Unsymmetrical Trimethine Cyanine Dyes: Synthesis, Optical Properties, and Evaluations as Inhibitors of Protein Arginine Methyl Transferases

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UNSMMETRICAL TRIMETHINE CYANINE DYES: SYNTHESIS, OPTICAL PROPERTIES, AND EVALUATION AS INHIBITORS OF PROTEIN ARGinine METHYL TRANSFERASES

by

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Under the Direction of Professor Maged M. Henary

ABSTRACT

Carbocyanine dyes are a class of organic compounds that possess two nitrogen containing heterocycles that act as electron donors and acceptors connected by a conjugated methine bridge. This thesis will present the synthetic methodology of symmetrical and unsymmetrical trimethine cyanine dyes in three chapters. The first chapter is a review on the synthesis and application of unsymmetrical cyanine dyes. The second will describe the synthesis of unsymmetrical trimethine cyanine dyes and how their optical properties differ from symmetrical dyes. The third chapter will not only discuss the synthetic procedure for synthesis of symmetrical trimethine cyanine dyes, but also will show how varying the N-alkyl substituents and hydrophobicity of the heterocycles affects the dyes interaction with and ability to be used as inhibitors for protein arginine methyltransferases (PRMTs). Several synthesized compounds have displayed lower IC₅₀ values for the inhibition of PRMT1 and PRMT5 comparable to that of current inhibitors.

INDEX WORDS: Cyanine Dye, Synthesis, Absorption, Fluorescence, Quantum yield, Stokes Shift, Lipinski Rule of Five, PRMT, IC₅₀, Imaging
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DEDICATION

This thesis is dedicated to my parents, Jeff and Ellen Levitz, for their support and guidance since day one.
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1 SYNTHESIS AND APPLICATIONS OF UNSYMMETRICAL CYANINE DYES, A REVIEW

1.1 Introduction to Cyanine Dyes

In 1856, C. H. G. Williams synthesized the first cyanine dye by heating quinoline with \(N\)-amyl lepidinium iodide in ammonia. The compound produced displayed a “magnificent blue color,” thus the Latin word cyanos, meaning blue, provided the general cyanine dye name.\(^1\) This vast class of dyes shows absorption that covers a wider range of the electronic spectrum, from the ultraviolet to the infrared, than any other class of dyes. Due to their extreme sensitivity to light, the inconceivable value of cyanine dyes was not discovered until almost 20 years later when H. W. Vogel used them to increase sensitivity of the photographic plate.\(^2\) Since then cyanine dyes have been used in an incredible amount of applications including but not limited to laser printing,\(^3\) pH sensors,\(^4\) fluorescence imaging in vivo,\(^5\) data storage,\(^6\) labels for nucleic acid detection,\(^7\)\(^8\) and medicine.\(^10\)

Cyanine dyes are distinguished from other dyes in that they possess two nitrogen containing heterocycles that are connected by a conjugated methine bridge as shown by the general structure in Figure 1.\(^11\)\(^-\)\(^14\)

![Figure 1. General Structure of Cyanine Dyes](image)

Their names depend on the how many methine groups are found in the bridge connecting the two heterocycles. Dye 1 represents the general structure of cyanine dyes. Dyes containing \(n = 0, 1, 2,\) and 3, are classified as mono-, tri-, penta-, and hepta-, respectively. There are multiple well described routes to synthesis of monomethine and trimethine cyanines,\(^15\) but pentamethine and heptamethine
cyanines have one general route. This route is through the condensation of methylsubstituted quaternized heterocyclic compounds with an α,ω dialdehyde or equivalent.\textsuperscript{13,15-18}

Their narrow absorption bands and high extinction coefficients are important properties that have allowed them to be used in various applications over the last 140 years. Monomethine and trimethine cyanines typically absorb in the 500-600 nm range of the electronic spectrum and the addition of each double bond to the methine chain causes a bathochromic shift of about 100 nm resulting in absorption of 600-700 nm for pentamethine, and 700-800 nm for heptamethine cyanines, well into the infrared region.

One synthesis route for unsymmetrical dyes is to condense two different quaternary ammonium salts with the polymethine chain linker in one pot. This route involves a difficult separation of two symmetrical dyes from the unsymmetrical dye which are all very similar. There are numerous synthetic routes for the synthesis of unsymmetrical monomethine cyanine dyes, but only two main routes for unsymmetrical trimethine, pentamethine, and heptamethine cyanines. These routes are known as the aldehyde and hemicyanine methods and have less difficult purifications than the one pot method condensation of two salts with linker. In addition a thioether method can be used to synthesize trimethine cyanines with a methyl group at the meso-carbon. This chapter will explore the synthesis of these unsymmetrical dyes with less difficult purification.
1.2 Synthesis of Monomethine Cyanine Dyes

Monomethine cyanine dyes typically absorb in the visible region. This is the most common type of unsymmetrical cyanine dye as they are the easiest type of unsymmetrical cyanine dye to synthesize. The majority of monomethine dyes are unsymmetrical as the synthesis is based on the condensation of two quaternary ammonium salts and there is no methine linker used. Unsymmetrical monomethine cyanines are the best non-covalent binding nucleic acid labels due to their very high binding constants, large molar absorptivities and quantum yields, and the generation of high fluorescence signals upon binding.\(^{19}\) Two commercially available unsymmetrical monomethine cyanines based on the oxazole yellow structure, YO-PRO-1 and YOYO-1 shown in Figure 3, are among the most popular intercalating dyes and have recently been used for DNA and chromatin imaging with super-resolution fluorescence microscopy.\(^{20}\)

![YO-PRO-1](image)

![YOYO-1](image)

**Figure 3. Commercially Available Unsymmetrical Monomethine Cyanine Dyes**

One synthesis route to monomethine cyanines is through the heating of sulfobetaines derived from N-alkylheterocyclic compounds and a quaternary salt of 2- or 4-methyl compounds under basic conditions as shown in Equation 1.\(^{21}\)
The addition of the methyl to compounds 5a-d increased yields from 21% in 2 hours using traditional approaches to 59% in 18 min with the use of 252 W microwave radiation (Equation 3).

Equation 1. Synthesis of Monomethine Cyanine Dyes Utilizing Sulfobetaines

A second approach to the synthesis of monocyanines involves the reaction of a ketone such as 7-hydroxy-4-methyl(\(H\))coumarin with a 2- or 4-methyl quaternary salt in the presence of a piperidine catalyst as shown in Equation 2.

Equation 2. Synthesis of Monomethine Cyanine Dyes Utilizing Ketones

These compounds 7a-d are effective antibiotics against Bacillus stearothermophilus, Serratia species, and Pseudomonas species; however these novel dyes are ineffective toward fungal infections. The addition of the methyl to compounds 7c and 7d has been shown to increase antimicrobial activity compared to other compounds tested.

Previously the synthesis of monomethine cyanine dyes was reported in low yields, but recently Yi Le Fu et al. have described a simple microwave assisted and solvent free preparation using the thioether method, a common approach to the synthesis of monomethine dyes. This process has increased yields from 21% in 2 hours using traditional approaches to 59% in 18 min with the use of 252 W microwave radiation (Equation 3).
The thioether method used for microwave synthesis was modified by Deligiorev et al. to synthesize a neutral monomethine 13 cyanine using an environmentally friendly melt method as shown in Equation 4.\(^\text{24}\)

An example of the reaction performed by Zanotti et al. using the thioether method to condense benzoxazole salts with benzothiazole and pyridinium salts to form monomethine dyes is shown in Equation 6. These dyes bind both yeast cell surface-displayed and soluble single-chain variable antibody fragments with nanomolar \(K_d\) values.\(^\text{26}\)
1.3 Synthesis of Tri-, Penta-, and Hepta-methine Cyanine Dyes

The synthesis of tri-, penta-, and hepta-methine unsymmetrical cyanine dyes is performed through two main synthesis routes that are very similar. The aldehyde method involves the addition of a short chain with an aldehyde to a quaternary ammonium salt for reaction with a second salt to form unsymmetrical dye. The hemicyanine method involves the synthesis of hemicyanine or “half dye” and its purification from any dye that forms. Hemicyanines are sometimes hydrolyzed into aldehydes for reaction with the second salt to prevent symmetrical dye formation. The thioether method that has been used for synthesis of monomethine dyes has been modified to synthesize trimethine cyanines containing a meso-methyl group and could be utilized for unsymmetrical synthesis.

1.3.1 Thioether Method for Trimethine Cyanine Dyes

Although uncommon, the thioether that is used for monomethine dye synthesis can also be used for trimethine cyanine dyes. This route has been described by Brooker et al. for the synthesis of symmetrical trimethine cyanine dyes with a methyl substitution on the meso carbon, but could also be used to form unsymmetrical dyes as shown in Equation 8.
In addition the meso methyl group in 22 can be condensed with aldehydes, such as 2,3,6,7-tetrahydrobenzo[i]quinolizine-9-carboxaldehyde 23 under basic conditions to furnish meso-substituted unsymmetrical trimethine cyanines as shown in Equation 9.

Equation 8. Synthesis of Meso-substituted Trimethine Cyanine Dyes

1.3.2 The Aldehyde Method

The aldehyde method has been used for the synthesis of trimethine, pentamethine and heptamethine cyanine dyes. The process involves the addition of a short chain with a terminal aldehyde to the quaternary ammonium salt which can then be reacted with a second salt to form unsymmetrical dye. The formation of the aldehyde is the key step in the synthesis of unsymmetrical cyanine dyes using this method. A pure aldehyde will allow only one product to be formed during the dye condensation making for much easier purification. There are a few different versions for trimethine and pentamethine cyanines, but only one for heptamethine.

For trimethine cyanine dyes, the most common version of the aldehyde method involves the use of a Vilsmeier reagent formed through the reaction of phosphorous oxychloride with N,N-dimethylformamide at 0°C. This aldehyde is then reacted with a quaternary ammonium salt to form dye.\textsuperscript{28-30}
Another aldehyde method for trimethine cyanine dyes was described by Meguellati, et al. In this method, the quaternary ammonium salt 29 is first converted to a Fischer base 30 under basic conditions. The Fischer base 30 is then reacted with (chloromethylene)dimethylammonium chloride in dichloromethane followed by dissolution in aqueous K$_2$CO$_3$ solution to form the aldehyde 31 as seen in Scheme 2. The ester was cleaved to a carboxylic acid for attachment of 34 to one strand of peptide nucleic acid (PNA) while the Fischer base 32 was attached to another strand of PNA. When the two strands combined to form G-quadruplex DNA dye 33 formed and fluorescence could be detected.\textsuperscript{31}

\textbf{Scheme 2. Trimethine Cyanine Dye Synthesized Upon Formation of G-Quadruplex DNA}\textsuperscript{31}
One of the most common purposes for the synthesis of unsymmetric trimethine cyanine dyes is for conjugation to one side of the dye. Hua et al. have utilized the aldehyde method to synthesize numerous trimethine cyanine dyes for use in solar cells. Through Sonogashira coupling, the halogen on the aldehyde 35 is converted to a trimethylsilane protected alkyne 36. This alkyne is then deprotected.

Scheme 3. Synthesis of Starburst-Triphenylamine and Fluoranthene-based Trimethine Cyanine Dyes

Dyes 32,33
and a second Sonogashira coupling reaction is performed to attach a polycyclic aromatic system. The dye $39$ is then formed by condensation of the aldehyde $38$ with a quaternary ammonium salt to form a dye sensitized solar cell. In 2009, starburst triphenylamine-based dyes $39a$ were synthesized for use as quasi-solid-state dye-sensitized solar cells. A starburst triphenylamine group was added as an electron donor through “click chemistry” improving photovoltaic performance. In addition, this group may inhibit aggregation, thereby increasing stability of the solar cells. The same chemistry, shown in Scheme 3, was utilized to synthesize fluoranthene-based cyanine dyes $39b$.

1.3.2.1 Synthesis of Penta- and Hepta-methine Cyanine Dyes Using the Aldehyde Method

Pentamethine cyanines typically absorb in the visible to near infrared region and are usually blue in color. The aldehyde methods for pentamethine cyanine dyes are similar to those of trimethine cyanines. One route, described by Peng et al. is to react quaternary ammonium salt $42$ with $1,1,3,3$-tetrethoxypropane $43$ in the presence of hydrochloric acid to form aldehyde $44$ as shown in Scheme 4. This aldehyde $44$ was synthesized for the purpose of imaging cellular viscosity and shows a 10-fold fluorescence increase in glycerol compared to ethanol.

