 4 equivalence of TBACl (green).



Scheme 23. Structures of methyl-quaternized BODIPY dyes

The response of **M1** and **BOD1** to higher concentrations of chloride (mM region) was thought to be a result of deprotonation by anions. Comparison of absorbance change of **M1** in the presence of excess chloride shows that the final spectrum (excess chloride added to protonated **M1**) is quite similar to chloride-bound non-protonated macrocycle (Figure 45). This may show that anions act as base to deprotonate pyridinium, thus let to the increase in emission in mM-region of the spectrum. To understand this further, we synthesized two methyl quaternized model compounds, methyl-pyridinium BODIPY (**BOD-Q-1**) and methyl-quinolinium BODIPY (**BOD-Q-2**). These dyes are essentially non-emissive with a PET process taking place between BODIPY core and quaternized electron poor pyridinium/quinolinium moieties. Since they lack a protonation site we expect to see no response related to anion basicity. Surprisingly however, an increase in the emission of both of these fluorophores was observed. The increase is not as promising as in **M1** and **BOD1** considering the dynamic range and fluorescence intensity but still the response is in mM concentration region and can be seen by naked eye. In both quaternized BODIPYs a bathochromic shift in UV-Vis absorbance accompanies an increase in extinction coefficient (Table 6, Figure 46). Quantum yield increases obviously upon addition of TBACl (Table 6). The effect of TBACl is more promising in the case of **BOD-Q-2**, the quantum yield increases from 0.01 to 0.54. On the other hand, the maximum response ranges are smaller compared to **M1**, the values for maximum concentrations of TBACl that induces a fluorescent response are approximately 60 mM for **BOD-Q-1** and 8 mM for **BOD-Q-2**.

Regarding these results, we suggest the contribution of a further process in the recognition event, namely charge transfer interaction between anion and electron deficient pyridinium or quinoliniums. Such charge transfer interactions between anions and electron deficient naphthalenediimide systems were extensively analyzed by Saha *et al.*¹⁸⁷ Here, we argue the possible contribution of such processes in blockage of PET process.

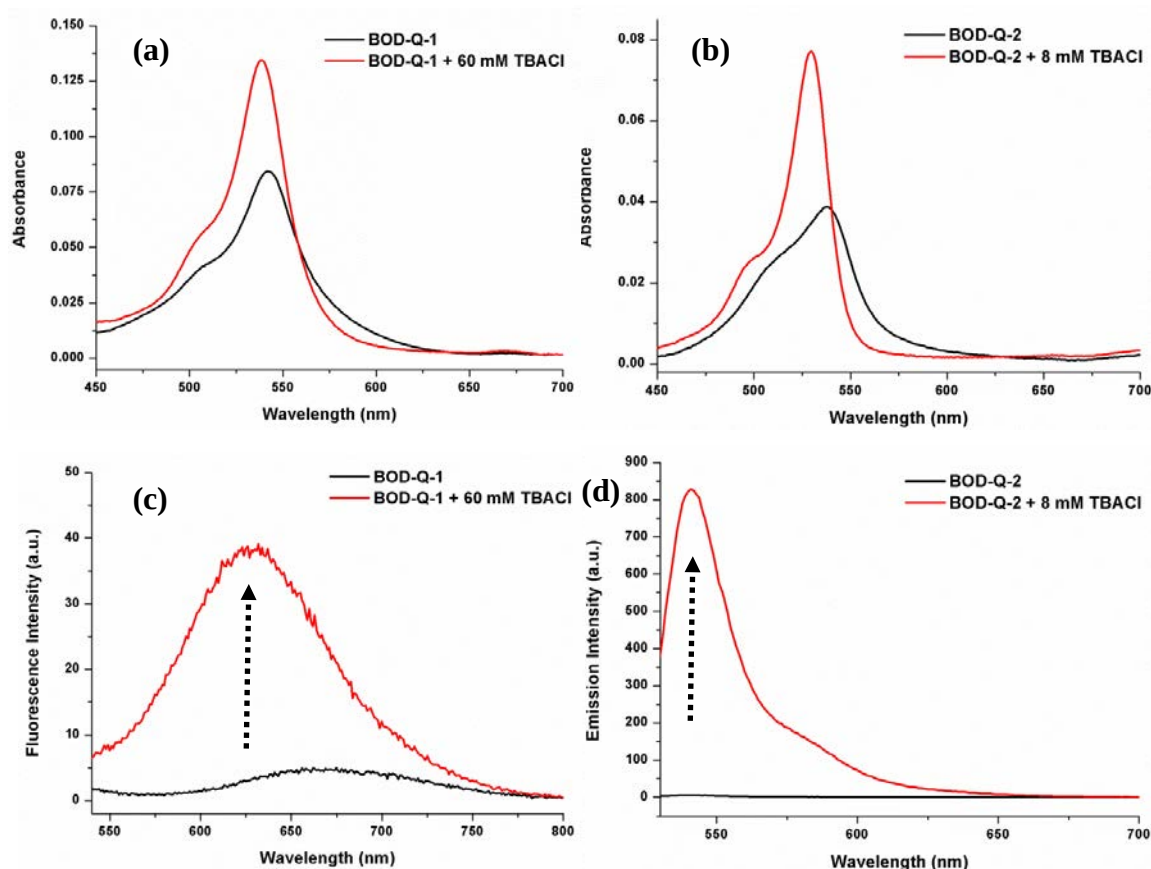


Figure 46. UV-Vis Absorbance and emission spectra of **BOD-Q-1** and **BOD-Q-2** before and after TBACl addition in CH_2Cl_2 .

5.4.5. Conclusions

In this work we have designed a chloride sensor by rationally combining the knowledge of protonation-induced PET in pyridine BODIPY dyes with the strong and selective chloride binding ability of the triazolophane. Two different processes involved in the recognition and emission sensitivity provide an unexpectedly large

dynamic range (0.2 μM -80 mM). The first of these processes is the simple binding to the triazolophane pocket, which provides a sensitivity to chloride at parts per billion concentrations as low as 0.2 μM . At higher chloride concentrations, collision induced reduction was thought to be effective in the turn-on sensing process. This phenomenon was studied in the literature with a number of different quinolinium dyes and other organic compounds.^{42,43,44} The possible explanation for the emission increase in the higher concentration regime could be the basicity of anions since acetate and fluoride also react at high concentrations. For long term biological application purposes, these competing anions may not be as interfering on account of the fact that their concentrations are much lower in the body. For compound **M1**, although the slope of response is different in the sub-micromolar and millimolar regions, the macrocycle is responsive to chloride practically between 0.2 μM - 80 mM ranges which matches the chloride level in most acidic biological compartments. Enhancement in quantum yields is 875 fold and both the excitation and the bright emission is in visible region of the spectrum. To increase the dynamic range of fluorophores for the detection of Zn^{2+} , Zhu *et al.* suggest multiple binding pockets with different affinities for Zn^{2+} .^{188,189} This method allows a wider dynamic range since saturation of two different sites rather than one determines the detection range. The same analogy is present in our case with a slightly different strategy. Here, the large optical response to a wide range of chloride concentrations is offered by two different processes rather than two binding sites. The concentration dependency of the two processes differ a lot, which enables the extended recognition range for chloride. With the knowledge obtained in this work, second-generation PET sensors with water solubility or the ones with appropriate carriers (i.e. micelles, soluble nanoparticles) can be designed for biological applications.

Although the sensor presented here is worked in organic solvent, further modification of such system may open a path in certain applications. For instance, long term biological relevance stems from the fact that, although the chloride concentrations in plasma, airways and some early endosomal pathways are known, there is no accurate data for late endosomal compartments, which are thought to have an acidity below pH 5.5. Furthermore, there is evidence that a number of diseases

related to poor protein processing and sorting or to exocytosis, which normally takes place in these late endosomes and lysosomes may be related to Cl^- channelopathies. As such, deficiencies in H^+/Cl^- exchangers within these compartments need to be more deeply understood. In addition to acidic eukaryotic organelles, chloride channels are known to have crucial roles in the maintenance of acid resistance for some bacteria that are able to survive in very harsh media with pH values below 3.⁵² A chloride-dependent promoter in some gram-positive bacteria was also identified which is thought to be induced in highly acidic and chloride-rich environments such as in the human stomach.¹⁹⁰ Genes associated with this promoter are expressed at low pH and high chloride levels. For these various reasons, developing chemosensors to determine chloride concentrations at low pH would be beneficial.

The importance of sensing chloride level in acidic media was mentioned above. The acid resistance in bacteria, ion-transport and subsequent control of protein processing, sorting, and cell volume regulation are associated with chloride concentration in acidic media. Developing a sensor that is stable in acidic conditions and that shows a chloride response within the desired concentration range is highly important for understanding such biological processes and for developing therapeutic systems for related diseases i.e. cystic fibrosis.

It is interesting to note that, the change in pK_a upon chloride binding can be used for ion co-transport across a membrane using the concept of facilitated transport. Namely, a proton gradient can be generated across a bilayer using chloride concentration difference. As it can be seen in Table 7, chloride binding increases the pK_a of pyridine within the structure of macrocycle. In this case, protonation becomes easier. When, the macrocycle is moved to a chloride-poor environment, dissociation of chloride would be expected to release proton as well, since the pK_a drops under these conditions. A similar exchange process is actually believed to be happening in acidic organelles to regulate pH. These concepts are yet to be analyzed both in live media with natural channels and synthetic ones. The use of optimized version (both for biological relevancy and chloride and proton binding properties) of the acid-chloride responsive macrocycle in those systems can be a way to understand and perform such facilitated anion co-transport mimicry.

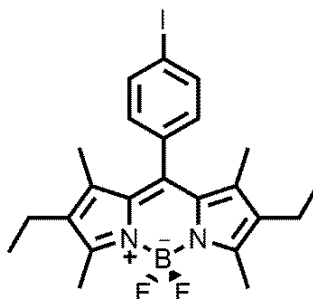
5.5. Experimental Details

5.5.1. Spectroscopy

5.5.1.1. Fluorescence Spectra and Quantum Yield Determination

2 μM solutions of **M1**, **M2**, **BOD1**, **BOD2** were prepared in CH_2Cl_2 . Emission spectra were recorded with excitation at 524 nm and with both excitation and emission slit widths set to 3 nm. To these solutions, different stock solutions of TFA (2 M, 10 M or 13 M TFA) were added in 2 – 4 μl aliquots until there were no further spectroscopic changes. For **M1** and **BOD1**, 30 mM of TFA is enough for more than 90% quenching of initial fluorescence. For **M2**, 500 mM TFA was required to achieve the same outcome. For anion titrations different stock solutions of TBACl (0.6 mM, 10 mM, 0.5 M, 2 M), TBABr (10 mM, 2 M), TBAI (10 mM, 2 M), TBAF (1 M), TBAOAc (1 M), TBAHSO₄ (0.5 M) were used.

For quantum yield measurements, absorbance values of all the compounds (**BOD 1**, **BOD 2**, **M1**, **M1**•H⁺, **M1**•H⁺•Cl⁻, **M2**) were between 0.05 – 0.15 to avoid self quenching (quartz cells with 1 cm path length were used). As a reference compound, 10-(4-iodophenyl)-2,8-diethyl-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2] diazaborinin-4-ium-5-uide, was used with a known quantum yield (0.78) in CHCl_3 (Scheme 24).¹⁶⁴



Scheme 24. Structure of the BODIPY compound used as the reference for the quantum yield calculations

The following equation was used for quantum yield calculation,

$$Q = Q_R (I/I_R)(A_R/A) \times (n^2/n_R^2) \quad \text{Equation 5}$$

where Q_R is the quantum yield of the reference BODIPY compound (0.78), I and I_R are the integrated areas of the emission spectra for sample and reference, respectively; A and A_R represent absorbance values at the excitation wavelength (524 nm) for sample and standard assuming a path length of 1 cm; and n and n_R refer to refractive indices of the solvents in which the sample and standard compounds were dissolved, respectively. All samples were dissolved in CH_2Cl_2 with a refractive index of 1.42 whereas the reference compound was dissolved in CHCl_3 ($n_R = 1.49$). The integrated areas were determined using *Origin* between 534 – 700 nm.

5.5.1.2. Binding Constant Determination

All binding constants were determined in CH_2Cl_2 . The ΔG values were determined using *Sivvu* by global fitting of the data. Binding constants in the presence and absence of TFA were calculated using the formula:

$$\Delta G = -RT \ln K \quad \text{Equation 6}$$

For the purposes of data fitting, the ΔG value for ion pairing of TBACl was taken to be $-27.7 \text{ kJ / mol}^{191}$ and dilution was taken into consideration in the software. The error was estimated by randomly omitting approximately 20% of the data points multiple times and recalculating the ΔG value. *Sivvu* output from the fitting is given in the following plots for each sample together with the associated error analyses. In each of the figures, the extinction coefficient (molar absorptivity) spectra of each absorbing species, the raw absorbance spectra for the titration within the wavelength range used in the calculation, speciation curves relative to the composition of each solution examined (solution number) and an error contour plot are presented. Red colors in the contour plot identify areas of greatest deviation.

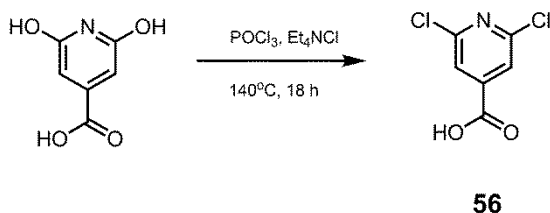
The ΔG for the dissociation of TFA into its acid, H^+ , and conjugate base was determined to be -17.79 kJ/mol in CH_2Cl_2 by monitoring UV-Vis absorptions during dilution of TFA in CH_2Cl_2 . The data were analyzed using *Sivvu* software. For any titrations that involve TFA, the -17.79 kJ/mol value was used as the ΔG and its

ionization was included in the fitting analysis. The binding affinity of the trifluoroacetate anion (TFA^-) with **M1** was estimated from titration of the tetrabutylammonium (TBA^+) salt of this anion with **M1**, the dissociation constant obtained by this method is -30.72 kJ/mol. The ion pairing between TFA^- and TBA^+ was also estimated from the titration spectra obtained upon the addition of TBACl to TFA and TBACl, and is found to be -30.94 kJ/mol.

5.5.1.3. Reversibility-Stability Experiment

In order to understand if the compound is still intact after TFA addition, a deprotonation experiment was performed with a base and UV-Vis spectral change was analyzed. To a solution of **M1** ($10\text{ }\mu\text{M}$) was added excess amounts of TFA (44 mM) to prepare $\text{M1}\cdot\text{H}^+$. Restoration of the initial spectrum was attained upon addition of a base, Et_3N . The base solution was prepared by diluting 1 ml of Et_3N with 9 ml of CH_2Cl_2 (1.4 M). The original spectrum was obtained after addition of an excess amount of (excess with respect to TFA) the Et_3N solution.

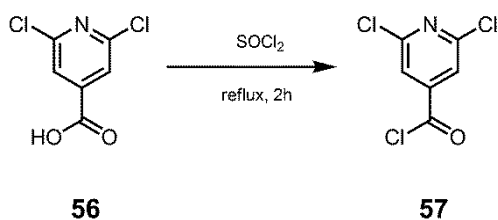
5.5.2. Synthesis



Synthesis of Compound 56:¹⁹² Citrazinic acid (5 g , 32.3 mmol) and tetraethylammonium chloride (5.34 g , 32.3 mmol) were suspended in phosphorous(V) oxychloride (10 ml). The reaction mixture was stirred at $140\text{ }^\circ\text{C}$ for 18 h . Upon cooling to RT, the mixture was poured over ice (75 g) and stirred for an additional 2 h . A brown solid product was obtained by filtering (5.03 g , 81%).

^1H NMR ($\text{DMSO}-d_6$, 400 MHz , $\delta\text{ ppm}$) 7.82 (s, 2H ; ArH).

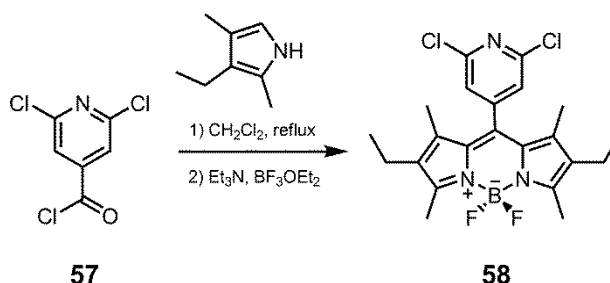
^{13}C NMR ($\text{DMSO}-d_6$, 400 MHz , $\delta\text{ ppm}$) 164.1 , 150.6 , 145.1 , 123.3 .



Synthesis of Compound 57: 2,6-Dichloroisonicotinic acid (1.2 g, 6.3 mmol) was suspended in thionyl chloride (SOCl₂, 10 ml) and the mixture was refluxed for 2 h. After cooling to RT, SOCl₂ was evaporated under vacuum to produce a brown solid (1.31 g, quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.83 (s, 2H; ArH).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 165.4, 152.2, 144.6, 123.0.

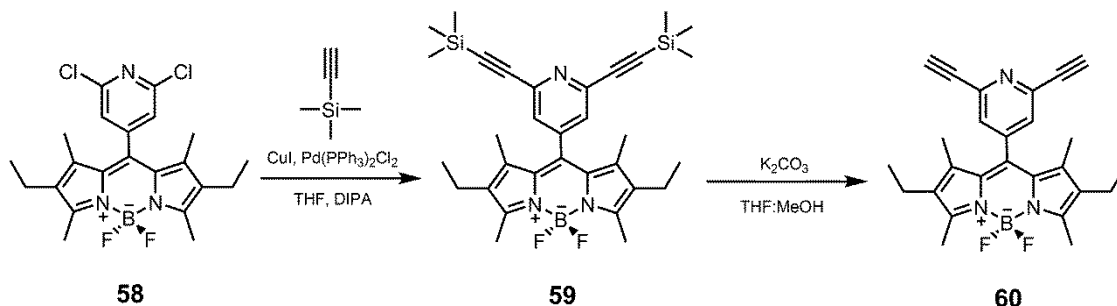


Synthesis of Compound 58: 2,6-Dichloroisonicotinoyl chloride (750 mg, 3.56 mmol) was dissolved in dichloromethane (CH₂Cl₂, 100 ml) and the solution was sparged with argon for 20 min. 2,4-Dimethyl-3-ethyl pyrrole (2.2 eq., 966 mg, 1.06 ml) was added and the solution was refluxed for 3.5 h. After cooling to RT, triethylamine (4 ml) was added to the reaction mixture. Subsequently, BF₃•OEt₂ (4 ml) was added and the reaction mixture was stirred for 1 h at RT. After washing with H₂O, the organic layer was collected and dried over Na₂SO₄. The crude product was purified by flash silica column chromatography using CH₂Cl₂/hexanes (50/50; v/v) as the mobile phase affording a red solid (530 mg, 33%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.29 (s, 2H; ArH), 2.51 (s, 6H; ArCH₃), 2.30 (q, 4H, J = 7.4 Hz; ArCH₂), 1.41 (s, 6H; ArCH₃), 0.98 (t, 6H, J = 7.4 Hz; CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 155.8, 151.5, 149.8, 137.3, 134.0, 132.8, 129.3, 123.1, 17.1, 14.5, 12.7, 12.4.

MS (HiResESI) Cald for $C_{22}H_{24}BCl_2F_2N_3$ (M-H)⁻ 448.1334; Found 448.1346 (M-H)⁻



Synthesis of Compound 59: Compound **58** (350 mg, 0.78 mmol) was dissolved in tetrahydrofuran (30 ml) and diisopropylamine (10 ml). Argon was sparged through the solution for 20 min prior to the addition of CuI (15 mg, 0.078 mmol) and Pd(PPh₃)₂Cl₂ (33 mg, 0.047 mmol). The flask was sealed and trimethylsilylacetylene (1.1 ml, 7.8 mmol) was added using a syringe. The reaction was stirred for 12 h at RT. The crude product was extracted with ethyl acetate then water and the organic layer was collected and dried over Na₂SO₄. The product was purified by column chromatography (SiO₂) using CH₂Cl₂/hexanes (2/1; v/v) as the mobile phase resulting in an orange solid (375 mg, 84%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.37 (s, 2H; ArH), 2.51 (s, 6H; ArCH₃), 2.29 (q, 4H, J = 7.4 Hz; CH₂), 1.39 (s, 6H; ArCH₃), 0.97 (t, 6H, J = 7.4 Hz; CH₃), 0.24 (s, 18H, CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 155.5, 145.5, 144.6, 138.1, 134.9, 133.9, 130.0, 126.7, 102.8, 97.5, 17.5, 15.0, 13.0, 12.9, 0.4.

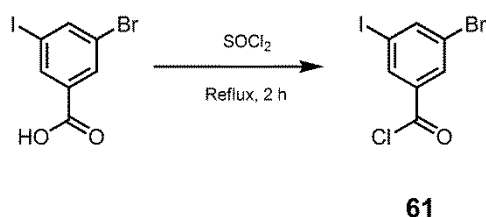
MS (HiResESI) Cald for $C_{32}H_{42}BF_2N_3Si_2$ (M-H)⁻ 572.2906; Found 572.2902 (M-H)⁻

Synthesis of Compound 60: Compound **59** (200 mg, 0.35 mmol) was dissolved in a mixture of THF (15 ml) and MeOH (15 ml). K₂CO₃ (145 mg, 1.05 mmol) was added, and the reaction was stirred at RT. After 15 min, TLC showed that all the starting compound was consumed. The reaction was quenched with saturated NH₄Cl (aq) and extracted with CH₂Cl₂. The organic layer was collected, dried over Na₂SO₄ and evaporated *in vacuo*. The product was purified by column chromatography using EtOAc/hexanes (1/1; v/v) as the mobile phase to afford an orange solid (150 mg, quantitative).

^1H NMR (CDCl_3 , 500 MHz, δ ppm) 7.44 (s, 2H; ArH), 3.22 (s, 2H; CH), 2.51 (s, 6H; ArCH₃), 2.28 (4H, $J = 7.4$ Hz; CH₂), 1.36 (s, 6H; ArCH₃), 0.97 (t, 6H, $J = 7.4$ Hz; CH₃).

^{13}C NMR (CDCl_3 , 500 MHz, δ ppm) 155.3, 145.5, 143.6, 137.5, 133.9, 133.7, 129.5, 126.9, 81.5, 79.1, 30.3, 17.0, 14.5, 12.6, 12.4.

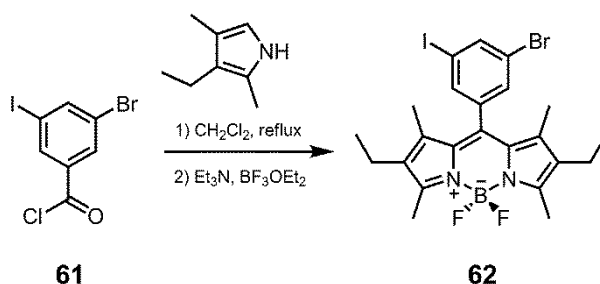
MS (HiResESI) Cald for $\text{C}_{26}\text{H}_{26}\text{BF}_2\text{N}_3$ (M-H)⁻ 428.2114; Found 428.2131 (M-H)⁻.



Synthesis of Compound 61: 3-Bromo-5-iodobenzoic acid (1 g, 3.06 mmol) was suspended in SOCl_2 (10 ml) and refluxed for 2 h. The reaction mixture was cooled to RT and the SOCl_2 was evaporated to dryness to yield the product (1.05 g, quantitative).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.33 (s, 1H; ArH), 8.18 (s, 1H; ArH), 8.14 (s, 1H; ArH).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 165.8, 146.0, 138.4, 136.0, 133.2, 123.5, 94.3.



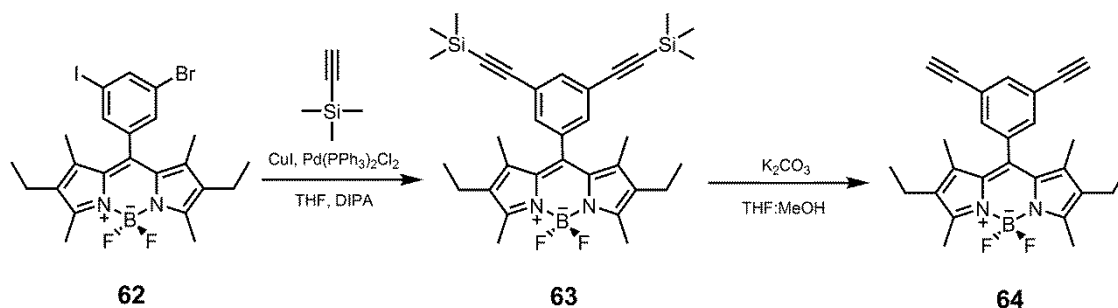
Synthesis of Compound 62: 3-Bromo-5-iodobenzoyl chloride, **61** (1.04 g, 3.01 mmol) was dissolved in CH_2Cl_2 (100 ml) and degassed with argon for 20 min. 2,4-Dimethyl-3-ethyl pyrrole (2.1 eq., 0.85 ml) was added and the solution was refluxed for 3.5 h. After cooling to RT, Et_3N (4 ml) was added followed by $\text{BF}_3 \cdot \text{OEt}_2$ (4 ml) and the reaction mixture was stirred for 1 h at RT. The reaction mixture was washed with H_2O and the organic layer was collected and dried over Na_2SO_4 . The product

was purified by flash column chromatography (SiO₂) using CH₂Cl₂:hexanes (50/50; v/v) as the mobile phase to produce a red solid (735 mg, 42%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.98 (t, 1H, J = 1.5 Hz; ArH), 7.61 (t, 1H, J = 1.5 Hz; ArH), 7.44 (t, 1H, J = 1.5 Hz; ArH), 2.50 (s, 6H; ArCH₃), 2.29 (q, 4H, J = 7.8 Hz; ArCH₂), 1.36 (s, 6H, ArCH₃), 0.98 (t, 6H, J = 7.6 Hz; CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 154.8, 140.1, 139.4, 137.9, 136.0, 135.8, 133.4, 131.0, 130.3, 123.4, 94.5, 17.1, 14.6, 12.6, 12.3.

MS (HiResESI) Cald for C₂₃H₂₅BBBrF₂IN₂ (M-H)⁻ 583.0233; Found 583.0203 (M-H)⁻.



Synthesis of Compound 63: Compound **62** (280 mg, 0.48 mmol) was dissolved in THF (30 ml) and diisopropylamine (10 ml) and then purged with argon (20 min). To the solution was added CuI (18 mg, 0.096 mmol) and Pd(PPh₃)₂Cl₂ (40 mg, 0.058 mmol). The flask was sealed and 10 equivalents of trimethylsilylacetylene (0.68 ml, 4.8 mmol) was added using a syringe. The reaction was stirred for 12 h at RT. The reaction mixture was extracted with EtOAc and the organic layer was collected and dried over Na₂SO₄. The product was purified by column chromatography using EtOAc/hexanes (1/4; v/v) as the mobile phase (orange solid, 270 mg, 98%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.66 (t, 1H, J = 1.4 Hz; ArH), 7.32 (d, 2H, J = 1.4 Hz; ArH), 2.50 (s, 6H; ArCH₃), 2.28 (q, 4H, J = 7.3 Hz; ArCH₂), 1.35 (s, 6H; ArCH₃), 0.97 (t, 6H, J = 7.3 Hz; CH₃), 0.22 (s, 18H; CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 154.3, 138.1, 136.2, 135.6, 133.0, 131.4, 130.5, 124.5, 103.1, 96.4, 86.0, 17.1, 14.6, 12.6, 12.3, 0.8.

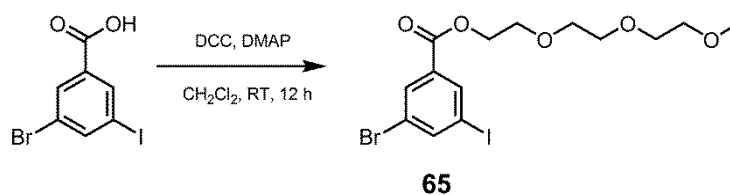
MS (HiResESI) Cald for C₃₃H₄₃BF₂N₂Si₂ (M-H)⁻ 571.2954; Found 571.2964 (M-H)⁻

Synthesis of Compound 64: Compound **63** (275 mg, 0.48 mmol) was dissolved in THF (15 ml) and MeOH (15 ml). K₂CO₃ (199 mg, 1.44 mmol) were added and the reaction was stirred at RT. After 15 min all the starting compound was consumed. The reaction was quenched with saturated NH₄Cl (aq) and extracted with CH₂Cl₂. The organic layer was collected, dried over Na₂SO₄ and evaporated *in vacuo*. The product was purified by column chromatography using acetone/hexanes (1/3; v/v) as the mobile phase resulting in a pure orange solid (190 mg, 92%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.70 (s, 1H; ArH), 7.40 (s, 2H; ArH), 3.14 (s, 2H; CH), 2.51 (s, 6H; ArCH₃), 2.28 (q, 4H, *J* = 7.3 Hz; ArCH₂), 1.33 (s, 6H; ArCH₃), 0.97 (t, 6H, *J* = 7.3 Hz; CH₃).

¹³C NMR (CDCl₃, 500 MHz, δ ppm) 154.5, 138.0, 137.2, 136.5, 135.8, 133.2, 132.2, 130.5, 123.6, 110.0, 81.7, 79.2, 17.1, 14.6, 12.6, 12.2.

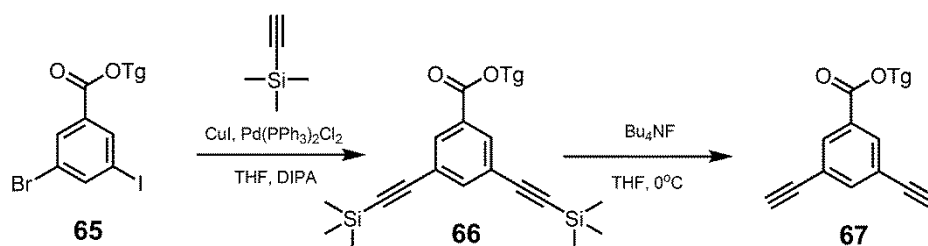
MS (HiResESI) Calcd for C₂₇H₂₇BF₂N₂ (M-H)⁻ 427.2162; Found 427.2139 (M-H)⁻.



Synthesis of Compound 65: 3-Bromo-5-iodobenzoic acid (2 g, 6.12 mmol), 4-dimethylamino pyridine (374 mg, 3.06 mmol) and triethylene glycol monomethyl ether (1.23 ml, 7.65 mmol) were dissolved in THF (25 ml). N,N'-dicyclohexylcarbodiimide (1.4 g, 6.73 mmol) was added and the reaction mixture was stirred at RT for 12 h. The white solids were removed by vacuum filtration and the solvent was evaporated from the filtrate. The crude product was purified by column chromatography using EtOAc/hexanes (1/1; v/v) as the mobile phase affording a colorless liquid (2.69 g, 93%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 8.27 (d, 1H, *J* = 1.8 Hz; ArH), 8.11 (d, 1H, *J* = 1.6 Hz; ArH), 8.00 (d, 1H, *J* = 1.8 Hz; ArH), 4.45 (m, 2H; OCH₂), 3.79 (m, 2H; OCH₂), 3.68-3.61 (m, 6H; OCH₂), 3.51 (m, 2H; OCH₂), 3.34 (s, 3H; OCH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 163.8, 143.8, 137.2, 133.3, 132.0, 122.9, 93.9, 71.9, 77.0, 76.7, 71.9, 70.6, 69.0, 64.8, 59.0.



Synthesis of Compound 66: Compound **65** (1.5 g, 3.2 mmol) was dissolved in THF (30 ml) and diisopropylamine (10 ml). Argon was purged through the solution for 20 min after which CuI (61 mg, 0.32 mmol) and Pd(PPh₃)₂Cl₂ (135 mg, 0.192 mmol) were added. The flask was sealed and trimethylsilylacetylene (2.8 ml, 18.2 mmol) was added by a syringe. The reaction was stirred for 12 h at RT, extracted with CH₂Cl₂ and the organic layer was collected and dried over Na₂SO₄. The product was purified by column chromatography using EtOAc:hexanes (1/2; v/v) as the mobile phase to produce a light brown liquid (1.42 g, 97%).

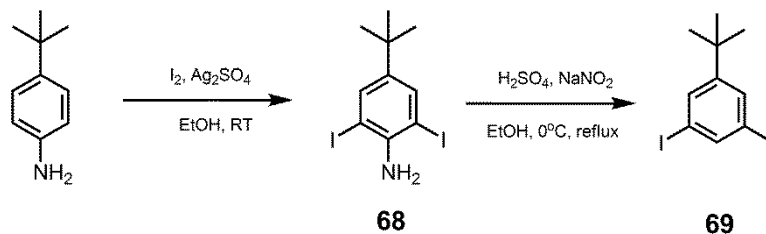
¹H NMR (CDCl₃, 400 MHz, δ ppm) 8.0 (d, 2H, J = 1.6 Hz; ArH), 7.70 (t, 1H, J = 1.6 Hz; ArH), 4.45 (m, 2H; OCH₂), 3.80 (m, 2H; OCH₂), 3.68-3.61 (m, 6H; OCH₂), 3.52 (m, 2H; OCH₂), 3.34 (s, 3H; OCH₃), 0.22 (s, 18H; CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 165.1, 139.1, 132.7, 130.5, 123.8, 102.9, 96.1, 71.9, 70.6, 69.1, 64.4, 59.0, 0.2.

Synthesis of Compound 67:¹⁹³ Compound **66** (1.5 g, 3.26 mmol) was dissolved in THF (50 ml) and the mixture was cooled to 0 °C using an ice bath. Tetrabutylammonium fluoride (8.17 ml, 1M in THF) was added slowly. The reaction was stirred at 0 °C for 10 min until all the starting compound was consumed. The reaction mixture was filtered through a silica pad and the product was purified by column chromatography (SiO₂) using EtOAc/hexanes (1/1; v/v) as the mobile phase affording a yellow oily liquid (949 mg, 92%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 8.09 (s, 2H; ArH), 7.79 (s, 1H; ArH), 4.45 (m, 2H; OCH₂), 3.80 (m, 2H; OCH₂), 3.67-3.61 (m, 6H; OCH₂), 3.49 (m, 2H; OCH₂), 3.34 (s, 3H; OCH₃), 3.12 (s, 2H; CH).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 164.9, 139.3, 133.3, 130.8, 123.0, 81.9, 78.9, 71.9, 70.6, 69.0, 64.5, 59.0.



Synthesis of Compound 68¹⁹⁴: 4-*Tert*-butylaniline (3 g, 3.2 ml, 20.1 mmol) was dissolved in ethanol (80 ml). I_2 (10 g, 41 mol) and Ag_2SO_4 (12.5 g, 41 mol) were added and the mixture was stirred for 1 h at RT. After completion, the solid precipitate was removed by filtration, the ethanol was evaporated and the crude product was extracted with CH_2Cl_2 and washed with 5% NaOH solution. The organic layer was washed with water and then dried over Na_2SO_4 . The product was purified by column chromatography using CH_2Cl_2 /hexanes (1/3; v/v) as mobile phase to generate a brown oily liquid (5.75 g, 71%).

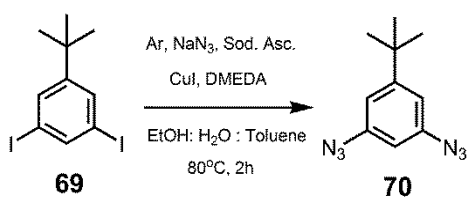
^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.60 (s, 2H; ArH), 4.45 (b, 2H; NH_2), 1.23 (s, 9H; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 144.6, 143.8, 136.5, 81.7, 33.7, 31.3.

Synthesis of Compound 69¹⁹⁵: Compound **68** (5.75 g, 14.3 mmol) was dissolved in ethanol (50 ml), the reaction mixture was cooled to 0°C using an ice bath after which 1 ml of concentrated H_2SO_4 was added. Subsequently, NaNO_2 (3.5 g) was added quickly and the mixture was refluxed for 24 h. The reaction was neutralized with NaOH solution and extracted with CHCl_3 . The organic layer was collected, dried over Na_2SO_4 and evaporated *in vacuo*. The product was purified by column chromatography using hexanes as mobile phase to afford a white solid (2.8 g, 51%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.89 (t, 1H, $J = 1.5$ Hz; ArH), 7.68 (d, 2H, $J = 1.5$ Hz; ArH), 1.30 (s, 9H; CH_3).

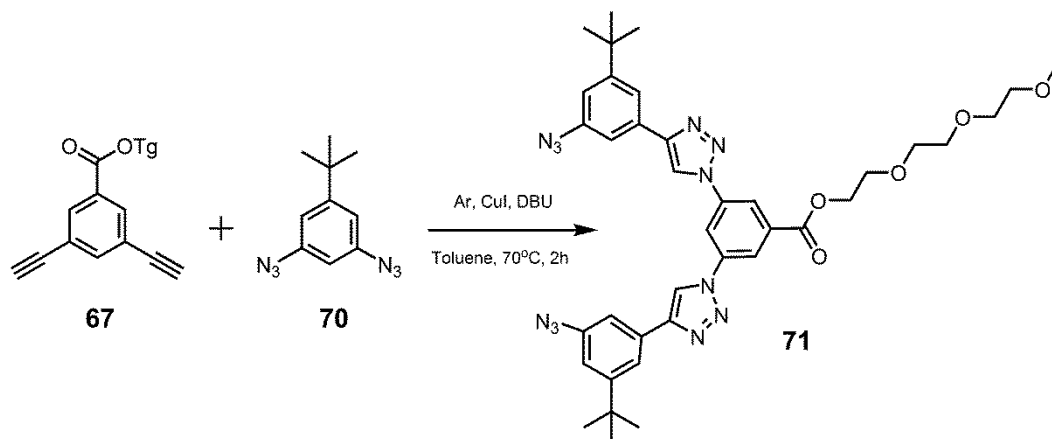
^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 155.6, 142.2, 134.1, 94.9, 34.9, 31.0.



Synthesis of Compound 70¹⁹⁶: Compound **69** (2.32 g, 6 mmol), NaN₃ (1.17 g, 18 mmol), sodium ascorbate (134 mg, 0.6 mmol), CuI (228 mg, 1.2 mmol) and N¹,N²-dimethylethane-1,2-diamine (193 μ l, 1.8 mmol) were added to a previously degassed 33 ml solvent mixture (ethanol:water:toluene; 7:3:1; v/v). The reaction was refluxed for 2 h under argon and monitored by TLC. After all the starting compound was consumed in 2 h, the reaction was cooled to RT. The crude product was purified by column chromatography using hexanes as the mobile phase producing a yellow oily liquid (980 mg, 76%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 6.83 (d, 2H, J = 1.5 Hz; ArH), 6.54 (t, 1H, J = 1.5 Hz; ArH), 1.33 (s, 9H; CH₃).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 155.1, 141.3, 113.0, 106.9, 35.1, 31.1.

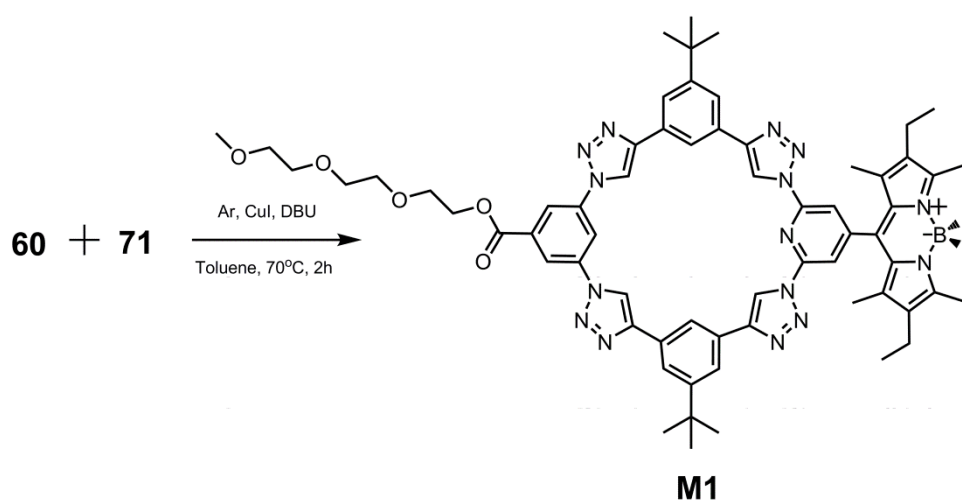


Synthesis of Compound 71: Compound **67** (160 mg, 0.53 mmol) and compound **70** (1.02 g, 5.3 mmol) were dissolved in toluene (15 ml). The solution was degassed with argon for 20 min and charged with three equivalents of 1,8-diazabicycloundec-7-ene (DBU, 250 μ l, 1.6 mmol) and CuI (50 mg, 0.26 mmol). The mixture was stirred at 70°C for 2 h under argon. After cooling to RT the crude product was purified by column chromatography using hexanes/acetone (2/1; v/v) to afford a yellow oily liquid (110 mg, 45%).

^1H NMR (CD_2Cl_2 , 400 MHz, δ ppm) 8.70 (t, 1H, $J = 1.6$ Hz; ArH), 8.54 (s, 2H; ArH), 8.47 (d, 2H, $J = 1.6$ Hz; ArH), 7.63 (t, 2H, $J = 1.6$ Hz; ArH), 7.37 (t, 2H, $J = 2.0$ Hz; ArH), 7.14 (t, 2H, $J = 1.8$ Hz; ArH), 4.52 (m, 2H; OCH_2), 3.87 (m, 2H; OCH_2), 3.71 (m, 2H; OCH_2), 3.66 (m, 2H; OCH_2), 3.59 (m, 2H; OCH_2), 3.47 (m, 2H; OCH_2), 3.25 (s, 3H; OCH_3), 1.40 (s, 9H; CH_3).

^{13}C NMR (CD_2Cl_2 , 500 MHz, δ ppm) 165.7, 155.4, 146.9, 141.49, 137.9, 131.4, 128.9, 128.1, 126.2, 118.8, 116.5, 114.1, 108.4, 71.8, 70.7, 70.5, 69.1, 64.6, 58.5, 35.3, 30.8.

MS (HiResESI) Calcd for $\text{C}_{38}\text{H}_{44}\text{N}_{12}\text{O}_5$ ($\text{M}+\text{I}$) $^-$ 875.2603; Found 875.2574 ($\text{M}+\text{I}$) $^-$.



