SYNTHESIS AND CHARACTERIZATION OF NEAR-INFRARED HEPTAMETHINE
INDOCYANINE DYES AND INTERCALATED NANOCOMPOSITES

by

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Abstract

This thesis describes the synthesis and characterization of a number of novel near-infrared heptamethine indocyanine dyes containing various aromatic chromophores. A known heptamethine indocyanine dye functionalized with a carboxylic acid moiety was employed as a parent dye for subsequent derivatization. Aromatic compounds with terminal hydroxyl groups including a bimetallic cationic organoiron-containing complex, and various anthracene, pyrene, and thiophene derivatives were reacted with the parent dye via ester condensation reactions. Structural analysis of the newly prepared heptamethine indocyanine dyes was accomplished using one- and two-dimensional nuclear magnetic resonance and infrared spectroscopy, and electrospray ionization mass spectrometry. These dyes exhibited high molar absorptivity based on the UV-visible/near-infrared spectral data. Fluorescence emission spectral data was used to determine the relative quantum yield. The formation of H-aggregates was observed in water at low concentrations, but was not present in methanol.

In addition, the intercalation of a heptamethine indocyanine dye and indolenine dye into lithium hectorite was reported. This was accomplished by taking advantage of the exfoliating and re-stacking properties of the host, hectorite. The resulting layered nanocomposites were characterized via powder X-ray diffraction, infrared spectroscopy, thermogravimetric analysis and scanning electron microscopy. X-ray diffraction analysis confirmed interlayer expansion. Scanning electron microscopy was employed for surface morphological characterization. Thermal analysis of the resulting intercalated nanocomposites demonstrated greater thermal stability compared to pure hectorite. In addition, the nanocomposites generally exhibited enhanced thermal stability compared to the non-intercalated heptamethine indocyanine dye.
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**List of Symbols and Abbreviations**

The following is a list of abbreviations and symbols employed in this thesis, most of which are in common use in the chemical literature.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
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<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>Abs.</td>
<td>absorbance</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>br.</td>
<td>broad (in a spectrum)</td>
</tr>
<tr>
<td>cm⁻¹</td>
<td>wavenumbers (in infrared spectroscopy)</td>
</tr>
<tr>
<td>¹³C NMR</td>
<td>carbon-13 nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>Cp</td>
<td>cyclopentadienyl</td>
</tr>
<tr>
<td>d</td>
<td>doublet (in a spectrum)</td>
</tr>
<tr>
<td>d₆</td>
<td>6 deuterium</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N’-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane, CH₂Cl₂</td>
</tr>
<tr>
<td>DCU</td>
<td>N,N’-dicyclohexylurea</td>
</tr>
<tr>
<td>DIC</td>
<td>N,N’-diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
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<tr>
<td>EDC</td>
<td>N-Ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride</td>
</tr>
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<td>Eq.</td>
<td>equation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization (in mass spectrometry)</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>electrospray ionization-mass spectrometry</td>
</tr>
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<td>Et</td>
<td>ethyl</td>
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<td>FT-IR</td>
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<td>gCOSY</td>
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<td>gradient double quantum correlation spectroscopy</td>
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<td>high performance liquid chromatography</td>
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<td>Hz</td>
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<td>ICT</td>
<td>intramolecular charge transfer</td>
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<td>infrared spectroscopy</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant (in spectroscopy; Hz)</td>
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<td>litre</td>
</tr>
<tr>
<td>M</td>
<td>molar (mol-L⁻¹)</td>
</tr>
<tr>
<td>m</td>
<td>multiplet (in a spectrum)</td>
</tr>
<tr>
<td>m/z</td>
<td>mass-to-charge ration (in a mass spectrum)</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
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</tbody>
</table>
mg  milligram (10^{-3} g)

MHz  megahertz

min  minutes

mL  millilitre (10^{-3} L)

mmol  millimole (10^{-3} mole)

mol  mole (6.022·10^{-23} particles)

NaOAc  sodium acetate

NIR  near-infrared

nm  nanometer

NMR  nuclear magnetic resonance

ppm  parts per million

q  quartet (in a spectrum)

ROMP  ring opening metathesis polymerization

r.t.  room temperature

s  singlet (in a spectrum)

SEM  scanning electron microscopy

S_{RN1}  unimolecular radical-nucleophilic substitution

t  triplet (in a spectrum)

T_d  onset thermal decomposition temperature (in TGA)

TEM  transmission electron microscopy

TGA  thermogravimetric analysis

TOCSY  total correlation spectroscopy

UV-vis  ultraviolet visible

XRD  X-ray diffraction
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$\lambda_{\text{abs}}$</td>
<td>absorbance maximum wavelength</td>
</tr>
<tr>
<td>$\lambda_{\text{em}}$</td>
<td>fluorescence emission maximum wavelength</td>
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<tr>
<td>$\lambda_{\text{ex}}$</td>
<td>fluorescence excitation maximum wavelength</td>
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Acknowledgements

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Chapter 1  Introduction

1.1  Development of polymethine cyanine dyes

Cyanine dyes, a class of polymethine dyes, are fluorescent molecules that consist of two nitrogen heterocyclic centers, functioning as both electron acceptors and donors that are connected by either a mono- or polymethine chain (dye 1.1, Figure 1.1).\textsuperscript{1-3} The dye’s polymethine bridge consists of a conjugated chain with \( x \) generally ranging from 0 to 10, as shown in Figure 1.1.\textsuperscript{1-3} The conjugation of \( \pi \)-electrons are distributed between the electron donor and electron acceptor groups results in a delocalized cationic charge across the polymethine bridge. Cyanine dyes are named according to the number of methine groups (\( x = 0, 1, 2, 3 \)) in their polymethine chain.\textsuperscript{1} For instance cyanine dye 1.1 with a 7 carbon linker (\( x = 3 \)) would be described as heptamethine cyanine. If \( x = 0, 1, \) or 2, they would be named as monomethine, trimethine, and pentamethine cyanine, respectively.

![Figure 1.1  Basic skeleton of a polymethine cyanine dye.](image)

Generally, monomethine cyanine dyes exhibit absorption in the visible region and each addition of a vinylene unit (CH=CH) in the polyene-chain causes a red shift (bathochromic; shifts to longer wavelength) of about 100 nm.\textsuperscript{4} The most extensively studied polymethine cyanine dyes include those derived from indolenine (1.1a), benzoxazole (1.1b), benzothiazole (1.1c), 2-quinoline (1.1d), and 4-quinoline (1.1e) (Figure 1.2).\textsuperscript{5} In addition, the spectral characteristics and electronic properties of heptamethine cyanine dyes can be modified by: (i) altering the polymethine chain length, (ii) incorporating terminal substituents with electron-donating or electron-accepting capabilities, and (iii) substituting the chlorine atom on the central
cyclohexenyl ring with different nucleophiles that contain electron-donating or electron-accepting groups.

![Chemical structures of indolenine, benzoxazole, benzothiazole, 2-quinoline, and 4-quinoline](image)

**Figure 1.2** Examples of heterocyclic rings (1.1a-e) that have been used to make polymethine cyanine dyes.

### 1.2 History of cyanine dyes

The first reported cyanine dye was synthesized by C. H. Greville Williams in 1856. Williams unintentionally discovered a vibrant blue-coloured dye (1.2) (Figure 1.3) from reacting crude quinoline with 1-iodopentane in excess ammonia. Later, it was shown that the crude quinoline contained its 4-methyl homolog, lepidine as an impurity, which was actually the heterocycle that participated in the reaction to form dye 1.2 (Figure 1.3). The name ‘cyanine’, originating from the Greek word *kýanos* (meaning dark blue), was originally applied to one compound based on Williams’ discovery of the intensely blue-coloured dye 1.2 and has since extended to this group of dyes.

![Chemical structure of cyanine dye 1.2 and lepidine](image)

**Figure 1.3** Structure of the first reported cyanine dye synthesized in 1856.

However, cyanine was not useful for dyeing fabrics due to its photoinstability. Later in 1873, H. W. Vogel found that cyanine could be used as photosensitizers in photography. This finding quickly gave rise to syntheses of a number of cyanine dyes with improved
photosensitizing properties in silver halide photography.\textsuperscript{8,9} Since then, the class of cyanine dyes has grown exponentially\textsuperscript{10,11} and have been used in a wide variety of applications such as photodynamic therapy\textsuperscript{11}, nonlinear optics\textsuperscript{12}, optical data storage\textsuperscript{11}, laser materials\textsuperscript{11}, and in photovoltaic and solar cells\textsuperscript{13,14}. Additionally, in the past two decades there has been a dramatic increase in research on fluorescent cyanine dyes, due to their applications in biologically related disciplines.\textsuperscript{9} They have been employed in biological applications as fluorescent probes for biomolecular labeling\textsuperscript{1,15,16}, organelle stains\textsuperscript{17,18}, labels for tracing neuronal pathways\textsuperscript{19}, and antitumor agents\textsuperscript{20}.

1.3 Naturally occurring polymethine cyanine dyes

There are some well-known chiral polymethine cyanine dyes such as 1.3 and 1.4 (Figure 1.4) that have been isolated from natural products such as Beta vulgaris (from the vegetable red beets) and Amanita muscaria (from the toadstool, fly agaric), respectively, during the late 1960s and 1970s.\textsuperscript{21–23} It was discovered that these natural dyes, Betanin (1.3, from red beets) and Musca-aurin I (1.4, from the toadstool fly agaric) contain chiral pentamethine cyanine moieties (Figure 1.4).\textsuperscript{24} It is the Betanin and Musca-aurin I that give the intensely red-purple colour in red beets and orange-red colour in the toadstool fly agaric fungi, respectively.\textsuperscript{24}

![Figure 1.4](image)

**Figure 1.4** Two examples of chiral cyanine dyes Betanin and Musca-aurin I found in natural products.
1.4 Synthesis of polymethine cyanine dyes

Within the last two decades there has been a renewed interest in the study of cyanine dyes that absorb between 600 – 1200 nm, the near-infrared (NIR) region of the electromagnetic spectrum, stemming from their use in biological applications.\textsuperscript{9,11} Numerous synthetic procedures for preparing cyanine dyes of diverse molecular structures, in particular mono- and trimethine cyanine dyes have been established.\textsuperscript{11,25} A classical method to prepare polymethine cyanine dyes involves the stepwise condensation of nucleophilic heterocycles containing an activated methyl group (aza-heterocycles, e.g. compound 1.5) and an unsaturated \(\alpha,\omega\)-dialdehyde (polyene-chain precursor) or its equivalent derivative (such as compound 1.6) in the presence of a catalyst (Scheme 1.1).\textsuperscript{11,25–28} It is very common for the bisaldehyde (\(\alpha,\omega\)-dialdehyde) equivalent to be derivatized as a Schiff base (\(R^1R^2C=NR^3\)).\textsuperscript{29} For instance, in Scheme 1.1 the quaternized heterocycle 1.5 acts as a nucleophile in its exocyclic methylene base form, 1.5', which reacts with the polyene-chain precursor, amidine 1.6, to afford the pioneering heptamethine cyanine dye 1.7, known as indocyanine green (ICG) (full mechanism shown in Appendix A.1).

![Scheme 1.1](image.png)

**Scheme 1.1** General synthesis of a polymethine cyanine dye 1.7.

Indocyanine green, 1.7, a classic NIR fluorescent heptamethine cyanine dye, has been used for fluorescent imaging in humans since the late 1950s and is still currently being employed in the medical field.\textsuperscript{30,31} The medical field uses near-infrared fluorescence imaging (between 700-1000 nm) due to its low absorption and decreased auto-fluorescence from biological tissues.
in the NIR spectral range. From this, the use of NIR fluorophores can enhance tissue depth penetration because light scattering decreases with increasing wavelength. This reduces background interference and improves image sensitivity. ICG has a low toxicity and has been used for over 50 years in clinical liver function testing and in cardiology for determining cardiac blood flow. It is one of the least toxic imaging agents administered to humans. Moreover, ICG is a U.S. Food and Drug Administration (FDA)-approved cyanine dye for NIR fluorescence imaging in clinical ophthalmic retinal and choroidal angiography since the 1970s. Fox and Wood were the first to describe the physical and physiological properties of ICG in 1960. For instance, ICG absorbs and emits light at 785 nm and 815 nm, respectively, in aqueous solution and at 805 nm and 835 nm, respectively, in blood. The inclusion of the sulfonate group enhances its water solubility and gives it amphiphilic properties, which is advantageous for biological applications. As a result, ICG binds very quickly to blood plasma proteins. The design and applicability of ICG has been a significant milestone in the development of heptamethine cyanine dyes.

Similar to numerous organic dyes, ICG exhibits low fluorescence emission efficiency as a result of internal conversion (transition from a higher to lower electronic state, in which no photons are emitted). It also has limited photochemical and photophysical stability in aqueous media, plasma, and blood for prolonged periods and is prone to photobleaching. Although ICG is successfully used in medical applications such as active tumor targeted imaging, its broad applicability is mainly limited by the difficulty of making derivatives from a lack of reactive moieties. This lack of functionality confines its use in targeting only specific tumor cell types. Despite these limitations, ICG has notable biocompatibility, which has paved the way for developing cyanine dye derivatives with functional groups for further reactivity. Furthermore, various modifications of indocyanine green have been developed to enhance the
chemical and photostabilities. For instance, Patonay, Strekowski, and coworkers were able to overcome these shortcomings by introducing a chlorocyclohexenyl ring into the polymethine chain (compound 1.8 in Figure 1.5). The incorporation of the cyclohexenyl ring enhances the rigidity of the polymethine bridge, which increases the solubility, fluorescence emission efficiency (quantum yield) and photostability. The modified structure also provides a site for further functionalization at the central ring, as shown in Figure 1.5, and is known as a ‘convertible cyanine dye’.

![Chlorocyclohexenyl ring](image)

Figure 1.5  Heptamethine cyanine dye 1.8 containing a chlorocyclohexenyl ring on the polymethine chain.

The aforementioned general synthetic method to prepare cyanine dyes is not compatible with a wide range of functional groups on the aza-heterocycles. Only reactive groups such as sulfonic and carboxylic acids are fully inert toward the reagents and condensation reaction conditions. Consequently, the development of a ‘convertible cyanine dye’ circumvents the traditional preparation and provides an alternative pathway to mono-functionalized dyes by direct substitution of the central chlorine atom. Thus, it can be used as a precursor to the targeted functionalized cyanine dye. Therefore, utilizing this new strategy chemically modified water-soluble heptamethine cyanine dyes with aryl- or alkyl-ether, -thioether, or -amine, linkages have been prepared. This chloro-substituted heptamethine cyanine dye is prepared with the classic step-wise condensation reaction, as shown in Scheme 1.2. The chlorine atom in the central meso-position can easily be substituted by various nucleophiles, thus allowing for post-
synthetic chemical transformations. This synthetic method has become a cornerstone in subsequent synthetic work by Strekowski and other researchers. From this, a vast majority of heptamethine cyanine dyes contain a central six-membered carbocyclic subgroup incorporated into the polymethine chain. Due to their growing use in a wide variety of applications, the appeal to this class of fluorophores stems from their facile syntheses, relative stability, high molar extinction coefficients, moderate to high fluorescence intensity, narrow spectrum width, and broad wavelength tunability.

Scheme 1.2 Synthesis of a ‘convertible’ heptamethine cyanine dye 1.8.

Unfortunately, polymethine dyes in solution have been known to degrade in the presence of light and air (specifically, molecular oxygen). The main cause that leads to photodegradation involves the reaction of singlet oxygen ($^1O_2$) with dye. Different structural changes have been made to improve the chemical and photostability of polymethine cyanine dyes. In particular, enhancing the rigidity of the polymethine chain prevents radiationless internal conversion (electron decays by non-radiative processes such as heat) and subsequent cis-trans photoisomerization. The increased rigidity provides decreased conformational freedom of the molecules which in turn inhibits photoisomerization. Additionally, modifications that enable
durability depend on reducing the chromophore’s intermolecular interaction by varying the ion-pair and/or steric properties.

There are generally two primary ways to shift the absorption bands: (1) adjust the length of the π-conjugated polymethine chain and (2) introduce terminal substituents that have their own π-electron systems.\textsuperscript{53} If the length of the polymethine bridge is increased, the wavelength of excitation and emission maxima shifts to a longer wavelength, usually by 100 nm with every single vinylene unit.\textsuperscript{54} However, lengthening the polymethine chain tends to result in decreased photochemical stability and fluorescence quantum efficiency. Thus, it is the tendency of cyanine dyes that absorb light greater than 700 nm, to undergo photodegradation.\textsuperscript{54} The fluorescence quantum yield, which is the number of emitted photons relative to the number of absorbed photons, is a very important characteristic of a fluorophore.\textsuperscript{55} Dyes with the large quantum yields exhibit the brightest fluorescent emissions.

In 2005, Peng \textit{et al.} synthesized amine-substituted heptamethine cyanine dyes (1.14a,b) through modification of the central cyclohexenyl ring in dye 1.13 (Scheme 1.3).\textsuperscript{45}

![Scheme 1.3](image)

\textbf{Scheme 1.3  Synthesis of amine-substituted heptamethine cyanine dyes.}

The study indicated that the novel dyes 1.14a,b absorb at much shorter wavelengths resulting in significantly large Stokes’ shifts (≥ 140 nm) (Figure 1.6) and as a result, enhanced fluorescence
than that of its precursor chloro-substituted dye 1.13.\textsuperscript{45} The authors suggest that the high Stokes’ shift may be attributed to an excited-state intramolecular charge transfer (ICT).

![Stokes’ shift](image)

**Figure 1.6** Absorption (black) and emission (blue) spectra of heptamethine cyanine dyes 1.13 (left) and 1.14a (right).\textsuperscript{1}

A Stokes’ shift, which is the distance between the excitation and emission wavelengths, is an important aspect in detecting fluorescence emission in biological applications.\textsuperscript{56} The reasoning behind this is fluorophores with very small Stokes’ shifts can cause self-quenching and errors in fluorescence measurements by excitation light and scattered light.\textsuperscript{55,57} Additionally, increased background signal tends to be exhibited in fluorophores with very small Stokes’ shifts.\textsuperscript{56} On the other hand, fluorophores with large Stokes’ shifts are easily distinguishable due to their greater separation between the emission and excitation wavelengths. As a result, these dyes tend to exhibit stronger fluorescence. The study reported by Peng and coworkers\textsuperscript{45} is especially important in biological applications because prior to this study, polymethine cyanine dyes had considerably smaller Stokes’ shifts typically between 20-30 nm. Ideally, polymethine cyanine dyes used as fluorescent probes for labelling of biomolecules should have a large Stokes’ shift.

\textsuperscript{1} Figure 1.6 Modified with permission from Journal of American Chemical Society, 127, X. Peng, F. Song, E. Lu, Y. Wang, W. Zhou, J. Fan, Y. Gao, “Heptamethine Cyanine Dyes with Large Stokes Shift and Strong Fluorescence: A Paradigm for Excited-State Intramolecular Charge Transfer”, 4170-4171. Copyright (2005) American Chemical Society.
and good quantum yield.\textsuperscript{1} Therefore, the large Stokes’ shift and enhanced fluorescence of the amine-substituted dyes \textbf{1.14} make them more suitable as fluorescent probes compared to previously reported cyanine dyes.\textsuperscript{45}

A widely used criterion to identify an ICT state is whether dyes have a strong solvatochromism.\textsuperscript{58} This is because absorption and emission solvatochromic effects are induced by intramolecular charge transfer.\textsuperscript{59} A solvatochromism can be defined as the influence of solvent on spectral properties of compounds, specifically the change in wavelength.\textsuperscript{60,61} The aforementioned study reported by Peng and coworkers\textsuperscript{45}, showed that amine-substituted heptamethine cyanine dye \textbf{1.14a} exhibited negative solvatochromism (blue-shifted) in the absorption spectrum from water to acetone. Solvatochromism is caused by differences in dipole moment between the ground and excited states of the compounds.\textsuperscript{11} If the ground state is more stabilized than the excited state resulting from increasing solvent polarity, then negative solvatochromism occurs.\textsuperscript{11} The opposite is true for positive solvatochromism (red-shifted). The authors attributed the negative solvatochromism to hydrogen bonding interactions between the dye molecules and solvent. Furthermore, the results also showed that these amine-substituted heptamethine cyanine dyes \textbf{1.14} were substantially blue-shifted and exhibited lower molar extinction coefficients but with greater quantum yields. Since it is advantageous to have fluorescent probes absorb light at longer wavelengths in the NIR region and have considerably high molar extinction coefficients, ongoing studies devoted to designing new NIR amine-substituted cyanine dyes with enhanced optical properties are taking place. In addition, since this study was reported, amine-substituted heptamethine cyanine dyes have been developed as fluorescent sensors for metal ions and pH values.\textsuperscript{62–66}
1.5 Aggregation effects of polymethine cyanine dyes

Polymethine cyanine dyes have the tendency to possess strong intermolecular van der Waals-like attractive forces causing the dye molecules to undergo self-association. In comparison to the monomeric species, the dimeric and higher-order aggregates exhibit distinct changes in the UV-visible to near-infrared absorption band in solution. From observing the spectral shifts, different structures such as dimers form as a result of aggregation and it depends on how the carbocyanine substituents interact with one another that result in the formation of different types of aggregates (Figure 1.7). Aggregates exhibiting a hypsochromic shift (towards the blue) compared to the monomer band are termed as H-aggregates (‘H’ for hypsochromic). Conversely, aggregates showing a bathochromic shift (towards the red) compared to the monomer band are denoted as J-aggregates (‘J’ is named after Jelly, who was among the first to investigate these shifts). It is widely accepted that both H and J-aggregates are made up of parallel dye molecules that are stacked end-to-end forming a head-to-tail arrangement (J-dimer) or stacked plane-to-plane forming a sandwich-type arrangement (H-dimer) (Figure 1.7).