![Scheme 4. Synthesis of Unsymmetric Pentamethine Cyanine Dyes Utilizing the Tetraethoxypropane Aldehyde Method](image)

Briks et al. have described aldehyde methods for pentamethine and heptamethine that are similar to the formation of a Vilsmeier reagent for trimethine cyanine dyes using chemicals analogous to
DMF but with longer chains. A dimethylamino derivative of the quaternary ammonium salt 47a and 47b is formed by reaction of the salt with 3,3-dimethylaminoacrolein and 1-(dimethylamino)-5-formyl-1,3-butadiene for pentamethine and heptamethine, respectively. The dimethylamino derivative 47 is then converted to an aldehyde 48 in a sodium hydroxide solution as shown in Scheme 5. Dyes 49a and 49b are then formed by condensing aldehydes 48a and 48b with a second salt in acetic anhydride to form penta- and hepta-methine cyanines respectively.

1.3.3 Hemicyanine Method

Another process used to synthesize tri-, penta- and hepta-methine cyanine dyes is the hemicyanine method. This process is much like the one used to synthesize symmetrical cyanines dyes, except that the reaction is performed at a lower temperature, for a shorter time, and a different ratio of salt to reagent is used. Synthesis of symmetrical dyes is carried out in one step where the quaternary ammoni-
um salt is used in a 2:1 ratio to the linker which is \(N,N\)-diphenylformamidine, malonaldehyde dianil hydrochloride or glutacondianil hydrochloride for tri-, penta- and hepta-methine cyanines respectively. For unsymmetrical cyanine dyes, a multistep process beginning with the reaction of quaternary ammonium salt with linker in a 1:1 ratio is carried out. This key step forms a hemicyanine or “half dye”. The problem with this method is preventing the formation of dye which is usually accomplished by carrying out the first step for a short period of time at lower temperatures than what are used for dye formation. Following this procedure the trimethine cyanine dye 52 was synthesized in 50% yield as described in Scheme 6.\(^{28}\)

Gerowska et al. utilized this process to synthesize unsymmetric trimethine cyanines with a halogen on one side. As shown previously in Scheme 3, through Sonogashira coupling, the halogen was converted to a triple bond for click labeling of azide nucleotides. Click labeled probes such as the one formed from dye 52 have been shown to perform better than probes labeled with esters of cyanine dyes, possibly due to the shorter linker between the dye and DNA.\(^{28}\)

![Scheme 6. Synthesis of Unsymmetric Trimethine Cyanine Dyes Utilizing Hemicyanine Method\(^{28}\)](image)

Ge et al. employed the hemicyanine method to synthesize a number of unsymmetric trimethine cyanine dyes 53a-d containing both benzoxazole and benzothiazole groups, seen in Figure 4, and showing \emph{in vitro} antiprotozoal activity against \emph{P. falciparum} and \emph{T. cruzi}. Symmetric dyes containing only benzoxazole groups were not as active against the protozoa revealing that the benzothiazole moieties are important for antiprotozoal activity.\(^{29}\)
Figure 4. Unsymmetric Trimethine Cyanine Dyes Showing Antiprotozoal Activity

Song et al. performed the hemicyanine method to design dyes 56 containing one carboxylic acid group for bioconjugation as shown in Scheme 7. The carboxylic acid was then converted to the active NHS ester for protein labeling on bovine serum albumin (BSA). These novel unsymmetric cyanines were shown to have better labeling performance due to the hydrophobic groups on one end and hydrophilic sulfo-groups on the other end.

Scheme 7. Synthesis of Unsymmetric Trimethine Cyanine Dye for Bovine Serum Albumin Labeling

Pentamethine and heptamethine cyanines undergo the hemicyanine method similarly to trimethine cyanines. Rather than N,N-diphenylformamide, malonaldehyde dianil hydrochloride and glutarodianil hydrochloride are used for the formation of pentamethine and heptamethine hemicyanines, respectively, as shown in Scheme 8. Jung et al. synthesized pentamethine dye 59a. This dye was then activated with N-hydroxysuccinamide to form the NHS activated dye to be used for difference gel electrophoresis and found it to be indistinguishable from commercially available dyes used for this purpose and stable in N,N-dimethylformamide for years.
Scheme 8. Synthesis of Unsymmetrical Penta- and Hepta- methine Cyanine Dyes Utilizing the Hemicyanine Method\textsuperscript{37}

While synthesis of unsymmetric trimethine cyanine dyes is becoming more prevalent, the synthesis of unsymmetric penta- and hepta-methine cyanine dyes is still uncommon, particularly heptamethine cyanines. Wang \textit{et al.} utilized the hemicyanine method to synthesize a series of pentamethine cyanine dyes (Scheme 9) for BSA labeling similar to the dyes 56 synthesized by Song \textit{et al.} for the same purpose.\textsuperscript{38} In this preparation quaternary ammonium salt 54 was refluxed with malonaldehyde dianil hydrochloride in a mixture of acetic anhydride and acetic acid. After purification by column chromatography, the hemicyanine intermediate 60 was heated with a second quaternary ammonium salt at 120°C for 40 min. The dyes 61\textit{a-c} were then purified by reverse phase column chromatography using a methanol/water mixture as the eluent.
1.3.4 Microwave Assisted Solid-Phase Synthesis

As microwave synthesis becomes more popular due to the ability to avoid time-consuming reaction steps and increase purity, Lopalco et al. designed a solid-phase synthesis approach to the hemicyanine method for tri-, penta-, and hepta-methine cyanines using microwave assisted synthesis. As shown in Scheme 10, this solid-phase method first reduces reaction time by synthesizing quaternary ammonium salts at 150°C in acetonitrile. Next, the alkylated indolenines are reacted with resin bound polymethine imines 63a-c in a step accelerated by microwave irradiation. The resin allows for any symmetrical impurity to be cleaved before the second carboxylated indolium salt was added. The resin
bound hemicyanines 64 are then reacted with another quaternary ammonium salt synthesizing dyes 65 which are no longer resin bound.

Scheme 10. Solid-Phase Synthesis of Unsymmetrical Cyanine Dyes

1.4 Conclusion

Cyanine dyes are highly modifiable compounds that have wide-ranging applications from cancer imaging to nucleic acid detection. They are generally non-toxic, stable and exhibit exceptional biocompatibility making their cellular use tremendously appealing. Due to the diverse functionality associated with them a large number have cyanines have been synthesized for various applications.
2 SYNTHESIS AND OPTICAL PROPERTIES OF UNSYMMETRICAL TRIMETHINE CYANINE DYES

2.1 Introduction

The majority of cyanine dyes are symmetrical in that the nitrogen containing heterocycles on both sides of the methine chain and the alkyl groups connected to the nitrogens are the same. Until the mid-1900s, it was widely accepted that an unsymmetrical cyanine dye would absorb halfway between the absorption of the parent symmetrical dyes.\textsuperscript{27} Brooker \textit{et al.} showed that if the basicities of the nitrogen containing heterocycles are not identical, or if the relative stabilities of the two forms are not different, the absorption would not be at the midpoint.\textsuperscript{27} If the basicity of the two nuclei is not equal the absorption should be found at a shorter wavelength than the intermediate position. As the difference in basicity is continually increased, the absorption maximum gets further from the midpoint, and this deviation increases with an increasing length of the polymethine chain.\textsuperscript{27} Although many monomethine unsymmetrical cyanine dyes have been made, there is a lack in the literature of trimethine cyanine dyes with indolenine moieties and those with substitutions on the benzene ring of the indolenine are sporadic.

When designing new dyes for various applications, it is important to understand how altering the substituents on the heterocycles affects the optical properties of the dyes. These changes can be made to improve solubility or increase binding and permeability, but structural modifications also cause changes in absorption and fluorescence. Herein we report the synthesis of a series of unsymmetrical trimethine cyanine dyes made to determine how these substitutions affect the optical properties of the dyes. Molar absorptivities (ε) will be calculated using the Beer-Lambert law and fluorescence quantum yields (φ) will be determined by a relative method.\textsuperscript{45} Stokes shift and absorption maxima will be compared to those of the symmetrical dyes.

A common application of cyanine dyes is for imaging and labeling \textit{in vivo}. Lipinski’s Rule of Five is used to evaluate if a chemical compound has properties that would make it orally active in humans.\textsuperscript{46}
These properties include molecular weight, hydrogen bond donors and acceptors, a partition coefficient, and polar surface area.\textsuperscript{47} Partition coefficients, such as $\log P$, are ratios of concentrations of a compound in a mixture of two immiscible phases. $\log D$ is much like $\log P$, but it also takes into account positions that can be fully protonated or deprotonated such as sulfonate or carboxylate groups. Based on $\log D$, it can be speculated as to where a compound will distribute in biological tissues. The Lipinski Rule of Five properties of each dye were investigated and the $\log D$ value of each dye was predicted to give a better idea of how each substituent affects partitioning of the dye and where it will distribute in biological tissues.

2.2 Methods

2.2.1 Chemicals and Instruments

Most reagents were purchased from Sigma-Aldrich and were used without purification. Absorption spectra were recorded on a Cary 3G UV-Visible Spectrophotometer (Santa Clara, CA) in DMSO for dyes and 0.1 M NaOH/water solution for fluorescein standard using VWR disposable two-sided polystyrene cuvettes. Fluorescence (LIF) emission analyses were performed using a K2 Multifrequency Phase Spectrofluorometer (ISS Inc., Champaign, IL) in DMSO for dyes and 0.1 M NaOH/water solution for fluorescein standard using Sigma-Aldrich disposable polystyrene fluorimeter cuvettes. Excitation was achieved with a 300 watt Excelitas Short Arc Xenon Lamp (Fremont, CA) at 645 nm. Slit widths were set to 2 mm and integration time of 3 seconds. Microsoft Excel 2010 was used for all calculations.

2.2.2 Stock Solutions

Stock solutions of the dyes and standard were prepared by weighing the solid on a 5-digit analytical balance in an amber vial and adding DMSO or 0.1 M NaOH solution via a class A volumetric pipette. The vials were vortexed for 20 seconds and then sonicated for 5 minutes to ensure complete dissolution. The stock solutions were stored in a dark freezer at 4°C when not in use.
2.2.3 Method of Determining Molar Absorptivity and Fluorescence Quantum Yield

Stock solutions were used to prepare six dilutions of dyes and the standard with concentrations ranging from 2 µM-6 µM using a class A volumetric pipette in order to maintain absorption between 0.1 and 1.0. The dye solutions were diluted ten-fold for fluorescence in order to minimize inner filter effect. For the standard, fluorescence concentrations were 0.02-0.06 µM and the area under the curve was then multiplied by 10. The absorbance spectrum of each sample was measured in triplicate using the Cary 3G UV-Visible Spectrophotometer from 400-800 nm. The spectra were then normalized at 400 nm. The emission spectrum of each sample was measured in triplicate using the ISS K2 spectrofluorometer with a 465 nm excitation wavelength ($\lambda_{\text{exc}}$).

For molar absorptivity, the absorbance at the wavelength of maximum absorbance ($\lambda_{\text{max}}$) was determined and the absorbance of each sample at $\lambda_{\text{max}}$ was plotted as a function of dye concentration ($C$). The linear regression equation was computed using Microsoft Excel software version 14.

The fluorescence quantum yields were determined relative to the fluorescein standard utilizing the gradient from the plot of integrated fluorescence intensity versus absorbance (Grad), refractive index ($\eta$) and the published quantum yield of the standard ($\phi_S$), as per Equation 9. In this equation, the subscripts S and D refer to the standards and the dyes, respectively.

$$\phi_D = \phi_S \cdot \text{Grad}_D / \text{Grad}_S \cdot \eta_D^2 / \eta_S^2$$

Equation 9. Quantum Yield Equation

2.2.4 Hydrophobicity Studies

Hydrophobic characteristics of each dye were assessed by acquiring absorbance spectra in varying ratios of methanol-buffer mixture (in the range of 2-100% methanol) using phosphate buffer pH 7.4. The concentration of methanol was slowly increased until there was no apparent changes at which point the spectra overlapped.
2.2.5 **Method of Predicting Lipinski Rule Violations**

Molecular weight was calculated without the counterion. Hydrogen bond donors and acceptors and \( \log D \) were predicted using MarvinSketch 5.9 (ChemAxon, Budapest, Hungary). Predicted \( \log D \) values were derived from the calculations by Viswanadhan,\(^{48}\) Klopman,\(^{49}\) and the PHYSPROP database at pH 7.4 and the three were averaged.

2.3 **Results and discussion**

2.3.1 **Synthesis of Unsymmetric Trimethine Cyanine Dyes**

![Scheme 11. Synthesis of Unsymmetrical Trimethine Cyanine Dyes](image)
As shown in Scheme 1, the synthesis of quaternary indolium salts 70 began with a Fischer indole synthesis by reaction of 4-substituted phenylhydrazines with 3-methyl-2-butanone in boiling glacial acetic acid. 2-methylbenzothiazole, 2-methylbenzoxazole, and 1,1,2-trimethylbenz[e]indole 70e were purchased from Sigma-Aldrich. The substituted indolines 70a-e and benzothiazoles (the dimethyl groups in 70 are replaced by a sulfur atom) were quaternized through $S_{N}2$ reaction with alkyl halides in boiling acetonitrile to form salts 71 and 72. Benz[c,d]indole quaternary ammonium salts were synthesized as previously described by our lab to form salts 73 and 77. The key step in the formation of unsymmetrical trimethine cyanine dyes is the formation of the aldehyde 69 through a Vilsmeier reaction.