Synthesis of Compound M1: Compound **60** (42 mg, 0.1 mmol) and compound **71** (75 mg, 0.1 mmol) were dissolved in toluene (75 ml) and degassed with argon for 30 minutes. This solution was then added dropwise (over one hour) to a degassed toluene solution (200 ml) containing DBU (150 μl) and CuI (catalytic amount). The reaction mixture was heated to 70°C during this addition. After addition was completed, the reaction was stirred for an additional hour at 70°C and then cooled to RT. The crude product was purified by column chromatography (SiO_2) using CH_2Cl_2 as the initial mobile phase which was changed to hexanes/acetone (1/1; v/v) after the fast-migrating byproducts were collected. Fractions containing the macrocycle product were subjected to additional column chromatography using CH_2Cl_2 :acetone (9:1; v/v) as mobile phase to afford a red waxy solid (40 mg, 34%).

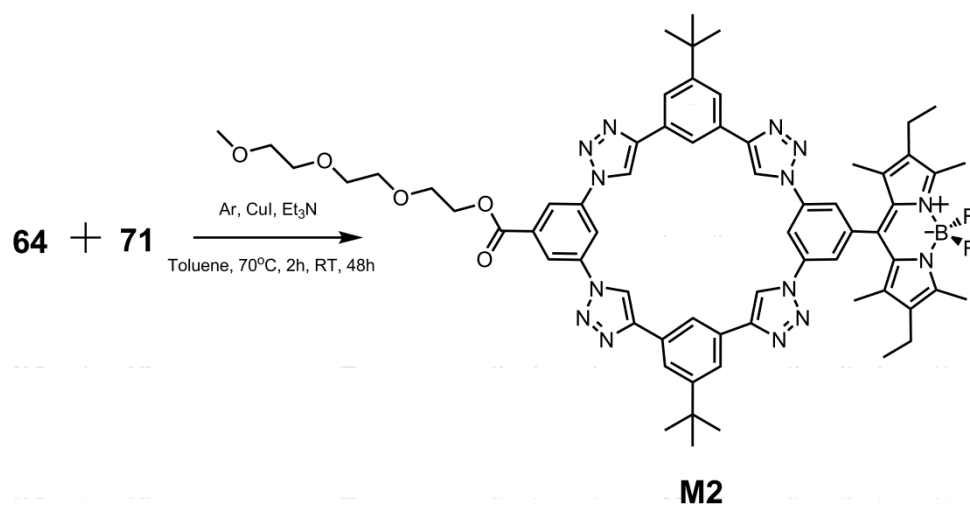
^1H NMR (CD_2Cl_2 , 500 MHz, δ ppm) 10.34 (s, 1H; ArH), 10.14 (s, 1H; ArH), 9.69 (s, 2H; ArH), 8.40-7.90 (m, 11H; ArH), 4.42-3.30 (m, 15H; OCH_2), 2.60-2.14 (m, 10H;

ArCH₃ + ArCH₂), 1.61-1.20 (m, 22H; ArCH₃ + CH₃), 0.89 (s, 6H; ArCH₃); To increase the peak resolution tetrabutylammonium salt of chloride was added to the NMR solution.

¹H NMR (For **M1**+Cl⁻, CD₂Cl₂, 500 MHz, δppm) 10.85 (s, 2H; ArH), 10.58 (s, 2H; ArH), 9.40 (s, 1H; ArH), 8.94 (s, 2H; ArH), 8.78 (s, 1H; ArH), 8.49 (s, 2H; ArH), 8.44 (s, 2H; ArH), 8.26 (s, 2H; ArH), 4.60 (m, 2H; OCH₂), 3.95 (m, 2H; OCH₂), 3.78 (m, 2H; OCH₂), 3.71 (m, 2H; OCH₂), 3.66 (m, 2H; OCH₂), 3.55 (m, 2H; OCH₂), 3.36 (s, 3H; OCH₃), 3.31 (TBACl), 2.57 (s, 6H; ArCH₃), 2.37 (q, 4H; ArCH₂), 1.68 (TBACl), 1.60 (s, 6H; ArCH₃), 1.54 (s, 18H; CH₃), 1.45 (TBACl), 1.03 (TBACl+6H).

¹³C NMR (M+Cl; CD₂Cl₂, 500 MHz, δppm) 167.4, 156.5, 155.4, 151.9, 150.6, 148.0, 146.6, 145.6, 139.1, 136.7, 135.6, 133.5, 130.7, 129.8, 129.1, 126.0, 124.5, 122.4, 120.9, 118.5, 115.7, 114.5, 106.4, 71.8, 70.5, 69.6, 63.8, 59.2, 35.6, 29.7, 29.1, 16.9, 14.3, 12.3, 11.6.

MS (HiResESI) Cald for C₆₄H₇₀BF₂N₁₅O₅ (M+I)⁻ 1304.4796; Found 1305.4746 (M+I)⁻.

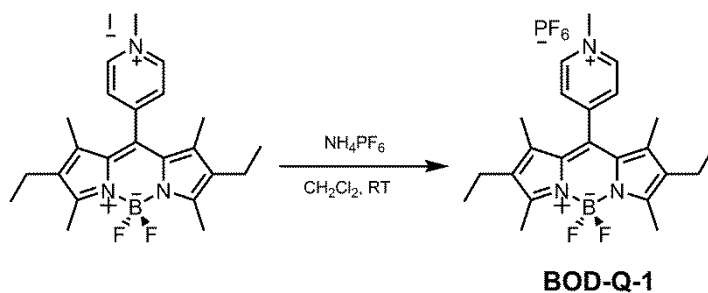


Synthesis of Compound M2: Compound **64** (43 mg, 0.1 mmol) and compound **71** (75 mg, 0.1 mmol) were dissolved in toluene (75 ml) and degassed with argon for 30 minutes. This solution was then added dropwise (over one hour) to a degassed toluene solution (200 ml) containing Et₃N (50 μl) and CuI (30 mg). The reaction mixture was heated to 70°C during this addition. After addition was completed, the reaction was stirred for an additional two hours at 70° C and then cooled to RT.

Additional amounts of Et₃N (55 μ l) and CuI (30 mg) were added and the mixture was stirred for a further 48 h. The crude product was subjected to an initial round column chromatography (SiO₂) using CH₂Cl₂ as the mobile phase followed by hexanes/acetone (1/1; v/v) after the fast-migrating byproducts were collected. The compound collected was washed with THF and subjected to a second round of column chromatography (SiO₂) using CH₂Cl₂ as the mobile phase to remove BHT (2,6-dimethyl-4-tert-butylphenol) originating from the THF used in the previous purification procedure to afford a red oily liquid (19 mmol, 22 mg, 19%).

¹H NMR (For **M2**+Cl⁻, CD₂Cl₂, 500 MHz, δ ppm) 10.41 (s, 2H; ArH), 10.37 (s, 2H; ArH), 9.17 (s, 1H; ArH), 9.03 (s, 1H; ArH), 8.73 (s, 2H; ArH), 8.65 (s, 2H; ArH), 8.34 (s, 2H; ArH), 8.12 (s, 2H; ArH), 4.57 (m, 2H; OCH₂), 3.92 (m, 2H; OCH₂), 3.73 (m, 2H; OCH₂), 3.67 (m, 2H; OCH₂), 3.65 (m, 2H; OCH₂), 3.59 (m, 2H; OCH₂), 3.33 (s, 3H; OCH₃), 3.31 (TBACl), 2.52 (s, 6H; ArCH₃), 2.32 (q, 4H; ArCH₂), 1.68 (TBACl), 1.53 (s, 6H; ArCH₃), 1.49 (s, 18H; CH₃), 1.45 (TBACl), 1.03 (TBACl+6H).

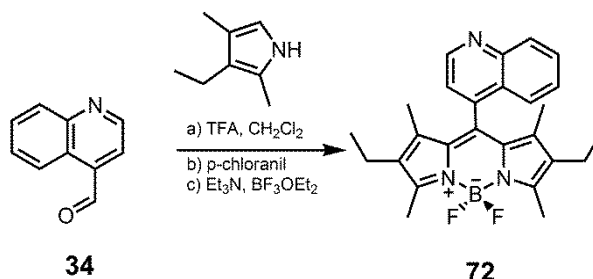
MS (HiResESI) Cald for C₆₅H₇₁BF₂N₁₄O₅ (M+I)⁻ 1303.4849; Found 1303.4788 (M+I)⁻.



Synthesis of Compound BOD-Q-1: Methyl-quaternized pyridine BODIPY¹⁹⁷ (30 mg, 57.4 μ mol) was dissolved in CH₂Cl₂ (3 ml). Ammonium hexafluorophosphate (150 mg, 915 μ mol) was added and the reaction mixture was stirred at RT for 12 h after which the reaction mixture was washed with H₂O and the organic layer was collected and dried over Na₂SO₄ to obtain a non-emissive red solid (31 mg, quantitative).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 8.89 (d, 2H, $J = 6.44$ Hz; ArH), 7.95 (d, 2H, $J = 6.18$ Hz; ArH), 4.50 (s, 3H; CH_3), 2.46 (s, 6H; ArCH_2), 2.43 (q, 4H, $J = 8.63$ Hz; CH_2), 1.26 (s, 6H; ArCH_3), 0.89 (t, 6H, $J = 7.52$ Hz; CH_3).

MS (HiResESI) Calcd for $\text{C}_{23}\text{H}_{29}\text{BF}_2\text{N}_3^+$ 396.2417; Found 396.2311, $\Delta = 26.8$ ppm.

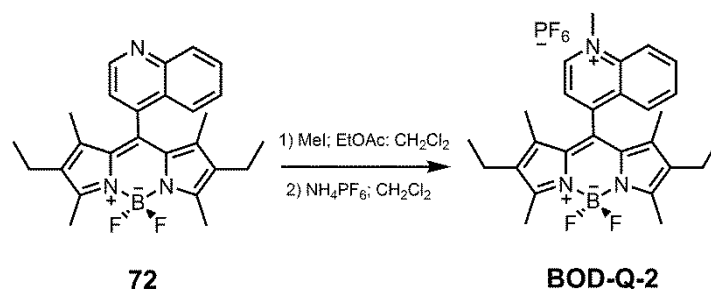


Synthesis of Compound 72: Quinoline-5-carboxaldehyde, compound **34** (472 mg, 3 mmol) was dissolved in CH_2Cl_2 (100 ml) and degassed with argon for 20 min. 2,4-Dimethyl-3-ethyl pyrrole (2.2 eq., 0.89 ml) and a catalytic amount of trifluoroacetic acid (TFA) was added and the solution was stirred for at RT. After 12 h, *p*-chloranil (0.74 g, 3 mmol) was added and the reaction was stirred for 1 h followed by addition of Et_3N (4 ml) then by $\text{BF}_3\cdot\text{OEt}_2$ (4 ml) and the reaction mixture was stirred for an additional 1 h at RT. The reaction mixture was washed with H_2O and the organic layer was collected and dried over Na_2SO_4 . The product was purified by flash column using CH_2Cl_2 as the mobile phase to produce a red solid (323 mg, 25%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 9.04 (d, 1H, $J = 4.32$ Hz; ArH), 8.40 (d, 1H, $J = 8.4$ Hz; ArH), 7.85 (m, 1H; ArH), 7.77 (m, 1H; ArH), 7.52 (m, 1H; ArH), 7.41 (d, 1H, $J = 4.32$ Hz; ArH), 2.55 (s, 6H; ArCH_3), 2.24 (q, 4H, $J = 7.52$ Hz; CH_2), 1.00 (s, 6H; ArCH_3), 0.95 (t, 6H, $J = 7.52$ Hz; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 190.2, 154.8, 150.3, 148.3, 142.7, 137.8, 134.7, 133.3, 130.4, 129.8, 127.9, 127.2, 125.1, 121.2, 17.0, 14.5, 12.6, 11.3.

MS (HiResESI) Calcd for $\text{C}_{26}\text{H}_{29}\text{BF}_2\text{N}_3^+$ 432.2417; Found 432.2463, $\Delta = 10.60$ ppm.



Synthesis of Compound BOD-Q-2: Compound **72** (90 mg, 0.21 mmol) was dissolved in EtOAc and CH₂Cl₂ (5:5 ml) and an excess amount of iodomethane (1 ml) was added. The reaction was stirred at RT for 12 h. The resulting precipitate was filtered to obtain the red iodide salt of quinolinium BODIPY (30 mg, 25%). This compound (7 mg) was dissolved in a minimum volume of CH₂Cl₂. Ammonium hexafluorophosphate (20 mg, 122 μmol) was added and the solution was stirred at RT for 12 h, washed with H₂O and the organic layer was collected and dried over Na₂SO₄ to obtain non-emissive red solid (7.2 mg, quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.38 (d, 1H, *J* = 5.82 Hz; ArH), 8.40 (d, 1H, *J* = 8.76 Hz; ArH), 8.22 (m, 2H; ArH), 7.90 (m, 2H; ArH), 4.76 (s, 3H; CH₃), 2.50 (s, 6H; ArCH₃), 2.17 (q, 4H, *J* = 7.44 Hz; CH₂), 0.95 (s, 6H; ArCH₃), 0.90 (t, 6H, *J* = 7.48 Hz; CH₃).

MS (HiResESI) Calcd for C₂₇H₃₁BF₂N₃⁺ 446.25736; Found 436.26234, Δ = 11.2 ppm.

Preparation of tetrabutylammonium trifluoroacetate. Trifluoroacetic acid (154 ml, 2 mmol) was added to a 1 M tetrabutylammonium hydroxide (2 ml) solution in methanol. The mixture was stirred at RT for 3 h. Water formed during the reaction was removed by evaporating to dryness (quantitative, white solid).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 3.21 (t, 8H, *J* = 8.13 Hz; NCH₂), 1.60 (m, 8H; CH₂), 1.38 (m, 8H; CH₂), 0.94 (t, 12H, *J* = 7.28 Hz; CH₃).

CHAPTER 6

Integrated Logic Gate for Some Function: Self Reporting Activatable Photosensitizer

Sundus Erbas-Cakmak, Yusuf Cakmak, Engin U. Akkaya

6.1 Objective

Compared to silicon-based logic operations chemical logic gates are still in their formative years. They can perform quiet complex tasks such as multiplexer/demultiplexer, flip-flop logics, subtracter/adder or key-pad-lock systems. Even, some smart oligonucleotide-base constructs playing simple games such as Tic-Tac-Toe have been reported, integration of one functional operation to another for further purpose is still to be improved. In the research presented in this section of the thesis, integration of two chemical logic gates is proposed to concetinate an acid activatable photosensitizer to the activity reporting moiety through singlet oxygen mediated information relay.

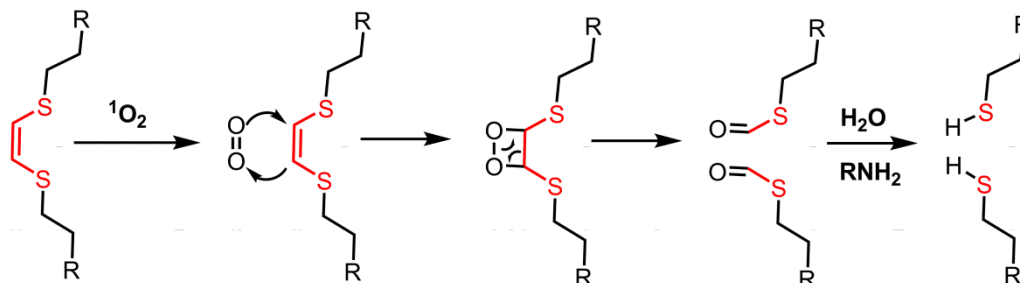
6.2 Introduction

Complexity of biochemical reaction networks usually result from the cascade of reactions that control or are controlled by other reactions in the network. This enables signal relay among functional biological modules and provides intricate emerging processes. Same analogy does apply to silicon-based logic operations. Complex digital computational processes are indeed show similar level of dependency of series of simple logic operations from which sophisticated algorithms can be effectively produced.

In chemical logic gates, physical integration of simple logic gates has been reported. Nevertheless, the functional relations between each independent modules or concetenated logic gates as a whole have not been done so far. To enable such

purposeful relation, photodynamic action and chemical sensor approach is used in this work. Since the output of a PDT action is $^1\text{O}_2$, a reaction based sensing of this output to report the activity of the first action is aimed.

$^1\text{O}_2$ is known to react readily through addition reactions. Reaction of $^1\text{O}_2$ with diene produces endoperoxide and when the olefins are reactants then the product is a hydroperoxide. Electron rich olefins are also susceptible to $^1\text{O}_2$ attack through 2+2 cycloaddition reaction producing dioxetanes.¹⁹⁸ This product is usually decomposes spontaneously. $^1\text{O}_2$ -olefin reaction is used in literature to create various cleavable systems in which PDT agents are used as $^1\text{O}_2$ generators. Among them, the reaction with (Z)-1,2-bis(alkylthio)ethene is chosen to build a $^1\text{O}_2$ related logic gate in this research since this is reported to have quite higher reaction rate and the relatively easy synthesis.¹⁹⁹ The reaction of this linker with $^1\text{O}_2$ is shown in Scheme 26. Following the initial addition of $^1\text{O}_2$ to the double bond, unstable dioxethane is formed. Spontaneous decomposition of this group produces S-alkyl methanethioate. In aqueous solution with the aid of amines this group is hydrolyzed to thiols.

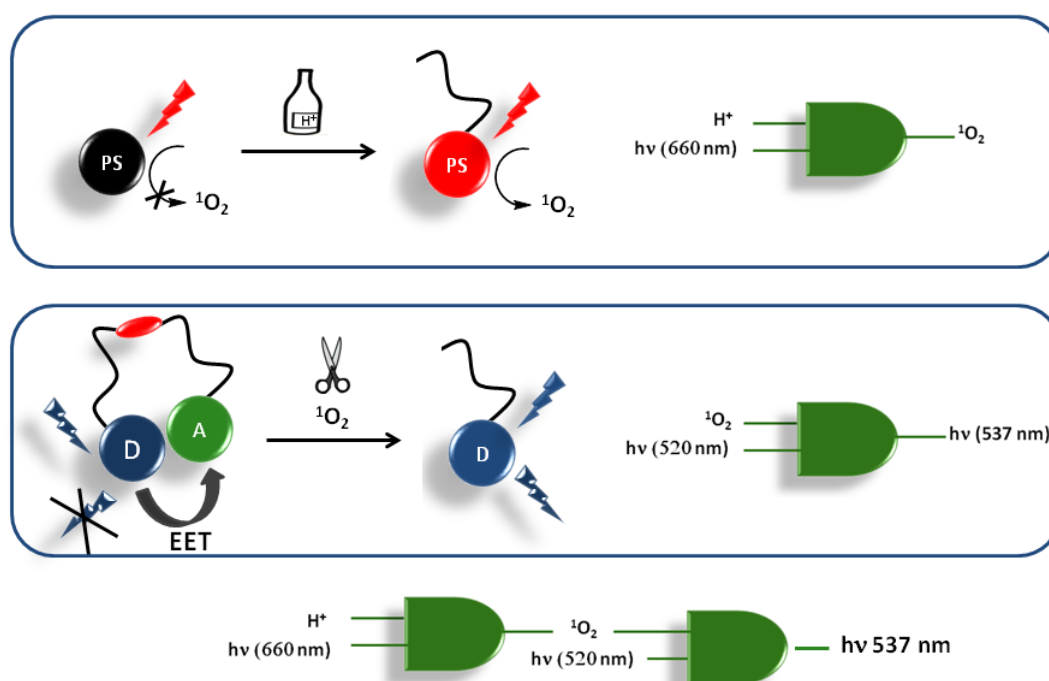


Scheme 25. Mechanism of reaction of $^1\text{O}_2$ with electron rich olefins.

This cleavage is used to separate the energy transfer modules (donor and acceptor dyes) in the project to report the presence of $^1\text{O}_2$. The pH-activatable photosensitizer is chosen based on the work presented in chapter 4. The photosensitizer has a highly applicable pKa value (6.92). In its deprotonated form, the singlet oxygen generation efficiency of the photosensitizer decreases substantially due to diminished excitability at the wavelength of light source and due to some other deactivation pathways involved in deprotonated case.

6.3 Design of the Functionally Integrated Logic Gates

The functional relations between two different logic gates are designed as follows. The first logic operation is an AND logic gate that builds a pH-activatable photosensitizer. ICT character of the photosensitizer enables a hypsochromic shift of the absorption of the PS upon protonation and the spectral change enables an enhanced excitation at the wavelength of LED light source. The requirement of light (660 nm) and acid for the singlet oxygen generation create the first AND logic operation (Scheme 27).

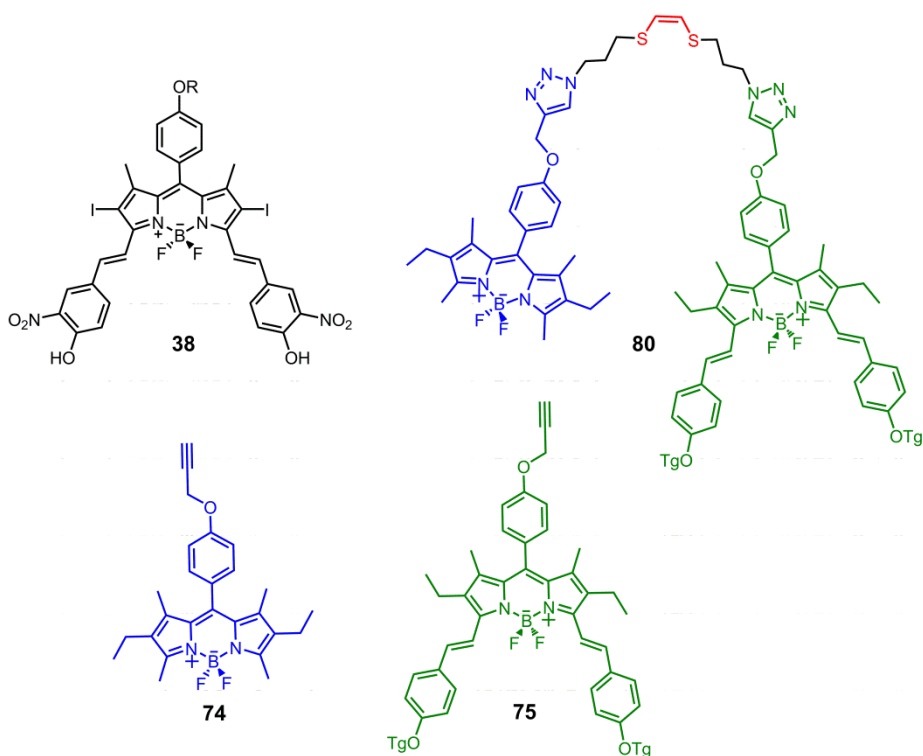


Scheme 26. Representative illustration of pH-activatable photosensitizer (AND logic gate 1) and release of FRET donor upon reaction with $^1O_2$ (AND logic gate 2). **A** represents FRET acceptor, **D** represents FRET donor and **PS** represents photosensitizer. The output of the first logic gate ($^1O_2$) is an input for the next which concatenates two gates functionally.

In the second AND logic gate construct, a Förster-type energy transfer system is designed with the BODIPY dye (compound **74**) being the energy donor and a distyryl-BODIPY dye (compound **75**) as energy acceptor. Energy transfer from the former dye decreases its emission intensity at 537 nm. The two dyes are attached to one another with a special linker, a (Z)-1,2-bis(alkylthio)ethene group that is

reported in literature to be cleaved by singlet oxygen efficiently. Presence of the $^1\text{O}_2$ input liberates the FRET donor and upon excitation with the light (520 nm), an enhanced emission from the donor BODIPY dye is expected to be observed. Since the $^1\text{O}_2$ is the output of the initial AND logic gate, the two gates are concatenated. Physical proximity between two logic gates is obtained by embedding both molecules within same micellar construct. The semi-chemical structures of the molecules and the individual energy transfer modules are shown in Scheme 27. Modules are used to analyze the energy transfer process in compound **80**. As an alternative second logic, glutathione is used as an input due to its $^1\text{O}_2$ scavenging property to construct an INHIBIT logic gate.

By the mechanism explained above, an activatable photosensitizer generates $^1\text{O}_2$ in slightly acidic media upon irradiation with 660 nm light and reports its activity in the form of $^1\text{O}_2$ reaction to separate FRET modules of compound **80**. The end product is an emission at 537 nm from the FRET donor.



Scheme 27. Chemical structures of photosensitizer (compound **38**), singlet oxygen reporter (compound **80**) and individual FRET modules (compound **74**, the FRET donor and compound **75**, the FRET acceptor). The $^1\text{O}_2$ reactive group is highlighted with red. Complete chemical structures are given in the experimental part.

6.4 Results and Discussion

6.4.1 Construction of First AND Gate

The photophysics, pH characteristics and photodynamic activity of the photosensitizer (compound **38**) are extensively studied in chapter 4 of this thesis. Aqueous solution of this compound within Cremephor EL micelle has a pKa value of 6.92 and displays a large hypsochromic spectral shift in absorption upon protonation from 720 nm to 649 nm. These properties make the compound an excellent candidate for pH-activatable photosensitizer for use in tumor therapy. By choosing an excitation wavelength which selectively excites protonated form of the PS, the PDT activity can be controlled with this input.

Figure 47 displays the photodynamic activity of the micelle encapsulated compound **38** in various conditions in aqueous solution. Singlet oxygen generation is followed by the decrease in the absorption of trap molecule (see chapter 4, experimental part) which bleaches as a result of the reaction with $^1\text{O}_2$. As it is obviously seen from the decrease in the absorption at 378 nm, $^1\text{O}_2$ generation is more pronounced in slightly acidic solution (pH $\sim$ 6.0) owing to the spectral match with the light of excitation. There is almost no decrease in absorption in slightly basic (pH $\sim$ 7.5) solutions containing photosensitizer. There may be other reasons for the poor $^1\text{O}_2$ generation efficiency of the PS in basic solutions since the emission of the compound upon excitation at the maximum absorption wavelength decreases as well. There may be other deactivation pathways beside spectral mismatch that acts on the phenolate form to quench the fluorescence and decrease the $^1\text{O}_2$ generation efficiency. Such systems usually have strong charge transfer character and the non-radiative transitions may dominate as also observed by the quenching of the fluorescence.

The requirement of light for $^1\text{O}_2$ generation is also observed since under any conditions trap absorption does not significantly change unless the light is irradiated. To check the stability of the $^1\text{O}_2$ trap under experimental conditions, two experiments are performed. Trap under acidic and basic aqueous solutions do not decompose as followed by the electronic absorption spectra.

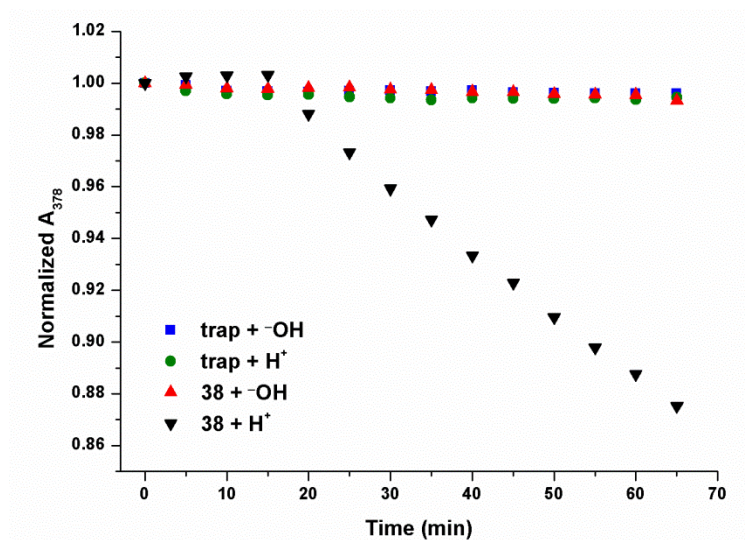


Figure 47. Relative $^1\text{O}_2$ generation efficiency of micellar compound **38** ($7.5\ \mu\text{M}$) in aqueous solution in the presence (black, triangle) and absence (red, triangle) of the acid as followed by the absorption change of the trap at 378 nm. The change in the trap absorption in slightly basic (blue, square) and acidic (green, circle) solutions ($\text{pH} \sim 7.5$ and 6.0 respectively). In the first 15 min, the samples were kept under dark and for the following 50 min; the samples were irradiated with 660 nm light using LED array.

With the use of the results obtained from PDT experiments an AND logic is constructed. Inputs are acid and light of 660 nm wavelength and output is $^1\text{O}_2$ generation efficiency. Output values are determined from the percent decrease of the trap absorption. Values are taken from data after samples are kept for 15 min under dark or after irradiated for 15 min with light of 660 nm. 1% decrease in absorption of the trap is determined as threshold for the logic gate (Figure 48). Electronic absorption spectra and the photophysical properties of the compound **38** (both protonated and deprotonated forms) are given in Figure 50 and Table 8 respectively. The compound is essentially non-emissive in its phenolate form.

6.4.2 Construction of Second AND Gate and Concetination

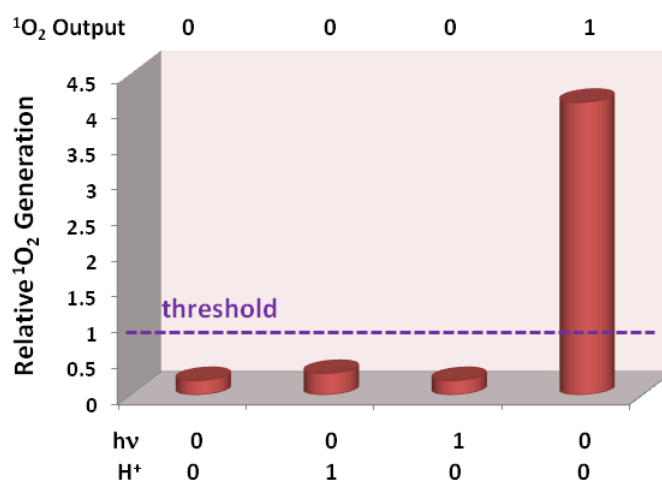


Figure 48. AND logic construct out of compound **38** where output is $^1\text{O}_2$ as measured by % decrease in trap absorbance within 15 min (under dark or with 660 nm irradiation). Inputs are 660 nm light and acid and threshold is determined to be 1.

Once the $^1\text{O}_2$ generation efficiency of the photosensitizer is controlled with an AND logic, the second part of the integrated-logic construct is developed. The inputs of this part are light of 520 nm and $^1\text{O}_2$. The latter input is provided by the PDT action in the first logic operation. To analyze the integration, two molecules (compound **38** and **80**) are incorporated together within the micelle with a mole ratio of approximately 1:1. The concentrations are estimated by comparison of the absorption values with the THF absorptivities.

Comparison of the emission spectra of the compound **80** with the modules shows that the emission of the FRET donor part (compound **74**) at 537 nm is quenched considerably in compound **80** as also verified with the decrease in the quantum yield of this part from 0.96 to 0.14 (Figure 49, Table 8). On the other hand, FRET acceptor module can not be excited at 520 nm to produce emission at 670 nm while compound **80** shows intense emission at this wavelength upon excitation at 520 nm. Both of these spectroscopic results indicate an efficient FRET process between the modules with a calculated FRET efficiency of 0.85%. The lack of unit efficiency in this process is probably due to poor spectral overlap between donor-acceptor moieties. Excitation spectra also confirms that the acceptor emission is a product of

excitation of both donor and acceptor moieties (Figure 50b). In contrast, excitation spectra of compound **75** does not display any peaks around 524 nm when emission at 670 nm is followed.

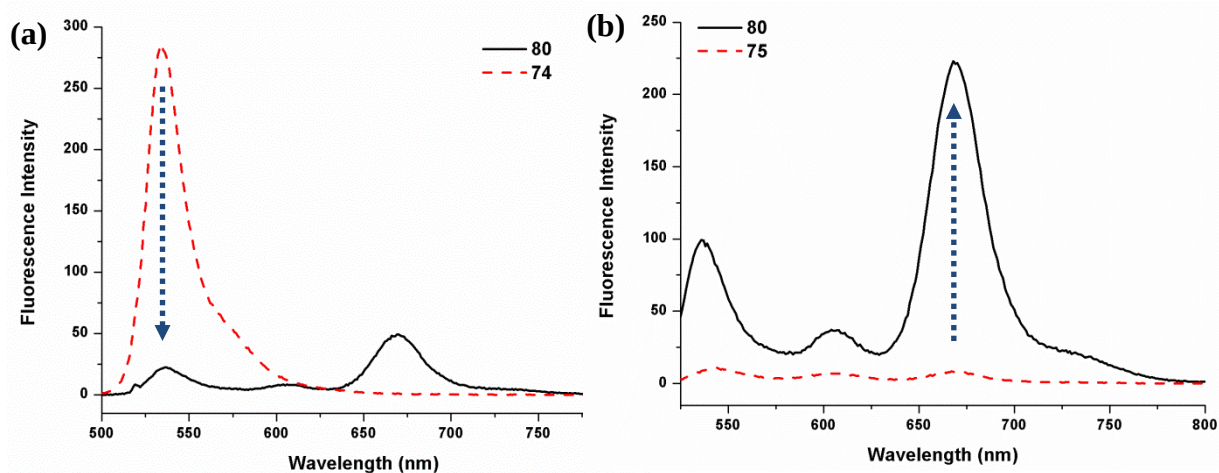


Figure 49. Comparison of emission spectra of equally absorbing compounds **80** (black, solid) and **74** (red, dashed) (a) excited at 520 nm and compounds **80** (black, solid) and **75** (red, dashed) (b) excited at the same wavelength in THF.

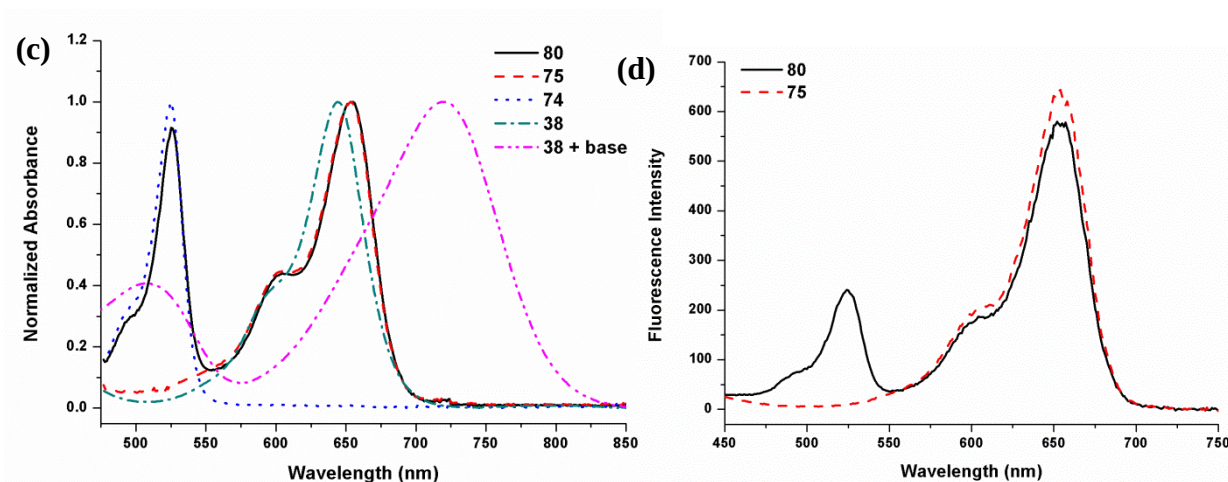


Figure 50. Electronic absorption spectra of compounds **80** (black, solid), **75** (red, dashed), **74** (blue, dot), **38** (green, dashed-dot), **38** in the presence of piperidine (purple, dashed, dotdot) in THF (a), comparison of excitation spectra of equally absorbing compounds **75** (red, dashed) and **80** (black, solid) for emission at 670 nm in THF (b).

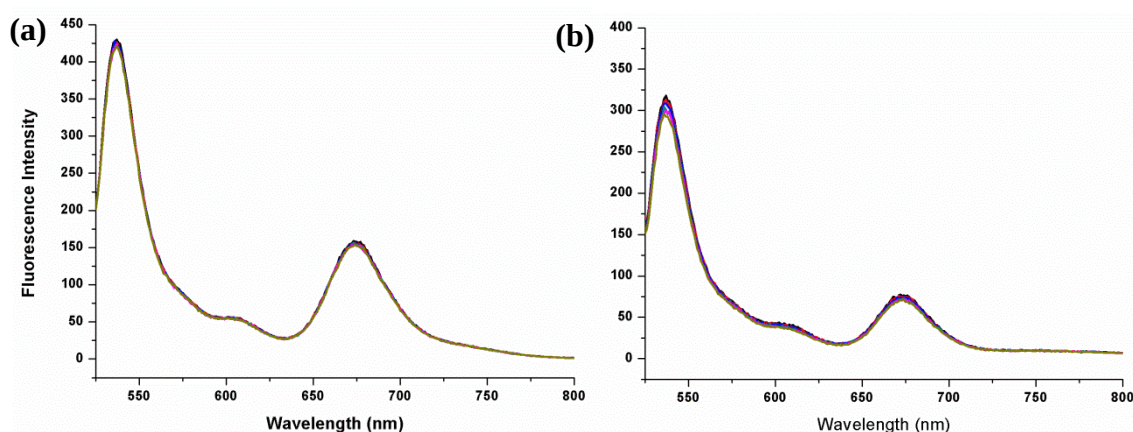


Figure 52. Change in the emission spectra of compound **80** ($0.75\ \mu\text{M}$) in the presence of compound **38** ($0.75\ \mu\text{M}$) in slightly acidic (a) and basic (b) aqueous solutions under dark. The spectrum is recorded by excitation at 520 nm.

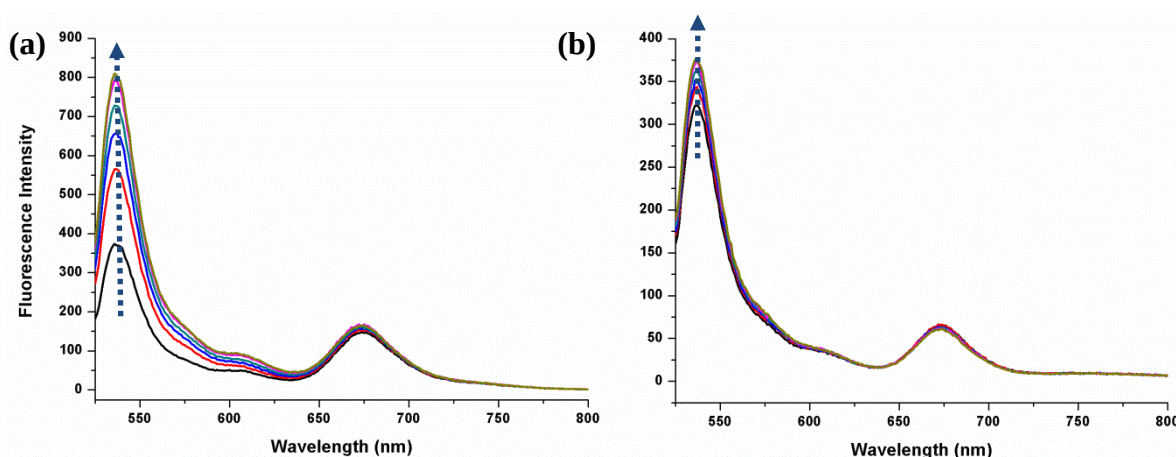


Figure 51. Change in the emission spectra of compound **80** ($0.75\ \mu\text{M}$) in the presence of compound **38** ($0.75\ \mu\text{M}$) in slightly acidic (a) and basic (b) aqueous solutions upon irradiation with 660 nm light for 50 min. The spectrum is recorded by excitation at 520 nm.

Since the PS produces singlet oxygen only in the presence of acid, acidic conditions are interpreted as the presence of $^1\text{O}_2$ in the second logic gate. Liberation of the FRET donor upon reaction of compound **80** with $^1\text{O}_2$ is expected. The samples kept under dark do not display any change in the emission of the FRET donor module of the compound **80** at 537 nm (Figure 51). Likewise, under slightly basic conditions, no significant change in the emission is observed since $^1\text{O}_2$ is not generated

efficiently under these conditions (Figure 52b). However, when there is acid to activate the PS, the emission intensity of the donor at 537 nm increases more than 2-folds indicating the liberation from the energy acceptor module (Figure 52a). Quantum yield of the donor emission within the micellar construct increases from 0.14 to 0.29 and the rate of increase in the emission seems to be almost saturated (Figure 54). Yet the final quantum yields are far from the quantum yield of the free compound **74** and the excitation spectra shows that the emission at 670 nm can still be collected by excitation at donor absorption (Figure 53). This shows that the FRET process is still effective to some extent due to remaining uncleaved compound **80**. This result may be due to decrease in dissolved oxygen within the solution after certain time which decreases the reaction rate. In real biological media, the oxygen flow within the blood and tissue are usually under homeostasis and essentially constant. Comparing the quantum yields of the free compound **74** and the compound **80** after 50 min light irradiation, 18% cleavage of the FRET module can be estimated. This is still enough to obtain an intense fluorescence output.

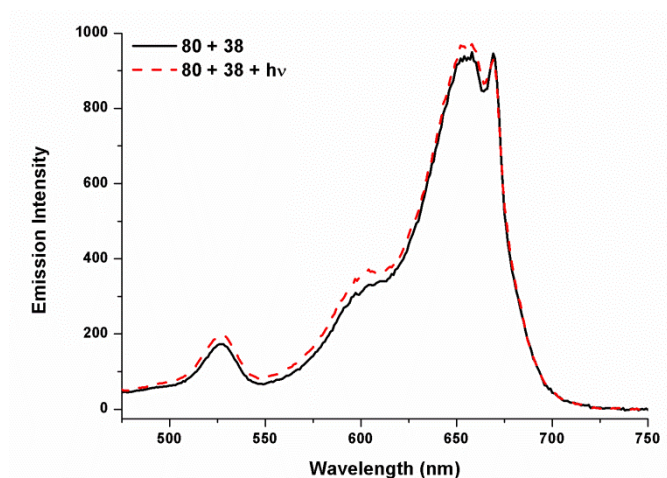


Figure 53. Excitation spectra of micellar construct of compound **80** and **38** in aqueous media before and after irradiation with 660 nm light. The light is irradiated for 50 min and excitation spectra is recorded for emission at 670 nm.

Overall results for the fluorescence enhancement in the presence acid or light of 660 nm are given in Figure 54. As it is obviously depicted, the singlet oxygen mediated fluorescence enhancement at 537 nm is apparently more pronounced in the presence

of $^1\text{O}_2$ which is efficiently produced in the presence of 660 nm light in acidic aqueous media.

