Design, Synthesis, And Characterization Of Novel Hydrophilic Fluorene-based Derivatives For Bioimaging Applications

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DESIGN, SYNTHESIS, AND CHARACTERIZATION OF NOVEL HYDROPHILIC FLUORENE-BASED DERIVATIVES FOR BIOIMAGING APPLICATIONS

by

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ABSTRACT

In this work, hydrophilic fluorene-based derivatives that contain ethylene oxide substituents, have been synthesized and characterized for potential use as new fluorophores for bioimaging applications and for fluorescence sensing of heavy metals. Symmetrical and unsymmetrical fluorene derivatives based on structural types of acceptor-π-acceptor, acceptor-π-donor, and donor-π-donor were characterized by TGA, UV-vis absorption, fluorescence emission, lifetime, anisotropy, and two-photon absorption (2PA) cross section. They were found to possess high thermal stability, high photostability, high fluorescence quantum yields, and generally large two-photon absorption cross sections, making them quite suitable for new probes in single-photon absorption and two-photon absorption fluorescence microscopy imaging.

Novel hydrophilic fluorene derivatives were synthesized from fluorene in multiple steps employing the metal-catalyzed Heck coupling reaction, the Stille reaction, the Sonogashira reaction, the Ullmann condensation reaction, and “click” chemistry. To increase the hydrophilicity of the new compounds, ethylene oxide substituents were utilized for to impart water solubility. An alternative alkylation methodology using ethylenedioxy tosylates was introduced for the synthesis of ethylene oxide-containing fluorene derivatives. Several of these hydrophilic derivatives were incubated into various cell lines as new probes for both conventional and two-photon absorption fluorescence bioimaging. These compounds were biocompatible, exhibiting low cytotoxicity as determined by cell viability studies, and displayed colocalization for selected cellular organelles.
In addition, hydrophilic bis(1,2,3-triazolyl)fluorene derivatives were found to exhibit sensitive fluorescence responses in the presence of certain heavy metal, and were selective for sensing zinc and mercury over other a number of other metal ions relevant to living cells or other biological environments. The UV-vis absorption and fluorescence emission spectra of the complexes exhibited a blue-shifted absorption and emission for selective metal chelation upon binding to zinc and mercury(II) ions, resulting in an approximately two-fold enhanced fluorescence response. Fluorescence titration studies revealed that the complexes of 1:2 and 1:3 ligand to metal formed with binding constant values of $10^8$ and $10^{14}$ for zinc and mercury ions, respectively.

Finally, preliminary experiments were performed to explore the possibility of employing select hydrophilic fluorene-based derivatives in the synthesis of hydrophilic fluorescent gold nanoparticles. Although results are very preliminary, the aim is to use such materials for other biomedical applications, such as surface enhanced scattering resonance and noninvasive photothermal therapy to diagnose and to treat cancers.

Thus, this research had led to the discovery of alternative methodologies for synthesis of hydrophilic fluorene derivatives by alkylation with alkyl tosylates and synthesis of hydrophilic fluorescent molecule capped gold nanoparticles. Furthermore, several novel hydrophilic fluorene-based derivatives were synthesized and characterized for their linear and nonlinear photophysical properties, and are now available for further examination of their bioimaging and sensing applications.
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<tr>
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<tr>
<td>°C</td>
<td>Degree Celcius</td>
</tr>
<tr>
<td>¹H-NMR</td>
<td>Proton Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>1PT</td>
<td>Single-photon</td>
</tr>
<tr>
<td>2PFM</td>
<td>Two-photon Fluorescence Microscopy</td>
</tr>
<tr>
<td>2PT</td>
<td>Two-photon</td>
</tr>
<tr>
<td>¹³C-NMR</td>
<td>13-Carbon Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>A</td>
<td>Acceptor</td>
</tr>
<tr>
<td>ab</td>
<td>absorption</td>
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<tr>
<td>ACN</td>
<td>Acetonitrile</td>
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<tr>
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<tr>
<td>CDCl₃</td>
<td>Deuterated Chloroform</td>
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<tr>
<td>δ (GM)</td>
<td>Two-photon Absorption Cross-Section</td>
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<td>δ (ppm)</td>
<td>Chemical Shift</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
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<tr>
<td>D</td>
<td>Donor</td>
</tr>
<tr>
<td>ΔEg</td>
<td>Energy Gap</td>
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<td>Time Exposed</td>
</tr>
<tr>
<td>DCB</td>
<td>Dichlorobenzene</td>
</tr>
<tr>
<td>DIC</td>
<td>Differential Interference Contrast</td>
</tr>
<tr>
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<td>Description</td>
</tr>
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</tr>
<tr>
<td>DMF</td>
<td>Dimethyl Formamide</td>
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<tr>
<td>DMSO</td>
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<td>Ethylene Diamino Tetracarboxylic Acid</td>
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<td>Food and Drug Administration</td>
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<td>fs</td>
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</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GFP</td>
<td>Green Fluorescence Protein</td>
</tr>
<tr>
<td>GM</td>
<td>Gopper-Mayer Unit (1GM = $10^{-50}.cm^4.s.photon^{-1}.mol^{-1}$)</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HH</td>
<td>Horizon-Horizon</td>
</tr>
<tr>
<td>HV</td>
<td>Horizon-Vertical</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
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<td>HRMS</td>
<td>High Resolution Mass Spectroscopy</td>
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xxxi
<table>
<thead>
<tr>
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<td>I</td>
<td>Intensity</td>
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<tr>
<td>IC</td>
<td>Internal Conversion</td>
</tr>
<tr>
<td>ILCT</td>
<td>Intra Ligand Charge Transfer</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>ISC</td>
<td>Intersystem Crossing</td>
</tr>
<tr>
<td>K</td>
<td>Binding Constant</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>Wavelength</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>M^+</td>
<td>Molecular Ion</td>
</tr>
<tr>
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<td>Maximum</td>
</tr>
<tr>
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</tr>
<tr>
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<td>Mass Spectroscopy</td>
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<tr>
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<td>Number Aperture</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>(\phi)</td>
<td>Quantum Yield</td>
</tr>
<tr>
<td>(\phi_{FL})</td>
<td>Fluorescence Quantum Yield</td>
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<tr>
<td>Acronym</td>
<td>Interpretation</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>Φ_D</td>
<td>Quantum Yield of Single-Photon Decomposition</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
</tr>
<tr>
<td>PCT</td>
<td>Photoinduced Charge Transfer</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly Ethylene Glycol</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per Billion</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per Million</td>
</tr>
<tr>
<td>r.a.</td>
<td>Relative Abundance</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>S₀</td>
<td>Ground State</td>
</tr>
<tr>
<td>S₁</td>
<td>First Excited State</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface-enhanced Raman scattering</td>
</tr>
<tr>
<td>τ</td>
<td>Lifetime</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>Ts</td>
<td>Tosyl (4-methylbenzene sulfonyl)</td>
</tr>
<tr>
<td>VH</td>
<td>Vertical-Horizon</td>
</tr>
<tr>
<td>vs</td>
<td>versus</td>
</tr>
<tr>
<td>VV</td>
<td>Vertical-Vertical</td>
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CHAPTER ONE: INTRODUCTION

1.1. The Goal of This Research

The ultimate goal of this research is to design, synthesis, and characterize hydrophilic fluorene-based derivatives as new two-photon absorbing molecules for bioimaging and sensing applications. From the recent literature, one can see that fluorene derivatives have significant potential for use as multiphoton absorbing fluorescent probes relative to other classes of organic molecules.1-3 Hydrophilic fluorene-based derivatives, containing polyethylene glycol substituents for solubility in aqueous media in the cellular environment, are desired for their high quantum yield, large two-photon absorption (2PA) cross sections, and suitability for nondestructive imaging for biological studies. On the other hand, the design of novel fluorene-based derivatives that sense and detect heavy metal ions like zinc4-9 and mercury10-13 are intriguing for important biological and environmental applications. In addition, the design and synthesis of hydrophilic fluorene-based derivatives for complexing with gold nanoparticles may be of interest for several biomedical applications, including the diagnosis and treatment of cancerous cells,14,15 and for noninvasive photothermal therapy.16-18 Hence, the aim of the research in this dissertation is also to explore hydrophilic fluorene-based derivatives as new bioimaging agents in conventional confocal and two-photon fluorescence microscopy.

1.2. Single-Photon Absorption and Two-Photon Absorption

In general, matter absorbs and, in some cases, emits light upon irradiation. Fluorescence emission occurs at a longer wavelength relative to the linear absorption
wavelength. Some natural minerals, such as calcite and willemite, fluorescence upon UV-vis light irradiation. Traces of certain transition metals contribute to this fluorescence. Since the 16th century, this phenomenon was observed in the family *Leguminosae* of the tropical hardwood narra tree (*Pterocarpus indicus*), which is native to Philippine and the Mexican kidneywood shrub (*Eysenhardtia polysastachya*). Isoflavones were identified as the pigments responsible for a striking blue fluorescence when the wood was placed into water and irradiated. This fluorescent solution has been proposed for medicinal treatment for liver and kidney ailments.\(^9\)

Recently, a natural auto fluorescent chromophore of the Green Fluorescence Protein (GFP) was isolated from the Pacific Northwest jellyfish (*Aequorea victoria*) by Shimonura in 1960, while the chromophore structure was elucidated in the 1980s (Fig. 1).\(^{20}\)

![Figure 1. Structure of the GFP Chromophore.](image)

In Figure 1, R and R’ are amino acid sequences (part of a large protein, typically >200 amino acids). The GFP chromophore emits a greenish light with a quantum yield of 0.8, and has gained wide spread use as a fluorescent marker for *in vitro* and *in vivo* imaging in cell biology. However, a synthetic GFP chromophore analog, where R and R’ were a propyl and methyl group, respectively, displayed a quantum yield of only 0.01, far too low for any practical bioimaging applications. This may be due to free rotation of the phenolic ring
adjacent to the C=C, quenching the fluorescence in this molecule. This highlights the sophistication and complexity of nature and factors contributing to fluorescence emission.

A Jablonski electronic energy diagram (Fig. 2) illustrates fluorescence emission upon light irradiation.21

![Jablonski Electronic Excitation Energy Diagram](image)

**Figure 2. The Jablonski Electronic Excitation Energy Diagram.**

Here $S_0$, $S_1$, and $S_2$ are ground state, first excited state, and second excited state, respectively; $T_1$ is the first triplet state. Fluorescence results in emission of a photon upon radiative deactivation of the $S_1$ to $S_0$ state, while phosphorescence results in emission upon deactivation of $T_1$ to $S_0$. The lifetime of the excited state $S_1$ is typically $10^{-7}$-$10^{-10}$ s; intersystem crossing (ISC) is $10^{-10}$-$10^{-4}$ s; internal conversion (IC) is $10^{-11}$-$10^{-9}$ s; and the lifetime of the excited state $T_1$ is $10^{-4}$-$1$ s.

A simple one-photon absorption process is presented in Figure 3.
Figure 3. One-photon Absorption.

Two-photon absorption, a process of simultaneous absorption of two photons upon light excitation, is depicted in Figure 4.

Figure 4. Two-photon Absorption.

Two-photon absorption was predicted by Goppert-Meyer\textsuperscript{22} in 1931. However, it was only applied for practice in the 1990s for two-photon excitation fluorescence microscopy.\textsuperscript{23-29} The introduction of multiphoton fluorescence excitation has shown tremendous advantage over one-photon fluorescence excitation in terms of three-dimensional localization in the focal volume of a laser beam and in greater penetration in tissues and cells, such as 1 mm for live imaging (Fig. 5).
Moreover, because multiphoton excitation occurs at a longer wavelength (in the visible and near-IR region), as the result, photodamage to the object (cell imaging) is lessened in comparison with one-photon excitation (i.e., excited by a shorter wavelength, typically in the UV region). Two-photon fluorescence excitation has been employed in the detection of biomolecules for enhancing sensitivity and selectivity.\textsuperscript{30,31}

\subsection*{1.3. Design of Multiphoton Absorbing Materials}

Recently, development of new multiphoton absorbing materials with larger two-photon cross sections has received attention for bioimaging applications\textsuperscript{32,33} and for photodynamic therapy.\textsuperscript{34} Some important photophysical properties for a new molecule are the absorption and emission range (UV-vis or near-IR), the quantum yield, the lifetime, the photostability (photobleaching), and the two-photon absorption (2PA) cross section. In general, a molecule for multiphoton absorption consists of a core chromophore ($\pi$-electron bridge) along with donor (electron-releasing) and/or acceptor (electron-withdrawing) groups.
linked by a conjugated π-system. A sp-hybridized carbon conjugated π-system makes a molecule more rigid as the triple bond has fixed rotation. Design of the first type (symmetrical) “pull-pull” or “push-push” of molecule involves a quadrupolar construct containing electron donor or acceptor groups to change the molecular quadrupole moment based on the concept that the symmetric charge transfer of a conjugated system upon excitation enhances values of 2PA cross sections. The second type (asymmetrical) “push-pull” molecule is dipolar or octupolar, with the concept that the charge transfer between a donor and an acceptor upon excitation and accounts for a larger 2PA cross section. Recently, a computational approach based on the molecular orbital theory of the excited state HOMO-LUMO gap has been used to assist in the design of a new class of organic molecules. A high extent of π-conjugation reduced the value of the band gap, e.g., a small band gap (ΔEg = 1 eV) was found for polyacetylene (Table 1).

Table 1. The HOMO-LUMO Gap of Some Organic Molecules

<table>
<thead>
<tr>
<th>Compound</th>
<th>ΔEg (eV)</th>
<th>Color</th>
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<tr>
<td>Naphthalene</td>
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<td>White</td>
</tr>
<tr>
<td>Anthracene</td>
<td>3.6</td>
<td>White</td>
</tr>
<tr>
<td>Perylene</td>
<td>3.0</td>
<td>Yellow</td>
</tr>
<tr>
<td>Tetracene</td>
<td>2.8</td>
<td>Orange</td>
</tr>
<tr>
<td>Naphthacene</td>
<td>2.8</td>
<td>Orange</td>
</tr>
<tr>
<td>Pentacene</td>
<td>2.2</td>
<td>Orange Red</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>1.8</td>
<td>Orange Red</td>
</tr>
<tr>
<td>Polyacetylene</td>
<td>1.0</td>
<td>Red/Blue</td>
</tr>
</tbody>
</table>

1.4. Fluorene-based Derivatives as New Fluorophores

As shown in previous work, fluorene derivatives are generally known as fluorophores that exhibit high quantum yields, high photostability, excellent thermal stability, and large multiphotons absorption cross sections relative to other organic materials.
derivatives can be functionalized with a variety of substituents of acceptor (A) and donor (D) at carbons 2 and/or 7 (X and Y), and hydrophobic or hydrophilic groups (R) at carbon 9. A typical A-π-D motif is shown (Fig. 6).

Figure 6. Fluorene-based Derivatives as Fluorophores.

Here X is an acceptor such as a benzothiazole, a 1,2,3-triazole, a cyano, or a nitro, while Y is a donor like a diphenylamino or a hydroxy moiety; and R is an alkyl or an ethylene oxide substituent, thereby tailoring the molecule’s solubility.

Fluorene-based derivatives also exhibit low cytotoxicity in relative to other organic chromophores. However, because fluorene-based derivatives, like other hydrophobic organic molecules, have limited solubility in polar solvents such as methanol or aqueous media, there is a need for hydrophilic fluorene-based derivatives that are soluble in aqueous media, such as that in human body or cells, and suitable for various bioimaging applications.

1.5. Hydrophilic versus Hydrophobic Derivatives

Since aqueous media is used for bioimaging, to increase the hydrophilicity of fluorene-based derivative, a polar group such as a carboxylic acid (CO₂H), an alcohol or phenol (ROH), or a sulfonic acid (SO₃H) can be utilized as a substituent. However, polyethylene glycol (PEG) is also known for its water soluble,⁵¹ biocompatibility⁵²,⁵³ low cellular toxicity⁵⁴, and is approval by the U. S. F.D.A. for human use. PEG was employed for
bioconjugation of certain liposomes to increase its circulation time.\textsuperscript{55} It is logical that fluorene-based derivatives should contain polyethylene glycol substituents to improve their water solubility for a number of biological applications. Moreover, the hydrophilicity of fluorene-based molecules is greatly increased by the number of ethylene oxide unit substituents. If the ethylene oxide unit substituents are large enough, the molecule may be completely soluble in water.

1.6. The Role of Heavy Metals in Biological Studies

Heavy metal ions can disturb normal biological functions and evoke cellular stress responses at high levels (0.01 ppm) in the cellular environment.\textsuperscript{56,57} Among the heavy metal ions, zinc is the second most abundant heavy metal ion in vivo and is an essential component of many protein scaffolds and enzymes in cells.\textsuperscript{58,59} The human body contains approximately 3000 zinc-proteins,\textsuperscript{60} and the zinc ion was recognized as a main mineral nutrition for cells by the work of Rauline on the fungus \textit{Aspergillus niger}.\textsuperscript{61,62} Zinc is a major regulatory ion in a number of ion channels and receptors, in neural signal transmission, in metalloenzyme regulation, and in the metallobolism of cells, exhibiting a binding constant of $3.2 \times 10^{13}$ M\textsuperscript{-1} for zinc metallothionein (Fig. 7).\textsuperscript{63-65}
Zinc also plays an important role in various biological processes, such as suppressing apoptosis, and in neurophysiology, such as inducing the formation and aggregation of β-amyloid that is related to Alzheimer’s disease.66 A high concentration of Zn$^{2+}$ is known to be a neurotoxin, and excess zinc in the brain has been implicated in neuropathology. Zinc release contributes to hypoglycemia-induced neuronal death.67 A zinc sensing molecule is quite important for biological studies and for disease diagnosis.68

On a similar note, mercury is a nonessential heavy metal that is highly toxic to cells.69 It is known that mercury is a severe neurotoxic, genotoxic, and immunotoxic species in mammals. Mercury has been found to reduce the chlorophyll content in the leaves of plants.70
Furthermore, mercury can induce aberrations in microtubules, ion channels, and mitochondria.\textsuperscript{71} Hence, methods for the sensitive and selective detection of mercury are a matter of great interest.

1.7. Design of Fluorescence Sensing Molecules for Metal Ion Detection

Design of a molecule for sensing of heavy metal ions, such as zinc \textit{in vivo} or mercury in contaminated sources, involves high selectivity for zinc or mercury over other relevant metal ions. Since the Zn\textsuperscript{2+} ion is inert with a closed shell of 3d\textsuperscript{10} in its electronic configuration, fluorometric methods have been employed as a technique of choice to detect zinc ions for various applications.\textsuperscript{72-74} New fluorescent sensing molecules for metal ion detection should have high affinity, bind rapidly and reversibly, be nontoxic with high fluorescence quantum yield, and high photostability. To date, zinc ion sensing molecules employed aryl sulfonamides of 8-aminoquinoline,\textsuperscript{75} fluoresceins,\textsuperscript{76-78} porphyrin-based derivatives,\textsuperscript{79} and calix[4]arene.\textsuperscript{80} Calixarene, a macrocyclic molecule of obligophenols linked by methylene bridges, was introduced recently as a sensing agent for various heavy metal ions.\textsuperscript{81} Current mercury sensing molecules employed include rhodamine-based,\textsuperscript{82} fluorescein-based,\textsuperscript{83,84} a perylene derivative,\textsuperscript{85} calyx[4]arenediazacrown ether,\textsuperscript{86} and porphyrine-based dyes.\textsuperscript{87} Few of these were designed for two-photon excitation and many have limited photostability.

In addition to zinc and mercury, cadmium and lead are contaminants that can alter the immune response of organisms at level of 10 ppb.\textsuperscript{88,89} Silver, which is employed in dental and medical materials, can induce cellular signaling at high levels.\textsuperscript{90} A calixarene-based molecule was also utilized to detect silver ions.\textsuperscript{91} Since multiphoton microscopy was
introduced and possesses a number of significant advantages over single-photon excitation, new metal ion sensing molecules which utilized an aza-crown ether linkage was reported that exhibited a large 2PA cross sections, though the synthetic complexity of this system is high and little is known about its photostability. Thus, new metal sensing 2PA fluorescent probes are needed. Designing of fluoroionophore is consisted a chromophore, a spacer of hydrocarbon, a binding group usually a nitrogen-heterocyclic or crown ether moiety (Fig. 8). The fluorescence can be enhanced or quenched upon binding to metal.

![Figure 8. A Conceptual Design of Chromoionophore](image.png)

1.8. Fluorene-based Derivatives for Fluorescence Sensing of Metal Ions

As demonstrated in previous studies, fluorene derivatives are known for good photophysical properties, high luminescence quantum yields, large 2PA cross sections, and excellent photostability. Hydrophilic fluorene-based derivatives that are soluble in aqueous media as new fluorescent metal ion sensors for selected metal ions, such as zinc and mercury, are potentially quite important for biological and environmental sensing applications. This research detailed herein reports the investigation of three hydrophilic fluorene-based derivatives for highly selective sensing of significant heavy metal ions.

1.9. Gold Nanoparticles in Biomedical Application

Gold colloid and gold nanoparticles were studied extensively since the 1850s for new optical materials. Recently, gold nanoparticles are shown low toxicity materials
that can be used for bioimaging studies\textsuperscript{97} and for noninvasive phototherapy.\textsuperscript{98} Fluorene-based derivatives that contain gold nanoparticles can be explored for new bioimaging probes and for diagnostic of treatment of cancers.

1.10. Design of Fluorescent Gold Nanoparticles as a Bioimaging Agent

Cancer has been a major cause of death in the 21\textsuperscript{st} century. By definition, cancer is the uncontrolled growth of cells with loss of differentiation which, upon metastasis, spreads to other tissues and organs. As a result, cancer can be life threatening to the body and must be treated. Cancer phototherapy and photothermal therapy use light irradiations to kill cancerous cells. A bioimaging agent is also used to stain or to incubate cells during the process of cancer treatment. The design of new molecules for diagnostic bioimaging or cancer treatment is intriguing for a number of biomedical applications. Figure 9 illustrates the concept for a fluorescent gold nanoparticle-based probe that contains a chromophore, such as a fluorene derivative, a spacer, such as hydrophobic hydrocarbon chain, and binding groups, like thiol moieties, to which gold nanoparticles can be linked.

![Figure 9. Design of Fluorescent Molecule Capped Gold Nanoparticles.](image)

New bioimaging materials such as hydrophilic fluorene-based capped gold nanoparticles may serve as new therapeutic probes, particularly since gold nanoparticles have been employed in photothermal therapy as a promising solution.\textsuperscript{99} Herein is reported preliminary experiments to design such a composite material.
CHAPTER TWO: RESULTS AND DISCUSSION

2.1. Hydrophilic Fluorene-based Derivatives for Bioimaging

The goal of this work was to synthesize a series of novel hydrophilic fluorene-based derivatives for bioimaging applications, and perform comprehensive molecular and photophysical characterization of the new compounds. The structure of symmetrical and unsymmetrical fluorene-based derivatives was varied by use of (a) donor and acceptor groups, (b) sp-hybridized carbon and sp$^2$-hybridized carbon conjugated π-systems, and (c) the number of ethylene oxide units in substituents.

2.1.1. Synthesis of Hydrophilic Fluorene-based Derivatives

Fluorene-based derivatives were synthesized containing the following structural motifs: acceptor-π-acceptor 6a, 6b (Scheme 1), acceptor-π-donor 8a, 8b, 9a (Scheme 4), 9b-d (Scheme 5), 8e-g (Scheme 7), 10 (Scheme 8), 8i (Scheme 9), and donor-π-donor 5e, 5f, 7g (Scheme 11).

2.1.1.1. Synthesis of Acceptor-π-Acceptor Fluorene-based Derivatives

In Scheme 1, fluorene 1 was iodinated to produce 2,7-diiodofluorene 2a in 70% yield using iodine (0.8 molar eq) and iodic acid in acetic acid at 70 °C. In general, the iodination of fluorene by iodine and iodic acid can yield 2-iodofluorene (Eq. 3) and 2,7-diodofluorene (Eq. 4), depending on the molar stoichiometry ratios of fluorene, iodine, and iodic acid. In this reaction, iodic acid was used as an oxidant to form [I$^-$] species$^{100}$ (Eq. 1) which the aromatic ring (nucleophile) attacked, affording a substitution product (Eq. 2).