The aldehyde is then reacted in acetic anhydride with each quaternary ammonium salt for one hour at 100°C to yield 13 novel unsymmetrical dyes 74-76 and 79 as shown in Scheme 11 and Equation 10. Efficient synthesis yields pure dyes, but those needing purification were purified by column chromatography using various methanol/dichloromethane ratios (1-5% methanol in dichloromethane). Dyes with multiple charges 74a,b,j tend to have lower yields due to the use of reverse phase column chromatography for purification because the dyes have positive and/or negative charges that can make them to stick to the column.

Compound 75c was chosen as a representative for complete chemical characterization and analysis. $^1$H NMR spectra of compound 75c measured in DMSO-$d_6$ can be seen in Figure 5. The triplet that is not fully resolved at 8.04 ppm corresponds to the meso-proton $H_{i}$ of the methine chain. This is known because the $J$ coupling constant of this proton and the doublets at 6.81 ppm, $H_{g}$, and 6.29 ppm,
$H_e$ is 12.8 Hz. $H_g$ corresponds to the proton further downfield as it is closer to the sulfur atom. The coupling constant above 12 suggests that the bonds in the methine chain all show trans character. The doublet at 8.09 ppm corresponds to $H_h$ as it is the closest to the sulfur. The doublet at 7.90 ppm corresponds to $H_h$ as it is close to a nitrogen atom, but also close to the sulfur. The triplets at 7.64 ppm and 7.51 ppm are the final two from the phenyl ring of the benzothiazole heterocycle corresponding to $H_j$ and $H_k$, respectively. Looking at the phenyl ring of the indolenine heterocycle, the doublet at 7.59 ppm corresponds to $H_a$ as it has the most influence from the nitrogen atom. The multiplet at 7.39 ppm corresponds to $H_b$ and $H_d$, and the triplet at 7.22 ppm corresponds to $H_c$ as it is the furthest triplet from any polar atoms. The singlet at 3.56 corresponds to the protons on the methyl side chain, and the singlet at 1.65 ppm corresponds to the dimethyl groups on the indolenine heterocycle. The signals at 4.44 ppm, 1.75 ppm, and 1.45 ppm correspond to the protons of the three internal carbons on the butyl side chain and can be specified as closest, middle and furthest from the nitrogen, respectively, based on their splitting patterns. The triplet at 0.95 ppm corresponds to the protons on the terminal carbon of the butyl side chain.
**Figure 5.** $^1$H NMR spectrum of 75c in DMSO-$d_6$ at 25°C

$^{13}$C NMR spectrum of 75c measured in DMSO-$d_6$ shows all 6 aliphatic carbons below 50 ppm as seen in Figure 6. Of the 18 other expected peaks, 16 are seen. The remaining two would are most likely ipso carbons and would need more scans to show up. Liquid chromatography coupled with a UV detector as depicted in Figure 7 shows one peak meaning the compound is very pure. High resolution mass spectrometry (Figure 8) shows a peak at 399.2061 which is within the acceptable range from the calculated value of 389.2046, confirming the compound. All final dyes were characterized using the same techniques as described for 75c.
Figure 6. $^{13}$C NMR spectrum of 75c in DMSO-$d_6$ at 25°C

Figure 7. Liquid Chromatography of 75c


2.3.2 Optical properties

Dye 74d was dissolved in numerous solvents that all of the dyes were soluble at a constant concentration of 3 μM. The effect of differing the solvent on absorption was investigated using acetonitrile, DMSO, ethanol and methanol and can be seen in Figure 9. The dyes had the highest absorption in ethanol and absorbed at the longest wavelength in DMSO. Methanol and acetonitrile showed no difference from each other. The dyes were best soluble in DMSO and therefore DMSO was chosen as the solvent for the studies as shown in Figure 9.

Figure 9. Solvent effects on the absorption of 74d A) full spectrum B) zoomed in to λ<sub>max</sub>
Figure 10 shows an example of the absorption of dye 74a at various concentrations tested. These absorption values were used to determine the molar absorptivity using Beer’s Law. The absorption shifts caused by the different heterocycles can be seen in Figure 11. The absorption $\lambda_{\text{max}}$ of trimethine cyanine dyes containing two indolenine bases is usually 552-553 nm in DMSO, and the fluorescence $\lambda_{\text{max}}$ is usually 565 nm. The most notable shifts come from dyes whose heterocycles have some interaction with the conjugation. For instance, the oxygen in benzoxazole heterocycles has more interaction with the conjugated methine chain than the sulfur in benzothiazole heterocycles does. As shown in Table 1, the observed chemical shift of the meso-proton in proton NMR is most likely due to electronegativity from the surrounding atoms. Oxygen is more electronegative than sulfur and therefore pulls more electrons from the methine chain shifting the meso-proton to 8.34 ppm. Sulfur is only slightly

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<td>75b</td>
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more electronegative than carbon and shows a meso-proton shift of 8.40 ppm corresponding to an absorption $\lambda_{\text{max}}$ of 549 nm. corresponding to the smaller meso-proton shift and shift in $\lambda_{\text{max}}$. The benz[c,d]indole heterocycle shows the largest shift in both the meso-proton and $\lambda_{\text{max}}$, 8.91 ppm and 672 nm, respectively. This is due to the increased conjugation in the heterocycle pulling more electrons from the methine chain. Figure 12 shows the electrostatic maps of these dyes next to their proton NMR to give a visual representation of how the heterocycles interact differently with the methine chain.

![Figure 12. Shift of meso-proton due to changing heterocycles](image)

Optical properties for the dyes can be seen in Table 2. The dyes containing bromine substituted indolenine 74j and 74f, benz[e]indole 74i, and benzoxazole 75b show $\lambda_{\text{max}}$ directly between where symmetrical dyes of the two heterocycles would absorb. This is because the heterocycles are equivalent in basicity to one another, but the bromine in dye 74f did cause an increase in molar absorptivity. The
λ_{max} of the dye containing an electron donating methoxy substitution on the indolenine 74g and benz[c,d]indole 76a was not in the expected position directly in between the λ_{max} of the two symmetric dyes the center showing that the methoxy group affects the basicity of the heterocycle more than the bromine does. This dye 74g also showed lower molar absorptivity and the lowest quantum yield due to the electron donating nature of the methoxy group introducing electron density back into the system. Dyes 74a-c all have two unsubstituted indolenine heterocycles and show molar absorptivity in the range of 120,000-127,000 M^{-1}cm^{-1}. This shows that the heterocycle has more effect on molar absorptivity than the side chain. The inclusion of a sulfonate group on the heterocycle increases the molar absorptivity. The molar absorptivity of dyes containing benzothiazole and benzoazole heterocycles is also slightly lower than those with two indolenine heterocycles. The addition of a benz[c,d]indole heterocycle causes a shift over 100 nm and makes the dye blue in color, but also causes a decrease in molar absorptivity. Adding two benz[c,d]indole heterocycles shifts absorption to the near-infrared region showing a λ_{max} of 758 nm. These shifts are of importance to imaging because it allows the fluorescence of trimethine cyanine dyes to be closer to the

<table>
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<tr>
<th>Dye</th>
<th>λ_{max}</th>
<th>E_{max}</th>
<th>Stokes shift</th>
<th>%QY (+/- .02%)</th>
<th>ε (M^{-1}cm^{-1}) (+/- 2%)</th>
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NIR range where there is less autofluorescence from biological tissue, but comes at the price of lower quantum efficiency. Lee et al. have shown that the benz[c,d]indole moiety causes a large decrease in fluorescence lifetime.\textsuperscript{54} This is probably due to the planar nature of these heterocycles allowing for easier stacking. The dye containing a benzoazole heterocycle \textbf{75b} had a blue shift further from the NIR region, it also had an increased stokes shift of over 20 nm. As mentioned before this is likely due to the electronegativity of the oxygen atom.

The majority of the dyes have a quantum yield around 10%. Dye \textbf{74f} with the bromine substituent on the heterocycle was slightly higher at 12.5%. This may be due to its higher molar absorptivity. The benzoazole containing dye \textbf{74b} was also close to 10%, but the benzothiazole dye was lower around 7%. Benzoazole dyes tend to have higher quantum yield due to the lone pair of the oxygen stabilizing the excited state, while the sulfur atom in benzothiazole dyes causes the opposite effect.

Another interesting property of the dyes is their apparent aggregation as seen in Figure 13. Cyanine dyes are known to aggregate, but in our study of unsymmetric trimethine cyanines, as the concentration of each sample was increased the ratio of the two peaks remained constant suggesting that the spectral representation of these compounds does not show aggregation in DMSO. In addition the second absorption maxima at the lower wavelength are not present in the dyes with a benzothiazole or benzoazole moiety. The second peak has been attributed to the more pronounced H-band associated with dyes containing two dimethyl-indolenine heterocycles.

\subsection*{2.3.3 Hydrophobicity}

Cyanine dyes are typically very soluble in DMSO, therefore hydrophobicity studies were carried out in methanol-buffer mixtures. As the polarity of the solvent decreased with increasing methanol concentration from 2\% to 100\% a general amplification of the monomeric band was observed. As the polarity decreases there is also an increase of the ratio of monomer band to the aggregate band up to
30% methanol when the ratio becomes steady. The increased absorbance is related to the defragmentation of aggregate to form monomers. As reported by Beckford et al., this aggregation is predominantly due to a plane-to-plane arrangement. Although this aggregation is still seen in unsymmetrical dyes at higher polarities the aggregation is not as obvious compared to symmetrical dyes. This is likely due to the two different heterocycles not stacking as easily as heterocycles that are the same, i.e. two benzothiazole heterocycles stacking together better than a benzothiazole and an indolenine heterocycle stack. In addition, these dyes all include one methyl side chain, so the effect of increasing the length and subsequent hydrophobicity of only one side would be expected to have a smaller effect on aggregation. Examples of this can be seen in Figure 13. Increasing the hydrophobicity of the dye by changing indolenine to benzene to benz[e]indolenine to benz[c,d]indolenine increases the amount of aggregation seen in higher polarity solvent.

![Figure 13. Absorption spectra of cyanine dyes as a function of increasing solvent hydrophobicity (% v/v methanol to buffer) at constant dye concentration at constant dye concentration of 10 μM (A) 74d (B)74i (C) 76a](image)

To show that the vast majority of hydrophobic effects are due to the heterocycle, an unsymmetrical dye 79 containing two benz[c,d]indole heterocycles with one methyl and one butyl side chain was synthesized as shown in Equation 11. The hydrophobic effects of this dye were compared to the dye reported by Beckford et al. and as shown in Figure 14 there is almost no hydrophobic change observed from changing one butyl group to a methyl group.
Changes in the hydrophobic nature of these dyes can make them less biocompatible with regard to uptake and biodistribution in vivo. Table 2 shows that altering the heterocycle from indolenine to benze[e]indolenine to benz[c,d]indolenine causes a red shift in absorption but also a decrease in quantum efficiency. A possible way to counter this decrease in quantum efficiency is through the use of unsymmetrical dyes. By changing only one heterocycle biocompatibility may be retained while only losing a small portion of the dye’s quantum efficiency. The improved solubility of these less hydrophobic dyes also increases their attractiveness as biological probes. These increases in hydrophobicity can be predicted through log D values as shown in Table 3.

2.3.2 LogD

The Lipinski Rule properties for each dye can be seen in Table 3. The molecular weight was calculated without the counterion as it would be lost in in vivo applications. The only dyes that didn’t meet the Lipinski Rule for molecular weight were 74h and 74j due to the addition of heavy substituents, such as bromine, to the heterocycle already containing the trimethylammonium side chain. None of the dyes contain any hydrogen bond donors. All of the dyes meet both rules for hydrogen bond donors and
acceptors. They also all meet polar surface area requirements. Three dyes 74e,f,i, had log D above the Lipinski Rule value, therefore 8 of the 13 dyes synthesized meet all the Lipinski rules. One common use of trimethine dyes is for cell staining. According to Burton et al.\(^5\)\(^6\) an incremental increase in cell permeability is seen with an increase in partition coefficient, so it would be expected that the dyes with the highest log D shown in Table 3 would have the best cell permeability. The dyes with a butyl substituents 74d,f,g,i and propylphenyl 74e substituents on them have high log D values, especially the butyl substituent on the benz[e]indole moiety. The dyes with the lowest log D values are those with more than one charge 74a,b,h,j and the benzoxazole moiety 75b. It would be expected that these dyes would have a lower probability of crossing the cell membrane, but may be more suitable for other applications.