Table 8. Photophysical characterization of compounds **38**, **74**, **75** and **80**.

Compound	$\lambda_{\text{abs}}^{\text{a}}$	$\lambda_{\text{em}}^{\text{b}}$	ϵ	$\Phi_{\text{F}}^{\text{c}}$
38	645 ^a	667 ^a	40000 ^a	0.13
	720 ^b	- ^b	30000 ^b	-
74	525	535	70000	0.96
75	654	670	76000	0.50
80	525	537	85000	0.14
	654	670	93000	0.56

^a values in THF; ^b values in piperidine-added THF; ^c for emission at 537 nm Rhodamine 6G (water, $\phi_{\text{F}} = 0.95$, $n = 1.333$) and for emission at 670 nm Cresyl Violet (MeOH, $\phi_{\text{F}} = 0.66$, $n = 1.329$) are used as reference compounds for quantum yield calculations.

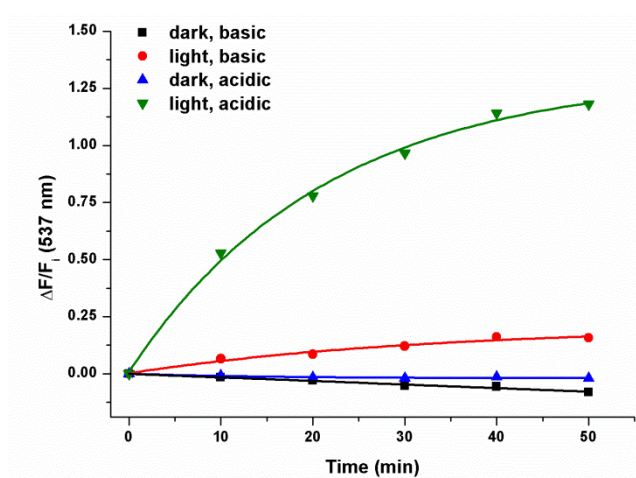


Figure 54. Fluorescence change at 537 nm of the micellar compounds **38** and **80** in D_2O in the presence of different combinations of acid-base and dark-light conditions. Light is supplied by a 660 nm LED source. All four samples are excited at 520 nm.

In order to make sure that the resultant increase is not a product of light solely, in other words to test the stability of the compound **80** under light illumination and same experimental conditions, a micelle containing only this compound is tested in aqueous solution. The results show that the increase in the emission of the donor in

the absence of the photosensitizer is significantly less efficient (Figure 55) indicating that the main contributor of the enhancement at 537 nm is $^1\text{O}_2$.

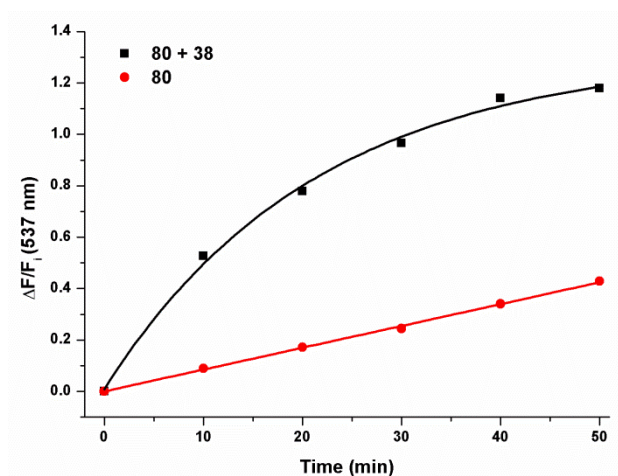


Figure 55. Comparison of fluorescence enhancement of micellar compound **80** (0.75 μM) in the presence (black, square) and absence (red, dot) of photosensitizer (compound **38**) in D_2O .

Considering all the results above, the second AND logic is constructed. Since the excitation with 520 nm is required for the second logic gate, the absence of this light is accepted as 0 outputs (no excitation). The results are depicted with a given threshold of 0.3 in Figure 56.

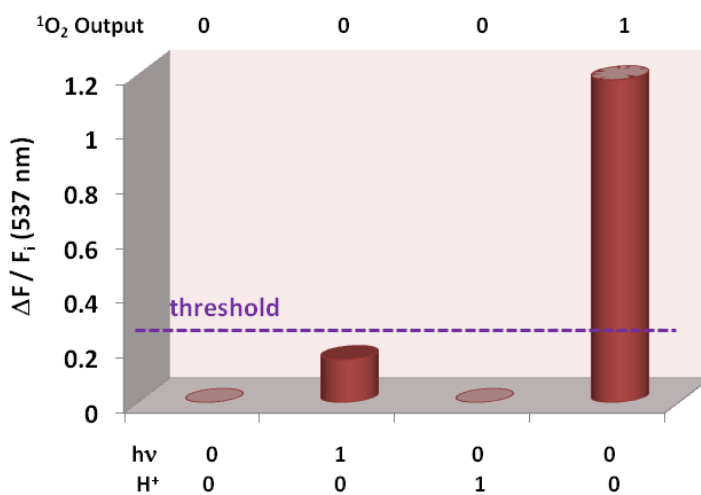


Figure 56. Construction of second AND logic gate with the inputs light (520 nm) and acid and the output is fold increase in emission at 537 nm. The threshold is determined as 0.3.

Although light is accepted as an input of a logic gate in a number of different researches in literature, this input may seem trivial since the excitation is required

anyway for any fluorescence process. For that reason, an alternative input is adopted for the second gate, glutathione. Although increased level of GSH in tumor tissue is used for the activation of PS in the research presented in chapter 4, the high level of this molecule resist PDT action because of its singlet oxygen scavenging property. Since in the former project the PS is expected to be activated in the extracellular medium, the increase in the concentration of this molecule is not that high to interfere with PDT action. To suppress the PDT deactivating property of GSH at high concentration, scientists use chemicals to decrease the biological production of this molecule.²⁰⁰ In this project, GSH is used as input because of its role as singlet oxygen scavenging. For improved action of produced $^1\text{O}_2$, GSH level should be low. The results of the fluorescence enhancement at 537 nm under different conditions are given in Figure 57.

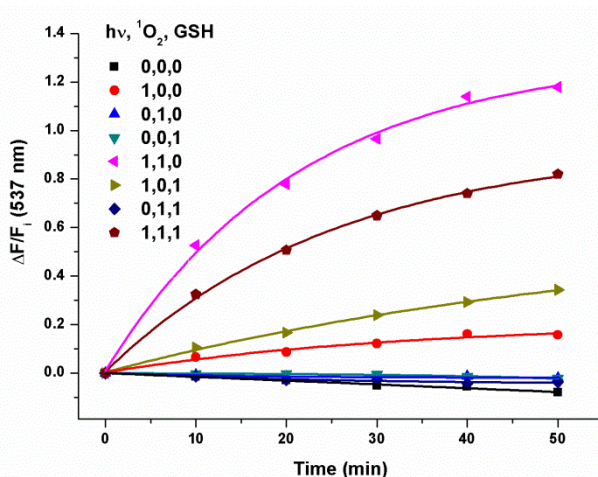


Figure 57. Comparison of fold increase in micellar compound **80** and **38** ($0.75 \mu\text{M}$) in the presence of different combinations of GSH, singlet oxygen (acid) or 660 nm light in aqueous solution. The fluorescence spectra are recorded by excitation at 520 nm.

Results in Figure 57 and 58 shows that presence of GSH interferes with fluorescence enhancement. This data is read as follows, GSH act as a $^1\text{O}_2$ scavenger and decrease the level of this molecule and thus rate of fluorescence enhancement is diminished. The lack of quantitative decrease in the fluorescence enhancement may be due to limited diffusion of GSH molecule to the interior of the micelle within the experimental time period (50 min). Yet, appropriate choice of threshold still allows the construction of an INHIBIT logic gate out of these results.

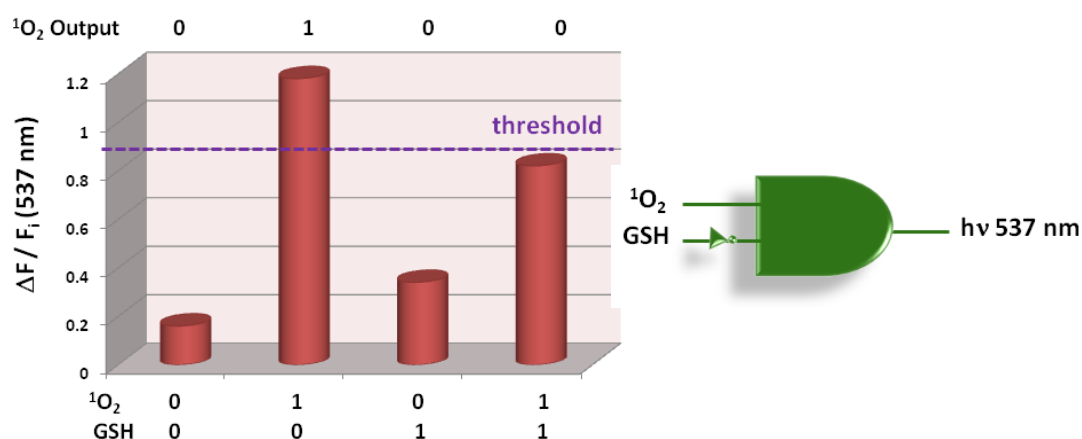


Figure 58. INHIBIT logic gate with the inputs $^1\text{O}_2$ and GSH and the output is emission at 537 nm.

Considering all, two different integrated logic gates with a meaningful function are presented in this research. An activatable photosensitizer with valuable pH-dependent activity constructs the first AND logic gate and the output of this logic ($^1\text{O}_2$) is used in the second logic gate as an input to release the FRET donor and restore its emission. Together with the $^1\text{O}_2$ input use of light (520 nm) makes an AND logic gate while use of GSH enables the development of an INHIBIT logic gate. The representations of both integrated logics are given in Figure 59. With these results, for the first time, logic gates are integrated for some functional intention. An activatable and self-reporting photosensitizer is introduced with the concept of logic gate concatenation.

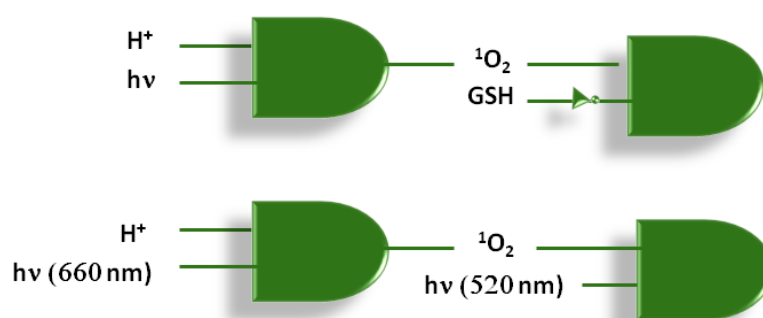


Figure 59. Representation of two different integrated logic gate constructs

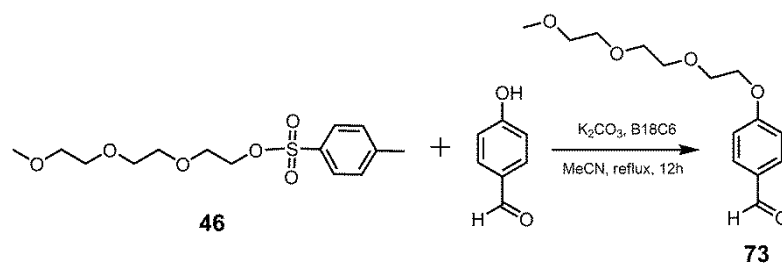
6.5 Experimental Details

Details of quantum yield calculations, lifetime measurements, micelle preparations are given in previous chapters, chapter 3, 4 and 5.

6.5.1 PDT and Fluorescence Measurements

$^1\text{O}_2$ dependent degradation of water soluble trap, 2,2'-(anthracene-9,10-diylbis(methylene)dimalonic acid was used to measure photodynamic activity since the absorption of this compound at 378 nm decreases indicating the generation of $^1\text{O}_2$. Corresponding reaction is given in the experimental section of Chapter 4. Measurements were performed using 660 nm LED and samples were irradiated with the light source from a 2 cm distance. All samples were aerated for 5 min prior to experiments. D_2O was used for the solutions since the lifetime of $^1\text{O}_2$ in this solution is higher. Relative $^1\text{O}_2$ generation efficiencies in different conditions were determined by taking the percent decrease of the trap absorption in a given time. Micellar constructs were prepared as described in experimental part of Chapter 4 and 1:1 ratio of compound **80** and compound **38** were used during micelle formation. pH of the solutions were adjusted by using NaOH and HCl solutions to slightly acidic (around 6) and slightly basic (around 7.5) values. The concentrations in micelle were determined by comparing the absorptivities of these compounds in THF solution.

6.5.2 Synthesis

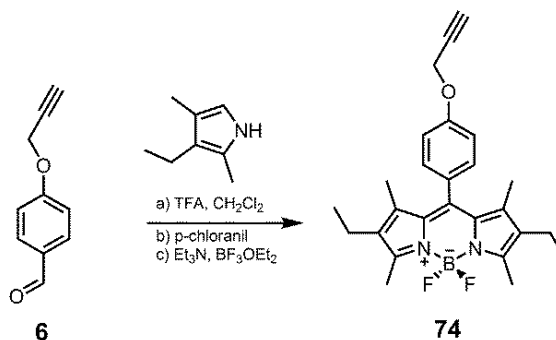


Synthesis of Compound 73: 4-hydroxybenzaldehyde (1.23 g, 10 mmol) and compound **46** (4.8 g, 15 mmol) were dissolved in 50 ml acetonitrile. K_2CO_3 (3.2 g, 22.4 mmol) and a few crystals of benzo-18-crown-6 were added. The reaction was

refluxed until all 4-hydroxybenzaldehyde was consumed. The solvent was evaporated in vacuo, extracted with water and CH₂Cl₂. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure. The product was purified by silica gel column chromatography using CH₂Cl₂/Hexane (75:25, v/v). Fraction containing compound **73** was collected then the solvent was removed under reduced pressure (2.1 g, 7.8 mmol, 78%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 9.80 (s, 1H), 7.75 (d, J =7.42 Hz, 2H, ArH), 6.94 (d, J =7.81 Hz, 2H, ArH), 4.13 (m, 2H; OCH₂), 3.80 (m, 2H; OCH₂), 3.70-3.40 (m, 8H; OCH₂), 3.27 (s, 3H, OCH₃).

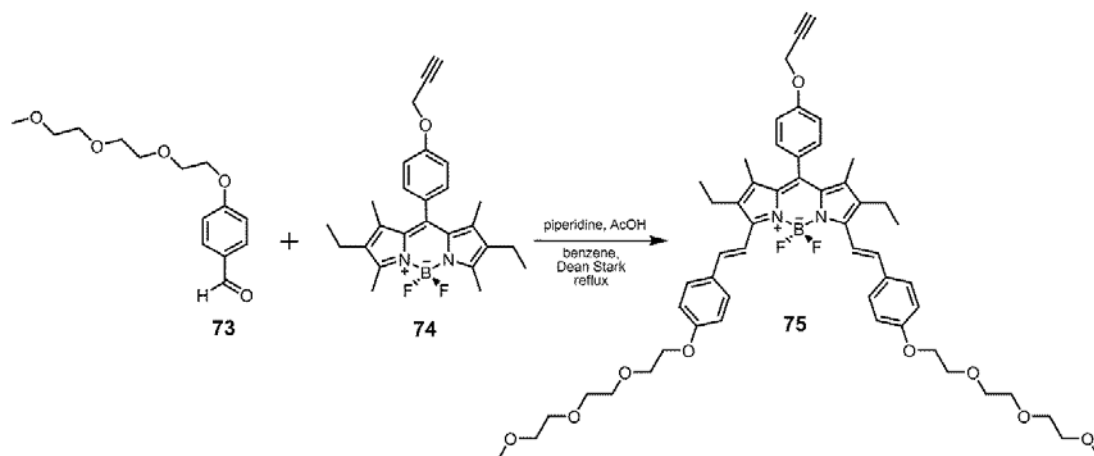
¹³C NMR (CDCl₃, 400 MHz, δ ppm) 190.8, 163.9, 131.9, 130.1, 114.9, 71.9, 70.9, 70.7, 70.6, 69.5, 67.8, 59.0.



Synthesis of Compound 74: CH₂Cl₂ (300 ml) was purged with Ar for 30 min. 4-(prop-2-yn-1-yloxy) benzaldehyde **6** (1 g, 6.24 mmol) and 2,4-dimethyl-3-ethyl pyrrole (1.54 g, 1.7 ml, 12.6 mmol) were added. 3 drops of trifluoroacetic acid was added. The reaction mixture was stirred at room temperature for 12h. Then, tetrachloro-1,4-benzoquinone (1.54 g, 6.24 mmol) was added and the reaction mixture was stirred at room temperature for 45 min. Then triethyl amine (8 ml) and boron trifluoride diethyl etherate (8 ml) were added sequentially. After stirring at room temperature for 30 min, it was extracted with water. Organic layer was dried with Na₂SO₄ and evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃/Hexane (75:25, v/v) as mobile phase. Fraction containing compound **74** was collected then the solvent was removed under reduced pressure (400 mg, 0.92 mmol, 15%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.21 (d, J = 8.72 Hz, 2H; ArH), 7.10 (d, J = 8.72 Hz, 2H; ArH), 4.78 (d, J = 2.40 Hz, 2H; OCH_2), 2.57 (t, J = 2.44 Hz, 1H; CH), 2.55 (s, 6H; ArCH_3), 2.32 (q, J = 7.56 Hz, 4H; ArCH_2), 1.33 (s, 6H; ArCH_3), 1.0 (t, J = 7.60 Hz, 6H; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 158.0, 153.6, 140.0, 138.4, 132.8, 131.1, 129.8, 128.9, 115.6, 78.1, 75.8, 56.0, 17.1, 14.6, 12.5, 11.8.

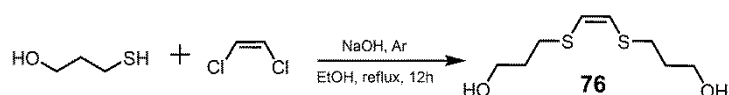


Synthesis of Compound 75: Compound **73** (112 mg, 0.42 mmol) and compound **74** (70 mg, 0.17 mmol) were dissolved in benzene (40 ml). Piperidine (0.4 ml) and acetic acid (0.4 ml) were added. The reaction mixture was refluxed using Dean Stark apparatus until all BODIPY was consumed. After the reaction was completed, it was extracted with CH_2Cl_2 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/MeOH (98:2, v/v). Fraction containing compound **75** was collected then the solvent was removed under reduced pressure (90 mg, 0.1 mmol, 59%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.68 (d, 2H, J = 16.77 Hz; ArH), 7.59 (d, 4H, J = 8.72 Hz; ArCH_3), 7.25-7.18 (d + d, 2H + 2H; ArH), 7.13 (d, 2H, J = 8.64 Hz; ArH), 6.97 (d, 4H, J = 8.72 Hz; ArH), 4.80 (d, 2H, J = 2.32 Hz; OCH_2), 4.20 (t, 4H, J = 4.68 Hz; OCH_2), 3.80-3.60 (m, 8H; OCH_2), 3.60 (m, 4H; OCH_2), 3.40 (s, 6H; OCH_3), 2.61 (q, 4H, J = 7.36 Hz; ArCH_2), 1.40 (s, 6H; ArCH_3), 1.15 (t, 6H, J = 7.40 Hz; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 159.4, 158.0, 150.4, 138.7, 137.7, 135.3, 133.6, 133.3, 129.9, 129.2, 128.7, 118.2, 115.6, 114.9, 78.1, 75.9, 72.0, 70.9, 70.7, 70.6, 69.7, 67.6, 59.0, 56.1, 18.4, 14.1, 11.7.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{54}\text{H}_{65}\text{BF}_2\text{N}_2\text{NaO}_9^+$ 957.46434 $[\text{M}+\text{Na}]^+$; found: 957.47300 $[\text{M}+\text{Na}]^+$, $\Delta = 9.0$ ppm.

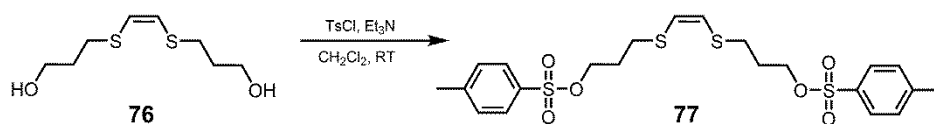


Synthesis of Compound 76: Argon was purged in EtOH (10 ml) for 15 min. NaOH (0.88 g, 22 mmol) and 3-mercaptopropanol (2 g, 1.86 ml, 22 mmol) were added and the reaction was stirred at 0°C ice bath for 30 min while the flask was sealed. Then, cis-1,2-dichloroethylene (1.04 g, 0.8 ml, 11 mmol) in degassed EtOH (1 ml) was added and the reaction mixture was refluxed for 18 h. After cooling to RT, it was extracted with petroleum ether and brine. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/Hexanes (50:50, v/v) as mobile phase. Fraction containing compound **76** was collected then the solvent was removed under reduced pressure (2 g, 9.6 mmol, 87%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 6.12 (s, 2H), 3.73 (t, $J = 6.08$ Hz, 4H; OCH_2), 2.84 (t, $J = 7.04$ Hz, 4H; SCH_2), 2.57 (b, 2H; OH), 1.88 (m, 4H; CH_2).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 123.9, 60.9, 32.7, 30.8.

HRMS (TOF-ESI): m/z calcd for $\text{C}_8\text{H}_{16}\text{NaO}_2\text{S}_2^+$: 231.04839 $[\text{M}+\text{Na}]^+$; found: 231.05234 $[\text{M}+\text{Na}]^+$, $\Delta = 17.0$ ppm.



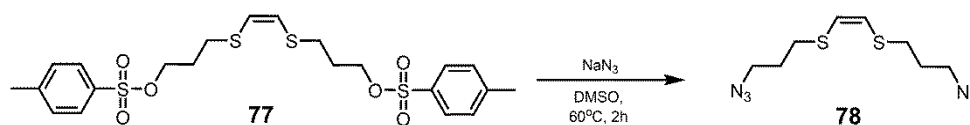
Synthesis of Compound 77: (Z)-3,3'-(ethene-1,2-diylbis(sulfaneyldi))dipropen-1-ol, compound **76** (1.7 g, 8.16 mmol) was dissolved in 30 ml CH_2Cl_2 and 3.4 ml Et_3N . In a dropper, p-toluene sulfonyl chloride (3.11 g, 16.32 mmol) was dissolved in CH_2Cl_2 10 ml and was added to the previous solution dropwise while the reaction mixture

was being cooled with ice bath. It was stirred for 12h. After the extraction with water, organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. The product was purified by silica gel column chromatography using CHCl₃ as mobile phase. Fraction containing compound **77** was collected, then the solvent was removed under reduced pressure (white solid, 5.7 mmol, 2.95 g, 70%).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 7.80 (d, 4H, J = 8.48 Hz; ArH), 7.37 (d, 4H, J = 8.48 Hz; ArH), 5.98 (s, 2H; ArH), 4.14 (t, 4H, J = 5.93 Hz; OCH₂), 2.73 (t, 4H, J = 7.00 Hz; SCH₂), 2.48 (s, 6H, ArCH₃), 1.95 (m, 4H; CH₂).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 144.9, 132.9, 129.9, 127.9, 123.9, 68.3, 29.9, 29.6, 21.7.

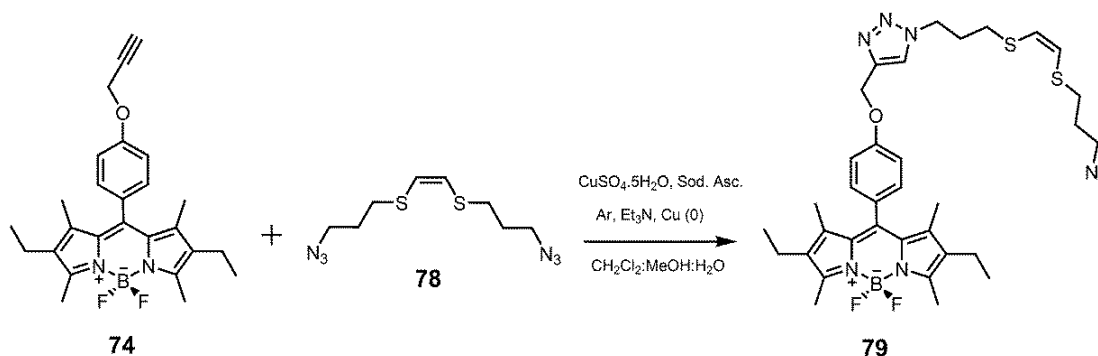
HRMS (TOF-ESI): m/z calcd for C₂₂H₂₈NaO₆S₄⁺ 539.06609 [M+Na]⁺, found: 539.07170 [M+Na]⁺, Δ = 10.0 ppm.



Synthesis of Compound 78: Compound **77** (200 mg, 0.38 mmol) was dissolved in 10 ml DMSO and sodium azide (247 mg, 3.8 mmol) was added to the reaction mixture. It was stirred 2h at 60 oC. After cooling to RT, it was extracted with EtOAc and brine. Organic layer was collected and dried with Na₂SO₄, evaporated under reduced pressure. (yellow oil, 3.8 mmol, 98 mg quantitative).

¹H NMR (CDCl₃, 400 MHz, δ ppm) 6.10 (s, 2H), 3.45 (t, J = 6.57 Hz, 4H; NCH₂), 2.81 (t, j = 6.96 Hz, 4H; SCH₂), 1.89 (m, 4H; CH₂).

¹³C NMR (CDCl₃, 400 MHz, δ ppm) 49.9, 37.6.

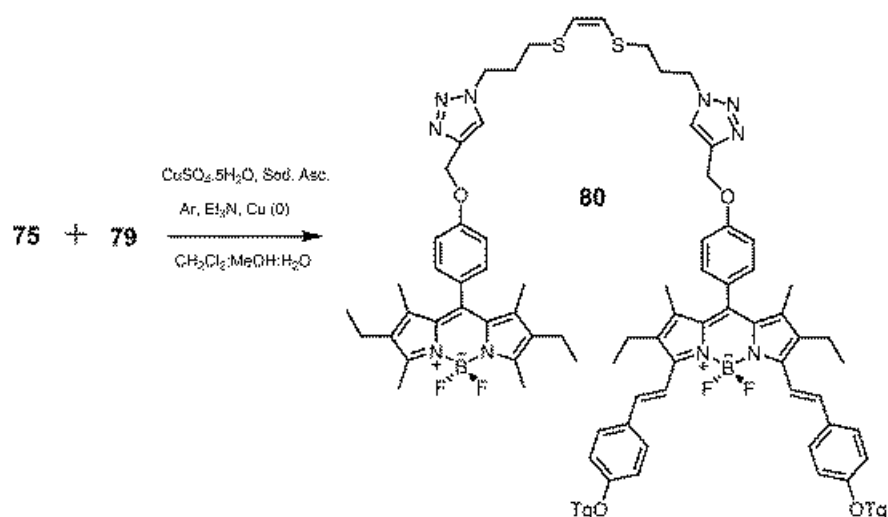


Synthesis of Compound 79: Compound **74** (80 mg, 184 μ mol) and compound **78** (476 mg, 1.84 mmol) were dissolved in CH_2Cl_2 (6 ml) and MeOH (3 ml). Flask was covered with aluminium folio to avoid light and Ar was purged for 15 min. Saturated solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (100 μ l) and sodium ascorbate (100 μ l) were added. Catalytic amount of Cu (0) was added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, it was extracted with CH_2Cl_2 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/Hexanes (50/50; v/v) as mobile phase. Fraction containing compound **79** was collected, then the solvent was removed under reduced pressure (94 μ mol, 65 mg, 51%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.71 (s, 1H; ArH), 7.20 (d, J = 8.72 Hz, 2H; ArH), 7.12 (d, J = 8.56 Hz, 2H; ArH), 6.15 (d, J = 8.32 Hz, 1H; CH), 6.06 (d, J = 8.37 Hz, 1H; CH), 5.26 (s, 2H, OCH_2), 4.55 (t, J = 6.76 Hz, 2H; NCH_2), 3.46 (t, J = 6.53 Hz, 2H; NCH_2), 2.84 (t, J = 6.97 Hz, 2H; SCH_2), 2.75 (t, J = 6.76 Hz, 2H; SCH_2), 2.53 (s, 6H; ArCH_3), 2.30 (m, 2H + 4H; CH_2 + ArCH_2), 1.82 (m, 2H; CH_2), 1.33 (s, 6H; ArCH_3), 1.0 (t, J = 7.49 Hz, 6H; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 158.7, 153.6, 143.7, 140.0, 138.4, 132.7, 131.1, 129.6, 128.5, 127.0, 125.4, 123.2, 122.7, 115.3, 62.1, 49.6, 48.4, 31.1, 30.7, 30.2, 29.4, 17.1, 14.6, 12.5, 11.8.

HRMS (TOF-ESI): m/z calcd for $\text{C}_{34}\text{H}_{43}\text{BF}_2\text{N}_8\text{NaOS}_2^+$ 715.29546 $[\text{M}+\text{Na}]^+$, found: 715.30182 $[\text{M}+\text{Na}]^+$, Δ = 8.89 ppm.



Synthesis of Compound 80: Compound **79** (30 mg, 43 μmol) and compound **75** (35 mg, 37 μmol) were dissolved in CH_2Cl_2 (6 ml) and MeOH (3 ml). Flask was covered with aluminium folio to avoid light and Ar was purged for 15 min. Saturated solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (100 μl) and sodium ascorbate (100 μl) were added. Catalytic amount of Cu (0) was added. The reaction mixture was stirred for 12 h at room temperature. After the reaction was completed, it was extracted with CH_2Cl_2 and water. Organic layer was collected and dried with Na_2SO_4 , evaporated under reduced pressure. The product was purified by silica gel column chromatography using EtOAc/MeOH (90/10; v/v) as mobile phase. Fraction containing compound **80** was collected, then the solvent was removed under reduced pressure (25 μmol , 40 mg, 68%).

^1H NMR (CDCl_3 , 400 MHz, δ ppm) 7.75-7.65 (s + s + d, 1H + 1H + 2H; ArH), 7.58 (d, $J = 8.80$ Hz, 4H; ArH), 7.25-7.15 (d + d + d, 2H + 2H + 2H; ArH), 7.15-7.08 (d + d, 2H + 2H; ArH), 6.96 (d, $J = 8.76$ Hz, 2H; ArH), 6.11 (s, 2H; CH), 5.29 (s, 2H; OCH_2), 5.27 (s, 2H; OCH_2), 4.55 (m, 2H + 2H; NCH_2), 4.20 (t, $J = 5.0$ Hz, 4H, OCH_2), 3.90 (m, 4H; OCH_2), 3.78 (m, 4H; OCH_2), 3.72-3.60 (m, 8H; OCH_2), 3.59 (m, 4H; OCH_2), 3.40 (s, 6H; OCH_3), 2.77 (m, 4H, SCH_2), 2.61 (m, 4H; CH_2), 2.53 (s, 6H; Ar CH_3), 2.30 (m, 4H + 4H; Ar CH_2), 1.38 (s, 6H; Ar CH_3), 1.34 (s, 6H; Ar CH_3), 1.17 (t, $J = 7.36$ Hz, 6H; CH_3), 1.00 (t, $J = 7.60$ Hz, 6H; CH_3).

^{13}C NMR (CDCl_3 , 400 MHz, δ ppm) 159.4, 158.7, 153.6, 150.4, 143.8, 140.0, 138.7, 138.4, 137.7, 135.4, 133.6, 133.2, 132.7, 130.5, 130.0, 129.6, 128.9, 128.7, 128.6,

124.1, 124.0, 123.2, 118.1, 115.4, 115.3, 114.9, 71.9, 70.9, 70.7, 70.6, 69.7, 67.6, 62.0, 59.0, 48.4, 30.7, 30.3, 18.4, 17.1, 14.6, 14.1, 12.5, 11.9, 11.7.

HRMS (TOF-ESI): m/z calcd for $C_{88}H_{108}B_2F_4N_{10}NaO_{10}S_2^+$ 1649.77058 $[M+Na]^+$,
found: 1649.78090 $[M+Na]^+$, $\Delta = 6.26$ ppm.

CHAPTER 7

CONCLUSION

This thesis involves four different projects related to functional manufacture of logic gates to obtain controlled photodynamic action (Chapters 3, 4, 6) and in another context, a fluorescent chemosensor designed for selective detection of chloride anion (Chapter 5).

Considering the time arrow of science, things are getting smaller and smarter as the knowledge grows immensely. More is known about molecular and genetic basis of diseases and therapies are more personalized accordingly. In parallel, technology products and organic devices are further becoming intelligent. Chemical logic gates increase the flexibility in functionality of the Boolean logic operations, and allow the creation of demanding complex works for different purposes. The missing link between the design of any kind of complex logic operation and assignment of real function is addressed to be reached in the projects of this thesis.

The first of these projects (Chapter 3) is a proof of principle demonstration of use of a demultiplexer (DEMUX) logic operation within an operational framework. The logic gate enables the selection of one from a set of outputs by means of an address input. In the project, acid is chosen as an address input, in order to mimick the tumor microenvironment, and the outputs are assigned to be emission and photodynamic action. Acid controlled change of the roles of FRET donor and acceptor modules either activates or quenches one of the modules thus an output of the form of emission or singlet oxygen is selected accordingly. This enables the construction of a theranostic device with a promising logic gate construct which, once optimized accordingly, can find a real biological application.

The project presented in Chapter 4 aims to carry the logic operation to a biologically meaningful arena. Photosensitizer is optimized rationally to have response to acid of biological concentrations and glutathione. The latter input glutathione is reported to be elevated in cancer cells. Acid induced change in the absorption of the photosensitizer allows an increase of extinction at the wavelength of excitation and

activates it. However, an energy acceptor conjugated to PS via a reducible disulfide bond still deactivates it. Presence of the GSH enables the reduction of the bond and the photosensitizer becomes free. Activity of the PS is shown to be increased in the presence of both inputs in aqueous solution, hence, an AND logic gate is created for the development of a highly selective photodynamic therapy action.

The third project (Chapter 5) is related to detection of chloride anion concentration in acidic and neutral media. As a start, the response of fluorescent BODIPY-triazolophane construct is shown to have response in organic solvent. An intriguing result is obtained considering the dynamic range of detection which ranges from micromolar to millimolar concentrations. The possible reasons of this large detection range and roles of other binding behaviour are investigated with a number of different control compounds lacking chloride binding pocket.

The final project of this thesis (Chapter 6) is also related to applicability of molecular logic gates. The aim of the project is to integrate two simple logic gates such that the nature of each gate and the relay of the information from one to other have a practical meaning. An AND logic gate is used to control activity of a photosensitizer with acid and light. The output of this logic, which is singlet oxygen, is used by another logic gate to enhance the emission either in the form of another AND gate or an INHIBIT gate depending on the choice of inputs. By means of this concatenation, two gates are communicating with one another such that an activity of an activatable photosensitizer is monitored by the increase in the emission of a fluorescent reporter nearby within the micellar structure.

Taking everything into consideration, this thesis successfully assigns functions to simple (AND) or more complex logic gates (DEMUX, integrated logic gates) and gives birth to new utilization for this nano-devices. In addition, a selective fluorescent sensor with a quite large dynamic response is introduced which can be optimized to work in aqueous solutions and find real applications.

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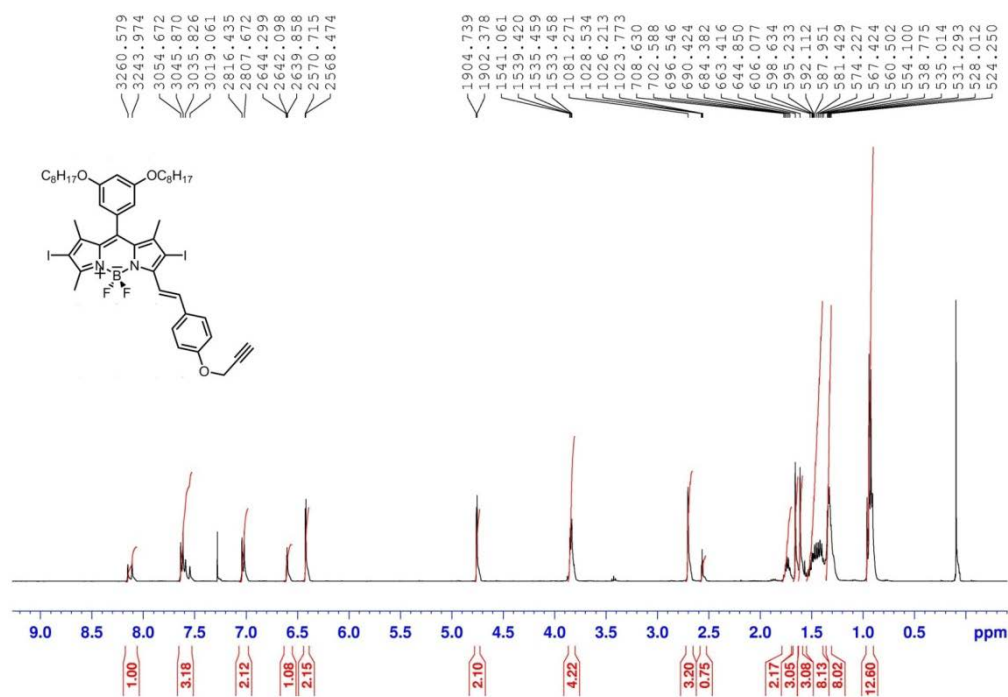
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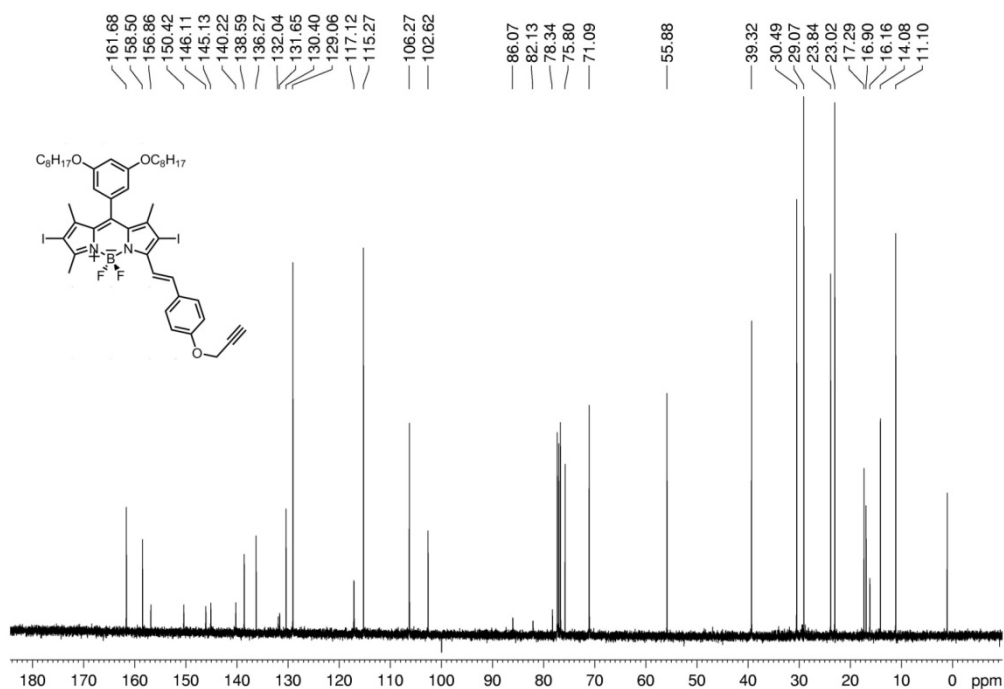
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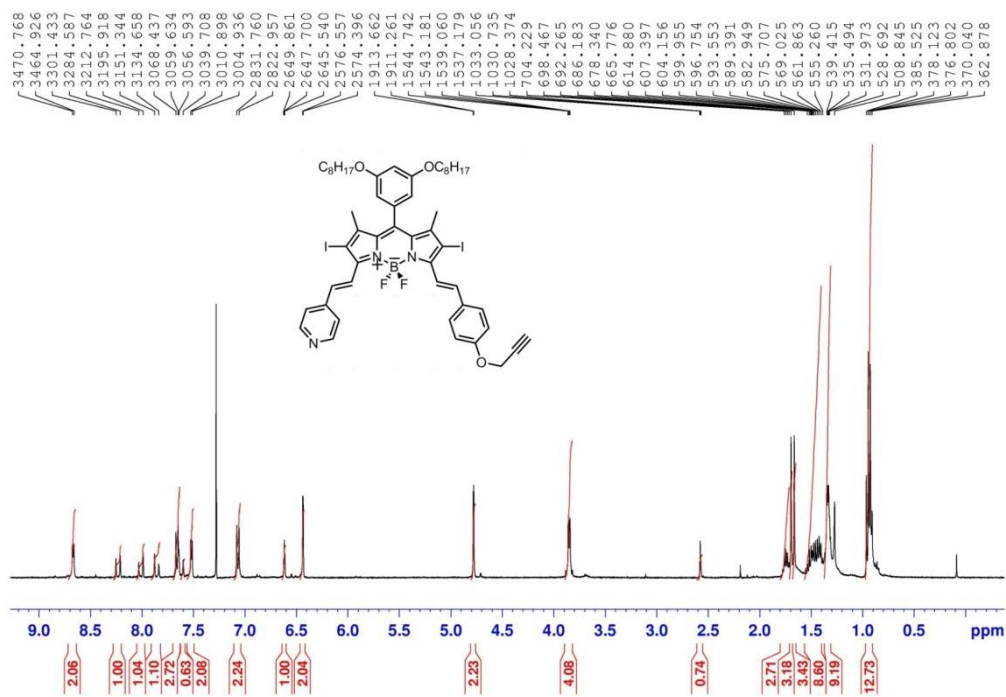
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NMR SPECTRA