Additionally, H-aggregates exhibit both a broad absorption band and negligible or low fluorescence compared to that of the monomer. On the other hand, J-aggregates are characterized by an intense narrow absorption band and exhibit enhanced fluorescence in comparison to the monomer. Therefore, it is preferential to have J-aggregates form, as opposed to H-aggregates if enhanced fluorescence emission efficiency is desired. Extensive studies on H- and J-aggregates
have suggested that these aggregates in solution, exist as a one-dimensional array that can be assembled in a ladder-type, staircase-type, or brickwork-type arrangement (Figure 1.8).\textsuperscript{11,74} The formation of aggregates depends on both the cyanine dye’s structure and its environment such as solvent polarity, concentration, pH, micellar, microemulsion, and temperature parameters.\textsuperscript{11}

![Figure 1.8](image)

**Figure 1.8** Schematic representation of the different types of arrangements that the dye aggregates exist in.\textsuperscript{2}

Dyes in the aggregated state in solution are especially susceptible to photodegradation, consequently, numerous studies have been devoted to designing new cyanine dyes with decreased aggregation in solution.\textsuperscript{51} For instance, aggregation can be inhibited by incorporating sterically bulky groups, such as cyclodextrin\textsuperscript{75}, and/or attaching sulfonate or sulfonato alkyl moieties\textsuperscript{27} to the heterocyclic rings. The steric interactions between the dyes inhibits aggregation because their molecular orbitals poorly overlap. Cyanine dyes that contain two alkyl sulfonate substituents, one attached to each nitrogen atom on the end-heterocyclic rings, shield the dye from intermolecular interactions with other dye molecules because of they are in a constant dynamic motion.\textsuperscript{51} Additional sulfonate moieties on the terminal aromatic rings further stabilize the dye and thus decrease aggregation. The negatively charged sulfonic group provides a sphere of solvation, which increases its solubility in polar solvents and thus causes decreased aggregation.

There are certain drawbacks with respect to cyanine dyes in terms of low or negligible fluorescence emission efficiency due to the formation of H-aggregates and low photochemical

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and thermal stability. This is particularly a concern in certain applications requiring photoactive dyes such as organic light emitting diode (OLED) devices.\textsuperscript{76} Recent studies have demonstrated that layered solids, such as clay minerals, can be employed as host-systems for luminescent guest molecules, which will be discussed in the following section.\textsuperscript{76} Such composite materials have been shown to enhance thermal and photophysical stabilities.\textsuperscript{76,77}

### 1.6 Intercalated dye-nanocomposite materials

Intercalation generally refers to the reversible inclusion of guest species into layered host materials while preserving the structural features of the host.\textsuperscript{78} The preparation of intercalated complexes using layered silicate lattices is a well-known method for the synthesis of organic/inorganic nanocomposite materials.\textsuperscript{77} Compounds with suitable structural features upon intercalation can maintain and/or exhibit particular properties such as photocatalysis, photoluminescence, photochromism, and optical nonlinearity.\textsuperscript{77} The most commonly used layered silicates are the clay minerals montmorillonite, hectorite, and saponite, which are all part of the smectite family (Figure 1.9).\textsuperscript{79} A valuable feature of layered lattices such as montmorillonite, saponite, and hectorite, is their ability to expand.\textsuperscript{77} This allows for the intercalation of guest molecules into the host system, which generally leads to their highly ordered molecular arrangement. Smectites have high surface area and reactivity, excellent adsorption abilities, and high cation exchange capacities all of which are useful for industrial applications.\textsuperscript{79}
Smectites, for example hectorite, consist of an octahedral (O) sheet primarily comprised of Mg$^{2+}$ with a minor amount of Li$^+$ ions, that is sandwiched between two silica tetrahedral (T) sheets (Figure 1.10). Together these three negatively charged sheets, referred to as “TOT” form a layer approximately 10 Å thick. The interlayer between the TOT layers (Figure 1.10) contains exchangeable sodium or lithium ions, depending on the hectorite, and variable amounts of water. When smectites are suspended in aqueous solutions, expansion occurs between the layers of the clay mineral. More specifically, because of the interlayer spacing and weak electrostatic/van der Waals forces between layers, other molecules can be intercalated between them, leading to an even greater interlayer expansion. As a result, this leads to the formation of intercalated clay nanocomposites. This intercalation process will be described in more detail in Chapter 3.
Figure 1.10  Schematic drawing of a hectorite structure. For each Li$^+$ entering an octahedral site (‘structural Li’), in substitution for a Mg$^{2+}$, one Li$^+$ goes into the interlayer position (‘exchangeable Li’), for layer charge compensation. Black and orange circles are for Si$^{4+}$ cations, white circles are for oxygen atoms, and blue circles represent OH$^-$.

Several studies have investigated the insertion of 1,1’-diethyl-2,2’-cyanine bromide (dye 1.15, Figure 1.11) a pseudo-isocyanine dye (PIC), into various silicate host lattices and its subsequent J-aggregation. J-aggregates of cyanine dyes have attracted a considerable amount of attention for their advantageous optical properties and their applications in spectral sensitization of photographic processes. Cyanine dyes that form J-aggregates exhibit useful photofunctions such as sensitizing and non-linear optical properties.

![Schematic drawing of a hectorite structure](Image)

Figure 1.11  Structure of pseudoisocyanine dye 1.15 (PIC).

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Ogawa and coworkers investigated the adsorption and aggregate formation of PIC cations on montmorillonite and saponite for aqueous suspensions and cast films. The study showed that PIC cations can be structured in a controlled manner by employing layered host lattices as organizing media. Furthermore, the authors proposed that the formation of the J-aggregates on the clay minerals is useful for studying optical properties of the aggregates due to their stable and restrained microstructure. As aforementioned, intercalation of various compounds into clay minerals is an effective method to prepare nanocomposite materials with ordered molecular arrangements. Ogawa and coworkers took advantage of this method by intercalating and PIC into smectite clay minerals. The UV-vis spectral results indicated that different types of aggregates were formed when intercalated into different magadiite clay minerals.

Dye molecules intercalated into host lattices can have improved electro-optical properties. In addition, enhanced fluorescent quantum efficiencies may be observed by strategic tuning of guest-host interactions. Furthermore, studies have shown that organic molecules bound within the interlamellar spaces of the clays minerals are often characterized by enhanced thermal and chemical stability. For instance a nanocomposite clay was recently prepared by intercalation of a luminescent polyhedral oligomeric silsesquioxane (POSS) into saponite (Scheme 1.4). The luminescent POSS (IRIS3-POSS-NH2) was obtained by linking a cyanine dye (IRIS3COOH) onto the POSS cage (Scheme 1.4). The study showed that the intercalated nanocomposite (IRIS3-POSS-NH3/SAP) exhibited high quantum efficiency and improved thermal and photochemical stability compared to that of the pure dye IRIS3-POSS-NH2. The authors attribute the enhanced photoluminescence to the dye’s limited mobility and aggregation. These findings are important as the active layers in hybrid light emitting diode (HLED) devices can be made from the IRIS3-POSS-NH3/SAP sample.
Previously our research group has prepared a number of examples of organic and organometallic monomers, polymers, and dendrimers containing azo dye moieties that are active in the UV-visible region. The incorporation of various photo-active fluorophores, such as near-infrared dyes, into organic and organometallic compounds will allow us to significantly extend the absorbance region and introduce fluorescence properties. As a result, the preparation of these new photo-active materials can open up the doors for their use in a wide variety of applications.

In this thesis, the synthesis, characterization, and investigation of the optical properties of novel heptamethine indocyanine dyes functionalized with various chromophores will be discussed in chapter 2. In addition, the preparation of novel nanocomposites through the intercalation of a heptamethine cyanine dye and indolenine dye into hectorite have been investigated and will be discussed in chapter 3.

Scheme 1.4  Schematic representation of IRIS3-POSS-NH$_2$ compound (A) and its intercalation in a saponite clay (B).\textsuperscript{4}

Chapter 2  Synthesis of Water-Soluble Heptamethine Cyanine Dyes

There are a limited number of reported studies that investigate the spectral properties of NIR polymethine cyanine dyes. Very few literature reports discuss the preparation and photophysical properties of heptamethine cyanine dyes containing esterified mercapto-alkyl or aryl moieties. As the importance of preparing NIR fluorescent cyanine dyes for their use in a wide variety of biological and industrial applications increased, novel water-soluble heptamethine cyanine dyes with distinctive structural features were developed. The intent was to structurally characterize and investigate their optical properties. Thus, various aromatic chromophores containing terminal aliphatic alcohols with varying chain lengths were esterified with the mercaptobenzoic acid moiety of a heptamethine cyanine dye (XI), as shown in Scheme 2.1.

Scheme 2.1  General synthesis of an esterified heptamethine indocyanine dye (XII) from its acid precursor (XI) and generic chromophore.

It is worthy to note that symmetrical nature of the prepared heptamethine indocyanine dyes was an important part of their design. Studies accomplished by Strekowski and coworkers show that the symmetry factor facilitates the synthetic method by minimizing unwanted by-products and also allows in general, simple purification methods for these complex molecules.
2.1 Synthetic route to the formation of a bimetallic organoiron-containing heptamethine cyanine dye

The starting material 2,3,3-trimethylindolenine 2.1 was quaternized to sulfonate 2.2 by reacting with 1,3-propane sultone, following a procedure slightly modified from that of Hammer and coworkers (Scheme 2.2). The authors reported that the crude product crystals were filtered and washed with acetone. However, in our hands the extremely viscous crude product hardened into an intractable sticky-solid material. To combat this problem, the crude product was slightly cooled and washed several times with acetone. The product was mostly insoluble in acetone, and completely soluble in methanol. Therefore, a mixture of acetone and methanol was used for recrystallization. Furthermore, in order to increase the number of crystals formed during recrystallization, the product remained submersed in its recrystallization solvent and was cooled in a refrigerator overnight. Employing this modified procedure afforded a very pure product with high yield. The formation of dye 2.2 was confirmed from its \( ^1H \) NMR results (Appendix B.1), which matched previously reported spectral data.

![Scheme 2.2: Synthesis of indolinium salt 2.2.](image)

Studies have shown that polymethine cyanine dyes containing a chlorocyclohexenyl ring in the polymethine chain can enhance the photostability and fluorescence efficiency (Figure 2.1). Furthermore, the meso-substituted chlorine atom on the cyclohexene ring provides an excellent reactive site for the dye, allowing for the substitution with various nucleophiles. Accordingly, the ease of functionalization of such compounds can be
very useful for the synthesis of a variety of polymethine dyes. Therefore, this feature was taken into consideration for the functionalization of new heptamethine cyanine dyes, which will described later.

![Chemical structure](image.png)

$n = \text{typically 5 or 6}$

**Figure 2.1** General structure of a heptamethine cyanine dye with a central chlorocylohexenyl ring (XIII).

The heptamethine chain of indocyanine dye 2.4 occurred through a condensation reaction of indolinium salt 2.2 and iminium salt 2.3 in the presence of the mild base sodium acetate (Scheme 2.3). A simple crystallization was employed rather than using the reported preparative reverse phase HPLC to purify dye 2.4. The crystallization of dye 2.4 from diethyl ether was reported by Strekowski et al. However, the crude product only partially dissolved into diethyl ether while heating, resulting in the presence of minor impurities in the final product. Modifications to this reported procedure are subsequently discussed. To increase the crude product’s solubility in diethyl ether, a small amount of anhydrous ethanol was added to the heated diethyl ether/product mixture. In turn, this improved both the crystallization method and overall purity. Prior to crystallization, the crude product was first immersed in diethyl ether and sonicated to reduce the size of any larger particles and thus facilitate its dissolution into the diethyl ether/ethanol solvent mixture. During filtration, a minimal amount of N,N-dimethylformamide (DMF) dissolved in diethyl ether was added to help solubilize any remaining impurities. Immediately after the addition of DMF, it was necessary to wash the product numerous times with diethyl ether to prevent the product from dissolving into DMF. The resulting red-bronze powder was obtained in both high yield and purity.
Evidence for the formation and purity of compound 2.4 was observed in the $^1$H NMR spectrum (Appendix B.1), which identically matched the $^1$H NMR values that have been previously reported. For instance, characteristic resonances of methine protons in the polymethine chain appear as doublets at 8.26 ppm and 6.52 ppm ($J = 14.1$ Hz). Additionally, the larger vicinal coupling constant of 14.1 Hz indicates that the methane protons reside in a trans configuration. UV-vis/NIR absorbance and fluorescence emission of dye 2.4 were measured in 100% methanol ($\lambda_{\text{abs}} = 782$ nm and $\lambda_{\text{em}} = 801$ nm, respectively) and were consistent with literature results. Additionally, the thermal stability, glass transition temperature, and surface morphology of compound 2.4 were measured using TGA and SEM, respectively. These results will be discussed and compared to their corresponding intercalated sample in section 3.1 of chapter 3.

The meso-chloro substituent of dye 2.4 was displaced upon treatment with 4-mercaptobenzoic acid to form carboxylic acid-functionalized NIR dye 2.5, following a modified previously reported procedure (Scheme 2.4). The carboxylic acid group was introduced in dye 2.5 to synthesize a number of ester derivatives.
Scheme 2.4 Synthesis of NIR dye 2.5.

Modifications of the previously reported procedure\textsuperscript{111,112} were employed in the synthesis of dye 2.5 are subsequently described. Rather than allowing the reaction mixture to stand during the entire time as reported,\textsuperscript{111} the reaction was stirred at the beginning and near the end to help improve the overall yield. It is worthy to note that continual stirring under nitrogen atmosphere throughout the entire reaction was also attempted. However, the resulting product contained around 50\% of the starting material chloro-dye 2.4 and the yield decreased significantly due to the chemical instability of the thioether linkage.\textsuperscript{31,45} Therefore, we found it was necessary to periodically stir the reaction under N\textsubscript{2}(g) for only a short duration in order to obtain an optimal yield and high purity. Additional nucleophiles, such as 3-mercaptopropionic acid and 6-aminocaproic acid were also used to react with NIR dye 2.4. However, both resulting products had significant amounts of impurities and only a minor percent contained the actual expected product. As a result, 4-mercaptobenzoic acid (illustrated in Scheme 2.4) was selected as the preferable nucleophile due to its convenient reaction conditions and ease of isolation of the corresponding product.

Polymethine cyanine dyes containing a meso-substituted chloro group are susceptible to nucleophilic attack by good single-electron donor nucleophiles because of their electrophilic electron-deficient \( \pi \)-systems and stabilization of the resultant anion. The mechanism for the displacement of the central chlorine atom involves an \( S_{RN1} \) mechanistic pathway (Equations 1-
It is initiated by single-electron-transfer (SET) from the nucleophile, in this case the benzenethiolate anion of 4-mercaptobenzoic acid, to the cationic π-system of the dye (R-Cl), which results in the formation of two radicals (Equation 1). Subsequently, (R-Cl)\(^+\) fragments into the free radical cation intermediate R\(^+\) and anionic chlorine leaving group (Equation 2). The radical cation R\(^+\) reacts with the incoming nucleophile (Nu\(^-\)) to form a radical nucleophile intermediate (R-Nu)\(^-\), thus propagating the chain reaction (Equation 3). Next, this radical nucleophile adduct (R-Nu)\(^-\) transfers one electron to the initial cationic dye (R-Cl)\(^+\) to afford the cationic nucleophilic substituted product (R-Nu)\(^+\) and another radical intermediate (R-Cl)\(^-\) (Equation 4). Termination occurs when all of the reactants have been used up in the reaction.

\[
\begin{align*}
(R-\text{Cl})^+ + \text{Nu}^- & \rightarrow (R-\text{Cl})^- + \text{Nu}^- \quad \text{(Eq. 1)} \\
(R-\text{Cl})^- & \rightarrow R^+ + \text{Cl}^- \quad \text{(Eq. 2)} \\
R^+ + \text{Nu}^- & \rightarrow (R-\text{Nu})^- \quad \text{(Eq. 3)} \\
(R-\text{Nu})^- + (R-\text{Cl})^+ & \rightarrow (R-\text{Nu})^+ + (R-\text{Cl})^- \quad \text{(Eq. 4)}
\end{align*}
\]

Furthermore, in reactions with good single-electron donors, the SET process is supported by polar aprotic solvents such as DMF or DMSO. Consequently, the success of the reaction in DMF supports the S\(\text{RN}_1\) chain mechanism. Additionally, when the reaction conditions of Scheme 2.4 were carried out in the absence of N\(_2\)(g) and allowed to stand for 24 h only a minimal amount of product was isolated. Presumably, the presence of oxygen acted as radical scavenger, inhibiting the chain reaction mechanism.

Successful product formation of dye 2.5 was confirmed from the \(^1\)H NMR results which were consistent with literature values.\(^{111}\) Although \(^1\)H NMR resonances are cited in the literature, a complete \(^1\)H and \(^{13}\)C NMR assignment of NIR dye 2.5 has yet to be published. Since dye 2.5 will later be used to prepare new heptamethine cyanine dyes (Section 2.3), it was essential to assign all of its proton and carbon atoms in order to facilitate \(^1\)H and \(^{13}\)C NMR interpretation of
its derivatives. Therefore, in addition to collecting $^1$H and $^{13}$C one-dimensional (1-D) NMR, $^1$H-$^1$H gdqCOSY, $^1$H-$^{13}$C gHSQC ($^1$J$_{CH}$ couplings), and $^1$H-$^{13}$C gHMBC ($^2$J$_{CH}$ and $^3$J$_{CH}$ couplings) two-dimensional (2-D) NMR spectra were also collected. The gHSQC NMR spectral data was significantly helpful for the carbon NMR assignments, with the exception of the quaternary carbon atoms. Table 2.1 summarizes a complete $^1$H and $^{13}$C NMR assignment of dye 2.5, based on the interpretation of the 1-D and 2-D NMR spectra (Appendix B.1).

Table 2.1 $^1$H and $^{13}$C NMR assignment of NIR dye 2.5 in DMSO-$d_6$ at 400 MHz.

<table>
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<tr>
<th>No.</th>
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<th>$^1$H (ppm)</th>
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<tr>
<td>23</td>
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In addition to the proton resonances matching those that have been previously reported, the resonance of the methine proton (H4 in Table 2.1) shifts from 8.26 ppm, which is observed for the chloro-substituted derivative 2.4, to 8.54 ppm for the aryl-thioether-substituted dye 2.5.
This downfield shift is a result of replacing the chlorine atom with a more electron-withdrawing aryl-thioether group, thus causing the methine proton to be more deshielded than its chlorinated derivative. Additionally, two new proton resonances corresponding to the aromatic protons (H₉ and H₁₀) bound to the mercaptobenzoic acid moiety appear at 7.86 and 7.38 ppm, respectively (Figure 2.2).