Equation 1
\[2\text{I}_2 + \text{HIO}_3 + 5\text{H}^+ \leftrightarrow 5[\text{I}^+] + 3\text{H}_2\text{O}\]  \hspace{2cm} (1)

Equation 2

\[\text{Ar-H} + \text{I}^+ \leftrightarrow \text{Ar-I} + \text{H}^+\] \hspace{2cm} (2)

Equation 3

\[5\text{C}_{13}\text{H}_{10} + 2\text{I}_2 + \text{HIO}_3 + 5\text{H}^+ \leftrightarrow 5\text{C}_{13}\text{H}_9\text{I} + 3\text{H}_2\text{O}\] \hspace{2cm} (3)

Equation 4

\[5\text{C}_{13}\text{H}_{10} + 4\text{I}_2 + 2\text{HIO}_3 + 10\text{H}^+ \leftrightarrow 5\text{C}_{13}\text{H}_8\text{I}_2 + 6\text{H}_2\text{O}\] \hspace{2cm} (4)

The resulting product 2a was identical to literature reports upon comparison of the mp and the NMR spectroscopic data.\(^{101}\) A method for the alkylation of fluorene derivatives can utilize tosylates of polyethylene glycol, synthesized from commercial reagents, as a good leaving group (Fig. 9).\(^{102}\)

![Figure 10. The Mechanism of Reaction of Fluorene Derivative with Alkyl Tosylate.](image)

Compound 2a underwent alkylation with 2-(2-ethoxy ethoxy)ethyl-1-(4-methylbenzenesulfonylate) 3a and 2-(2-(2-ethoxy ethoxy)ethoxy)ethoxy)methyl-1-(4-methylbenzenesulfonylate) 3b (Scheme 2) in DMSO at room temperature to obtain compounds 4a and 4b in 55 and 40 % yield, respectively. Compounds 3a and 3b were synthesized in 95 and 90 % yields, respectively, utilizing a nucleophilic substitution reaction of 2-(2-ethoxy ethoxy)ethanol and tetraethylene glycol methyl ether with 4-methylbenzenesulfonyl chloride.
in pyridine at 5 °C for a period of 3 h. The NMR spectra of compounds 3a and 3b were identical to those reported in the literature.\textsuperscript{103,104} Compound 6a was synthesized in 68 % yield by the Sonogashira cross-coupling reaction (Appendix B1) of compound 4a with compound 5a.\textsuperscript{105,106} The \textsuperscript{1}H NMR spectrum of compound 6a showed signals at 8, 7.8, and 7.3 δ whose integration represent a total of 22 aromatic protons. Compound 5a (Scheme 3) was synthesized from a condensation reaction of 4-iodobenzoic acid with 2-amino thiophenol to form the first intermediate, 2-(4-iodophenyl)benzothiazole. This was then transformed to a second intermediate 2-(4-(1-trimethylsilyl)ethynylphenyl)benzothiazole by the Sonogashira reaction with trimethylsilylacetylene in THF in 75 % yield. Finally, this was hydrolyzed in 80 % yield with a solution of 5 % potassium hydroxide in methanol at room temperature for 30 min. The mp of compound 5a was identical to the literature value.\textsuperscript{107} The \textsuperscript{1}H NMR spectrum of compound 5a showed a signal at 3.4 δ for a terminal alkyne. The \textsuperscript{13}C NMR spectrum of compound 6a showed signals at 90 and 92.5 ppm for sp-carbons in this molecule. The HRMS analysis of compounds 5a and 6a gave molecular ions at 235.0449 and 864.3049, respectively, which were consistent with the formulas of C\textsubscript{15}H\textsubscript{19}NS and C\textsubscript{55}H\textsubscript{48}O\textsubscript{4}N\textsubscript{2}S\textsubscript{2}. Compound 6b was synthesized in 60 % yield via Heck coupling reaction\textsuperscript{108} (Appendix B2) of compound 4b with 2-(4-ethenylphenyl)benzothiazole 5b, which was synthesized from a procedure reported in the literature.\textsuperscript{46} Signals were present in compound 6b at 7.4, 7.8, and 8 δ which integrated for a total of 26 aromatic protons. The HRMS analysis of compound 6b confirmed the molecular ion at 1017.4177 for the formula C\textsubscript{61}H\textsubscript{64}O\textsubscript{8}N\textsubscript{2}S\textsubscript{2}. 

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Scheme 1. Synthesis of Acceptor-π-Acceptor Derivatives 6a and 6b

Scheme 2. Synthesis of 3a-c

Synthesis of Acceptor-π-Donor Fluorene-based Derivatives

Scheme 4 shows the formation of 2-iodofluorene 2b, prepared in 78 % yield by monoiiodination of fluorene using 0.4 molar eq of iodine (Eq. 3). The mp and the NMR spectrum of compound 2b were identical upon comparison with the values from the literature. Alkylation of compound 2b with 2-(2-ethoxy ethoxy)ethyl tosylate 3a was conducted to obtain 2-iodo-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 4c in 60 % yield. Nitration then led to 2-iodo-7-nitro-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 5c in 50 % yield. Heck coupling reaction of compound 5c with 5b resulted in compound 6c in 60 % yield. 6c was then reduced with hydrazine on 10 % Pd/C in a mixture of ethanol and THF at 70 °C to obtain compound 7a in 95 % yield. Alkylation of compound 7a with ethyl bromoacetate in DMF yielded compound 8a in 75 % yield. Finally, compound 8b was synthesized in 62 % yield by an Ullmann condensation reaction of 7a with iodobenzene. The 1H NMR spectrum of compound 8a showed signals at 4.21 and 1.26 δ resulting in an integration representing a total of 8 protons (ethoxy groups and amino methylene groups) and 6 protons of two ethoxy moieties in this molecule. The 13C NMR spectrum of compound 8a showed a signal at 170.86 ppm corresponding to ester carbons. Signals were present at 6.6, 7, 7.3, 7.5, and 8.0 δ which were integrated for a total of 26 aromatic protons in 8b. The HRMS of compounds 8a and 8b confirmed molecular ions at 821.383 and 801.3821 for the corresponding formulas C_{48}H_{56}O_{8}N_{2}S and C_{52}H_{52}O_{4}N_{2}S, respectively. Finally, compound 9a was obtained in 40 % yield by a hydrolysis reaction of compound 8a with sodium hydroxide in methanol at 70 °C. The MS of 9a a signal at 764 for a molecular ion of a formula of C_{44}H_{48}O_{8}N_{2}S.
Scheme 4. Synthesis of Acceptor-π-Donor Derivatives 8a, 8b, and 9a

Scheme 5 illustrates that bis(1,2,3-triazolyl) fluorene derivatives 9b-d were synthesized from 7a and 7b by “click” chemistry, a cycloaddition reaction of an alkyne and an azide (Appendix B3). Click reactions are more practical and reliable for large thermodynamic driving forces of greater than 20 Kcal.mol⁻¹. Compounds 7a and 7b underwent alkylation with propargyl bromide in DMF at 95 °C to obtain compounds 8c and 8d, respectively. Click reactions of compounds 8c and 8d with 1-azidobutane and 1-azido-11-undecanethiol (Scheme 5), utilizing a copper(I) catalyst, gave compounds 9b, 9c, and 9d in
72, 65, and 60 % yields, respectively. 1-Azidobutane was prepared by a nucleophilic substitution reaction of 1-bromobutane with sodium azide in DMSO at 95 °C for 24 h. The NMR spectrum of 1-azidobutane was identical to a literature report. Likewise, 1-azido-11-undecanethiol was synthesized in 80 % overall yield from 1-bromo-11-undecanol in 3 steps (Scheme 6). The first step was a nucleophilic substitution reaction of 1-bromo-11-undecanol with sodium azide at 80 °C in acetonitrile for 20 h, yielding the first intermediate 1-azido-1-undecanol in 97% yield. This intermediate was reacted with 4-methylbenzenesulfonyl chloride in pyridine at 5 °C for 3 h to yield the second intermediate, 1-azido-11-undecanyl tosylate, in 92 % yield. Lastly, a nucleophilic substitution reaction of the tosylate with thiourea at 70 °C for 10 h, followed by hydrolysis with sodium hydroxide at 100 °C led to 1-azido-11-undecanethiol. The NMR data of 1-azido-11-undecanethiol were also identical with values from the literature.

Scheme 5. Synthesis of Acceptor-π-Donor Derivatives 9b-d
Compounds 8e-g were synthesized in Scheme 7. First, compound 8e was prepared in 3 steps from the Sonogashira coupling reaction of 5c with 5a at room temperature to obtain 6e in 78 % yield, and then it was reduced by hydrazine on 10 % Pd/C to obtain 7c in 90 % yield. Compound 7c underwent alkylation and amine arylation with propargyl bromide and iodobenzene, respectively, to obtain 8e and 8f in 70 and 67 % yields, respectively. The \textsuperscript{1}H NMR spectrum of 8e showed signals at 6.9, 7.3, 7.9, and 8.0 \( \delta \) which were integrated for a total of 14 aromatic protons. Signals were present at 4.13 and 2.68 \( \delta \) which integrated corresponding to 4 aminomethylene protons and 2 alkyne protons, respectively, in 8f. In addition, the \textsuperscript{13}C NMR spectrum of compound 8f showed signals at 78.96 and 73.1 ppm which were consistent with the propargyl functionality in this molecule. The HRMS analysis of 8e and 8f gave molecular ions at 722.3172 and 798.03485, respectively, which consistent with formulas C\textsubscript{46}H\textsubscript{46}O\textsubscript{4}N\textsubscript{2}S and C\textsubscript{52}H\textsubscript{50}O\textsubscript{4}N\textsubscript{2}S, respectively. Compound 6f was synthesized in 60 % yield from the Stille reaction (Appendix B4) of compound 5c with (2-tri-n-butylstannyl)benzothiazole (which was prepared according to the literature).\textsuperscript{110} Likewise, 7d was synthesized in 85 % yield by reduction of 6f with hydrazine on 10 % Pd/C in a mixture of ethanol and THF at 70 \( ^\circ \)C. Finally, compound 8g was synthesized in 60 % yield from the
Ullmann condensation reaction of 7d with 4-iodoanisole. The $^1$H NMR spectrum of 8g showed signals at 3.8, 6.9, 7.1, 7.4, 7.5, 7.9, and 8.1 $\delta$ that were integrated for a total of 6 methoxy protons and 18 aromatic protons, respectively. The HRMS analysis of 8g gave a molecular ion at 758.3383, consistent with the formula C$_{46}$H$_{50}$O$_6$N$_2$S.

Scheme 7. Synthesis of Acceptor-$\pi$-Donor Derivatives 8e-g

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Fluorene-based derivative 10, with the acceptor-$\pi$-donor archetype (Scheme 8), was synthesized in 5 steps from 2-iodo-7-nitro-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 5c. The first step involved the Sonogashira reaction of 5c with trimethylsilylacetylene at room temperature for 12 h, resulting in 6g in 70 % yield, which was then hydrolyzed with potassium hydroxide to obtain 7e in 90 % yield. Click chemistry was then utilized to
synthesize 8h in 80 % yield from a click reaction of 7e with azidobenzene in dioxane, using copper(I) iodide catalyst at room temperature for 24 h. The $^{13}$C NMR spectrum of 7e showed a signal at 148 ppm for the presence of a carbon in the new 1,2,3-triazole moiety.

Azidobenzene was synthesized from phenylhydrazine by utilizing a procedure reported by Lindsay and Allen.$^{120}$ The IR spectrum of azidobenzene showed a strong absorption band at 2125 cm$^{-1}$ for an azide functionality. The NMR spectra of azidobenzene were identical upon comparison with the NMR data reported in the literature.$^{121}$ Compound 9e was synthesized in 90% yield via reduction of 8h with hydrazine on 10 % Pd/C at 70 °C. Finally, 10 was synthesized in 60 % yield by the Ullmann condensation reaction of 9e with iodobenzene in 1,2-dichlorobenzene at 180 °C for 24 h. The $^1$H NMR spectrum of 10 had signals at 7.03, 7.25, and 7.75 δ which were integrated for a total of 22 aromatic protons. The HRMS analysis of 10 gave a molecular ion at 708.3669, consistent with the formula C$_{45}$H$_{48}$N$_4$O$_4$.

Scheme 8. Synthesis of Acceptor-π-Donor Derivative 10
Compound $8i$ (Scheme 9) was prepared in 4 steps from 2-iodofluorene $2b$. The first step involved bromination of $2b$ with N-bromosuccinimide in propylene carbonate at $80^\circ$C to obtain 2-iodo-7-bromofluorene $3d$ in 80 % yield. The bromoiodofluorene was alkylated with $3a$ to obtain 2-iodo-7-bromo-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene $4e$ in 60 % yield. The mp and the NMR spectrum of compound $3d$ to literature values.$^{122}$ The last steps involved the Sonogashira reactions of $4e$ consecutively with $5a$ and $7f$, affording $8i$ in 31 % overall yield. Compound $6h$ was synthesized from $5a$ by a Sonogashira cross-coupling reaction selective for an iodoarene due to its higher reactivity versus bromoarene (the dissociation energy of C-I and C-Br bonds are 65 and 81 Kcal.mol$^{-1}$ respectively).$^{123}$ 4-Ethynyl-N,N-dimethylbenzenamine ($7f$, Scheme 10) was synthesized from an iodination reaction of N,N-dimethylaniline with iodine in aqueous sodium bicarbonate solution$^{124}$ to obtain intermediate 4-iodo-N,N-dimethylbenzenamine in 70 % yield. 4-Iodo-N,N-dimethylbenzenamine was reacted with trimethylsilylacetylene using PdCl$_2$(PPh$_3$) and CuI as catalysts$^{125}$ to obtain a second intermediate, 4-(2-trimethylsilylethynyl)-N,N-dimethylbenzenamine, in 70 % yield. Finally, the later product was hydrolyzed with a solution of potassium hydroxide, producing compound $7f$ in 80 % yield. The NMR spectra of $7f$ were identical with the literature.$^{126}$ The $^1$H NMR spectrum of $8i$ showed signals at 6.7, 7.4, 7.8, and 8.1 $\delta$ which were integrated for a total of 18 aromatic protons. The $^{13}$C NMR spectrum of compound $8i$ showed signals at 92 and 88.1 ppm which were consistent with the presence of two sp-carbons in the new molecule. The HRMS analysis of compound $8i$ gave a molecular ion at 774.3485 for a formula of C$_{50}$H$_{50}$O$_4$N$_2$S.
Scheme 9. Synthesis of Acceptor-π-Donor Derivative 8i

Scheme 10. Synthesis of 7f

2.1.1.3. Synthesis of Donor-π-Donor Fluorene-based Derivatives

Fluorene-based derivatives structural type donor-π-donor compounds 5e, 5f, and 7g (Scheme 11) were also synthesized. First, 5e was prepared in 72% yield from the Sonogashira reaction of 4f with 4-ethynyl-N,N-dimethylbenzenamine 7f catalyzed by dichlorobis(triphenylphosphine)palladium(II) in THF at room temperature. The $^1$H NMR spectrum of 5e showed signals 7.4 and 6.7 $\delta$ which were integrated for a total of 14 aromatic protons. The $^{13}$C NMR spectrum of 5e revealed signals at 91.46, 88.08, and 51.12 ppm, assignable to two alkyne carbons and an amimo carbon, respectively. The HRMS analysis of 5e gave a molecular ion at 1361.7881 for a formula of C$_{75}$H$_{112}$O$_2$N$_2$. Compound 5f was synthesized in 62% yield from the Ullmann condensation of 4a with diphenylamine at 180 $^0$C for 24 h. The $^1$H NMR spectrum of 5f showed signals at 7, 7.2, and 7.5 $\delta$ which were
integrated for a total of 26 aromatic protons. The HRMS analysis of compound 5f gave a molecular ion at 732.3960, consistent with the formula C_{49}H_{52}O_{4}N_{2}. Finally, 7g was synthesized in 3 steps from 4a. The first step involved the Sonogashira coupling reaction of 4a with trimethylsilylacetylene to obtain intermediate 5g in 75 % yield. Hydrolyzed of 5g resulted in a second intermediate 6i in 95 % yield. Lastly, a second Sonogashira reaction of 6h with 4-iodophenol produced 7g in 58 % yield. The IR spectrum of 7g showed a broad absorption at 3273 cm\(^{-1}\) for phenolic moieties. The \(^1\)H NMR spectrum of 7g showed signals at 6.84 and 7.27 δ which were integrated for a total of 14 aromatic protons. The HRMS analysis of 7g gave a molecular ion at 630.2975 which was consistent with a formula of C_{41}H_{42}O_{6}. Bis-phenol derivative 7g has pK\(_1\) = 10.6 and pK\(_2\) = 11.6, determined by a spectrophotometric method and calculated by the Henderson-Hasselbalch equation.\(^{127,128}\) This compound has a high quantum yield value (\(\phi_{Fl} = 0.95\)) for the phenolic neutral form (at pH < 10). However, the anionic form of its phenolate anion (at pH = 11 and above) was nonfluorescence (\(\phi_{Fl} = 0\)) which can be further investigated for its fluorescent sensing of pH at basic media.
2.1.1.4. Synthesis of Fluorescent Gold Nanoparticles

Scheme 12 shows that 11a and 11b were synthesized from reactions of 9b and 9c with gold nanoparticles. First, gold nanoparticles were synthesized according to the literature, then reacted with 9b and 9c to yield hybrid composites 11a and 11b, respectively.

Scheme 12. Synthesis of Fluorescent Gold Nanoparticles
2.1.2. Thermogravimetric Analysis

The new hydrophilic fluorene-based derivatives generally displayed high thermostabilities (Figs. 11 - 24). In general, fluorene-based derivatives decomposed at temperatures above 300 °C with a low percentage weight loss (< 5 %). For example, 6b and 8b (Figs. 13 and 15) exhibited a very high decomposition temperature (above 400 °C). A sp-hybridized carbon conjugated π-system was relatively less percentage weight loss than its analog of a sp²-hybridized carbon conjugated π-system (Figs. 13, 15, 16). A higher number of ethylene oxide units in the substituents had slightly lower thermostability than is hydrocarbon analogs (Fig. 23).

Figure 11. The TGA of 5e.

Figure 12. The TGA of 5f.
Figure 13. The TGA of 6a and 6b.

Figure 14. The TGA of 7g.

Figure 15. The TGA of 8b and 8f.
Figure 16. The TGA of $8c$ and $8e$.

Figure 17. The TGA of $8a$.

Figure 18. The TGA of $8c$ and $8d$. 
Figure 19. The TGA of 8g.

Figure 20. The TGA of 8i.

Figure 21. The TGA of 9a.
Figure 22. The TGA of 9b.

Figure 23. The TGA of 9c and 9d.

Figure 24. The TGA of 10.
2.1.3. Linear Photophysical Characterization of Hydrophilic Fluorene-based Derivatives

2.1.3.1. Ethylene Oxide Unit Substituents Effect on Solubility

In general, the hydrophilicity of new fluorene-based derivatives increases when a larger number of ethylene oxide units are present in the molecules. For example, by introduction of diethylene oxide units (n = 2) into fluorene-based derivatives make them soluble in polar organic solvents like ethyl acetate. However, tetraethylene oxide substituents (n = 4, 6b) gave good solubility only in highly polar organic solvents like THF, DMSO, and in alcohols. A higher degree of polyethylene oxide unit substitution (n = 10) gave water soluble molecules (5e and 9d).

2.1.3.2. Photostability of Hydrophilic Fluorene-based Derivatives

Photodecomposition analysis (ΦD) of fluorene-based derivatives was determined by the absorption method utilizing linear (one-photon) excitation at 405 nm, and calculated according to the literature. In general, a sp-hybridized carbon conjugated π-system has value one order of magnitude smaller photodecomposition quantum yield (i.e., is one order of magnitude more photostable) in comparison to its sp²-hybridized conjugated π-system analog (Table 1).

2.1.3.3. UV-vis Absorption and Fluorescence Emission of Fluorene-based Derivatives

UV-vis absorption and fluorescence emission spectra of hydrophilic fluorene-based derivatives were recorded (Figs. 25 - 41). As expected, dipolar derivatives displayed a larger Stokes shift when the polarity of solvent increased compared to quadrupolar derivatives. A red shift was observed for the UV-vis absorption and fluorescence emission of hydrophilic fluorene-based derivatives when the conjugated π-system in the molecules increased.
However, a sp²-hybridized carbon conjugated π-system had a slight bathochromic shift (15-25 nm) relative to its sp-hybridized conjugated π-system analog (Tables 2 and 3). This could be due to its electronic delocalization in triple bond is weaker than its analog of the double bond respectively.

2.1.3.4. Quantum Yield of Hydrophilic Fluorene-based Derivatives

The fluorescence quantum yields\textsuperscript{130} of hydrophilic fluorene-based derivatives in THF, toluene, and DMSO were determined and tabulated (Tables 2 and 3). The UV-vis absorption and fluorescence emission of compounds 6a and 6b, 8b and 8f, 8c, and 8e showed a blue-shift both in absorption and emission for sp-hybridized carbon conjugated π-system analogs vs. sp²-hybridized carbon conjugated π-system analogs.

Table 2. Linear Photophysical Characterization of Fluorene-based Derivatives in THF

<table>
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<tr>
<th>Solvent</th>
<th>Tetrahydrofuran</th>
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<tr>
<td>Compound</td>
<td>ϕ \text{Fl}</td>
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<tr>
<td>5e</td>
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<tr>
<td>5f</td>
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<tr>
<td>6a</td>
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<td>6b</td>
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<td>8a</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>8e</td>
<td>0.50</td>
</tr>
<tr>
<td>8f</td>
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Table 3. Linear Photophysical Characterizations of Fluorene-based Derivatives in Toluene and DMSO

<table>
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</tr>
<tr>
<td>8i</td>
<td>0.95</td>
<td>0.76</td>
</tr>
<tr>
<td>9a</td>
<td>0.95</td>
<td>64</td>
</tr>
</tbody>
</table>

<sup>a</sup> ε = 97000 M<sup>-1</sup>.cm<sup>3</sup>, λ<sub>ab</sub> = 357 nm, λ<sub>em</sub> = 411 nm in acetonitrile
<table>
<thead>
<tr>
<th></th>
<th>9b</th>
<th>9c</th>
<th>9d</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>φ</td>
<td>0.92</td>
<td>0.93</td>
<td>0.95</td>
<td>0.82</td>
</tr>
<tr>
<td>λ&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>1.15</td>
<td>1.54</td>
<td>1.13</td>
<td>0.71</td>
</tr>
<tr>
<td>λ&lt;sub&gt;em&lt;/sub&gt;</td>
<td>5.4</td>
<td>5.2</td>
<td>5.1</td>
<td>3.4</td>
</tr>
<tr>
<td>ε</td>
<td>408</td>
<td>407</td>
<td>407</td>
<td>371</td>
</tr>
<tr>
<td>λ&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>501</td>
<td>504</td>
<td>505</td>
<td>401</td>
</tr>
<tr>
<td>λ&lt;sub&gt;em&lt;/sub&gt;</td>
<td>93</td>
<td>97</td>
<td>98</td>
<td>30</td>
</tr>
<tr>
<td>ε</td>
<td>418</td>
<td>415</td>
<td>417</td>
<td>367</td>
</tr>
<tr>
<td>λ&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>655</td>
<td>650</td>
<td>651</td>
<td>439</td>
</tr>
<tr>
<td>λ&lt;sub&gt;em&lt;/sub&gt;</td>
<td>237</td>
<td>235</td>
<td>234</td>
<td>72</td>
</tr>
</tbody>
</table>

a: φ = 0.43, λ<sub>ab</sub> = 382 nm, λ<sub>em</sub> = 516 nm in water. b: ε = 85000 M<sup>-1</sup>.cm<sup>-1</sup>, λ<sub>ab</sub> = 357 nm, λ<sub>em</sub> = 423 nm in MeOH.

2.1.3.5. Anisotropy of Fluorene-based Derivatives

Fluorescence anisotropy analysis of hydrophilic fluorene-based derivatives aided in assigning various electronic transitions, e.g., corresponding to transition from the ground state (S<sub>0</sub>) to the first excited state (S<sub>1</sub>), and are shown in Figs. 25 - 41. The hydrophilic fluorene-based derivatives typically displayed values of anisotropy from 0.30 - 0.35 at λ<sub>ab</sub><sub>max</sub>.

2.1.4. Nonlinear Optical Properties of Hydrophilic Fluorene-based Derivatives

Two-photon absorption cross sections of hydrophilic fluorene-based derivatives with acceptor-π-acceptor, acceptor-π-donor, and donor-π-donor archetypes were determined, generally by a femtosecond two-photon upconverted fluorescence technique (Figs. 25-41).
Figure 25. Normalized Absorbance, Emission, Anisotropy, and 2PA of 6a in THF.

Figure 26. Normalized Absorbance, Emission, Anisotropy, and 2PA of 6b in THF.
Figure 27. Normalized Absorbance, Emission, Anisotropy, and 2PA of 5f in THF.

Figure 28. Normalized Absorbance, Emission, Anisotropy, and 2PA of 7g in THF.
Figure 29. Normalized Absorbance, Emission, Anisotropy, and 2PA of 5e in THF.

Figure 30. Normalized Absorbance, Emission, Anisotropy, and 2PA of 8a in Toluene.
Figure 31. Normalized Absorbance, Emission, Anisotropy, and 2PA of 8b in Toluene.

Figure 32. Normalized Absorbance, Emission, Anisotropy, and 2PA of 8c in Toluene.
Figure 33. Normalized Absorbance, Emission, Anisotropy, and 2PA of 8e in Toluene.

Figure 34. Normalized Absorbance, Emission, Anisotropy, and 2PA of 8f in Toluene.
Figure 35. Normalized Absorbance, Emission, Anisotropy, and 2PA of 8g in Toluene.

Figure 36. Normalized Absorbance, Emission, Anisotropy, and 2PA of 8i in Toluene.
Figure 37. Normalized Absorbance, Emission, Anisotropy, and 2PA of 9a in THF.

Figure 38. Normalized Absorbance, Emission, Anisotropy, and 2PA of 9b in THF.
Figure 39. Normalized Absorbance, Emission, Anisotropy, and 2PA of \(9c\) in THF.

Figure 40. Normalized Absorbance, Emission, Anisotropy, and 2PA of \(9d\) in THF.
2.1.4.1. The Strength of Donor and Acceptor Groups

Linear and nonlinear absorption properties of hydrophilic fluorene derivatives, possessing different electron donor and acceptor groups, were compared. A 1,2,3-triazole moiety in 10 is a weak acceptor (Fig. 41) while a hydroxy moiety is a relatively strong donor (Fig. 28), showing an increase in the fluorescence quantum yield. Moreover, an increase the strength of a donor, such as a methoxy substituent in compound 8g, contributed to the red shift of its UV-vis absorption and fluorescence emission spectra, and also resulted in an increased 2PA cross section (Fig. 35).

2.1.4.2. Quadrupolar versus Dipolar Molecules

Quadrupolar fluorene-based derivatives displayed a greater value of 2PA near the visible region (Figs. 25 and 26), however, dipolar fluorene-based derivatives exhibited a
greater 2PA at longer wavelengths in the near-IR region (Figs. 30, 35 - 40).