### Table 3. Summary of Lipinski Rule Properties

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<th>Dye</th>
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<th>H bond donors</th>
<th>H bond acceptors</th>
<th>Polar Surface Area (Å(^2))</th>
<th>Log D</th>
<th>Meets Criteria</th>
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2.4 Conclusions

A series of 13 novel unsymmetrical trimethine cyanine dyes was synthesized in very good yield, purified, and characterized by \(^1\)H NMR, \(^{13}\)C NMR, liquid chromatography, and mass spectrometry. Their optical properties including absorption, fluorescence, stokes shift, molar absorptivity, and quantum yield
were studied. Solvent effects were evaluated and DMSO was determined to be the best solvent for studying the optical properties. Dyes with electron withdrawing substitutions on the heterocycle show increased molar absorptivity while those with electron donating groups show lower molar absorptivity. These changes in molar absorptivity are likely also the cause for parallel changes in quantum yield. Unsymmetrical dyes can be used to fine tune absorption wavelengths without the loss of other favorable properties such as quantum yield. Unsymmetrical dyes were shown to not aggregate as much as symmetric dyes most likely due to the inability of the different heterocycles to stack as well. Trimethine cyanine dyes could make good oral drugs as the majority of them meet Lipinski’s Rule of Five.
3 SYNTHESIS AND EVALUATION OF TRIMETHINE CYANINE DYSES AS PROTEIN ARGinine METHYL TRANSFERASE INHIBITORS AND IMAGING AGENTS

3.1 Introduction to Protein Arginine Methyltransferase

Arginine differs from other amino acids in that its side chain contains more potential hydrogen bond donors than other amino acids. A common post-translational modification (PTM) of arginine is methylation. This is achieved by a family of enzymes called protein arginine methyltransferases (PRMTs). A three-dimensional model of rat PRMT1 can be seen in Figure 15. This family of nine enzymes found in mammalian genomes has important roles in signal transduction, gene transcription, DNA repair, mRNA splicing, and many other biological activities that affect cell growth, proliferation and differentiation. PRMTs catalyze three types of arginine methylation as shown in Figure 16. The first is monomethylation which is done by all PRMTs. Type III PRMTs only monomethylate. Monomethylation is a precursor of dimethylation which can be asymmetric (Type I) or symmetric (Type II). PRMT1 and PRMT5 are the major asymmetric and symmetric arginine methyltransferases, respectively. The other
PRMTs have fewer substrates and more specialized roles. Although alterations are generally not found in PRMTs, various cancers including breast cancer, prostate cancer, and leukemia have been linked to overexpression of these enzymes making them viable targets for therapeutic strategies. PRMT1 may be responsible for leukemia development.

![Figure 16. Types of Methylation on Arginine Residues](image)

The prominent asymmetric arginine methyltransferase, PRMT1, accounts for over 90% of methylarginine residues in mammalian cells. Its activity at arginine 3 on histone H4 has been positively correlated with increasing tumor grade and can be used to predict the risk of reoccurrence of prostate cancer. PRMT1 has also been associated with telomeric repeat-binding factor 2, a component that protects telomeres. It is widely accepted that the deregulation of telomeric maintenance and the DNA repair pathway has a major role in carcinogenesis. Inhibition of this pathway may stimulate the accumulation of DNA damage. Overexpression of another target of PRMT1, the oestrogen receptor, has been implicated in the pathogenesis of breast cancer. Two studies in mice have also shown that PRMT1 may be responsible for leukemia development. These studies suggest that inhibitors specific for PRMT1 may have a therapeutic effect on leukemia as well as anti-cancer activity.
The key type II arginine transferase, PRMT5,\textsuperscript{66} shows oncogene-like properties in its ability to repression the expression of tumor suppressor genes. Higher levels of PRMT5 have been seen in transformed cells and mice with no PRMT5 show a slowed cell growth, while those overexpressing PRMT5 show cellular hyperproliferation.\textsuperscript{67} Epithelial-mesenchymal transition, a normal process by which cells become less adhesive more mobile causes cancerous cells to become metastatic.\textsuperscript{59} PRMT5 plays an important role in epithelial-mesenchymal transition.

Although PRMT has only recently been linked to cancer\textsuperscript{59}, early studies have been quite circumstantial. The field of small-molecule PRMT inhibitors is still in its early stages, but the knowledge gained from these initial studies allows for the prompt evaluation of tricarbocyanines \textit{in vivo} efficacy as PRMT inhibitors.\textsuperscript{59}

The first non-nucleoside specific inhibitors of PRMTs were discovered in 2004 through random screening, some of which are shown in Figure 17.\textsuperscript{68} Stilbamidine and AMI-1 have IC\textsubscript{50} values of 57 μM and 8.8 μM, respectively.\textsuperscript{68} It remains difficult to develop selective and potent PRMT inhibitors due to PRMTs highly conserved binding pockets.\textsuperscript{70} An important characteristic of carbocyanines is their absorption and fluorescence in the near-IR range which offers a great advantage for \textit{in vivo} fluorescent imaging with minimal background fluorescence from biomolecular autofluorescence. Relative changes in the absorption or emission spectra of cyanine dyes caused by binding of biomolecules can be studied spectrophotometrically.\textsuperscript{14} Previously, our lab has synthesized a series of trimethine dyes using the
indolenine, benz[e]indole, and benz[c,d]indole heterocycles to test for PRMT1 binding. Through this series it has been shown that trimethine cyanine dyes inhibit PRMT1 in a noncompetitive, reversible fashion, bind 3 regions on PRMT (Figure 18), accumulate in the nucleus of HeLa cells (Figure 19), and can be seen under visible microscope (Figure 19A) or by fluorescence (Figure 19B). These dyes can be a powerful tool in revealing mechanisms and functions of PRMTs.

![Figure 18. Probable binding regions of trimethine cyanine dyes on PRMT](image)

![Figure 19. Accumulation of Trimethine Cyanine Dye in the Nucleus of Hela Cells Under (A) Visible Microscope and (B) Fluorescence](image)

There has only been one study done using carbocyanines as PRMT inhibitors. This study which yielded good results tested symmetrical dyes containing indolenine, benz[e]indole, and benz[c,d]indole heterocycles. In order to further decrease IC_{50} values, this study began by creating unsymmetrical dyes with one indolenine heterocycle and one benzothiazole heterocycle in hopes that removal of one of the
dimethyl groups may allow for better binding. From this initial study, it was determined that the benzo-benzothiazole heterocycle could be important to PRMT inhibition and therefore benzothiazole containing symmetrical dyes similar to the dyes previously published were synthesized. Each modification will be discussed in detail throughout the rest of this chapter and the anti-PRMT activity will be compared with data from our previous study.\textsuperscript{53}

### 3.2 Unsymmetric Trimethine Cyanine Dyes for PRMT Inhibition

![Scheme 12. Synthesis of Unsymmetric Dyes for PRMT Inhibition Screening](image)

This research concentrates on the development of unsymmetrical benzothiazole-containing hydrophobic trimethine cyanine dye derivatives utilizing ethyl, butyl and phenylpropyl halides for nitrogen alkylation. Initial synthesis for this work involved the development of a series of unsymmetric trimethine cyanine dyes as described in Scheme 11 and Scheme 12. Quaternization of commercially obtained benzothiazole and synthesized indolenine containing compounds proceeds through an \textit{S\textsubscript{N}2} reaction with alkyl halides using either acetonitrile or no solvent. These quaternary ammonium salts are then reacted with aldehyde in acetic anhydride at 100°C for 1 hr. The workup for these dyes involves precipitation in diethyl ether and decanting the solvent, before washing with ether and filtering. Efficient synthesis gives good yield and high purity compounds, but those needing additional purification were purified by column chromatography (4% methanol in dichloromethane).
Synthesized trimethine cyanine dyes were screened for anti-PRMT1 and PRMT5 activity to compare against the indolenine based series. Typical anti-PRMT screening assays include recombinantly expressed His-tagged PRMT \[^{14}\text{C}\text{-labeled AdoMet, and a histone H4 peptide containing the 20-amino acid sequence on the N-terminal tail of histone H4. A 30 mL sample is heated at 30^\circ\text{C} and the degree of diminishment of PRMT activity is used to evaluate the potency of the compounds. Dyes showing promising activity from the screening were supplemented to IC_{50} studies.}

Initial screening assays were done on the unsymmetrical cyanine dyes. Results from the anti-PRMT activity and IC\(_{50}\) studies are shown in Table 4. These data show that unsymmetrical dyes containing benzothiazole moieties, specifically 82a and 82c, inhibited PRMTs well, while dyes with benzoxazole moieties 75b (Scheme 11) show less inhibition. The dyes with additional charges 74a,b,h (Scheme 11) did not show any PRMT inhibition concluding that additional cations on the cyanine core decrease the efficacy of PRMT inhibition.

After screening, the three dimensional structure and log D values of these dyes at pH 7.4, shown in Figure 19, were predicted to better draw a correlation between structure and activity. All of the dyes have molecular weight less than 500 and polar surface area less than 140 Å. All dyes with potent inhibition had log D values above 4 and the dyes with extra charges had log D values of 3 and below. In addition, the oxygen atom in the benzoxazole containing dye led to a log D below 2. Compound 82a was the

<table>
<thead>
<tr>
<th>Compound</th>
<th>Remaining PRMT1 activity</th>
<th>Remaining PRMT5 activity</th>
<th>PRMT1 IC(_{50}) (µM)</th>
<th>PRMT5 IC(_{50}) (µM)</th>
<th>Ratio of IC(<em>{50}) PRMT1/IC(</em>{50}) PRMT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>74a</td>
<td>1.23</td>
<td>0.710</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>74b</td>
<td>1.08</td>
<td>0.640</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>74c</td>
<td>1.13</td>
<td>0.510</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>74d</td>
<td>1.17</td>
<td>0.400</td>
<td>&gt;20</td>
<td>13.9</td>
<td>&gt;1.43</td>
</tr>
<tr>
<td>74e</td>
<td>0.452</td>
<td>0.302</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>74f</td>
<td>0.155</td>
<td>0.340</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>74h</td>
<td>1.08</td>
<td>0.800</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>75b</td>
<td>0.573</td>
<td>0.680</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td><strong>82a</strong></td>
<td>0.012</td>
<td>-0.049</td>
<td>6.11</td>
<td>5.42</td>
<td>1.13</td>
</tr>
<tr>
<td><strong>82b</strong></td>
<td>0.629</td>
<td>0.277</td>
<td>37.5</td>
<td>10.7</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>82c</strong></td>
<td>0.484</td>
<td>0.236</td>
<td>9.46</td>
<td>11.92</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*nd = not determined

first unsymmetrical benzothiazole containing dye tested and showed excellent inhibition with IC$_{50}$ values of 6.11 μM and 5.42 μM for PRMT1 and PRMT5, respectively. This dye led to the designing of a second series of symmetrical dyes containing the benzothiazole heterocycle with higher log D values. The next synthetic step based on these predictions and the screening results was to design benzothiazole containing symmetrical trimethine cyanines based on our previous set of compounds.\(^\text{53}\)
Figure 20. Three Dimensional Structures and Log D values of Unsymmetric Trimethine Cyanines
3.3 Symmetric Trimethine Cyanine Dyes for PRMT Inhibition

![Diagram showing the synthesis of symmetric cyanine dyes](image)

**Scheme 13. Synthesis of Unsubstitued Symmetrical Trimethine Cyanine Dyes for PRMT Inhibition**

The synthesis of symmetrical benzothiazole containing trimethine cyanine dyes begins with the alkylation of 2-methylbenzothiazole 83 with alkyl halides by refluxing without solvent. The corresponding salts are then washed with ether to remove impurities. Individual salts 84a-c are then reacted with triethyl orthoformate in acetic anhydride for two hours. The reaction is allowed to cool to room temperature and the solution is poured into ether. The ether is decanted and the solid is washed with ether several times before filtering the dyes. No column chromatograph purification was needed and these dyes were synthesized in yields ranging from 66%-72%. All dyes were fully characterized as discussed in Chapter 1. Screening assays, using the same procedure as described before, revealed that dye 85a showed the best PRMT1 inhibition for this series corresponding to a 27.21 μM IC₅₀ value as shown in Table 5. Dye 85c showed the lowest remaining activity for PRMT5 which is expected to be an IC₅₀ value below 10 based on a comparison of the screening activity of 85a and 85c for PRMT5. This shows that increasing the length and/or size of the N-alkyl side chain has a better effect on PRMT5 than PRMT1. Due to our previous results indicating that compounds with higher log D values show better inhibition, the next step was to synthesize symmetrical dyes with increased hydrophobicity by adding a bromine atom.
Table 5. Anti-PRMT Activity of Benzothiazole-containing Symmetrical Dyes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Remaining PRMT1 activity</th>
<th>Remaining PRMT5 activity</th>
<th>PRMT1 IC\textsubscript{50} (µM)</th>
<th>PRMT5 IC\textsubscript{50} (µM)</th>
<th>Ratio of IC\textsubscript{50} PRMT1/IC\textsubscript{50} PRMT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>85a</td>
<td>0.144</td>
<td>0.353</td>
<td>27.21</td>
<td>12.65</td>
<td>2.15</td>
</tr>
<tr>
<td>85b</td>
<td>0.173</td>
<td>0.331</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>85c</td>
<td>0.186</td>
<td>0.323</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

*nd = not determined

Figure 21. Three Dimensional Structures and Log D values of Symmetric Trimethine Cyanines

![Figure 21](image)

Scheme 14. Synthesis of Bromine Substituted Benzothiazole Containing Trimethine Cyanine Dyes

Dye 85b with an N-butyl side chain was chosen for further modification due to its moderate activity for both PRMT1 and PRMT5. Bromine was added to increase log D from 3.92 to 5.46. To observe whether there was a spacial factor, bromine atoms were added in two different positions. Symmetrical trimethine cyanine dyes were synthesized by the same method described in Scheme 13, only starting with 5-bromo-2-methylbenzothiazole 86a and 6-bromo-2-methylbenzothiazole 86b. Because these compounds are solids and not liquid like unsubstituted 2-methylbenzothiazole, the alkylation of 86a-b
was carried out in boiling acetonitrile. The pure dyes were synthesized in very good yield over 75%. As shown in Table 6, the bromine substitution decreased anti-PRMT activity, possibly due to electrostatic interactions caused by the electronegativity of the bromine atom, shown in Figure 22. Previous results indicated that dyes containing benz[e]indole heterocycles acted as potent PRMT inhibitors.