![Diagram of NIR dye 2.5 and 2.4]

**Figure 2.2  Comparison of a selected region in \(^1\)H NMR spectra of NIR dyes 2.4 and 2.5.**

Proton NMR assignments were confirmed from the gdqCOSY and TOCSY 2-D NMR spectra. For instance, correlations between protons that are directly coupled to one another were obtained from the gdqCOSY NMR spectrum (Figure 2.3). It was observed that the methylene proton resonance at 2.00 ppm (H₂₂) \(^1\)J couples with the N-CH₂ proton resonance at 4.34 ppm (H₁₉) and the methylene protons adjacent to the sulfonate group at 2.56 ppm (H₁₈). To facilitate the assignment of the aromatic protons, the 2-D TOCSY spectrum of NIR dye 2.5 was obtained (Appendix B.1). This allowed for the observation of \(^1\)H-\(^1\)H correlations among protons that are not directly coupled, but belong to the same spin system.¹¹⁴
Figure 2.3  gedqCOSY 2-D NMR spectrum (400 MHz, DMSO-d$_6$) of dye 2.5.

$^3$J$_{CH}$ and $^4$J$_{CH}$ couplings were observed in the gHMBC NMR spectrum (Figure 2.4). For instance, the imine quaternary carbon resonance appears at 172.1 ppm (C$_1$) and $^3$J$_{CH}$ couples to the methylene protons adjacent to the nitrogen atom at 4.34 ppm (H$_{19}$). Additionally, the 2-D NMR spectrum shows $^3$J$_{CH}$ coupling of the carbonyl resonance at 166.8 ppm (C$_2$) with its nearby aromatic protons at 7.86 ppm (H$_9$). The C=O stretch of the arene carboxylic acid group appears at 1703 cm$^{-1}$ in the FT-IR spectrum of dye 2.5 (Appendix B.2). Altogether, these spectral results indicate the replacement of the central chlorine atom with the aryl-thioether moiety of 4-mercaptobenzoic acid.
Figure 2.4  Selected region of the gHMBC NMR spectrum (400 MHz, DMSO-$d_6$) of dye 2.5.

Our research group has published numerous papers that incorporate cationic $\eta^6$-dichloroarene-$\eta^5$-cyclopentadienyliron complex XIV (Figure 2.5) into various complexed materials. The incorporation of cationic cyclopentadienyliron moieties is advantageous because it can enhance solubility and facilitate nucleophilic aromatic substitution reactions due to the intense electron-withdrawing ability of the iron center.

Figure 2.5  Structure of cationic $\eta^6$-dichloroarene-$\eta^5$-cyclopentadienyliron complex.

In addition to the presence of a carboxylic acid reactive site, complex 2.6 (structure is shown in Scheme 2.5) was selected in the synthetic route to an organoiron-containing NIR
heptamethine indocyanine dye due to the cationic cyclopentadienyliron moieties. Moreover, incorporating the cationic cyclopentadienyliron substituents can facilitate nucleophilic aromatic substitution for future polymerization reactions. Additionally, the di-iron complex 2.6 was substituted with a long alkyl chain to minimize steric bulk, thus facilitating its reactivity with NIR dye 2.5. The Steglich-type esterification reaction of previously reported bimetallic complex 2.6 with 1,12-dodecanediol formed organoiron-containing complex 2.7, following a modified procedure recently published by the Abd-El-Aziz research group (Scheme 2.5).\(^{93}\)

\[
\begin{align*}
\text{Cl} & \quad \text{Fe}^+\text{PF}_6^- \\
\text{HO} & \quad \text{O} \\
\text{DMAP} & \quad \text{CH}_2\text{Cl}_2, \text{dry DMSO} \\
\text{DCC} & \quad \text{r.t. 5 days}
\end{align*}
\]

**Scheme 2.5** Synthesis of organoiron-containing bimetallic complex 2.7.

Modifications, which include using a different carbodiimide derivative and purification process, were employed to obtain a highly pure product. For instance, \(N\)-Ethyl-\(N^\prime\)-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was used as a coupling reagent, which forms a water-soluble urea (1-[3-(dimethylamino)propyl]-3-ethylurea) as a byproduct. EDC is more suitable as opposed to the use of \(N, N^\prime\)-dicyclohexylcarbodiimide (DCC), which instead forms insoluble dicyclohexylurea (DCU) and so poses separation problems. Additionally, an excess amount of 1,12-dodecanediol was used to ensure that complex 2.6 reacted completely. Most of the excess 1,12-dodecanediol was filtered out after the crude product was dissolved into acetone. The water-soluble urea was removed once the product was precipitated into an aqueous
solution of 10% HCl and ammonium hexafluorophosphate (NH₄PF₆), vacuum filtered, and then washed several times with water. The pure product was obtained in 87% yield. Although this is lower than the reported yield of 96%⁹³, the isolated product was obtained in higher purity without the presence of DCU. This was determined by NMR spectral analysis, which is subsequently discussed.

Evidence for the esterification of complex 2.6 was confirmed from the results of the ¹H and ¹³C NMR spectral data of complex 2.7 (Appendix B.1). For ease of future NMR interpretation and ¹H and ¹³C assignment for any derivatives of complex 2.7, gdqCOSY and gHSQC 2-D NMR spectra were also collected for 2.7 (Appendix B.1). The Abd-El-Aziz research group has previously reported the synthesis and characterization of complex 2.7.⁹³ However, after its publication in 2010, we continued in trying to improve its synthetic methodology and purification by replacing the DCC carbodiimide coupling reagent with EDC carbodiimide coupler. This modified procedure allowed for a better separation and the product obtained did not require extensive purification. While the NMR assignments of complex 2.7 were reported in our previous publication, certain aliphatic resonances overlapped with the DCU byproduct. However, the product obtained from this modified synthesis allowed for these aliphatic proton and carbon resonances to be easily resolved. Since the urea byproduct of EDC is water-soluble and can be easily removed in the purification process, this new methodology eliminates the appearance of the byproduct in the NMR spectra. As a result, this allowed for the complete assignment of every proton and carbon atom in complex 2.7. For example, the assignments of proton and carbon atoms 11 and 12 (Table 2.2) were determined based on gHSQC and gdqCOSY 2-D NMR spectral data. In the gHSQC spectrum, ¹JCH coupling is observed between the resonances of H₁₂ and C₁₂ at 3.36 ppm and 60.7 ppm, respectively, and the resonances of H₁₁ and C₁₁ at 3.98 ppm and 64.0 ppm, respectively (Figure 2.6). Additionally, the
gdqCOSY spectrum indicated $^{3}J_{\text{HH}}$ coupling between the $–\text{CH}_{2}\text{OH}$ protons (H$_{12}$ at 3.36 ppm) and the adjacent methylene protons (H$_{15}$) at 1.39 ppm (Figure 2.7). It also confirmed the $^{3}J_{\text{HH}}$ coupling between the $–\text{COCH}_{2}$ protons (H$_{11}$) at 3.98 ppm and its neighbouring methylene protons (H$_{18}$) at 1.54 ppm. The remaining NMR assignments of complex 2.7 were determined in a similar fashion.

Table 2.2  $^1$H and $^{13}$C NMR assignment of complex 2.7 in DMSO-$d_6$ at 400 MHz.

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Figure 2.6 Select region of gHSQC spectrum (400 MHz, DMSO-\(d_6\)) displaying \(^1J_{CH}\) correlations of protons/carbons 11 and 12 in complex 2.7.

Figure 2.7 Select region of gdqCOSY spectrum (400 MHz, DMSO-\(d_6\)) displaying \(^3J_{HH}\) correlations of protons 11, 18, 12, and 15 in complex 2.7.
Following a similar method to complex 2.7, the synthesis of the novel NIR dye 2.8 occurred through an esterification reaction of the carboxylic acid moiety on dye 2.5 with the terminal hydroxyl group on di-iron complex 2.7 (Scheme 2.6). EDC was used as the carboxylic acid coupling reagent in the presence of the catalyst, DMAP, in a solvent mixture of dichloromethane (DCM) and dry dimethylsulfoxide (DMSO). The esterification mechanism of the bimetallic NIR dye 2.8 is postulated in Scheme 2.7.\textsuperscript{126}

Scheme 2.6 Synthesis of novel bimetallic NIR dye 2.8.
*Note: all arrows are double-headed arrows.

Scheme 2.7  Steglich-type esterification mechanism of NIR dye 2.5 with complex 2.7.

The polar aprotic solvent dichloromethane was used as the main solvent in the esterification reaction due to its easy removal and moderate solubility. Small aliquots of dry
DMSO were added to the stirring reaction mixture to ensure complete dissolution into DCM. The reaction was stirred under nitrogen for five days to maximize product formation. Furthermore, the reaction took place in the dark to prevent decomposition of the cationic organoiron moieties. The addition of ammonia hexafluorophosphate provided a counterion to the cationic organoiron moieties, which helped significantly reduce the product’s solubility in water, despite the presence of the sulfonate groups, thus facilitating precipitation. Conveniently, the majority of DMSO was removed when the product was precipitated into the aqueous solution. Even though a majority of the product was insoluble in the aqueous solution, a small amount of product remained water-soluble. By taking advantage of the differing solubilities between the starting materials and product, different organic solvents were used to wash the crude product and remove a significant amount of any remaining starting materials. Since dye 2.5 is soluble in methanol and complex 2.7 is not, the green product was washed several times with methanol at room temperature and then decanted to remove any of the unreacted dye 2.5. The remaining methanol insoluble product was dried *in vacuo*. Since the organoiron complex 2.7 is acetone-soluble, a minimal amount of acetone was added in attempt to dissolve the relatively purified product. The addition of acetone did indeed dissolve most of the product, which was subsequently re-precipitated into 10% HCl(aq) containing NH₄PF₆. However, when the \(^1\)H NMR of the NIR dye-complex 2.8 was obtained, the resonances corresponding to the diiron complex (2.7) protons displayed considerably higher integrations than expected. Therefore, as evident in the \(^1\)H NMR spectrum, the product still contained unreacted di-iron complex 2.7 starting material. It is noteworthy that a small amount of residue remained when the product was dissolved in acetone. Based on this observation, it was hypothesized that the acetone used to dissolve the product contained enough water in it to dissolve a majority of the product. To test this, deuterated acetone obtained from a newly opened bottle was added to the product and
subsequently decanted, and then set aside for $^1$H NMR analysis. The remaining acetone-insoluble residue was dried in vacuo and analyzed via $^1$H NMR spectroscopy in DMSO-$d_6$. The corresponding $^1$H NMR results showed decreased integration values of the proton resonances corresponding to the organometallic complex 2.7. Additionally, the resonances in $^1$H NMR spectrum of the acetone-soluble fraction consisted mainly of complex 2.7, with only a very small amount of the NIR dye resonances. Based on these preliminary $^1$H NMR results, it can be concluded that the product, NIR dye 2.8, is mainly insoluble in anhydrous acetone, and thus an acetone wash was used to initially remove impurities.

1-D and 2-D NMR spectroscopy (complete NMR spectra are given in Appendix B.1) were used to confirm the structure of bimetallic NIR dye 2.8. Proton and carbon atoms were assigned in a similar fashion to that of complex 2.7 and dye 2.5. For instance, the appearance of a new CH$_2$ proton resonance at 4.18 ppm in the $^1$H NMR spectrum indicates that they are bound next to an electronegative atom. This chemical shift is consistent with the expected proton NMR shift of the -CH$_2$OH protons (complex 2.7), which should become more deshielded because of the newly formed ester bond. Due to the increased electron-withdrawing effect of the ester carbonyl, the -CH$_2$O- protons become more electron poor, thus causing a downfield shift in the proton resonance (Figure 2.8). This occurrence was indeed observed based on the observation of the -CH$_2$O proton resonance shifting from 3.36 (complex 2.7) to 4.18 ppm (dye 2.8), as seen in Figure 2.8.
Figure 2.8  Comparison of selected region in the $^1$H NMR spectra (400 MHz, DMSO-$d_6$) of dye 2.8 (A) and complex 2.7 (B).

In addition to the $^1$H NMR results, the formation of the ester bond bridging the dye (2.5) to the organoiron complex (2.7) was also evident in the $^1$H-$^{13}$C gHMBC NMR spectrum. For instance, a $^3J_{CH}$ correlation between the carbonyl resonance at 165.1 ppm and the -CH$_2$O- proton resonance at 4.18 ppm was observed (Figure 2.9). Additionally, the $^3J$ correlation between the dye’s carbonyl carbon and the two neighboring aromatic protons at 7.87 ppm was also apparent in the gHMBC spectrum (Figure 2.9). The remaining proton and carbon NMR resonances of NIR dye 2.8 (listed in Experimental Section 2.4.3) remained relatively unchanged compared to those of the corresponding starting materials, complex 2.7 and dye 2.5. Based on the NMR interpretation of dye 2.8, the product still contains minor amounts of impurities.
Figure 2.9  Select region of gHMBC spectrum displaying the carbonyl $^{3}J_{\text{CH}}$ correlations in NIR dye 2.8.

The FT-IR spectrum (Appendix B.2) of NIR dye 2.8 displays a carbonyl stretch at 1715 cm$^{-1}$ corresponding to the ester carbonyl group. As expected, this C=O IR stretch shifted to a higher vibrational frequency compared to its carboxylic acid derivative, dye 2.5, which displays a C=O stretch at 1703 cm$^{-1}$. In general, carboxylic acids will have lower vibrational frequencies compared to their ester derivatives due to inter- and intramolecular hydrogen bonding. Therefore, the IR spectral result is consistent with the general trend in frequency shift between carboxylic acids and esters.

Polymerization of NIR dye 2.8 was attempted and preliminary results provide evidence of successful polymerization, which will be discussed later. The condensation polymerization of complexed NIR dye 2.8 took place via metal-mediated nucleophilic aromatic substitution of the terminal chloro groups bound to the cationic arene cyclopentadienyliron moieties with the nucleophilic thiol groups of 4,4’-thiobisbenzenethiol, to afford NIR polymer 2.9 (Scheme 2.8). In order to reduce the precipitated product’s water solubility, it was necessary to cool the mixture overnight in the refrigerator.
Scheme 2.8  Metal-mediated nucleophilic aromatic substitution of NIR dye 2.8.

Previously, the Abd-El-Aziz research group reported the synthesis of an organoiron complexed polyphenylenesulfide derivative, using a 1,4-dichlorobenzene-organoiron complex and 4,4’thiobisbenzenethiol as a dinucleophile. In the published results, part of the evidence given for successful polymerization came from the $^1$H NMR spectral data. In particular, the
cyclopentadienyl (Cp) resonance shifted from 5.47 ppm (complexed monomer) to 5.13 ppm (complexed polymer). Additionally, broadening of the \textsuperscript{1}H NMR resonances occurred, which is characteristic for polymers. Based on these previous findings, evidence for polymerization was observed in the \textsuperscript{1}H NMR spectrum of polymer 2.9 (Appendix B.1). After polymerization, the Cp resonance became more electron-rich from the arene-thiol substitution causing an upfield shift from 5.28 ppm (monomer 2.8) to either 5.17 or 5.03 ppm (polymer 2.9). Since both an upfield shift of the Cp protons and resonance broadening are observed in its \textsuperscript{1}H NMR spectrum, suggesting successful polymerization of dye 2.8. However, due to the difficulty in preparing and purifying the monomer 2.8, polymer 2.9 was obtained in a small quantity. Consequently, only a \textsuperscript{1}H NMR spectrum of polymer 2.9 could be obtained, and these should be considered to be preliminary results. In future work, the weight average molecular weight of polymer 2.9 will be determined using either gel permeation chromatography (GPC) or light scattering. Optimization of the polymerization reaction conditions and purification methods are currently ongoing and may be reported in future work.

2.2 Synthesis of NIR heptamethine dye 2.5 derivatives substituted with various chromophores and/or polymerizable substituents

Various chromophores and polymerizable substituents containing an aliphatic hydroxyl group were functionalized with heptamethine cyanine dye 2.5. Investigating the effect of varying the substituents of NIR dye 2.5 on the UV-vis-NIR absorption and fluorescence emission spectra was the primary objective for the syntheses of these compounds. In addition, the possibility of further functionalizing these compounds for potential polymerization can be determined depending on the changes in their photophysical properties. The synthesis of these new NIR dyes occurred by utilizing the carboxylic acid group of NIR dye 2.5 (Figure 2.10) and functionalizing it with various substituted alcohols \textit{via} esterification reactions.
NIR dye 2.10 was prepared by reacting anthracene-9-methanol with NIR dye 2.5 in the presence of DCC and DMAP (Scheme 2.9). Since NIR dye 2.5 is insoluble in diethyl ether, it was assumed that the product, dye 2.10, would also be insoluble in diethyl ether. Therefore, precipitation of the crude product was attempted by slowly adding the product mixture in DMF to a stirring solution of diethyl ether. However, this method proved unsuccessful and no precipitate formed. An alternative isolation method, which followed the same as dye 2.5 was employed instead. First, the product was concentrated in vacuo and then the crude mixture was slowly crystallized by adding dropwise an diethyl ether/methanol (~20:1) mixture with continuous stirring. Subsequently, an iridescent green powder was obtained through vacuum filtration.

Scheme 2.9  Synthesis of anthracene-containing NIR dye 2.10.
The structure of NIR dye 2.10 was characterized via $^1$H/$^{13}$C 1-D and 2-D NMR spectroscopy (Appendix B.1). The gHSQC spectrum was interpreted in conjunction with the gHMBC spectrum for both carbon and proton assignments to help distinguish between the aromatic resonances of the product. Shows a select region of the $^1$H NMR spectrum of dye 2.10 displaying the aromatic and highly deshielded aliphatic resonances. The resonances corresponding to the aromatic protons on the anthracene moiety appear at 8.68 ppm (H$_A$), 8.41 ppm (H$_B$), 8.10 ppm (H$_C$), and 7.55-7.49 ppm (H$_E$, H$_E'$). Additionally, as shown in Figure 2.11, the anthracene resonances at 8.41 ppm (H$_B'$) and 7.55-7.49 ppm (H$_E$, H$_E'$) overlap with the methine (H$_B$; 8.46 ppm) and aromatic resonances (H$_E''$, H$_E'''$, H$_F$, H$_G$; 7.50-7.49 ppm) of the heptamethine indocyanine moiety. As a result, it was quite challenging for the specific assignment of each of the aromatic protons in dye 2.10.

![Diagram of dye 2.10](image)

**Figure 2.11** Selected region in the $^1$H NMR spectrum of dye 2.10 displaying aromatic and highly deshielded aliphatic resonances.

However, despite the challenge with having overlapping NMR resonances, the combined interpretation of the 2-D NMR spectra of dye 2.10 along with previously assigned $^1$H/$^{13}$C
resonances of 9-anthracenemethanol (retrieved from SDBS\textsuperscript{5}), were utilized for determining these NMR assignments. For instance, a downfield shift occurs for the -CH\textsubscript{2}OH resonance of the anthracene moiety from 5.47 ppm (-CH\textsubscript{2}OH) to 6.32 ppm (CH\textsubscript{2}O-), which suggests that the methylene protons were successfully incorporated into NIR dye 2.5. It is notable to mention that the exact -CH\textsubscript{2}O- proton assignment was obtained from the gHSQC spectral data, which displays \textsuperscript{1}J coupling between the carbon and proton resonance at 59.0 ppm and 6.32 ppm, respectively. The alkoxy carbon resonance at 59.0 ppm is consistent with the expected shift of an alkoxy group bound next to an arene moiety appearing between 65-55 ppm. Evidence for the esterification is further supported in the gHMBC spectrum, which displays \textsuperscript{3}J correlations between the carbonyl resonance at 165.1 ppm (C\textsubscript{1} in Figure 2.12) and the methylene and aromatic resonances at 6.32 ppm (H\textsubscript{J}) and 7.79 ppm (H\textsubscript{D}), respectively (Figure 2.12).

\textsuperscript{5} Spectral Database for Organic Compounds (SDBS); NMR spectra; SDBS No.: 12305; RN 1468-95-7; http://riodb01.ibase.aist.go.jp/sdbs/ (accessed September 12, 2012).
confirmed its structure by displaying the molecular ion peak (M-Na)\(^-\) at \(m/z\) 1005.3 (\(m/z\) calculated for \(C_{58}H_{57}N_2O_8S_3\): 1005.3).