2.1.4.3. Sp-hybridized Carbon Conjugated π-System vs Sp²-hybridized Carbon Conjugated π-System

An extended conjugated π-system of a fluorene-based derivative exhibited red shifted UV-vis absorption and fluorescence emission spectra relative to shorter conjugation analogs, and an increase in the 2PA cross section values, e.g., 8b and 8e vs. 5f and 10 (Figs. 31 and 33 vs. Figs. 27 and 41). For dipolar molecules, such as 8b and 8f (Figs. 31 and 34) or 8c and 8e (Figs. 32 and 33), there were little differences in the 2PA cross sections value of a sp²-hybridized carbon conjugated π-system in comparison to its analogous sp-hybridized carbon conjugated π-system. For molecules of high symmetry, this is likely due to the fact that 2PA is forbidden at low energy (long wavelength) while it is allowed at higher energy. In addition, dipolar molecules often have low-energy charge-transfer transitions that result in 2PA in normally two-photon forbidden (one-photon allowed) transitions.

For quadrupolar molecules, such as 6a and 6b (Figs. 26 and 27), a higher value of the 2PA cross section for a sp²-hybridized carbon conjugated π-system vs. its sp-hybridized carbon conjugated π-system analog was observed. This may be due to a large contribution of 2PA cross sections from the charge transfer (CT) and change in the quadrupole moment that enhances the corresponding transition dipole moments in the excited state upon photoexcitation. It is likely that an alkene-π-bridge may enables a higher polarizability or hyperpolarizability density since the double bond is longer than the triple bond (1.3 Å vs. 1.2 Å), hence greater electronic delocalization and extent of charge transfer transition towards the second-order response to increase its 2PA cross sections.
A figure of merit, $F_M$, that accounts not only for the 2PA cross section and fluorescence quantum yield but, importantly, for the compound’s photostability (i.e., photodecomposition quantum yield) is defined in Eq. 5. The $F_M$ values for several fluorene derivatives are presented in Table 4, along with values for Rhodamine B and Rhodamine 6G (see notes under the table).

Equation 5

$$F_M = \frac{\delta_{2PA} \Phi_{Fl}}{\Phi_D} \tag{5}$$

Table 4. Two-photon Absorption Cross Sections and Figure of Merit of Hydrophilic Fluorene-based Derivatives in THF/Toluene

| Solvent | THF | | Solvent | Toluene | |
|---------|-----|---------|---------|--------|
| Compound | $\lambda_{ex}$ (nm) | $\delta$ (GM) | $a$,$b$ $F_M, 10^{-6}$ | | $\lambda_{ex}$ | $\delta$ | $F_M, 10^{-6}$ |
| $5e$ | 690 | 1507 | 31.4 | $8a$ | 690 | 357 | 1.5 |
| | 820 | 48 | 1 | | 800 | 211 | 0.9 |
| $5f$ | 740 | 10 | 0.7 | $8b$ | 690 | 208 | 2.4 |
| | 6a | 690 | 579 | 157 | | 820 | 75 | 0.8 |
| | 820 | 11 | 3 | | | | |
| | $6b$ | 690 | 1799 | 63.3 | | 820 | 195 | 0.8 |
| | 820 | 94 | 3.3 | $8c$ | 690 | 409 | 1.7 |
| | 710 | 59 | 3.3 | | 780 | 117 | 3.4 |
| $7g$ | 820 | 306 | 0.2 | $8f$ | 700 | 170 | 130 |
| | 800 | 342 | 5.7 | | 820 | 255$^c$ | 195 |
| $9b$ | 750 | 124 | 0.7 | $8g$ | 690 | 85 | 8.9 |
| | 820 | 406 | 2.4 | | 840 | 180 | 18.9 |
| $9d$ | 820 | 390 | 3.7 | $8i$ | 690 | 749 | 93.6 |
| | 710 | 39 | 5.4 | | 820 | 125 | 15.6 |
| $10$ | | | | | | | |

a: Rhodamine B: $\delta_{2PA_{max}} = 263$ GM at 700 nm; $\Phi_{Fl} = 0.40$ in methanol$^{135}$; $\Phi_D(532$nm$) = 8 \times 10^{-7}$; $F_M = 130 \times 10^6$. b: Rhodamine 6G: $\delta_{2PA_{max}} = 132$ GM at 700 nm; $\Phi_{Fl} = 0.94$ in methanol; $\Phi_D(532$ nm$) = 1.3 \times 10^{-6}$; $F_M = 95 \times 10^6$. c: $\delta = 260$ GM by Z-scan method$^{136}$
2.1.5. Cell Imaging with Select Hydrophilic Fluorene-based Probes

Figures 42 - 45 show images of HTC 116 cells incubated with 20 μM solution of probes 5e, 6a, 8b, and 10 for 1 h. All images were collected at 60 x using an oil immersion objective with index-matching oil. Differential Interference Constrain (DIC) images were exposed for 400 ms; single-photon fluorescence images were exposed for 150 ms; 2-photon fluorescence was excited at 700 nm; and 3D images were obtained by overlaying of a series of X-Y plane two-photon fluorescence images.

The images of HTC 116 cells incubated with probe 5e (Fig. 42, grids are 5 μm in the lower right image) showed the dye 5e entered the cells with ease as the cell exhibited good morphology. Indeed, cell viability experiments were conducted with high concentrations of the dyes (up to 50 μM), a concentration higher than that required for imaging. However, cells were found alive for several hours without changing their morphology.
The images of the HTC 116 cells incubated with probe 6a (Fig. 43, grids are 5 μm in the lower right image) illustrate that probe 6a penetrated peripheries of the cellular membranes with a certain degree of localization in certain organelles.
The images of HTC 116 cells incubated with probe 8b (Fig. 44, grids are 5 μm in the lower right image) showed that probe 8b preferred peripheries of cellular membranes and exhibited a lesser degree of localization in certain organelles.
Figure 44. The Images of the HTC 116 Cells Incubated Probe 8b.

The images of HTC 116 cells incubated probe 8i (Fig. 45), where the scale bars are 10 μm (lower left and upper right) and the grids are 5 μm (lower right), showed that dye 8i also penetrated into the cells with a higher degree of localization in certain organelles.
Figure 45. The Images of the HTC 116 Cells Incubated Probe 8i.

Figure 46 showed the colocalization study of probe 5e(20 μ M) and LysoTracker Red (75 nM) incubated 1h in HCT 116 towards lysosome. The single-photon images of cells exposed 200 ms for 5e (left) and LysoTracker Red (middle left). The DIC image exposed 550 ms (middle right), and the overlapped images of 5e and LysoTracker Red (right). The colocalization coefficient was 0.98 and calculated by the Slide book program.
In general, the images of HTC 116 cells showed that hydrophilic fluorene-based derivatives can be employed as new fluorescent probes for single-photon and two-photon fluorescence excitation. The fluorescent probes dispersed well into cell membranes for a bright fluorescence contrast and imaging for 2PA at 700 nm and above.

2.2. Hydrophilic Fluorene-based Derivatives for Metal Ion Sensing

Compounds 9b, 9c, and 9d were investigated for sensing of selective metal ions, since bis(1,2,3-triazole) moieties in these compounds may enhance metal chelation.\(^{137,138}\)

2.2.1. Response towards Metals

The metal ion complexes of compounds 9b-d were screened by UV-vis absorption and fluorescence emission spectrophotometry for selectivity sensing of metals. The fluorescence response factor \((F/F_0)\) was measured by comparison of the maximum integrated area of the corrected spectrum of the metal complex, i.e., metal and ligand, \((F)\) with that area
of the dye, i.e., ligand, (9b-d) at initial stage (F₀). The dye concentration was 4x10⁻⁶ M in ethanol. The relevant ions of alkali, alkaline earth, and transition metals with concentrations varying from 10⁻⁶ to 10⁻⁴ M in ethanol (Table 5) can be divided to group A, consisting of Ba, Ca, Ce, Cs, Hg(I), K, La(III), Li, Mg, Mn(II), Na, and Sn(II) that showed no fluorescence response; group B, consisting of Al, Au, Bi(III), Cr(III), Cu(I), Cu(II), Eu(III), Fe(II), In(III), and Ni (Fig. 47) that displayed a slight decrease in fluorescence; group C, consisting of Co(III), Fe(III), Ir(III), Pd, Pt(III), Rh(III), Ru(III), and Ti(IV) that exhibited a fluorescence quenching response; and group D, consisting of Ag, Cd, Hg, Pb(II), and Zn where an enhanced fluorescence response was observed (Fig. 48).

Table 5. Selected Metals for UV-vis and Fluorescence Sensing towards Compounds 9b-d

<table>
<thead>
<tr>
<th>Li</th>
<th>Na</th>
<th>Mg</th>
<th>Al</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>Ca</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ti</td>
<td>V</td>
<td>Cr</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>Fe</td>
<td>Co</td>
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<tr>
<td></td>
<td>Ni</td>
<td>Cu</td>
<td>Zn</td>
</tr>
<tr>
<td>Sr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cs</td>
<td>Ba</td>
<td>La</td>
<td></td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ir</td>
<td>Pt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Au</td>
<td>Hg</td>
<td>Pb</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lanthanides</td>
<td></td>
<td>Ce</td>
<td>Eu</td>
</tr>
</tbody>
</table>

Bi
A typical quenching of the fluorescence emission spectrum of compound 9b upon addition of iron(III) (Fig. 49) or palladium in ethanol (Fig. 50) are shown.
Figure 49. Fluorescence Spectra of 9b upon Addition of Iron(III) in Ethanol.

Figure 50. Fluorescence Spectra of 9b upon Addition of Palladium in Ethanol.
The UV-vis absorption and fluorescence emission spectra of the complexes of 9b-d with zinc (Figs. 51 - 54), mercury(II) (Figs. 55, 56), silver (Figs. 57, 58), lead(II) (Fig. 59), and cadmium (Fig. 60) showed moderately enhanced fluorescence responses, and a blue-shift that may be due to the change of the HOMO-LUMO gap of the complex relative to the dye alone (Fig. 61).\textsuperscript{73}

![Figure 51. UV-vis Absorption Spectra of 9b upon Addition of Zinc in Ethanol.](image1)

![Figure 52. Fluorescence Emission Spectra of 9b upon Addition of Zinc in Ethanol.](image2)
Figure 53. UV-vis Absorption Spectra of 9c upon Addition of Zinc in Ethanol.

Figure 54. Fluorescence Emission Spectra of 9c upon Addition of Zinc in Ethanol.

Figure 55. UV-vis Absorption Spectra of 9c upon Addition of Mercury in Ethanol.
Figure 56. Fluorescence Emission Spectra of \(9c\) upon Addition of Mercury in Ethanol.

Figure 57. UV-vis Absorption Spectra of \(9c\) upon Addition of Silver in Ethanol.

Figure 58. Fluorescence Emission Spectra of \(9c\) upon Addition of Silver in Ethanol.
Figure 59. Fluorescence Emission Spectra of 9b upon Addition of Lead in Ethanol.

Figure 60. Fluorescence Emission Spectra of 9c upon Addition of Cadmium in Ethanol.

Figure 61. HOMO-LUMO Diagram of the Chromophore and its Metal Complex.
2.2.2. Linear Photophysical Properties

Linear photophysical characterizations of probes 9b-d with metals are presented in Table 6.

Table 6. Linear Photophysical Characterization of 9b-d and their Metal Complexes

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ethanol</th>
<th>Tetrahydrofuran</th>
<th>Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>$\phi_{FI}$</td>
<td>$\lambda_{ab\text{ max}}$</td>
<td>$\lambda_{em}$</td>
</tr>
<tr>
<td>9b</td>
<td>0.32</td>
<td>400</td>
<td>609</td>
</tr>
<tr>
<td>9c</td>
<td>0.31</td>
<td>401</td>
<td>612</td>
</tr>
<tr>
<td>9d</td>
<td>0.31</td>
<td>403</td>
<td>610</td>
</tr>
<tr>
<td>9b+Zn</td>
<td>0.51</td>
<td>385</td>
<td>542</td>
</tr>
<tr>
<td>9c+Zn</td>
<td>0.41</td>
<td>386</td>
<td>545</td>
</tr>
<tr>
<td>9d+Zn</td>
<td>0.45</td>
<td>384</td>
<td>537</td>
</tr>
<tr>
<td>9b+Hg</td>
<td>0.46</td>
<td>382</td>
<td>540</td>
</tr>
<tr>
<td>9c+Hg</td>
<td>0.47</td>
<td>383</td>
<td>530</td>
</tr>
<tr>
<td>9d+Hg</td>
<td>0.45</td>
<td>382</td>
<td>521</td>
</tr>
<tr>
<td>9b+Ag</td>
<td>0.53</td>
<td>392</td>
<td>582</td>
</tr>
<tr>
<td>9c+Ag</td>
<td>0.53</td>
<td>395</td>
<td>571</td>
</tr>
<tr>
<td>9d+Ag</td>
<td>0.50</td>
<td>392</td>
<td>560</td>
</tr>
</tbody>
</table>

2.2.3. Response with Solvents

The UV-vis absorption and fluorescence emission of complexes of 9a-c with zinc and mercury were investigated in acetonitrile, ethanol, THF, and aqueous acetonitrile (Figs. 62 - 66). The UV-vis absorption and fluorescence emission spectra of the complexes of 9b-d with metal ions were similar in organic solvents, however, as expected, a larger Stokes shift in the fluorescence emission was observed when the polarity of the solvent increased.
Figure 62. Normalized Absorbance, Emission Spectra of $9b$ and Its Complexes in Ethanol.

Figure 63. Normalized Absorbance, Emission Spectra of $9c$ and Its Complexes in ACN.

Figure 64. Normalized Absorbance, Emission Spectra of $9d$ and Its Complexes in ACN.
2.2.4. Binding Constants of Metal Complexes

The binding constants of the complexation of \(9b-c\) with metals were determined by a fluorescence titration method (Figs. 67 - 75).
Figure 67. The Linear Fit of 9b upon Addition of Zinc in Ethanol.

Figure 68. The Linear Fit of 9c upon Addition of Zinc in Ethanol.

Figure 69. The Linear Fit of 9d upon Addition of Zinc in Ethanol.
Figure 70. The Linear Fit of 9b upon Addition of Mercury in Ethanol.

Figure 71. The Linear Fit of 9c upon Addition of Mercury in Ethanol.

Figure 72. The Linear Fit of 9d upon Addition of Mercury in Ethanol.
Figure 73. The Linear Fit of 9b upon Addition of Silver in Ethanol.

Figure 74. The Linear Fit of 9c upon Addition of Silver in Ethanol.

Figure 75. The Linear Fit of 9d upon Addition of Silver in Ethanol.
Table 7. Binding Constant Values of 9b-d with Metals in Ethanol.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zn</th>
<th>Hg</th>
<th>Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log K</td>
<td>n</td>
<td>log K</td>
</tr>
<tr>
<td>9b</td>
<td>8.0</td>
<td>2</td>
<td>14.5</td>
</tr>
<tr>
<td>9c</td>
<td>8.9</td>
<td>2</td>
<td>16.4</td>
</tr>
<tr>
<td>9d</td>
<td>9</td>
<td>2.1</td>
<td>16.5</td>
</tr>
</tbody>
</table>

From Table 7, the complexation of 9b-d suggested that a 2:1 metal:ligand for zinc ion and 3:1 metal:ligand for mercury(II) and silver ions occurred. These results are consistent with literature reports of 2:1 metal:ligand for cobalt-quercitin,139 zinc-p-tert-butylcalix[4]arene,140 and lithium-cryptand141 complexes.

2.2.5. Reversibility of the Complex

Complexes of 9a-c with metal ions formed rapidly (within minutes upon addition of metal ions), were generally stable for several hours, and were reversible, since addition of EDTA demonstrated that the metal can be displaced to release the uncomplexed (free) dyes (Eq. 6 and Figs. 76 - 78).

Equation 6

\[
[Dye-metal] + EDTA \leftrightarrow [EDTA-metal] + Dye
\] (6)
Figure 76. Fluorescence Emission of 9b/Zinc Complex toward EDTA in Ethanol.

Figure 77. Fluorescence Emission of 9b/Mercury Complex toward EDTA in Ethanol.

Figure 78. Fluorescence Emission of 9b/Silver Complex toward EDTA in Ethanol.
2.2.6. Two-photon Absorption Cross Sections of Metal Complexes

Two-photon absorption cross sections of 9b, 9c, and their corresponding complexes with zinc, mercury, and silver were determined under fs excitation and shown in Figs. 70 and 71. 2PA cross section values of the complexes decreased relative to the free dyes 9b and 9c. This observation may be due to a change in the transition dipole moment\textsuperscript{50} of a photoinduced charge transfer molecule\textsuperscript{39} (PCT) upon complexation with metal, or the presence of intraligand charge-transfer (ILCT) induction from interaction of a bound cation with an electron-donating group (such as an amino functionality) in 9b and 9c, consistent to that reported in the literature.\textsuperscript{142,143} These potential explanations are hypothetical and require more extensive work to elucidate, a subject for future investigations.

Figure 79. 2PA Cross Sections of 9b and its Complexes in Ethanol.
2.3. Hydrophilic Fluorene-based Derivatives Complexed to Gold Nanoparticles

The UV-vis absorption of 11a and 11b (complexes of gold nanoparticles and 9c and 9d, respectively) showed an absorption at 520 nm, quite typical for gold nanoparticles (Figs. 81, 82). The fluorescence emission of nanoparticle complexes 11a and 11b also showed a blue shift relative to the uncomplexed dyes with 2PA cross sections of 300 GM at 820 nm. The Raman scattering spectra of composites 11a and 11b showed strong scattering bands as evidence of the surface plasmon of gold nanoparticles (Fig. 83). TEM imaging of nanoparticle composites 11a and 11b revealed a particle size of 15-20 nm (Figs. 84, 85).
Figure 81. Compound 9c and Fluorescent Gold Nanoparticle Composite 11a in THF.

Figure 82. Normalized Absorbance, Emission, and 2PA Cross Section of Composites 11a and 11b in THF.

Figure 83. The Raman Scattering Spectra of Composites 11a and 11b.
Two-photon cross sections of composites 11a and 11b (Figs. 86 and 87) showed a decrease in the 2PA cross sections for fluorescent gold nanoparticle composites in comparison to (uncomplexed) 9c and 9d, respectively.
Figure 86. 2PA Cross Sections of 9c and 11a in THF.

Figure 87. 2PA Cross Sections of 9d and 11b in THF.
3.1. Materials and General Experimental Procedures

Chemicals and solvents from commercial sources were used as received. 2-(4-ethenylphenyl)benzothiazole $5b$, $46$ (2-tri-n-butylstannyl)benzothiazole$^{110}$ were prepared according to reported procedures. Metal cations (Al, Ca, Cd, Eu(III), Fe(II), Hg(II), Mg, Pb(II), Zn) were used as perchlorate salts, (Co(III), Cu(II), Pd, Rh(II)) were used as acetate salts, (Au, Ba, Cu(I), Hg(I), Ir(III-V), La(III), Li, Ni, Pt(III), Ru(III), Sn(II), Sn(IV), Ti(IV), V(III)) as were used as chloride salts, (Ag, Bi(III), Cr(III), In(III), Mn(II)) were used as nitrate salts, ceric ammonium sulfate, cesium fluoride, and rhenium iodide were used to prepare metal ion stock solutions.

Caution! Safety precautions were taken when azides were synthesized since azides are potentially explosive. This was performed by heating at moderate temperatures and by working on a small scale.

Thermal stabilities were determined using a TGA (TA Instruments Model Q5000) with the heating rate of $5 - 10 \degree C$ per minute. The mp was recorded on a Mel-Temp apparatus. NMR spectra were recorded in deuterated chloroform or DMSO-d$_6$ on a Mercury 300 MHz or a Varian 500 MHz spectrometer. High resolution mass spectrometry (HRMS-ESI) data was provided by the Mass Spectrometry Laboratory at the University of Florida. The silica gel for chromatography was obtained from Silicycle with particle size of 40 - 63 $\mu$m. The pH of sample and buffer solutions$^{144}$ was recorded on an Accumet Basic pH meter (Model AB15 Fisher Scientific). TEM imagines of samples on copper grids were recorded on a JEOL (Model JEM-1011) TEM. IR spectra were recorded on a Perkin-Elmer Spectrum One
FTIR spectrometer. UV-vis absorption spectra were obtained with a Hewlett-Packard (Model 8453) diode-array spectrophotometer in 1.0 cm quartz cells. Fluorescence emission spectra were obtained with a PTI (Model MD-5020) Quantamaster fluorimeter equipped with a 75 W continuous Xe-arc lamp as a light source. Quantum yields and fluorescence anisotropy of the sample were determined according to the literature.\textsuperscript{130} The quantum yield of sample was measured in triplicate by a comparison of the integrated area of the corrected emission spectrum of the sample with that of a known standard, such as 9,10-diphenylanthracene ($\phi_r = 0.95$ in cyclohexane) or rhodamine 6G ($\phi_r = 0.94$ in methanol), and was calculated from Eq. 7.

Equation 7

$$\phi = \phi_r \frac{I_{OD}}{I_r OD_r} \frac{n^2}{n_r^2}$$\hspace{1cm}(7)$$

where $I$ and $I_r$ is the corrected integrated fluorescence intensity of the sample and the standard; $OD$ and $OD_r$ is the optical density (absorbance) of the sample and the standard; and $n$ and $n_r$ is the refractive index of the sample and the standard, respectively.

Single-photon photodecomposition quantum yield ($\Phi_D$) at 405 nm was determined from Equation 8 according to the literature.\textsuperscript{145}

Equation 8

$$\Phi_D = \frac{[A(\lambda, 0) - A(\lambda, t)]N_A}{10^3 \epsilon I (1 - 10^{-A_{ave}}) \Delta t}$$\hspace{1cm}(8)$$

where $A(\lambda, 0)$ and $A(\lambda, t)$ is the absorbance at a corresponding wavelength $\lambda$ of the initial state ($t = 0$) and at time $t$, respectively; $A_{ave}$ is the average value of $A(\lambda, 0)$ and $A(\lambda, t)$; $N_A$ is the Avogadro number; $\epsilon$ is the molar absorption coefficient of the sample; $I$ is the intensity of the irradiation light; and $\Delta t$ is the exposure time in seconds.
Fluorescence anisotropy (A) of the hydrophilic fluorene-based derivatives were recorded in poly THF (M_n = 250) using two polarizers for excitation and emission (Fig. 88), and calculated using Equation 9.

Figure 88. A Schematic Fluorescence Anisotropy Experimental Setup.

Equation 9

\[ A = \frac{I_{VV} - \frac{I_{HV}}{I_{HH}} I_{VH}}{I_{VV} + 2 \frac{I_{HV}}{I_{HH}} I_{VH}} \]  

where \( I_{VV} \) is the emission intensity of vertical-vertical direction; \( I_{HV} \) is horizon-vertical direction; \( I_{VH} \) is the vertical-horizon; and \( I_{HH} \) is the horizon-horizon direction.

Two-photon absorption (2PA) cross sections, \( \delta \ (1 \text{GM} = 10^{-50} \text{cm}^4 \text{s. photon}^{-1} \text{mol}^{-1}) \) were measured by a fluorescence method, using a Ti:sapphire, mode-locked, femtosecond Coherent Mira 900F laser system (Fig. 89). The pulse width of the laser was 200 fs with a repetition rate of 76 MHz and 700 mW of average power. The 2PE spectrum was digitally processed, and the average of the two-photon upconverted fluorescence emission \( <F> \) estimated. The numerical estimation of the 2PA cross section \( \delta \) was performed by comparison with a known reference and calculated using Equation 10.
Equation 10

\[ \delta_{2PA} = \delta_r \frac{<F_r> \phi_r C_r n_r^2}{<F> \phi C n^2} \]

where \( r \) describes the reference; \(<F>\) is the average fluorescence intensity integrated from two-photon upconverted fluorescence emission spectrum; \( n \) is the refractive index of the solvent; \( C \) is the concentration; \( \phi \) is the quantum yield, and \( P \) is the incident power into the sample.

3.2. UV-vis Absorption and Fluorescence Emission of Metal Complexes

UV-vis absorption and fluorescence emission titrations of 9b-d upon complexing metal ions were performed in ethanol. A 3.0 mL dye solution was prepared and the corresponding metal ion stock solution was added. The resulting solution was agitated and spectra were recorded. Experiments were performed triplicate for a comparison of data.
analysis. The basic equations for complexation of the ligand-metal are shown below (Eqs. 11-14).

Equation 11

$$D + nM \rightleftharpoons C$$  \hspace{1cm} (11)

Equation 12

$$K = \frac{[C]}{[D][M]^n}$$  \hspace{1cm} (12)

Equation 13

$$[D]_i = [D] + [C]$$  \hspace{1cm} (13)

Equation 14

$$[M]_i = [M] + n[C]$$  \hspace{1cm} (14)

where \(D\) is the dye molecule (ligand); \(M\), metal ion; \(C\), complex (D-M); \(n\), molar stoichiometry number; \(K\), binding constant; \([D]_i\), total concentration of the dye molecule at initial state; \([M]_i\), total concentration of the metal at initial state; \([D]\), \([M]\), \([C]\), concentrations of the dye, metal ion, and complex, respectively. The binding constant \(K\) of the metal complex was determined by Equation 15 (Appendix C) with the approximation of concentration of free metal equal to its total concentration \(([M] \approx [M]_i)\).\textsuperscript{148-150}

Equation 15

$$\frac{F - F_o}{F_m - F} = \frac{[C]}{[D]} = K[M]^n$$  \hspace{1cm} (15)

where \(F_o\), \(F\), and \(F_m\) are the corrected fluorescence emission intensity of complex respectively at initial, \(t\), and the final state where the complex was fully formed upon addition of metal ion. The binding constant \(K\) can be determined from the plot of the linear regression of
log[(F-F₀)/(Fₘ-F)] against log[M] in Equation 16, derived from Equation 15, to obtain the intercept as log K with slope n.