### Table 6. PRMT Inhibition of Bromine Substituted Benzothiazole Containing Trimethine Cyanine Dyes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Remaining PRMT1 activity</th>
<th>Remaining PRMT5 activity</th>
<th>PRMT1 IC₅₀ (µM)</th>
<th>PRMT5 IC₅₀ (µM)</th>
<th>Ratio of IC₅₀ PRMT1/IC₅₀ PRMT5</th>
</tr>
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<tbody>
<tr>
<td>88a</td>
<td>0.488</td>
<td>0.662</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>88b</td>
<td>0.544</td>
<td>0.656</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

*nd = not determined

![Figure 22. Three Dimensional Structures and Log D values of Bromine Substituted Symmetric Trimethine Cyanines](image)

**Scheme 15. Synthesis of Napthothiazole Containing Symmetrical Trimethine Cyanine Dyes**

Napthothiazole structurally mimics the benz[e]indole heterocycle that was used in our previous study. To further modify this structure the same systematic set of N-alkyl side chains were incorporated as the initial series of symmetric dyes discussed earlier. As described in Scheme 15 alkyl
halides were refluxed in acetonitrile with 2-methylnaptho[2,1-d]thiazole 89 to form quaternary ammonium salts 90a-c. Dyes 91a-c were formed in 50-70% yield following the same procedure as described in Schemes 13 and 14. Dye 91c with the phenylpropyl substituent had a lower yield due to the precursor quaternary ammonium salt 90c not being completely pure. As shown in Table 7, dye 91a-c containing napthothiazole substituents with ethyl side chains significantly diminished PRMT1 suggesting that this heterocycle is advantageous. The instrument for IC\textsubscript{50} determinations was not sensitive enough to determine the actual IC\textsubscript{50} for this dye, but it is expected to be between 1 and 2 µM. Dye 91b also had excellent anti-PRMT1 activity from the screening assay and has better selectivity toward PRMT1 than dye 91a. Three dimensional structures and log D values for these compounds can be seen in Figure 23. In this series we see a decrease in PRMT5 potency with increasing side chain length, the opposite of what was seen with benzothiazole heterocycles. This probably has to do with the size of the compound getting too large to fit in the binding region.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Remaining PRMT1 activity</th>
<th>Remaining PRMT5 activity</th>
<th>PRMT1 IC\textsubscript{50} (µM)</th>
<th>PRMT5 IC\textsubscript{50} (µM)</th>
<th>Ratio of IC\textsubscript{50} PRMT1/IC\textsubscript{50} PRMT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>91a</td>
<td>0.023</td>
<td>0.166</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>nd</td>
</tr>
<tr>
<td>91b</td>
<td>0.071</td>
<td>0.191</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>nd</td>
</tr>
<tr>
<td>91c</td>
<td>nd</td>
<td>nd</td>
<td>5.21</td>
<td>&gt;10</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

*nd = not determined

Figure 23. Three Dimensional Structures and Log D values of Napthothiazole Containing Symmetric Trimethine Cyanines
3.4 Conclusion

PRMTs have been linked to a variety of cancers, therefore the designing of potent small molecule inhibitors is highly desired. Trimethine cyanine dyes containing substituted and unsubstituted benzothiazole heterocycles and hydrophobic substituents were synthesized and several possessed single digit micromolar potency for PRMT1 and PRMT5 inhibition. Cyanine dyes tend to have long-wavelength absorption and fluorescence properties allowing them to be used as probes in vivo. The compound containing napthothiazole heterocycles with ethyl substituents had the highest potency for PRMT1 and PRMT5. Bromination of the benzothiazole heterocycle decreased potency. Compounds with multiple charges show no anti-PRMT activity. Through the synthesis and analysis of these compounds new routes should be explored including the synthesis of unsymmetric compounds with a napthothiazole heterocycle and the addition of meso substitutions. Overall, this study provides unique photoactive chemical molecules for both PRMT inhibition and imaging.
4 CONCLUSIONS

The synthesis of 25 symmetrical and unsymmetrical trimethine cyanine dyes and their characterization was reported and will be indicated in the experimental section. It has been shown that the addition of electron withdrawing substituents to the heterocycle can increase molar absorptivity and quantum efficiency of cyanine dyes. Unsymmetrical dyes can be used to fine tune absorption wavelengths without the loss of other favorable properties such as quantum yield and may be used to counteract the aggregation of cyanine dyes. Hydrophobic cyanine dyes containing ethyl substituents have shown the highest potency for PRMT1 and PRMT5. Dyes with multiple charges show no anti-PRMT activity. Through the work presented in this thesis it has been shown that cyanine dyes are a comprehensive class of compounds that can be modified to interact in a selective fashion for a multitude of applications spanning the breadth of chemistry and biology even outside of their niched use as probes for fluorescent labeling.
5 EXPERIMENTAL

5.1 Chemicals and Instruments

Most reagents were purchased from Sigma-Aldrich and were used without purification. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometer. High resolution mass spectra (HRMS) were obtained using a Waters Q-TOF micro (ESIQ-TOF) mass spectrometer.

2-(1,3,3-trimethylindolin-2-ylidene)acetaldehyde, 69: Phosphorous oxychloride was added dropwise to cold $N,N$-dimethylformamide, not allowing the temperature to go above 5°C to form a Vilsmeier-Haack reagent. The Vilsmeier-Haack reagent was added dropwise to 1,2,3,3-tetramethyl-1H-indolium iodide and the reaction was refluxed for one hour. The solution was then cooled to room temperature, poured into a cold solution of sodium perchlorate in methanol and left in the freezer to crystallize overnight. The solid was collected by vacuum filtration and dissolved in chloroform. The chloroform solution was then heated with a 20% sodium hydroxide/water solution for 24 hours. The organic phase was then washed with water three times and dried with sodium sulfate. The solvent was then removed by rotary evaporation to leave a reddish-orange solid 2; mp 104-106°C, 26% yield (lit mp 116°C, 60% yield).

5.1 Indoles

5-methoxy-2,3,3-trimethyl-3H-indole, 70b: A mixture of 4-methoxyphenylhydrazine hydrochloride and 3-methyl-2-butanone was refluxed in glacial acetic acid for 24 hrs. Acetic acid was then removed by rotary evaporation and the solution was diluted with dichloromethane. Sodium bicarbonate was added to neutralize any remaining acid and the 5-methoxy-2,3,3-trimethyl-3H-indole 70b was extracted three times with 50 mL dichloromethane, dried with magnesium sulfate, and concentrated under vacuum to form a brown oil; 91% yield (lit 95% yield).

sodium 2,3,3-trimethyl-3H-indole-5-sulfonate, 70c: was synthesized by our group as previously reported.
5-bromo-2,3,3-trimethyl-3H-indole, 70d: was synthesized by a similar procedure to 70b; 91% yield (lit 98% yield\textsuperscript{72}).

5.2 Quaternary Ammonium Salts

2,3,3-trimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium bromide, 71a: was synthesized by our group as previously reported.\textsuperscript{5}

3-(2,3,3-trimethyl-3H-indol-1-ium-1-yl)propane-1-sulfonate, 71b: A mixture of 2,3,3-trimethyl-3H-indole and 1,3-propane sultone were refluxed in toluene for 18 hrs. The reaction was cooled to room temperature and the product was filtered and washed with ether and acetone; mp 118-120\textdegree C, 41% yield (lit mp 126-128\textdegree C, 87% yield\textsuperscript{73}).

1-ethyl-2,3,3-trimethyl-3H-indol-1-ium iodide, 71c: A mixture of 2,3,3-trimethyl-3H-indole and iodobutane were refluxed in toluene for 72 hrs. The reaction was cooled to room temperature and the solution was poured into cold ether and the precipitate was filtered; mp 217-221\textdegree C, 82% yield (lit mp 165-166\textdegree C\textsuperscript{53}).

1-butyl-2,3,3-trimethyl-3H-indol-1-ium iodide, 71d: was synthesized by a similar procedure to 71c; mp 116-119\textdegree C, 80% yield (lit mp 102\textdegree C, 83% yield\textsuperscript{53}).

2,3,3-trimethyl-1-(3-phenylpropyl)-3H-indol-1-ium bromide, 71e: was synthesized by a similar procedure to 71c; mp 156-158\textdegree C, 86% yield; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}6): \textit{δ} 7.98 (t, \textit{J} = 5.2 Hz, 1 H), 7.84 (t, \textit{J} = 5.2 Hz, 1 H), 7.46 (m, 2 H), 7.29 (m, 5 H), 4.51 (t, \textit{J} = 8.0 Hz, 2 H), 2.81 (m, 5 H), 2.17 (m, 2 H), 1.53 (s, 6 H); \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}6): \textit{δ} 14.3, 22.0, 28.8, 31.7, 47.4, 54.1, 115.4, 123.5, 126.0, 128.2, 128.3, 128.8, 129.3, 140.6, 141.0, 141.8, 196.6; HRMS: Calcd for C\textsubscript{20}H\textsubscript{24}N\textsuperscript{+} m/z 278.1904, obsd m/z 278.1915.

5-bromo-1-butyl-2,3,3-trimethyl-3H-indol-1-ium iodide, 71f: was synthesized by a similar procedure to 71c; mp 176-178\textdegree C, 78% yield; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}6): \textit{δ} 0.93 (t, \textit{J} = 7.2 Hz, 3 H), 1.43 (m, 2 H), 1.55 (s, 6 H), 1.80 (m, 2 H), 2.85 (s, 3 H), 4.45 (t, \textit{J} = 7.2 H, 2 H), 4.43 (t, \textit{J} = 7.6 Hz, 2 H), 7.83 (d, \textit{J} = 8.4 Hz,
$^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 0.93 (t, $J = 7.2$ Hz, 3 H), 1.43 (m, 2 H), 1.53 (s, 6 H), 1.80 (m, 2 H), 2.80 (s, 3 H), 3.86 (s, 3 H), 4.43 (t, $J = 7.6$ Hz, 2 H), 7.13 (dd, $J = 10.8$ Hz, 1 H), 7.50 (s, 1 H), 7.89 (d, $J = 9.2$ Hz, 1 H); $^{13}$C NMR (100 MHz, MeOD-$d_6$): $\delta$ 12.44, 19.14, 21.61, 29.09, 47.93, 53.81, 55.78, 108.43, 114.50, 116.04, 133.49, 143.54, 160.71, 192.31; HRMS: Calcd for C$_{16}$H$_{24}$NO$^+$ m/z 246.1852, obsd m/z 246.1857.

2,3,3-trimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate bromide, 71h: was synthesized by our group as previously reported.5

3-butyl-1,1,2-trimethyl-1H-benzo[e]indol-3-ium iodide, 71l: was synthesized by a similar procedure to 71c; mp 142-145°C, 87% yield (lit mp 127-129°C, 90% yield$^{22}$).

5-bromo-2,3,3-trimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium bromide, 71j: was synthesized by our group as previously reported.$^{24}$

2,3-dimethylbenzo[d]thiazol-3-ium iodide, 72a: was synthesized by a similar procedure to 71c; mp 217-219°C, 84% yield (lit mp 218-220°C, 88% yield$^{75}$).