The synthesis of NIR dye 2.11 occurred via an esterification reaction following the same esterification reaction conditions and isolation method described previously for NIR dye 2.10 (Scheme 2.10). The crude product was isolated as a dark green powder. The structure of dye 2.11 was confirmed via 1-D and 2-D NMR spectroscopy (Appendix B.1).

Scheme 2.10  Esterification of NIR dye 2.5 with 1-pyrenemethanol.

Due to the extensive conjugation in dye 2.11, exact NMR assignments for each individual proton and carbon atom was challenging, so some resonances are listed as peak ranges rather than single peaks (NMR signals are listed in Experimental Section 2.4.3 for NIR dye 2.10). With the exception of the carbonyl carbon resonance (165.0 ppm), proton and carbon signals of dye 2.11 corresponding to the NIR dye moiety (2.5) either remain unchanged or only vary slightly compared to those observed in dye 2.5. Although most of the aromatic proton couplings could not be determined directly from the \(^1\)H NMR spectrum, their \(^1\)H-\(^1\)H connectivities were observed in the gdqCOSY NMR spectrum of NIR dye 2.11. The NMR assignments of the aromatic proton resonances of the pyrenemethanol (PyMeOH) moiety appear at 8.37, 8.30, 8.26-8.23, 8.21-8.19, 8.20-8.13, and 8.06 ppm (Figure 2.13). These proton assignments of the aromatic resonances were achieved based on \(^3\)J correlations between these
protons (from gdqCOSY spectrum, Figure 2.13) along with identifying which carbon atoms $^1J$ couple to the protons (from gHSQC spectrum, Figure 2.14). For example, the gdqCOSY spectrum shows $^3J$ correlations between $H_b$, $H_d$ and $H_k$ (Figure 2.13). Furthermore, protons $H_b$ and $H_k$ also have the same coupling constant of 9.3 Hz, which provides additional support for their $^3J_{HH}$ correlation to one another. As well, the gHSQC spectrum displays their $^1J$ coupling to carbon resonances at 125.6, 125.5, and 126.4 ppm, respectively (Figure 2.14). These carbon shifts appear in the same relative order as the corresponding carbon atoms in 1-pyrenemethanol, which appear at 125.1 (b), 125.0 (d), and 125.5 (k) in the $^{13}$C NMR spectrum (in DMSO-$d_6$) of 1-pyrenemethanol (Appendix B.1).

Figure 2.13  Selected region of gdqCOSY NMR spectrum of dye 2.11 displaying $^3J_{HH}$ correlations between aromatic hydrogen atoms from PyMeOH moiety.
Figure 2.14  Selected region of gHSQC NMR spectrum of dye 2.11 displaying $^1J$ coupling of aromatic hydrogens from PyMeOH moiety.

In the formation of an ester bond derived from an alcohol and a carboxylic acid, one would expect that the alkoxy group would shift downfield since the oxygen attached to the CH$_2$ group is bound to an electron-withdrawing carbonyl functionality. This downfield shift is evident in the NMR spectra of dye 2.11, which shows that the proton and carbon resonances of CH$_2$-O in 1-pyrenemethanol shift from 5.24 ppm to 6.01 ppm and 61.3 ppm to 64.9 ppm, respectively, when esterified with NIR dye 2.5. A comparison between a selected region of the $^1$H NMR spectra of NIR dye 2.11 and 1-pyrenemethanol (Appendix B.1) illustrating the downfield shift of the CH$_2$-O protons is displayed in Figure 2.15. Additionally, the disappearance of the hydroxyl proton resonance (5.52 ppm) of 1-pyrenemethanol is evident in the $^1$H NMR spectrum of dye
2.11 (Figure 2.15), which also supports the formation of the ester functionality. The gHMBC spectrum also confirmed the ester group formation of dye 2.11 by showing $^3J$ correlations between the arene protons (7.89 ppm), the CH$_2$-O (6.01 ppm), and the ester carbonyl (165.0 ppm) (Figure 2.16). Furthermore, FT-IR spectral results (Appendix B.2) also supported the ester linkage formation in dye 2.11 by displaying a shift in the C=O frequency from a carboxylic acid at 1703 cm$^{-1}$ to an ester at 1713 cm$^{-1}$. The structure of NIR dye 2.11 was also characterized by ESI mass spectrometry showing its molecular ion peak (M-Na$^-$) at $m/z$ 1029.4 ($m/z$ calculated for C$_{60}$H$_{57}$N$_2$O$_8$S$_3$: 1029.3) (Appendix B.3).

![Esterified pyrene moiety in dye 2.11](image)

**Figure 2.15** Selected regions of $^1$H NMR spectra (400 MHz, DMSO-$d_6$) of NIR dye 2.11 (top) and 1-pyrenemethanol (bottom) displaying a shift of the methylene protons.
Esterification of NIR dye 2.5 with 1-pyrenebutanol affording NIR dye 2.12, took place under the same reaction conditions as NIR dye 2.10 (Scheme 2.11). The crude product 2.12 was isolated as a green powder following the same method described previously for dye 2.10.

Scheme 2.11  Synthesis of pyrene-containing NIR dye 2.12.

1-D and 2-D NMR spectral data (Appendix B.1) of NIR dye 2.12 were obtained, conducted in a similar fashion as NIR dye 2.11. Based on the spectral data from the gHSQC, gHMBC and gdqCOSY NMR, proton and carbon resonances most likely corresponding to the ester moiety in dye 2.11.
product were identified (refer to Experimental Section 2.4.3 for NIR dye 2.12). The formation of the ester linkage was confirmed in the NMR spectra. Specifically, a downfield shift from 3.47 ppm (1-pyrenebutanol) to 4.31 ppm (dye 2.11) of the CH$_2$-O protons appears in the $^1$H NMR spectrum (Figure 2.17). Additionally, the disappearance of the hydroxyl proton resonance (4.40 ppm) of 1-pyrenebutanol is evident in the $^1$H NMR spectrum of dye 2.12 (Figure 2.17), which also supports the formation of the ester functionality. The distinction between the proton resonances of the N-CH$_2$ (4.32 ppm) and the CH$_2$-O (4.31 ppm) groups was obtained from the gHSQC NMR spectral data (Figure 2.18). A selected region of the gHSQC spectrum is shown in Figure 2.18, which indicates that the carbon resonances at 42.8 (N-CH$_2$) and 64.3 (CH$_2$-O) ppm $^1J$ couple to the proton resonances at 4.32 and 4.31 ppm, respectively.

![Figure 2.17](image)

**Figure 2.17**  Comparison of methylene protons in selected region of $^1$H NMR spectra (400 MHz, DMSO-$d_6$) of NIR dye 2.12 (top) and 1-pyrenebutanol (bottom).
Figure 2.18  Selected region of gHSQC spectrum of NIR dye 2.12 displaying $^1J$ couplings of CH$_2$-O and N-CH$_2$ groups.

The $^{13}$C NMR spectral data also confirms the formation of the ester, which is observed in the carbonyl’s shift from 166.8 ppm to 165.2 ppm of the carboxylic acid to the ester functionality, respectively. It was expected that a $^3J$ correlation would be observed between the CH$_2$-O group and the ester carbonyl in the gHMBC spectrum. However, this was indistinguishable in the gHMBC spectrum of dye 2.12. Despite this discrepancy, the molecular ion peak (M-Na$^-$) at 1071.2 $m/z$ (calculated for C$_{63}$H$_{63}$N$_2$O$_8$S$_3$: 1071.4 $m/z$) was obtained from the ESI mass spectrum of dye 2.12 (Appendix B.3), thus confirming its structure. Additionally, the FT-IR spectral data (Appendix B.2) further confirms the formation of the ester functionality of dye 2.12 by displaying a shift in the C=O frequency from the carboxylic acid at 1703 cm$^{-1}$ to the ester at 1713 cm$^{-1}$. 
3-Thiophenemethanol and 3-thiopheneethanol were incorporated into NIR dye 2.5 to form NIR-absorbing monomers 2.13 and 2.14 (Scheme 2.12 and Scheme 2.13, respectively). Not only was the purpose to investigate UV-vis/NIR absorption and fluorescence emission for these thiophene-containing monomers, but their preparation was also intended for potential electrochemical polymerization of the thiophene-containing moiety. NIR dye 2.13 was synthesized though an esterification reaction of NIR dye 2.5 with 3-thiophenemethanol following the same reaction conditions and isolation method described previously for NIR dye 2.10 (Scheme 2.12).

![Scheme 2.12 Esterification of NIR dye 2.5 with 3-thiophenemethanol.](image)

The structure of NIR dye 2.13 was characterized by 1-D and 2-D NMR spectroscopy in a similar fashion described previously for NIR dyes (Appendix B.1). In particular, the 2-D NMR spectral data provided the proton and carbon assignments for the 3-thiophenemethanol moiety. For instance, the gHSQC spectral data indicates that the CH₂-O protons (H_d, 5.26 ppm in Figure 2.19) of the thiophene moiety \(^1J\) couple to the carbon resonance at 61.6 ppm (Figure 2.19), which indicates that a downfield shift occurred for the methylene protons. The gHSQC spectrum in Figure 2.19 also shows the resonances of the aromatic protons on the thiophene ring appear at 7.15 ppm (H_b), 7.51 (H_c), and 7.57 (H_d) and their corresponding carbon atoms appear at 127.6, 126.8, and 124.6 ppm, respectively. Additionally, the ester linkage in dye 2.13 was confirmed
from the gHMBC NMR spectrum by showing $^3J$ correlations between the methylene protons of the thiophene moiety (5.26 ppm), the aromatic protons near the ester group (7.91 ppm), and the carbonyl carbon (165.0 ppm) (Figure 2.20). The other proton and carbon resonances of the heptamethine indocyanine moiety of dye 2.13 either remain unchanged or only vary slightly compared to those described previously for dye 2.5. Infrared spectroscopy also supported the formation of the ester linkage with a carbonyl stretching frequency of 1713 cm$^{-1}$ (Appendix B.2). The structure of NIR dye 2.13 was also characterized by ESI mass spectrometry showing its molecular ion peak (M-Na$^-$) at $m/z$ 911.6 ($m/z$ calculated for $C_{48}H_{51}N_2O_8S_4$: 911.3) (Appendix B.3).

Figure 2.19  Selected regions of the gHSQC NMR spectrum of NIR dye 2.13 displaying $^1J_{CH}$ coupling of the thiophene moiety.
Figure 2.20  Selected region of gHMBC NMR spectrum of NIR dye 2.13 displaying $^3J_{CH}$ correlations.

The esterification of NIR dye 2.5 with 3-thiopheneethanol, affording NIR dye 2.14, took place under the same reaction conditions as NIR dye 2.10 (Scheme 2.13). The crude product 2.14 was isolated as a dark green powder following the same method described for dye 2.10.

Scheme 2.13  Synthesis of thiophene-containing NIR dye 2.14.

The structure of NIR dye 2.14 was characterized with 1-D and 2-D NMR spectroscopy (Appendix B.1). The NMR spectral data is very similar to that of NIR dye 2.13, since dye 2.14
only differs by an additional methylene group in the aliphatic chain. The aromatic proton and corresponding carbon resonances of the thiophene moiety (dye 2.14) appear at 7.03, 7.39, and 7.24 ppm and 128.5, 125.9, and 121.9 ppm, respectively. The gdqCOSY spectral data in Figure 2.21 shows a $^3J$ correlation between the CH$_2$-O protons at 4.38 ppm (H$_a$) and the adjacent methylene protons at 2.98 ppm (H$_e$). The other aliphatic $^3J_{HH}$ correlations corresponding to the NIR dye moiety are also shown in the gdqCOSY spectrum in Figure 2.21. Even though the NCH$_2$- resonance at 4.34 ppm (H$_b$) overlaps with the CH$_2$-O resonance (H$_a$), its NMR assignment could still be determined from the gdqCOSY and gHSQC NMR spectral data. For instance, a $^3J_{HH}$ correlation between the resonances at 4.34 (H$_b$) and 2.00 (H$_r$) ppm was obtained from the gdqCOSY spectrum (Figure 2.21). Additionally, $^1J_{CH}$ coupling was observed between proton resonances at 4.38 ppm (H$_a$) and 4.34 (H$_b$) ppm and their corresponding carbon resonances at 64.6 and 42.8 ppm, respectively, thus further distinguishing the difference between the CH$_2$-O and NCH$_2$- groups (Figure 2.22). Additionally, the ester linkage in dye 2.14 was confirmed from the gHMBC NMR spectrum by showing $^3J$ correlations between the methylene protons of the thiophene moiety (4.38 ppm), the aromatic protons near the ester group (7.85 ppm), and the carbonyl carbon (165.0 ppm) (Figure 2.23). Infrared spectral data also supported the formation of the ester linkage in dye 2.14 by displaying a shift in the carbonyl stretching frequency from 1703 cm$^{-1}$ to 1715 cm$^{-1}$ (Appendix B.2). Additionally, the ESI mass spectrum of dye 2.14 (Appendix B.3) confirmed its structure by displaying the molecular ion peak (M-Na$^-$) at $m/z$ 925.8 ($m/z$ calculated for C$_{49}$H$_{53}$N$_2$O$_8$S$_4$: 925.3 $m/z$).
Figure 2.21  Selected region of gdqCOSY NMR spectrum of NIR dye 2.14 displaying $^3J_{HH}$ correlations between aliphatic protons.

Figure 2.22  Selected region of gHSQC NMR spectrum of NIR dye 2.14 displaying $^1J_{CH}$ couplings of CH$_2$-O and NCH$_2$- moieties.
Norbornene-containing monomers can undergo ring-opening metathesis polymerization (ROMP) using catalysts such as Grubbs’ catalyst to form uniform high molecular weight polymers. It is useful to incorporate norbornene into monomers since the norbornene backbone of the respective polymer can provide a high tear strength and resistance to chemical side reactions.\textsuperscript{92} Therefore, the synthesis of norbornene-containing NIR dye 2.15 (Scheme 2.14) was intended as a pilot monomer for the preparation of NIR dye-containing polymers via ring-opening metathesis polymerization. This monomer, NIR dye 2.15, was prepared by reacting dye 2.5 with endo,exo-5-norbornene-2-methanol in the presence of the carbodiimide, DIC, and DMAP (Scheme 2.14).
Precipitation of the product that had been dissolved in methanol into diethyl ether was attempted, but proved unsuccessful. As a result, the solvent mixture had been removed in vacuo, and the crude product was obtained as a viscous liquid. 1-D and 2-D NMR spectroscopy was used to confirm the formation of dye 2.15. However, the NMR spectra indicated the presence of impurities and thus purification was attempted. For instance, the product was suspended in a mixture of diethyl ether and acetone (20:1) and sonicated with the intent of solubilizing the impurities by taking advantage of the different solvent polarities. Unfortunately, this purification method resulted in removing only some of the impurities, and increased the amount of others. As a result, NMR analysis is not carried out for this product, but only for the crude product.

Structural analysis of the norbornene-containing NIR dye 2.15 was accomplished via one- and two-dimensional NMR spectroscopy (Appendix B.1). Evidence that supports the formation of dye 2.15 was observed in the gHMBC spectrum (Figure 2.24), which displays $^3J$ correlations between both the exo CH$_2$-O isomer (4.27 and 4.21 ppm) and endo CH$_2$-O isomer (3.95 and 3.72 ppm), the aromatic protons near the ester group, and both the exo/endo carbonyl carbons (165.1/165.0 ppm, respectively). The distinction between the endo and exo norbornene-CH$_2$O protons was determined based on previously reported NMR assignments.$^{128}$
Figure 2.24  gHMBC NMR spectrum of NIR dye 2.15 displaying $^3J$ correlations between the exo and endo methylene protons, the Ar-CH protons, and the carbonyl carbon.

The structure of the ester product of NIR dye 2.15 was also characterized by ESI mass spectrometry showing its molecular ion peak (M-Na)$^-$ at $m/z$ 921.9 ($m/z$ calculated for C$_{51}$H$_{57}$N$_2$O$_8$S$_3$: 921.3). However, the relative intensity of the (M-Na)$^-$ peak is 1/3 the intensity of the peak at $m/z$ 658.6 corresponding to a smaller fragmented ion (Figure 2.25). This intensity difference may indicate that the fragment corresponding to the peak at 658.6 is more stable compared the (M-Na)$^-$ peak.

The NMR spectral data indicates a large percentage of impurities and an excess of unreacted exo,endo-5-norbornene-2-methanol starting material. Since purification was unachievable, the excess 5-norbornene-2-methanol employed in the reaction was not removed. Furthermore, the combination of both methylene resonances of unreacted exo,endo-5-norbornene-2-methanol and the esterified norbornene product 2.15 are very complicated due to the mixture of endo/exo isomers. As a result, exact NMR assignments were not obtained. Altogether, continual investigation into the optimization of its synthetic methodology and
puration process needs to be carried out in order for the ROMP reaction of monomer 2.15 to take place.

![ESI mass spectrum (negative ion mode) of NIR dye 2.15 illustrating select fragmentation](image)

**Figure 2.25** ESI mass spectrum (negative ion mode) of NIR dye 2.15 illustrating select fragmentation

### 2.3 Spectral properties of novel ester derivatives of heptamethine cyanine dye 2.5

UV-vis/NIR absorption and fluorescence emission spectral data were collected for NIR dye 2.5 ester derivatives in order to understand their optical properties. The UV-vis/NIR absorption ($\lambda_{\text{abs}}$) and fluorescence emission ($\lambda_{\text{em}}$) of the dyes were measured with uniform concentrations of 1.0x10^{-6} M in methanol. Additionally, the absorption spectra of NIR dyes 2.8, 2.10-2.12, and 2.14 were also obtained with the same concentration (1x10^{-6} M) in pure water to investigate aggregation formation and solvent effects. The data pertaining to the spectral properties of the NIR dyes are presented in Table 2.3.
The molar absorption coefficients were calculated using the Beer-Lambert law as follows,

$$A = \varepsilon bc$$  \hspace{1cm} (Eq. 5)

where $A$ denotes the absorbance at each compound’s maximum wavelength, $\varepsilon$ is the molar absorption coefficient, $b$ denotes the path length of the cuvette which was held constant at 1 cm, and $c$ is the concentration of the dyes in solutions. Table 2.3 lists the estimated relative quantum yields. These calculations were carried out for the sole purpose of comparing numerical values of dyes 2.5, 2.8, 2.10-2.14 relative to one another.

A relative quantum yield is determined by measuring the fluorescence spectrum and comparing its integrated intensity with the same quantity for a reference standard with a known absolute quantum yield. This measurement can be obtained from absorption and emission spectra. Therefore, due to the ease in obtaining the relative quantum yield, these measurements were calculated for dyes 2.5, 2.8, 2.10-2.14 using the equation shown below:

$$\Phi_{f,i} = \Phi_{f,\text{st}} \frac{F_i f_i(\lambda_{\text{ex},i}) n_{i}^2}{F_{\text{st}} f_{\text{st}}(\lambda_{\text{ex},\text{st}}) n_{\text{st}}^2}$$ \hspace{1cm} (Eq. 6)

where $\Phi_{f,i}$ and $\Phi_{f,\text{st}}$ are the fluorescence quantum yields of the sample and the standard, respectively. The subscripts $i$ and $\text{st}$ denote sample and standard and the subscript $\text{ex}$ excitation. The known quantum yield of the standard, $\Phi_{f,\text{st}}$, is typically obtained from the literature. $F_i$ and $F_{\text{st}}$ are the integrated intensities (area; in units of photons) of sample and standard spectra, respectively. The refractive indices of the sample and the standard solution are $n_i$ and $n_{\text{st}}$, respectively. $f_i(\lambda_{\text{ex}})$ and $f_{\text{st}}(\lambda_{\text{ex}})$ are absorption factors, which give the fraction of the excitation light ($\lambda_{\text{ex}} = 760$ nm) absorbed by the dye (Equation 7). $f(\lambda_{\text{ex}})$ is related to absorbance $A(\lambda_{\text{ex}})$ and therefore is also related to the molar absorption coefficient $\varepsilon$ of the dye at the excitation wavelength, the concentration $c$ and to the path length $l$ (Equation 7).

$$f(\lambda_{\text{ex}}) = 1 - \tau(\lambda_{\text{ex}}) = 1 - 10^{-A(\lambda_{\text{ex}})} = 1 - 10^{-\varepsilon(\lambda_{\text{ex}})cl}$$ \hspace{1cm} (Eq. 7)
Generally, it is best to determine the quantum yield using two standards, both with known quantum yields, and calculate the relative quantum yield using equation 6, which would give two separate quantum yields for each test sample. The average of the two would then be taken, and thus providing a more accurate relative quantum yield that may be compared to other relevant quantum yields reported in the literature. However, due to limitations with respect to instrument accessibility, calibration using two reference samples was not obtained. Additionally, the quantum yield of the standard (NIR dye 2.4) was obtained in methanol, whereas the literature value was reported in a phosphate buffer solution at pH of 7.4. As a result, the relative quantum yields provided in Table 2.3 should be interpreted only as relative to each other, rather than as absolute values. These relative values are still useful because they provide numerical values that can be used to compare against one another.
Table 2.3  Spectral properties of varying substituent (R) groups attached to the carboxyl group of heptamethine cyanine dye 2.5 in methanol.