Equation 16

\[ \log \frac{F - F_0}{F_m - F} = \log K + n \log[M] \]  

(16)

3.3. Cell Incubation and Imaging

The epithelial colorectal carcinoma cell line HCT 116, was purchased from ATCC (America Type Culture Collection, Manassas, VA, USA). All cells were incubated in Dulbecco’s modified Eagle’s Medium (DMEM, Invitrogen, Carlsbad, CA, USA), supplemented with 10 % fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA, USA), 100 units/mL penicillin-streptomycin (Atlanta Biologicals, Lawrenceville, GA, USA) and incubated at 37 °C in a 95 % humidified atmosphere containing 5 % CO₂. The HCT 116 cells were placed onto poly-D-lysine coated glass cover slips in 24-well plates (ca. 30,000 cells per well). The cells were grown for 36 h before being incubated with fluorescent probes. Stock solutions of fluorescent probes in water or DMSO were prepared as 1 mM to 2 mM solutions, and then they were diluted to 20 µM dye solutions with the complete growth medium, Dulbecco’s modification of Eagle’s medium (DMEM). The dye solutions were freshly prepared and placed over the cells for 1 h. After incubation with a fluorescent probe, the cells were washed with the PBS solution (3 to 5 times) and fixed using a 3.7 % formaldehyde solution for 15 min at 37 °C. To each well, 0.5 mL of a solution of NaBH₄ (1 mg/mL) in the PBS solution (which was added a few drops of 6N NaOH solution) was added and kept for 15 min, and then replaced by an additional 0.5 mL of NaBH₄. At the end of this period, the plates were rinsed twice with PBS solution, and then with water. Finally, the glass
coverslips were mounted by Prolong Gold mounting media for microscopy. Imaging by DIC involved 400 ms exposure, 1PA fluorescence was exposed 150 ms, and 2PA fluorescence excitation was performed at 700 nm. The cell titer 96 aqueous one solution reagent for HTC 116 cells MTS viability assay was obtained from Promega.

For fluorescence imaging, an inverted microscope/spinning disk confocal system (Olympus IX80 DSU) equipped with an EMCCD (Hamamatsu) was utilized, where the output of a filtered 100 W mercury lamp was employed as the light excitation source. A customized filter cube (Ex 377/50, DM 409, Em 460/50) was used. Two-photon fluorescence microscopy (2PFM) images were collected on a modified Olympus Fluoview FV300 microscope system coupled to a tunable laser with excitation at or above 700 nm. A long-pass emission filter (cutoff 690 nm) was placed in the microscope scanhead to avoid background irradiance from the excitation source. Consecutive layers, separated by approximately 0.15 µm, were recorded to create a 3D reconstruction from overlaying X-Y plane two-photon fluorescence images. The two-photon induced fluorescence was collected with a 60x microscope objective (UPLANSAPO 60x, NA= 1.35, Olympus). Single-photon images of probe 5e and LysoTracker Red in colocalization study used custom made filter cube (Ex 377/50, DM 409, Em 525/40) and Texas Red filter cube (Ex 562/40, DM 593, Em 624/40).

3.4. Preparation of 2a

Fluorene (1.70 g, 10 mmol) was weighed and dissolved in acetic acid (40 mL) at 40°C in a 250 mL flask. To this solution, 2.3 g of iodine (2.30 g, 9 mmol), sulfuric acid (2 mL, 36 mmol), and a solution of iodic acid (1.30 g, 7 mmol) in water (4 mL) were added. The resulting solution was heated to 70°C for 1 h. At the end of this period, the solution
discolored and a white precipitate formed. The resulting mixture was allowed to cool to room temperature, and then poured into water (200 mL). The precipitate was collected by vacuum filtration, washed with a solution of 2 % NaHCO₃, water, and then dried under reduced pressure. The crude produce was recrystallized from ethyl acetate, and then dried under vacuo to obtain 2,7-diiodofluorene (3.0 g, 7 mmol) as a white crystal with mp of 216 - 217 °C (70 % yield). ¹H NMR (500 MHz, CDCl₃, δ): 7.89 (s, Ar-H, 2H), 7.75 (m, Ar-H, 2H), 7.52 (m, Ar-H, 2H), 3.85 (m, CH₂, 2H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 144.84, 140.39, 136.05, 134.16, 121.60, 92.47 (C-I), 39.90 (CH₂).

3.5. Preparation of 3a

To a stirring solution of 2-(2-ethoxy ethoxy)ethanol (13.50 g, 100 mmol) in dry pyridine (30 mL) at 5 °C in a 250 mL flask submerging in an ice-bath, 4-methylbenzenesulfonyl chloride (19.0 g, 100 mmol) was added in small portions in 30 minutes to maintain the temperature at 5 - 10 °C. The resulting solution was stirred at 5 °C for 3 h. At the end of this period, a white precipitate formed and the mixture was poured into a cold solution of hydrochloric acid (25 mL) in water (100 mL). The resulting solution was transferred into a separatory flask, and then diethyl ether (50 mL) was added. The ether phase was then removed. The aqueous phase was then extracted twice with portions of diethyl ether (100 mL). The ether phases were then combined, washed with a solution of 1 % sodium bicarbonate (150 mL), and then water. The ether phase was dried by anhydrous sodium sulfate and evaporated in vacuo to obtain compound 3a (27.0 g, 95 mmol) as a colorless liquid (95 % yield). ¹H NMR (500 MHz, CDCl₃, δ): 7.80 (d, Ar-H, 2H), 7.30 (d, Ar-H, 2H), 4.18 (m, SO₃CH₂, 2H), 3.70 (m, SO₃CCH₂O, 2H), 3.48-3.58 (m, OCH₂, 6H), 2.44 (s, PhCH₃,
3H), 1.17 (m, CH₃, 3H). $^{13}$C NMR (500 MHz, CDCl₃, δ (ppm)): 144.83, 132.90, 129.80, 128.0, 70.80 (SO₃CH₂), 69.70, 69.28, 68.60, 66.61, 21.60 (Ph-CH₃), 15.12 (CH₃).

3.6. Preparation of 3b

Tetraethyleneglycol methyl ether (10.5 g, 50 mmol) was utilized and methylene chloride employed to extract to obtain 3b (16.0 g, 48 mmol) in 96 % yield as a colorless liquid. $^1$H NMR (500 MHz, CDCl₃, δ): 7.80 (d, Ar-H, 2H), 7.35 (d, Ar-H, 2H), 4.15 (m, SO₃CH₂, 2H), 3.54-3.72 (m, OCH₂, 14H), 3.36 (s, OCH₃, 3H), 2.45 (s, Ph-CH₃, 3H). $^{13}$C NMR (500 MHz, CDCl₃, δ (ppm)): 144.80, 132.97, 129.74, 128.00, 71.91 (SO₃CH₂), 70.73, 70.67, 70.59, 70.52, 70.46, 69.25, 68.66, 59.05 (OCH₃), 21.70 (Ph-CH₃), 15.12 (CH₃).

3.7. Preparation of 4a

2,7-Diiodofluorene (2.50 g, 6 mmol) was weighed and dissolved in dry DMSO (30 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10 minutes, and then potassium iodide (0.1 g, 0.6 mmol) and potassium hydroxide (1.00 g, 18 mmol) were added. To this solution, 2-(2-ethoxy ethoxy)ethyl tosylate 3a (5.40 g, 18 mmol) was added dropwise in 10 minutes. The resulting solution was stirred overnight and then it was poured into water (50 mL) to which methylene chloride (50 mL) was added. The organic phase was then removed, and the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, dried with sodium sulfate, and then evaporated under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (7:3) as the eluent to obtain 2,7-diiodo-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 4a (2.0 g, 3.1 mmol) as a white solid with mp of 77 - 78°C (55 % yield). $^1$H NMR (500 MHz, CDCl₃, δ): 7.78 (s, Ar-H, 2H), 7.75 (d, Ar-H, 2H), 7.22
(d, Ar-H, 2H), 3.40-3.45 (m, OCH2, 8H), 3.35 (m, 4H), 3.20 (m, 4H), 2.75-2.79 (m, CH2CH2O, 4H), 2.35-2.38 (m, CH3CH2O, 4H), 1.10-1.15 (m, CH3, 6H). 13C NMR (500 MHz, CDCl3, δ (ppm)): 150.78, 139.12, 136.52, 132.50, 93.27 (C-I), 70.13, 69.68, 66.76, 66.65, 51.68 (C9), 39.44 (CH2CH2O), 15.14 (CH3). HRMS calcd for C25H32O4I2, 650.3354; found 650.0384.

3.8. Preparation of 4b

Compound 3b was utilized to synthesis of 2,7-diiodo-9,9-di-2-(2-(2-ethoxyethoxy)ethoxy)ethoxy)methylfluorene 4b as a colorless liquid (40 % yield). 1H NMR (500MHz, CDCl3, δ): 7.73 (s, Ar-H, 2H), 7.66-7.68 (m, Ar-H, 2H), 7.22-7.25 (m, Ar-H, 2H), 3.52-3.61 (m, OCH2, 16H), 3.37-3.41 (m, 10H), 3.19-3.21 (m, OCH3, 4H), 2.74-2.77 (m, CH2CH2O, 4H), 2.32-2.34 (m, CH3CH2O, 4H). 13C NMR (500 MHz, CDCl3, δ (ppm)): 150.78, 139.13, 136.47, 132.42, 121.50, 93.25 (C-I), 71.91 (OCH2), 70.64, 70.53, 70.48, 70.42, 70.36, 66.74, 59.08 (OCH3), 51.68 (C9), 39.45 (CH2CH2O). HRMS calcd for C31H44O8I2, 798.4938; found 798.1119.

3.9. Preparation of 2-(4-(1-Trimethylsilyl)ethynylphenyl)benzothiazole

2-(4-iodophenyl)benzothiazole was synthesized from the literature46 (mp of 154 - 155 0C). 1H NMR (500 MHz, CDCl3, δ): 8.07-8.09 (d, Ar-H, 1H), 7.82-7.93 (m, Ar-H, 5H), 7.50-7.53 (m, Ar-H, 1H), 7.40-7.48 (m, Ar-H, 1H). 13C-NMR (500 MHz, CDCl3, δ (ppm)): 167.53 (C=N), 154.25 (sp2 C-N), 138.89, 135.67, 133.45, 129.31, 126.71, 125.95, 123.98, 121.95, 120.02, 97.92 (C-I).
2-(4-Iodophenyl)benzothiazole (3.40 g, 10 mmol) was weighed and dissolved into a solution of triethylamine (60 mL) and THF (20 mL) in a 250 mL flask. The solution was degassed with nitrogen for 10 minutes, and then PdCl2P(Ph3)2 (0.35 g, 5 mmol), CuI (0.20 g, 10 mmol), and trimethylsilylacetylene (1.50 g, 15 mmol) were added. The resulting solution was stirred overnight and then it was poured into water (100 mL) to which methylene (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with portions methylene chloride (150 mL), and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was evaporated under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (8:2) as the eluent to obtain 2-(4-(2-trimethylsilyl)ethynylphenyl)benzothiazole (2.30 g, 7.5 mmol) as a yellow solid with mp of 61 - 63°C (75 % yield). 1H NMR (500 MHz, CDCl3, δ): 7.80-7.82 (m, Ar-H, 3H), 7.65 (d, Ar-H, 1H), 7.35-7.38 (m, Ar-H, 2H), 7.22-7.25 (m, Ar-H, 1H), 7.15-7.18 (m, Ar-H, 1H), 0.00-0.12 (m, Si(CH3)3, 9H). 13C NMR (500 MHz, CDCl3, δ (ppm)): 167.21 (C=N), 154.20 (sp2-C-N), 135.20, 133.40, 132.62, 132.37, 131.79, 128.07, 127.36, 126.40, 125.50, 123.19, 121.67, 104.44 (sp-C), 97.19 (sp-C), 0.60 (Si(CH3)3).

3.10. Preparation of 2-(4-Ethynylphenyl)benzothiazole 5a

2-(4-(1-Trimethylsilyl)ethynylphenyl)benzothiazole (1.85 g, 6 mmol) was weighed and dissolved in THF (25 mL) in a 100 mL flask, and then a solution of 5 % KOH in methanol (25 mL) was added. The resulting solution was stirred for 30 minutes at room temperature, and then it was poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with
methylene chloride (50 mL). The organic phases were combined, washed with water, and
dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude
product which was further purified on a silica gel column using hexane and ethyl acetate
(7:3) as the eluent to obtain 2-(4-ethynylphenyl)benzothiazole 5a (1.20 g, 4.8 mmol) as a
white solid with mp of 123 - 125 °C (80 % yield). ¹H NMR (500 MHz, CDCl₃, δ): 8.00-8.08
(m, Ar-H, 3H), 7.82-7.85 (d, Ar-H, 1H), 7.50-7.58 (m, Ar-H, 2H), 7.40-7.45 (m, Ar-H, 1H),
7.25-7.29 (m, Ar-H, 1H), 3.15 (s, alkyne proton, 1H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)):
166.95, 154.12, 135.14, 133.73, 132.72, 127.37, 126.51, 125.48, 124.66, 123.40, 121.69,
83.05 (sp-C), 79.54 (sp-C). HRMS calcd for C₁₅H₁₉NS, 235.3028; found 235.0449.

3.11. Preparation of 6a

Compound 4a (0.65 g, 1 mmol) was weighed and dissolved in THF (10 mL) and
triethylamine (10 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10
minutes. To this solution, PdCl₂P(Ph₃)₂ (0.10 g, 0.17 mmol), CuI (0.06 g, 0.3 mmol),
compound 5a (0.56 g, 2.4 mmol), and a magnetic stirring bar were added. The resulting
solution was stirred under nitrogen atmosphere for 24 h at room temperature. At the end of
this period, it was poured into water (50 mL) to which methylene chloride (40 mL) was
added. The organic phase was removed, and the aqueous phase was extracted 3 times with
methylene chloride (40 mL). The organic phases were combined, washed with water, and
dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude
product which was further purified on a silica gel column using hexane and ethyl acetate
(1:1) as the eluent to obtain 6a (0.50 g, 0.7 mmol) as a yellow solid with mp of 162 - 163 °C
(68 % yield). ¹H NMR (500 MHz, CDCl₃, δ): 8.00-8.06 (m, Ar-H, 6H), 7.81-7.86 (d, Ar-H,
2H), 7.55-7.67 (m, Ar-H, 9H), 7.55 (d, Ar-H, 1H), 7.45 (m, Ar-H, 2H), 7.35-7.38 (m, Ar-H, 2H), 3.25-3.37 (m, OCH2, 8H), 3.20 (m, 4H), 2.85-2.86 (m, CH2CH2O), 4H), 2.38-2.41 (m, CH3CH2O, 4H), 1.07-1.09 (m, CH3, 6H). 13C NMR (500 MHz, CDCl3, δ (ppm)): 167.18 (C=N), 154.17(sp C-N), 149.54, 140.21, 135.15, 133.17, 132.15, 131.63, 127.51, 126.60, 126.50, 125.90, 125.70, 123.35, 122.08, 121.69, 120.11, 92.56 (sp-C), 89.82 (sp-C), 70.13, 69.67, 66.88, 66.62, 51.42 (C9), 39.95 (CH2CH2O), 15.10 (CH3). HRMS calcd for C55H48O4N2S2, 865.1148; found 864.3049.

3.12. Preparation of 6b

Compound 5b was prepared from the literature.37 1H NMR (500 MHz, CDCl3, δ): 8.07-8.09 (m, Ar-H, 3H), 7.91-7.93 (d, Ar-H, 1H), 7.51-7.55 (m, Ar-H, 3H), 7.40 (m, Ar-H, 1H), 6.78-6.94 (m, CH=CH2, 1H), 5.87-5.90 (d, C=CH2, 1H), 5.37-5.39 (d, C=CH2, 1H). 13C NMR (500 MHz, CDCl3, δ): 167.69 (C=N), 154.19 (sp2 C-N), 140.15, 136.05, 135.03, 132.89, 127.83, 127.77, 126.81, 126.38, 125.20, 123.19, 121.62, 115.67.

Compound 4a (0.80 g, 1 mmol) was weighed and dissolved into a mixture of DMF (15 mL) and triethylamine (3 mL) in a 50 mL flask. A magnetic stirring bar was added and the solution was degassed with nitrogen for 10 minutes. To this solution, palladium acetate (0.08 g, 0.4 mmol), tri(o-tolyl)phosphine (0.20 g, 0.7 mmol), and compound 5b (0.57 g, 2.4 mmol) were added. The resulting solution was then refluxed at 90 °C for 40 h. At the end of this period, the solution was allowed to cool to room temperature, and then it was poured into water (50 mL) to which methylene chloride (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and then dried over sodium sulfate. The
filtrate was removed under reduced pressure to obtain a crude product which was further
purified on a silica gel column using ethyl acetate and THF (1:1) as the eluent to obtain
compound 6b (0.6 g, 0.6 mmol) as a yellow-green solid with mp of 82 – 83 °C (60 % yield).

$^1$H NMR (500 MHz, DMSO, $\delta$): 8.02-8.08 (dd, Ar-H, 8H), 7.90 (s, Ar-H, 2H), 7.82-7.85 (m,
Ar-H, 6H), 7.65 (d, Ar-H, 2H), 7.45-7.57 (m, Ar-H, 8H), 3.09-3.39 (m, OCH$_2$, 34H), 2.69-
2.72 (m, CH$_2$CH$_2$O, 4H). $^{13}$C NMR (500 MHz, DMSO, $\delta$ (ppm)): 167.43 (C=N), 154.12 (sp$^2$
C-N), 150.41, 140.80, 140.23, 136.64, 134.89, 132.07, 128.21, 128.11, 128.04, 127.95,
127.85, 127.69, 127.50, 123.73, 123.55, 123.25, 123.11, 121.90, 121.67, 71.64, 70.16, 70.13,
69.95, 67.85, 59.52 (OCH$_3$), 51.26 (C9), 40.00 (CH$_2$CH$_2$O). HRMS calcd for C$_{61}$H$_{64}$O$_8$N$_2$S$_2$,
1017.3052; found 1017.4177.

3.13. Preparation of 2b

Fluorene (1.70 g, 10 mmol) was weighed and dissolved in acetic acid (20 mL) at 40
°C in a 250 mL flask. To this solution, iodine (1.08 g, 0.4 mmol), sulfuric acid (1 mL, 1.8
mmol), and a solution of iodic acid (0.6 g, 0.3 mmol) in water (2 mL) were added. The
resulting solution was heated to 70 °C for 1 h. At the end of this period, the solution
discolored and it was allowed to cool to room temperature. The solution was poured into
water (200 mL), and the precipitate was collected by vacuum filtration. It was washed with a
solution of 2 % NaHCO$_3$, water, and then dried under reduced pressure. The crude produce
was recrystallized from methanol, and then dried under vacuo to obtain compound 2b (2.3 g
0.8 mmol) as a white solid with mp of 124 - 126 °C (78 % yield). $^1$H NMR (500 MHz,
CDCl$_3$, $\delta$): 7.90-7.95 (s, Ar-H, 1H), 7.70-7.78 (dd, Ar-H, 2H), 7.54-7.55 (m, Ar-H, 2H),7.35-
7.40 (dd, Ar-H, 2H), 3.89 (m, CH$_2$, 2H). $^{13}$C NMR (500 MHz, CDCl$_3$, $\delta$ (ppm)): 145.47,
3.14. Preparation of \(3c\)

Poly(ethylene glycol) methyl ether (M$_n$ = 550, 27.50 g, 50 mmol) was utilized and methylene chloride and methanol (95:5) were used as the eluent to obtain compound \(3c\) (28 g) as a colorless liquid (yield 92 %). \(^1\)H NMR (500 MHz, CDCl$_3$, \(\delta\)): 7.78 (d, Ar-H, 2H), 7.32 (d, Ar-H, 2H), 4.17 (m, SO$_3$CH$_2$, 2H), 3.55-3.67 (m, OCH$_2$, 40H), 3.35 (s, OCH$_3$, 3H), 2.42 (s, Ph-CH$_3$, 3H). \(^13\)C NMR (500 MHz, CDCl$_3$, \(\delta\) (ppm)): 144.80, 132.91, 129.80, 127.95, 71.90 (SO$_3$CH$_2$), 70.70, 70.57, 70.50, 70.47, 69.31, 69.20, 68.63, 59.04 (OCH$_3$), 21.62 (PhCH$_3$).

3.15. Preparation of \(4c\)

2-Iodofluorene \(2b\) (1.80 g, 6 mmol) was utilized and hexane and ethyl acetate (7:3) was used as the eluent to obtain 2-iodo-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene \(4c\) (1.90 g, 3.6 mmol) as a white crystal with mp of 49 - 50 °C (60 % yield). \(^1\)H NMR (500 MHz, CDCl$_3$, \(\delta\)): 7.78 (s, Ar-H, 1H), 7.70-7.75 (m, Ar-H, 2H), 7.22-7.25 (m, Ar-H, 2H), 7.10-7.15 (dd, Ar-H, 2H), 3.35-3.43 (m, OCH$_2$, 8H), 3.18-3.20 (m, OCH$_2$, 4H), 2.75-2.78 (m, CH$_2$CH$_2$O, 4H), 2.38-2.42 (m, CH$_3$CH$_2$O, 4H), 1.13-1.18 (m, CH$_3$, 6H). \(^13\)C NMR (500MHz, CDCl$_3$, \(\delta\) (ppm)): 152.89, 150.12, 147.80, 146.0, 137.75, 137.45, 133.63, 123.66, 123.01, 120.05, 119.01, 95.54 (C-I), 70.12, 69.59, 66.75, 66.64, 52.24 (C9), 39.19 (CH$_2$CH$_2$O), 15.06 (CH$_3$). HRMS calcd for C$_{25}$H$_{33}$O$_4$I, 524.4383; found 524.1417.

3.16. Preparation of \(4d\)
Compound 3c was utilized, and methylene chloride and methanol (95:5) was used as the eluent to obtain an oily liquid 4d (40 % yield). $^1$H NMR (500 MHz, CDCl$_3$, $\delta$): 7.75 (s, Ar-H, 1H), 7.65-7.68 (m, Ar-H, 2H), 7.38-7.42 (m, Ar-H, 2H), 7.32-7.35 (m, Ar-H, 2H), 3.55-3.65 (m, 50H), 3.38-3.42 (s, 6H), 2.65-2.68 (m, CH$_2$CH$_2$O, 4H), 2.32-2.36 (m, CH$_3$CH$_2$O, 4H). $^{13}$C NMR (500 MHz, CDCl$_3$, $\delta$ (ppm)): 148.33, 145.31, 137.09, 136.46, 133.33, 129.46, 125.20, 124.48, 120.12, 118.47, 116.94, 89.62, 68.87, 68.49, 68.36, 67.66, 67.61, 67.58, 67.53, 67.50, 63.81, 56.05 (OCH$_3$), 48.39 (C9), 36.46 (CH$_2$CH$_2$O). HRMS calcd for C$_{47}$H$_{77}$O$_{16}$I, 1025.0207; found 1024.9921.

3.17. Preparation of 5c

2-Iodo-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 4c (0.53 g, 1 mmol) was weighed and dissolved in a mixture of acetic acid (2 mL) and acetic anhydride (2 mL) in a 50 mL flask. To this solution, nitric acid (0.30 g, 3 mmol) was added and stirred overnight. The resulting solution was poured into water (50 mL), and then methylene chloride (40 mL) was added. The organic phase was removed, and then the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and then dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate as the eluent to obtain 2-iodo-7-nitro-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 5c (0.30 g, 0.5 mmol) as a light yellow solid with mp of 55 - 56 °C in 50 % yield. $^1$HNMR (500 MHz, CDCl$_3$, $\delta$): 8.15-8.17 (m, Ar-H, 2H), 7.80-7.82 (s, 1H), 7.75 -7.78 (m, Ar-H, 2H), 7.35-7.38 (m, 1H), 3.38-3.42 (m, 4H), 3.28-3.32 (m, 4H), 3.15-3.19 (m, OCH$_2$, 4H), 2.80-2.88 (dd, CH$_2$CH$_2$O, 4H), 2.40-2.48 (dd, CH$_3$CH$_2$O, 4H), 1.10-1,15 (m, CH$_3$, 6H). $^{13}$C NMR (500
MHz, CDCl₃, δ(ppm)): 151.30, 148.32, 140.07, 139.46, 136.27, 132.45, 128.01, 127.82, 123.52, 121.65, 119.92, 92.64 (C-I), 70.07, 69.67, 66.89, 66.60, 51.37 (C9), 39.55 (CH₂CH₂O), 15.10 (CH₃). HRMS calcd C₂₅H₃₂NIO₆ for 569.4359; found 569.1268.

3.18. Preparation of 5d

Compound 5d was obtained as a yellowish liquid (40 % yield). ¹H NMR (500 MHz, CDCl₃, δ): 8.25-8.30 (m, Ar-H, 2H), 7.75-7.82 (m, Ar-H, 3H), 7.50-7.55 (m, Ar-H, 1H), 7.50 (m, Ar-H, 1H), 3.4--3.75 (m, 72H), 3.35-3.42 (s, 6H), 2.78-2.82 (d, CH₂CH₂O, 4H), 2.38-2.42 (d, 4H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 149.85, 147.06, 144.47, 142.85, 134.74, 134.04, 130.05, 120.78, 120.0, 117.15, 116.0, 92.51 (C-I), 74.35, 74.10, 73.84, 69.16, 68.95, 68.75, 68.66, 68.55, 68.45, 68.33, 68.16, 67.79, 67.66, 66.41, 56.10 (OCH₃), 49.22 (C9), 36.23 (CH₂CH₂O). HRMS calcd for C₄₇H₇₆O₁₈NI, 1070.0183; found 1070.8651.