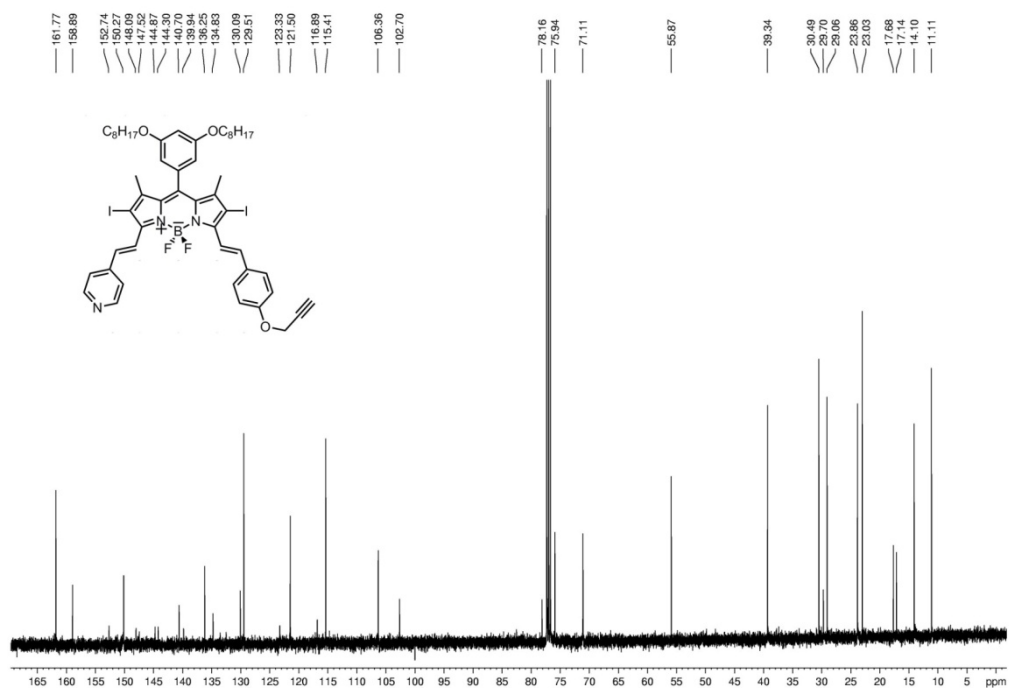
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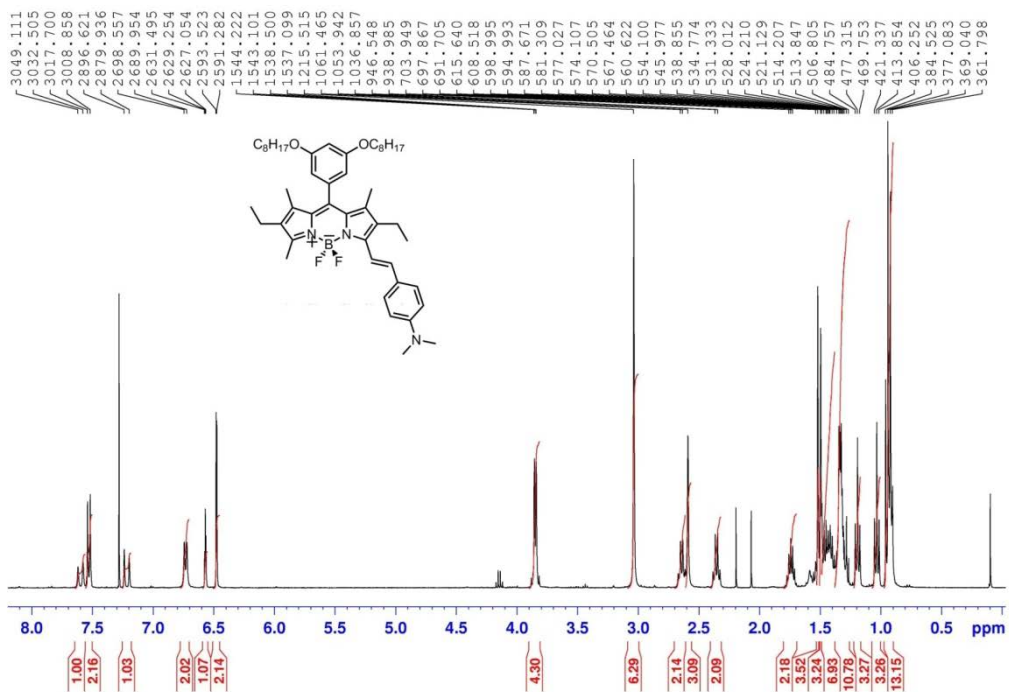
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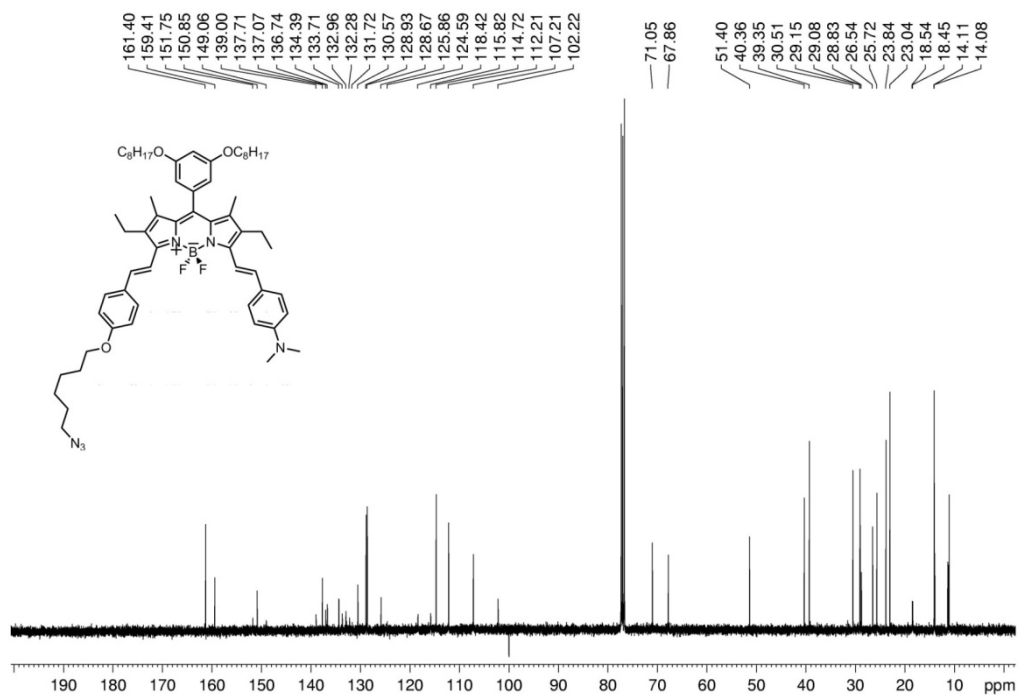
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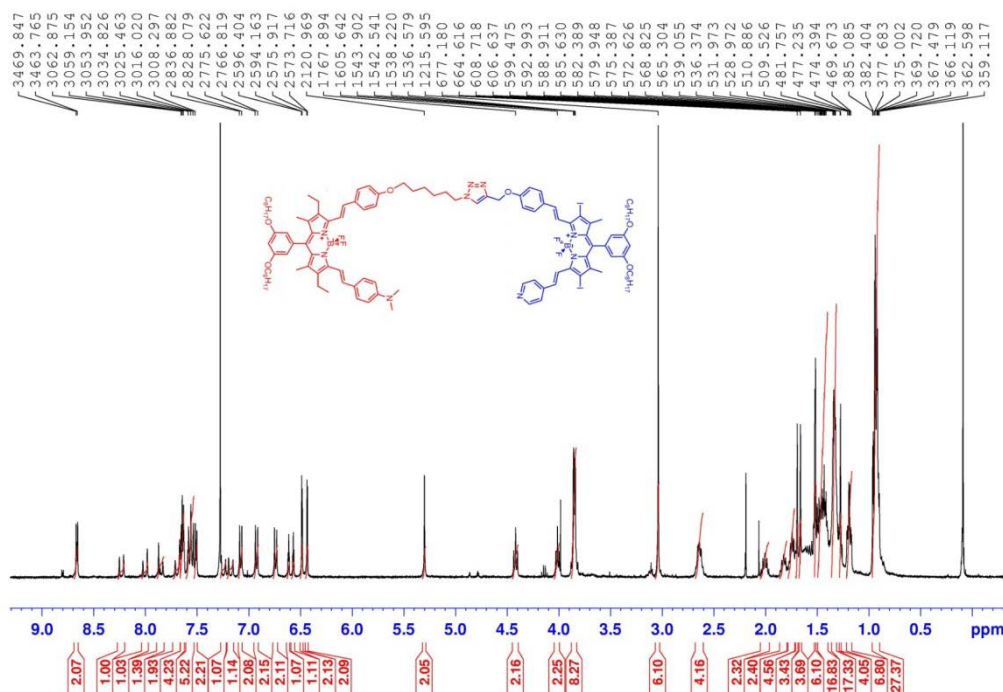
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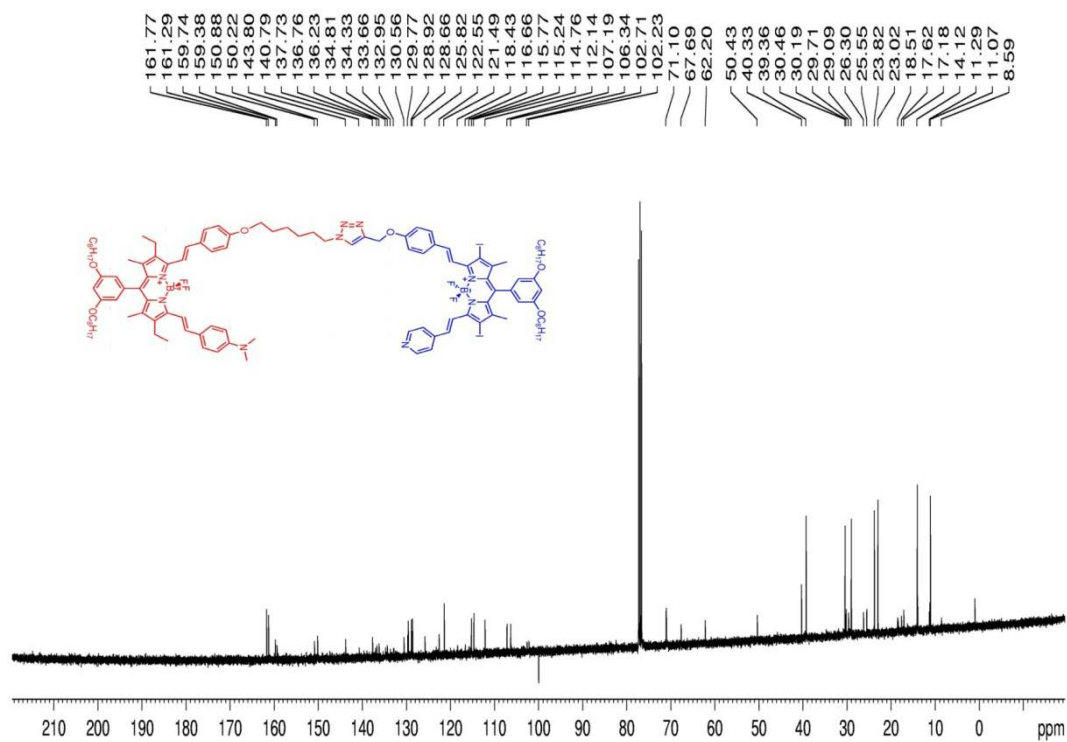
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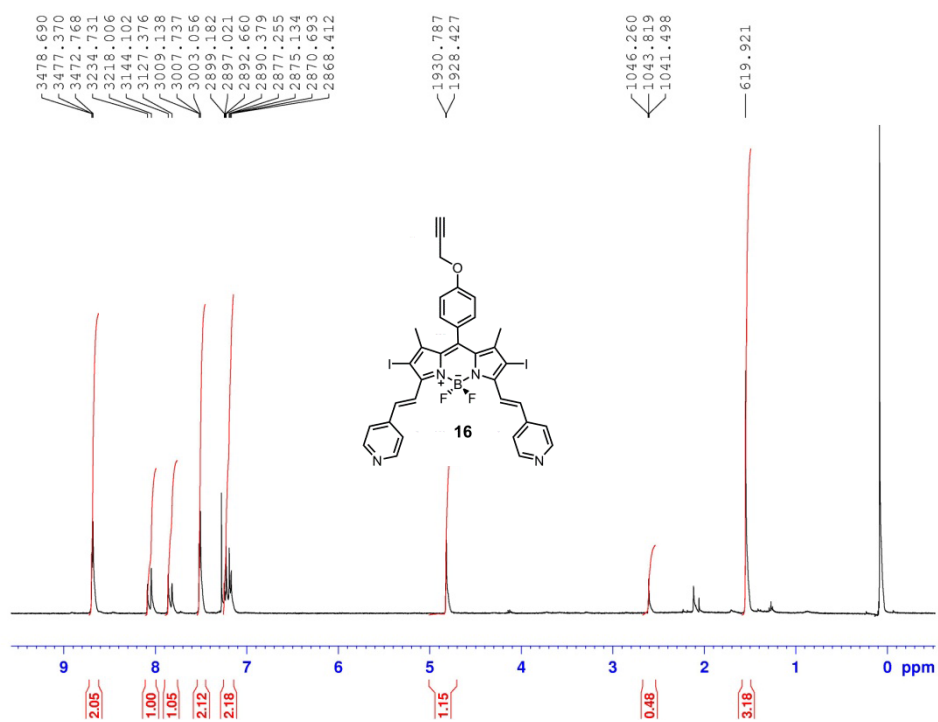
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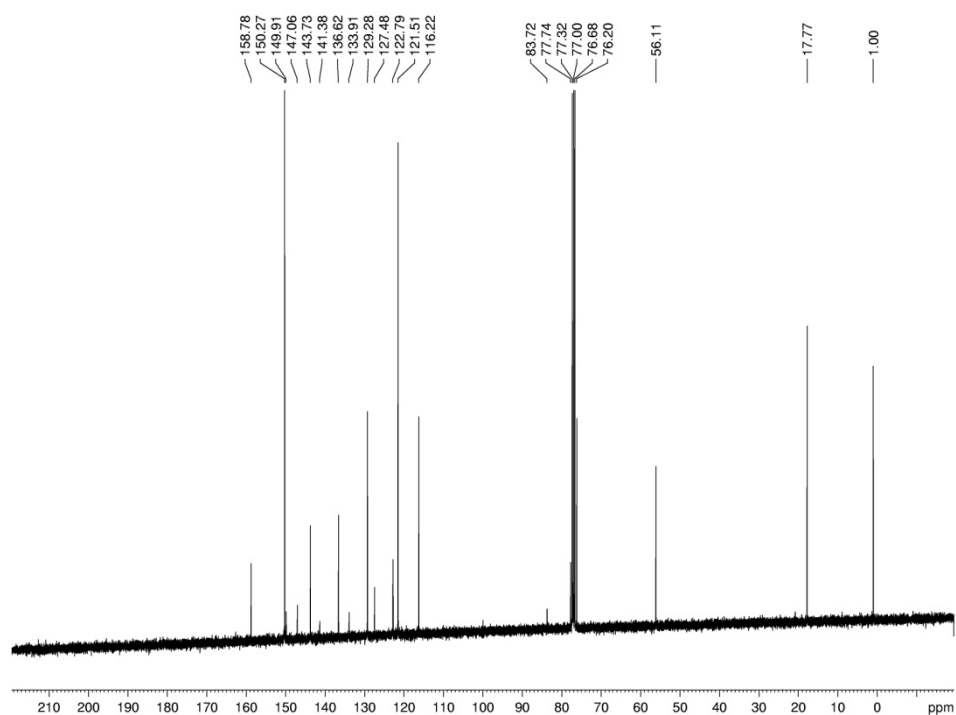
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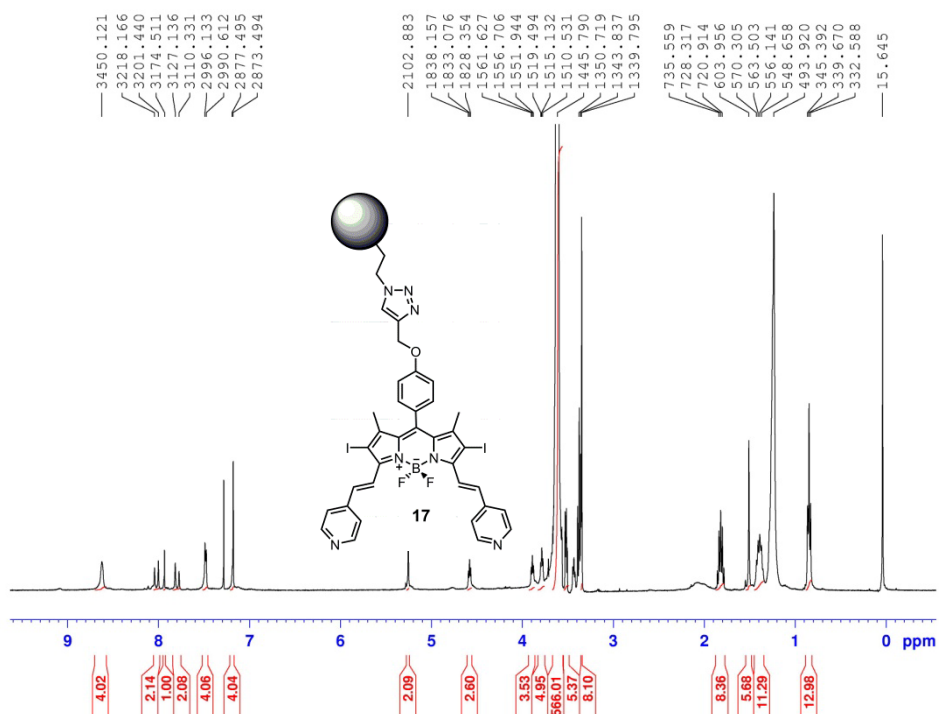
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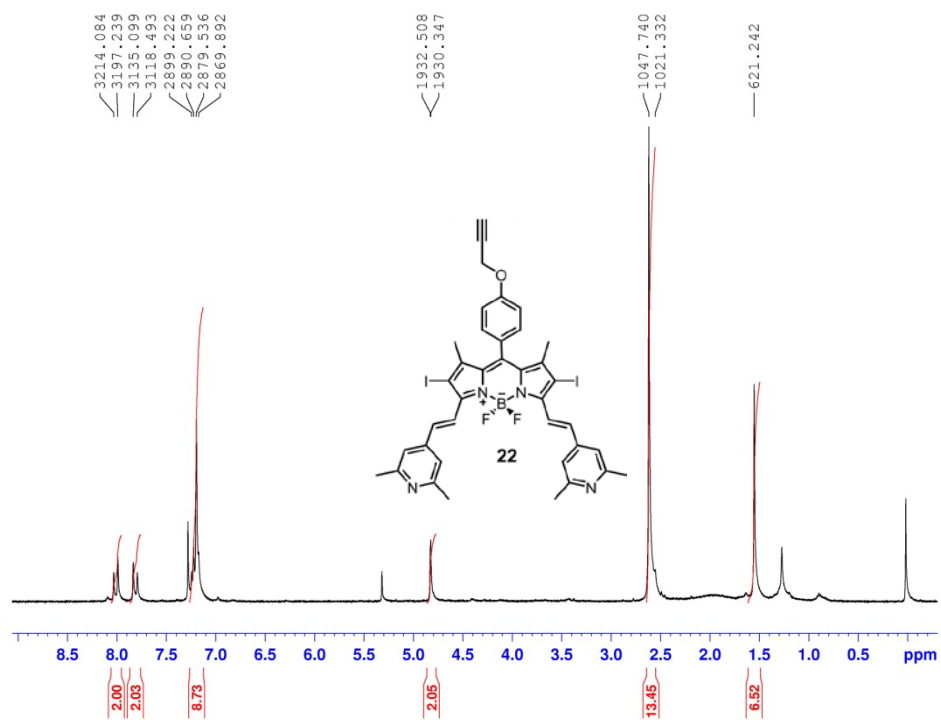
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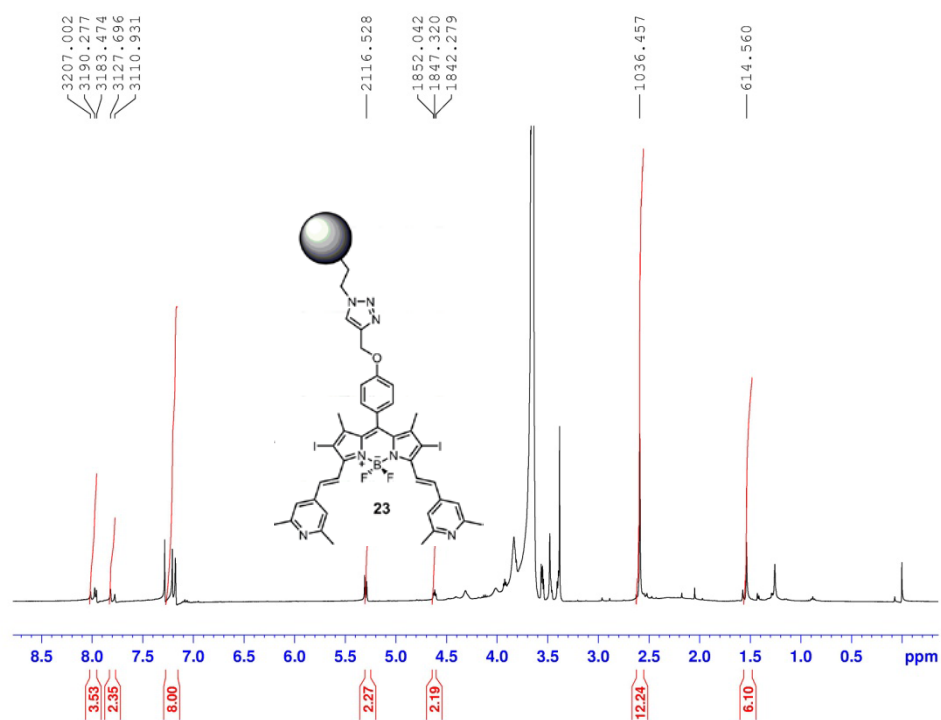
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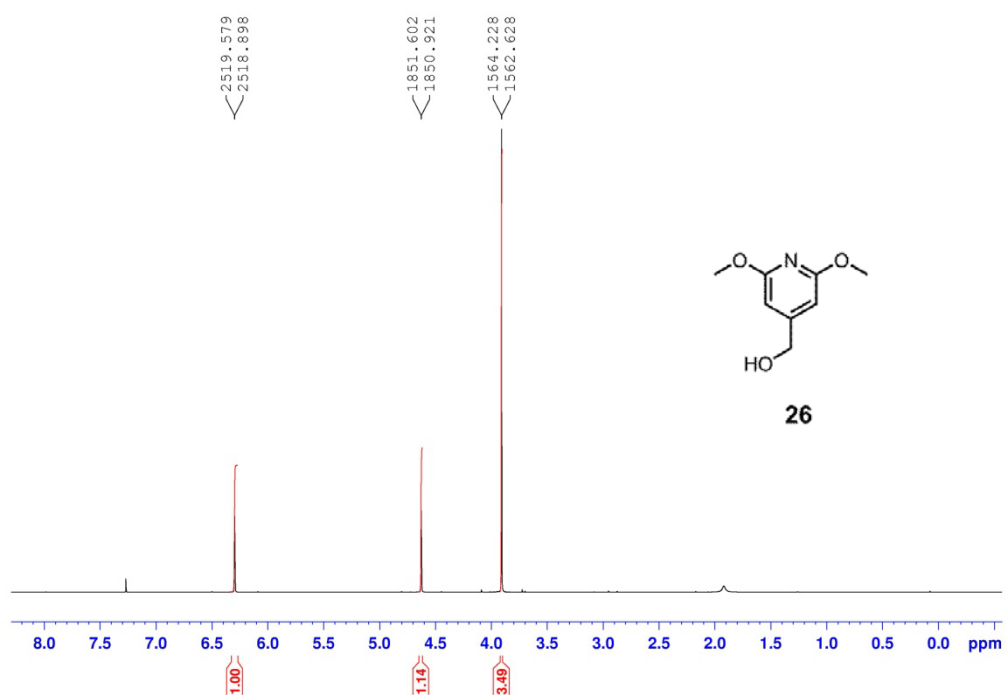
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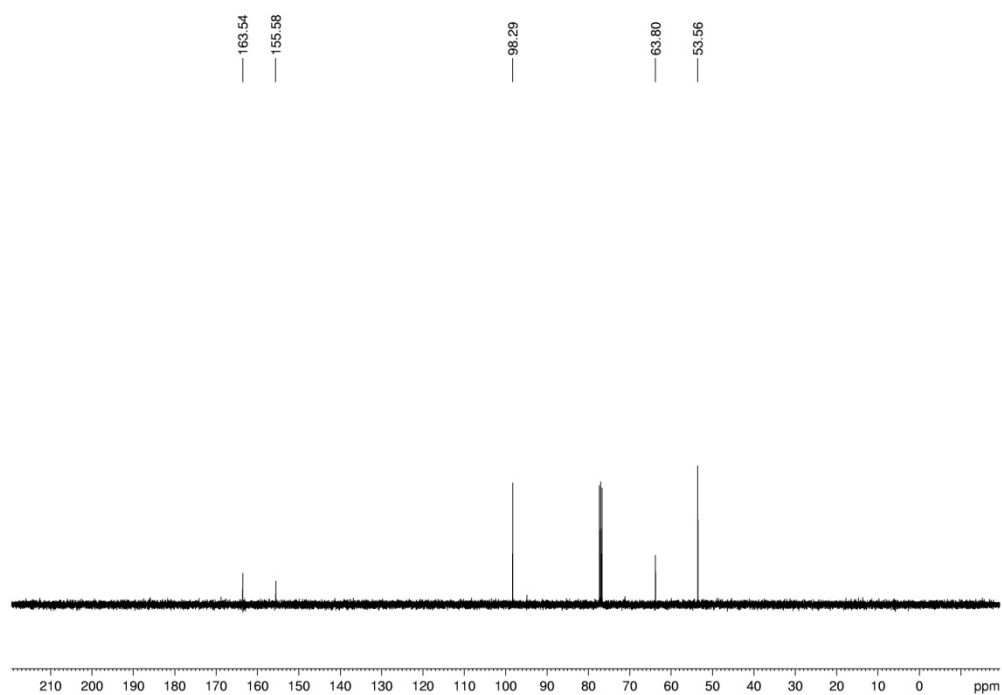
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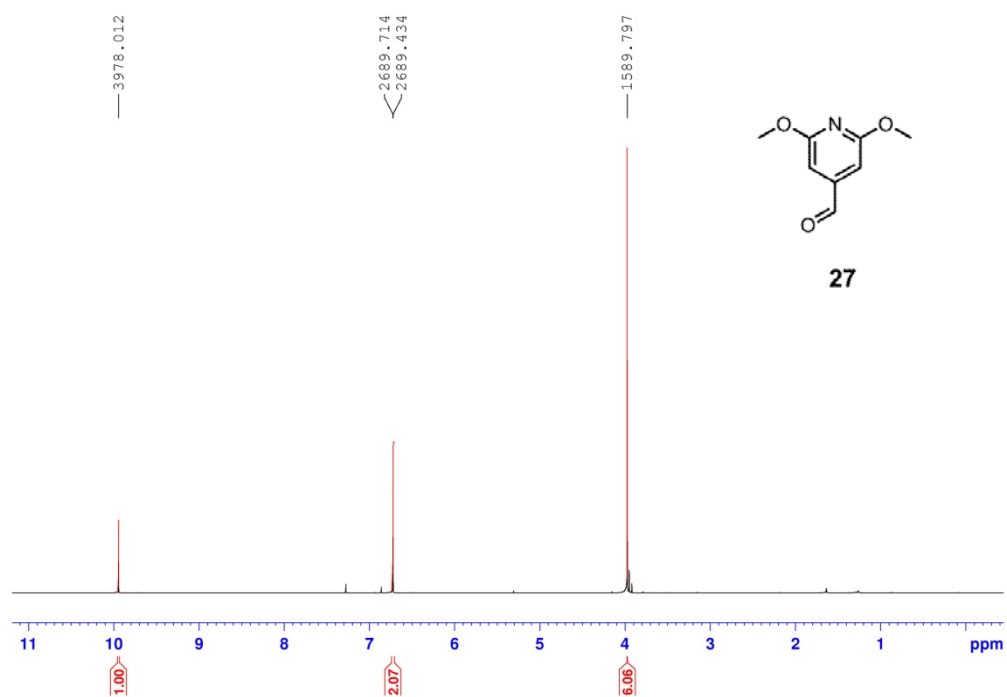
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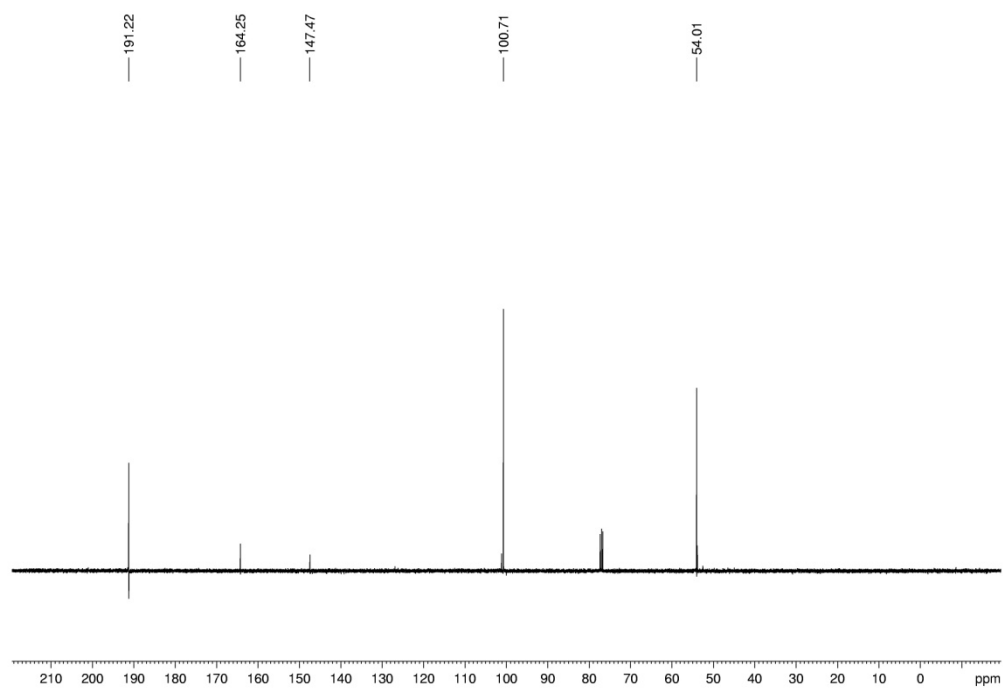
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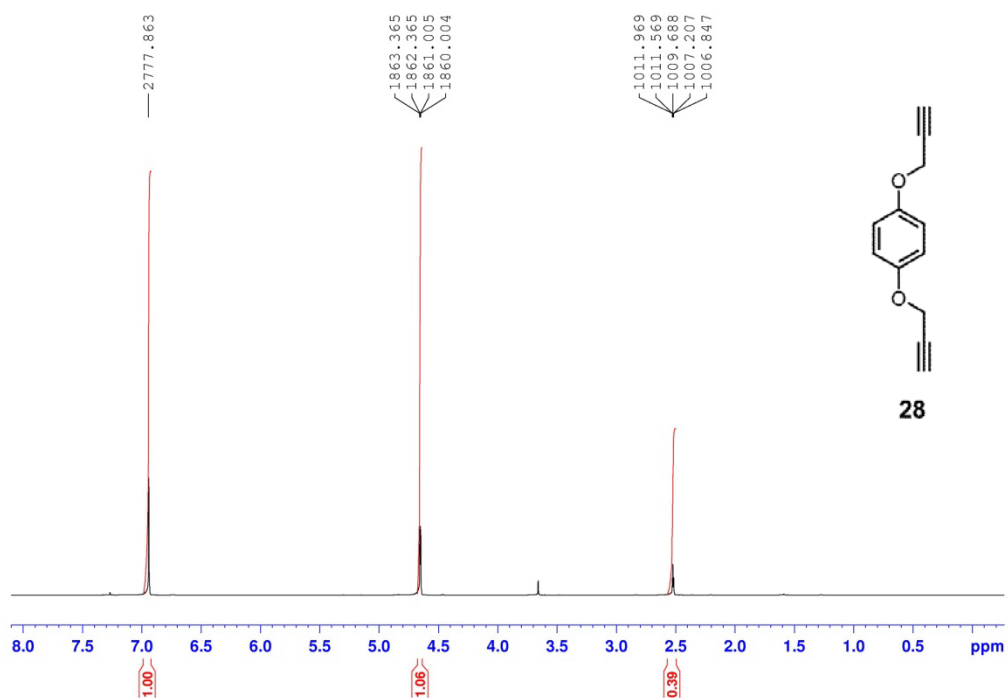
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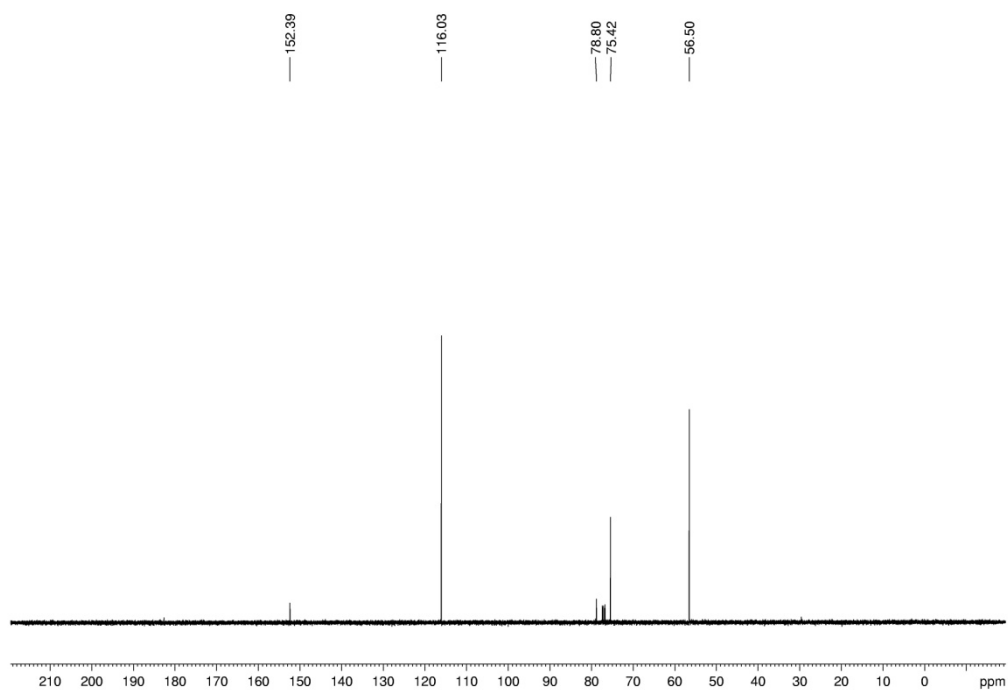
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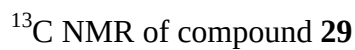
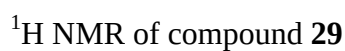
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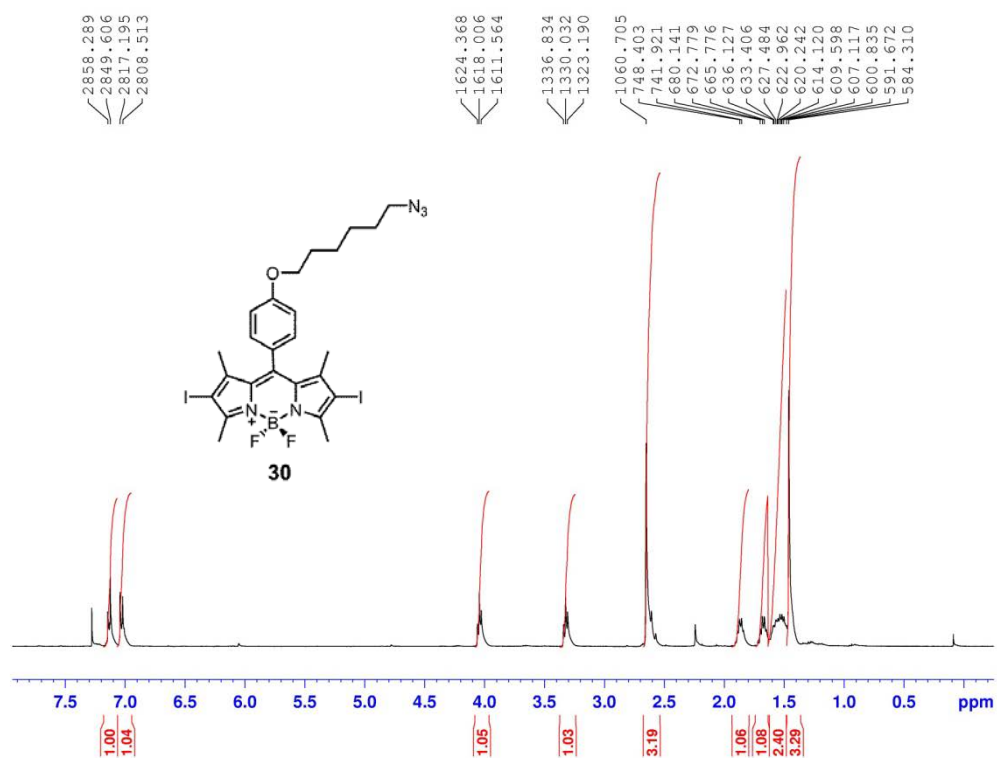


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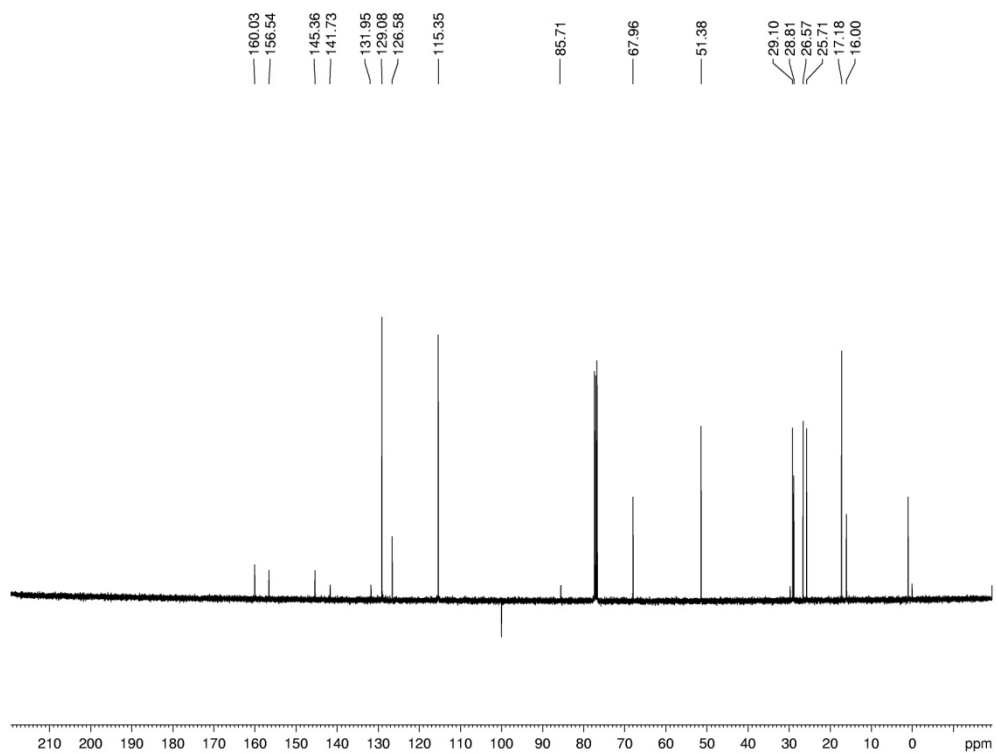


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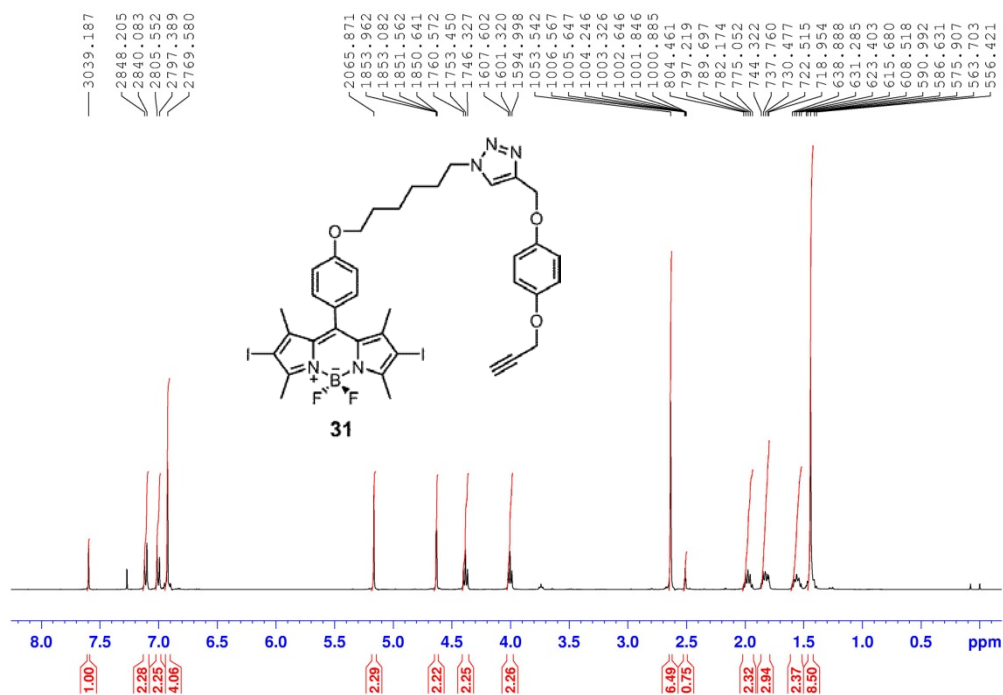