![Dye structure](image)

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<th>$\lambda_{\text{em}}$ (nm)$^a$</th>
<th>Stokes Shift (nm)</th>
<th>$\varepsilon \times 10^{-5}$ (M$^{-1}$cm$^{-1}$)$^b$</th>
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</table>

*a. Excitation was at 760 nm.*

*b. Absorption coefficients were measured at each compound’s respective $\lambda_{\text{max}}$, and calculated according to the Beer-Lambert law, where $b = 1.0 \text{ cm}$ and $c = 1 \times 10^{-6} \text{ M}$.

*c. NIR dye 2.4 was used as a quantum yield standard ($\Phi_F = 0.065$) in PBS (pH=7.4), and was corrected to $\Phi_F = 0.12$ in methanol.*

Absorption and fluorescence emission spectra were measured in methanol solutions with 1x10$^{-6}$ M concentration at room temperature. All of the dyes showed no apparent aggregate formation in these conditions. A bathochromic shift (> 10 nm) in the maximum wavelength...
absorption was observed for the dyes containing the more electron-donating thiol-substituents (2.5-2.13) compared to the non-substituted Cl-counterpart, dye 2.4 (tabulated spectral results in Table 2.3). These results are consistent with a previous study of similar heptamethine cyanine dyes containing different nucleophiles at the meso position.108 Specifically, a dye containing a meso-substituted sulfur atom exhibited a bathochromic shift of 12 nm higher than the non-substituted chloro-counterpart.108 A maximum wavelength absorption band at 793 nm was observed for carboxylic acid dye 2.5 and a maximum wavelength fluorescence emission band at 812 nm, which is similar to previously published results.111,130 Esterification reactions of the carboxylic acid with various conjugated substituents resulted in slightly red-shifted UV-vis/NIR absorption and emission spectra. This also led to either smaller or unchanged Stokes shifts ranging between 15 and 19 nm (Table 2.3). It is common for fluorophores that have relatively small Stokes shifts to exhibit lower quantum yields. This general trend was observed in the fluorescence emission spectra of the NIR dyes (2.5, 2.8, and 2.10-2.14) which exhibited lower relative quantum yields ranging between 0.051 and 0.098 (Figure 2.26) in comparison the chloro-precursor 2.4. The maximum wavelength absorptions of the esterified heptamethine cyanine dyes range from 795 to 798 nm and did not change significantly compared to the parent NIR dye 2.5. This suggests that the replacement of various linkers on NIR dye 2.5 did not affect the excited state intramolecular charge transfer (ICT). Additionally, the change in maximum wavelengths observed in the emission spectra (λ_em ranged from 812 to 816 nm) was negligible (Figure 2.26). Since the absorbance and emission wavelength maxima were not significantly altered among the ester derivatives of NIR dye 2.5 and NIR dye 2.5 suggests that the ground-state interaction between the ester substituents on the sulfur-bridging benzene moiety and the heptamethine chain was minimal in all of them. All of the dyes exhibited high molar absorption coefficients (ε) ranging between 1.2 and 1.9 x 10^5 M^-1 cm^-1 in methanol at 1.0 x 10^-6 M
concentrations. However, it was observed that the dyes substituted with longer aliphatic linkers, specifically dyes 2.8 and 2.12, exhibited lower molar absorption coefficients compared to the other dyes containing either methylene or ethylene linkages (dyes 2.10, 2.11, 2.13, 2.14). For instance, the pyrene-butyl substituent of dye 2.12 exhibited a decreased molar absorption coefficient from $1.5 \times 10^5 \text{M}^{-1}\text{cm}^{-1}$ (2.11) to $1.3 \times 10^5 \text{M}^{-1}\text{cm}^{-1}$ (2.12) and a decrease in the relative quantum yield. However, there were no significant differences observed between thiophene-containing NIR dyes 2.13 and 2.14. This indicates that the difference of one methylene linker does not affect the overall photophysical properties of these particular dyes.

![Figure 2.26](image)

**Figure 2.26**  *Comparison of fluorescence intensities of NIR dyes 2.5, 2.8, 2.10-2.14.*

As aforementioned in chapter 1, dye aggregation or self-association is enhanced in cyanine dyes when there are strong intermolecular van der Waals interactions, hydrophobic interactions, or hydrogen bonding.\textsuperscript{11} The delocalization of positive charge on the nitrogen atom in polymethine cyanine dyes results in increased intermolecular van der Waals interactions, thus increasing their tendency towards aggregation. Cyanine dyes are known to form aggregates in aqueous media in high and even low concentrations, as well in higher concentrations in organic
solvents. As mentioned previously in chapter 1, different structures such as dimers form as a result of aggregation, and it depends on how the carbocyanine substituents interact with one another that result in the formation of either H-aggregates (hypsochromic shift) or J-aggregates (bathochromic shift). H-aggregates are observed if a blue shift in the absorption spectrum occurs compared to that of the monomer and exhibit broader absorption bands. Conversely, J-aggregates are observed if there is a red shift in the absorption spectrum compared to that of the monomer and exhibit narrow absorption bands.

This aggregation phenomenon was investigated for dyes 2.8, 2.10-2.12, and 2.14 by measuring the absorption of each dye dissolved in water and comparing the shifts in wavelength maxima to their absorption spectra in methanol (Figure 2.27 and Table 2.4).

![Figure 2.27 UV-vis/NIR absorption comparison of H-aggregate formation in select NIR dyes in 100% water solutions.](image-url)
Table 2.4  Comparison of UV-vis/NIR absorption spectral results measured in water and methanol (MeOH).

<table>
<thead>
<tr>
<th>Dye</th>
<th>MeOH $\lambda_{abs}$ (nm)</th>
<th>Water $\lambda_{abs}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>797</td>
<td>788</td>
</tr>
<tr>
<td>2.12</td>
<td>797</td>
<td>790</td>
</tr>
<tr>
<td>2.11</td>
<td>796</td>
<td>788</td>
</tr>
<tr>
<td>2.10</td>
<td>798</td>
<td>788</td>
</tr>
<tr>
<td>2.14</td>
<td>797</td>
<td>790</td>
</tr>
</tbody>
</table>

Altogether, the appearance of the blue-shifted absorption bands at 788 and 790 nm (Table 2.4) for dyes 2.8, 2.10-2.12, and 2.14 observed in the water medium is indicative of the formation of aggregates and that the dyes exhibit solvent-sensitivity. Furthermore, the absorption spectra obtained in a methanol medium suggests that aggregation is inhibited, which results in primarily the monomeric dye. The degree of aggregation and to which extent dimers or higher order aggregates form requires further investigation. Thus, future work is needed to gain insight into the degree of H-aggregation of the dyes and whether or not changes in molar absorptivities between the monomers and aggregates are solvent- and/or concentration-dependent.

2.4 Experimental

2.4.1 General considerations

All reagents were purchased from Sigma-Aldrich, Alfa Aesar, or VWR, and used without further purification. Phosphorus oxychloride (POCl$_3$) was stored under N$_2$(g) upon opening. All solvents were HPLC grade from Fisher Scientific and used without further purification. Compound 2.2 and complex 2.6 were prepared according to previously established procedures.$^{3,31,118}$ All reactions and complexes containing an $\eta^6$-chlorobenzene-$\eta^5$-
cyclopentadienylin(II) hexafluorophosphate moiety were kept in the dark to prevent decomposition.

2.4.2 Characterization

All NMR spectral data was collected on a Varian Mercury Plus Spectrometer (400 MHz), with an ATB tunable multinuclear probe with a gradient channel in dimethyl sulfoxide-$d_6$ (DMSO-$d_6$) at room temperature. Chemical shifts are referenced to residual solvent peaks and coupling constants are reported in Hz. Infrared spectroscopy was performed on an IRPrestige-21 FT-IR by Shimadzu with a MIRacle ATR by PIKE Technologies. All absorption measurements were performed on methanol solutions unless otherwise specified, in 1.0 cm$^2$ quartz cuvettes at 22 ±1 °C, and were measured on a Shimadzu UV-2550 UV-vis spectrophotometer. Fluorescence emission spectra were measured by Jessica Pilfold at the University of British Columbia Vancouver campus and measured on a Cary Eclipse fluorescence spectrophotometer with the excitation set to 760 nm and with excitation and emission slit widths of 5.0 nm. Fluorescence measurements were performed on methanol solutions in 1.0 cm$^2$ quartz at 22 ±1 °C. Electrospray ionization mass spectra in negative mode were obtained on a Waters/Micromass LCT time-of-flight mass spectrometer equipped with an electrospray ion source, using methanol solutions of the products. All mass spectra were measured by Dr. Yun Ling at the University of British Columbia Vancouver campus.

2.4.3 Synthesis

**Indolinium inner salt 2.2.** Used a modified procedure reported by Flanagan et al.$^{12}$ Dissolved 2,3,3-trimethylindolenine, 2.1, (5.00 mL, 31.2 mmol) and 1,3-propane sultone (4.10 mL, 46.8 mmol) into toluene (25.0 mL), stirred and heated to reflux for 18 h. The resulting
viscous product mixture was cooled to room temperature, vacuum filtered, and washed numerous times with acetone. The solid crude product was recrystallized in anhydrous denatured ethanol and diethyl ether (1:1) and the resulting crystals were left to form overnight in the refrigerator. It is important to note that in order for the crude product to fully dissolve into the recrystallizing solvent, heating and sonication was carried out. The crystals were subsequently collected via vacuum filtration and washed several times with acetone to afford a pale pink powder: 65 % yield. \( ^1 \text{H NMR (400 MHz, DMSO-d}_6 \text{)} \delta: 8.05 (m, 1H), 7.82 (m, 1H), 7.62 (m, 2H), 4.66 (t, 8.0 Hz, 2H) 2.83 (s, 3H), 2.63 (t, 6.5 Hz, 2H), 2.15 (m, 2H), 1.53 (s, 6H).

**Iminium salt 2.3.** This procedure has been previously reported by Flanagan et al.\(^3\) Anhydrous DMF (13.0 mL, 17.0 mmol) was cooled to 0 °C, followed by adding phosphorus oxychloride (11.0 mL, 12.0 mmol) dropwise via a pressure-equalizing addition funnel to the cooled DMF and subsequently stirred for 30 minutes. Next, cyclohexanone (5.5 mL, 5.3 mmol) was added to the reaction mixture and refluxed for 1 hour. After the red-orange reaction mixture was cooled to room temperature, a mixture of aniline (9 mL) and anhydrous denatured ethanol (1 L) was subsequently added dropwise and stirred for 1 hour at room temperature. The resulting dark purple product mixture was poured into an ice cold 10% HCl solution (1 L) and placed into a fridge overnight to allow the formation of crystals. The resulting purple-black powder was isolated via vacuum filtration, washed twice with ice cold water and twice with diethyl ether, and allowed to dry in vacuo: 75 % yield. \( ^1 \text{H NMR (400 MHz, DMSO-d}_6 \text{)} \delta: 8.52 (s, 2H), 7.62 (d, 7.9 Hz, 4H), 7.45 (m, 4H), 7.26 (m, 2H), 2.76 (t, 6.0 Hz, 4H), 1.84 (m, 2H).

**NIR dye 2.4.** Used a modified procedure reported by Flanagan et al.\(^3\) Combined indolinium salt 2.2 (1.69 g, 6.00 mmol), iminium salt 2.3 (1.08 g, 3.00 mmol), and dry sodium acetate (0.600 g, 7.00 mmol) in anhydrous denatured ethanol (60 mL) and refluxed under \( \text{N}_2 \text{(g)} \) for 4 h. The solvent was removed in vacuo and the crude product was purified by the following:
approximately 25 mL of diethyl ether was added to the solid product and triturated via sonication. Subsequently, the product/diethyl ether mixture was heated and anhydrous denatured ethanol was added dropwise until most of the product dissolved, then cooled to room temperature, and placed in an ice bath to allow crystallization. The green-bronze product was subsequently filtered in vacuo through a sintered glass crucible. Approximately 2 mL of DMF was added to the crucible containing the filtered product followed by the immediate addition of diethyl ether and then stirred in the crucible. Next, the product was washed several times with diethyl ether to ensure the removal of DMF and subsequently dried in vacuo to afford a bronze-coloured powder: 89 % yield. \( ^1 \text{H} \text{NMR (400 MHz, DMSO-}d_6 \text{)} \delta: 8.26 (d, 14.1 \text{ Hz, } 2H), 7.62 (d, 7.3 \text{ Hz, } 2H), 7.53 (d, 8.0 \text{ Hz, } 2H), 7.42 (t, 7.3 \text{ Hz, } 2H), 7.27 (t, 7.5 \text{ Hz, } 2H), 6.52 (d, 14.2 \text{ Hz, } 2H), 4.38 (t, 7.5 \text{ Hz, } 4H), 2.75 (t, 5.8 \text{ Hz, } 4H), 2.57 (t, 6.8 \text{ Hz, } 4H), 2.03 \text{(quint, 6.9 Hz, } 4H), 1.84 (m, 2H), 1.67 (s, 12H). \text{ } ^{13} \text{C NMR (101 MHz, DMSO-}d_6 \text{)} \delta: 172.21, 147.97, 143.20, 142.11, 141.13, 128.62, 126.56, 125.06, 122.47, 111.55, 101.98, 48.95, 47.77, 42.85, 27.52, 26.00, 23.47, 20.57. \text{ UV-vis/NIR: } \lambda_{\text{abs}} = 783 \text{ nm. Fluorescence: } \lambda_{\text{em}} = 804 \text{ nm.}

NIR dye 2.5. The procedure was modified from an established method. \text{^111,112 NIR dye 2.4} (0.147 g, 0.200 mmol) and 4-mercaptobenzoic acid (0.093 g, 0.600 mmol) were combined with DMF (10 mL) and stirred under \text{N}_2(g) \text{ for approximately 2 minutes. The reaction was allowed to stand for 24 h with a continual flow of } \text{N}_2(g). \text{ The product was precipitated by adding a mixture of anhydrous denatured ethanol/diethyl ether (1:20) dropwise to the product mixture while stirring continuously. Immediately after, the product was vacuum filtered through a sintered glass crucible, washed several times with diethyl ether, and allowed to dry in vacuo overnight: 64 % yield. \( ^1 \text{H} \text{NMR (400 MHz, DMSO-}d_6 \text{)} \delta: 8.54 (d, 14.0 \text{ Hz, } 2H), 7.86 (d, 8.6 \text{ Hz, } 2H) 7.52 (d, 7.6 \text{ Hz, } 2H), 7.49 (d, 8.6 \text{ Hz, } 2H), 7.39 (d, 7.6 \text{ Hz, } 2H), 7.37 (d, 8.6 \text{ Hz, } 2H) 7.22 (t, 7.6 \text{ Hz, } 2H), 6.55 (d, 14.0 \text{ Hz, } 2H), 4.34 (t, 6.8 \text{ Hz, } 4H), 2.82 (t, 6.0 \text{ Hz, } 4H), 2.55 (t, 6.8 \text{ Hz,}
4H), 2.00 (quint., 6.8 Hz, 4H), 1.93 (t, 6.0 Hz, 2H), 1.39 (s, 12H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 172.1, 166.8, 147.1, 144.8, 143.0, 142.1, 141.2, 133.6, 130.5, 128.6, 127.8, 125.4, 125.0, 122.4, 111.5, 102.33, 48.8, 47.8, 42.9, 27.2, 26.0, 23.5, 20.6. IR, v/cm$^{-1}$: 1703 (C=O). UV-vis/NIR: $\lambda_{abs} = 793$ nm. Fluorescence: $\lambda_{em} = 812$ nm.

**Bimetallic complex 2.7.** The procedure was modified from an established method. Combined valeric bimetallic complex 2.6 (3.18 g, 3.00 mmol) with 1,12-dodecandiol (1.19 g, 6.90 mmol), EDC (0.880 g, 4.60 mmol), and DMAP (0.562 g, 4.60 mmol) into CH$_2$Cl$_2$ (40 mL) and dry DMSO (12 mL). The reaction mixture was flushed with N$_2$(g) and stirred in the dark for 5 days. CH$_2$Cl$_2$ was removed *in vacuo* and excess 1,12-dodecandiol was removed by dissolving the crude product into acetone, vacuum filtering and collecting the filtrate. Subsequently, acetone was removed *in vacuo* and the product was precipitated into a solution of 10% hydrochloric acid (150 mL) and ammonium hexafluorophosphate (0.978 g, 6.00 mmol). The final product was collected via vacuum filtration, washed several times with water, followed by diethyl ether, and dried *in vacuo* overnight: 78% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.38 (d, 8.8 Hz, 4H), 7.27 (d, 8.8 Hz, 4H), 6.81 (d, 6.8 Hz, 4H), 6.43 (d, 6.8 Hz, 4H), 5.28 (s, 10H), 4.32 (m, 1H) 3.98 (m, 2H), 3.36 (m, 2H), 2.44 (m, 2H), 2.16 (m, 2H), 1.67 (s, 3H), 1.54 (m, 2H), 1.39 (m, 2H), 1.23 (br. s, 16H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 172.8, 151.2, 146.2, 131.9, 129.3, 120.1, 103.6, 86.8, 79.4, 76.4, 64.0, 60.7, 45.1, 36.0, 32.6, 29.8, 29.1-29.0, 28.1, 27.0, 25.5.

**NIR complex 2.8.** Combined complex 2.7 (0.737 g, 0.617 mmol), NIR dye 2.5 (0.350 g, 0.411 mmol), EDC (0.158 g, 0.822 mmol), and DMAP (0.100 g, 0.822 mmol) into a mixture of CH$_2$Cl$_2$ (12 mL) and dry DMSO (3 mL). The reaction mixture was flushed under N$_2$(g) and stirred in the dark for 5 days. CH$_2$Cl$_2$ was removed *in vacuo* and the crude product was precipitated into a solution of 10% HCl (50 mL) and ammonium hexafluorophosphate (0.201 g,
Subsequently, the precipitate was collected via vacuum filtration, washed with a minimal amount of ice cold water, followed by diethyl ether, and dried in vacuo. The crude product was rinsed several times with methanol and any unreacted amount of NIR dye 2.5 was dissolved into methanol and removed by decanting the methanol layer. Next, the product was rinsed numerous times with dry acetone and any unreacted amount of complex 2.7 was dissolved into acetone and removed by decanting the acetone layer. The remaining product was dissolved into a minimal amount of DMF, precipitated into a mixture of 10% HCl (40 mL) containing NH₄PF₆ (0.201 g, 1.20 mmol), vacuum filtered, and dried in vacuo overnight: 30 % yield. 

**1H NMR (400 MHz, DMSO-d₆)** δ: 8.51 (d, 13.5 Hz, 2H), 7.87 (d, 8.0 Hz, 2H), 7.51 (m, 2H), 7.48 (m, 2H), 7.38 (m, 8H), 7.27 (d, 6.8 Hz, 4H), 7.21 (t, 7.7 Hz, 2H), 6.81 (d, 6.8 Hz, 4H), 6.54 (d, 14.2 Hz, 2H), 6.43 (d, 6.8 Hz, 4H), 5.28 (s, 10H), 4.34 (m, 4H), 4.18 (t, 6.4 Hz, 2H), 3.95 (m, 2H), 2.81 (m, 4H), 2.57 (t, 6.8 Hz, 4H), 2.43 (m, 2H), 2.15 (m, 2H), 2.00 (m, 4H), 1.92 (m, 2H), 1.67 (s, 3H), 1.37 (br. s, 12H), 1.23 (br. s, 20H). 