3-butyl-2-methylbenzo[d]thiazol-3-ium iodide, 72c: was synthesized by a similar procedure to 71c; mp 115-116°C, 94% yield (lit mp 114-115°C, 67% yield$^{76}$).

1-butyl-2-methylbenzo[c,d]indol-1-ium iodide, 73a: was synthesized by our group as previously reported.$^{53}$

3-butyl-2-methylbenzo[d]thiazol-3-ium iodide, 81b: was synthesized by a similar procedure to 71c; mp 115-116°C, 94% yield (lit mp 114-115°C, 67% yield$^{76}$).
6-bromo-3-butyl-2-methylbenzo[d]thiazol-3-ium iodide, 81c: was synthesized by a similar procedure to 71c; 60% yield; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 0.93 (t, J = 7.2 Hz, 3 H), 1.44 (m, 2 H), 1.79 (m, 2 H), 3.20 (s, 3 H), 4.69 (t, J = 7.6 Hz, 2 H), 8.07 (d, J = 9.2 Hz, 1 H), 8.30 (d, J = 9.2 Hz, 1 H), 8.71 (s, 1 H).

3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide, 84a: was synthesized by a similar procedure to 71c; mp 193-195°C, 87% yield (lit mp 196°C, 83% yield$^{39}$).

3-butyl-2-methylbenzo[d]thiazol-3-ium iodide, 84b: was synthesized by a similar procedure to 71c; mp 115-116°C, 94% yield (lit mp 114-115°C, 67% yield$^{76}$).

5-bromo-3-butyl-2-methylbenzo[d]thiazol-3-ium iodide, 87a was synthesized by a similar procedure to 71c; 49% yield; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 0.94 (t, J = 7.2 Hz, 3 H), 1.46 (m, 2 H), 1.80 (m, 2 H), 3.22 (s, 3 H), 4.69 (t, J = 7.6 Hz, 2 H), 7.99 (d, J = 8.8 Hz, 1 H), 8.39 (d, J = 8.8 Hz, 1 H), 8.71 (s, 1 H). $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 14.01, 17.54, 19.73, 30.24, 49.80, 119.16, 121.26, 127.50, 131.50, 132.80, 140.66, 178.50.

6-bromo-3-butyl-2-methylbenzo[d]thiazol-3-ium iodide, 87b: was synthesized by a similar procedure to 71c; 60% yield; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 0.93 (t, J = 7.2 Hz, 3 H), 1.44 (m, 2 H), 1.79 (m, 2 H), 3.20 (s, 3 H), 4.69 (t, J = 7.6 Hz, 2 H), 8.07 (d, J = 9.2 Hz, 1 H), 8.30 (d, J = 9.2 Hz, 1 H), 8.71 (s, 1 H). $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 14.01, 17.73, 19.68, 30.25, 49.76, 120.04, 123.15, 126.79, 128.93, 131.58, 142.47, 179.06.

3-ethyl-2-methylnaphtho[2,1-d]thiazol-3-ium iodide, 90a: was synthesized by a similar procedure to 71c; 75% yield; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 1.51 (t, J = 7.2 Hz, 3 H), 3.30 (s, 3 H), 4.88 (q, J = 7.2 Hz, 2 H), 7.87 (m, 2 H), 8.30 (d, J = 8.0 Hz, 1 H), 8.43 (m, 3 H).

3-butyl-2-methylnaphtho[2,1-d]thiazol-3-ium iodide, 90b: was synthesized by a similar procedure to 71c; 90% yield; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 0.95 (t, J = 7.2 Hz, 3 H), 1.49 (q, J = 7.2 Hz, 2 H), 1.884 (m, 2 H), 3.31 (s, 3 H), 4.83 (t, J = 7.2 Hz, 2 H), 7.86 (m, 2 H), 8.30 (d, J = 7.6 Hz, 1 H), 8.41 (m, 3 H).
5.3 Unsymmetrical Trimethine Cyanine Dyes

2,3-(3,3-dimethyl-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3H-indol-1-ium bromide, 74a: A mixture of 69 and 70a were heated at 100°C for one hour in acetic anhydride. The solution was allowed to cool to room temperature and then poured into cold diethyl ether. The liquid was decanted and more ether and the solid was collected by vacuum filtration. The dye was purified by reverse phase column chromatography (95% methanol/water). This compound was obtained in 54% yield; mp 188-190°C; ¹H NMR (400 MHz, MeOD-d6): δ 8.59 (t, J = 13.6, 1 H), 7.52 (m, 3 H), 7.46 (m, 2 H), 7.42 (d, J = 13.6 Hz, 1 H), 7.35 (m, 2 H), 6.89 (m, 2 H), 4.28 (t, J = 8 Hz, 2 H), 3.86 (t, J = 8, 2 H), 3.78 (s, 3 H), 3.28 (s, 9 H), 2.36 (t, J = 8 Hz, 2 H), 1.81 (s, 6 H), 1.78 (s, 6 H); ¹³C NMR (100 MHz, MeOD-d6): δ 176.06, 173.91, 151.16, 142.60, 141.73, 140.96, 140.57, 128.67, 128.61, 125.75, 125.11, 122.20, 122.01, 111.25, 110.63, 104.02, 102.38, 63.02, 52.67, 49.41, 49.01, 40.40, 31.06, 27.13, 26.71, 20.93. HRMS: Calcd for C₃₀H₄₁N₅₂⁺ m/z 221.6645, obsd m/z 221.6626.

3-(3,3-dimethyl-2-(3-(1,3,3-trimethyl-3H-indol-1-ium-2-yl)allylidene)indolin-1-yl)propane-1-sulfonate, 74b: was synthesized by a similar procedure to 74a. The dye was purified reverse phase column chromatography (0.5% HCl/methanol). This compound was obtained in 38% yield; decomp 168°C; ¹H NMR (400 MHz, MeOD-d6): δ 8.59 (t, J = 7.6 Hz, 1 H), 7.55 (d, J = 6.8 Hz, 2 H), 7.48 (t, 6.8 Hz, 3 H), 7.34 (m, 3 H), 6.60 (d, J = 6.8 Hz, 1 H), 6.50 (d, J = 6.8 Hz, 1 H), 4.382 (t, J = 7.2 Hz, 2 H), 3.71 (s, 3 H), 3.30 (t, J = 6.8 Hz, 2 H), 2.27 (t, J = 7.2 Hz, 2 H), 1.78 (s, 12 H); ¹³C NMR (100 MHz, MeOD-d6): δ 175.42, 174.57, 150.91, 142.71, 141.86, 140.76, 128.64, 128.53, 125.36, 125.25, 122.04, 121.93, 111.04, 110.90, 102.78, 102.47, 49.22, 49.19, 42.57, 30.39, 26.95, 26.77, 22.75. HRMS: Calcd for C₂₇H₃₂N₄O₃S m/z 465.2167, obsd m/z 465.2231.

2-(3-(1-ethyl-3,3-dimethylindolin-2-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide, 74c: was synthesized by a similar procedure to 74a. The dye was purified by chromatotron (1% methanol/dichloromethane). This compound was obtained in 25.6% yield; mp 139-141°C; ¹H NMR (400
MHz, MeOD-\textit{d}_6): \delta 1.44 (t, J = 7.2 Hz, 3 H), 1.78 (s, 12 H), 3.70 (s, 3 H), 4.23 (m, 2 H), 6.50 (m, 2 H), 7.33 (t, J = 7.6 Hz, 2 H), 7.37 (d, J = 7.6 Hz, 2 H), 7.46 (t, J = 7.2 Hz, 2 H), 7.56 (m, 1 H), 7.57 (m, 1 H), 8.57 (t, J = 13.2 Hz, 1 H); \textsuperscript{13}C NMR (100 MHz, MeOD-\textit{d}_6): \delta 11.26, 26.79, 26.82, 30.49, 38.91, 49.18, 49.26, 102.02, 102.37, 110.80, 110.85, 121.98, 122.18, 125.32, 125.35, 128.54, 128.62, 140.68, 140.92, 141.53, 142.70, 150.82, 174.23, 175.23. HRMS: Calcd for C\textsubscript{26}H\textsubscript{31}N\textsubscript{2} + m/z 371.2487, obsd m/z 371.2473.

2-(3-(1-butyl-3,3-dimethylindolin-2-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3\textsubscript{H}-indol-1-ium iodide, \textit{74d}: was synthesized by a similar procedure to \textit{74a}. The dye was purified by column chromatography (95% dichloromethane/methanol). This compound was obtained in 20.5% yield; mp 115-117\textdegree C; \textsuperscript{1}H NMR (400 MHz, MeOD-\textit{d}_6): \delta 1.03 (t, J = 7.6 Hz, 3 H), 1.55 (m, 2 H), 1.77 (s, 12 H) 1.84 (m, 2 H), 3.72 (s, 3 H), 4.19 (t, J = 7.6 Hz, 2 H), 6.60 (d, J = 7.2 Hz, 2 H), 7.31 (t, J = 7.2 Hz, 2 H), 7.37 (d, J = 7.6, 2 H), 7.46 (t, J = 7.6 Hz, 2 H), 7.56 (dd, J = 6.8 Hz, 2 H), 8.55 (t, J = 6.8 Hz, 1 H); \textsuperscript{13}C NMR (MeOD-\textit{d}_6) \delta 12.95, 19.86, 26.91, 27.03, 29.33, 30.87, 43.90, 49.15, 49.22, 102.56, 102.70, 110.90, 111.11, 122.04, 122.19, 125.31, 128.56, 128.61, 140.71, 140.83, 142.01, 142.70, 150.69, 174.50, 175.13. HRMS: Calcd for C\textsubscript{28}H\textsubscript{35}N\textsubscript{2} + m/z 399.2795, obsd m/z 399.2810.

2-(3-(3,3-dimethyl-1-(3-phenylpropyl)indolin-2-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3\textsubscript{H}-indol-1-ium bromide, \textit{74e}: was synthesized by a similar procedure to \textit{74a}. The dye was purified by column chromatography (4% methanol/dichloromethane). This compound was obtained in 60.2% yield; mp 134-135\textdegree C; \textsuperscript{1}H NMR (400 MHz, MeOD-\textit{d}_6): \delta 1.75 (s, 6 H), 1.77 (s, 6 H), 2.17 (m, 2 H), 2.84 (t, J = 7.6 Hz, 2 H), 3.71 (s, 3 H), 4.16 (t, J = 7.6 Hz, 2 H), 6.21 (d, J = 13.6 Hz, 1 H), 6.29 (d, J = 13.6 Hz, 1 H), 7.22 (d, J = 8.0 Hz, 1 H), 7.31 (m, 4 H), 7.34 (m, 3 H), 7.36 (m, 3 H), 7.48 (m, 2 H), 7.57 (t, J = 6.8 Hz, 2 H), 8.51 (t, J = 13.6 Hz, 1 H); \textsuperscript{13}C NMR (100 MHz, MeOD-\textit{d}_6): \delta 22.82, 26.75, 26.89, 28.53, 30.49, 32.26, 43.02, 49.22, 102.19, 102.42, 110.87, 110.90, 121.99, 122.13, 125.32, 125.41, 126.05, 128.25, 128.31, 128.56, 140.71, 140.76, 141.87, 142.66, 150.61, 174.41, 175.29. HRMS: Calcd for C\textsubscript{33}H\textsubscript{37}N\textsubscript{2} + m/z 461.2952, obsd m/z 461.2939.
2-(3-(5-bromo-1-butyl-3,3-dimethylindolin-2-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide, 74f: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 86% yield; mp 141-143°C; 1H NMR (400 MHz, DMSO-d6): \( \delta \) 0.94 (t, J = 7.2 Hz, 3 H), 1.41 (m, 2 H), 1.69 (m, 14 H), 3.68 (s, 3 H), 4.07 (t, J = 7.2 Hz, 2 H), 6.47 (d, J = 13.6 Hz, 1 H), 6.55 (d, J = 13.6 Hz, 1 H), 7.33 (t, J = 7.2 Hz, 1 H), 7.37 (d, J = 8.4 Hz, 1 H), 7.50 (m, 2 H), 7.61 (dd, J = 6.4 Hz, 1 H), 7.65 (d, J = 7.2 Hz, 1 H), 7.91 (s, 1 H), 8.32 (t, J = 13.6 Hz, 1 H); 13C NMR (100 MHz, DMSO-d6): \( \delta \) 14.28, 20.00, 27.63, 27.83, 29.48, 32.11, 44.16, 49.30, 49.56, 102.64, 104.26, 112.30, 113.60, 117.61, 122.91, 126.06, 126.21, 129.09, 131.81, 141.24, 141.88, 143.03, 143.25, 150.16, 173.24, 176.61. HRMS: Calcd for C28H34BrN2+ m/z 479.1900, obsd m/z 479.1881.