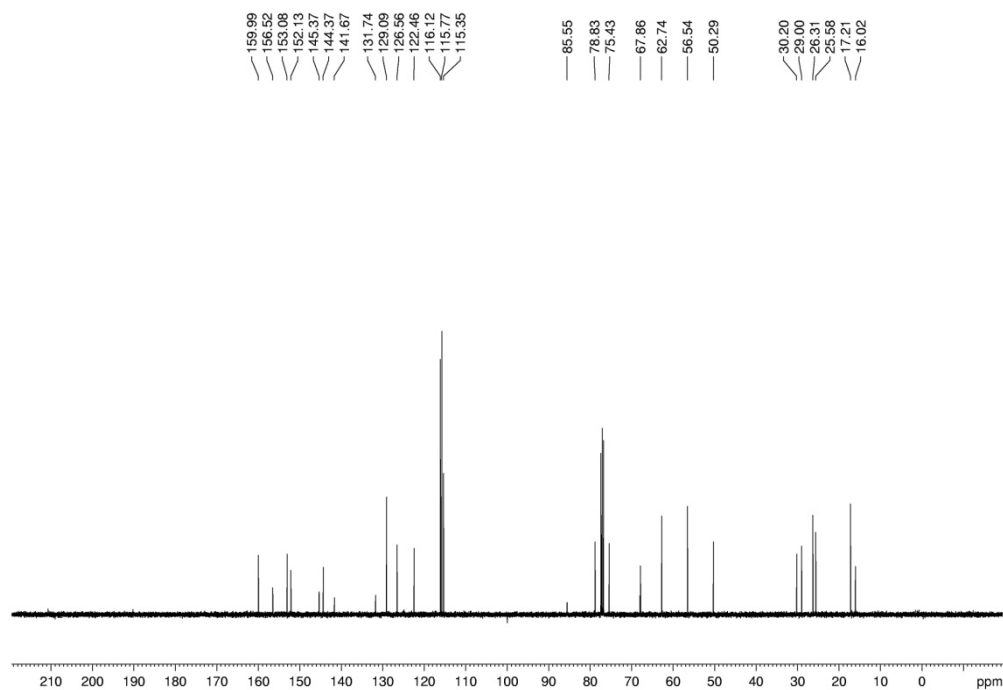
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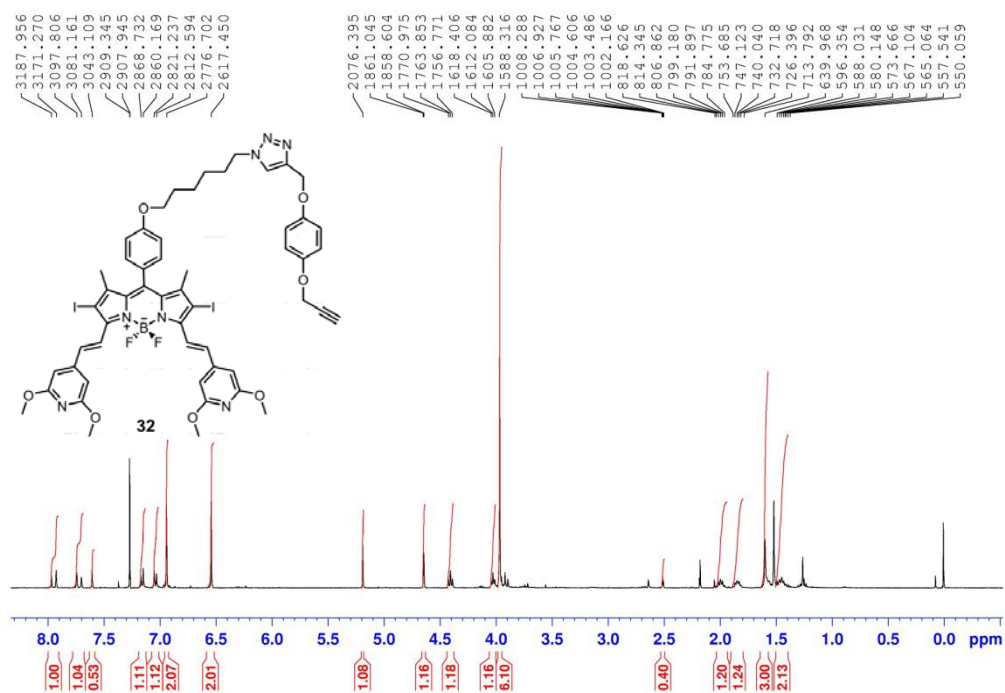
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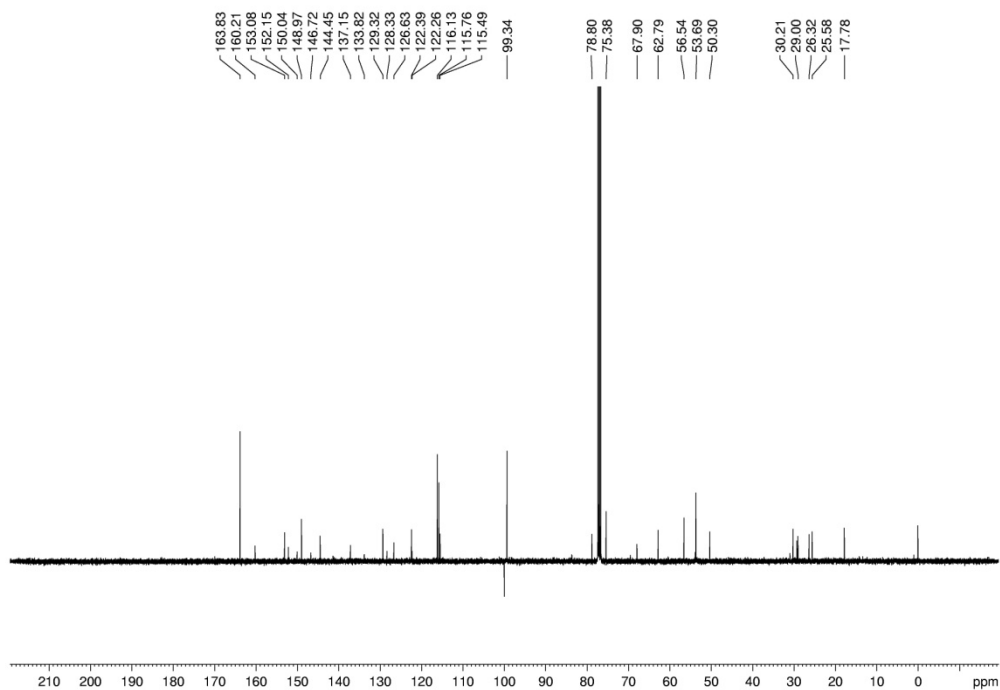
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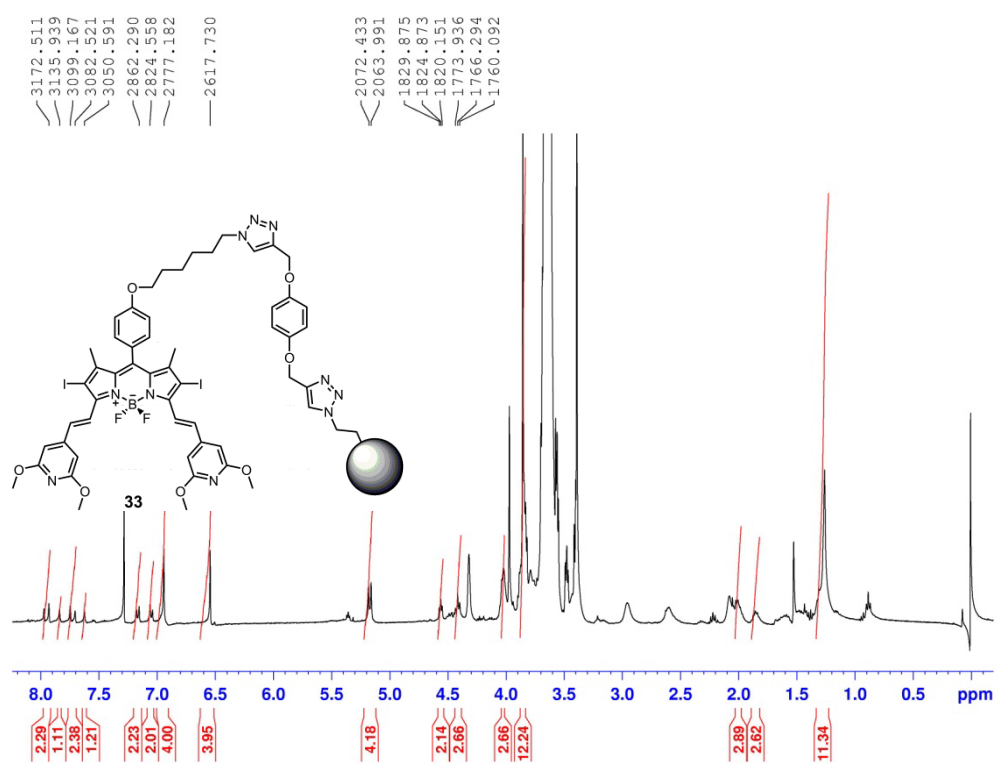
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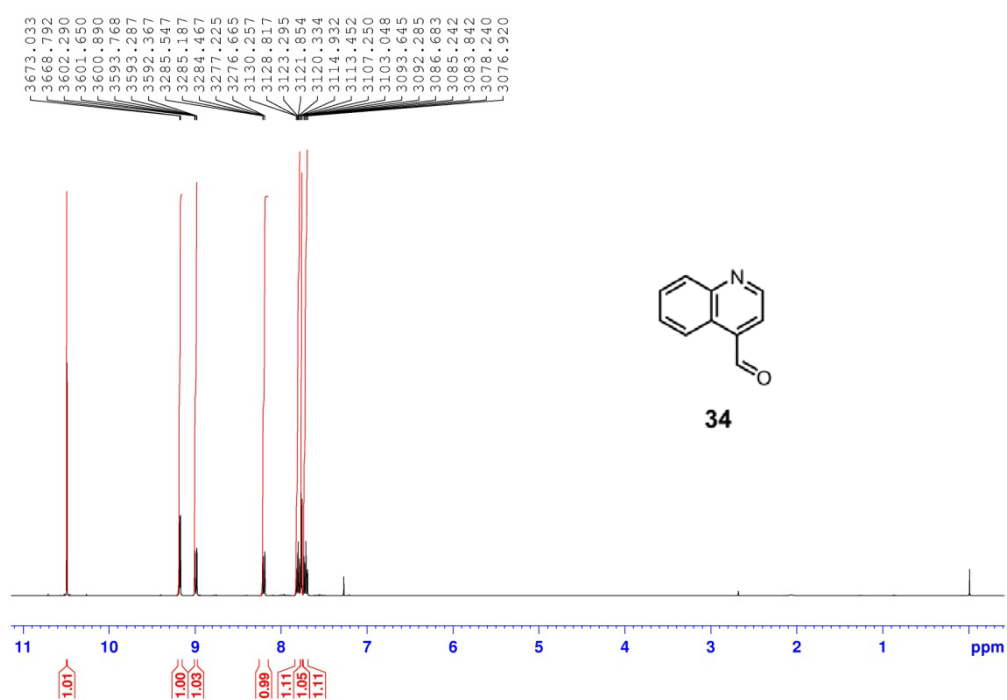
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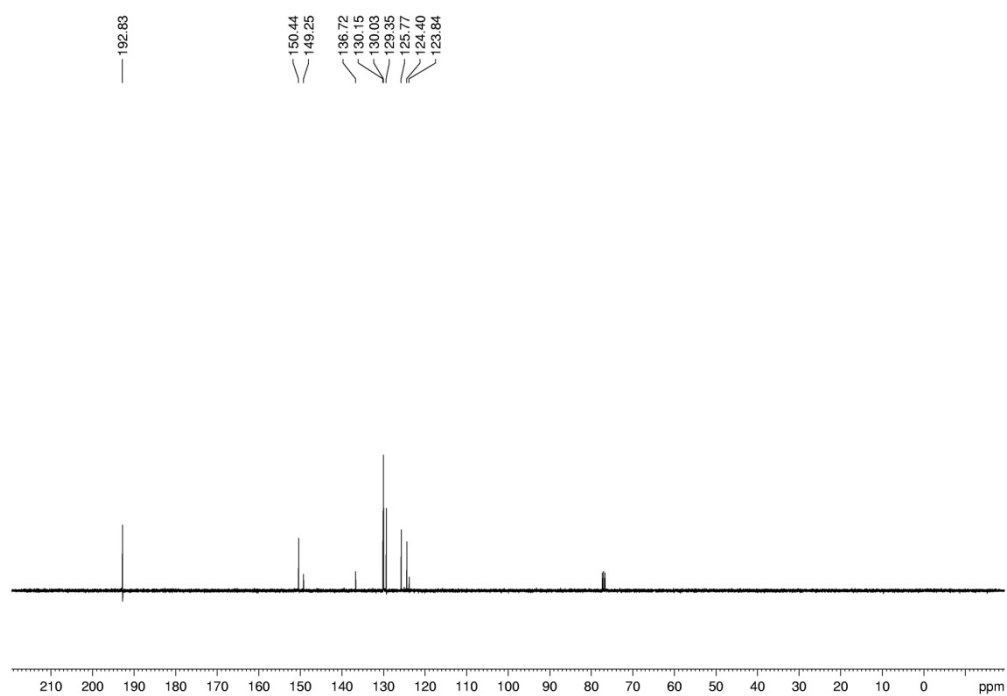
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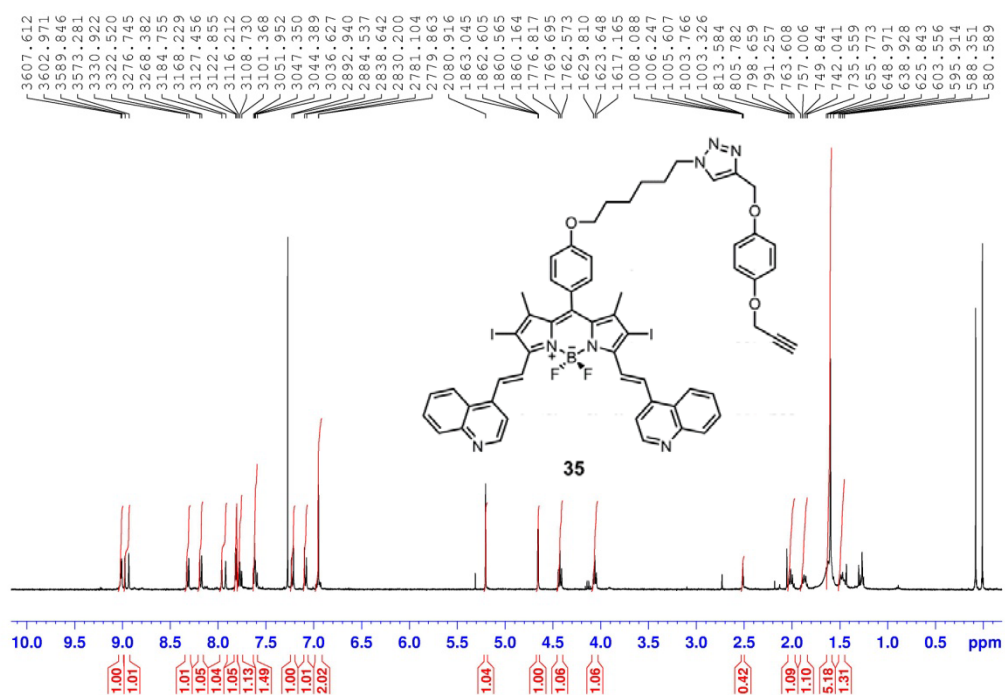
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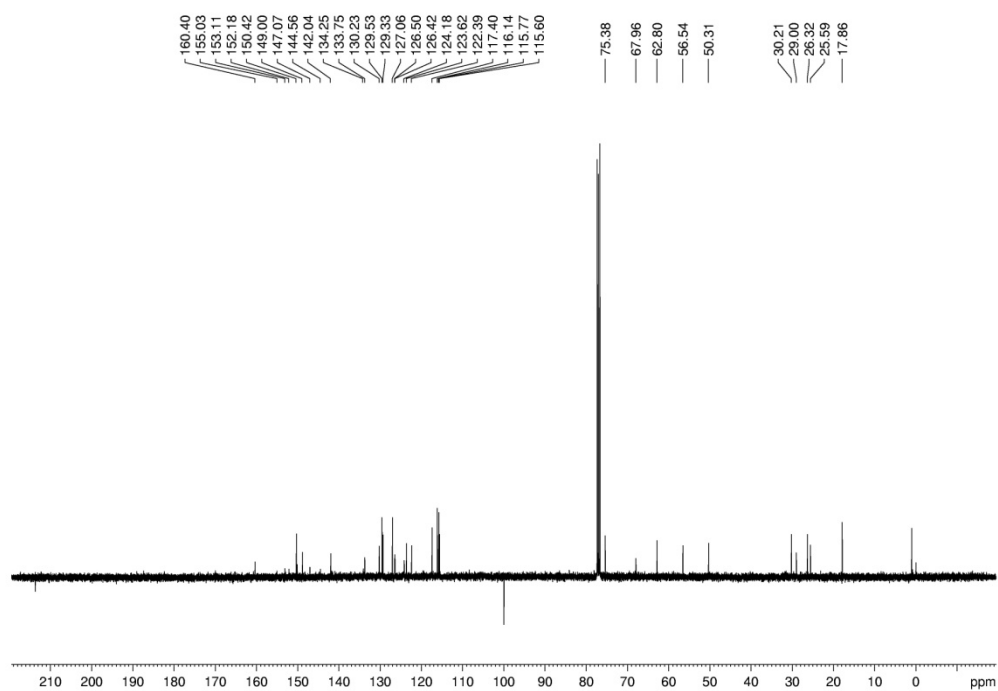
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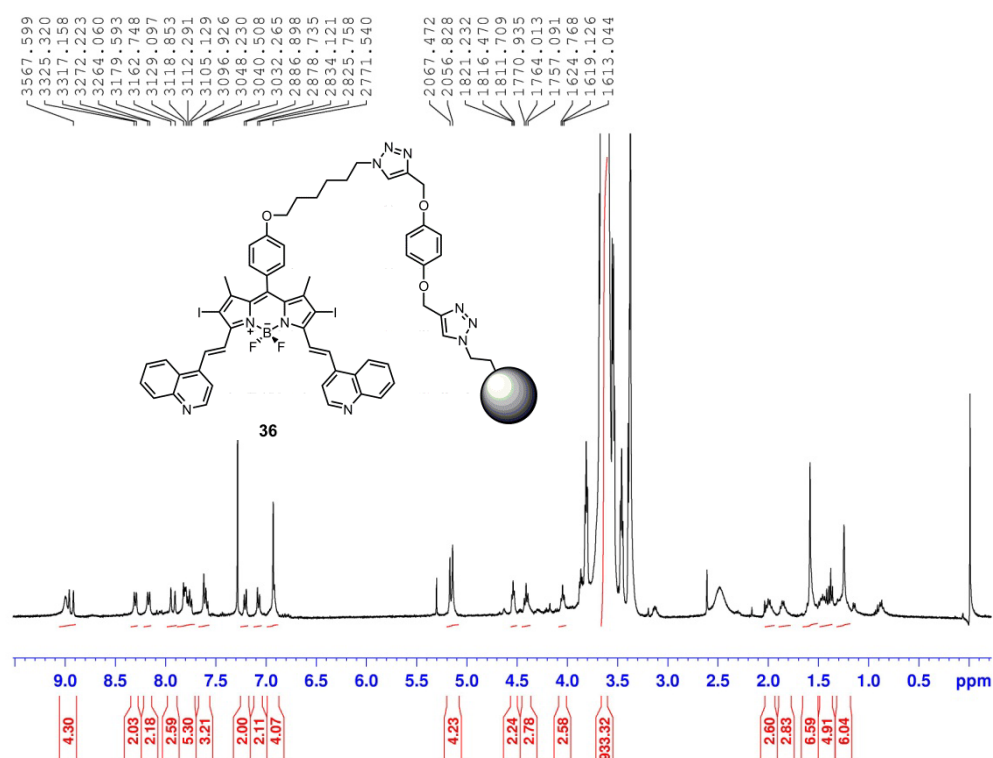
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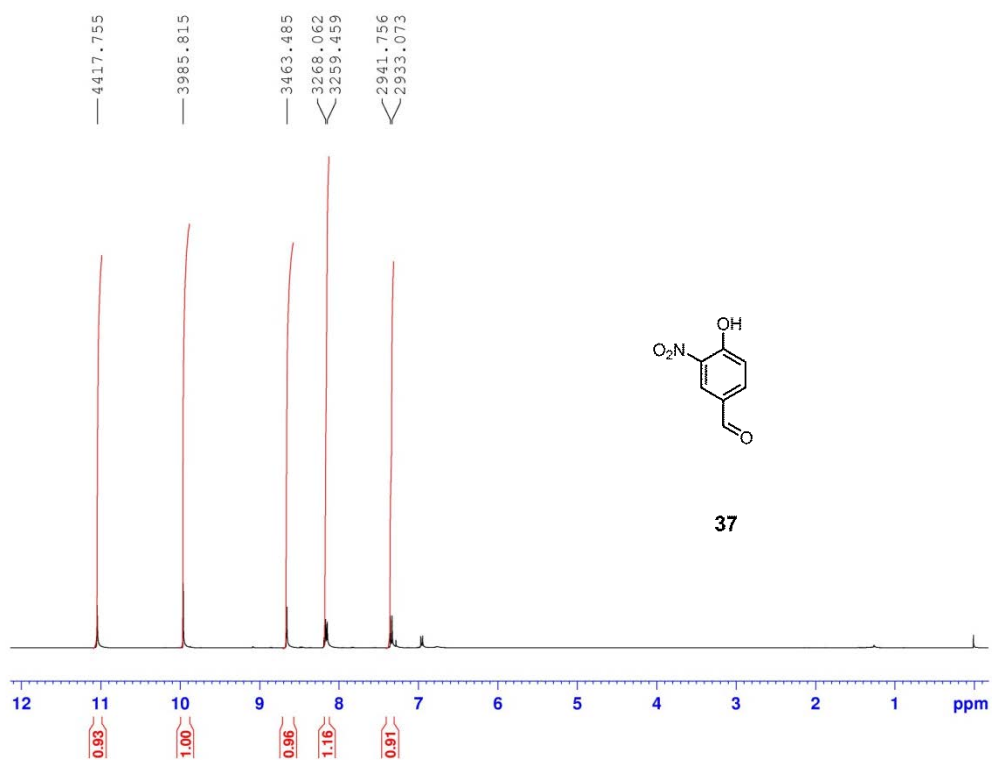
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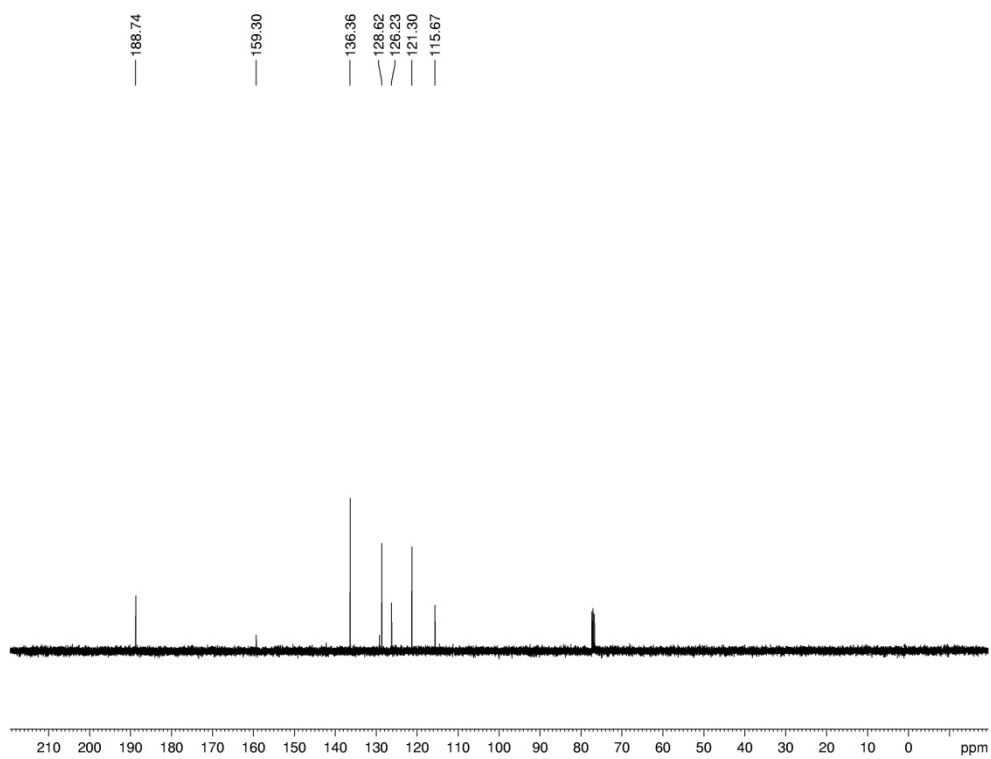
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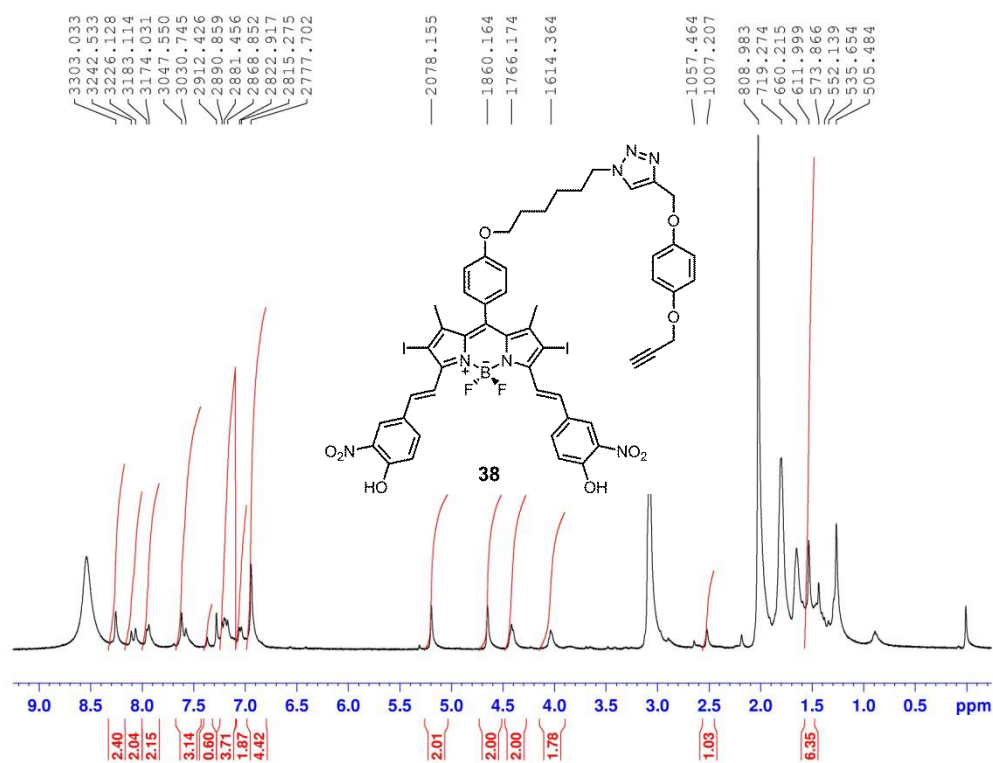
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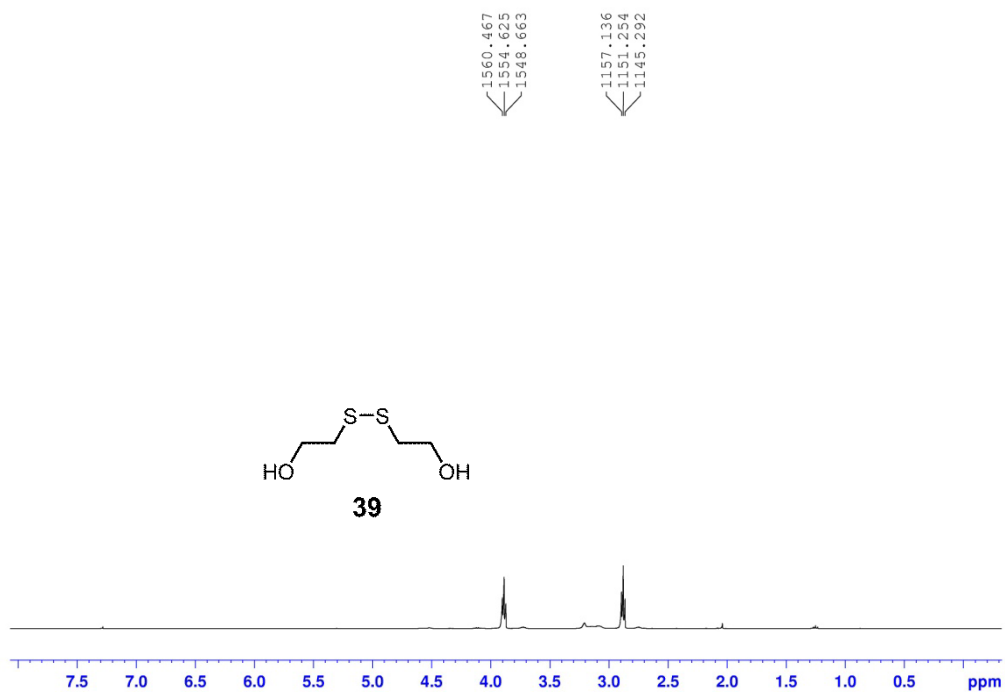
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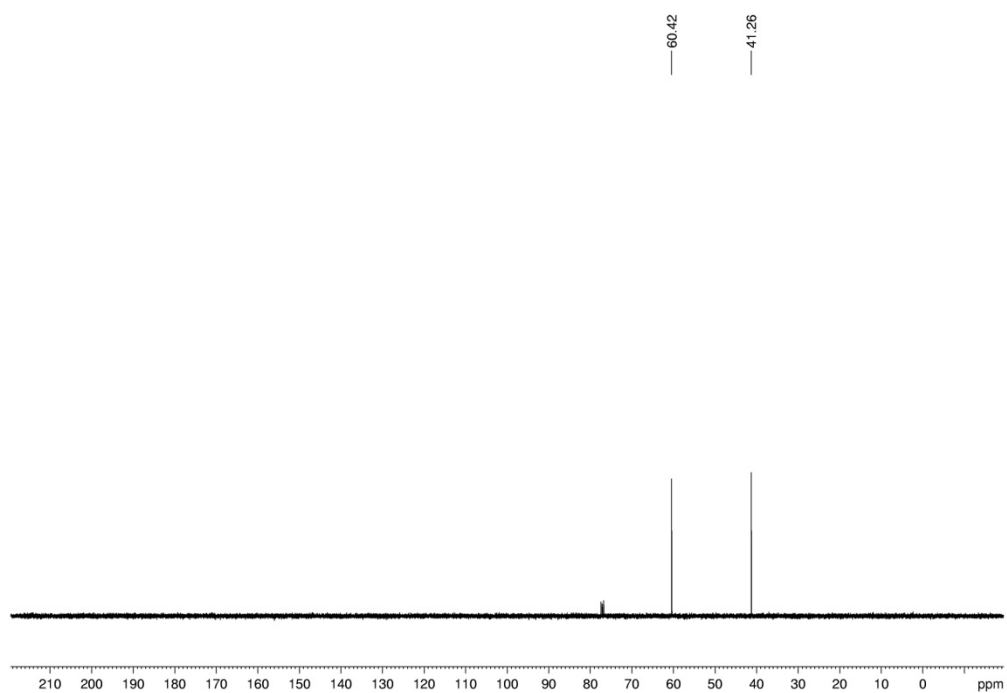
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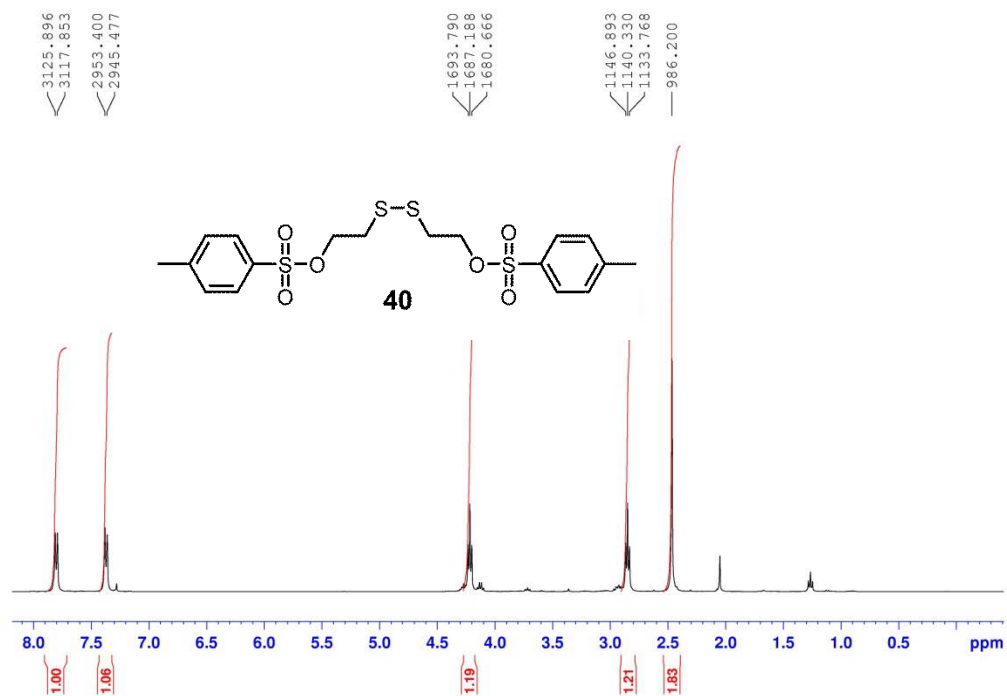
¹H NMR of compound **38** + AcOH



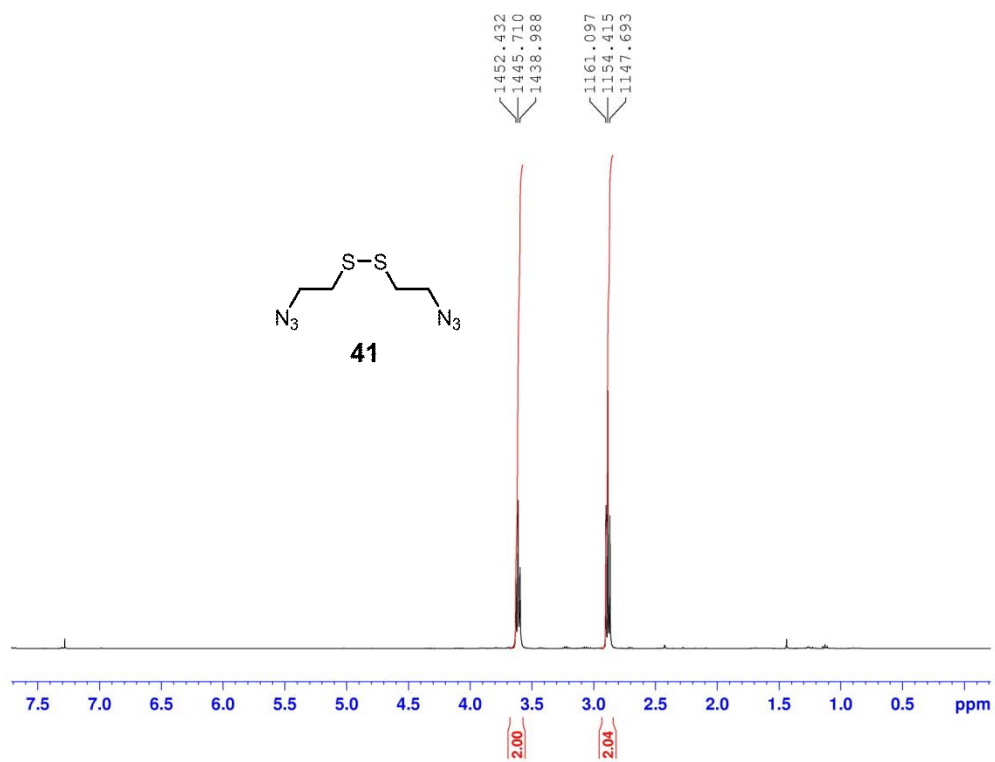
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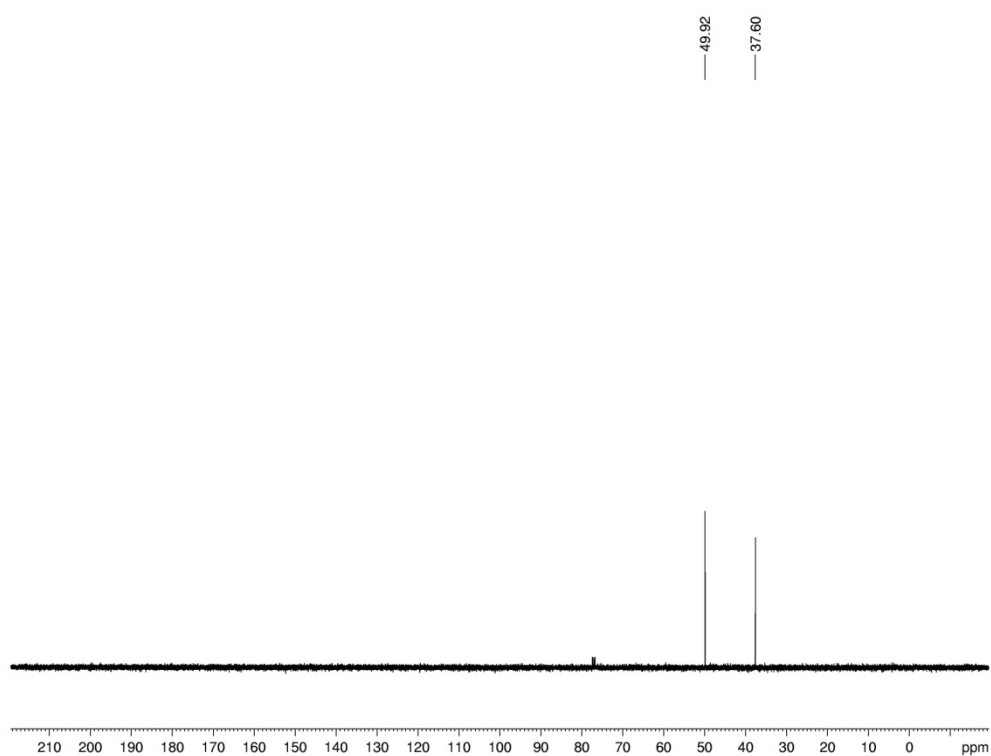
^{13}C NMR of compound **39**