3.19. Preparation of 6c

2-Iodo-7-nitro-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 5b (2.0 g, 3.5 mmol) was weighed and dissolved into 30 mL of a mixture of DMF (25 mL) and triethylamine (5 mL) in a 50 mL flask. The solution was degassed with nitrogen for 10 minutes, then palladium acetate (0.04 g, 0.17 mmol), tri(o-tolyl)phosphine (0.1 g, 0.33 mmol), and 2-(4-ethenylphenyl)benzothiazole ⁴⁶ (1.0 g, 4.2 mmol) were added. The resulting solution was heated to 90°C for 40 h. At the end of this period, the solution was allowed to cool to room temperature and then poured into water (200 mL) to which methylene chloride (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude
product. It was purified on a silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain 2-nitro-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 6c (1.40 g, 2 mmol) as a yellow solid with mp of 145 – 147 °C (60 % yield). $^1$H NMR (500 MHz, CDCl$_3$, $\delta$): 8.12-8.15 (dd, Ar-H, 2H), 8.02-8.09 (dd, Ar-H, 3H), 7.83-7.88 (m, Ar-H, 1H), 7.75-7.78 (dd, Ar-H, 2H), 7.65-7.70 (m, Ar-H, 3H), 7.55-7.57 (m, Ar-H, 1H), 7.48-7.52 (m, Ar-H, 1H), 3.05-3.38 (m, Ar-H, 1H), 7.22-7.30 (m, Ar-H, 2H), 1.0-1.04 (m, CH$_3$, 6H).

$^{13}$C NMR (500 MHz, CDCl$_3$, $\delta$ (ppm)): 167.95 (C=N), 154.22 (sp$^2$ C-N), 151.90, 151.80, 147.63, 146.45, 139.66, 138.34, 138.08, 135.75, 133.80, 129.89, 128.90, 128.01, 127.16, 127.05, 126.95, 126.43, 124.85, 123.70, 123.21, 121.65, 121.55, 119.88, 118.94, 70.15, 69.61, 66.86, 66.61, 51.95 (C9), 39.45 (CH$_2$CH$_2$), 15.05 (CH$_3$). HRMS calcd for C$_{40}$H$_{42}$O$_6$N$_2$S, 678.8416; found 678.2827.

3.20. Preparation of 6d

Yield 70%. $^1$H NMR (500 MHz, CDCl$_3$, $\delta$): 8.20-8.22 (dd, 2H), 8.02-8.10 (dd, 3H), 7.85-7.90 (m, 1H), 7.70-7.75 (d, 2H), 7.62-7.68 (dd, Ar-H, 3H), 7.55-7.57 (d, Ar-H, 1H), 7.45-7.48 (m, Ar-H, 1H), 7.30-7.35 (m, Ar-H, 1H), 7.22-7.25 (m, Ar-H, 2H), 3.40-3.62 (m, 66H), 2.70-2.80 (d, CH$_2$CH$_2$O, 4H), 2.38-2.41 (m, CH$_3$CH$_2$O, 4H). $^{13}$C NMR (500 MHz, CDCl$_3$, $\delta$ (ppm)): 167.90, 154.01, 151.7, 151.05, 147.06, 146.10, 140.00, 138.20, 138.10, 135.02, 132.89, 130.02, 129.80, 129.50, 128.07, 127.55, 126.80, 125.99, 125.80, 124.00, 123.80, 123.50, 123.20, 122.00, 120.00, 119.50, 119.00, 71.89, 71.00, 70.76, 70.63, 70.52, 70.32, 70.26, 70.04, 66.80, 59.01 (OCH$_3$), 51.89 (C9), 39.80 (CH$_2$CH$_2$O). HRMS calcd for C$_{54}$H$_{70}$O$_{14}$N$_2$S, 1003.212; found 1002.9711.
3.21. Preparation of 7a

2-Nitro-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 6c (1.20 g, 1.8 mmol) was weighed and dissolved in a mixture of ethanol (25 mL) and THF (25 mL) in a 200 mL flask. The resulting solution was degassed with nitrogen for 10 minutes, and then 10 % palladium on carbon (0.12 g) was added. The solution was heated to 60 °C under nitrogen atmosphere. To this resulting mixture, hydrazine hydrate (0.50 g, 10 mmol) was added dropwise in 10 minutes. The solution was then refluxed at 70 °C for 20 h. At the end of this period, the solution was allowed to cool to room temperature and then filtered using a short silica gel plug, and washed with THF (100 mL). The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using ethyl acetate and THF (4:1) as the eluent to obtain 2-amino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7a (1.0 g, 1.6 mmol) as a yellow solid with mp of 185 – 188 °C (90 % yield). IR (neat, cm⁻¹): 3400, 3340, 3230 (-NH₂). ¹H NMR (300 MHz, CDCl₃, δ): 7.95-8.05 (m, Ar-H, 3H), 7.82-7.85 (d, Ar-H, 1H), 7.58-7.62 (d, Ar-H, 2H), 7.30-7.50 (m, Ar-H, 6H), 7.10-7.18 (m, Ar-H, 2H), 6.56-6.58 (d, Ar-H, 1H), 3.85 (s, NH₂, 2H), 3.25-3.38 (m, OCH₂, 8H), 3.15-3.18 (m, 4H), 2.62-2.78 (m, CH₂CH₂O, 4H), 2.21-2.42 (m, CH₃CH₂O, 4H), 1.02-1.12 (m, CH₃, 6H). ¹³C NMR (CDCl₃, 300 MHz, δ (ppm)): 167.87 (C=N), 154.15 (sp² C-N), 151.70, 148.62, 146.76, 141.86, 140.55, 135.01, 134.65, 132.05, 131.24, 131.04, 128.13, 127.05, 126.87, 126.61, 126.54, 126.05, 123.33, 121.82, 121.10, 121.05, 119.10, 114.35, 110.15, 70.41, 70.00, 67.37, 66.89, 51.06 (C9), 40.40 (CH₂CH₂O), 15.48 (CH₃). HRMS calcd for C₄₀H₄₄N₂O₄S, 648.8586; found 648.3016.

3.22. Preparation of 7b
Yield 90 % as a light yellow liquid. IR (neat, cm⁻¹): 3447, 3355, 3236 (-NH₂), 2870, 1602, 1468, 1354, 1293, 1248, 1101 (C-O), 1031, 958, 871, 822, 762. \(^1\)H NMR (500 MHz, CDCl₃, δ): 8.10-8.15 (m, Ar-H, 3H), 7.90-7.93 (d, Ar-H, 1H), 7.65-7.68 (d, Ar-H, 2H), 7.40-7.58 (m, Ar-H, 6H), 7.18-7.20 (d, Ar-H, 1H), 6.79 (s, Ar-H, 1H), 6.70 (d, Ar-H, 1H), 3.95-4.02 (s, 2H), 3.50-3.70 (m, OCH₂, 68H), 3.35-3.45 (m, OCH₃,10H), 2.78-2.80 (m, CH₂CH₂O, 4H), 2.30-2.42 (d, CH₃CH₂O, 4H). \(^{13}\)C NMR (500 MHz, CDCl₃, δ (ppm)):
167.79, 154.23, 148.48, 140.37, 135.01, 134.50, 132.27, 131.50, 131.21, 128.02, 127.84, 126.96, 126.50, 126.20, 125.55, 123.85, 121.81, 121.50, 121.15, 121.05, 119.50, 115.48, 114.85, 109.80, 71.92, 70.77, 70.54, 70.46, 70.30, 70.00, 67.04, 59.10 (OCH₃), 50.73 (C9), 40.05 (CH₂CH₂O). HRMS calcd for C₅₈H₈₀O₁₄N₂S, 1065.3204; found 1065.6587.

3.23. Preparation of 8a

2-Amino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7a (0.65 g, 1 mmol) was weighed and dissolved into DMF (15 mL). The solution was degassed with nitrogen for 10 minutes, and then potassium carbonate (0.50 g, 3.6 mmol), ethyl bromoacetate (0.50 g, 3 mmol) were added. The resulting solution was heated under nitrogen atmosphere to 95 °C for 20 h. At the end of this period, the solution was allowed to cool to room temperature and poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain 0.61 g of 2-N,N-bis(2-ethylacetate)amino-9,9-di-2-(2-ethoxy
ethoxy)ethylfluorene 8a (0.61 g, 0.7 mmol) as a yellow solid with mp of 103 - 105 °C (75 % yield). $^1$H NMR (500 MHz, CDCl$_3$, δ): 8.02-8.08 (m, Ar-H, 3H), 7.82-7.85 (d, Ar-H, 1H), 7.58-7.62 (m, Ar-H, 3H), 7.40-7.58 (m, Ar-H, 3H), 7.30-7.38 (d, Ar-H, 2H), 7.25 (d, Ar-H, 1H), 7.10-7.12 (d, Ar-H, 1H), 6.52-6.59 (m, Ar-H, 2H), 4.10-4.18 (m, CO$_2$CH$_2$, NCH$_2$, 8H), 3.30-3.38 (m, OCH$_3$, 8H), 3.10-3.18 (m, 4H), 2.65-2.78 (m, CH$_2$CH$_2$O, 4H), 2.22-2.43 (m, CH$_3$CH$_2$O, 4H), 0.98-1.02 (m, CH$_3$, 6H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 170.86 (CO$_2$C$_2$H$_5$), 167.98 (C=N), 154.11 (sp$^2$ C-N), 150.55, 150.15, 148.35, 148.25, 148.08, 140.47, 140.27, 140.13, 135.01, 134.65, 132.03, 131.25, 131.01, 127.94, 126.67, 126.35, 126.16, 125.87, 125.32, 122.28, 122.18, 121.96, 120.75, 120.45, 118.37, 112.12, 106.42, 70.06, 69.66, 67.05, 66.54, 61.28, 53.83 (NCH$_2$), 50.89 (C9), 39.92 (CH$_2$CH$_2$O), 15.05 (CH$_3$), 14.27 (CH$_3$). HRMS calcd for C$_{48}$H$_{56}$O$_8$N$_2$S, 821.039; found 820.3751.

3.24. Preparation of 9a

Compound 8a (0.41 g, 0.5 mmol) was weighed and dissolved into a solution of 30 % aqueous methanol (20 mL). To this solution, sodium hydroxide (0.12 g, 3 mmol) was added and refluxed overnight. At the end of this period, the resulting solution was allowed to cool to room temperature and then it was poured into water (30 mL) to which HCl was added to neutralize this solution. The solution was transferred into a separatory flask, and then methylene chloride (40 mL) was added. The organic phase was removed and the aqueous phase was extracted 3 times with portions of methylene chloride (100mL). The organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purification on a silicagel column using methanol and methylene chloride (3:7) as the eluent.
to obtain compound 9a as a light yellow solid with mp of 110 – 114 °C in 40 % yield. \(^1\)H NMR (500 MHz, CDCl\(_3\), \(\delta\)): 7.98-99 (d, Ar-H, 1H), 7.8-7.9 (m, Ar-H, 2H), 7.62-7.65 (m, Ar-H, 2H), 7.45-7.55 (m, Ar-H, 3H), 7.30-7.35 (m, Ar-H, 3H), 7.15-7.18 (d, Ar-H, 2H), 7.05-7.08 (d, Ar-H, 1H), 6.58-6.59 (s, Ar-H, 1H), 6.50 (m, Ar-H, 1H), 4.10-4.15 (s, NCH\(_2\), 4H), 3.10-3.43 (m, OCH\(_2\), 12H), 2.65-2.75 (d, CH\(_2\)CH\(_2\)O, 4H), 2.2-2.3 (m, CH\(_3\)CH\(_2\), 4H), 1.26-1.32 (m, CH\(_3\), 6H), 1.14-1.16 (m, CH\(_3\), 6H). \(^{13}\)C NMR (500 MHz, CDCl\(_3\), \(\delta\) (ppm)): 170.86 (CO\(_2\)C\(_2\)H\(_5\)), 167.98 (C=N), 154.11 (sp\(^2\) C-N), 150.55, 150.15, 148.35, 148.25, 148.08, 140.47, 140.27, 140.13, 135.01, 134.65, 132.03, 131.25, 131.01, 127.94, 126.67, 126.35, 126.16, 125.87, 125.32, 122.28, 122.18, 121.96, 120.75, 120.45, 118.37, 112.12, 106.42, 70.06, 69.66, 67.05, 66.54, 61.28, 53.83 (NCH\(_2\)), 50.89 (CH\(_2\)CH\(_2\)O), 39.92 (CCH\(_2\)CH\(_2\)O), 15.05 (CH\(_3\)), 14.27 (CH\(_3\)). HRMS calcd for C\(_{44}\)H\(_{48}\)O\(_8\)N\(_2\)S, 764.8886; found 764 (traces).

3.25. Preparation of \(8b\)

2-Amino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene \(7a\) (0.65 g, 1 mmol) was weighed and dissolved in 1,2-dichlorobenzene (10 mL) in a 50 mL flask. To this solution, potassium carbonate (0.7 g, 5 mmol), Cu powder (0.3 g, 5 mmol), 18-crown-6 (0.10 g), and iodobenzene (0.60 g, 2.9 mmol) were added. The mixture was refluxed under nitrogen atmosphere at 180 °C for 24 h. At the end of this period, the solution was allowed to cool to room temperature. The solution was then filtered using a short silica gel plug and washed with ethyl acetate (100 mL). The filtrate was removed under reduced pressure to obtain a crude product which was purified on a silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain compound \(8b\) (0.50 g, 0.6 mmol) as a yellow solid with mp of 44 – 45 °C (62 % yield). \(^1\)H NMR (500 MHz, CDCl\(_3\), \(\delta\)): 8.08 (d, Ar-H, 1H), 7.98 (d,
Ar-H, 2H), 7.92 (d, Ar-H, 1H), 7.48-7.52 (m, Ar-H, 4H), 7.41-7.45 (m, Ar-H, 4H), 7.23-7.28 (m, Ar-H, 4H), 7.10-7.15 (m, Ar-H, 4H), 7.01-7.08 (m, Ar-H, 4H), 6.72-6.78 (d, Ar-H, 1H), 6.63 (d, Ar-H, 1H), 3.35-3.41 (m, OCH2, 8H), 3.25-3.28 (m, 4H), 2.80-2.82 (m, CH2CH2O, 4H), 2.16-2.19 (m, CH3CH2O, 4H), 1.12-1.15 (m, CH3, 6H). 13C NMR (500 MHz, CDCl3, δ (ppm)): 167.90 (C=N), 154.05 (sp2 C-N), 150.11, 147.80, 147.70, 140.14, 139.94, 135.73, 134.53, 132.09, 129.78, 129.55, 129.31, 129.21, 128.12, 127.48, 126.12, 125.75, 124.03, 123.65, 123.48, 123.21, 123.12, 122.86, 121.95, 120.56, 119.55, 119.45, 70.11, 69.72, 67.28, 66.58, 50.96 (C9), 39.24 (CH2CH2O), 15.10 (CH3). HRMS calcd for C52H52O4N2S, 801.0538; found 800.3742.

3.26. Preparation of 8c

2-Amino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7a (0.65 g, 1 mmol) was weighed and dissolved into DMF (15 mL). The solution was degas with nitrogen for 10 minutes, and then potassium carbonate (0.5 g, 3.6 mmol), sodium iodide (0.30 g, 2 mmol), propargyl bromide (0.36 g, 3 mmol), and a magnetic stirring bar were added. The resulting solution was heated to 95° C for 20 h. At the end of this period, the solution was allowed to cool to room temperature and then poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed, and the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and then dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain 2-N,N-bispropargylamino-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 8c (0.56 g, 0.7 mmol) as a
yellow solid with mp of 151 – 152 °C in 70 % yield. \(^1\)H NMR (300 MHz, CDCl\(_3\), δ): 8.05-8.10 (m, Ar-H, 3H), 7.85-7.90 (d, Ar-H, 1H), 7.75-7.82 (m, Ar-H, 2H), 7.50-7.55 (m, Ar-H, 3H), 7.40-7.45 (m, Ar-H, 2H), 7.35-7.38 (m, Ar-H, 1H), 7.20-7.22 (d, Ar-H, 1H), 7.05-7.08 (d, Ar-H, 1H), 6.82-6.92 (m, Ar-H, 2H), 4.05-4.10 (s, 4H), 3.32-3.38 (m, OCH\(_2\), 8H), 3.10-3.18 (m, 4H), 2.65-2.78 (m, CH\(_2\)CH\(_2\)O, 4H), 2.30-2.38 (m, CH\(_3\)CH\(_2\)O, 4H), 1.02-1.08 (m, 6H). \(^{13}\)C NMR (CDCl\(_3\), 500 MHz, δ (ppm)): 166.75, 153.22, 149.61, 147.95, 146.83, 139.83, 139.29, 133.99, 133.86, 131.32, 131.14, 129.75, 126.92, 125.85, 125.66, 125.63, 125.32, 124.11, 122.11, 120.59, 119.77, 119.64, 118.10, 114.13, 109.57, 77.99, 72.03, 69.06, 68.65, 66.07, 65.56, 49.97 (C9), 39.94 (CH\(_2\)CH\(_2\)O), 38.59 (C-N), 14.05 (CH\(_3\)). HRMS calcd for C\(_{46}\)H\(_{48}\)O\(_4\)N\(_2\)S, 724.9562; found 724.3329.

3.27. Preparation of 8d

Compound 7b was utilized, then methylene chloride and methanol (95:5) was used as the eluent to obtain compound 8d as an oily liquid (65 % yield). \(^1\)H NMR (500 MHz, CDCl\(_3\), δ): 8.12-8.15 (m, Ar-H, 3H), 7.92-7.95 (d, 1H), 7.65-7.72 (d, Ar-H, 2H), 7.55-7.58 (m, Ar-H, 3H), 7.50-7.55 (m, Ar-H, 2H), 7.38-7.39 (d, Ar-H, 1H), 7.30-7.32 (d, Ar-H, 1H), 7.20-7.22 (d, Ar-H, 1H), 6.90-6.95 (m, 2H), 4.20 (m, 4H), 3.54-3.65 (m, 70H), 3.37-3.42 (m, 12H), 3.22 (m, 4H), 2.80 (m, CH\(_2\)CH\(_2\)O, 4H), 2.38-2.42 (m, CH\(_3\)CH\(_2\)O, 4H). \(^{13}\)C NMR (500 MHz, CDCl\(_3\), δ (ppm)): 167.77, 154.23, 150.35, 149.10, 148.00, 140.95, 140.28, 135.01, 132.33, 132.19, 128.03, 127.84, 127.16, 126.99, 126.87, 125.95, 125.23, 123.76, 121.80, 121.35, 119.62, 119.43, 115.78, 115.61, 110.25, 110.15, 79.50, 73.55, 71.91, 70.77, 70.65, 70.53, 70.45, 70.41, 70.27, 69.99, 58.90 (OCH\(_3\)), 50.97 (C9), 40.50 (CH\(_2\)CH\(_2\)O), 39.2 (C-N). HRMS calcd for C\(_{60}\)H\(_{76}\)O\(_{12}\)N\(_2\)S, 1049.3266; found 1048.7501.
3.28. Preparation of 1-Azidobutane

1-Bromobutane (5.50 g, 40 mmol) was weighed and dissolved in DMSO (50 mL) at room temperature in a 200 mL flask to which was added sodium azide (3.90 g, 60 mmol). The solution was then heated to 95 °C under nitrogen for 24 h. At the end of this period, the solution was allowed to cool to room temperature. A white precipitate formed and the resulting mixture was poured into water (100 mL). It was transferred into a separatory flask to which diethyl ether (50 mL) was added. The organic phase was then removed. The aqueous phase was then extracted 3 times with portion of diethyl ether (100 mL). The ether phases were then combined, washed with water, dried with sodium sulfate, then evaporated under reduced pressure to yield 1-azidobutane (3.0 g, 30 mmol) in 80 % yield as a colorless liquid. IR: 2090 cm⁻¹ (N₃). ¹H NMR (500MHz, CDCl₃, δ): 3.18-3.21 (m, 2H), 1.49-1.55 (m, 2H), 1.30-1.37 (m, 2H), 0.86-0.89 (m, 3H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 39.97 (C-N), 29.82, 18.87, 12.60 (CH₃).

3.29. Preparation of 1-Azido-11-Undecanol

1-Bromo-11-undecanol (5.00 g, 20 mmol) was weighed and dissolved into acetonitrile (125 mL) in a 500 mL flask. To this solution, sodium azide (2.00 g, 30 mmol) and a magnetic stirring bar were added. The resulting solution was refluxed at 80 °C under nitrogen for 20 h. At the end of this period, the solution was allowed to cool to room temperature, and then filtered and washed with acetonitrile (50 mL). The filtrate was evaporated under reduced pressure to obtain 1-azido-11-undecanol (4.20 g, 19 mmol) as a colorless liquid (97 % yield). The product was pure enough for the next reaction without further purification. ¹H NMR (500 MHz, CDCl₃, δ): 3.59-3.61 (m, 2H), 3.23-3.25 (m, 2H), 3.18-3.21 (m, 2H), 1.49-1.55 (m, 2H), 1.30-1.37 (m, 2H), 0.86-0.89 (m, 3H).
1.53-1.60 (m, 4H), 1.27-1.34 (m, 14H). $^{13}$C NMR (500 MHz, CDCl₃, δ (ppm)): 62.91 (OCH₂), 51.46 (C-N), 32.75, 29.54, 29.44, 29.40, 29.25, 29.12, 28.81, 26.69, 25.73.

3.30. Preparation of 1-Azido-11-Undecanyl Tosylate

1-Azido-11-undecanol (4.00 g, 19 mmol) was weighed and dissolved into pyridine (10 mL) in a 100 mL flask immersed in an ice bath at 5 °C. To this solution, 4-methylbenzenesulfonyl chloride (3.50 g, 18 mmol) was added in portions in 20 minutes. The resulting solution was stirred at 5 °C under nitrogen atmosphere for 3 h. At the end of this period, a white precipitate formed and the solution was poured into a cold solution hydrochloric acid (10 mL) in water (50 mL). The resulting solution was transferred into a separatory flask to which methylene chloride (40 mL) was added. The organic phase was removed, and the aqueous phase was extracted 3 times with portions of methylene chloride (100 mL). The organic phases were combined, washed with a solution of 1 % sodium bicarbonate (100 mL) and water, and then dried over sodium sulfate. The filtrate was removed under reduced pressure and under vacuo to obtain 1-azido-11-undecanyl tosylate (6.30 g, 18 mmol) as a colorless oily liquid (92 % yield). The product is pure enough for the next reaction without further purification. $^1$H NMR (500 MHz, CDCl₃, δ): 7.70-7.72 (d, Ar-H, 2H), 7.20-7.28 (d, Ar-H, 2H), 3.93-3.95 (m, 2H), 3.17-3.19 (m, 2H), 2.37-2.50 (s, 3H), 1.44-1.57 (m, 4H), 1.15-1.36 (m, 14H). $^{13}$C NMR (500 MHz, CDCl₃, δ (ppm)): 144.65, 133.19, 129.73, 127.91, 70.70 (SO₃C), 51.46 (C-N), 29.39, 29.34, 29.10, 28.89, 28.82, 28.79, 26.68, 25.31, 21.68.

3.31. Preparation of 1-Azido-11-Undecanethiol
1-Azido-11-undecanyl tosylate (6.00 g, 16 mmol) was weighed and dissolved into ethanol (40 mL) in a 250 mL flask, and a magnetic stirring bar. To this solution, thiourea (1.80 g, 24 mmol) was added and the solution was refluxed at 80 °C under nitrogen atmosphere for 10 h. At the end of this period, the solution was allowed to cool to room temperature, and then a solution of 10 % sodium hydroxide (60 mL) was added. The resulting solution was refluxed at 100 °C for 4 h. It was allowed to cool to room temperature, and neutralized with a solution of 10 % hydrochloric acid. The solution was transferred into a separatory flask to which methylene chloride (50 mL) was added. The organic phase was removed, and the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and then dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product as a white solid. The crude product was purified on a silica gel column using hexane and methylene chloride (7:3) as the eluent to obtain 1-azido-11-undecanethiol (3.00 g, 13 mmol) as a colorless liquid (80 % yield). IR (Perkin Elmer): 2098 cm⁻¹. ¹H NMR (500 MHz, CDCl₃, δ): 3.15-3.20 (m, 2H), 2.42-2.62 (m, 2H), 1.47-1.63 (m, 4H), 1.21-1.32 (m, 15H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 51.47 (C-N₃), 39.15 (C-S), 34.04, 29.45, 29.20, 29.05, 28.83, 27.72, 28.36, 26.71, 24.66.

3.32. Preparation of 9b

Compound 8c (0.36 g, 0.5 mmol) was weighed and dissolved into a solution of dioxane (15 mL) and triethylamine (1 mL) in a 50 mL flask at room temperature. The solution was degassed with nitrogen for 10 minutes, and then CuI (0.10 g, 0.5 mmol) and 1-azidobutane (0.20 g, 2 mmol) were added. The resulting solution was stirred under nitrogen
for 24 h. At the end of this period, the solution was poured into water (40 mL) to which methylene chloride (30 mL) was added. The organic phase was removed, and the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined and then dried with sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using ethyl acetate and THF (8:2) as the eluent to obtain compound 9b (0.33 g, 0.36 mmol) as an oily liquid in 72 % yield. \(^1\)H NMR (500 MHz, CDCl\(_3\), \(\delta\)):\(8.05-8.08\) (m, Ar-H, 4H), 7.82-7.85 (d, Ar-H, 1H), 7.62-7.65 (m, Ar-H, 2H), 7.40-7.45 (m, Ar-H, 6H), 7.30-7.35 (m, Ar-H, 1H), 7.20-7.22 (m, Ar-H, 1H), 7.05-7.10 (d, Ar-H, 1H), 6.85-6.90 (m, Ar-H, 2H), 4.65-4.70 (s, N-CH\(_2\), 4H), 4.22-4.28 (d, NCCH\(_2\), 4H), 3.30-3.38 (m, OCH\(_2\), 8H), 3.10-3.18 (m, OCH\(_2\), 4H), 2.75-2.78 (m, CH\(_2\)CH\(_2\)O, 4H), 2.22-2.30 (d, CH\(_3\)CH\(_2\)O, 4H), 1.82-1.89 (m, CH\(_2\), 4H), 1.26-1.35 (m, CH\(_2\), 4H), 1.09-1.13 (m, OCCH\(_3\), 6H), 0.91-0.95 (m, CH\(_3\), 6H). \(^{13}\)C NMR (500 MHz, CDCl\(_3\), \(\delta\) (ppm)):\(167.80\) (C=N), 154.25 (sp\(^2\) C-N), 150.98, 148.37, 145.0, 141.52, 140.77, 135.87, 134.65, 134.25, 132.17, 130.84, 127.98, 126.55, 126.06, 125.78, 123.25, 122.0, 121.75, 121.45, 118.85, 110.37, 108.89, 70.01, 69.81, 66.87, 66.47, 50.15 (C9), 49.85(C-N), 46.0 (C-N), 39.85 (CH\(_2\)CH\(_2\)O), 32.12, 19.95, 14.89 (CH\(_3\)), 13.67 (CH\(_3\)).