2-(3-(1-butyl-5-methoxy-3,3-dimethylindolin-2-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide, 74g: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 77% yield; mp 136-138°C; 1H NMR (400 MHz, DMSO-d6): \( \delta \) 0.94 (t, J = 7.2 Hz, 3 H), 1.40 (m, 2 H), 1.68 (s, 6 H), 1.69 (m, 2 H), 1.69 (m, 2 H), 3.61 (s, 3 H), 3.82 (s, 3 H), 4.12 (t, J = 7.8 Hz, 2 H), 6.38 (d, J = 13.6 Hz, 1 H), 6.47 (d, J = 13.6 Hz, 1 H), 7.00 (d, J = 8.4 Hz, 1 H), 7.26 (t, J = 7.2 Hz, 1 H), 7.32 (s, 1 H), 7.42 (m, 3 H), 7.61 (d, J = 7.2 Hz, 1 H), 8.29 (t, J = 13.6 Hz, 2 H); 13C NMR (100 MHz, DMSO-d6): \( \delta \) 14.27, 20.01, 27.81, 27.89, 29.75, 31.62, 44.43, 48.97, 49.74, 56.37, 102.34, 103.21, 109.69, 111.51, 113.05, 114.16, 122.81, 125.19, 128.98, 135.68, 140.76, 143.04, 143.26, 149.15, 158.57, 173.71, 173.98. HRMS: Calcd for C29H37N2O+ m/z 429.2901, obsd m/z 429.2913.

3,3-dimethyl-2-(3-(1,3,3-trimethyl-3H-indol-1-ium-2-yl)allylidene)-1-(3-(trimethylammonio)propyl)indoline-5-sulfonate bromide, 74h: was synthesized by a similar procedure to 74a. The dye was purified by recrystallization in acetonitrile. This compound was obtained in 22.5% yield; decomp 168°C; 1H NMR (400 MHz, MeOD-d6): \( \delta \) 8.59 (t, J = 1.2 Hz, 1 H), 7.91 (s, 1 H), 7.80 (d, J = 1.2 Hz, 1 H), 7.78 (d, J = 1.2 Hz, 1 H), 7.50 (m, 3 H), 7.74 (t, J = 7.2 Hz, 1 H), 6.75 (d, J = 7.2 Hz, 1 H), 6.67 (d, J = 7.2 Hz, 1 H), 4.24 (t, J = 7.6 Hz, 2 H), 3.79 (s, 3 H), 3.72 (t, J = 7.6 Hz, 2 H), 3.24 (s, 9H), 2.38 (m, 2
\[ H \], 1.80 (s, 6 H) 1.78 (s, 6 H); \(^{13}\text{C}\) NMR (100 MHz, MeOD-d6): \( \delta \) 176.85, 173.66, 151.23, 143.25, 142.45, 142.12, 142.12, 141.15, 140.36, 128.69, 126.83, 126.21, 122.10, 120.05, 111.62, 109.98, 104.66, 102.29, 63.06, 52.49, 49.75, 48.80, 40.94, 31.01, 27.09, 26.54, 20.83. HRMS: Calcd for C\textsubscript{30}H\textsubscript{40}N\textsubscript{3}O\textsubscript{3}S \(^{+}\) m/z 522.2785, obsd m/z 522.2783.

2-\{3-(3-butyl-1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)prop-1-en-1-yl\}-1,3,3-trimethyl-3H-indol-1-ium iodide, 74i: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 44% yield; mp 133°C; \(^1\text{H}\) NMR (400 MHz, DMSO-d6): \( \delta \) 0.96 (t, \( J = 7.2 \) Hz, 3 H), 1.48 (m, 2 H), 1.74 (s, 6 H), 1.78 (m, 2 H), 1.98 (s, 6 H), 3.67 (s, 3 H), 4.26 (t, \( J = 7.2 \) Hz, 2 H), 6.50 (d, \( J = 13.6 \) Hz, 1 H), 6.56 (d, \( J = 13.6 \) Hz, 1 H), 7.31 (m, 1 H), 7.45 (d, 2 H), 7.54 (t, \( J = 7.6 \) Hz, 1 H), 7.68 (m, 2 H), 7.79 (d, \( J = 8.4 \) Hz, 1 H), 8.09 (m, 2 H), 8.30 (d, \( J = 8.4 \) Hz, 1 H), 8.47 (t, \( J = 13.6 \) Hz, 1 H); \(^{13}\text{C}\) NMR (100 MHz, DMSO-d6): \( \delta \) 0.58, 14.31, 20.02, 27.54, 27.80, 29.95, 31.82, 44.37, 49.26, 51.09, 102.51, 103.17, 111.86, 112.24, 122.70, 122.91, 125.56, 127.86, 128.32, 129.05, 130.38, 130.93, 131.98, 133.76, 139.96, 140.98, 143.18, 149.49, 174.56, 175.89. HRMS: Calcd for C\textsubscript{32}H\textsubscript{37}N\textsubscript{2} \(^{+}\) m/z 449.2952, obsd m/z 449.2973.

2-\{3-(3-bromo-3,3-dimethyl-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)prop-1-en-1-yl\}-1,3,3-trimethyl-3H-indol-1-ium bromide, 74j: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 77% yield; mp 226°C; \(^1\text{H}\) NMR (400 MHz, DMSO-d6): \( \delta \) 1.69 (s, 6 H), 1.71 (s, 6 H), 2.13 (m, 2 H), 3.10 (s, 9 H), 3.59 (t, \( J = 6.8 \) Hz, 2 H), 3.71 (s, 3 H), 4.08 (t, \( J = 7.2 \) Hz, 2 H), 6.68 (t, \( J = 12.4 \) Hz, 2 H), 7.36 (t, \( J = 7.6 \) Hz, 1 H), 7.47 (m, 2 H), 7.55 (d, \( J = 8.0 \) Hz, 1 H), 7.67 (t, \( J = 7.6 \) Hz, 2 H), 7.93 (s, 1 H), 8.34 (t, \( J = 13.2 \) Hz, 1 H); \(^{13}\text{C}\) NMR (100 MHz, DMSO-d6): \( \delta \) 21.09, 27.54, 27.92, 32.37, 41.20, 49.16, 49.76, 53.05, 62.81, 102.58, 104.89, 112.60, 113.32, 117.50, 122.96, 126.26, 126.37, 129.14, 131.74, 141.38, 141.70, 142.98, 143.10, 150.36, 172.76, 176.08.

1,3,3-trimethyl-2-\{3-(3-methylbenzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl\}-3H-indol-1-ium iodide, 75a: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 67% yield; mp 223-225°C; \(^1\text{H}\) NMR (400 MHz, DMSO-d6): \( \delta \) 1.65 (s, 6 H), 3.55 (s, 3 H), 3.94 (s,
3 H), 6.24 (d, J = 12.8 Hz, 1 H), 6.78 (d, J = 13.6 Hz, 1 H), 7.21 (t, J = 7.6 Hz, 1 H), 7.39 (m, 2 H), 7.51 (t, J = 7.6 Hz, 1 H), 7.58 (d, J = 7.6 Hz, 1 H), 7.65 (t, J = 7.6 Hz, 1 H), 7.67 (d, J = 8.4 Hz, 1 H), 8.06 (m, 2 H); 13C NMR (100 MHz, DMSO-d6): δ 28.71, 34.51, 48.73, 99.92, 102.82, 110.96, 114.92, 122.72, 123.69, 124.54, 125.80, 126.46, 128.78, 128.87, 140.65, 142.36, 143.40, 147.64, 168.17, 172.12. HRMS: Calcd for C22H23N2S+ m/z 347.1577, obsd m/z 347.1588.

2-(-3-(3-butylbenzo[d]oxazol-2(3H)-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide, 75a: was synthesized by a similar procedure to 74a. The dye was purified by chromatotron (2% methanol/dichloromethane). This compound was obtained in 38% yield; mp 134°C; 1H NMR (400 MHz, DMSO-d6): δ 0.94 (t, J = 7.2 Hz, 3 H), 1.41 (m, 2 H), 1.66 (s, 6 H), 1.78 (m, 2 H), 3.54 (s, 3 H), 4.29 (t, J = 7.6 Hz, 2 H), 6.23 (d, J = 13.6 Hz, 1 H), 6.41 (d, J = 13.2 Hz, 1 H), 7.21 (t, J = 7.2 Hz, 1 H), 7.34 (d, J = 7.6 Hz, 2 H), 7.39 (t, J = 7.6 Hz, 1 H), 7.53 (m, 3 H), 7.81 (d, J = 8.0 Hz, 1 H), 7.87 (t, J = 8.0 Hz, 1 H), 8.34 (t, J = 13.2, 1 H); 13C NMR (100 MHz, DMSO-d6): δ 14.08, 19.83, 28.42, 30.21, 44.58, 48.73, 91.17, 99.24, 110.92, 111.92, 112.55, 122.67, 124.51, 126.38, 126.79, 128.83, 131.56, 140.61, 143.39, 147.17, 148.13, 162.40, 172.75; HRMS: Calcd for C25H29N2O+ m/z 373.2280, obsd m/z 373.2281.

3-butyl-2-(-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide, 75c: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 86% yield; mp >250°C; 1H NMR (400 MHz, DMSO-d6): δ 0.95 (t, J = 7.2 Hz, 3 H), 1.45 (m, 2 H), 1.65 (s, 6 H), 1.76 (m, 2 H), 3.56 (s, 3 H), 4.44 (t, J = 7.2 Hz, 2 H), 6.29 (d, J = 12.8 Hz, 1 H), 6.82 (d, J = 13.2 Hz, 1 H), 7.22 (t, J = 7.2 Hz, 1 H), 7.39 (m, 2 H), 7.51 (t, J = 7.6 Hz, 1 H), 7.59 (d, J = 7.2 Hz, 1 H), 7.64 (t, J = 7.2 Hz, 1 H), 7.90 (d, J = 8.4 Hz, 1 H), 8.06 (m, 2 H); 13C NMR (100 MHz, DMSO-d6): δ 14.15, 19.86, 28.73, 30.22, 47.10, 48.78, 100.19, 102.41, 111.04, 114.86, 122.73, 123.80, 124.61, 125.94, 126.44, 128.86, 140.77, 141.72, 143.37, 147.94, 167.67, 172.27. HRMS: Calcd for C25H29N2S+ m/z 389.2046, obsd m/z 389.2061.

2-(-3-(1-butylbenzo[c,d]indol-2(1H)-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide, 76a: was synthesized by a similar procedure to 74a. The dye was purified by column chromatography
(1% methanol/dichloromethane). This compound was obtained in 79% yield; mp 128-130°C; $^1$H NMR (400 MHz, MeOD-d6): $\delta$ 1.04 (t, J = 7.6 Hz, 3 H), 1.55 (m, 2 H), 1.85 (s, 6 H), 1.89 (m, 2 H), 3.84 (s, 3 H), 4.32 (t, J = 7.6 Hz, 2 H), 6.82 (d, J = 13.6 Hz, 1 H), 6.87 (d, J = 13.6 Hz, 1 H), 7.41 (t, J = 7.2 Hz, 1 H), 7.49 (m, 3 H), 7.64 (d, J = 7.6 Hz, 1 H), 7.68 (d, J = 7.6 Hz, 2 H), 7.77 (d, J = 8.4 Hz, 1 H), 7.95 (t, J = 7.6 Hz, 1 H), 8.23 (d, J = 8.4 Hz, 1 H), 8.38 (d, J = 7.2 Hz, 1 H), 8.91 (t, J = 13.6 Hz, 1 H); $^{13}$C NMR (100 MHz, MeOD-d6): $\delta$ 12.83, 14.03, 19.88, 23.30, 29.27, 31.06, 43.56, 65.49, 105.97, 106.89, 109.28, 111.55, 122.11, 122.52, 125.09, 126.08, 126.61, 128.67, 129.30, 129.54, 130.29, 131.16, 141.31, 141.44, 142.58, 149.23, 155.41, 175.92. HRMS: Calcd for C$_{29}$H$_{31}$N$_2$ $^+$ m/z 407.2482, obsd m/z 407.2499.