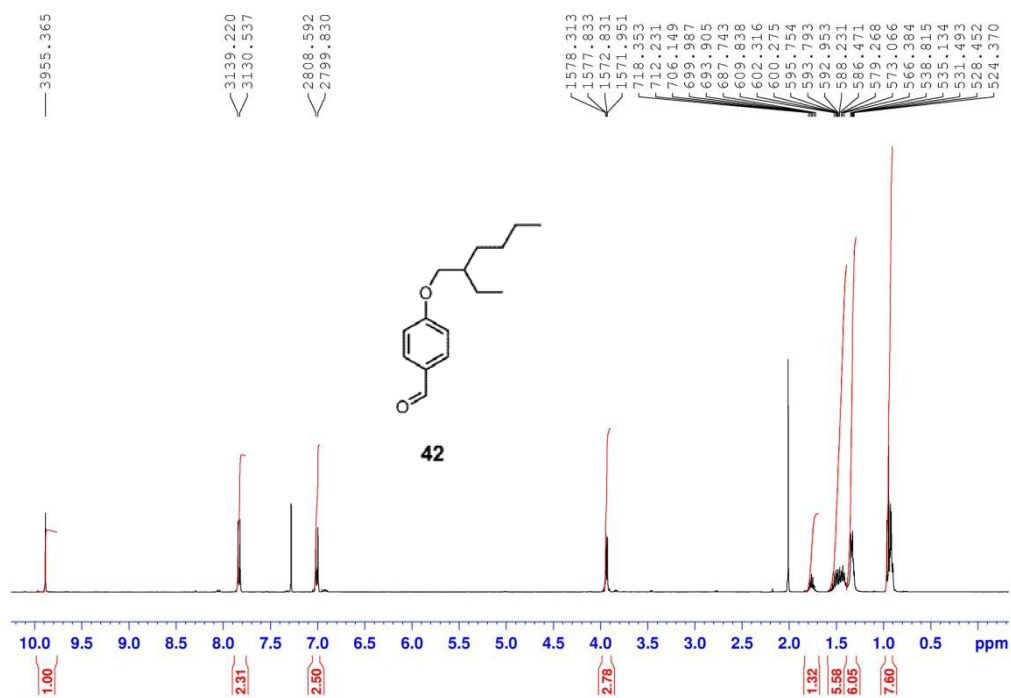
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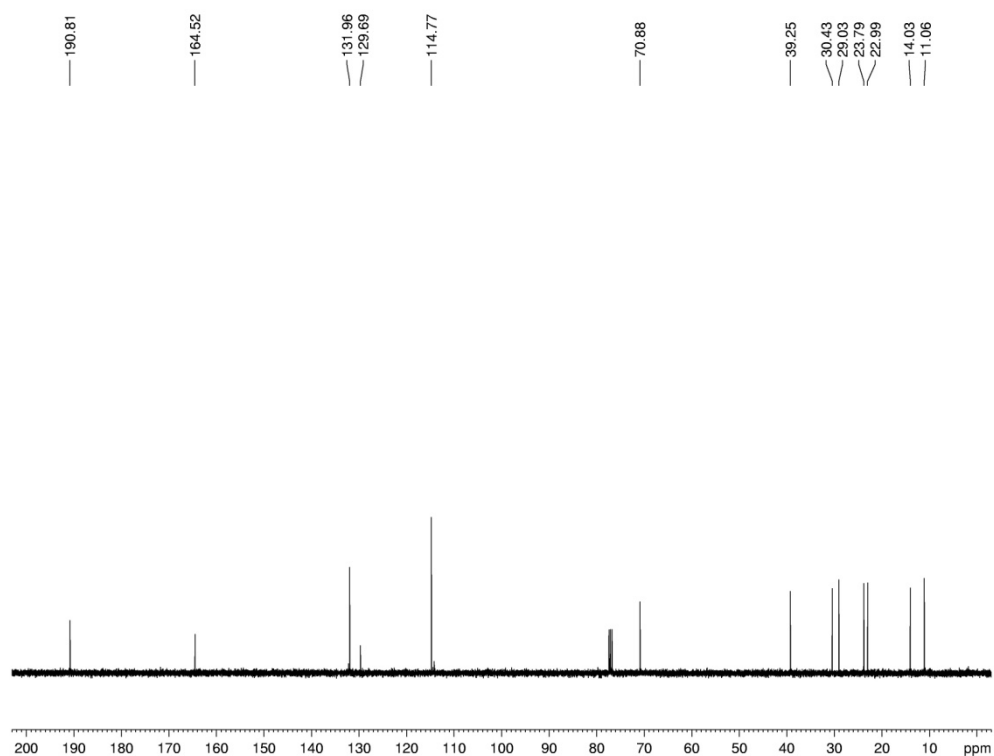
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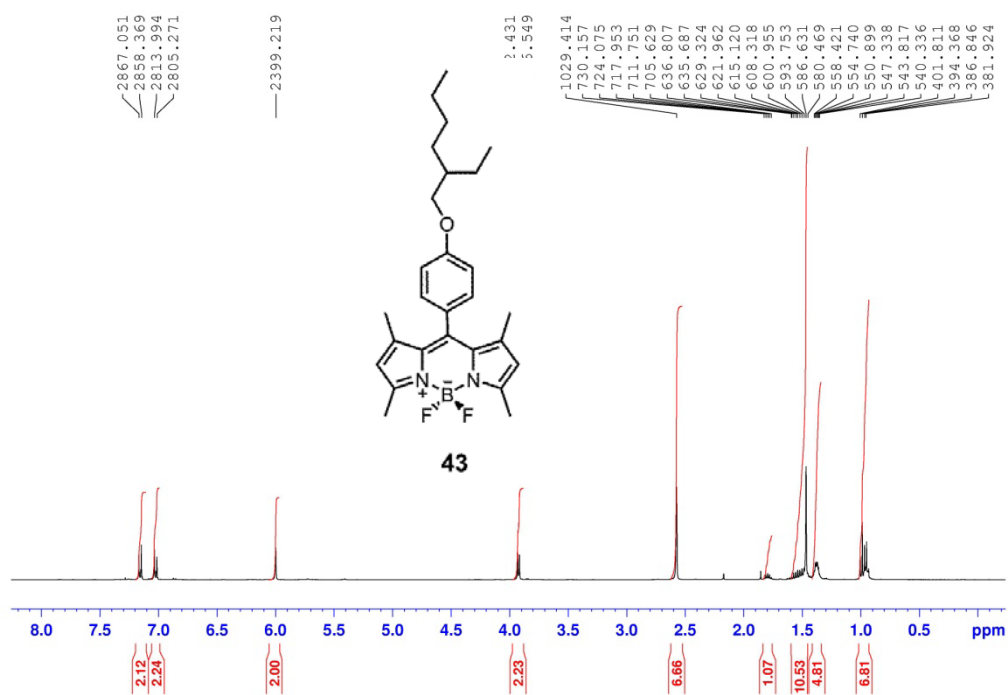
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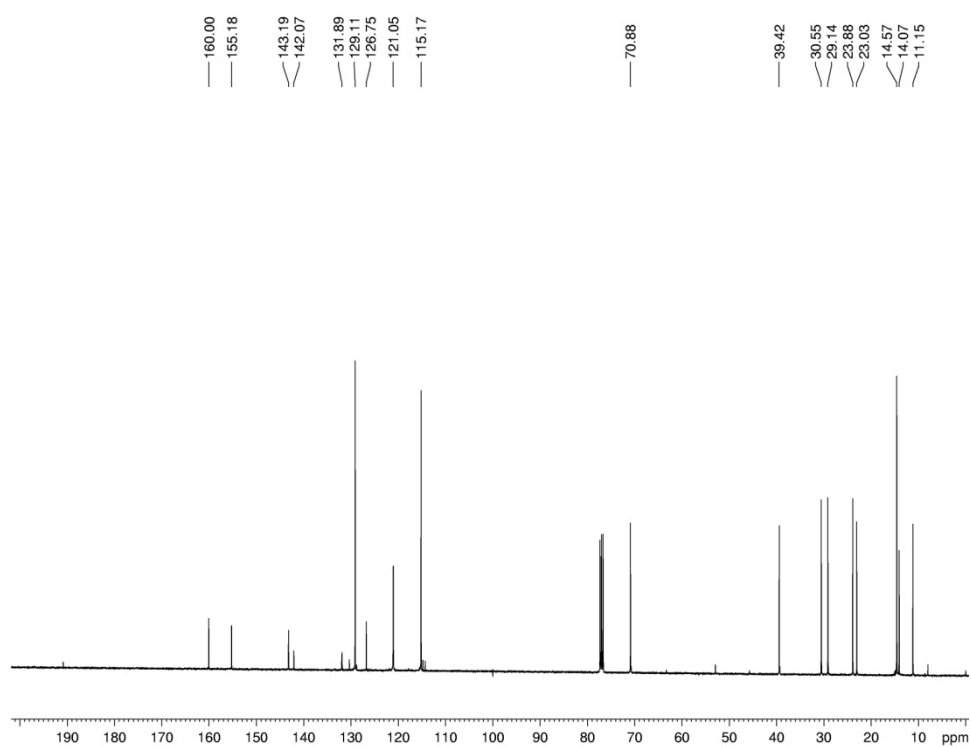
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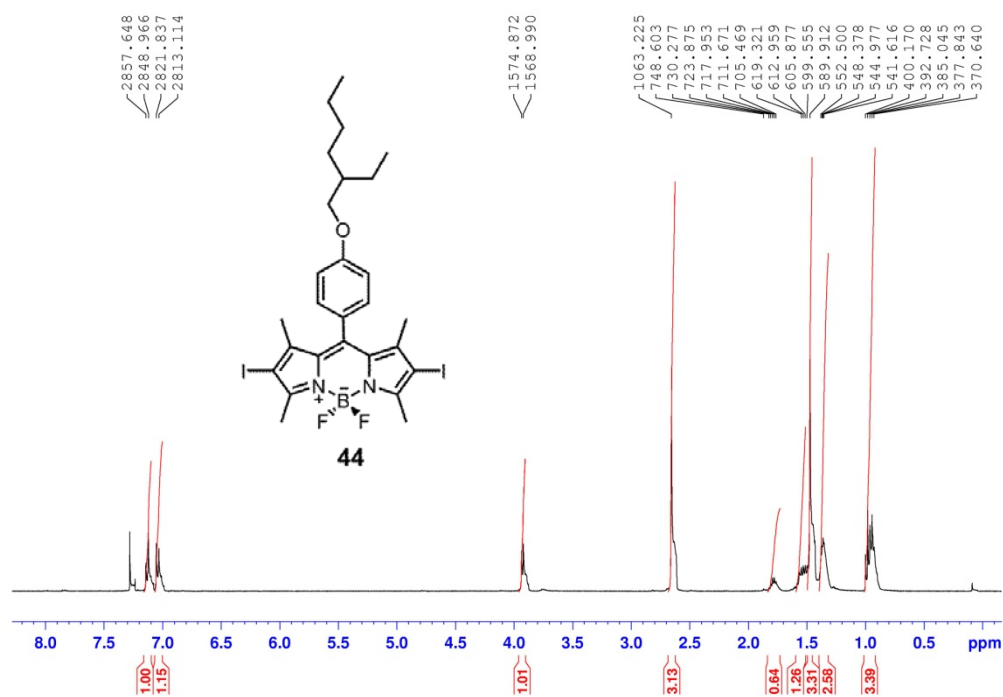
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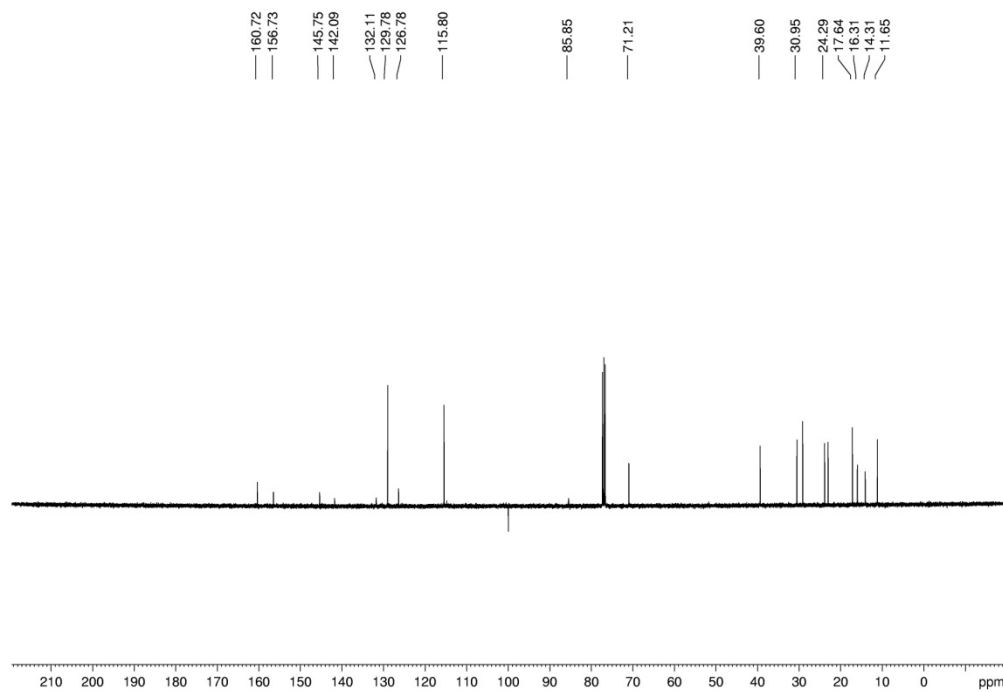
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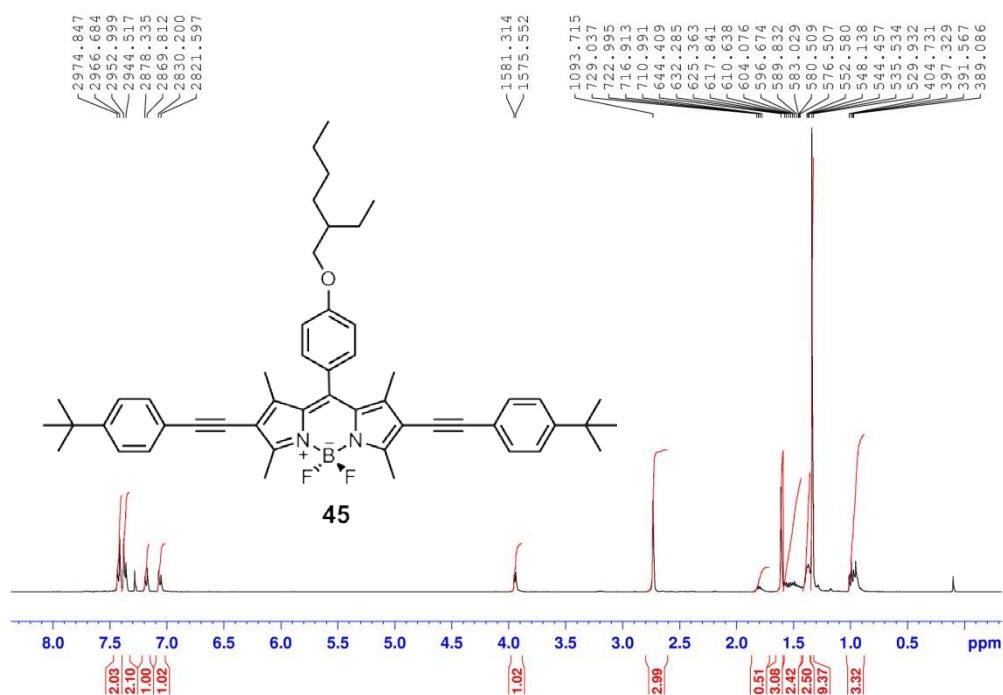
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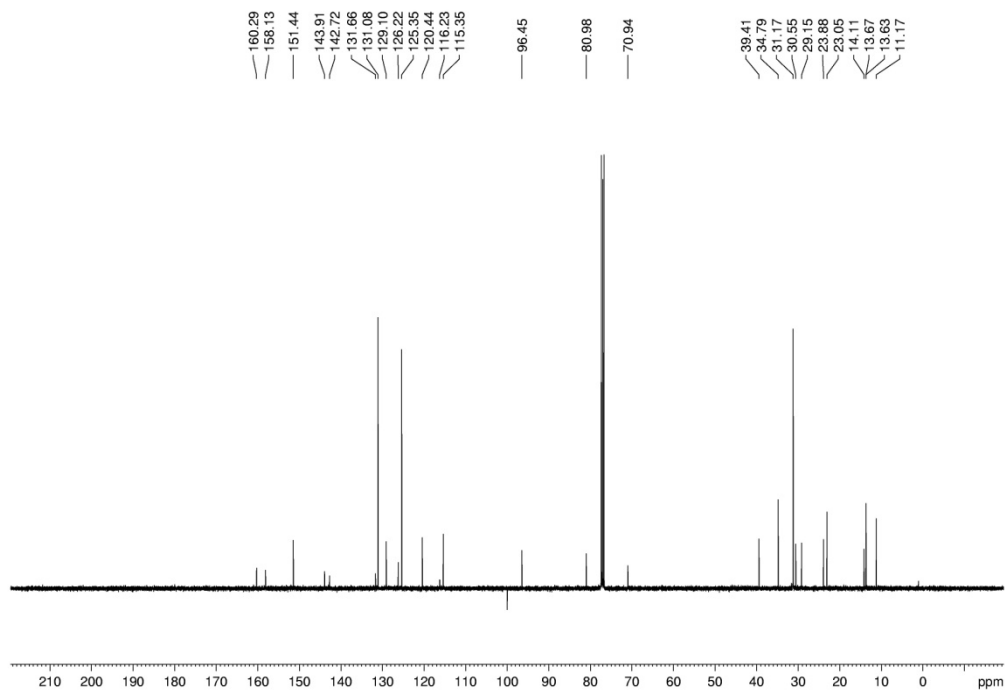
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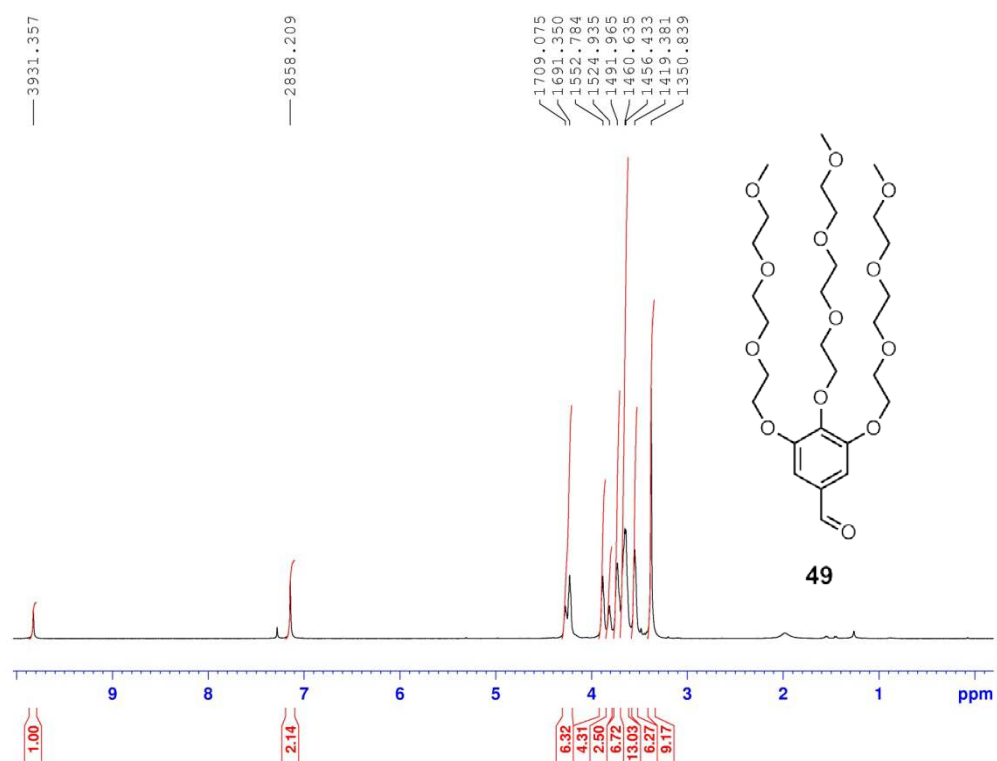
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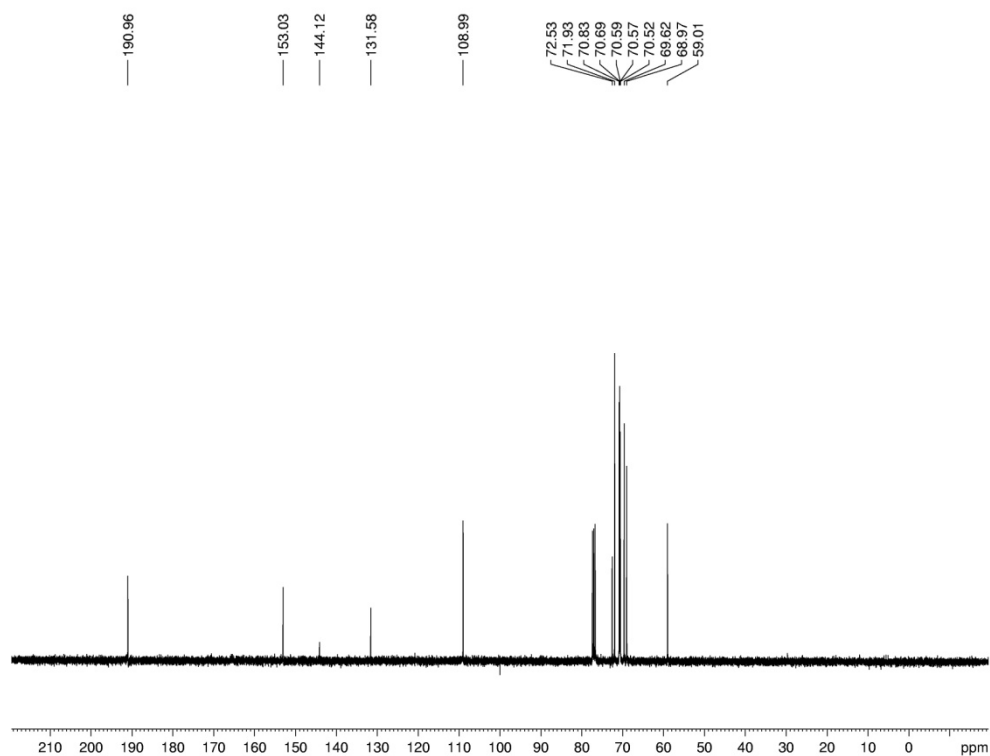
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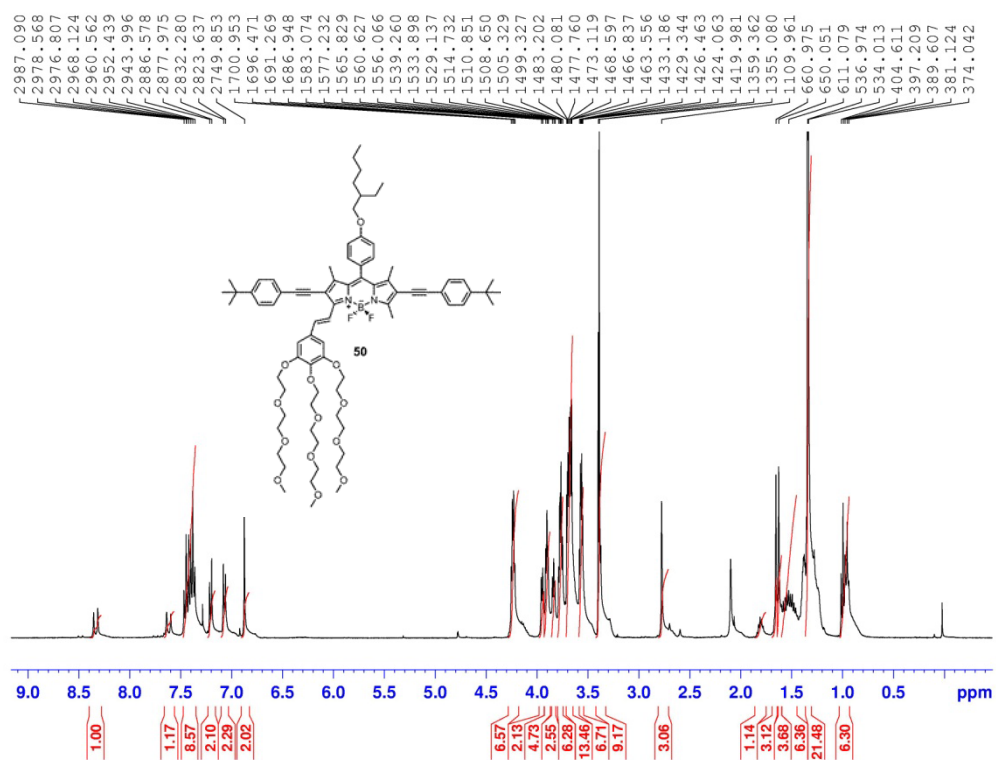
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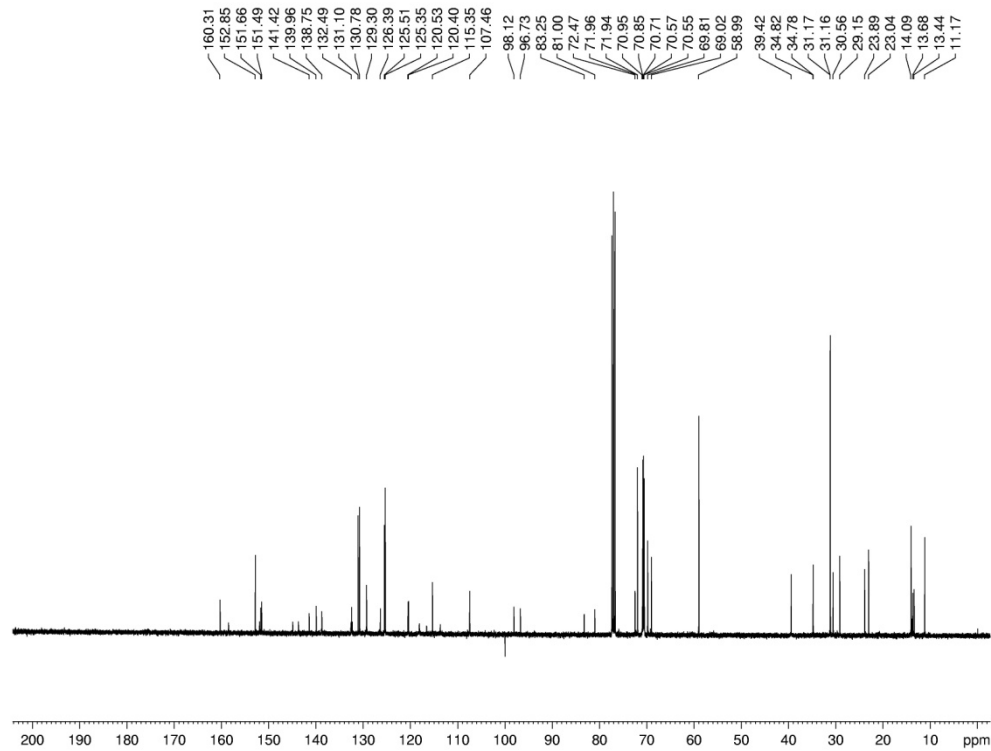
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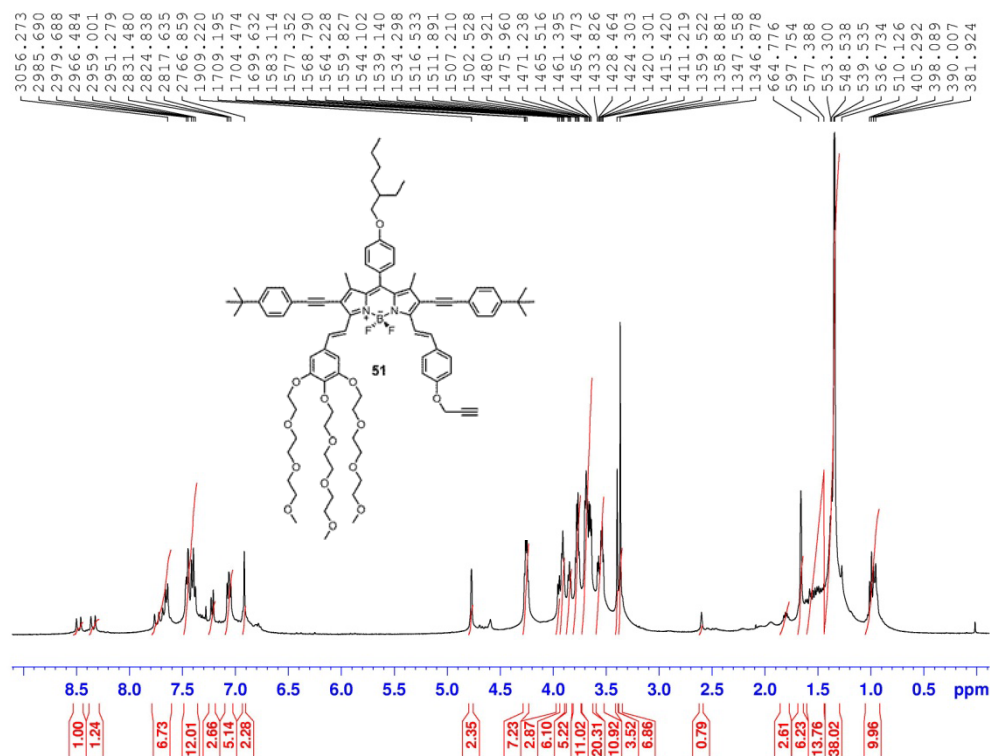
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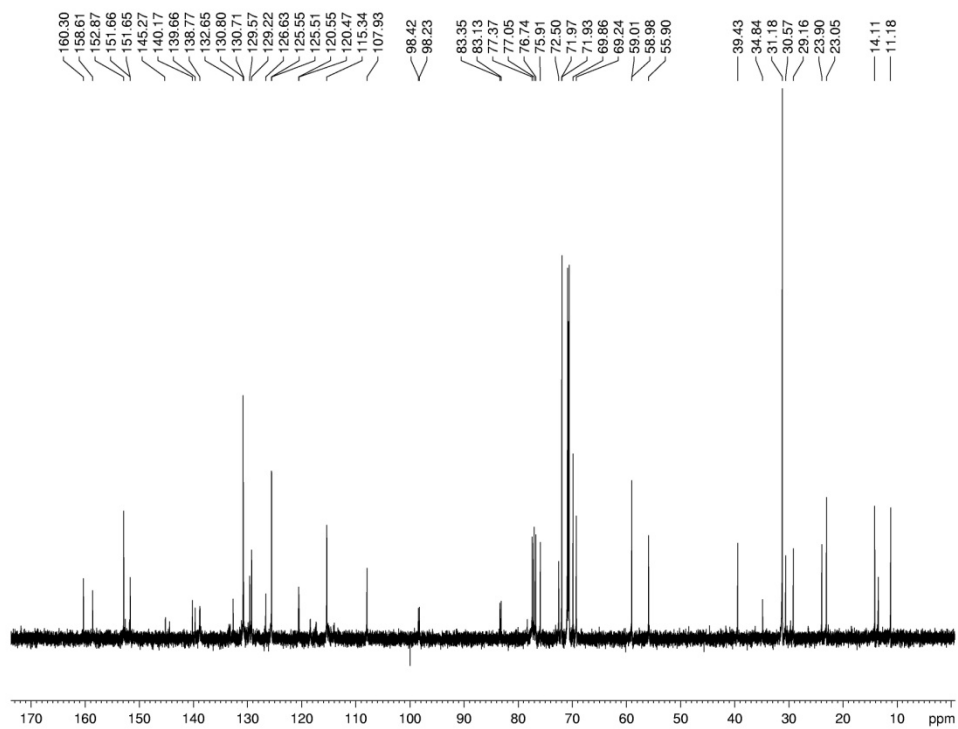
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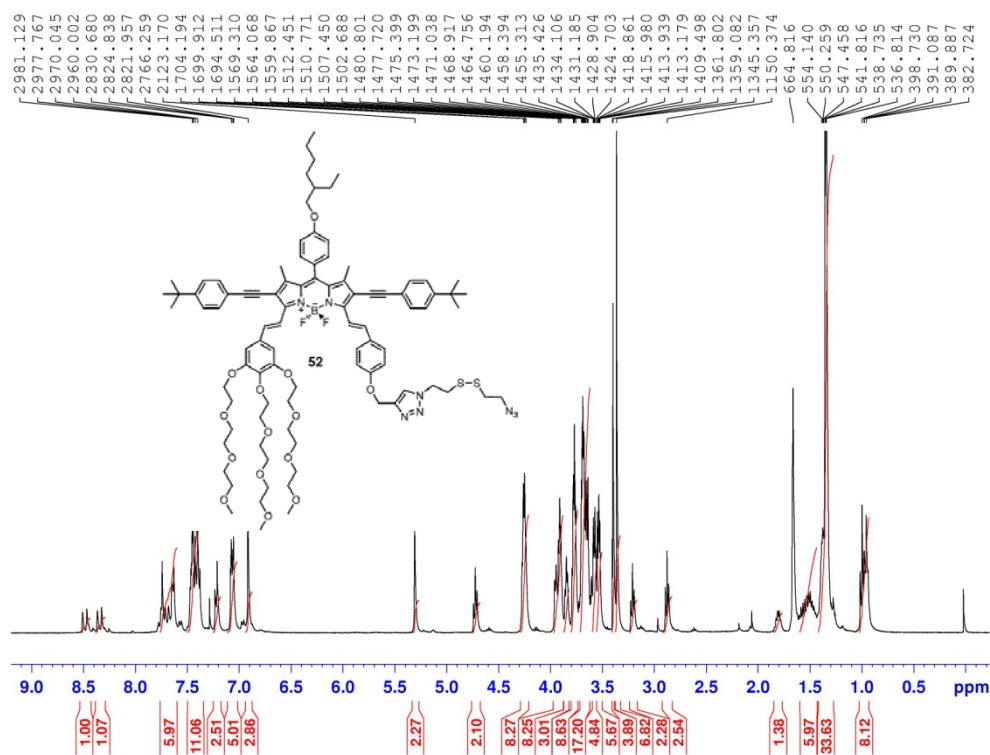
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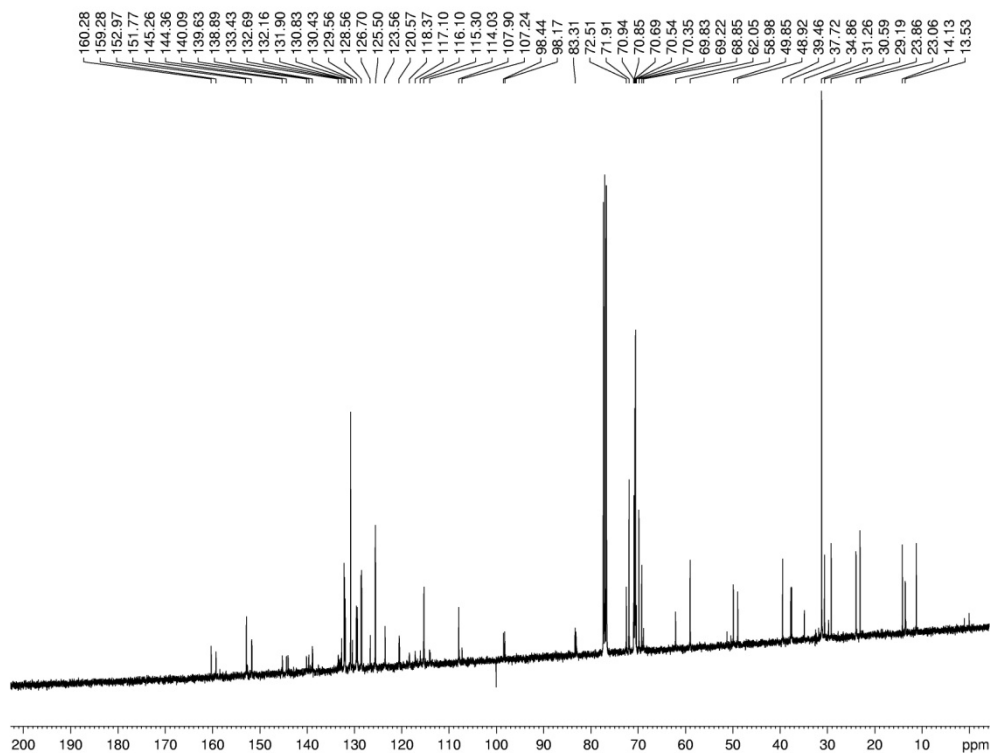
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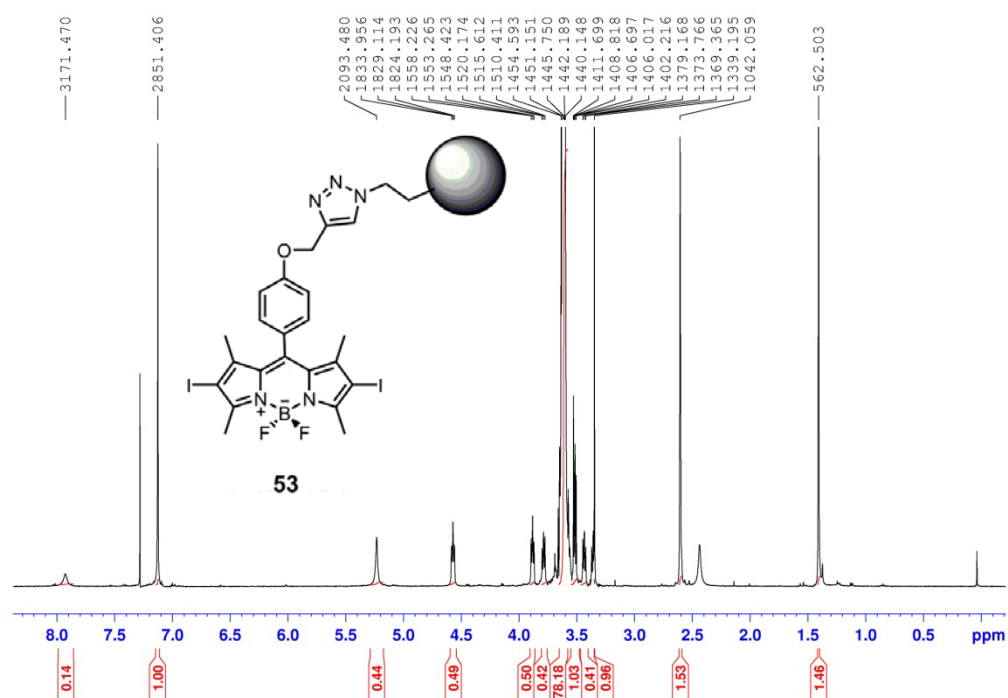
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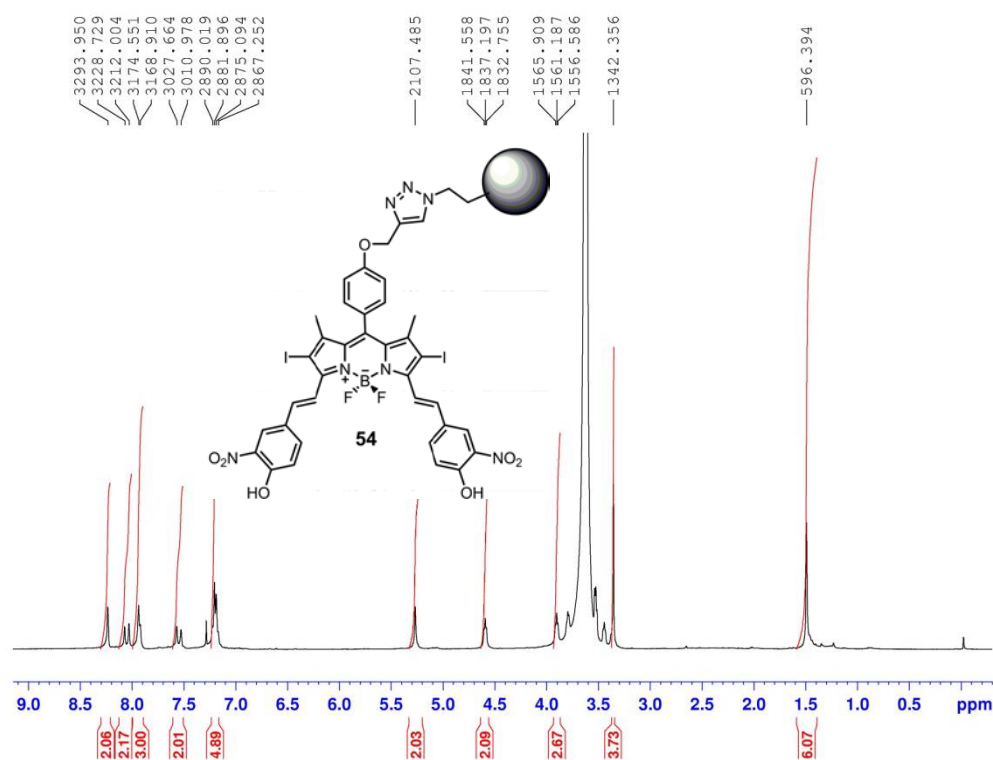
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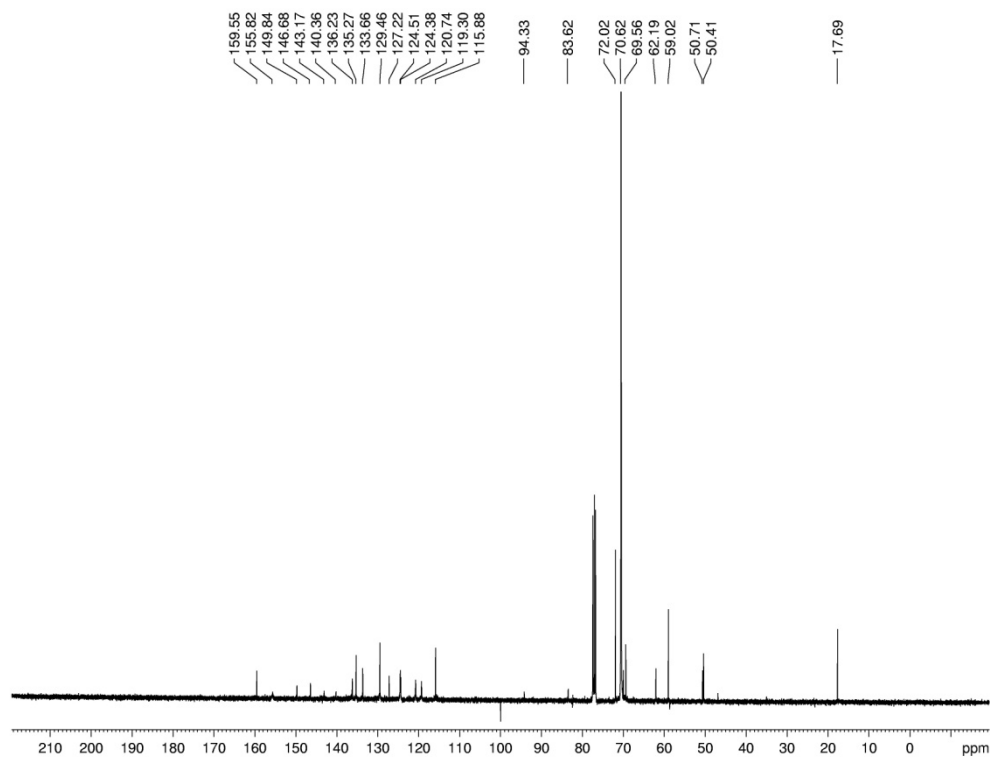
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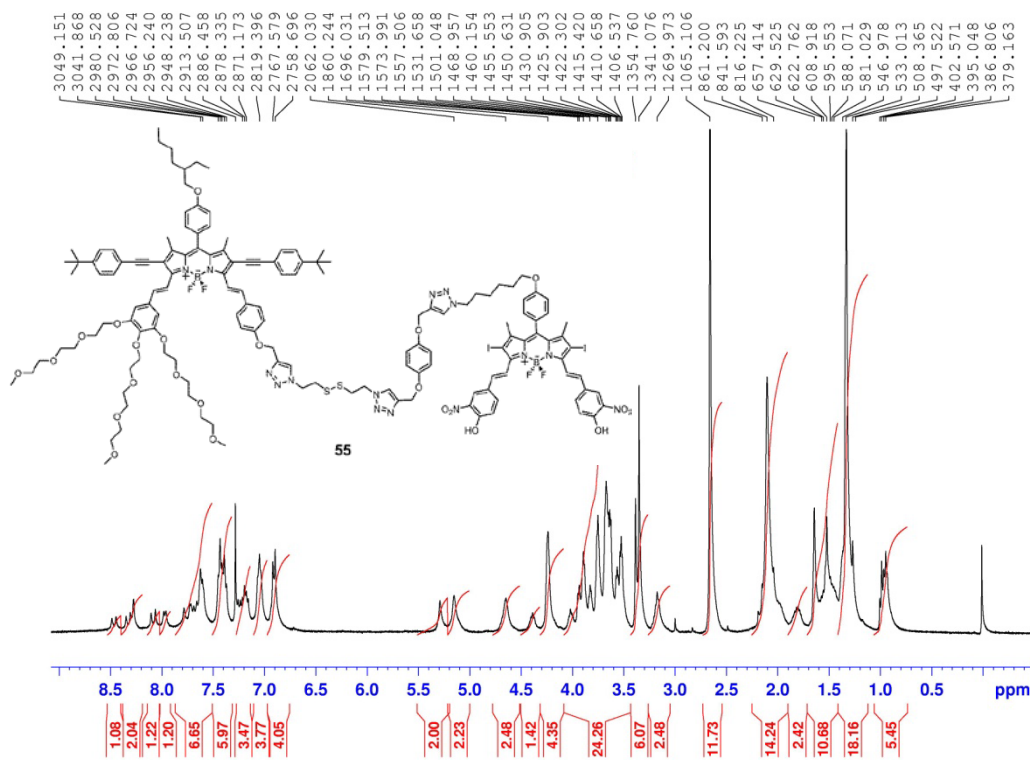
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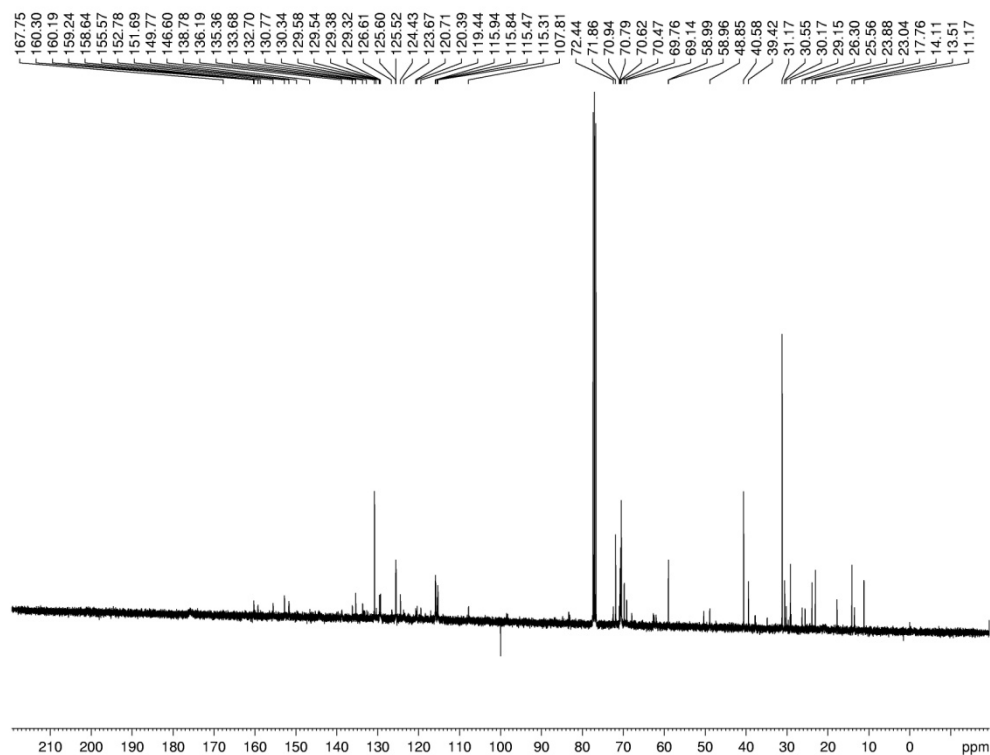
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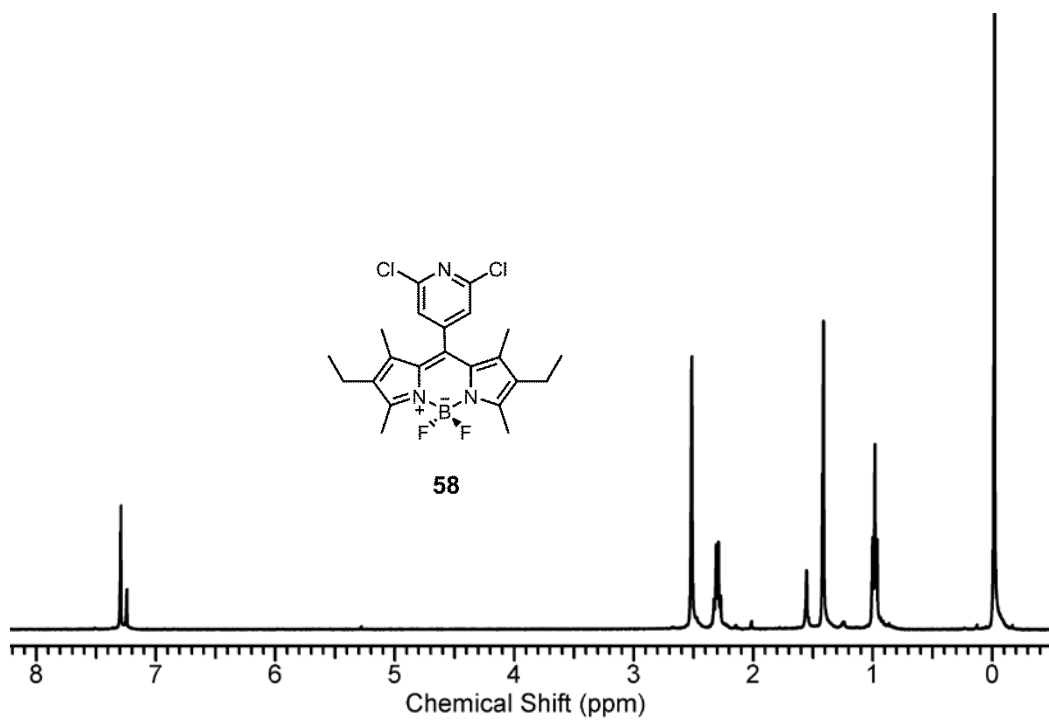
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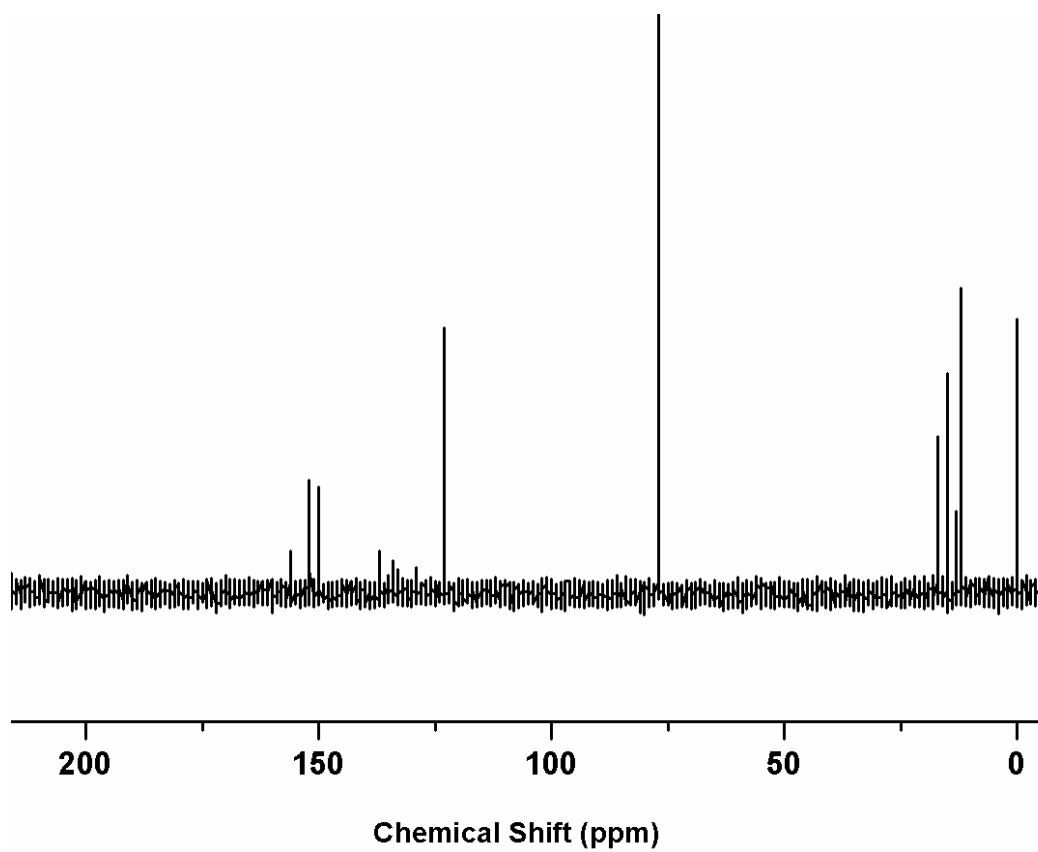
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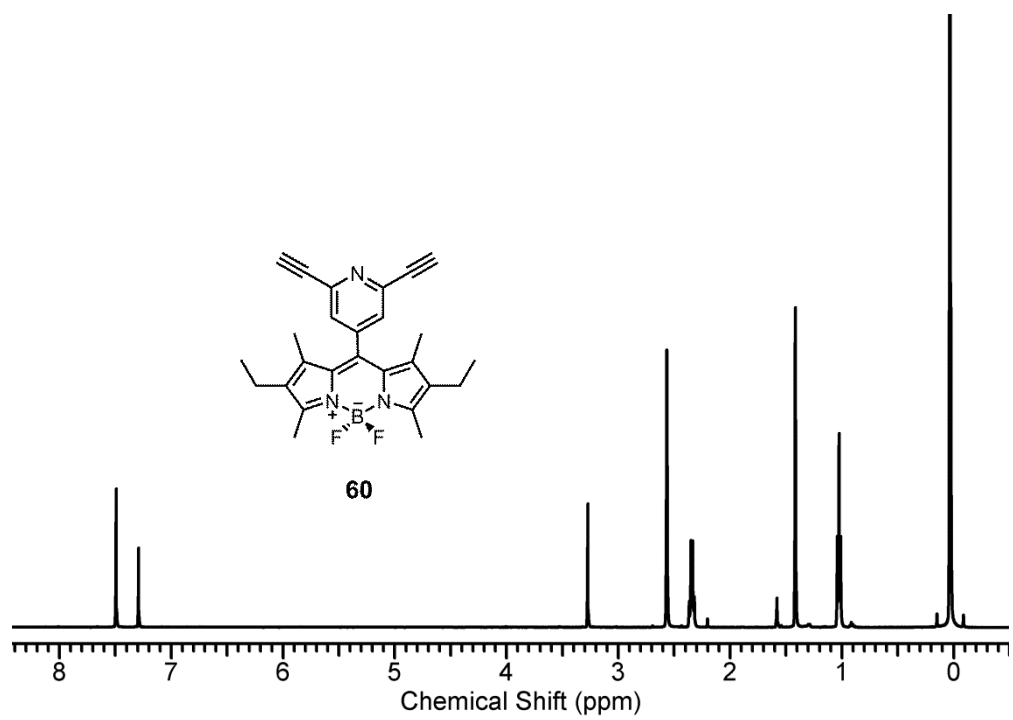
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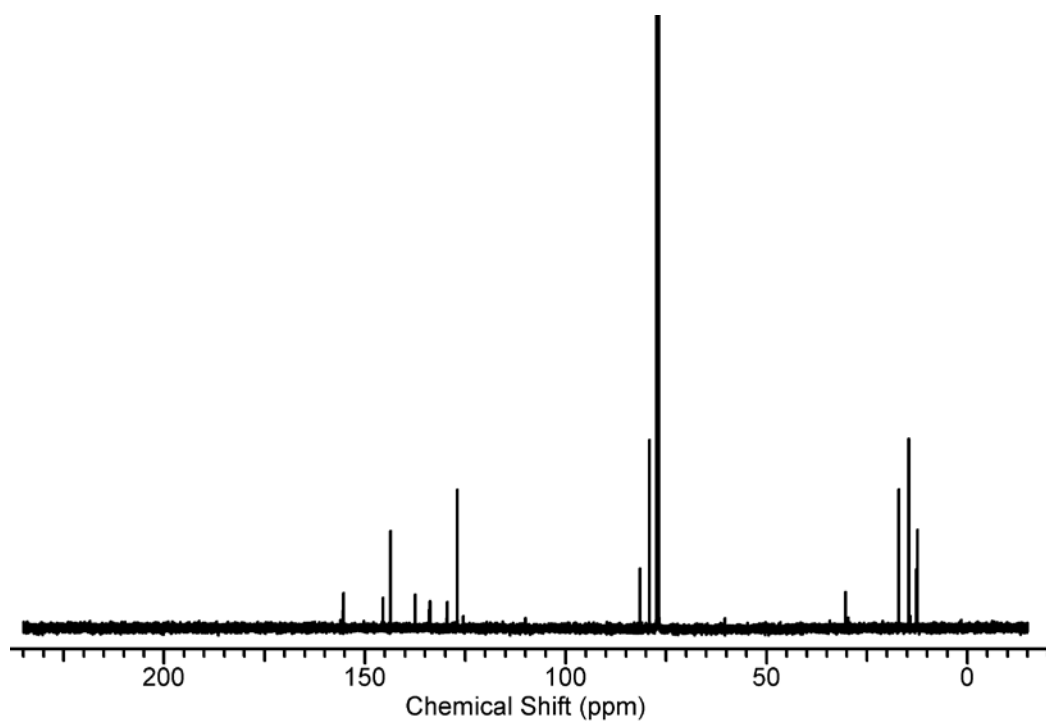
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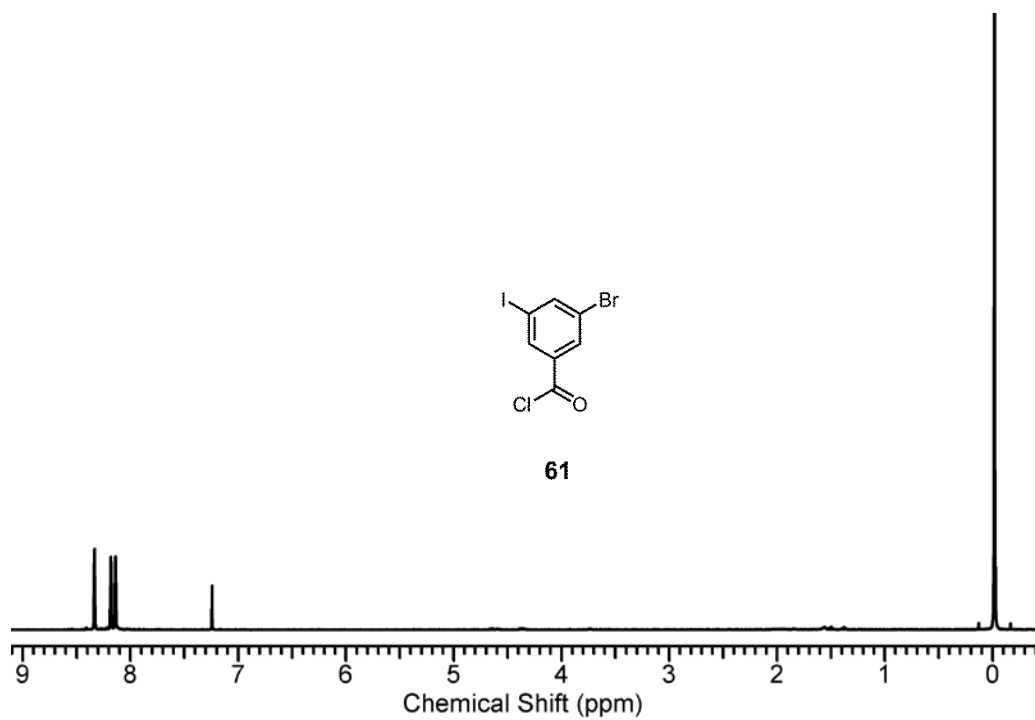
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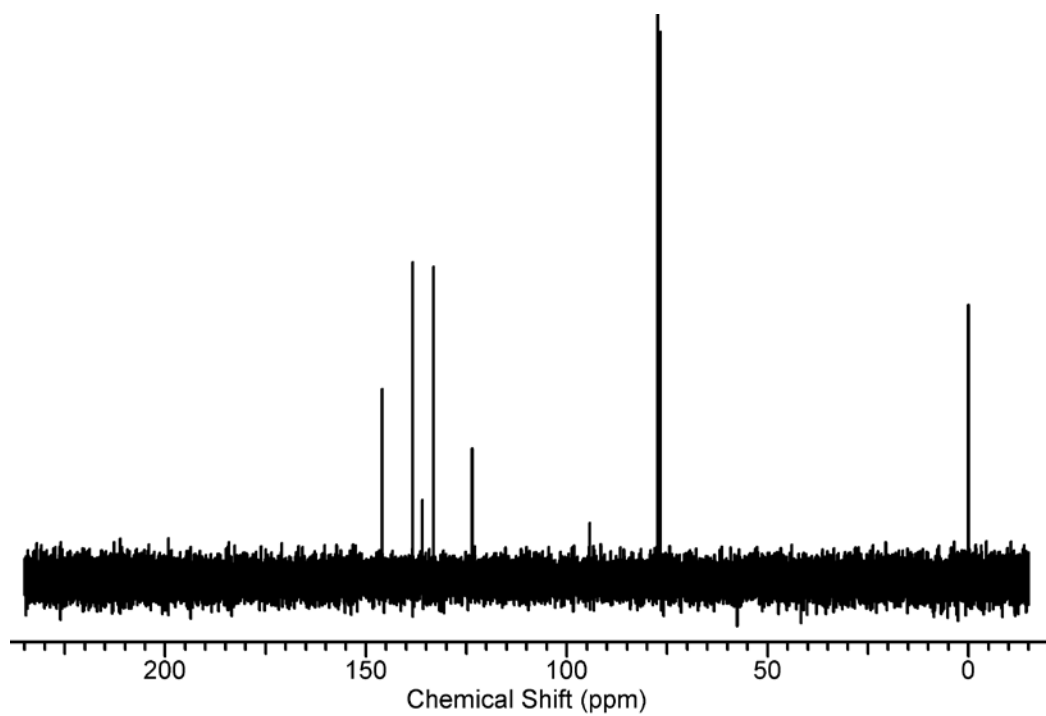
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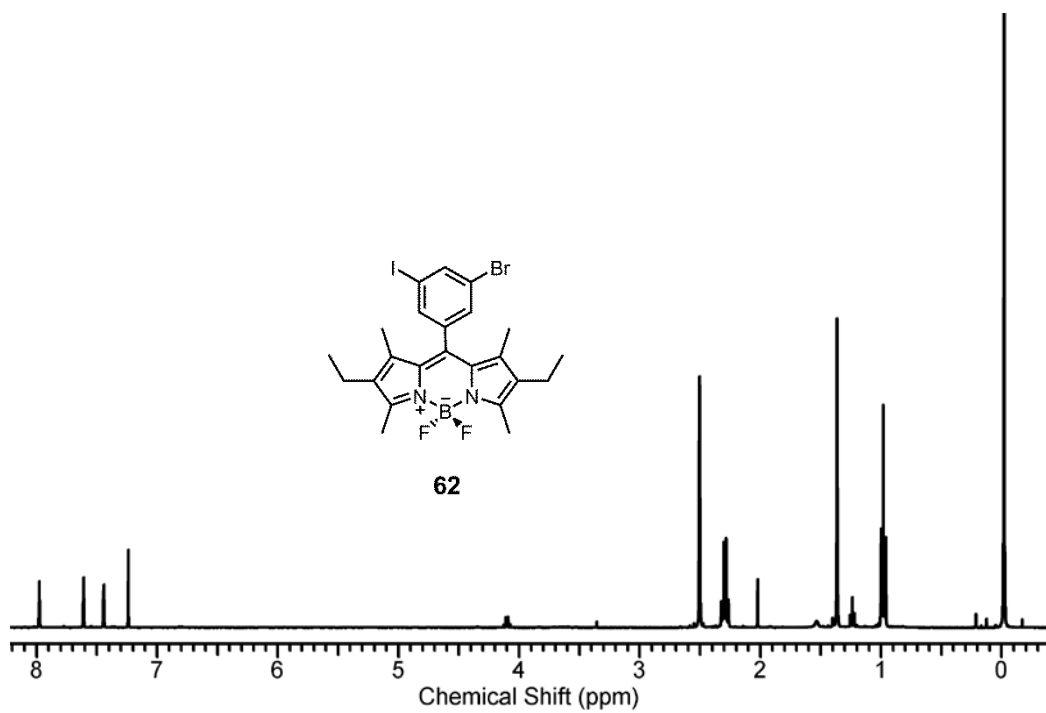
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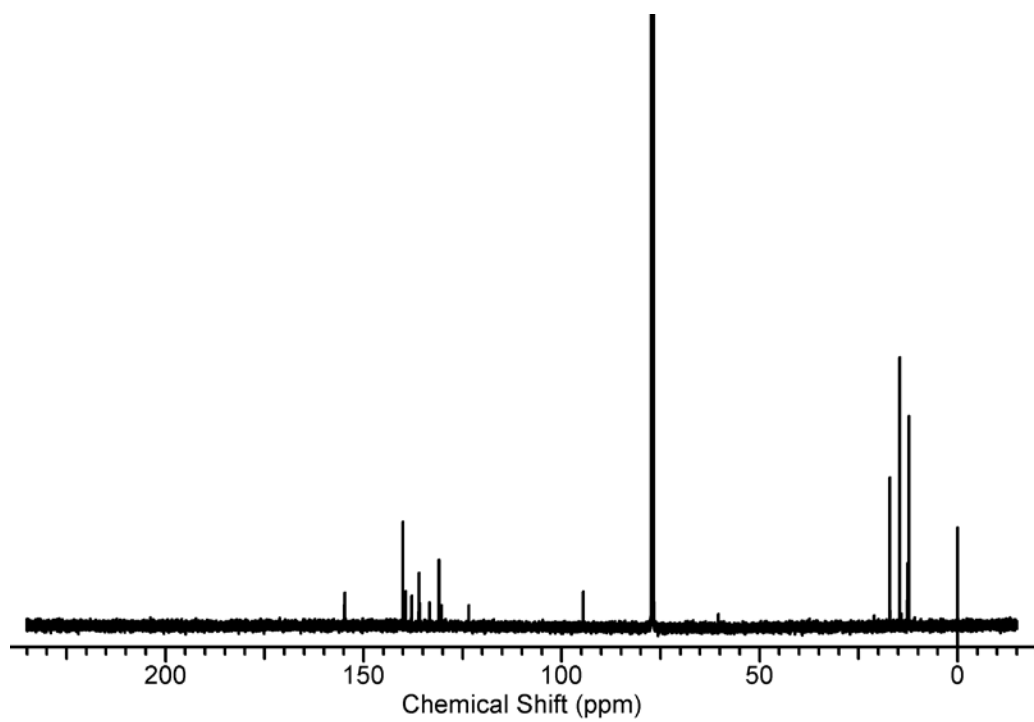
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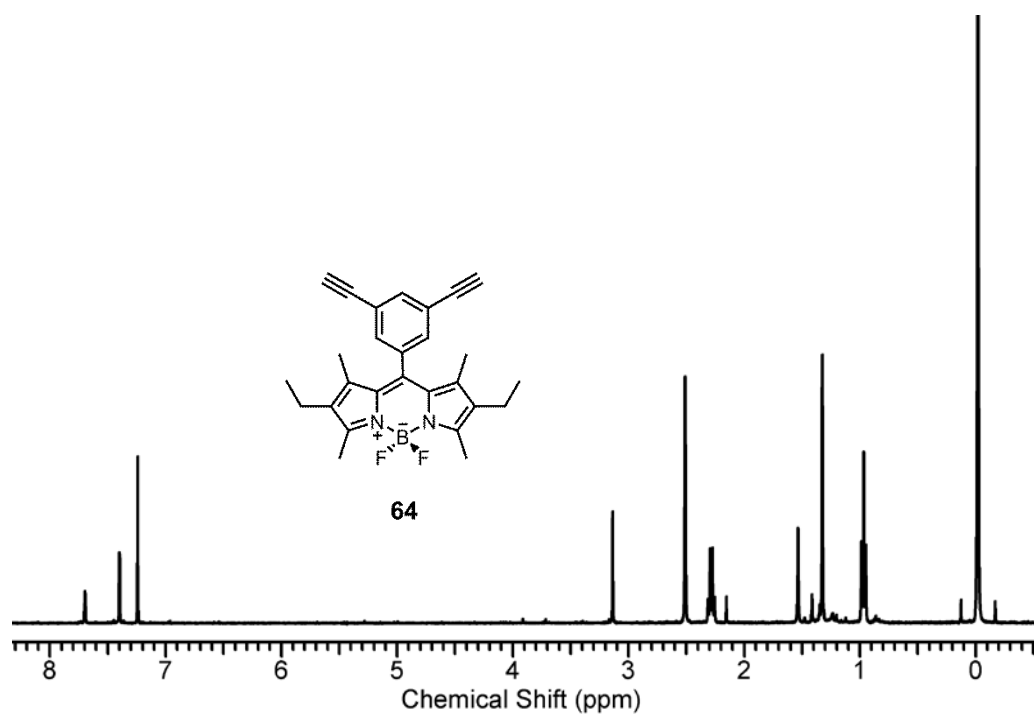
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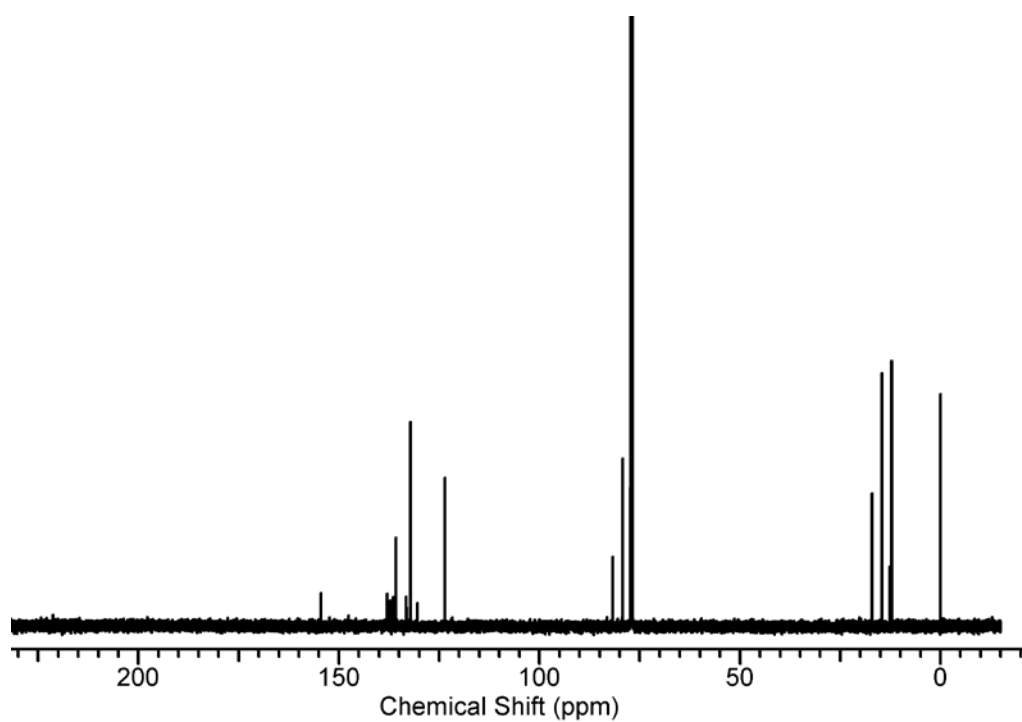
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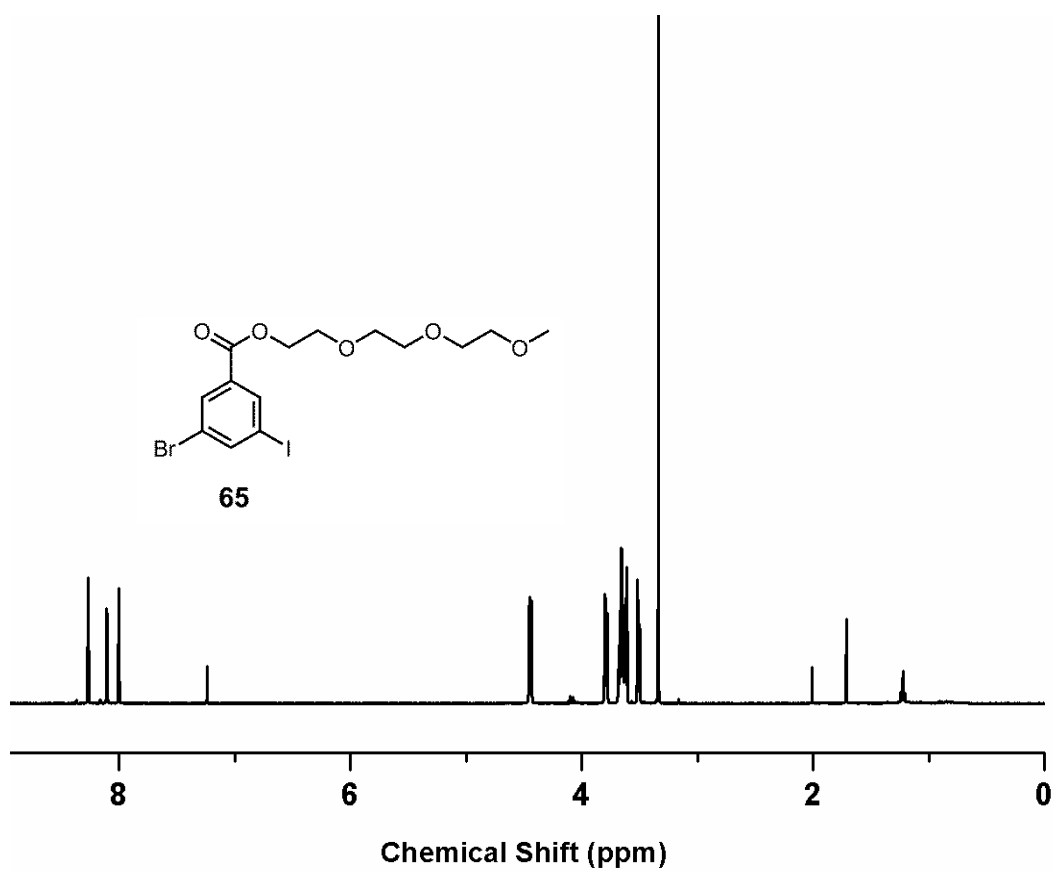
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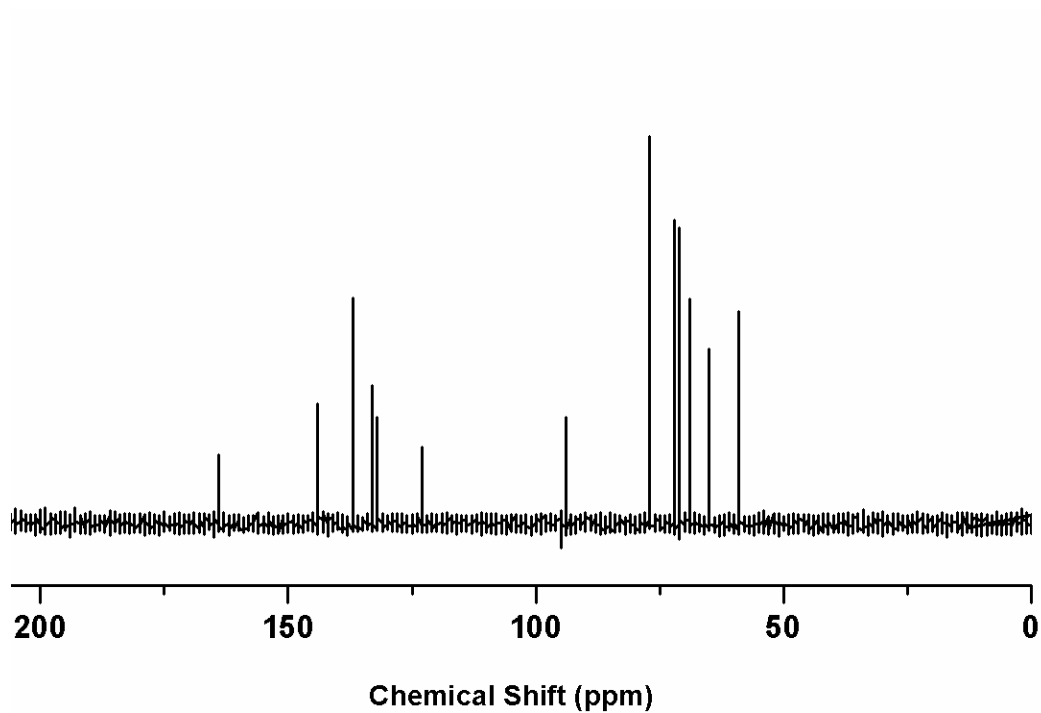
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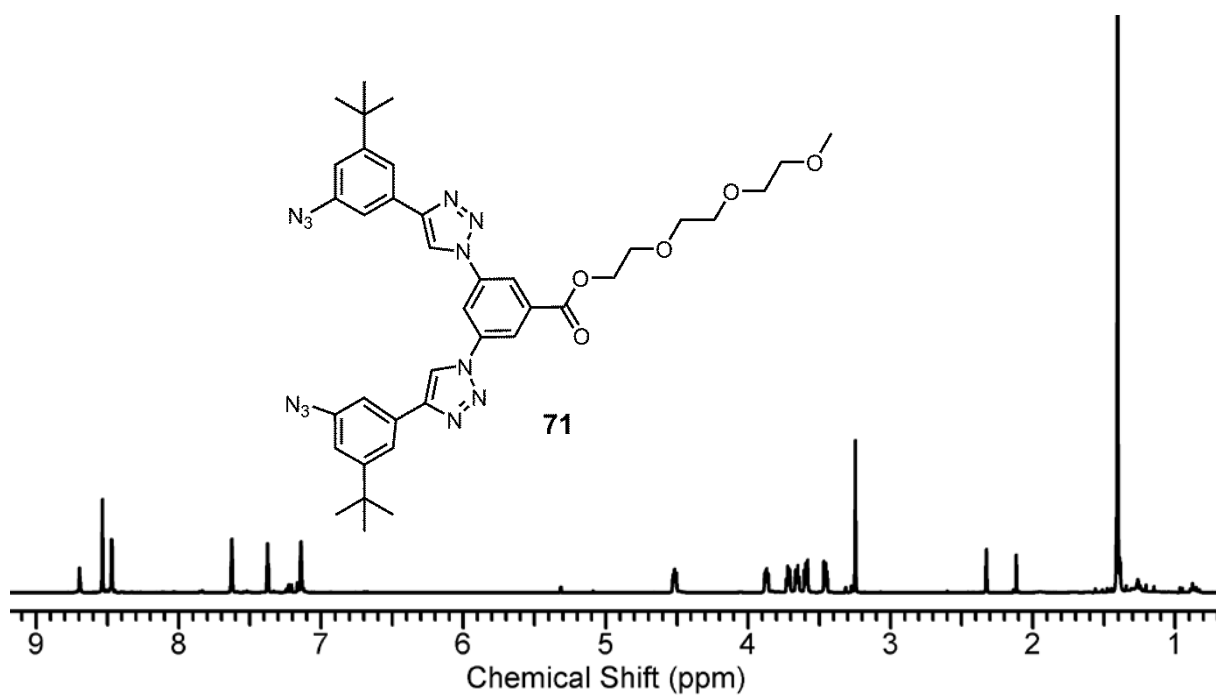
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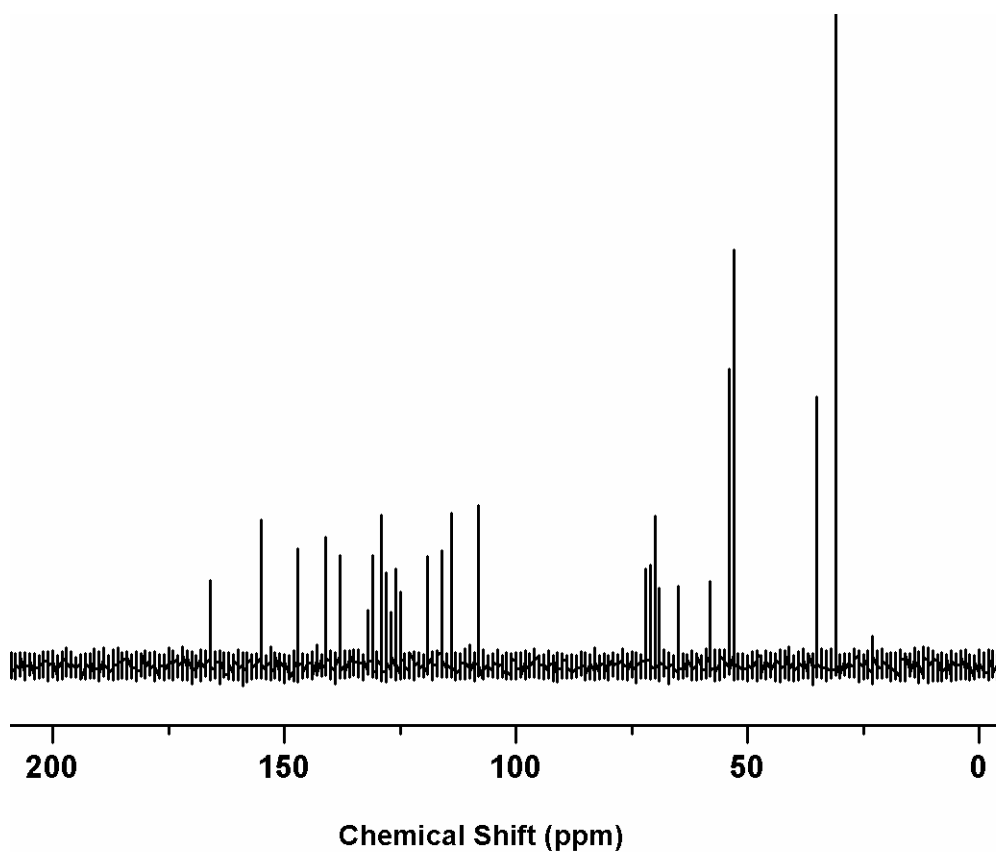
^1H NMR of compound **65**