HRMS calcd for C\(_{54}\)H\(_{66}\)O\(_4\)N\(_8\)S, 923.2266; found 922.4922.

3.33. Preparation of 9c

Compound 8a (0.36 g, 0.5 mmol) was weighed and dissolved in a solution of dioxane (15 mL) and triethylamine (1 mL) in a 50 mL flask at room temperature. The solution was degassed with nitrogen for 10 minutes, and then copper (I) iodide (0.10 g, 0.5 mmol) and 1-azido-11-undecanethiol (0.46 g, 2 mmol) were added into the solution and heated to 70 °C.
under nitrogen for 48 h. At the end of this period, the solution was poured into water (40 mL) to which methylene chloride (30 mL) was added. The organic phase was removed, and the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using ethyl acetate and THF (8:2) as the eluent to obtain compound 9c (0.35 g, 0.25 mmol) as an oily liquid in 50 % yield. $^1$H NMR (500 MHz, CDCl$_3$, $\delta$): 8.10-8.15 (m, Ar-H, 4H), 7.90-7.92 (d, Ar-H, 1H), 7.65-7.70 (d, Ar-H, 2H), 7.50-7.58 (m, Ar-H, 6H), 7.35-7.38 (m, Ar-H, 1H), 7.30-7.34 (m, Ar-H, 1H), 7.15-7.18 (d, Ar-H, 1H), 7.05-7.10 (m, Ar-H, 2H), 4.75-4.78 (s, NCH$_2$, 4H), 4.25-4.28 (m, NCCH$_2$, 4H), 3.39-3.41 (dd, OCH$_2$, 8H), 3.20-3.28 (m, OCH$_2$, 4H), 2.75-2.82 (m, CH$_2$CH$_2$O, 4H), 2.62-2.65 (m, 4H), 2.30-2.42 (dd, CH$_3$CH$_2$O, 4H), 1.82-1.85 (m, CH$_2$, 4H), 1.50-1.65 (m, CH$_2$, 10H), 1.20-1.28 (m, CH$_2$, 24H) 1.10-1.12 (m, CH$_3$, 6H). $^{13}$C NMR (500MHz, CDCl$_3$, $\delta$ (ppm)): 167.85 (C=N), 154.20 (sp$^2$ C-N), 151.05, 148.27, 145.11, 141.54, 140.15, 135.19, 134.27, 132.18, 130.47, 127.94, 126.70, 126.22, 125.14, 122.87, 122.15, 121.79, 121.05, 119.06, 112.58, 108.26, 70.06, 69.72, 66.83, 66.57, 51.58 (C9), 50.39 (C-N), 46.75 (C-N), 39.13 (CH$_2$CH$_2$O), 30.35, 29.48, 29.42, 29.25, 29.15, 29.03, 28.84, 28.51, 26.72, 26.53, 15.09 (CH$_3$). HRMS calcd for C$_{68}$H$_{94}$O$_4$N$_8$S$_3$, 1183.7218; found 1183.6347.

3.34. Preparation of 9d

Compound 8d (0.6 g) was utilized and 0.2 g of copper (I) complex$^{151}$ was employed. Purification was used methylene chloride and methanol (95:5) as the eluent to obtain a yellow liquid (40 % yield). $^1$H NMR (500 MHz, CDCl$_3$, $\delta$): 8.10-8.13 (m, Ar-H, 4H), 7.80-
7.85 (d, Ar-H, 1H), 7.62-7.64 (d, Ar-H, 2H), 7.42-7.58 (m, Ar-H, 6H), 7.35-7.38 (m, Ar-H, 1H), 7.25-7.28 (m, Ar-H, 1H), 7.18-7.20 (m, Ar-H, 1H), 6.90-6.98 (m, Ar-H, 2H), 4.70-4.75 (s, NCH\_2, 4H), 4.32-4.35 (m, NCCH\_2, 4H), 3.42-3.62 (m, OCH\_2, 58H), 3.35-3.42 (m, OCH\_2, 10H), 3.20-3.25 (m, OCH\_3, OCH\_2, 10H), 2.90-2.92 (m, 4H), 2.78-2.81 (m, CH\_2CH\_2O, 4H), 2.62-2.65 (m, 4H), 2.38-2.42 (m, 4H), 1.80-1.85 (m, CH\_2, 4H), 1.48-1.78 (m, CH\_2, 10H), 1.20-1.40 (m, CH\_2, CH\_3, 24H). \(^{13}\)C NMR (500MHz, CDCl\_3, \(\delta\) (ppm)): 167.89 (C=N), 154.25 (sp\(^2\) C-N), 151.11, 148.34, 145.01, 141.11, 140.21, 134.92, 134.62, 132.25, 130.05, 127.79, 127.15, 125.01, 123.15, 122.27, 121.88, 121.03, 119.29, 113.59, 108.47, 72.01, 71.10, 70.92, 70.52, 70.31, 70.11, 69.82, 66.58, 58.85 (OCH\_3), 50.78 (C9), 50.18 (C-N), 46.25 (C-N), 39.53 (CH\_2CH\_2O), 30.41, 30.35, 29.56, 29.40, 29.19, 28.79, 28.56,28.21, 27.76, 27.63, 27.41, 26.55. HRMS calcd for C\(_{74}\)H\(_{106}\)O\(_8\)N\(_8\)S\(_3\), 1331.8802; found 1332.3254.

3.35. Preparation of 6\(e\) 

2-Iodo-7-nitro-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 5\(c\) (1.70 g, 3 mmol) was weighed and dissolved into a mixture of THF (15 mL) and triethylamine (15 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10 minutes, then PdCl\(_2\)P(Ph\(_3\))\(_2\) (0.10 g, 0.14 mmol), CuI (0.06 g, 0.32 mmol) and 2-(4-ethynylphenyl)benzothiazole 5\(a\) (0.85 g, 3.6 mmol) were added. The resulting solution was stirred under nitrogen atmosphere for 12 h. At the end of this period, it was poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and
ethyl acetate (6:4) as the eluent to obtain 2-nitro-7-styrylbензотиазолил-9,9-di-2-(2-эфирно этилфенил)флуорен 6e (1.60 g, 2.4 mmol) as a light yellow solid with mp of 126 - 127 0C (68 % yield). 1H NMR (500 MHz, CDCl3, δ): 8.22-8.28 (m, Ar-H, 2H), 8.05-8.15 (m, Ar-H, 3H), 7.88-7.90 (d, Ar-H, 1H), 7.70-7.75 (m, Ar-H, 2H), 7.62-7.68 (m, Ar-H, 3H), 7.55-7.58 (d, Ar-H, 1H), 7.42-7.45 (m, Ar-H, 1H), 7.35-7.38 (m, Ar-H, 1H), 3.05-3.40 (t, OCH2, 12H), 2.75-2.85 (dd, CH2CH2O, 4H), 2.38-2.45 (m, CH3CH2O, 4H), 1.05-1.08 (m, CH3, 6H). 13C NMR (500 MHz, CDCl3, δ (ppm)): 167.57 (C=N), 154.25 (sp2 C-N), 152.03, 149.57, 140.01, 139.23, 135.17, 132.55, 132.13, 131.28, 130.67, 127.50, 126.79, 126.57, 126.49, 125.41, 123.35, 121.96, 121.90, 121.69, 121.47, 119.94, 112.13, 89.79, 70.13, 69.66, 66.82, 66.62, 51.64 (C9), 39.57 (CH2CH2O), 15.10 (CH3). HRMS calcd for C40H40O6N2S, 676.8258; found 676.2601.

3.36. Preparation of 7c

2-Nitro-7-styrylbензотиазолил-9,9-di-2-(2-эфирно этилфенил)флуорен 6d (1.20 g, 1.8 mmol) was weighed and dissolved in a mixture of ethanol (25 mL) and THF (25 mL) in a 200 mL flask at room temperature. The solution was degassed with nitrogen for 10 minutes, and then 10 % palladium on carbon (0.12 g) was added. The resulting solution was heated to 60 0C under nitrogen atmosphere. To this solution, hydrazine hydrate (0.50 g, 10 mmol) was added dropwise in 10 minutes, and then the solution was refluxed at 70 0C for 20 h. At the end of this period, the solution was allowed to cool to room temperature, and then it was filtered using a short silica gel plug, and washed with THF. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using ethyl acetate and THF (4:1) as the eluent to obtain 2-amino-7-styrylbензотиазолил-
9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7c (1.10 g, 1.7 mmol) as a yellow solid with mp of 126 – 127 °C (90 % yield). \(^1\)H NMR (300 MHz, CDCl\(_3\), \(\delta\)): 8.05-8.10 (m, Ar-H, 3H), 7.85-7.90 (d, Ar-H, 1H), 7.61-7.65 (m, Ar-H, 2H), 7.40-7.60 (m, Ar-H, 6H), 6.62-6.74 (d, Ar-H, 2H), 3.78-3.82 (s, NH\(_2\), 2H), 3.29-3.40 (m, OCH\(_2\), 8H), 3.16-3.23 (m, 4H), 2.71-2.80 (m, CH\(_2\)CH\(_2\)O, 4H), 2.27-2.40 (m, CH\(_3\)CH\(_2\)O, 4H), 1.05-1.10 (m, CH\(_3\), 6H). \(^1\)\(^3\)C NMR (CDCl\(_3\), 500 MHz, \(\delta\) (ppm)): 166.25 (C=N), 153.14 (sp\(^2\)-C-N), 150.23, 147.00, 145.94, 140.64, 134.10, 131.83, 131.01, 130.12, 129.62, 126.43, 125.43, 125.22, 125.16, 125.13, 124.33, 122.27, 120.65, 120.21, 118.43, 117.40, 113.44, 108.62, 92.15, 87.29, 69.08, 68.66, 65.94, 65.55, 49.80 (C9), 38.87 (CH\(_2\)CH\(_2\)O), 14.06 (CH\(_3\)). HRMS calcd for C\(_{40}\)H\(_{42}\)O\(_4\)N\(_2\)S, 646.8428; found 646.2859.

3.37. Preparation of 8e

2-Amino-7-styrylbenzothiazoly-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7c (0.65 g, 1 mmol) was weighed and dissolved into DMF (15 mL). The solution was degassed with nitrogen for 10 minutes, and then potassium carbonate (0.5 g, 3.6 mmol), sodium iodide (0.3 g, 2 mmol), propargyl bromide (0.36 g, 3 mmol) were added. The resulting solution was then heated at 95 °C for 20 h under nitrogen atmosphere. At the end of this period, the solution was allowed to cool to room temperature and poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was purified on silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain 2-(N,N-bispropargyl)amino-7-
styrynylbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 8e (0.51 g, 0.7 mmol) as a yellow solid with mp of 144.5 - 145.5 °C in 70 % yield. ¹H NMR (500 MHz, CDCl₃, δ): 8.01-8.10 (m, Ar-H, 3H), 7.85-7.90 (d, Ar-H, 1H), 7.42-7.61 (m, Ar-H, 7H), 7.35-7.38 (m, Ar-H, 1H), 6.85-6.90 (m, Ar-H, 2H), 4.10-4.15 (s, NCH₂, 2H), 3.31-3.38 (m, OCH₂, 8H), 3.13-3.20 (m, 4H), 2.68-2.78 (m, CH₂CH₂O, 4H), 2.25-2.38 (m, CH₃CH₂O, 4H), 1.06-1.09 (m, CH₃, 6H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 165.57 (C=N), 154.11 (sp² C), 150.67, 148.27, 148.11, 140.95, 135.27, 132.25, 132.06, 131.66, 131.45, 127.47, 126.47, 126.20, 129.98, 123.32, 121.68, 121.15, 119.92, 115.89, 114.75, 110.11, 93.85, 89.27, 78.96, 73.10, 70.10, 69.69, 67.01, 66.60, 51.12 (C⁹), 40.92 (NCH₂), 39.79 (CH₂CH₂O), 15.10 (CH₃).

HRMS calcd for C₄₆H₄₆O₄N₂S, 722.9404; found 722.3172.

3.38. Preparation of 8f

2-Amino-7-styrynlbenzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7c (0.65 g, 1 mmol) was weighed and dissolved in 1,2-dichlorobenzene (10 mL) in a 50 mL flask. To this solution, potassium carbonate (0.70 g, 5 mmol), Cu powder (0.3 g, 5 mmol), 18-crown-6 (0.1 g), and iodobenzene (0.60 g, 2.9 mmol) were added. The solution was refluxed at 180 °C for 24 h. At the end of this period, it was allowed to cool to room temperature, and filtered using a short silica gel plug. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain compound 8f (0.45 g, 0.6 mmol) as a yellow solid with mp of 145 – 146 °C in 67 % yield. ¹H NMR (500 MHz, CDCl₃, δ): 8.01-8.08 (m, Ar-H, 3H), 7.82-7.88 (d, Ar-H, 1H), 7.58-7.61 (m, Ar-H, 2H), 7.42-7.55 (m, Ar-H, 3H), 7.35-7.38 (m, Ar-H, 1H), 7.18-7.20 (m, Ar-H, 6H), 7.02-7.08 (m, Ar-H, 5H), 6.92-6.98 (m, Ar-H, 3H),
3.31-3.39 (m, OCH₂, 8H), 3.16-3.23 (m, 4H), 2.74-2.83 (m, CH₂CH₂O, 4H), 2.16-2.29 (m, CH₃CH₂O, 4H), 1.07-1.10 (m, CH₃, 6H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 167.73 (C=N), 154.25 (sp²-C-N), 151.52, 149.01, 148.0, 147.7, 140.15, 135.07, 144.06, 132.61, 132.17, 132.06, 131.65, 131.49, 128.75, 128.47, 126.73, 126.68, 126.35, 126.27, 126.13, 125.57, 122.1, 124.21, 123.89, 123.54, 122.0, 121.80, 121.70, 120.40, 118.65, 118.45, 118.14, 117.93, 92.27, 89.20, 70.11, 69.71, 67.16, 66.63, 51.14 (C9), 39.94 (CH₂CH₂O), 15.21 (CH₃). HRMS calcd for C₅₂H₅₀O₄N₂S, 799.3546; found 799.038.

3.39. Preparation of 6f

Compound 5c (0.60 g, 1 mmol) was dissolved in toluene (10 mL) in a 50 mL flask and the solution was degassed with nitrogen for 10 minutes. To this solution, tetrakis(triphenylphosphine) palladium (0.04 g) was added, and then followed by (2-tri-n-butylstannyl)benzothiazole¹⁰³ (0.70 g, 1.2 mmol). The solution was then refluxed at 110 °C for 12 h under nitrogen atmosphere. At the end of this period, it was allowed to cool to room temperature. The resulting solution was poured into water (50 mL), and then it was extracted by methylene chloride. The filtrate was removed under reduced pressure to obtain a crude produce which was further purified on a silica gel column using hexane and ethyl acetate (40:60) as the eluent to obtain 0.3 g of compound 5b (0.30 g, 0.52 mmol) as a yellow solid with mp of 64 – 65 °C (55 % yield). ¹H NMR (500 MHz, CDCl₃, δ): 8.35 (s, Ar-H, 1H), 8.30-8.32 (m, Ar-H, 1H), 8.25 (s, Ar-H, 1H), 8.12-8.18 (m, Ar-H, 2H), 7.95-7.98 (d, Ar-H, 1H), 7.85-7.92 (m, Ar-H, 2H), 7.51-7.57 (m, Ar-H, 1H), 7.22-7.25 (m, Ar-H, 1H), 3.30-3.39 (m, OCH₂, 4H), 3.22-3.25 (m, 4H), 3.15-3.20 (m, 4H), 2.80-2.90 (m, CH₂CH₂O, 2H), 2.80-2.82 (m, CH₂CH₂O, 2H), 2.55-2.58 (m, CH₃CH₂O, 4H), 1.07-1.10 (m, CH₃, 6H). ¹³C NMR (500
3.40. Preparation of 7d

Compound 6f (0.58 g, 1 mmol) was dissolved in a mixture of ethanol (5 mL) and THF (5 mL) in a 50 mL flask. The solution was degassed with nitrogen for 10 minutes, and then 10 % palladium on carbon (0.06 g) was added. The solution was heated to 60 °C under nitrogen atmosphere. To this solution, hydrazine hydrate (0.26 g, 6 mmol) was added dropwise in 10 minutes, and then the resulting solution was refluxed at 70 °C for 20 h. At the end of this period, the solution was allowed to cool to room temperature, and then it was filtered using a short silica gel plug. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (1:4) as the eluent to obtain 2-amino-7-benzothiazolyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7d (0.45 g, 0.8 mmol) as a yellow solid with mp of 131 - 132 °C in 85 % yield. $^1$H NMR (300 MHz, CDCl$_3$, δ): 8.01-8.04 (d, Ar-H, 2H), 7.92-7.95 (d, Ar-H, 1H), 7.84-7.85 (d, Ar-H,1H), 7.53-7.55 (d, Ar-H,1H), 7.42-7.46 (m, Ar-H, 2H), 7.32-7.35 (m, Ar-H, 1H), 6.75-6.78 (s, Ar-H, 1H), 6.61-6.65 (d, Ar-H, 2H), 3.25-3.36 (m, 10H), 3.11-3.18 (m, 4H), 2.65-2.78 (m, CH$_2$CH$_2$O, 4H), 2.42-2.46 (m, CH$_2$CH$_2$O, 2H), 2.27-2.33 (m, CH$_3$CH$_2$, 2H), 1.02-1.05 (m, CH$_3$, 6H).$^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 168.63 (C=N), 154.14 (sp$^2$ C-N), 151.75, 148.78, 146.49, 144.07, 134.89, 130.91, 130.37, 127.40, 126.25, 126.15, 125.25, 120.76, 121.79, 121.59, 121.55, 118.48, 114.84, 110.01, 70.09, 69.66, 66.99, 66.56,
Compound 7d (0.40 g, 0.7 mmol) was weighed and dissolved in 1,2-dichlorobenzene (10 mL) in a 50mL flask. To this solution, potassium carbonate (0.35 g, 2.5 mmol), Cu powder (0.15 g, 2.5 mmol), 18-crown-6 (0.1 g), and then 4-iodoanisole (0.52 g, 2.2 mmol) were added and heated to 180 °C for 24 h. At the end of this period, the resulting mixture was allowed to cool to room temperature, and then it was filtered using a short silica gel plug. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (1:1) as the eluent to obtain compound 8g (0.33 g, 0.4 mmol) as a yellow waxy solid in 60 % yield. ^1H NMR (500 MHz, CDCl₃, δ): 8.03-8.13 (dd, Ar-H, 3H), 7.92-7.95 (d, Ar-H, 1H), 7.62-7.64 (d, Ar-H, 1H), 7.48-7.51 (m, Ar-H, 2H), 7.36-7.39 (m, Ar-H, 2H), 7.01-7.10 (m, Ar-H, 3H), 7.0 (s, 1H), 6.85-6.92 (m, Ar-H, 5H), 3.83 (s, OCH₃, 6H), 3.36-3.43 (m, OCH₂, 8H), 3.22-3.29 (m, 4H), 2.83-2.88 (m, CH₂CH₂O, 4H), 2.38-2.44 (m, CH₃CH₂O, 2H), 2.25-2.30 (m, CH₃CH₂O, 2H), 1.03-1.08 (m, CH₃, 6H). ^13C NMR (500 MHz, CDCl₃, δ (ppm)): 168.25 (C=N), 156.00 (sp²-C-N), 154.10, 151.22, 149.46, 143.70, 140.83, 134.24, 131.93, 131.14, 127.38, 126.68, 126.62, 126.52, 124.25, 122.94, 121.84, 121.63, 121.23, 120.01, 119.42, 114.78, 70.06, 69.69, 67.19, 66.59, 55.56 (OCH₃), 51.23 (C9), 39.39 (CH₂CH₂O), 15.10 (CH₃). HRMS calcd for C₄₆H₅₀O₆N₂S, 758.9708; found 758.3383.
2-Iodo-7-nitro-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 5c (2.90 g, 5 mmol) was weighed and dissolved into a solution of triethylamine (20 mL) and THF (20 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10 minutes, and then PdCl₂P(Ph₃)₂ (0.18 g, 0.25 mmol) and 0.1 g of CuI (0.1 g, 0.5 mmol) were added and following up by trimethylsilylacetylene (0.75 g, 7.5 mmol). The resulting solution was stirred for 12 h at room temperature, and then it was poured into water (50 mL) to which methylene chloride (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then the organic phases were combined, washed with water and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (7:3) to obtain 2-nitro-7-(1-trimethylsilyl)ethynyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 6g (2.10 g, 0.4 mmol) as a yellow oily liquid (70 % yield). \(^1\)H NMR (500 MHz, CDCl₃, δ): 8.18-8.23 (d, Ar-H, 2H), 7.68-7.70 (d, Ar-H, 1H), 7.63-7.64 (d, Ar-H, 1H), 7.50 (s, Ar-H, 1H), 7.44-7.46 (d, Ar-H, 1H), 3.30-3.32 (m, OCH₂ 4H), 3.20-3.22 (m, 2H), 3.04-3.11 (m, 4H), 2.73-2.78 (d, CH₂CH₂O, 2H), 2.65-2.71 (d, CH₂CH₂O, 2H), 2.31-2.43 (d, CH₃CH₂O, 4H), 1.05-1.18 (m, CH₃, 6H), 0.20-0.32 (s, Si(CH₃)₃, 9H). \(^{13}\)C NMR (500 MHz, CDCl₃, δ (ppm)): 151.56, 151.27, 147.91, 146.59, 138.92, 132.72, 127.87, 121.91, 121.04, 120.49, 119.74, 119.49, 105.58, 96.82, 70.99, 70.71, 67.74, 67.34, 52.65 (C9), 40.07 (CH₂CH₂O), 16.06 (CH₃), 0.64 (Si(CH₃)₃). HRMS calcd for C₃₀H₄₁NO₆Si, 539.743; found 539.2725.

3.43. Preparation of 7e
Compound 6g (2.00 g, 3.8 mmol) was weighed and dissolved into THF (20 mL) in a 100 mL flask. To this solution, a solution of 5% potassium hydroxide in methanol (20 mL) was added and stirred for 30 minutes. At the end of this period, the resulting solution was poured into water (50 mL), and then methylene chloride (40 mL) was added. The organic phase was removed, and the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and then dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (7:3) as the eluent to obtain 2-nitro-7-ethynyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7e (1.60 g, 3.4 mmol) as a yellow solid with mp of 88 – 89 °C in 90% yield. ¹H NMR (500 MHz, CDCl₃, δ): 8.10-8.15 (m, Ar-H, 2H), 7.70-7.80 (m, Ar-H, 2H), 7.50-7.61 (m, Ar-H, 2H), 3.20-3.42 (t, OCH₂, sp-CH, 13H), 2.79-2.85 (m, CH₂CH₂O, 4H), 2.30-2.45 (m, CH₃CH₂O, 4H), 1.05-1.14 (m, CH₃, 6H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 150.99, 150.77, 147.40, 145.87, 138.67, 131.96, 127.37, 123.61, 122.94, 121.16, 120.27, 119.06, 83.60, 78.81, 70.12, 69.58, 66.77, 66.60, 52.10 (C9), 39.24 (CH₂CH₂O), 15.05 (CH₃). HRMS calcd for C₂₇H₃₃O₆N, 467.5698; found 467.2302.

3.44. Preparation of Azidobenzene

Phenyl hydrazine (3.40 g, 31 mmol) was weighed in a 30 mL beaker and then was added into a solution of hydrochloric acid (6 mL) and water (30 mL) in a 200 mL flask at 0-5 °C immersing in an ice bath. The resulting solution was stirring with a magnetic stirring bar and maintained at this temperature during the experimental. To this solution, diethyl ether (10 mL) was then added which was followed by the dropwise addition of a solution of
sodium nitrite (2.50 g, 36 mmol) in water (5 mL). The resulting mixture was allowed to stand at this temperature for an additional 15 minutes, and then diethyl ether (10 mL) was added. It was poured into a 100 mL separatory flask and extracted with portions of diethyl ether (45 mL). The organic layers were combined, dried over sodium sulfate, and then evaporated under reduce pressure to obtain a crude product. The crude produce was purified on a silica gel column using hexane as eluent to obtain azidobenzene (2.2 g, 18 mmol) of a colorless liquid in 60 % yield. IR (neat, cm⁻¹): 2125 (s, N₃), 2094 (s, N₃), 1594, 1492, 1294. ¹H NMR (500 MHz, CDCl₃, δ): 7.36-7.39 (m, Ar-H, 2H), 7.05-7.18 (m, Ar-H, 3H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 140.00, 129.78, 124.89, 119.04.