**13C NMR (101 MHz, DMSO-d₆)** δ: 172.1, 172.0, 165.1, 151.1, 146.7, 146.2, 144.6, 143.5, 142.0, 141.1, 133.5, 131.9, 130.2, 129.2, 128.5, 126.73, 125.4, 124.9, 122.3, 120.1, 111.4, 103.6, 102.3, 86.8, 79.3, 76.4, 64.5, 64.0, 48.6, 47.8, 45.0, 42.8, 35.9, 32.5, 29.7, 28.9, 28.0, 27.3, 27.08, 26.0, 25.4, 23.4, 20.5. IR, ν/cm⁻¹: 1715 (C=O). UV-vis/NIR: λₐ₉ = 797 nm. Fluorescence: λₐ₉ = 814 nm.

**NIR dye 2.9.** Combined NIR dye 2.8 (38.3 mg, 0.0190 mmol), 4,4’-thiobisbenzenethiol (6.8 mg, 0.019 mmol), and potassium carbonate (9.7 mg, 0.048 mmol) into DMF (0.5 mL) and flushed under N₂(g). The reaction was stirred in the dark for 2 days at room temperature. The resulting product mixture was precipitated into 10% HCl (40 mL) containing NH₄PF₆ (6.1 mg, 0.038 mmol) and filtered in vacuo. Yield: 7.8 mg. 

**1H NMR (400 MHz, DMSO-d₆)** δ: 8.51 (br. s), 7.95 (s, possible impurity), 7.86 (br. s), 7.54-7.27 (m), 7.09 (s, possible impurity), 6.96 (s, possible impurity), 6.81 (d, 6.8 Hz), 6.76-6.62 (m, impurity), 6.54 (br. s), 6.42 (br. s), 6.31 (br.
s), 5.28 (s, Cp starting material), 5.22 (br. s), 5.17 (br. s), 5.03 (br. s), 4.34 (br. m), 4.18 (br. s),
3.95 (br. s), 2.88 (s, DMF solvent), 2.83-2.67 (br. m), 2.77 (s), 2.72 (s, DMF solvent), 2.33 (s),
2.13 (br. s), 2.00 (br. s), 1.87 (br. s), 1.64 (br. s), 1.39 (br. s), 1.30 (s, impurity), 1.23 (br. s),

**NIR dye 2.10.** Dissolved NIR dye 2.5 (0.200 g, 0.235 mmol), anthracene-9-methanol
(48.9 mg, 0.235 mmol) and DMAP (43.0 mg, 0.352 mmol) into CH$_2$Cl$_2$ (7 mL) and DMF (5 mL)
and cooled to 0 °C. DCC (72.7 mg, 0.352 mmol) was dissolved in CH$_2$Cl$_2$ (1 mL) and added to
the cooled reaction mixture. The reaction was stirred at room temperature for 4 days. CH$_2$Cl$_2$
was removed *in vacuo* and a slow crystallization was induced by adding dropwise a mixture of
anhydrous denatured ethanol and diethyl ether (1:20, 22 mL total). The product was isolated *via*
vacuum filtration into a sintered glass crucible, was washed multiple times with diethyl ether,
and allowed to dry overnight *in vacuo*: 71% crude yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.68
(s, 1H), 8.46 (m, 2H), 8.41 (m, 2H), 8.10 (d, 8.4 Hz, 2H), 7.79 (d, 8.6 Hz, 2H), 7.55 (m, 2H),
7.48 (m, 6H), 7.38 (m, 2H), 7.32 (m, 2H), 7.21 (m, 2H), 6.49 (d, 14.0 Hz, 2H), 6.32 (s, 2H),
4.32 (m, 4H), 2.77 (m, 4H), 2.57 (m, 4H), 1.98 (m, 4H), 1.88 (m, 2H), 1.43 (br. s, 12H). $^{13}$C
NMR (101 MHz, DMSO-$d_6$) $\delta$: 172.0, 165.1, 146.6, 144.6, 143.8, 142.0, 141.1, 133.5, 130.9,
130.5, 130.3, 129.0, 128.95, 128.5, 126.9, 126.8, 126.4, 125.5, 125.3, 125.0, 124.0, 122.4, 111.5,
102.3, 59.0, 48.7, 47.8, 42.8, 27.3, 26.0, 23.4, 20.5. IR, $\nu$/cm$^{-1}$: 1709 (C=O). UV-vis/NIR: $\lambda_{abs} =$
798 nm. Fluorescence: $\lambda_{em} = 813$ nm. ESI-MS (M-Na)$^-$ m/z calcd for C$_{58}$H$_{57}$N$_2$O$_8$S$_3$: 1005.3,
found 1005.3.

**NIR dye 2.11.** Dissolved NIR dye 2.5, (0.200 g, 0.235 mmol), 1-pyrenemethanol (83.7
mg, 0.354 mmol) and DMAP (43.2 mg, 0.508 mmol) into CH$_2$Cl$_2$ (8 mL) and DMF (3.5 mL)
and cooled to 0 °C under N$_2$(g). DCC (85.2 mg, 0.413 mmol) was dissolved in CH$_2$Cl$_2$ (1 mL),
added to the cooled reaction mixture, and flushed under N$_2$(g). The reaction was stirred at room
temperature for 7 days. CH$_2$Cl$_2$ was removed *in vacuo* and a slow crystallization was induced by
adding dropwise a mixture of anhydrous denatured ethanol and diethyl ether (1:20, 23 mL total). The product was isolated via vacuum filtration into a sintered glass crucible, was washed multiple times with diethyl ether, and allowed to dry overnight in vacuo: 86% crude yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.47 (d, 14.0 Hz, 2H), 8.37 (d, 9.3 Hz, 1H), 8.30 (d, 7.8 Hz, 1H), 8.26-8.23 (m, 2H), 8.21-8.19 (d, 9.3 Hz, 1H), 8.20-8.14 (m, 3H), 8.06 (t, 7.8 Hz, 1H), 7.89 (d, 8.6 Hz, 2H), 7.48-7.44 (m, 4H), 7.38-7.34 (m, 4H), 7.20 (m, 2H), 6.5 (d, 14.0 Hz, 2H), 6.01 (s, 2H), 4.32 (m, 4H), 2.77 (m, 4H), 2.57 (m, 4H), 1.98 (m, 4H), 1.88 (m, 2H), 1.39-1.33 (br. s, 12H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$: 172.0, 165.0, 146.6, 144.6, 143.9, 142.0, 141.1, 133.5, 131.1, 130.6, 130.4, 130.1, 129.2, 128.9, 128.5, 128.1, 127.9, 127.7, 127.3, 126.5, 126.4, 125.6, 125.53, 125.51, 125.0, 124.7, 124.0, 123.7, 123.1, 122.3, 111.5, 102.3, 64.9, 48.7, 47.8, 42.8, 27.1, 26.0, 23.4, 20.5. IR, $\nu$/cm$^{-1}$: 1713 (C=O). UV-vis/NIR: $\lambda_{\text{abs}}$ = 796 nm. Fluorescence: $\lambda_{\text{em}}$ = 814 nm. ESI-MS (M-Na$^+$) $m/z$ calcd for $C_{66}H_{57}N_2O_8S_3$: 1029.3, found 1029.4.

**NIR dye 2.12.** Combined NIR dye 2.5 (0.300 g, 0.353 mmol), 1-pyrenebutanol (0.145 g, 0.529 mmol), and DMAP (64.6 mg, 0.529 mmol) into CH$_2$Cl$_2$ (12 mL) and dry DMSO (5.5 mL). The mixture was flushed under N$_2$(g), stirred, and cooled reaction mixture to 0 °C. Subsequently, DCC (0.145 g, 0.705 mmol) that had been dissolved into CH$_2$Cl$_2$ (1.5 mL) was added to the reaction mixture under N$_2$(g) and stirred at room temperature for 7 days. Dichloromethane was removed in vacuo and a mixture of anhydrous denatured ethanol (1 mL) and diethyl ether (20 mL) was slowly added to the product and then decanted. Next, diethyl ether (25 mL) was added to the remaining oily residual product, which was then sonicated, followed by decanting off the diethyl ether mixture. The addition of diethyl ether, sonication, and decanting was repeated until a precipitate formed. The dark green precipitate was isolated by vacuum filtration and dried in vacuo: 67% crude yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.49 (d, 14.0 Hz, 2H), 8.32 (d, 9.3 Hz, 1H), 8.28-8.19 (m, 2H), 8.17-8.12 (m, 2H), 8.09 (m, 2H), 8.03 (t, 7.75 Hz, 1H), 7.90 (m,
1H), 7.87 (m, 2H), 7.50 (m, 2H), 7.47 (m, 2H), 7.40-7.36 (m, 4H), 7.17-7.15 (m, 2H), 6.52 (d, 14.0 Hz, 2H), 4.32 (m, 6H), 3.31 (m, 2H), 2.80 (m, 4H), 2.57 (m, 4H), 1.99 (m, 4H), 1.89 (m, 2H), 1.84 (m, 4H), 1.43-1.31 (m, 12H). \(^{13}\)C NMR (101 MHz, DMSO-d\(_6\)) \(\delta\): 172.0, 165.2, 146.7, 144.6, 143.6, 142.0, 141.1, 136.7, 133.5, 130.9, 130.4, 130.2, 129.2, 128.5, 128.0, 127.4, 127.2, 126.7, 126.5, 126.1, 125.4, 124.9, 124.7, 124.2, 124.1, 123.4, 122.3, 111.5, 102.3, 64.3, 48.7, 47.8, 32.1, 28.1, 27.9, 27.3, 27.1, 26.0, 23.5, 20.5. IR, \(\nu/cm^{-1}\): 1713 (C=O). UV-vis/NIR: \(\lambda_{abs} = 797\) nm. Fluorescence: \(\lambda_{em} = 813\) nm. ESI-MS (M-Na\(^-\)) \(m/z\) calcd for \(C_{63}H_{63}N_2O_8S_3\): 1071.4, found 1071.2.

**NIR dye 2.13.** Dissolved NIR dye 2.5, (0.201 g, 0.240 mmol), 3-thiophenemethanol (83.7 mg, 0.354 mmol) and DMAP (43.2 mg, 0.508 mmol) into CH\(_2\)Cl\(_2\) (8 mL) and DMF (3.5 mL) and cooled to 0 °C under N\(_2\) (g). DCC (85.2 mg, 0.413 mmol) was dissolved in CH\(_2\)Cl\(_2\) (1 mL), added to the cooled reaction mixture, and flushed under N\(_2\) (g). The reaction was stirred at room temperature for 7 days. CH\(_2\)Cl\(_2\) was removed in vacuo and a slow crystallization was induced by adding dropwise a mixture of anhydrous denatured ethanol and diethyl ether (1:20, 23 mL total). The product was isolated via vacuum filtration into a sintered glass crucible, was washed multiple times with diethyl ether, and allowed to dry overnight in vacuo: 67% crude yield. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\): 8.52 (d, 14.0 Hz, 2H), 7.91 (d, 8.3 Hz, 2H), 7.57-7.48 (m, 4H), 7.38 (m, 4H), 7.20 (m, 2H), 7.14 (m, 1H), 6.54 (d, 14.0 Hz, 2H), 5.26 (s, 2H), 4.36 (m, 4H), 2.81 (m, 4H), 2.59 (m, 4H), 2.01 (m, 4H), 1.91 (m, 2H), 1.37 (br. s, 12H). \(^{13}\)C NMR (101 MHz, DMSO-d\(_6\)) \(\delta\): 172.0, 165.0, 146.7, 144.7, 143.8, 142.0, 141.1, 136.8, 133.6, 130.4, 128.5, 127.6, 126.8, 126.5, 125.5, 125.0, 124.6, 122.4, 111.5, 102.3, 61.6, 48.7, 47.8, 42.8, 27.2, 26.0, 23.5, 20.5. IR, \(\nu/cm^{-1}\): 1713 (C=O). UV-vis/NIR: \(\lambda_{abs} = 795\) nm. Fluorescence: \(\lambda_{em} = 813\) nm. ESI-MS (M-Na\(^-\)) \(m/z\) calcd for \(C_{48}H_{51}N_2O_8S_4\): 911.3, found 911.6.
**NIR dye 2.14.** Dissolved NIR dye 2.5 (0.217 g, 0.254 mmol), 2-(3-thienyl)ethanol (0.03 mL, 0.3 mmol) and DMAP (62.1 mg, 0.508 mmol) into CH₂Cl₂ (6 mL) and DMF (5 mL) and cooled to 0 °C. DCC (0.105 g, 0.508 mmol) was dissolved in CH₂Cl₂ (1 mL) and added to the cooled reaction mixture. The reaction was stirred at room temperature for 4 days. CH₂Cl₂ was removed *in vacuo* and a slow crystallization occurred by adding dropwise a mixture of anhydrous denatured ethanol and diethyl ether (1:20, 21 mL total). The product was isolated *via* vacuum filtration into a sintered glass crucible, was washed multiple times with diethyl ether, and allowed to dry overnight *in vacuo*: 72% crude yield. ¹H NMR (400 MHz, DMSO-d₆) δ: 8.51 (d, 14.0 Hz, 2H), 7.85 (d, 8.6 Hz, 2H) 7.52 (d, 7.6 Hz, 2H), 7.49 (d, 8.6 Hz, 2H), 7.39 (m, 4H), 7.24 (m, 1H), 7.22 (m, 2H), 7.02 (m, 1H), 6.54 (d, 14.0 Hz, 2H), 4.38 (m, 2H), 4.34 (m, 4H), 2.98 (t, 6.6 Hz, 2H), 2.82 (t, 6.0 Hz, 4H), 2.57 (t, 6.8 Hz, 4H), 2.00 (quint., 6.8 Hz, 4H), 1.93 (m, 2H), 1.37 (s, 12H). ¹³C NMR (101 MHz, DMSO-d₆) δ: 172.0, 165.1, 146.7, 144.7, 143.7, 142.1, 141.1, 138.2, 133.6, 130.3, 128.5, 126.6, 125.9, 125.5, 125.0, 122.4, 121.9, 111.5, 102.3, 64.6, 48.7, 47.8, 42.8, 28.9, 27.2, 26.0, 23.5, 20.5. IR, ν/cm⁻¹: 1715 (C=O). UV-vis/NIR: λₐₙ₅ = 797 nm. Fluorescence: λₑₐ₅ = 816 nm. ESI-MS (M-Na)⁻ m/z calcd for C₄₉H₅₃N₂O₈S₄: 925.3, found 925.8.

**NIR dye 2.15.** Dissolved NIR dye 2.5 (0.123 g, 0.144 mmol) and DMAP (35.2 mg, 0.288 mmol) into CH₂Cl₂ (10 mL). Next, *endo,exo*-5-norbornene-2-methanol (0.05 mL, 0.4 mmol) and DIC (0.06 mL, 0.4 mmol) were added to the stirring reaction mixture under N₂(g). The reaction was stirred for 4 days at room temperature. The resulting product mixture was concentrated *in vacuo* and collected as a viscous liquid: 36% yield. ESI-MS (M-Na)⁻ m/z calcd for C₅₁H₅₇N₂O₈S₃: 921.3, found 921.9.
Chapter 3  Intercalated Fluorophores into Hectorite: Precursory Results

In the past, various fluorophores have been intercalated and/or adsorbed onto inorganic layered clay minerals such as Li$_x$MoS$_2$\cite{133}, montmorillonites\cite{82,84}, hectorites\cite{134,135}, saponites\cite{76,85,136}, and laponites\cite{137}. There is generally two methods of intercalation of guest molecules into smectites (clay minerals): (1) a cation exchange with interlayer exchangeable cations and (2) the adsorption of polar molecules such as alcohols, amides, and ketones via ion-dipole interactions with cations in the interlayer and/or hydrogen bonding with oxygen atoms on the surface the silicate sheets.\cite{78} As a result, intercalated nanocomposites are prepared by utilizing the exfoliation/re-stacking properties of the hydrophilic clay mineral.\cite{138} For example, Scheme 3.1 gives a schematic representation of the intercalation of fluorescent heptamethine indocyanine dye 2.4 and indolenine dye 3.2 into hectorite. The dispersion of hectorite in water causes a disorganization of the layered structure, known as exfoliation.\cite{134} Subsequently, the addition of the dye solutions during exfoliation causes a displacement of the interlamellar water molecules with the dyes in the layered structure, followed by the re-stacking of the hectorite layers.\cite{139}

![Scheme 3.1](image)

**Scheme 3.1  Schematic representation of the intercalation of fluorescent heptamethine indocyanine dye 2.4 and indolenine dye 3.2 into hectorite.**

As aforementioned in chapter 1, there are a number of studies that have investigated the insertion of pseudoisocyanine (PIC) dyes\textsuperscript{76,78,79,82--85,140--142} and merocyanine dyes\textsuperscript{78} into various
layered lattices and very recently, the intercalation of a trimethine indocyanine dye into saponite clay. However, few studies have reported the intercalation of cyanine dyes into the layered matrices of hectorite. The intercalation of heptamethine cyanine dyes and indolenine dyes into hectorite has yet to be explored. This prompted us to select two highly polar, water-soluble dyes, including a heptamethine indocyanine dye (2.4) and a simplified indolenine dye (3.2) as guest molecules for their insertion into a layered silicate host (Scheme 3.1). Hectorite was selected as the layered silicate lattice due to its negligible content of Fe impurities, which are known to quench fluorescence and are abundant in other expandable aluminosilicates. In addition to its low iron content, hectorite has a large particle size, the layers of which easily stack when dye molecules are adsorbed in the interlayer spaces of the clay particles. Novel intercalated dye-clay nanocomposites have been prepared and characterized. The results discussed in this chapter are preliminary and provide a baseline for future work involving the preparation of novel clay nanocomposites containing heptamethine indocyanine dyes.

### 3.1 Intercalation of heptamethine indocyanine and indolenine dyes into hectorite: formation of nanocomposite materials

The synthesis of nanocomposites 3.1 and 3.3 followed similar methodology reported by Bissessur, and coworkers (Scheme 3.2 and Scheme 3.3). Water-soluble heptamethine indocyanine dye 2.4 and indolenine dye 3.2 were intercalated into hectorite by adding aqueous solutions of the dyes to an aqueous suspension of hectorite. In general, various mole ratios of 2:1, 4:1, and 5:1 of the dyes with respect to hectorite were used. It should be noted that aqueous solutions of the dyes were added dropwise to the stirring aqueous suspension of hectorite to ensure an even dispersion of dye into the layered clay material. Although previous intercalation studies have used sonication to facilitate the dissolution of the solute, this technique was not used since the dyes were highly water-soluble. The hectorite-dye suspensions were stirred
for 2 days, to ensure the inclusion of the dyes into layered hectorite. To ensure the removal of any remaining water molecules after centrifugation, the hectorite-dye slurry was freeze-dried for a period of two days. After centrifugation a small quantity of the nanocomposite sediments were cast as thin films onto glass plates and allowed to dry for characterization by powder X-ray diffraction (XRD).

Scheme 3.2  Intercalation of NIR dye 2.4 into hectorite.

As reported in the literature, the incorporation of various compounds into layered systems containing interlamellar water molecules is primarily driven by entropy.\textsuperscript{146–148} Two different concentrations of the hectorite aqueous suspensions (i.e. 0.01 g/mL and 0.005 g/mL) were utilized to determine whether or not the formation of the intercalation compound would be sensitive towards these varying concentrations. These concentrations were selected based on previous intercalation studies reported by Bissessur and co-workers.\textsuperscript{133,144,149} In addition, various mole ratios of dye with respect to hectorite were employed in order to characterize and investigate any noticeable differences in the intercalated materials, which will be discussed later.
3.2 General characterization of intercalated nanocomposites

Powder X-ray diffraction analysis (XRD) was carried out to determine the formation of the dye-hectorite nanocomposites and to investigate the intercalation effect of these nanomaterials. The interlayer distances of pristine hectorite and the nanocomposites are important for determining the degree of intercalation of fluorophores 2.4 and 3.2 into hectorite. Accordingly, the approximated interlayer distances of the nanocomposites were calculated from the (001) peak obtained from the XRD pattern, using Bragg’s equation,

\[ n\lambda = 2dsin\theta \]  
(Eq. 8)

where \( n \) is an integer, \( \lambda \) denotes the wavelength of the incident X-rays, \( d \) is the approximated interplanar (basal or interlayer) spacing of the crystal, and \( \theta \) denotes the angle of incidence. As aforementioned, an intercalation reaction occurs when the interlayer water molecules within the disordered hectorite are displaced by the incoming dye molecules, followed by the rearrangement into a layered material. As a result, an interlayer expansion takes place when the dyes are incorporated into the hectorite layers. The extent of interlayer expansion is determined by the change in the interlayer spacing between pristine hectorite and the nanocomposite.