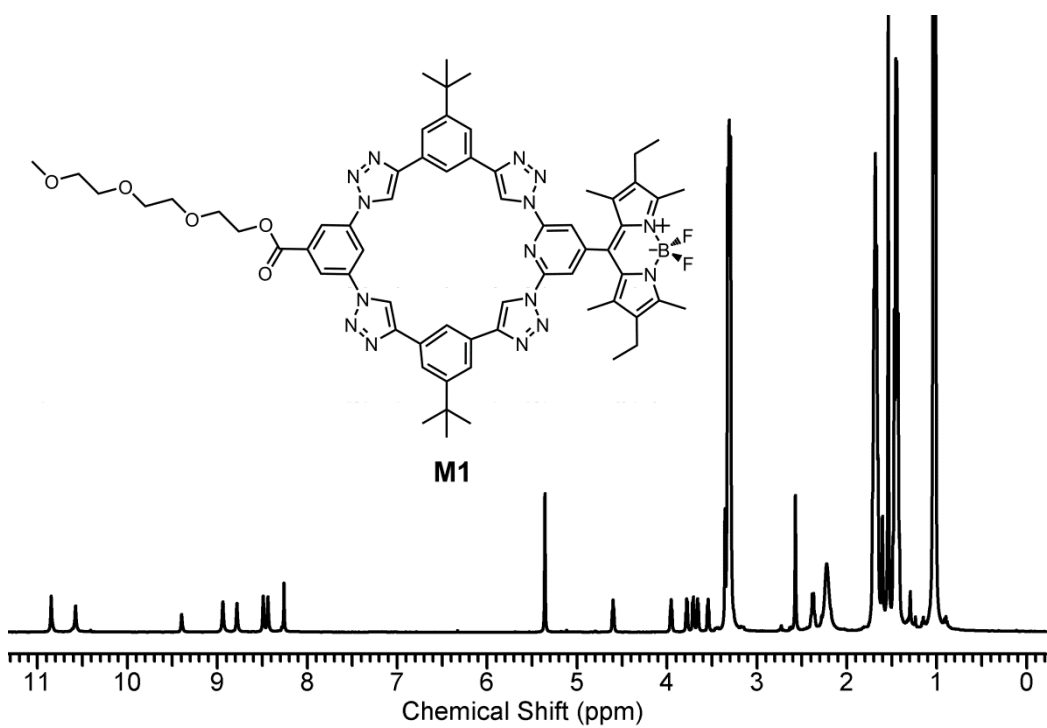
^{13}C NMR of compound **65**



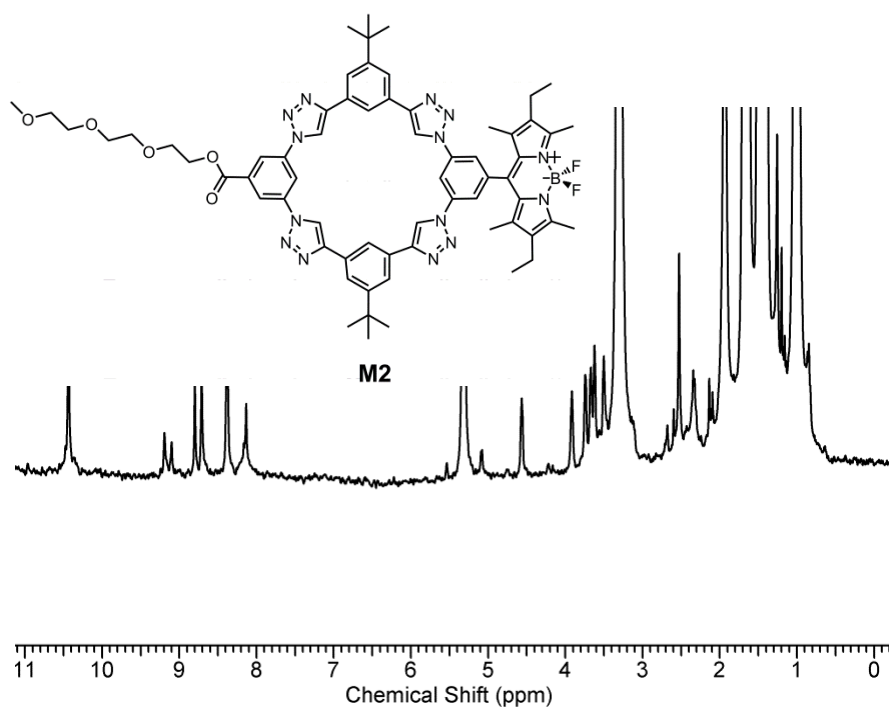
^1H NMR of compound **71**



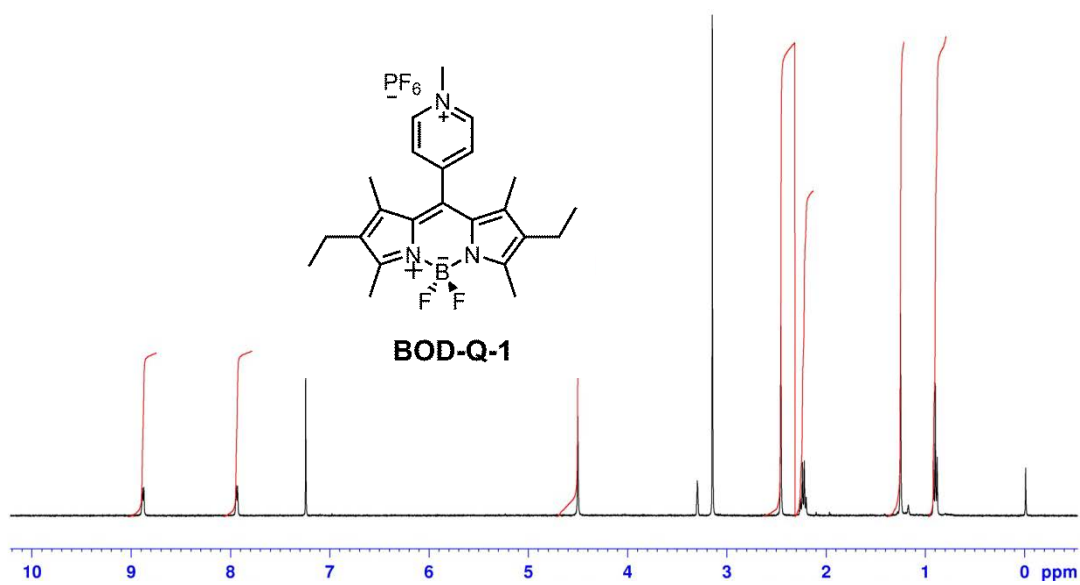
^{13}C NMR of compound **71**



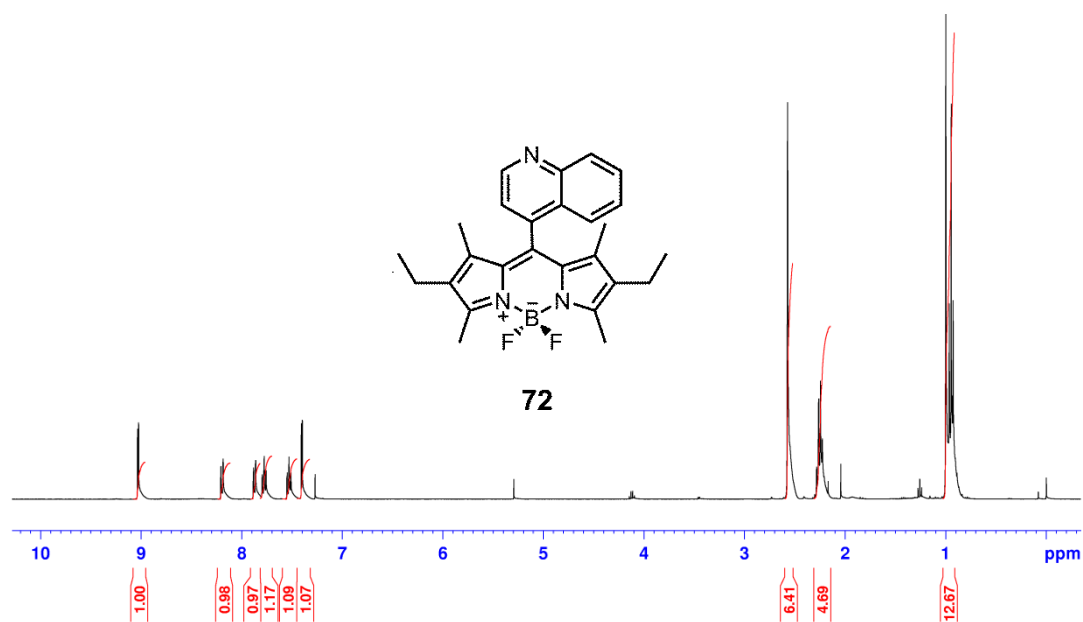
^1H NMR of compound **M1** titrated with TBACl