3.45. Preparation of 8h

2-Nitro-7-ethynyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 7e (1.50 g, 3 mmol) was weighed and dissolved in dioxane (15 mL) in a 50 mL flask. To this solution, triethylamine (1 mL), CuI (0.10 g, 0.5 mmol), and azidobenzene (0.50 g, 4.2 mmol) were added. The resulting solution was stirred under nitrogen atmosphere for 24 h. At the end of this period, the solution was poured into water (40 mL) to which methylene chloride (50 mL) was added. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then the organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product. It was purified on a silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain 8h (1.30 g, 2.4 mmol) as a light yellow solid with mp of 122 – 123 °C in 80 % yield. ¹H NMR (500 MHz, CDCl₃, δ): 8.38 (s, Ar-H, 1H), 8.30-8.38 (dd, Ar-H, 2H), 8.01-8.05 (s, Ar-H, 2H), 7.79-7.85 (m, Ar-H, 4H), 7.51-7.61 (m, Ar-H, 2H), 7.50-7.55 (m, Ar-H, 1H), 3.34-3.39 (m, OCH₂, 4H),
3.25-3.28 (m, 4H), 3.15-3.18 (m, 4H), 2.82-2.90 (m, CH₂CH₂O, 2H), 2.78-2.81 (m, CH₂CH₂O, 2H), 2.51-2.57 (m, CH₃CH₂O, 4H), 1.07-1.10 (m, CH₃, 6H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 151.56, 150.89, 148.03 (sp² C-N), 147.22, 146.34, 138.36, 137.22, 131.40, 129.91, 129.90, 128.99, 125.63, 123.65, 121.88, 120.90, 120.56, 119.98, 118.99, 118.19, 70.13, 69.59, 66.88, 66.57, 52.19 (C9), 39.39 (CH₂CH₂O), 15.02 (CH₃). HRMS calcd for C₃₃H₃₈O₆N₄, 586.6864; found 586.2785.

3.46. Preparation of 9e

Compound 8h (1.00 g, 1.7 mmol) was weighed and dissolved in a mixture of ethanol (20 mL) and THF (20 mL) in a 100 mL flask, and then 10 % palladium on carbon (0.10 g) was added. The solution was degassed with nitrogen for 10 minutes, and then it was heated to 60 °C under nitrogen atmosphere. To this solution, hydrazine monohydrate (0.50 g, 10 mmol) was added dropwise in 15 minutes. The resulting solution was refluxed at 70 °C under nitrogen atmosphere for 24 h. At the end of this period, the solution was allowed to cool to room temperature, and then it was filtered and washed with ethyl acetate (100 mL). The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using ethyl acetate as the eluent to obtain 9e (0.85 g, 1.5 mmol) as a yellow solid with mp of 156 - 157 °C in 90 % yield. ¹H NMR (500MHz, CDCl₃, δ): 8.12 (s, Ar-H, 1H), 7.82-7.86 (m, Ar-H, 4H), 7.60-7.75 (m, Ar-H, 5H), 6.45-6.50 (s, Ar-H, 1H), 6.40-6.42 (m, Ar-H, 1H), 3.80 (s, 2H), 3.30-3.42 (m, OCH₂, 8H), 3.21-3.25 (m, 4H), 2.70-2.80 (m, CH₂CH₂O, 4H), 2.30-2.40 (m, CH₃CH₂O, 4H), 1.02-1.08 (m, CH₃, 6H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 151.04, 148.87, 148.64, 141.25, 137.14, 132.0, 129.84, 128.75, 127.68, 125.11, 120.94, 120.92, 120.52, 120.28, 119.03, 117.43, 114.56, 110.30,
3.47. Preparation of 10

Compound 9e (0.50 g, 0.9 mmol) was weighed and dissolved in 1,2-dichlorobenzene (10 mL) in a 50 mL flask. To this solution, potassium carbonate (0.70 g, 5 mmol), Cu powder (0.30 g, 5 mmol), 18-crown-6 (0.1 g), and iodobenzene (0.60 g, 2.9 mmol) were added and heated to reflux at 180 °C under nitrogen atmosphere for 24 h. At the end of this period, the solution was allowed to cool to room temperature, and then it was filtered using a short silica gel plug. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (2:8) as the eluent to obtain 10 (0.40 g, 0.56 mmol) as a yellow solid with mp of 40 – 41 °C (60 % yield). 1H NMR (500 MHz, CDCl3, δ): 7.62-7.64 (d, Ar-H, 1H), 7.38-7.58 (m, Ar-H, 8H), 7.42-7.45 (m, Ar-H, 5H), 7.03-7.12 (m, aromatic, 8H), 3.38-3.45 (m, OCH2, 8H), 3.24-3.25 (m, 4H), 2.75-2.81 (m, CH2CH2O, 4H), 2.02-2.18 (m, CH3CH2O, 4H), 1.05-1.10 (m, CH3, 6H). 13C NMR (500 MHz, CDCl3, δ (ppm)): 150.58, 149.25, 147.82, 147.75, 145.17, 140.01, 136.02, 134.95, 130.17, 129.51, 129.27, 129.18, 128.98, 127.69, 126.69, 125.18, 124.18, 123.40, 122.84, 121.78, 120.61, 119.38, 118.81, 70.05, 69.72, 67.19, 66.58, 50.98 (C9), 39.22 (CH2CH2O), 15.13 (CH3). HRMS calcd for C45H48N4O4, 708.8989; found 708.3669.

3.48. Preparation of 3d

2-Iodofluorene (10.20 g, 35 mmols) was weighed and dissolved in propylene carbonate (120 mL) at 40 °C in a 250 mL flask. To this solution, N-bromosuccinimide (7.60 g, 42 mmol) was added in portions in 15 minutes. The solution was heated to 65 °C and
maintained at this temperature for 4 h. At the end of this period, the resulting solution was then heated to 80 °C for 30 minutes and allowed to cool to room temperature. The resulting mixture was poured into water (200 mL) to which methylene chloride (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (50 mL), and then organic phases were combined, wash with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product. It was recrystallized from ethyl acetate to obtain 2-iodo-7-bromofluorene (10.50 g, 28 mmol) as a white crystal with mp of 182 - 184 °C (80 % yield). \(^1\)H NMR (CDCl\(_3\), 500 MHz, \(\delta\)): 7.79 (s, Ar-H, 1H), 7.58-7.62 (m, Ar-H, 2H), 7.48-7.50 (m, Ar-H, 1H), 7.20-7.25 (m, Ar-H, 2H), 3.75-3.77 (m, CH\(_2\), 2H). \(^{13}\)C NMR (CDCl\(_3\), 500 MHz, \(\delta\) (ppm)): 145.02, 144.61, 140.30, 139.76, 136.00, 134.22, 130.14, 128.25, 121.54, 121.26, 121.21, 92.30 (C-I), 36.43 (CH\(_2\)).

3.49. Preparation of 4e

2-iodo-7-bromofluorene (2.30 g, 6 mmol) was utilized and hexane and ethyl acetate (7:3) was used as the eluent to obtain 2-iodo-7-bromo-9,9-di-2-(2-ethoxyethoxy)ethyfluorene 4e (2.20 g, 3.6 mmol) as a white solid with mp of 81 - 82 °C in 60 % yield. \(^1\)H NMR (500 MHz, CDCl\(_3\), \(\delta\)): 7.66 (s, Ar-H, 1H), 7.61 (d, Ar-H, 1H), 7.41-7.50 (dd, Ar-H, 3H), 7.40 (d, Ar-H, 1H), 3.38-3.40 (m, OCH\(_2\),48H), 3.28-3.30 (m, 4H), 3.15-3.18 (m, 4H), 2.65-2.70 (m, CH\(_2\)CH\(_2\)O, 4H), 2.25-2.30 (m, CH\(_3\)CH\(_2\)O, 4H), 1.05-1.10 (m, CH\(_3\), 6H). \(^{13}\)C NMR (500 MHz, CDCl\(_3\), \(\delta\) (ppm)): 151.02, 150.68, 139.05, 138.50, 136.53, 132.58, 130.64, 126.67, 121.82, 121.67, 121.25, 93.08, 70.12, 69.66, 66.77, 66.63, 51.77 (C9), 39.45 (CH\(_2\)CH\(_2\)O), 15.10 (CH\(_3\)). HRMS calcd for C\(_{25}\)H\(_{32}\)O\(_4\)IBr, 603.3354; found 602.0484.
3.50. Preparation of 6h

Compound 4e (0.60 g, 1 mmol) was weighed and dissolved in THF (10 mL) and triethylamine (10 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10 minutes, and then PdCl$_2$P(Ph)$_3$$_2$ (0.05 g, 0.07 mmol), CuI (0.03 g, 0.16 mmol), and compound 5a (0.30 g, 1.2 mmol) were added. The resulting solution was stirred under nitrogen atmosphere for 24 h. At the end of this period, it was poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (1:1) as the eluent to obtain 6h (0.40 g, 0.56 mmol) as a yellow solid with mp of 132 – 133 °C in 60 % yield. $^1$H NMR (500 MHz, CDCl$_3$, δ): 8.10-8.15 (m, Ar-H, 3H), 7.90-7.92 (d, Ar-H, 1H), 7.65-7.70 (m, Ar-H, 3H), 7.50-7.22 (m, Ar-H, 6H), 7.45-7.47 (m, Ar-H, 1H), 3.40-3.42 (m, OCH$_2$, 4H), 3.35-3.38 (m, 4H), 3.18-3.21 (m, 4H), 2.78-2.81 (m, CH$_2$CH$_2$O, 4H), 2.38-2.42 (m, CH$_3$CH$_2$O, 4H), 1.14-1.18 (m, CH$_3$, 6H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 167.62 (C=N), 154.21 (sp$^2$-C-N), 151.98, 148.67, 139.95, 139.23, 135.15, 133.05, 132.11, 132.00, 131.85, 131.60, 131.41, 131.21, 127.82, 127.52, 126.54, 126.05, 125.93, 125.84, 125.37, 123.50, 122.00, 121.05, 120.01, 92.35, 89.79, 70.02, 69.81, 67.05, 66.73,49.94 (C9), 39.87 (CH$_2$CH$_2$O), 15.08 (CH$_3$). HRMS calcd for C$_{40}$H$_{40}$O$_4$NSBr, 710.7263; found 710.1888.

3.51. Preparation of 4-Iodo-N,N-Dimethylbenzenamine
N,N-dimethylbenzenamine (14.70 g, 120 mmol) was weighed and dissolved in a solution of sodium carbonate (15 g, 140 mmol) in water (100 mL) at 10 °C in a 250 mL flask. To this solution, iodine (25.40 g, 100 mmol) was added in portions in 30 minutes, and then it was stirred for an additional of 30 minutes. At the end of this period, the resulting solution was poured into water (100 mL) to which methylene chloride (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (50 mL), and then the organic phases were combined, washed with water (100 mL), and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and ethyl acetate (7:3) as the eluent to obtain 4-iodo-N,N-dimethylbenzenamine (17 g, 69 mmol) as a solid with mp of 75 – 77 °C in 70 % yield. $^1$H NMR (500 MHz, CDCl$_3$, δ): 7.38-7.40 (m, Ar-H, 2H), 6.41-6.44 (m, Ar-H, 2H), 2.85-2.88 (m, NCH$_3$, 6H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 150.00, 137.55, 114.71, 77.44 (C-I), 40.39 (NCH$_3$).

3.52. Preparation of 4-(2-Trimethylsilylethynyl)-N,N-Dimethylbenzenamine

4-Iodo-N,N-dimethylbenzenamine (7.24 g, 30 mmol) was weighed and dissolved into triethylamine (60 mL) in a 250 mL flask. The solution was degassed with nitrogen for 10 minutes, and then PdCl$_2$P(Ph$_3$)$_2$ (0.60 g, 0.85 mmol) and copper(I) iodide (0.45 g, 2.4 mmol) were added. To this solution, trimethylsilylacetylene (4.30 g, 44 mmol) was added and the solution was stirred overnight. At the end of this period, the resulting solution was poured into water (100 mL) to which methylene chloride (50 mL) was added. The organic phase was removed, and then the aqueous phase was extracted 3 times with methylene chloride (40 mL). The organic phases were combined, washed with water, and dried over sodium sulfate.
The filtrate was removed under reduced pressure to obtain a crude product which was purified in a silica gel column using hexane and ethyl acetate (8:2) as the eluent to obtain 4-(2-trimethylsilylethylnyl)-N,N-dimethylbenzenamine (4.0 g, 19 mmol) as light yellow solid with mp of 88 – 89 °C in 70 % yield. $^1$H NMR (500 MHz, CDCl$_3$, δ): 7.08-7.12 (m, Ar-H, 2H), 6.35-6.37 (m, Ar-H, 2H), 2.73-2.75 (m, NCH$_3$, 6H), 0.00-0.16 (m, Si(CH$_3$)$_3$, 9H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 149.95, 132.90, 111.33, 109.61, 106.32, 90.94, 39.95 (NCH$_3$), 0.60 (Si(CH$_3$)$_3$).

3.53. Preparation of 4-Ethynyl-N,N-Dimethylbenzenamine 7f

4-(2-Trimethylsilylethylnyl)-N,N-dimethylbenzenamine (2.0 g, 10 mmol) was weighed and dissolved in THF (10 mL) in a 100 mL flask, and then of a solution of potassium hydroxide (1.0 g, 18 mmol) in methanol (10 mL) was added. The resulting solution was stirred for 30 minutes, and then it was poured into water (50 mL) to which methylene chloride (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water (50 mL), and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further purified in a silica gel column using hexane and ethyl acetate (7:3) as the eluent to obtain 4-ethynyl-N,N-dimethylbenzenamine 7f (1.10 g, 7.6 mmol) as a light yellow powder with mp of 51 - 52 °C in 80 % yield. $^1$H NMR (500 MHz, CDCl$_3$, δ): 7.22-7.23 (m, Ar-H, 2H), 6.46-6.49 (m, Ar-H, 2H), 2.83-2.84 (m, NCH$_3$, sp-CH, 7H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 150.38, 133.21, 111.68, 108.73, 84.87 (sp-C), 74.80 (sp-C), 40.18 (NCH$_3$).
3.54. Preparation of 8i

Compound 6h (0.71 g, 1 mmol) was weighed and dissolved in THF (10 mL) and triethylamine (10 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10 minutes, and then PdCl₂P(Ph₃)₂ (0.05 g, 0.07 mmol), CuI (0.03 g, 0.16 mmol), and compound 7f (0.22 g, 1.5 mmol) were added. The resulting solution was refluxed at 70 °C under nitrogen atmosphere for 48 h. At the end of this period, the solution was allowed to cool to room temperature, and poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was purified on a silica gel column using hexane and ethyl acetate (1:1) as the eluent to obtain compound 8i (0.4 g, 0.5 mmol) as a yellow solid with mp of 139-140 °C (52 % yield). ¹H NMR (500 MHz, CDCl₃, δ): 8.10-8.15 (m, Ar-H, 3H), 7.90-7.94 (d, Ar-H, 1H), 7.65-7.75 (m, Ar-H, 4H), 7.50-7.58 (m Ar-H, 4H), 7.41-7.45 (m, Ar-H, 4H), 6.62-6.65 (m, Ar-H, 2H), 3.42-3.45 (m, OCH₂, 4H), 3.32-3.38 (m, 4H), 3.18-3.20 (m, 4H), 3.02 (s, NCH₃, 6H), 2.78-2.80 (m, CH₂CH₂O, 4H), 2.41-2.43 (m, CH₃CH₂O, 4H), 1.14-1.16 (m, CH₃, 6H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 167.23 (C=N),154.15 (sp² C-N), 150.01, 149.75, 140.36, 138.27, 135.78, 133.09, 132.92, 132.63, 132.23, 132.13, 131.56, 127.89, 126.25, 126.15, 126.05, 125.99, 121.88, 121.61, 120.01, 111.85, 110.01, 92.27, 92.00, 89.61, 88.05, 70.10, 69.65, 66.87, 66.60, 51.26 (C9), 40.50 (C-N), 39.69 (CH₂CH₂O), 15.03 (CH₃). HRMS calcd for C₅₀H₅₀O₄N₂S, 775.016; found 774.3485.

3.55. Preparation of 4f
2,7-diiodofluorene 2a and compound 3c were utilized, and methylene chloride and methanol (95:5) was used as the eluent to obtain an oily liquid in 30 % yield. $^1$H NMR (500 MHz, CDCl$_3$, δ): 7.70 (s, Ar-H, 2H), 7.62-7.65 (d, Ar-H, 2H), 7.38-7.40 (d, Ar-H, 2H), 3.51-3.65 (m, OCH$_2$, 60H), 3.35-3.38 (s, OCH$_3$, 6H), 3.15-3.18 (m, 4H), 2.68-2.70 (m, CH$_2$CH$_2$O, 4H), 2.35-2.38 (m, 4H), 2.25-2.30 (m, CH$_2$CH$_2$O, 4H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 150.76, 139.11, 136.50, 132.45, 121.60, 93.23, 71.91, 70.63, 70.59, 70.55, 70.51, 70.40, 70.02, 69.80, 66.72, 59.05 (OCH$_3$), 51.23 (C9), 39.43 (CH$_2$CH$_2$O).

3.56. Preparation of 5e

Compound 4f (0.65 g, 1 mmol) was weighed and dissolved in THF (10 mL) and of triethylamine (10 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10 minutes, and then 0.1 g of PdCl$_2$P(Ph$_3$)$_2$ (0.1 g, 0.14 mmol), CuI (0.06 g, 0.3 mmol), and compound 7f (0.35 g, 2.4 mmol) were added. The resulting solution was stirred under nitrogen atmosphere for 24 h. At the end of this period, it was poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product. It was purified on a silica gel column using methylene chloride and methanol (95:5) as the eluent to obtain compound 5e (0.90 g, 0.7 mmol) as an oily liquid in 72 % yield. $^1$H NMR (500 MHz, CDCl$_3$, δ): 7.60-7.62 (d, Ar-H, 2H), 7.55-7.58 (s, Ar-H, 2H), 7.42-7.52 (m, Ar-H, 6H), 6.65-6.70 (d, Ar-H, 4H), 3.51-3.65 (m, OCH$_2$, 60H), 3.38-3.41 (m, OCH$_3$, 10H), 3.19-3.21 (m, 4H), 3.01 (s, 12H), 2.75-2.85 (m, CH$_2$CH$_2$O, 4H), 2.40-2.42 (m, 4H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 150.09, 149.12,
139.31, 132.75, 131.23, 126.11, 123.04, 120.0, 111.84, 109.88, 91.46, 88.08, 71.91, 70.55, 70.51, 70.49, 70.37, 70.0, 66.84, 59.06 (OCH$_3$), 51.12 (C9), 40.24 (C-N), 39.80 (CH$_2$CH$_2$O). HRMS calcd for C$_{75}$H$_{112}$O$_2$N$_2$, 1361.7112; found 1361.7881.

3.57. Preparation of 5f

2,7-Diiodo-9,9-diethyl-2-(2-ethoxy ethoxy)fluorene 4a (0.65 g, 1 mmol) was weighed and dissolved in 1,2-dichlorobenzene (10 mL) in a 50 mL flask. To this solution, potassium carbonate (0.70 g, 5 mmol), 0.3 g of Cu powder (0.3 g, 5 mmol), 18-crown-6 (0.1 g), and diphenylamine (0.51 g, 3 mmol) were added and heated to reflux at 180 $^\circ$C under nitrogen atmosphere for 24 h. At the end of this period, the resulting mixture was allowed to cool to room temperature, and then it was poured into a short silica gel plug and washed with ethyl acetate. The filtrate was removed under reduced pressure to obtain a crude product. It was purified on a silica gel column using hexane and ethyl acetate (8:2) as the eluent to obtain compound 5f (0.45 g, 0.6 mmol) in as a white powder with mp of 50 -52 $^\circ$C (62 % yield). $^1$H NMR (500 MHz, CDCl$_3$, $\delta$): 7.45-7.48 (d, Ar-H, 3H), 7.20-7.25 (m, Ar-H, 5H), 7.00-7.17 (m, Ar-H, 18H), 3.42-3.47 (m, OCH$_2$, 8H), 3.25-3.28 (m, 4H), 2.85-2.92 (m, CH$_2$CH$_2$O, 4H), 2.15-2.18 (m, CH$_3$CH$_2$O, 4H), 1.10-1.15 (m, CH$_3$, 6H). $^{13}$C NMR (500 MHz, CDCl$_3$, $\delta$ (ppm)): 150.22, 147.91, 146.80, 135.14, 129.42, 129.03, 124.18, 123.78, 123.52, 122.82, 122.48, 120.07, 70.04, 69.75, 67.41, 66.64, 50.94 (C9), 39.09 (CH$_2$CH$_2$O), 15.06 (CH$_3$). HRMS calcd for C$_{49}$H$_{52}$O$_4$N$_2$, 732.9608; found 732.3960.

3.58. Preparation of 5g

Compound 4a (0.65 g, 1 mmol) was weighed and dissolved in THF (10 mL) and triethylamine (10 mL) in a 100 mL flask. The solution was degassed with nitrogen for 10
minutes, and then PdCl₂P(Ph₃)₂ (0.1 g, 0.14 mmol), CuI (0.06 g, 0.3 mmol), and trimethylsilylacetylene (0.30 g, 3 mmol) were added. The resulting solution was stirred under nitrogen atmosphere for 24 h. At the end of this period, the solution was poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with 40 mL of methylene chloride, and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was purified on a silica gel column using hexane and ethyl acetate (8:2) as the eluent to obtain compound 5g (0.48 g, 0.7 mmol) as a dark yellow solid with mp of 61 -63 °C in 75 % yield. ¹H NMR (500 MHz, CDCl₃, δ): 7.38-7.40 (d, Ar-H, 2H), 7.33 (s, Ar-H, 2H), 7.32-7.35 (d, Ar-H, 2H), 3.21-3.25 (m, OCH₂, 4H), 3.13-3.15 (m, 4H), 2.97-2.98 (m, 4H), 2.50-2.53 (m, CH₂CH₂O), 4H), 2.18-2.21 (m, CH₃CH₂O, 4H), 0.95-0.97 (m, CH₃, 6H), 0.10 (s, Si(CH₃)₃, 18H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 149.81, 140.64, 122.82, 106.17, 95.53, 88.55 (sp-C), 86.56 (sp-C), 70.70, 70.24, 67.39, 67.20, 51.82 (C9), 40.20 (CH₂CH₂O), 15.75 (CH₃), 0.63 (Si(CH₃)₃).

3.59. Preparation of 6i

Compound 5g (0.47 g, 0.8 mmol) was weighed and dissolved in THF (10 mL) in a 100 mL flask, and then followed by a solution of 5 % potassium hydroxide in methanol (10 mL). The resulting solution was stirred for 30 minutes, and then it was poured into water (50 mL) to which methylene chloride (50 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water (50 mL), and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was further
purified on a silica gel column using hexane and ethyl acetate (7:3) as the eluent to obtain 2,7-diethynyl-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene 6i (0.34 g, 0.75 mmol) as a white solid with mp of 85 – 86 °C (95 % yield). $^1$H NMR (500 MHz, CDCl$_3$, δ): 7.42-7.45 (d, Ar-H, 2H), 7.35-7.39 (s, Ar-H, 2H), 7.30-7.32 (d, Ar-H, 2H), 3.41-3.43 (m, OCH$_2$, 4H), 3.32-3.35 (m, 4H), 3.17-3.20 (m, 4H), 2.70-2.75 (m, CH$_2$CH$_2$O, 4H), 2.35-2.40 (m, CH$_3$CH$_2$O, 4H), 1.10-1.15 (m, CH$_3$, 6H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 149.36, 140.24, 132.1, 127.0, 121.27, 120.01, 84.21 (sp-C), 77.80 (sp-C), 70.09, 69.63, 66.79, 66.60, 51.32 (C9), 39.48 (CH$_2$CH$_2$O), 15.10 (CH$_3$). HRMS calcd for C$_{29}$H$_{34}$O$_4$, 446.5852; found 446.2451.

3.60. Preparation of 7g

Compound 6i (0.36 g, 0.8 mmol) was weighed and dissolved THF (10 mL) and triethylamine (10 mL) in a 50 mL flask. The resulting solution was degassed with nitrogen for 10 minutes, and then PdCl$_2$P(Ph$_3$)$_2$ (0.10 g, 0.14 mmol), CuI (0.06 g, 0.3 mmol), and 4-iodophenol (0.55 g, 2.5 mmol) were added. The solution was stirred under nitrogen atmosphere for 24 h. At the end of this period, the resulting solution was poured into water (50 mL) to which methylene chloride (40 mL) was added. The organic phase was removed. The aqueous was extracted 3 times with methylene chloride (40 mL), and then organic phases were combined, washed with water, and dried over sodium sulfate. The filtrate was removed under reduced pressure to obtain a crude product which was purified on a silica gel column using methylene chloride and methanol (95:5) as the eluent to obtain compound 7g (0.30 g, 0.5 mmol) as a light yellow solid with mp of 52 – 53 °C (58 % yield). IR (neat, cm$^{-1}$): 3273 (OH), 2973, 2866, 1604, 1512, 1462, 1272, 1221, 1096, 823. $^1$H NMR(500 MHz, CDCl$_3$, δ): 7.62-7.70 (m, Ar-H, 3H), 7.50-7.58 (m, Ar-H, 7H), 6.81-6.83 (m, Ar-H, 4H),
6.15-6.18 (s, OH, 2H), 3.36-3.46 (m, OCH₂, 8H), 3.20-3.22 (m, 4H), 2.75-2.77 (m, CH₂CH₂O, 4H), 2.30-2.37 (m, CH₃CH₂O, 4H), 1.05-1.10 (m, CH₃, 6H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 156.32, 149.16, 139.60, 133.31, 132.0, 131.51, 128.45, 126.10, 122.73, 120.02, 115.70, 115.07, 90.35, 88.55, 70.08, 69.64, 66.91, 66.76, 51.14 (C9), 39.85 (CH₂CH₂O), 15.01 (CH₃). HRMS calcd for C₄₁H₄₂O₆, 630.7792; found 630.2975.