The intercalation of heptamethine indocyanine dye 2.4 and indolenine 3.2 into hectorite was verified from the powder X-ray diffraction patterns and compared to that of pure hectorite. The hectorite XRD pattern (graph A in Figure 3.1) showed a reflection at \( 2\theta = 7.70^\circ \) corresponding to an interlayer spacing of 11.48 Å.
Figure 3.1 Select XRD patterns of: (A) hectorite and nanocomposites (B) 3.1a and (C) 3.3b.
Table 3.1 Summary of XRD results for nanocomposites 3.1 and 3.3.

<table>
<thead>
<tr>
<th>Intercalation compound</th>
<th>Dye:hectorite (mol)</th>
<th>Interlayer spacing (Å)</th>
<th>Interlayer expansion(^b) (Å)</th>
<th>Crystallite size (Å)</th>
<th>Hectorite conc.(^a) (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1a</td>
<td>2:1</td>
<td>27.99</td>
<td>16.51</td>
<td>77</td>
<td>0.01</td>
</tr>
<tr>
<td>3.1b</td>
<td>2:1</td>
<td>26.64</td>
<td>15.16</td>
<td>61</td>
<td>0.005</td>
</tr>
<tr>
<td>3.1c</td>
<td>4:1</td>
<td>27.79</td>
<td>16.31</td>
<td>90</td>
<td>0.005</td>
</tr>
<tr>
<td>3.3a</td>
<td>2:1</td>
<td>16.81</td>
<td>5.33</td>
<td>63</td>
<td>0.01</td>
</tr>
<tr>
<td>3.3b</td>
<td>2:1</td>
<td>16.61</td>
<td>5.13</td>
<td>54</td>
<td>0.005</td>
</tr>
<tr>
<td>3.3c</td>
<td>5:1</td>
<td>16.57</td>
<td>5.09</td>
<td>54</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^a\) Hectorite concentration (conc.) is determined from the grams of hectorite per 1 millilitre of water

\(^b\) Interlayer expansion, defined as \(\Delta d\), was determined from XRD data as follows: \(\Delta d = d_{\text{intercalate}} - d_{\text{hectorite}}\)

The relatively large interlayer expansions of the nanocomposites confirms the genuine intercalation of the dyes into hectorite. For example, nanocomposite 3.1a has an observed interlayer expansion of 16.51 Å, with an approximated interlayer spacing, \(d\), of 27.99 Å (Figure 3.1, graph B). The large interlayer spacing of the intercalated dye-hectorite system suggests the dye molecule has non-planar-geometry with respect to the hectorite layers, which in turn causes a larger interlayer expansion of the layered host.\(^{133,146}\) The interlayer spacing is greater than that of a somewhat similar saponite nanocomposite containing a trimethine indocyanine dye attached to polyhedral oligomeric silsesquioxanes (POSS)\(^76\) (structure shown in Scheme 1.4 in chapter 1). From the XRD results in this previous study the authors report a basal spacing of 12.6 Å.\(^76\) Additionally, they estimated basal spacing measurements from a TEM micrograph, which gave interlayer spacing ranging from 13 to 20 Å. Based on the relatively wide range in basal spacing the authors postulate that the indocyanine-POSS dye molecules are confined in different spatial configurations in the saponite interlayer space. Further investigation will be required in the future to provide better insight into the structural arrangements of the dyes in the interlayer of hectorite.

There were no significant differences in the XRD results between nanocomposites 3.1a and 3.1b that were prepared with different concentrations of hectorite in water. This indicates
that the intercalation process was not highly sensitive to these differing concentrations of 0.01g/mL and 0.005 g/mL of hectorite in water. Therefore, either concentration are acceptable to use for future work.

The XRD results of the other nanocomposites 3.1b and 3.1c, as listed in Table 3.1, do not show any significant variations in their respective XRD patterns. However, the XRD of nanocomposite 3.1c shows a weak reflection at 2θ = 4.65°. Since this nanocomposite contained the greatest excess of dye molecules, it may correspond to a dye impurity. However, further investigation would be required in future work to confirm this. If there is too high of a mole ratio of dye to hectorite then there would be too many dye molecules to all be inserted into the interlayer, which would cause the dye molecules to be pushed to the outside of the layers.

Smaller interlayer spacing ranging from 5.33 Å to 5.09 Å was observed for nanocomposites 3.3a-c, respectively (Figure 3.1 graph C, Table 3.2). This spacing is consistent with the smaller size of indolenine dye 3.2 and suggests that the dye lies co-planar with respect to the hectorite layers. Similar to nanocomposites 3.1a-c, intercalation compounds 3.3a-c do not show significant differences between their respective XRD patterns.

The XRD patterns of the nanocomposites were also used to determine their crystallite sizes based on a simplified version of the Scherrer equation (Equation 9).\(^{133,150,151}\)

\[
D = \frac{K \cdot \lambda \cdot 57.3}{\beta^{1/2} \cdot \cos \theta}
\]

(Eq. 9)

where \(D\) (in Å) is the average crystallite size, \(\lambda\) is the wavelength of the CuK\(_{\alpha}\) radiation (1.541 Å), \(\beta^{1/2}\) denotes the peak width at half-height in degrees and \(\theta\) is the position of the peak (in degrees). \(K\) is the Scherrer constant, which varies depending on the crystallite shape.\(^{150,151}\) It is assumed that the crystallites are spherical crystals with cubic symmetry based on the literature, thus \(K = 0.9\).\(^{133,150}\) Additionally, the numerical value 57.3 denotes the conversion factor for radians to degrees. The largest crystallite size of 90 Å was observed for nanocomposite 3.1c
compared to the other crystallite sizes, which ranged from 77 Å to 54 Å for nanocomposites 3.1a to 3.3a-c, respectively (Table 3.2). All of the crystallite sizes show either nearly a two- or three-fold increase compared to that of pristine hectorite, which has a crystallite size of 38 Å. This increase suggests that after exfoliation the hectorite layers re-stack extremely well, creating a more ordered structure and resultant increase in the crystallite size of the nanocomposites.

Table 3.2  Crystallite sizes of the intercalated nanocomposites and pristine hectorite.

<table>
<thead>
<tr>
<th>Intercalated nanocomposite</th>
<th>Crystallite size (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1a</td>
<td>77</td>
</tr>
<tr>
<td>3.1b</td>
<td>61</td>
</tr>
<tr>
<td>3.1c</td>
<td>90</td>
</tr>
<tr>
<td>3.3a</td>
<td>63</td>
</tr>
<tr>
<td>3.3b</td>
<td>54</td>
</tr>
<tr>
<td>3.3c</td>
<td>54</td>
</tr>
<tr>
<td>Hectorite</td>
<td>38</td>
</tr>
</tbody>
</table>

Scanning electron microscopy (SEM) was used to examine the surface morphologies of the intercalated dyes into hectorite. SEM micrographs of nanocomposite 3.1a at various magnifications are shown as representative examples for nanocomposites 3.1 in Figure 3.2 and Figure 3.3. The significant changes in the micrograph of nanocomposite 3.1a compared to that of dye 2.4 suggest that dye 2.4 has successfully been intercalated into the hectorite layers. For instance, the SEM micrograph of nanocomposite 3.1a depicts layered sheets with a striated morphology (Figure 3.2 and Figure 3.3), as opposed to a granular one observed in that of dye 2.4 (Figure 3.2). Additionally, the striated nature of the individual layers in intercalation complex 3.1a suggests that the layers are aligned rather than oriented randomly. Moreover, these layered sheets appear to be flexible, as illustrated in the curvature of the layers in Figure 3.3, compared to the pure dye 2.4. For future studies, it may be useful to prepare uniformly distributed thin films of these nanocomposites in order to examine and compare their
morphological characteristics to their respective solid particulates. High resolution SEM and transmission electron microscopy (TEM) would be valuable instrumental analysis for this particular characterization.

Figure 3.2  SEM micrographs of nanocomposite 3.1a and dye 2.4 at magnification of x2.5k.
Figure 3.3  SEM micrographs of nanocomposite 3.1a at different magnifications.

The comparison of the SEM micrograph of intercalated nanocomposite 3.1a to that of pristine hectorite (Figure 3.4) provides evidence for the successful intercalation of dye 2.4 into hectorite. For instance, the cross-sectional view of pristine hectorite shows extremely-densely packed silicate layers, which according to the literature are approximately 1 nm thick and stacked in ~10 μm-sized multi-layered stacks. Conversely, uniformly-dispersed individual silicate layers are observed in the SEM image of nanocomposite 3.1a, which supports that an interlayer expansion occurred during the intercalation reaction. Moreover the SEM micrographs, indicate that the insertion of the dye molecules into hectorite resulted in an ordered molecular arrangement in the nanocomposites. These SEM results are consistent with the results obtained from the XRD patterns of the nanocomposites.
Figure 3.4 Comparison of SEM micrographs of hectorite (top left) and nanocomposite 3.1a (bottom left) at x600 magnification and their respective magnified regions at x2.5k magnification.

The significant changes in the micrograph of nanocomposite 3.3a compared to that of dye 3.2, suggests that dye 3.2 has successfully been intercalated into the hectorite layers (Figure 3.5 and Figure 3.6). For example, a layered and randomly oriented flaky/platelet morphology is depicted in the SEM micrograph of nanocomposite 3.3a as opposed to a dense and rod-like porous morphology observed in dye 3.2 (Figure 3.6). This change in morphology is characteristic of intercalated nanoclays, in which the individual nm-thick clay layers become completely separated to form platelet-like nanoparticles. These SEM results are consistent with the increased crystallite sizes as determined from the XRD patterns.
Figure 3.5  SEM micrographs of nanocomposite 3.3a and dye 3.2 at magnification of x600.

Figure 3.6  SEM micrographs of cross-sectional views of nanocomposite 3.3a and dye 3.2 at magnification of x2.5k.

Fourier transform infrared (FT-IR) attenuated total reflectance (ATR) spectroscopy was conducted on solid state samples to demonstrate the interlamellar spacing modification of hectorite by dyes 2.4 and 3.2. By comparing the different shifts the IR spectra of hectorite and fluorophores 2.4 and 3.2 (Appendix C.2 and Figure 3.9, respectively) were interpreted and used as a comparison for the characterization of the nanocomposites. Furthermore, band shifts in the infrared spectra of the intercalated materials confirm the successful intercalation of dyes 2.4 and 3.2 into hectorite. Additionally, there are no significant differences between the IR spectra of
nanocomposites 3.1a-c and for nanocomposites 3.3a-c. As a result, the IR spectra of nanocomposites 3.1a and 3.3a are shown in Figure 3.8 and Figure 3.9, respectively and are subsequently discussed as representative examples of nanocomposites 3.1 and 3.3, respectively.

The IR spectrum of nanocomposite 3.1a (Figure 3.8) shows a combination of characteristics very similar to that of dye 2.4 and hectorite (Figure 3.7). For instance, nanocomposite 3.1a exhibits IR bands at 3408 and 1624 cm\(^{-1}\) due to the stretching and bending vibrations for hydroxyl groups of water molecules within the hectorite interlayers. These OH bands have decreased relative intensities (relative with respect to the intensities other IR bands in the spectrum) compared to the relative intensities of hectorite. This may attributed to the general displacement of the water molecules in the interlayer with dye molecules, which would in turn decrease the concentration of water (Figure 3.8). It should be noted that the concentrations of the samples are not relevant in this particular comparison since it is the relative intensities of the IR bands that are being compared. The appearance of a strong, broad Si-O stretch observed in the IR spectrum of hectorite shifts from 969 cm\(^{-1}\) to 990 cm\(^{-1}\) for nanocomposite 3.1a as a result of reduced electrostatic interactions between the hectorite sheets. This is consistent with the XRD data, which indicates an expansion between the layered hectorite sheets as a result of intercalation, which in turn minimizes the interlamellar electrostatic interactions. In both of the IR spectra of hectorite and nanocomposite 3.1a, the IR bands around 699, and 437 cm\(^{-1}\) are attributed to the Si-O out-of-plane and in-plane bending, respectively.\(^{152}\) A comparison of the IR spectra of dye 2.4 and nanocomposite 3.1a indicates very little change between the vibrational frequencies of the dye and the intercalation compound. An exception is observed in the shift in the absorption band associated with the SO\(_3^+\) stretching shifting from 1129 cm\(^{-1}\) in dye 2.4 to 1143 cm\(^{-1}\) in nanocomposite 3.1a. This may be attributed to the interaction between the sulfonate groups and the hectorite, which minimizes the dye-dye inter- and intramolecular hydrogen
bonding interactions. Thus, the S=O double bond character is strengthened, which in turn increases the absorption frequency. Typical sp$^2$ and sp$^3$ C-H stretches appear around 3024 cm$^{-1}$ and 2975-2874 cm$^{-1}$, respectively. Due to the complex nature of the IR spectra of heptamethine cyanine dyes in the fingerprint region, IR vibrational frequencies can be challenging to identify as a consequence of overlapping IR bands.$^{153}$ However despite this, typical functional absorption bands were assigned in the intercalation compound. The C=C alkene and arene stretches appear between 1552-1510, 1453-1434 and 1395 cm$^{-1}$ and arene C-H out-of-plane bending (ortho-substitution) around 715 cm$^{-1}$. The IR bands around 1251-1170, and 1080-1020 cm$^{-1}$ correspond to the vibrational frequencies of the SO$_3^-$ moieties.

Figure 3.7  FT-IR-ATR spectrum of hectorite in the solid state.
Figure 3.8  FT-IR-ATR spectrum of nanocomposite 3.1a in the solid state.

Similar C-H and C=C vibrational frequencies observed in nanocomposite 3.1a are observed in the IR spectrum of nanocomposite 3.3c. Additionally, a characteristic carbonyl stretch of the carboxylic acid moiety appears at 1717 cm$^{-1}$, which is upshifted compared to that of dye 3.2 appearing at 1700 cm$^{-1}$ (Figure 3.9). This is attributed to the decreased intermolecular interactions between the dye aggregates upon intercalation. As a result, the C=O double bond character is strengthened, which in turn increases the absorption frequency by 17 cm$^{-1}$. Similarly, the OH stretch from the carboxylic acid moiety in dye 3.2 shifts up from 2835-3017 cm$^{-1}$ to ~2871-3035 cm$^{-1}$ in nanocomposite 3.3a. In general, most of the IR bands corresponding to the dye moiety for nanocomposite 3.3a are weaker in their intensities compared to dye 3.2, which is attributed to the decreased concentration of the dye molecules inserted into hectorite.
Figure 3.9 FT-IR spectra of dye 3.2 and nanocomposite 3.3c in the solid state.

As aforementioned in Chapter 1, intercalation of guest molecules into host clay lattices has been known to enhance their thermal stabilities with respect to the pure guest molecules. Specifically, the clay layers can provide greater insulation and obstruction against transportation of the volatile compounds produced from thermal decomposition. Materials with improved thermal stabilities are desirable for their use in a wide variety of applications in materials
Accordingly, thermal stabilities of the intercalated nanocomposites were investigated using thermogravimetric analyses (TGA) and compared to the corresponding dye and hectorite starting materials.

According to the TGA results, as summarized in Table 3.3, there were no significant differences between nanocomposites 3.1a to 3.1c. Therefore, investigation of the thermal stability of intercalation compound 3.1b is discussed as an example. The TGA thermal curve of nanocomposite 3.1b, shown in Figure 3.10, indicates thermal degradation occurred within four steps. Comparison of the TGA data of hectorite and dye 2.4, suggests weight loss of 2% with thermal stability up to 281°C can be attributed to the partial decomposition of the dye moiety. It is speculated that this initial onset weight loss is attributed to the loss of the polymethine chain, and aliphatic substituents, possibly due to the lower bond dissociation energies. Additionally, the weight loss of 5% (step 3 decomposition, Table 3.3) with respect the dye moiety is 54 °C greater degradation temperature compared to cyanine dye 2.4. Lastly, the final weight loss of 6.1% at a degradation temperature of 851 °C corresponds to the dehydroxylation of the silicate layers.154

Table 3.3 Summary of TGA results of nanocomposites 3.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dye:Hectorite (mol)</th>
<th>Hectorite conc.(^a) (M)</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(T_d) (°C) (% wt)</td>
<td>(T_d) (°C) (% wt)</td>
<td>(T_d) (°C) (% wt)</td>
<td>(T_d) (°C) (% wt)</td>
</tr>
<tr>
<td>3.1a</td>
<td>2:1</td>
<td>0.026</td>
<td>280 (4.5%)</td>
<td>378 (9.4%)</td>
<td>656 (2.6%)</td>
<td>815 (7.5%)</td>
</tr>
<tr>
<td>3.1b</td>
<td>2:1</td>
<td>0.013</td>
<td>281 (2.1%)</td>
<td>384 (8.7%)</td>
<td>630 (5.1%)</td>
<td>851 (6.1%)</td>
</tr>
<tr>
<td>3.1c</td>
<td>4:1</td>
<td>0.013</td>
<td>285 (2.6%)</td>
<td>389 (9.1%)</td>
<td>628 (8.4%)</td>
<td>814 (7.5%)</td>
</tr>
<tr>
<td>2.4</td>
<td>-</td>
<td>-</td>
<td>271 (18.8%)</td>
<td>427 (21.1%)</td>
<td>576 (45.3%)</td>
<td>-</td>
</tr>
<tr>
<td>H Hectorite</td>
<td>-</td>
<td>-</td>
<td>127 (3.7%)</td>
<td>723 (4.2%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) ‘conc.’ refers to concentration of hectorite aqueous suspension in mol/L (M).
In general, TGA of nanocomposites 3.3 indicate an apparent initial step around 150 °C corresponding to a weight loss of approximately 2% attributing to the desorption of co-intercalated water molecules (Figure 3.11). The loss of water molecules observed in the TGA thermal curve of pure hectorite is lower and is observed at 125 °C (weight loss of 5.57%), suggesting a greater amount of water molecules are present in nanocomposite 3.3 compared to that of hectorite. Interestingly, intercalated materials of NIR dye 2.4 in hectorite do not show this discernible step in their TGA thermal curves, which signifies that the co-inclusion of water molecules is not occurring in the latter materials. In addition to the initial step, nanocomposite 3.3 also exhibits four additional weight losses between 233 and 660 °C, corresponding to the stepwise decomposition of the organic functional groups in dye 3.2 (Table 3.4). A final step corresponding to a weight loss of 3.58% at 851 °C corresponds to the dehydroxylation of the silicate sheets. The incorporation of dye 3.2 into hectorite alters the decomposition pattern from two weight losses in dye 3.2 to four in nanocomposite 3.3, indicating a change in decomposition
temperatures. There are no significant changes between nanocomposites 3.3 and dye 3.2 in the initial thermal degradation step. This suggests that the intercalation of dye 3.2 into hectorite did not significantly alter the thermal stability of the nanocomposites compared to the pure dye.

Table 3.4  Summary of TGA results of nanocomposites 3.3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_d (°C) ) (% wt)</td>
<td>( T_d (°C) ) (% wt)</td>
<td>( T_d (°C) ) (% wt)</td>
<td>( T_d (°C) ) (% wt)</td>
<td>( T_d (°C) ) (% wt)</td>
</tr>
<tr>
<td>3.3a</td>
<td>229 (5.61%)</td>
<td>353 (4.64%)</td>
<td>529 (7.32%)</td>
<td>645 (6.06%)</td>
<td>843 (3.48%)</td>
</tr>
<tr>
<td>3.3b</td>
<td>244 (4.37%)</td>
<td>350 (4.48%)</td>
<td>551 (6.10%)</td>
<td>675 (3.52%)</td>
<td>868 (4.45%)</td>
</tr>
<tr>
<td>3.3c</td>
<td>212 (11.20%)</td>
<td>336 (4.23%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.2</td>
<td>236 (74.83%)</td>
<td>442 (29.13%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hectorite</td>
<td>127 (3.73%)</td>
<td>723 (4.17%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

![TGA thermal curve of nanocomposite 3.3a.](image)

Figure 3.11  TGA thermal curve of nanocomposite 3.3a.