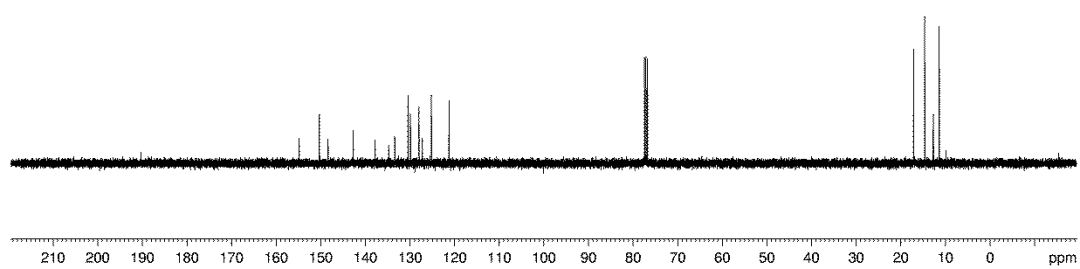
^1H NMR of compound **M2** titrated with TBACl



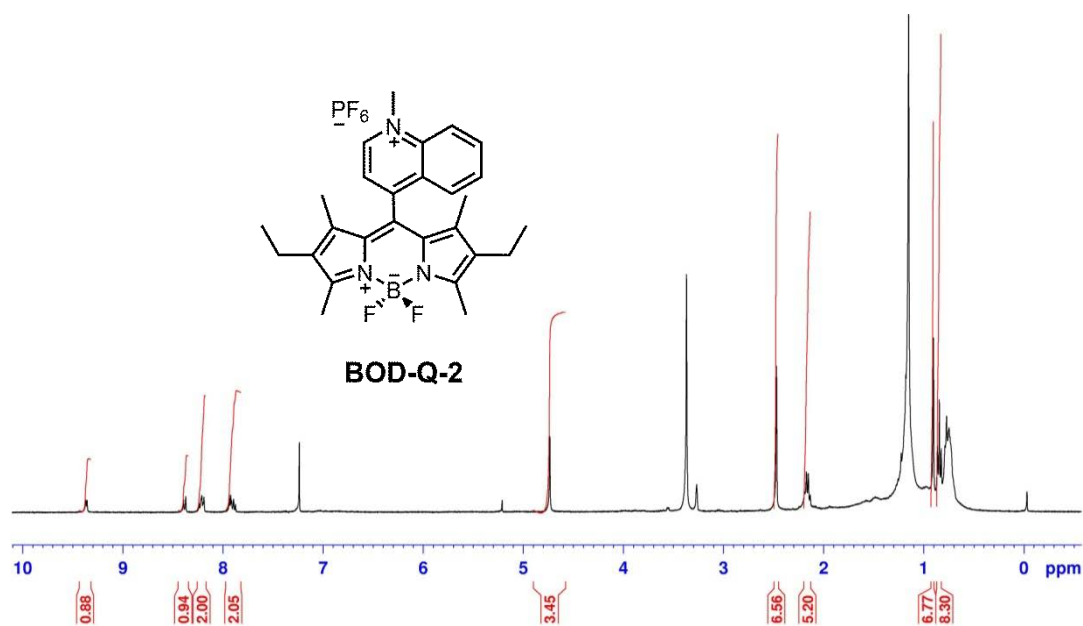
^1H NMR of compound **BOD-Q-1**



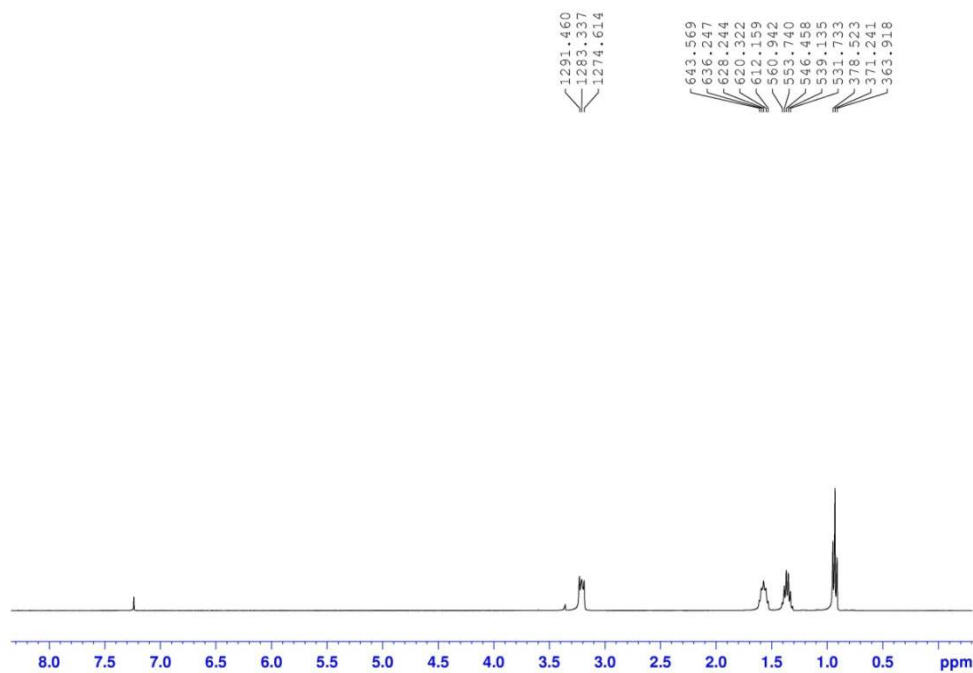
¹H NMR of compound 72



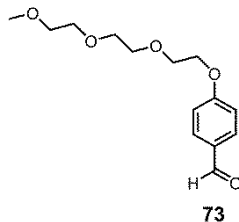
¹³C NMR of compound 72



¹H NMR of compound **BOD-Q-2**

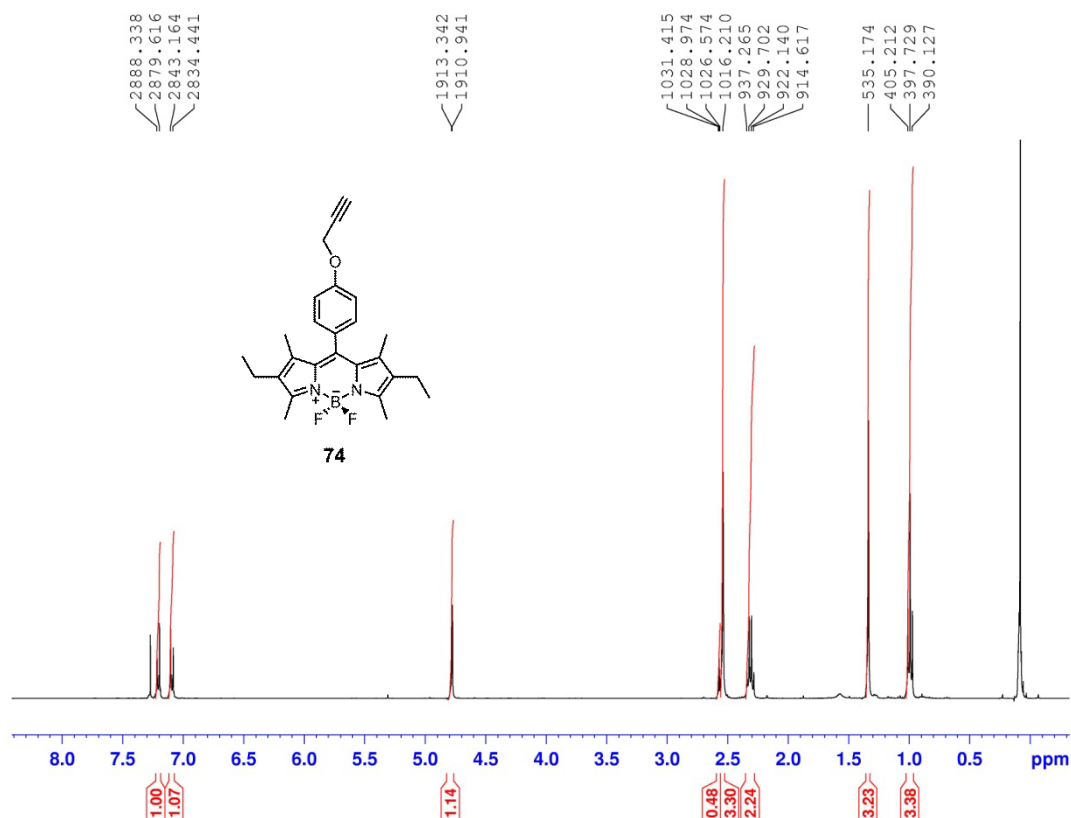


¹H NMR of compound **TBATFA**

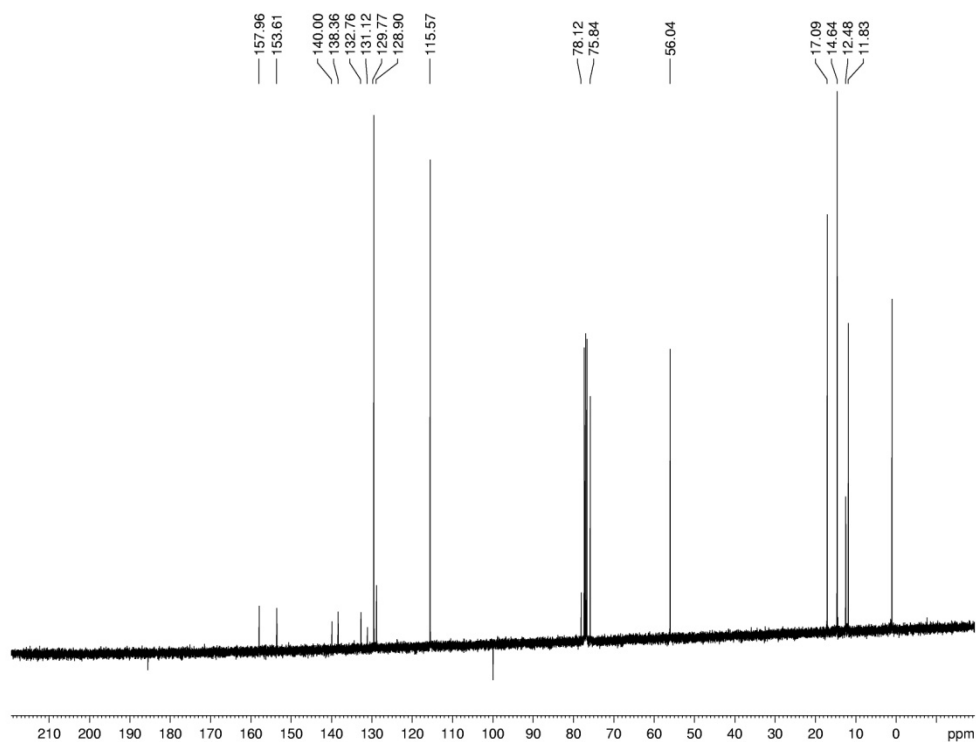


¹³C NMR spectrum (CDCl₃) of compound 10. The spectrum shows peaks at the following chemical shifts (ppm): 190.78, 163.86, 131.93, 130.06, 114.88, 71.93, 70.91, 70.67, 70.59, 69.48, 67.77, 59.03, and 25.00.

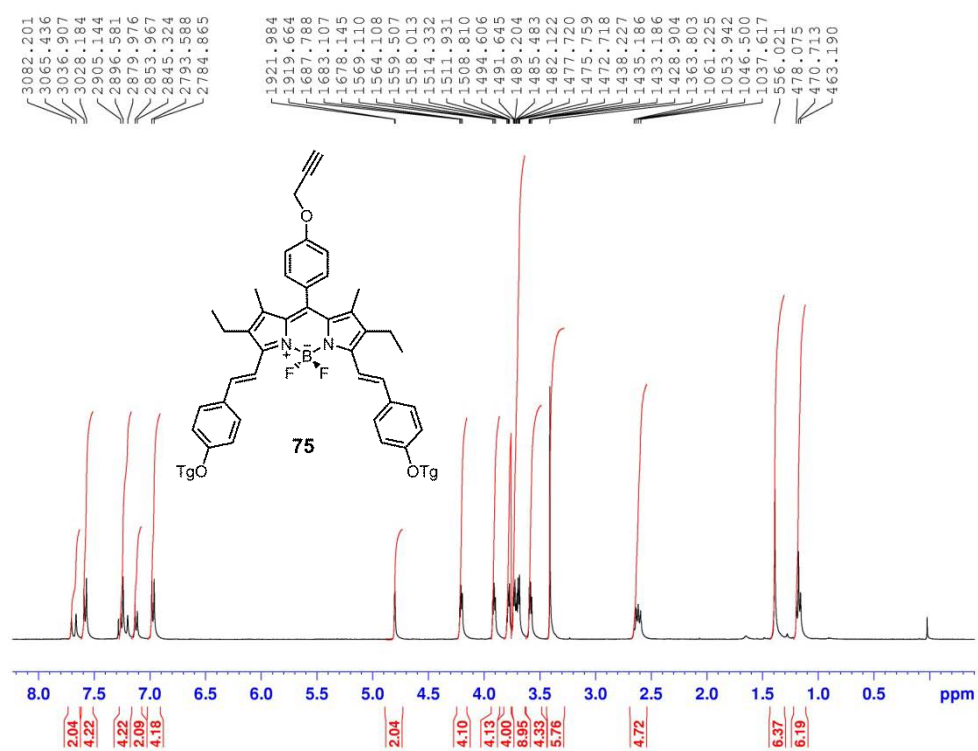
241



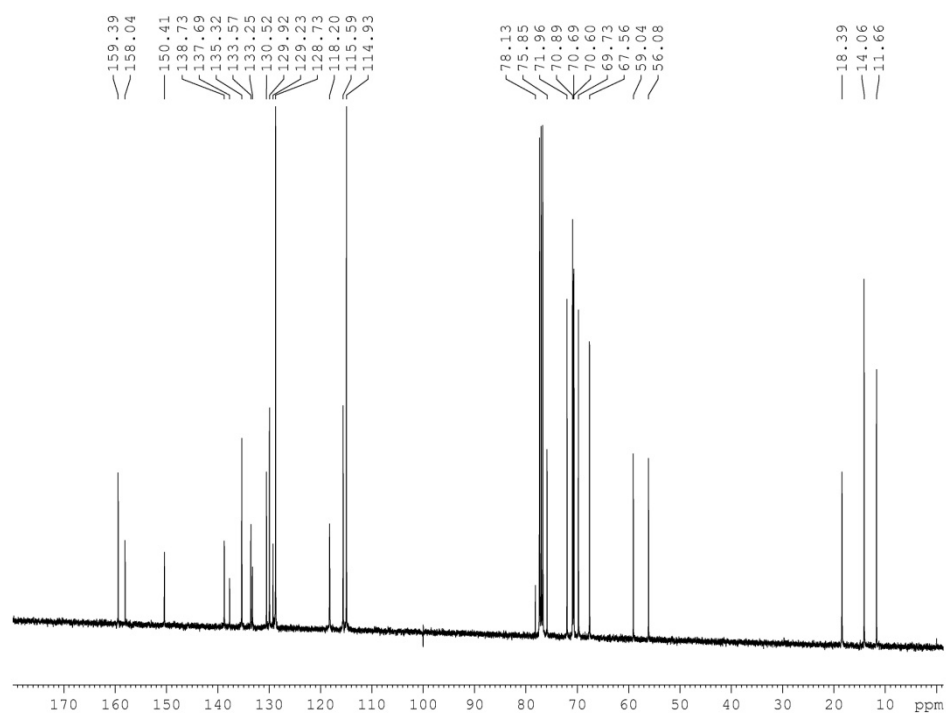
¹H NMR of compound 74



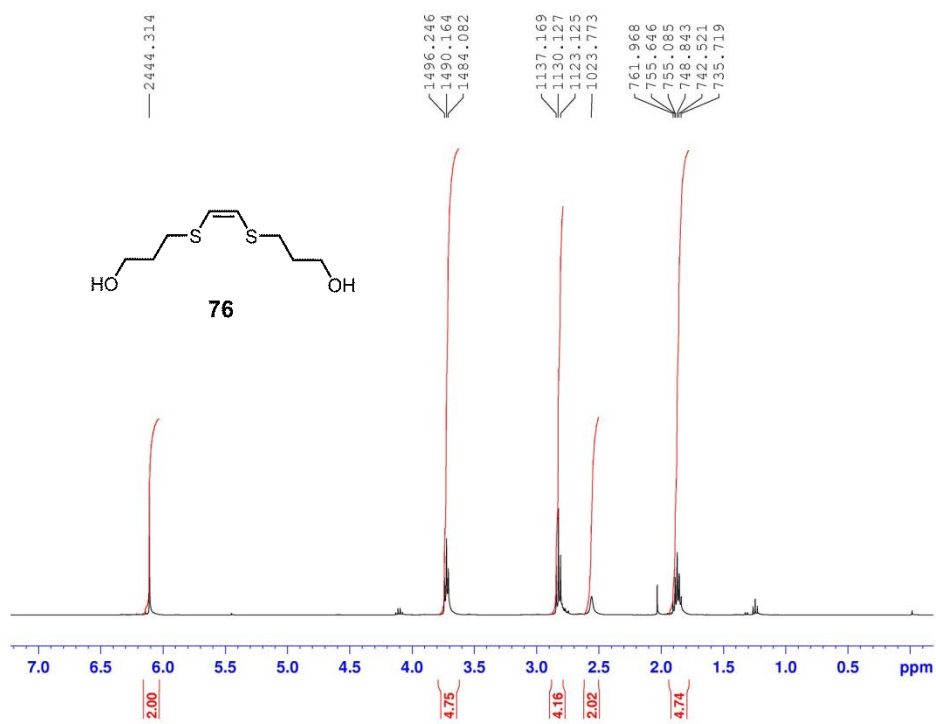
¹³C NMR of compound 74



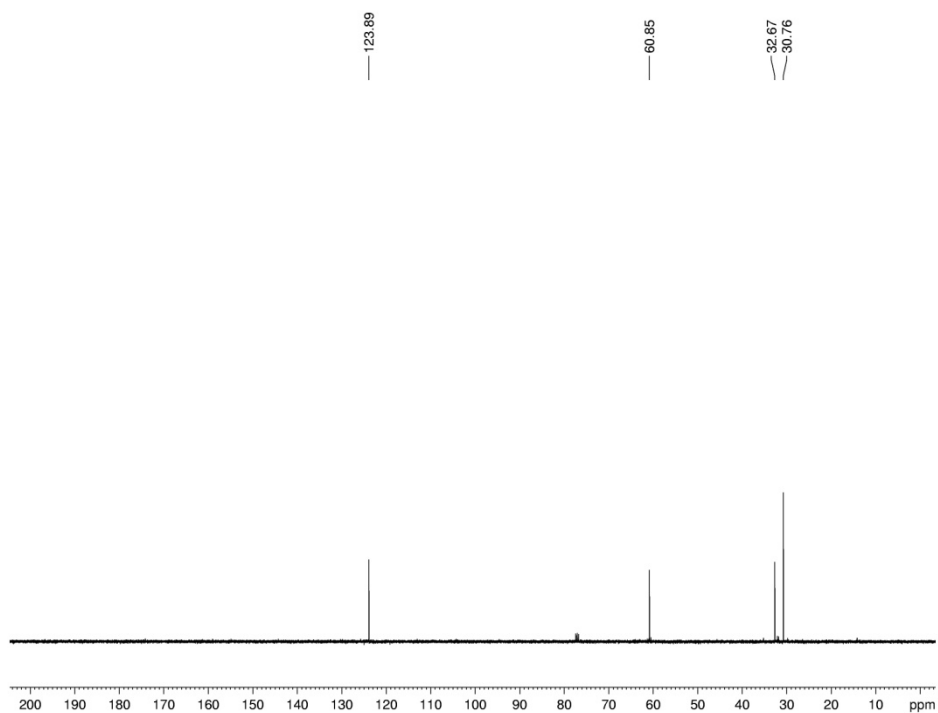
¹H NMR of compound 75



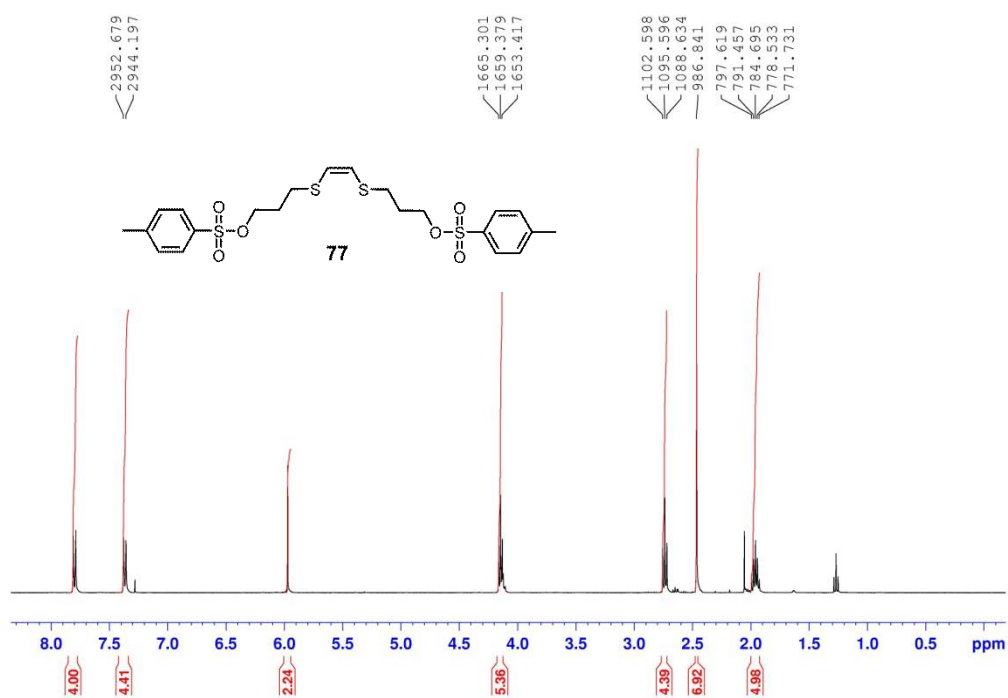
¹³C NMR of compound 75



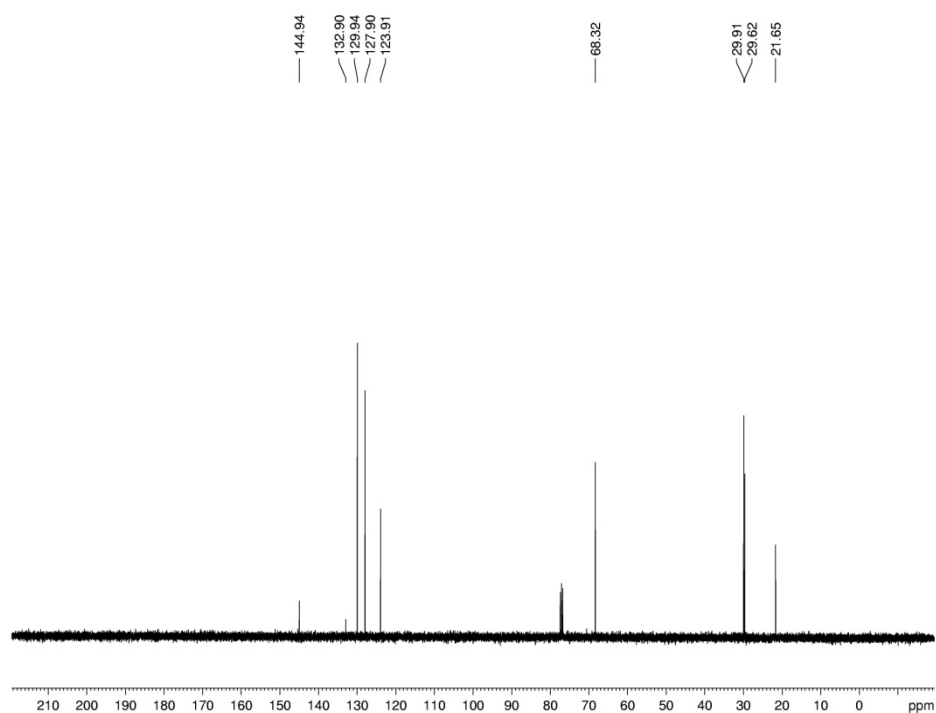
¹H NMR of compound **76**



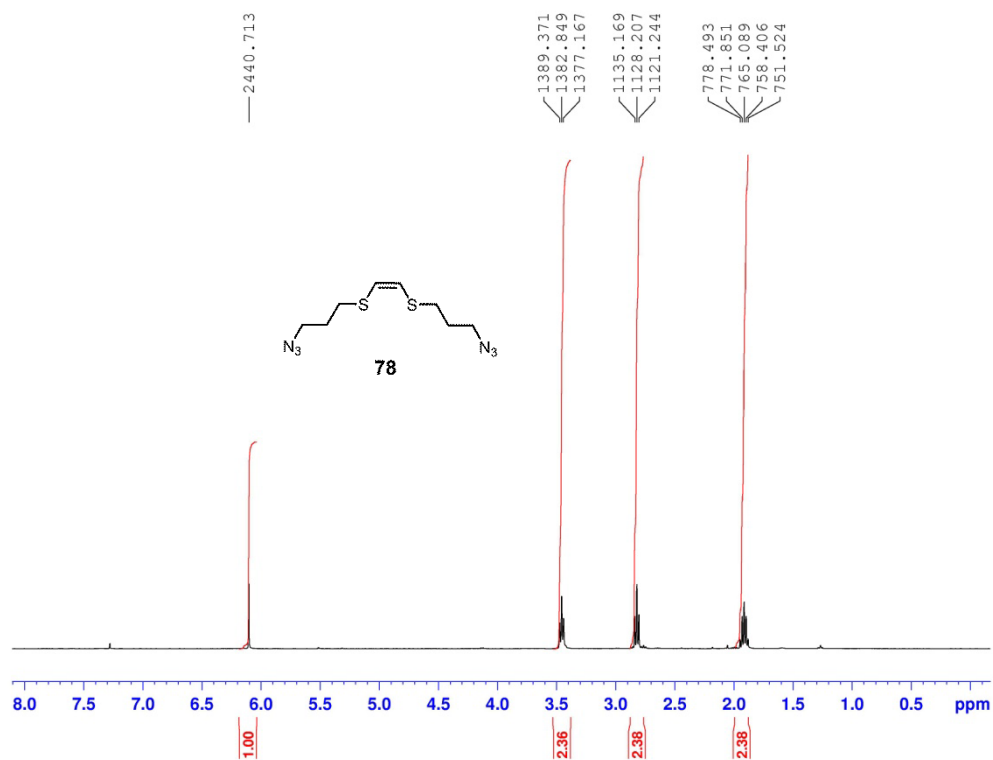
¹³C NMR of compound **76**



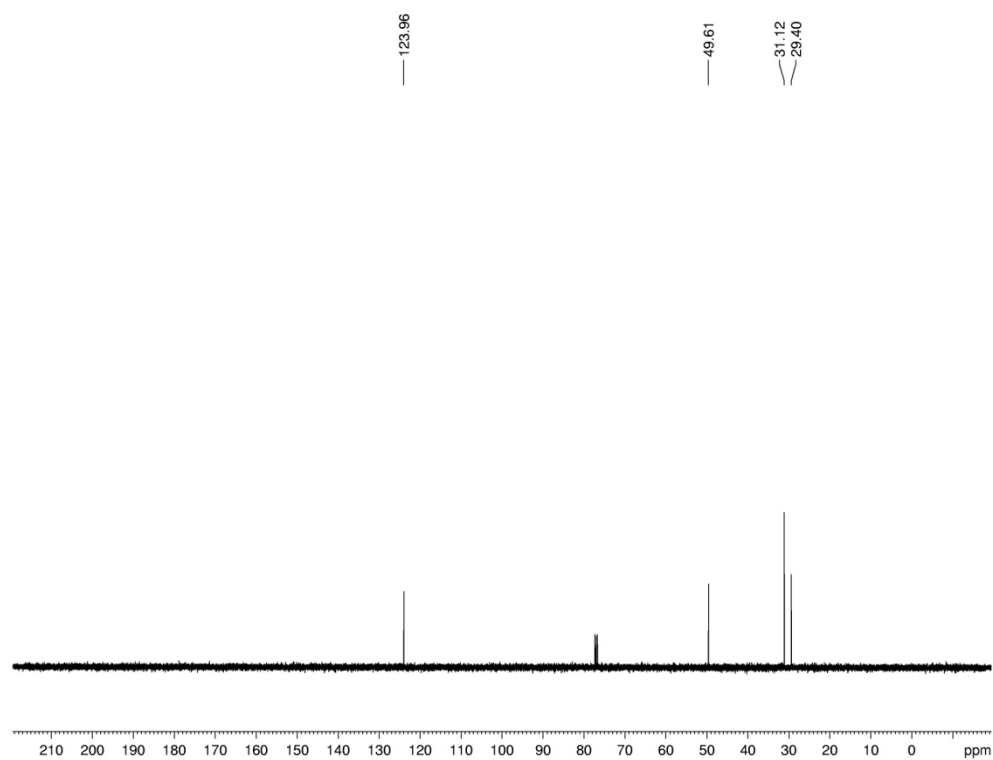
¹H NMR of compound 77



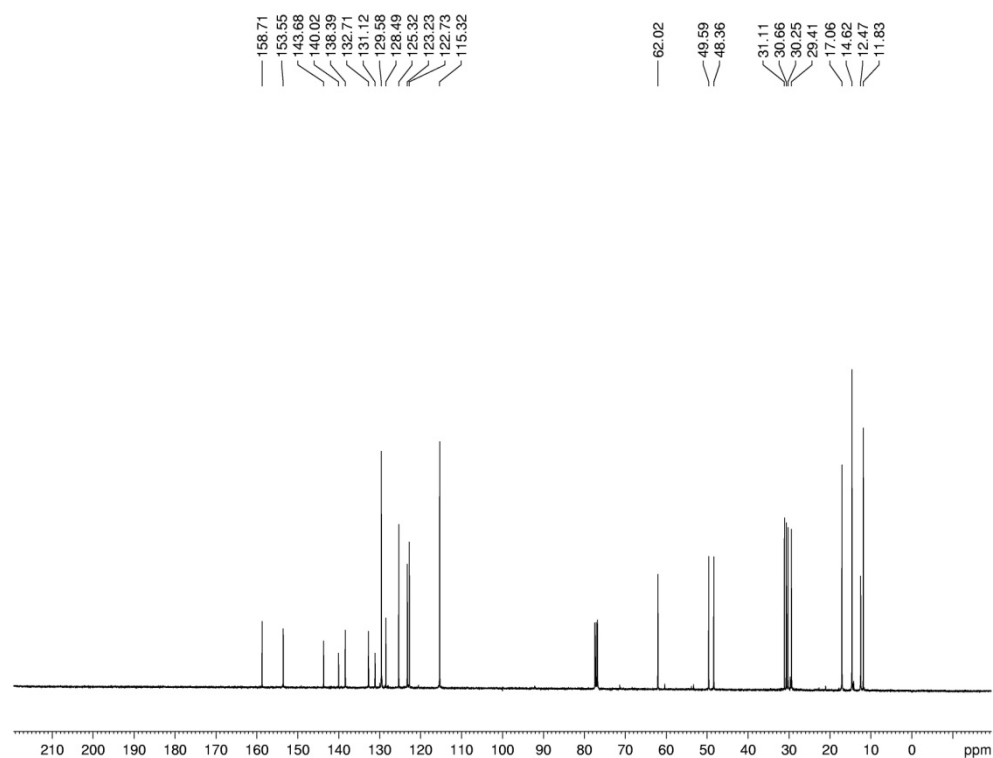
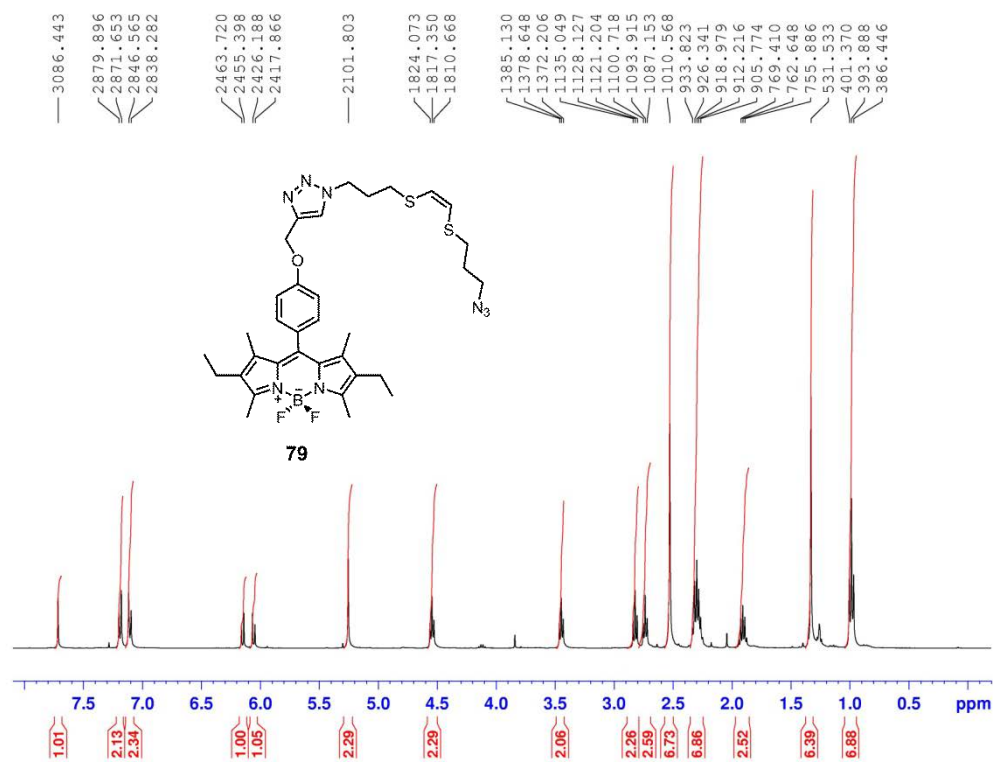
¹³C NMR of compound 77

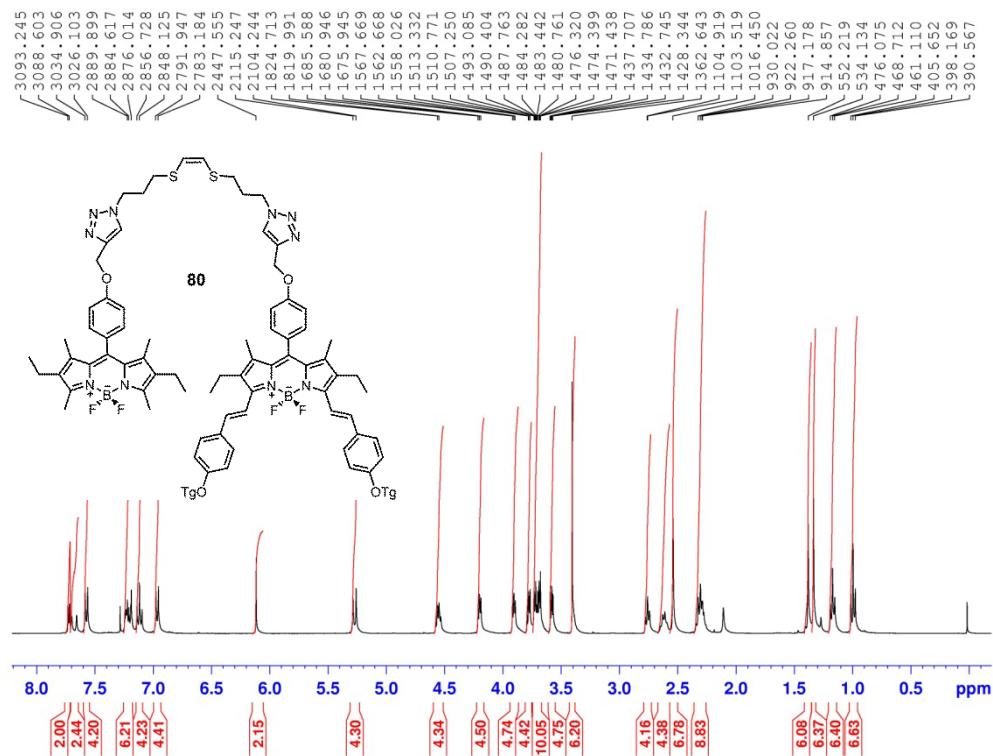


¹H NMR of compound **78**

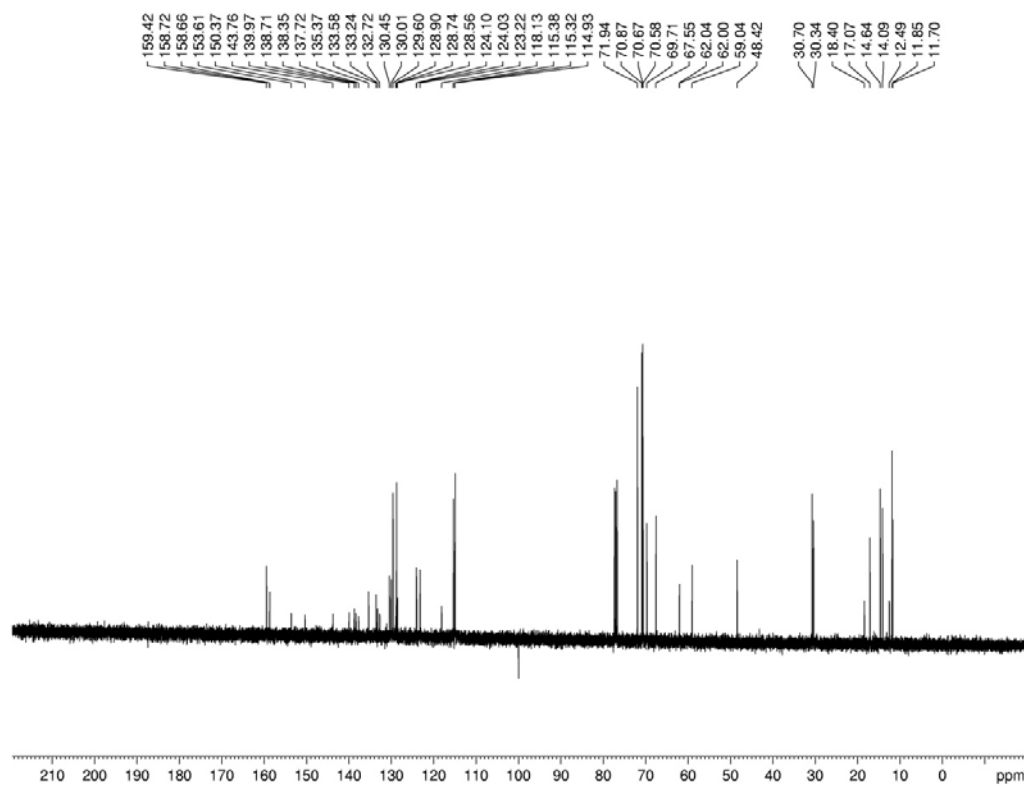


¹³C NMR of compound **78**





¹H NMR of compound **80**



¹³C NMR of compound **80**