3.61. Preparation of Gold Nanoparticles

Gold nanoparticles were prepared according to a procedure from the literature. The size of the gold nanoparticles was ca. 12 nm (Appendix D).

3.62. Preparation of Nanoparticle Composite 11a

To a 150 mL solution of gold nanoparticles in a 250 mL flask, 50 mL of a solution of 9c (2.5x10⁻⁴ M) in methylene chloride was added. The resulting solution was stirred for 1 h. The organic phase was separated and removed under reduced pressure to get a black waxy solid. It was washed with ethanol and dried in vacuo to obtain 11a. ¹H NMR (500 MHz, CDCl₃, δ): 8.00-8.02 (m, Ar-H, 4H), 7.82-7.84 (m, Ar-H, 1H), 7.56-7.58 (m, Ar-H, 2H), 7.30-7.48 (m, Ar-H, 7H), 7.20-7.21 (m, Ar-H, 1H), 7.09-7.11 (m, Ar-H, 1H), 6.87-6.89 (m, Ar-H, 2H), 4.60-4.61 (s, 4H), 4.22-4.24 (m, 4H), 3.16-3.58 (m, OCH₂, 12H), 2.70-2.74 (m, CCH₂O, 4H), 2.58-2.62 (m, CH₂CH₂O, 4H), 2.15-2.33 (m, CH₃CH₂O, 4H), 1.76-1.80 (m, 4H), 1.06-1.37 (m, CH₂, CH₃, 44H). ¹³C NMR (500 MHz, CDCl₃, δ (ppm)): 170.17 (C=N), 154.12 (sp² C-N), 149.10, 140.25, 134.47, 132.58, 130.79, 129.98, 127.98, 126.92, 125.86, 125.27, 124.02, 121.95, 120.62, 118.01, 69.04 (OCH₂), 68.69, 66.02, 65.56, 51.27 (C9), 49.79 (C-N), 46.25 (C-N), 39.47 (CCH₂O), 30.57, 29.31, 28.68, 28.43, 28.35, 28.18, 28.04, 27.49, 25.47, 14.67 (CH₃).
3.63. Preparation of Nanoparticle Composite \textit{11b}

Nanoparticle Composite \textit{11b} was prepared from \textit{9d} and gold nanoparticles to yield a black waxy solid. $^1$H NMR (500 MHz, CDCl$_3$, δ): 8.07-8.12 (m, Ar-H, 4H), 7.92-7.96 (m, Ar-H, 4H), 7.67-7.68 (d, Ar-H, 1H), 7.38-7.49 (m, Ar-H, 5H), 7.24-7.26 (m, Ar-H, 2H), 7.02-7.04 (m, Ar-H, 2H), 4.82-4.85 (s, C-N, 4H), 4.26-4.28 (m, C-N, 4H), 3.53-3.64 (m, OCH$_2$, 64H), 3.30-3.37 (m, OCH$_3$, OCH$_2$, 8H), 3.24-3.27 (m, 8H), 2.95-2.97 (m, 4H), 2.77-2.80 (m, CCH$_2$O, 4H), 2.62-2.72 (m, 4H), 2.38-2.42 (m, CH$_2$CH$_2$O, 4H), 1.57-1.75 (m, CH$_2$, 14H), 1.28-1.36 (m, CH$_2$, 24H). $^{13}$C NMR (500 MHz, CDCl$_3$, δ (ppm)): 167.55 (C=N), 153.80 (sp$^2$ C-N), 148.23, 141.75, 134.10, 131.35, 127.07, 126.10, 125.52, 122.35, 120.25, 69.85 (OCH$_2$), 69.75, 69.35, 69.10, 66.35, 62.01, 58.04, 50.35 (C9), 38.79 (CCH$_2$O), 30.25, 29.17, 28.98, 28.65, 28.47, 28.23, 28.18, 27.78, 25.89, 19.96.
CHAPTER FOUR: CONCLUSION

4.1. Novel Hydrophilic Fluorene-based Derivatives for Bioimaging Applications

In a summary, hydrophilic fluorene-based derivatives were synthesized and characterized by UV-vis absorption, fluorescence emission, anisotropy, fluorescence lifetime, and were found to possess high quantum yield, high photostability, and generally high 2PA cross sections. To increase the hydrophilicity of the new fluorene-based derivatives, ethylene oxide substituents were utilized for the design of water soluble or hydrophilic probes. The sp-hybridized carbon conjugated \( \pi \)-system exhibited higher photostability relative to its sp\(^2\)-hybridized carbon conjugated \( \pi \)-system analog, however, the \( \lambda_{\text{max}} \) of its UV-vis absorption and fluorescence emission occurred at shorter wavelengths in comparison to the sp\(^2\)-hybridized analog. In addition, it was observed that the sp-hybridized carbon \( \pi \)-conjugated system resulted in a lower 2PA cross section in a quadrupolar molecule. Quite significantly, these novel hydrophilic fluorene-based derivatives were proven to be biocompatible for single-photon and two-photon fluorescence cellular bioimaging. In addition, the new probes were found to exhibit low cytotoxicity to the cells they were incubated with, and displayed selectivity for certain organelles upon incubation.

4.2. Novel Hydrophilic Fluorene-based Derivatives for Selective Sensing of Metal Ions

Hydrophilic bis(1,2,3-triazolyl)fluorene-based derivatives exhibited selectivity in detecting zinc and mercury ions over other relevant metals, resulting in an approximately two-fold increase in fluorescence upon complexation. The complexes formed fast and reversibly with binding constants of \( 10^8 \) and \( 10^{14} \) for zinc and mercury, respectively. The
derivatives that exhibited this selectivity are good candidates for further study in living cells and other biological environments.

Hydrophilic fluorene-based derivatives were also found to be suitable ligands for the preparation of fluorescent gold nanoparticles, materials that should lend themselves for future investigation for diagnostic (SERS) and treatment of cancer by photothermal therapy.

4.3. Future Exploration for Novel Hydrophilic Fluorene-based Derivatives

From this study and the previous works of others, fluorene-based derivatives display a wide range of linear and nonlinear photophysical properties that are attractive for bioimaging applications. Further explorations are proposed in the areas listed below.

4.3.1. Strong Donor and Acceptor Groups

Since the strength of the donor and acceptor groups, as well as the extension of the conjugated \( \pi \)-system, in fluorene-based derivatives contributed to the absorption in the UV-vis region, to increase the absorption maximum to the near infrared region, an optimal window for many biological applications, a strong donor (X) such as an amino or an alkoxy and a strong acceptor (Y) like a cyano or a sulfonyle group can be incorporated.\(^{152-154}\)

4.3.2. Large Multiphoton Absorption Derivatives

Design and synthesis of new multiphoton absorbing molecules are emerging for nonlinear excitation fluorescence microscopy and for bioimaging applications.\(^{155-156}\) Therefore, obtaining high two-photon absorptivity must be considered for the design of new 2PA fluorescent probes. This may involve forming strong intermolecular interactions between donor and acceptor groups and between core \( \pi \)-electron systems,\(^ {157,158}\) as well as
employing heteroaromatic conjugated π-systems such as thiophene or squaraine functionalities.159-161

4.3.3. Fluorescence Sensing of Metal Ions In Vitro

Selected hydrophilic fluorene-based derivatives were shown to exhibit selective sensing of metal ions. These are promising for the detection of metal ions in vitro in living cells, particularly since fluorene-based derivatives generally have low toxicity to cells. To achieve this investigation, the first step is to incubate these hydrophilic fluorene-based derivatives into living cells to which various concentrations of relevant metal ions are added. One- or two-photon fluorescence imaging with these probes may provide a sensitive measure of the presence of metal ions in cellular systems.

4.4.4. Fluorescent Gold Nanoparticles for Bioimaging

As described in previous chapters, gold nanoparticles can be explored for bioimaging, especially in living cells.17,18,98 It is interesting that hydrophilic fluorene-based capped gold nanoparticles can be detected by conventional fluorescence microscopy as well as by darkfield microscopy in which only scattered light from nanoparticles is detected.162 A cytotoxicity assay must be performed for further investigation of the fluorescent gold nanoparticles for bioimaging applications.
APPENDIX A: IR, NMR, AND HRMS SPECTRA
Figure 90. The $^1$H-NMR Spectrum of 2a

Figure 91. The $^{13}$C-NMR Spectrum of 2a
Figure 92. The $^1$H-NMR Spectrum of $3a$

Figure 93. The $^{13}$C-NMR Spectrum of $3a$
Figure 94. The $^1$H-NMR Spectrum of 3b

Figure 95. The $^{13}$C-NMR Spectrum of 3b
Figure 96. The $^1$H-NMR Spectrum of 4a

Figure 97. The $^{13}$C-NMR Spectrum of 4a
Figure 98. The HRMS Spectrum of 4a

A 5. 4b

Figure 99. The $^1$H-NMR Spectrum of 4b
Figure 100. The $^{13}$C-NMR Spectrum of $4b$

Figure 101. The HRMS Spectrum of $4b$
A. 2-(4-Iodophenyl)benzothiazole

Figure 102. The $^1$H-NMR Spectrum of 2-(4-Iodophenyl)benzothiazole

Figure 103. The $^{13}$C-NMR Spectrum of 2-(4-Iodophenyl)benzothiazole
A 7. 2-(4-(1-Trimethylsilyl)ethynylphenyl)benzothiazole

Figure 104. The $^1$H-NMR Spectrum of 2-(4-(1-Trimethylsilyl)ethynylphenyl)benzothiazole

Figure 105. The $^{13}$C-NMR Spectrum of 2-(4-(1-Trimethylsilyl)ethynylphenyl)benzothiazole
Figure 106. The $^1$H-NMR Spectrum of 5a

Figure 107. The $^{13}$C-NMR Spectrum of 5a
Figure 108. The HRMS Spectrum of 5a

Figure 109. The $^1$H-NMR Spectrum of 6a
Figure 110. The $^{13}$C-NMR Spectrum of $6a$

Figure 111. The HRMS Spectrum of $6a$
A 10. 2-(4-Ethenylphenyl)benzothiazole

Figure 112. The $^1$H-NMR Spectrum of 2-(4-Ethenylphenyl)benzothiazole

Figure 113. The $^{13}$C-NMR Spectrum of 2-(4-Ethenylphenyl)benzothiazole
A 11. 6b

Figure 114. The $^1$H-NMR Spectrum of 6b

Figure 115. The $^{13}$C-NMR Spectrum of 6b
Figure 116. The HRMS Spectrum of 6b

A 12. 2b

Figure 117. The $^1$H-NMR Spectrum of 2b
Figure 118. The $^{13}$C-NMR Spectrum of $2b$

Figure 119. The $^1$H-NMR Spectrum of $3c$
Figure 120. The $^{13}$C-NMR Spectrum of $3c$

\[ \text{A 14. 4c} \]

Figure 121. The $^1$H-NMR Spectrum of $4c$
Figure 122. The $^{13}$C-NMR Spectrum of $4c$

Figure 123. The HRMS Spectrum of $4c$

Theoretical $[\text{M+H}]^+ = 525.1495$
$[\text{M+NH}_3]^+ = 542.1762$
$[\text{M+Na}]^+ = 547.1316$
Figure 124. The $^1$H-NMR Spectrum of $4d$

Figure 125. The $^{13}$C-NMR Spectrum of $4d$
Figure 126. The HRMS of 4d

A 16. 5c

Figure 127. The $^1$H-NMR Spectrum of 5c
Figure 128. The $^{13}$C-NMR Spectrum of 5c

Figure 129. The HRMS Spectrum of 5c
A 17. 5d

Figure 130. The $^1$H-NMR Spectrum of 5d

Figure 131. The $^{13}$C-NMR Spectrum of 5d
Figure 132. The HRMS of 5d

A 18. 6c

Figure 133. The $^1$H-NMR Spectrum of 6c
Figure 134. The $^{13}$C-NMR Spectrum of $6c$

Figure 135. The HRMS Spectrum of $6c$

Theoretical [M+H]$^+ = 679.2836$
[M+Na]$^+ = 701.2656$
A 19. 6d

Figure 136. The $^1$H-NMR Spectrum of 6d

Figure 137. The $^{13}$C-NMR Spectrum of 6d
Figure 138. The HRMS of $6d$

A 20. $7a$

Figure 139. The IR Spectrum of $7a$
Figure 140. The $^1$H-NMR Spectrum of 7a

Figure 141. The $^{13}$C-NMR Spectrum of 7a
Figure 142. The HRMS Spectrum of 7a

A 21. 7b

Figure 143. The IR Spectrum of 7b
Figure 144. The $^1$H-NMR Spectrum of 7b

Figure 145. The $^{13}$C-NMR Spectrum of 7b
Figure 146. The HRMS of 7b

A 22. 8a

Figure 147. The $^1$H-NMR Spectrum of 8a
Figure 148. The $^{13}$C-NMR Spectrum of $8a$

Figure 149. The HRMS Spectrum of $8a$
A 23. 9a

Figure 150. The $^1$H-NMR Spectrum of 9a

Figure 151. The $^{13}$C-NMR Spectrum of 9a
A 24. 8b

Figure 152. The $^1$H-NMR Spectrum of 8b

Figure 153. The $^{13}$C-NMR Spectrum of 8b
Figure 154. The HRMS Spectrum of $8b$

Figure 155. The $^1$H-NMR Spectrum of $8c$
Figure 156. The $^{13}$C-NMR Spectrum of $8c$

Figure 157. The HRMS Spectrum of $8c$
Figure 158. The $^1$H-NMR Spectrum of $8d$

Figure 159. The $^{13}$C-NMR Spectrum of $8d$
A 27. 1-Azidobutane

\[
\begin{align*}
\text{H}_3\text{C} & \hspace{1cm} \text{N}_3 \\
\end{align*}
\]
Figure 162. The $^1$H-NMR Spectrum of Azidobutane

Figure 163. The $^{13}$C-NMR Spectrum of 1-Azidobutane
A 28. 1-Azido-11-Undecanol

Figure 164. The $^1$H-NMR Spectrum of 1-Azido-11-Undecanol

Figure 165. The $^{13}$C-NMR Spectrum of 1-Azido-11-Undecanol
A 29. 1-Azido-11-Undecanyl Tosylate

Figure 166. The $^1$H-NMR Spectrum of 1-Azido-11-Undecanyl Tosylate

Figure 167. The $^{13}$C-NMR Spectrum of 1-Azido-11-Undecanyl Tosylate
A 30. 1-Azido-11-Undecanethiol

![IR Spectrum](image)

Figure 168. The IR Spectrum of 1-Azido-11-Undecanethiol

![NMR Spectrum](image)

Figure 169. The $^1$H-NMR Spectrum of 1-Azido-11-Undecanethiol
Figure 170. The $^{13}$C-NMR Spectrum of 1-Azido-11-Undecanethiol

A 31. 9b

Figure 171. The $^1$H-NMR Spectrum of 9b
Figure 172. The $^{13}$C-NMR Spectrum of $9b$

Figure 173. The HRMS Spectrum of $9b$
Figure 174. The $^1$H-NMR Spectrum of $9c$

Figure 175. The $^{13}$C-NMR Spectrum of $9c$
Figure 176. The HRMS Spectrum of 9c

A 33. 9d

Figure 177. The $^1$H-NMR Spectrum of 9d
Figure 178. The $^{13}$C-NMR Spectrum of 9d

Figure 179. The HRMS of 9d
Figure 180. The $^1$H-NMR Spectrum of 6e

Figure 181. The $^{13}$C-NMR Spectrum of 6e
Figure 182. The HRMS Spectrum of 6e

A 35. 7c

Figure 183. The $^{1}$H-NMR Spectrum of 7c
Figure 184. The $^{13}$C-NMR Spectrum of 7c

Figure 185. The HRMS Spectrum of 7c
A 36. \(8e\)

![Chemical structure of \(8e\)](image)

**Figure 186. The \(^1\)H-NMR Spectrum of \(8e\)**

![NMR spectrum of \(8e\)](image)

**Figure 187. The \(^{13}\)C-NMR Spectrum of \(8e\)**

![NMR spectrum of \(8e\)](image)
Figure 188. The HRMS Spectrum of 8e

A 37. 8f

Figure 189. The $^1$H-NMR Spectrum of 8f
Figure 190. The $^{13}$C-NMR Spectrum of $8f$

Figure 191. The HRMS Spectrum of $8f$

Theoretical [M+H]$^+$ = 799.3564
[M+Na]$^+$ = 821.3384
A 38. $6f$

**Figure 192.** The $^1$H-NMR Spectrum of $6f$

**Figure 193.** The $^{13}$C-NMR Spectrum of $6f$
Figure 194. The HRMS Spectrum of 6f

\[ \text{A 39. 7d} \]

Figure 195. The $^1$H-NMR Spectrum of 7d
Figure 196. The $^{13}$C-NMR Spectrum of Compound 7d

Figure 197. The HRMS Spectrum of 7d
Figure 198. The $^1$H-NMR Spectrum of $8g$

Figure 199. The $^{13}$C-NMR Spectrum of $8g$
Figure 200. The HRMS of Spectrum 8g

A 41. 6g

Figure 201. The $^1$H-NMR Spectrum of 6g
Figure 202. The $^{13}$C-NMR Spectrum of 6g

Figure 203. The HRMS Spectrum of 6g
Figure 204. The $^1H$-NMR Spectrum of 7e

Figure 205. The $^{13}C$-NMR Spectrum of 7e
Figure 206. The HRMS Spectrum of 7e

A 43. Azidobenzene

\[
\text{\includegraphics[width=0.5\textwidth]{azidobenzene.png}}
\]

Figure 207. The IR Spectrum of Azidobenzene
Figure 208. The $^1$H-NMR Spectrum of Azidobenzene

Figure 209. The $^{13}$C-NMR Spectrum of Azidobenzene
Figure 210. The $^1$H-NMR Spectrum of $8h$

Figure 211. The $^{13}$C-NMR Spectrum of $8h$
Figure 212. The HRMS of 8h

A 45. 9e

Figure 213. The $^1$H-NMR Spectrum of 9e
Figure 214. The $^{13}$C-NMR Spectrum of $9e$

Figure 215. The HRMS Spectrum of $9e$
Figure 216. The $^1$H-NMR Spectrum of 10

Figure 217. The $^{13}$C-NMR Spectrum of 10
Figure 218. The HRMS Spectrum of 10

A 47. 3d

\[
\text{Theoretical } [\text{M+H}]^+ = 709.3748 \\
[\text{M+Na}]^+ = 731.3568
\]

Figure 219. The $^1$H-NMR Spectrum of 3d
Figure 220. The $^{13}$C-NMR Spectrum of $3d$

A 48. $4e$

\[
\begin{align*}
\text{Figure 221. The $^1$H-NMR Spectrum of $4e$}
\end{align*}
\]
Figure 222. The $^{13}$C-NMR Spectrum of $4e$

Figure 223. The HRMS Spectrum of $4e$
A 49. $6h$

Figure 224. The $^1$H-NMR Spectrum of $6h$

Figure 225. The $^{13}$C-NMR Spectrum of $6h$
Figure 226. The HRMS Spectrum of $6h$

A 50. 4-Iodo-N,N-Dimethylbenzeneamine

\[
\begin{array}{c}
\text{H}_3\text{C} \\
\text{N} \\
\text{H}_3\text{C} \\
\text{I}
\end{array}
\]

Figure 227. The $^1$H-NMR Spectrum of 4-Iodo-N,N-Dimethylbenzeneamine
Figure 228. The $^{13}$C-NMR Spectrum of 4-Iodo-N,N-Dimethylbenzeneamine

A 51. 4-(2-Trimethylsilylethynyl)-N,N-Dimethylbenzeneamine

![Chemical Structure]

Figure 229. The $^1$H-NMR Spectrum of 4-(2-Trimethylsilylethynyl)-N,N-Dimethylbenzeneamine
Figure 230. The $^{13}$C-NMR Spectrum of 4-(2-Trimethylsilylethynyl)-N,N-Dimethylbenzeneamine

A 52. 4-Ethynyl-N,N-Dimethylbenzeneamine

Figure 231. The $^1$H-NMR Spectrum of 4-Ethynyl-N,N-Dimethylbenzeneamine
Figure 232. The $^{13}$C-NMR Spectrum of 4-Ethynyl-N,N-Dimethylbenzeneamine

A 53. $8i$

Figure 233. The $^1$H-NMR Spectrum of $8i$
Figure 234. The $^{13}$C-NMR Spectrum of $8i$

Figure 235. The HRMS Spectrum of $8i$
Figure 236. The $^1$H-NMR Spectrum of 4f

Figure 237. The $^{13}$C-NMR Spectrum of 4f
Figure 238. The $^1$H-NMR Spectrum of 5e

Figure 239. The $^{13}$C-NMR Spectrum of 5e
Figure 240. The HRMS Spectrum of 5e

A 56. 5f

Figure 241. The $^1$H-NMR Spectrum of 5f
Figure 242. The $^{13}$C-NMR Spectrum of $5f$

Figure 243. The HRMS Spectrum of $5f$
Figure 244. The $^1$H-NMR Spectrum of 5g

Figure 245. The $^{13}$C-NMR Spectrum of 5g
Figure 246. The $^1H$-NMR Spectrum of 6i

Figure 247. The $^{13}C$-NMR Spectrum of 6i
Figure 248. The HRMS Spectrum of 6i

A 59. 7g

Figure 249. The IR Spectrum of 7g
Figure 250. The $^1$H-NMR Spectrum of 7g

Figure 251. The $^{13}$C-NMR Spectrum of 7g
Figure 252. The HRMS Spectrum of $7g$

A 60. $11a$

Figure 253. The $^1$H-NMR Spectrum of $11a$
Figure 254. The $^{13}$C-NMR Spectrum of $11a$

A 61. $11b$

Figure 255. The $^1$H-NMR Spectrum of $11b$
Figure 256. The $^{13}$C-NMR Spectrum of $11b$
APPENDIX B: PROPOSED MECHANISMS OF METAL-CATALYZED REACTIONS
B1. The Sonogashira Reaction

Scheme 13. Mechanism of the Sonogashira Reaction

Main steps of the Sonogashira reaction\textsuperscript{163}:

1. Oxidative addition: formation of organopalladium halides.
2. Transmetalation: exchange ligand of organopalladium halides with organocopper acetylide.
3. Reductive elimination: formation of the alkyne and regeneration of the catalyst.
Main steps of the Heck reaction:\textsuperscript{164}:

1. Oxidative addition: formation of organopalladium halides
2. Insertion: addition of alkene into organopalladium complex
3. Reductive elimination: formation of the alkene and regeneration of the catalyst
Main steps of the click reaction:

1. Oxidative addition: formation of organocopper acetylide.
2. 1,3-Cycloaddition: addition of azide to organocopper complex.
3. Reductive elimination: formation of 1,2,3-triazole and regeneration of the catalyst.
B4. The Stille Reaction

Scheme 16. Mechanism of the Stille Reaction

Main steps of the Stille reaction\textsuperscript{165,166}:

1. Oxidative addition: formation of organopalladium complex.

2. Transmetalation: exchange ligands between organopalladium complex and organostannane.

3. Reductive elimination: formation of the alkene and regeneration of the catalyst.
APPENDIX C: DERIVATION OF THE EQUATION 15
The equation (12) can be rewritten to the equation (17) as bellows.

Equation 17

\[ K[M]^* = \frac{[C]}{[D]} \]  \hspace{1cm} (17)

At the initial state \((t = 0)\), the fluorescence response is given by the equation (18) where \(k\) is the coefficient constant.

Equation 18

\[ F_0 = k_D[D]_t \]  \hspace{1cm} (18)

Likewise, at the interval \(t\) state, the fluorescence response is obtained from the equation (19) since \(k_M = 0\) for metals.

Equation 19

\[ F = k_D[D] + k_C[C] + k_M[M] = k_D[D] + k_C[C] \]  \hspace{1cm} (19)

At the final state \((t = \infty)\), the fluorescence response is obtained from the equation (20) as bellows.

Equation 20

\[ F_m = k_C[C]_\infty = k_C[D]_t \]  \hspace{1cm} (20)

Where \(k_D, k_C, k_M\) are the corresponding constant of dye, complex, and metal respectively; for metal ions, \(k_M \approx 0\).

Now, substitution the equation (13) into the equations (18) and (20) obtain the equations (21) and (22) as bellows.

Equation 21
\begin{align*}
F_0 &= k_D([D] + [C]) = k_D[D] + k_D[C] \\
\text{Equation 22}
\end{align*}

\begin{align*}
F_m &= k_C([D] + [C]) = k_C[D] + k_C[C] \\
\text{Equation 22}
\end{align*}

Then subtraction the equation (19) to the equation (21) obtained the equation (23).

\begin{align*}
F - F_0 &= k_D[D] + k_C[C] - (k_D[D] + k_D[C]) = k_C[C] - k_D[C] = (k_C - k_D)[C] \\
\text{Equation 23}
\end{align*}

Likewise, the equation (24) obtained from the equation (22) substracts the equation (19).

\begin{align*}
F_m - F &= k_C[D] + k_C[C] - (k_D[D] + k_C[C]) = k_C[D] - k_D[D] = (k_C - k_D)[D] \\
\text{Equation 24}
\end{align*}

Finally, division of the equation (23) to the equation (24), and then substitution into equation (17) obtained the equation (15).

\begin{align*}
\frac{F - F_0}{F_m - F} &= \frac{(k_C - k_D)[C]}{(k_C - k_D)[D]} = \frac{[C]}{[D]} = K[M^n] \\
\text{Equation 15}
\end{align*}
APPENDIX D: TEM IMAGES OF GOLD NANOPARTICLES 11a AND 11b
Figure 257. The TEM Image of Gold Nanoparticles

Figure 258. The TEM Image of Composite 11a
Figure 259. The TEM Image of Composite $11b$
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