The variations in the thermal degradation steps of nanocomposites 3.1 and 3.3 in comparison to their non-intercalated starting materials, further supports the generation of a new material. Thus, thermal gravimetric analyses of the nanocomposites are consistent with the results obtained from the XRD patterns, SEM micrographs, and FT-IR spectra, which altogether indicate that intercalation has occurred.
Nanocomposites 3.1 and 3.3 were also examined using fluorescence spectroscopy; however, the fluorophore moieties from dyes 2.4 and 3.2 exhibited no observable fluorescence emission above the background signal for both nanocomposites. This was quite unexpected and is contrary to the anticipated enhanced fluorescence. The original intent of introducing these fluorophores into the layers of hectorite was to enhance their material properties such as optical, thermal, and mechanical properties. Since these types of dyes are known to aggregate in concentrated solutions, it was predicted that their intercalation into hectorite would lead to the individual separation and enhanced rigidity of the dye molecules. This was expected to eliminate dye-dye intermolecular interactions and inhibit dimerization and/or higher aggregate formation. As previously discussed in chapter 2, the hindrance of aggregation in particular, H-aggregate formation reduces the fluorescence quantum efficiency. Therefore, it is advantageous to prepare intercalated nanomaterials containing well-separated dye molecules with the anticipation of their enhanced fluorescence.

Previously, Bissessur and Wagner reported the intercalation of fluorophores, pyrene (pyr) and 8-anilino-1-napthalenesulfonic acid (ANS) into molybdenum disulfide (MoS$_2$). Despite the successful intercalation of these fluorophores into MoS$_2$, fluorescence emission was not observed for either intercalated nanocomposites. One possible rationale offered was the presence of solid aggregates within the host layers rather than individual molecules. From this, the authors indicate that these nanocomposites would experience self-quenching of their fluorescence emission. Additionally, the authors suggested the lack of fluorescence might be caused by the strong absorption by the MoS$_2$ layers. It was postulated that very little of the excitation light was reaching the intercalated fluorophores and that any fluorescence would have been efficiently absorbed by the MoS$_2$ layer. Based on this previous work, it is speculated that these nanocomposites may have experienced self-quenching and/or that the hectorite layers may have
been too densely packed, either of which would prevented the nanocomposite from exhibiting fluorescence. Future work should be directed towards casting the nanocomposite in thin layers, with a uniform distribution. This would eliminate the possible strong absorbance of the dense hectorite layers. Additionally, self-quenching could be reduced by lowering the ratio of dye to hectorite.

3.3 Experimental

3.3.1 General considerations

All reagents were purchased from Sigma-Aldrich, Alfa Aesar, or VWR, and used without further purification. All solvents were HPLC grade from either Fisher Scientific or Sigma Aldrich and used without further purification. Purified lithium hectorite (sample # IS2-59B) was obtained from Prof. Rabin Bissessur’s research group in the Department of Chemistry at the University of Prince Edward Island. The lithium hectorite sample IS2-59B had been prepared from the lithiation of sodium hectorite via cation exchange method, which has been previously reported.\textsuperscript{156} Prior to lithiation, the crude sodium hectorite (SHCa-1), purchased from Source Clays Repository, was purified according to previously established methodology.\textsuperscript{144} Heptamethine indocyanine dye 2.4 and indolenine dye 3.2 were prepared according to previously published procedures.\textsuperscript{3,31,54,157} Deionized water from a Milli-Q system was used in all experiments.

3.3.2 Characterization

Protocols were identical to those reported in section 2.4. Infrared spectroscopy was run on a Perkin-Elmer 1600 FT-IR series instrument. Fluorescence measurements were performed
on solids in 1 mm² quartz cuvettes at 22 ±1 °C. Fluorescence spectra were measured on a Photon Technologies International LS-100 luminescence spectrometer, with excitation and emission monochromator bandpasses set at 3 nm and varying excitation wavelengths, depending on the sample. Thermogravimetric analyses (TGA) were performed on a TA Instruments TGA Q500 with a balance purge flow rate of 40 mL N₂(g)/min and a sample flow rate of 60 mL/m air. Dynamic TGA was performed; therefore, the heating rate was varied. TGA thermal curves were measured by Oliver Xu at the University of Prince Edward Island. Powder X-ray diffraction (XRD) was run on a Bruker AXS D8 Advance diffractometer equipped with a graphite monochromator. Cu Kα radiation (λ = 1.5406 Å) was utilized and the data collection was carried out in air, at room temperature, using a scan range of 2–60°. Intercalated samples were run as thin films on glass substrates. XRD patterns were measured by Michael Cowper at the University of Prince Edward Island.

3.3.3 Synthesis of intercalated nanocomposites

General procedure for the synthesis of nanocomposites 3.1 and 3.3: Heptamethine indocyanine dye 2.4 (or indolenine dye 3.2 for nanocomposites 3.3) was dissolved into deionized water. Next, an aqueous suspension of hectorite was prepared and stirred for 2.5 h. Subsequently, the aqueous suspension of hectorite was added dropwise to the aqueous solution of the dye and stirred for 2 days. The product mixture was evenly distributed into centrifuge tubes and centrifuged for 45 minutes. Subsequently, the supernatant liquid was pipetted out and the remaining sediment was freeze-dried for 2 days to remove the excess water.

3.1a. Heptamethine indocyanine dye 2.4: 0.149g (0.200 mmol) into 15 mL water; hectorite: 37.9 mg (0.100 mmol) into 3.8 mL water (0.01 g/mL).
3.1b. Heptamethine indocyanine dye 2.4: 0.288 g (0.400 mmol) into 29 mL of water; hectorite: 76.6 mg (0.200 mmol) into 15 mL of water (0.005 g/mL).

3.1c. Heptamethine indocyanine dye 2.4: 0.400 g (0.555 mmol) into 40 mL of water; hectorite: 53.1 mg (0.139 mmol) into 10.6 mL of water (0.005 g/mL).

3.3a. Indolenine dye 3.2: 73.2 mg (0.200 mmol) into 7.3 mL water; hectorite: 39.3 mg (0.100 mmol) into 7.3 mL of water (0.01 g/mL).

3.3b. Indolenine dye 3.2: 0.142 g (0.400 mmol) into 14 mL of water; hectorite: 76.6 mg (0.200 mmol) into 15 mL of water (0.005 g/mL).

3.3c. Indolenine dye 3.2: 0.169 g (0.478 mmol) into 16 mL of water; hectorite: 42.5 mg (0.111 mmol) into 4.3 mL of water (0.01 g/mL).
Chapter 4  Conclusion and Future Work

A number of novel heptamethine indocyanine dyes containing various aromatic chromophores were synthesized and characterized using primarily NMR spectroscopy and mass spectrometry. These near-infrared dyes were found to possess photoactive characteristics. The molar absorption coefficients and fluorescence quantum yields of these fluorophores were dependent on the nature of the meso-substituted thiol moieties. All of the dyes exhibited high molar absorption coefficients ranging from $1.2$ to $1.9 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$. It was observed that the dyes substituted with longer aliphatic linkers, specifically dyes 2.8 and 2.12, exhibited lower molar absorption coefficients compared to the other dyes containing either methylene or ethylene linkages (dyes 2.10, 2.11, 2.13, 2.14). Additionally, the esterified heptamethine indocyanine dyes containing various aromatic chromophores resulted in slightly red-shifted UV-vis/NIR absorption and emission spectra in comparison to their corresponding non-substituted chloro-heptamethine cyanine parent. The esterified dye derivatives’ absorption and emission wavelength maxima were relatively constant. This characteristic may be useful for applications requiring photo-active compounds that require functionalization without significantly altering the wavelength maxima.

Aggregation was not observed in the absorption spectra obtained in a methanol medium (1x10$^{-6}$ M concentration), which indicates that in these particular conditions these dyes exist primarily in the monomeric form. On the other hand, in a water medium (1x10$^{-6}$ M concentration) blue-shifted absorption bands at 788 and 790 nm are observed compared to that in methanol, which is indicative H-aggregate formation. Due to their high molar absorptivities, water-solubility and their ability to be fine-tuned, these novel functionalized heptamethine indocyanine dyes may be potential candidates for use in industrial and biological applications.
Finally, the successful preparation of novel nanocomposites through the intercalation of a heptamethine cyanine dye or indolenine dye into hectorite was investigated. These intercalated nanocomposites were prepared by utilizing the exfoliation/re-stacking properties of the hydrophilic hectorite clay. Powder X-ray diffraction analyses indicated successful intercalation of the dye-hectorite nanocomposites. The intercalation of the dye molecules into the layered lattice of hectorite led to highly ordered molecular arrangement, as confirmed from the scanning electron micrographs. Furthermore, thermogravimetric analysis indicated that some of the intercalated dye nanocomposites exhibit enhanced thermal stabilities, which is useful for industrial applications.

Future Work

Future work involves optimization of the metal-mediated nucleophilic aromatic substitution polymerization reaction conditions of polymer 2.9. Additionally other polymerization techniques may be explored including ring-opening metathesis polymerization of norbornene-containing derivatives and electropolymerization of thiophene-containing derivatives. The degree of dye aggregation and to which extent dimers or higher order aggregates form will require further investigation. As a result, future work is needed to gain insight into the degree of H-aggregation of the dyes and whether or not changes in molar absorptivities between the monomers and aggregates are solvent- and/or concentration-dependent.

The intercalated dye-clay nanocomposites reported in this study provide a baseline for future work involving the preparation of novel clay nanocomposites containing heptamethine indocyanine dyes. Thus, continuing work involving the intercalation of NIR dyes such as 2.5, 2.8, 2.10-2.14 and polymers of dyes 2.8, 2.13, and 2.14 into hectorite clay may take place. In
addition, it may be useful to prepare uniformly distributed thin films of the nanocomposites in order to examine and compare their morphological characteristics to their respective solid particulates. High resolution SEM and transmission electron microscopy (TEM) would be valuable instrumental analysis for this particular characterization. Additionally, future work that involves casting the nanocomposite in thin layers with a uniform distribution may eliminate the possible strong absorbance of the dense hectorite layers and possibly provide suitable fluorescence emission spectra of the nanocomposites.
References


Appendices

Appendix A: Supplemental information from chapter 1

*Note: All arrows in mechanism are double-headed arrows.

Figure A.1  General condensation reaction mechanism for the formation of heptamethine cyanine dyes using NIR dye 2.4 as an example
Appendix B: Spectral data of dyes in chapter 2

B.1 NMR Spectra of Dyes in Chapter 2

Figure B.1.1 $^1$H NMR spectrum of indolenine dye 2.2 (400 MHz, DMSO-$d_6$)
Dye 2.3

NIR dye 2.4

Figure B.1.2 $^1$H NMR spectrum of polymethine dye 2.3 (400 MHz, DMSO-$d_6$)

Figure B.1.3 $^1$H NMR spectrum of NIR dye 2.4 (400 MHz, DMSO-$d_6$)
Figure B.1.4  $^{13}$C NMR spectrum of NIR dye 2.4 (101 MHz, DMSO-$d_6$)

Figure B.1.5  gdqCOSY NMR spectrum of NIR dye 2.4 (400 MHz, DMSO-$d_6$)
Figure B.1.6 gHSQC NMR spectrum of NIR dye 2.4 (400 MHz, DMSO-\textit{d}_6)

NIR dye 2.5

Figure B.1.7 \textsuperscript{1}H NMR spectrum of NIR dye 2.5 (400 MHz, DMSO-\textit{d}_6)
Figure B.1.8 $^{13}$C NMR spectrum of NIR dye 2.5 (101 MHz, DMSO-$d_6$)

Figure B.1.9 gdqCOSY NMR spectrum of NIR dye 2.5 (400 MHz, DMSO-$d_6$)
Figure B.1.10  TOCSY 2-D NMR spectrum of NIR dye 2.5 (400 MHz, DMSO-$d_6$)

Figure B.1.11  gHSQC NMR spectrum of NIR dye 2.5 (400 MHz, DMSO-$d_6$)
Figure B.1.12  gHMBC NMR spectrum of NIR dye 2.5 (400 MHz, DMSO-d$_6$)

Complex 2.7

Figure B.1.13  $^1$H NMR spectrum of bimetallic complex 2.7 (400 MHz, DMSO-d$_6$)
Figure B.1.14  $^{13}$C NMR spectrum of bimetallic complex 2.7 (101 MHz, DMSO-$d_6$)

Figure B.1.15  gdqCOSY NMR spectrum of bimetallic complex 2.7 (400 MHz, DMSO-$d_6$)
Figure B.1.16  
\[ \text{gHSQC NMR spectrum of bimetallic complex 2.7 (400 MHz, DMSO-}d_6) \]

NIR dye 2.8

Figure B.1.17  
\[ \text{\(^1\)H NMR spectrum of NIR dye 2.8 (400 MHz, DMSO-}d_6) \]
Figure B.1.18  $^{13}$C NMR spectrum of NIR dye 2.8 (101 MHz, DMSO-$d_6$)

Figure B.1.19  gdqCOSY NMR spectrum of NIR dye 2.8 (400 MHz, DMSO-$d_6$)
Figure B.1.20  gHSQC NMR spectrum of NIR dye 2.8 (400 MHz, DMSO-$d_6$)
Figure B.1.21  gHMBC NMR spectrum of NIR dye 2.8 (400 MHz, DMSO-\textit{d}_6)

Figure B.1.22  $^1$H NMR spectrum of polymer 2.9 (400 MHz, DMSO-\textit{d}_6)
NIR dye 2.10

Figure B.1.23  $^1$H NMR spectrum of NIR dye 2.10 (400 MHz, DMSO-d$_6$)

Figure B.1.24  $^{13}$C NMR spectrum of NIR dye 2.10 (101 MHz, DMSO-d$_6$)
Figure B.1.25  gdqCOSY NMR spectrum of NIR dye 2.10 (400 MHz, DMSO-$d_6$)

Figure B.1.26  gHSQC NMR spectrum of NIR dye 2.10 (400 MHz, DMSO-$d_6$)
Figure B.1.27  gHMBC NMR spectrum of NIR dye 2.10 (400 MHz, DMSO-$d_6$)

Figure B.1.28  $^1$H NMR spectrum of NIR dye 2.11 (400 MHz, DMSO-$d_6$)
Figure B.1.29 $^{13}$C NMR spectrum of NIR dye 2.11 (101 MHz, DMSO-$d_6$)

Figure B.1.30 gdqCOSY NMR spectrum of NIR dye 2.11 (400 MHz, DMSO-$d_6$)
Figure B.1.31  gHSQC NMR spectrum of NIR dye 2.11 (400 MHz, DMSO-$d_6$)

Figure B.1.32  gHMBC NMR spectrum of NIR dye 2.11 (400 MHz, DMSO-$d_6$)
Figure B.1.33 $^1$H NMR spectrum of 1-pyrenemethanol (400 MHz, DMSO-$d_6$)

Figure B.1.34 $^{13}$C NMR spectrum of 1-pyrenemethanol (101 MHz, DMSO-$d_6$)

Figure B.1.35 $^1$H NMR spectrum of NIR dye 2.12 (400 MHz, DMSO-$d_6$)
Figure B.1.36  $^{13}$C NMR spectrum of NIR dye 2.12 (101 MHz, DMSO-$d_6$)

Figure B.1.37  gdqCOSY NMR spectrum of NIR dye 2.12 (400 MHz, DMSO-$d_6$)
Figure B.1.38  gHSQC NMR spectrum of NIR dye 2.12 (400 MHz, DMSO-$d_6$)

Figure B.1.39  gHMBC NMR spectrum of NIR dye 2.12 (400 MHz, DMSO-$d_6$)
Figure B.1.40 $^1$H NMR spectrum of NIR dye 2.13 (400 MHz, DMSO-$d_6$)

Figure B.1.41 $^{13}$C NMR spectrum of NIR dye 2.13 (101 MHz, DMSO-$d_6$)
Figure B.1.42  gdqCOSY NMR spectrum of NIR dye 2.13 (400 MHz, DMSO-$d_6$)

Figure B.1.43  gHSQC NMR spectrum of NIR dye 2.13 (400 MHz, DMSO-$d_6$)
Figure B.1.44  gHMBC NMR spectrum of NIR dye 2.13 (400 MHz, DMSO-$d_6$)

NIR dye 2.14

Figure B.1.45  $^1$H NMR spectrum of NIR dye 2.14 (400 MHz, DMSO-$d_6$)
Figure B.1.46  $^{13}$C NMR spectrum of NIR dye 2.14 (101 MHz, DMSO-$d_6$)

Figure B.1.47  gdqCOSY NMR spectrum of NIR dye 2.14 (400 MHz, DMSO-$d_6$)
Figure B.1.48  gHSQC NMR spectrum of NIR dye 2.14 (400 MHz, DMSO-$d_6$)

Figure B.1.49  gHMBC NMR spectrum of NIR dye 2.14 (400 MHz, DMSO-$d_6$)
**Figure B.1.50**  
$^1$H NMR spectrum of NIR dye 2.15 (400 MHz, DMSO-$d_6$)

NIR dye 2.15

**Figure B.1.51**  
$^{13}$C NMR spectrum of NIR dye 2.15 (101 MHz, DMSO-$d_6$)
Figure B.1.52  gdqCOSY NMR spectrum of NIR dye 2.15 (400 MHz, DMSO-$d_6$)

Figure B.1.53  gHSQC NMR spectrum of NIR dye 2.15 (400 MHz, DMSO-$d_6$)
Figure B.1.54  gHMBC NMR spectrum of NIR dye 2.15 (400 MHz, DMSO-\textit{d}_6)
B.2 FT-IR ATR spectra of NIR heptamethine cyanine dyes in chapter 2 in the solid state

Figure B.2.1 FT-IR ATR spectrum of NIR dye 2.5

Figure B.2.2 FT-IR ATR spectrum of NIR dye 2.8
Figure B.2.3  FT-IR ATR spectrum of NIR dye 2.10

Figure B.2.4  FT-IR ATR spectrum of NIR dye 2.11
Figure B.2.5  FT-IR ATR spectrum of NIR dye 2.12

Figure B.2.6  FT-IR ATR spectrum of NIR dye 2.13
Figure B.2.7  FT-IR ATR spectrum of NIR dye 2.14
B.3 Electrospray ionization mass spectra of NIR dyes in chapter 2

Figure B.3.1 ESI mass spectrum of NIR dye 2.10

Chemical formula: C_{58}H_{57}N_{2}NaO_{8}S_{3}
Exact mass: 1028.32
Figure B.3.2  ESI mass spectrum of NIR dye 2.11

Chemical formula: $C_{60}H_{57}N_2NaO_8S_3$
Exact mass: 1052.32
Figure B.3.3  ESI mass spectrum of NIR dye 2.12

Chemical formula: $C_{63}H_{63}N_2NaO_8S_3$
Exact mass: 1094.36
Figure B.3.4  ESI mass spectrum of NIR dye 2.13
Figure B.3.5 ESI mass spectrum of NIR dye 2.14

Chemical formula: $C_{49}H_{53}N_2NaO_8S_4$
Exact mass: 948.26
B.4 Absorbance and emission spectra of NIR dyes in chapter 2

Figure B.4.1 Absorbance and emission spectra of NIR dye 2.5

Figure B.4.2 Absorbance and emission spectra of NIR dye 2.8
Figure B.4.3  Absorbance and emission spectra of NIR dye 2.10

Figure B.4.4  Absorbance and emission spectra of NIR dye 2.11
Figure B.4.5  Absorbance and emission spectra of NIR dye 2.12

Figure B.4.6  Absorbance and emission spectra of NIR dye 2.13
Figure B.4.7  Absorbance and emission spectra of NIR dye 2.14
Appendix C: Characterization data of intercalation compounds in chapter 3

C.1 Powder X-ray diffraction patterns not shown in chapter 3

![Graph of Powder X-ray diffraction pattern of nanocomposite 3.1b](image1)

**Figure C.1.1** Powder X-ray diffraction pattern of nanocomposite 3.1b

![Graph of Powder X-ray diffraction pattern of nanocomposite 3.1c](image2)

**Figure C.1.2** Powder X-ray diffraction pattern of nanocomposite 3.1c
Figure C.1.3  Powder X-ray diffraction pattern of nanocomposite 3.3a

Figure C.1.4  Powder X-ray diffraction pattern of nanocomposite 3.3c
C.2 FT-IR ATR spectrum of NIR dye not shown in chapter 3

Figure C.2.1 FT-IR ATR spectrum of heptamethine cyanine dye 2.4