1,3,3-trimethyl-2-(3-(1-(3-(trimethylammonio)propyl)benzo[cd]indol-2(1H)-ylidene)prop-1-en-1-yl)-3H-indol-1-ium bromide, 76b: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 38% yield; mp 134°C; $^1$H NMR (400 MHz, MeOD-d6): $\delta$ 1.77 (s, 6 H), 2.26 (m, 2 H), 3.10 (s, 9 H), 3.64 (m, 2 H), 3.92 (s, 3 H), 4.33 (t, J = 6.4 Hz, 2 H), 7.05 (d, J = 13.6 Hz, 1 H), 7.12 (d, J = 12.8 Hz, 1 H), 7.42 (t, J = 7.6 Hz, 1 H), 7.54 (t, J = 7.6 Hz, 1 H), 7.58 (d, J = 7.2 Hz, 1 H), 7.64 (d, J = 8.0 Hz, 1 H), 7.72 (t, J = 6.8 Hz, 1 H), 7.76 (m, 2 H), 8.00 (t, J = 7.6 Hz, 1 H), 8.28 (d, J = 8.4 Hz, 1 H), 8.36 (d, J = 7.2 Hz, 1 H), 8.73 (t, J = 13.6 Hz, 1 H); $^{13}$C NMR (100 MHz, MeOD-d6): $\delta$ 22.57, 28.09, 32.93, 40.93, 50.30, 52.99, 63.01, 107.73, 108.47, 109.39, 113.17, 122.44, 123.06, 125.23, 126.93, 127.19, 129.22, 129.80, 130.09, 130.23, 130.64, 131.41, 141.40, 142.09, 142.96, 148.87, 154.21, 176.40. HRMS: Calcd for C$_{31}$H$_{37}$N$_3$ $^+$ m/z 225.6488, obsd m/z 225.6170.

2-(3-(1-butylbenzo[cd]indol-2(1H)-ylidene)prop-1-en-1-yl)-1-methylbenzo[cd]indol-1-ium bromide, 79: was synthesized by a similar procedure to 74a. The dye was purified by precipitation from methanol with ether. This compound was obtained in 41% yield; mp >260°C; $^1$H NMR (400 MHz, DMSO-d6): $\delta$ 0.96 (t, J = 7.2 Hz, 3 H), 1.47 (m, 2 H), 1.83 (m, 2 H), 3.92 (s, 3 H), 4.36 (t, J = 7.2 Hz, 2 H), 7.19 (d, J = 13.2 Hz, 2 H), 7.72 (m, 4 H), 7.86 (d, J = 7.2 Hz, 2 H), 8.05 (m, 2 H), 8.34 (d, J = 7.6 Hz, 2 H), 8.65 (d, J = 7.2 Hz, 2 H), 9.16 (t, J = 13.2 Hz, 1 H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 0.59, 14.23, 20.14, 31.28, 31.85, 44.30, 55.40,
3-(3-phenylpropyl)-2-(3-(1,3,3-trimethylindolin-2-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium bromide, 82a: was synthesized by a similar procedure to 74a. The dye was purified by column chromatography (4% methanol/dichloromethane). This compound was obtained in 67% yield; mp 120-121°C; \(^1\)H NMR (400 MHz, MeOD-\(d_6\)): \(\delta\) 1.71 (s, 6 H), 2.24 (m, 2 H), 2.86 (t, \(J = 7.2\) Hz, 2 H), 3.61 (s, 3 H), 4.47 (t, \(J = 7.6\) Hz, 2 H), 6.15 (d, \(J = 12.8\) Hz, 1 H), 6.44 (d, \(J = 13.2\) Hz, 1 H), 7.27 (m, 5 H), 7.36 (m, 2 H), 7.42 (t, \(J = 7.6\) Hz, 1 H), 7.46 (m, 2 H), 7.62 (d, \(J = 8.0\) Hz, 2 H), 7.92 (d, \(J = 8.0\) Hz, 1 H), 8.16 (t, \(J = 13.2\) Hz, 1 H); \(^{13}\)C NMR (100 MHz, MeOD-\(d_6\)): \(\delta\) 27.64, 28.81, 32.01, 45.91, 99.36, 101.26, 110.02, 113.61, 121.86, 122.68, 124.36, 125.91, 126.12, 128.19, 128.26, 128.32, 128.37, 140.34, 141.29, 148.18, 167.74, 172.87 HRMS: Calcd for C\(_{30}\)H\(_{27}\)N\(_2\)S\(^+\) m/z 415.2169, obsd m/z 415.2172.

3-butyl-2-(3-(1,3,3-trimethylindolin-2-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide, 82b: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 86% yield; mp >250°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 0.95 (t, \(J = 7.2\) Hz, 3 H), 1.45 (m, 2 H), 1.65 (s, 6 H), 1.76 (m, 2 H), 3.56 (s, 3 H), 4.44 (t, \(J = 7.2\) Hz, 2 H), 6.29 (d, \(J = 12.8\) Hz, 1 H), 6.82 (d, \(J = 13.2\) Hz, 1 H), 7.22 (t, \(J = 7.2\) Hz, 1 H), 7.39 (m, 2 H), 7.51 (t, \(J = 7.6\) Hz, 1 H), 7.59 (d, \(J = 7.2\) Hz, 1 H), 7.64 (t, \(J = 7.2\) Hz, 1 H), 7.90 (d, \(J = 8.4\) Hz, 1 H), 8.06 (m, 2 H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 14.15, 19.86, 28.73, 30.22, 47.10, 48.78, 100.19, 102.41, 111.04, 114.86, 122.73, 123.80, 124.61, 126.44, 128.86, 140.77, 141.72, 143.37, 147.94, 167.67, 172.27. HRMS: Calcd for C\(_{25}\)H\(_{29}\)N\(_2\)S\(^+\) m/z 389.2046, obsd m/z 389.2061.

6-bromo-3-butyl-2-(3-(1,3,3-trimethylindolin-2-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide, 82c: was synthesized by a similar procedure to 74a. No purification was needed. This compound was obtained in 85% yield; mp >260°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 0.94 (t, \(J = 7.2\) Hz, 3 H), 1.43 (m, 2 H), 1.65 (s, 6 H), 1.71 (m, 2 H), 3.58 (s, 3 H), 4.39 (t, \(J = 7.2\) Hz, 2 H), 6.34 (d, \(J = 13.2\) Hz, 1 H), 6.78 (d, \(J = 12.8\) Hz, 2 H), 7.40 (d, \(J = 8.4\) Hz, 1 H), 8.06 (m, 2 H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 14.15, 19.86, 28.73, 30.22, 47.10, 48.78, 100.19, 102.41, 111.04, 114.86, 122.73, 123.80, 124.61, 126.44, 128.86, 140.77, 141.72, 143.37, 147.94, 167.67, 172.27. HRMS: Calcd for C\(_{25}\)H\(_{29}\)N\(_2\)S\(^+\) m/z 389.2046, obsd m/z 389.2061.
Hz, 1 H), 7.25 (t, J = 6.8 Hz, 1 H), 7.41 (m, 2 H), 7.60 (d, J = 7.2 Hz, 1 H), 7.81 (m, 2 H), 8.02 (t, J = 13.2 Hz, 1 H), 8.32 (s, 1 H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 14.16, 19.81, 28.63, 30.07, 47.14, 49.00, 100.87, 102.06, 111.35, 116.28, 118.25, 122.76, 124.95, 126.05, 127.96, 128.92, 131.57, 140.93, 141.21, 143.27, 148.30, 167.18, 172.97. HRMS: Calcd for C\(_{25}\)H\(_{28}\)BrN\(_2\)S\(^+\) m/z 467.1151, obsd m/z 467.1149.

3-ethyl-2-(3-(3-ethylbenzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide, 85a:
Quaternary ammonium salt 84a and triethyl orthoformate were heated in acetic anhydride for 2 hours. The solution was allowed to cool to room temperature and poured into ether. The ether was decanted and more ether was added three times before the solid was filtered. No purification was needed. This compound was obtained in 72% yield; mp >260°C (lit mp 267-269°C, 86% yield\(^{7b}\)); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 1.35 (t, J = 6.8 Hz, 6 H), 4.39 (d, J = 6.8 Hz, 4 H), 6.63 (d, J = 13.2 Hz, 2 H), 7.43 (t, J = 7.6 Hz, 2 H), 7.58 (t, J = 8.0 Hz, 2 H), 7.80 (m, 3 H), 8.01 (d, J = 7.6 Hz, 2 H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 13.08, 21.53, 41.94, 99.10, 113.84, 123.59, 125.67, 128.59, 141.27, 147.13, 164.64.

3-butyl-2-(3-(3-butylbenzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide 85b:
was synthesized by a similar procedure to 85a. No purification was needed. This compound was obtained in 66% yield; mp >260°C (lit mp 283-284°C\(^{7b}\)); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 0.94 (t, J = 7.2 Hz, 6 H), 1.43 (m, 4 H), 1.72 (m, 4 H), 4.34 (t, J = 7.2 Hz, 4 H), 6.62 (d, J = 12.8 Hz, 2 H), 7.43 (t, J = 7.6 Hz, 2 H), 7.58 (t, J = 7.6 Hz, 2 H), 7.78 (m, 3 H), 8.00 (d, J = 7.6 Hz 2 H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 13.70, 19.41, 29.44, 46.08, 98.78, 113.64, 123.06, 125.22, 128.08, 139.48, 141.26, 146.68, 164.62.

3-(3-phenylpropyl)-2-(3-(3-(3-phenylpropyl)benzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide 85c: was synthesized by a similar procedure to 85a. No purification was needed. This compound was obtained in 67% yield; mp 220-221°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.09 (t, J = 7.2 Hz, 4 H), 2.79 (m, 4 H), 4.38 (t, J = 7.2 Hz, 4 H), 6.43 (d, J = 12.0 Hz, 2 H), 7.26 (m, 12 H), 7.56 (t, J =6.8 Hz, 2 H), 7.69 (d, J = 7.2 Hz, 2 H), 7.76 (t, J = 12.8 Hz, 1 H), 7.99 (d, J = 7.2 Hz, 2 H); \(^{13}\)C NMR
60
(100 MHz, DMSO-d6): δ 28.57, 31.78, 45.82, 113.37, 122.89, 125.09, 125.94, 127.91, 128.05, 128.25, 140.51, 141.07. HRMS: Calcd for C_{35}H_{33}N_{2}S_{2} m/z 545.2080, obsd m/z 545.2061.

5-bromo-2-(3-(5-bromo-3-butylbenzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)-3-butylbenzo[d]thiazol-3-ium iodide, 88a: was synthesized by a similar procedure to 85a. No purification was needed. This compound was obtained in 77% yield; mp >260°C; 1H NMR (400 MHz, DMSO-d6): δ 0.94 (t, J = 7.2 Hz, 6 H), 1.43 (m, 4 H), 1.69 (m, 4 H), 4.33 (t, J = 7.2 Hz, 4 H), 6.63 (d, J = 12.8 Hz, 2 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.79 (t, J = 12.8 Hz, 1 H), 7.95 (d, J = 8.4 Hz, 2 H), 8.08 (s, 2 H); 13C NMR (100 MHz, DMSO-d6): δ 0.56, 14.20, 19.78, 29.91, 46.70, 100.02, 116.78, 121.59, 125.06, 125.10, 128.43, 143.14, 147.65. HRMS: Calcd for C_{25}H_{27}Br_{2}N_{2}S_{2} m/z 576.9977, obsd m/z 576.9983.

6-bromo-2-(3-(6-bromo-3-butylbenzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)-3-butylbenzo[d]thiazol-3-ium iodide, 88b: was synthesized by a similar procedure to 88a. This compound was obtained in 76% yield; mp >260°C; 1H NMR (400 MHz, DMSO-d6): δ 0.93 (t, J = 7.2 Hz, 6 H), 1.42 (m, 4 H), 1.69 (m, 4 H), 4.31 (t, J = 7.2 Hz, 4 H), 6.62 (d, J = 12.8 Hz, 2 H), 7.77 (m, 5 H), 8.08 (s, 2 H); 13C NMR (100 MHz, DMSO-d6): δ 0.56, 14.15, 19.83, 29.85, 46.80, 99.84, 115.73, 117.63, 125.91, 127.77, 131.34, 141.20, 147.52, 165.24. HRMS: Calcd for C_{25}H_{27}Br_{2}N_{2}S_{2} m/z 576.9977, obsd m/z 576.9983.

3-ethyl-2-(3-(3-ethylnaphtho[2,1-d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)naphtho[2,1-d]thiazol-3-ium iodide, 91a: was synthesized by a similar procedure to 88a. The dye was purified by chromatotron (4% methanol/dichloromethane). This compound was obtained in 70% yield; mp >260°C; 1H NMR (400 MHz, DMSO-d6): δ 1.40 (t, J = 6.4 Hz, 6 H), 4.47 (q, J = 6.0 Hz, 4 H), 6.47 (d, J = 12.4 Hz, 2 H), 7.55 (t, J = 7.6 Hz, 2 H), 7.93 (m, 5 H), 8.06 (d, J = 7.2 Hz, 2 H), 8.14 (d, J = 7.6 Hz, 2 H); 13C NMR (100 MHz, DMSO-d6): δ 13.47, 42.43, 99.21, 113.18, 121.40, 123.52, 126.75, 127.00, 129.07, 129.59, 130.64, 139.39, 145.86, 163.44. HRMS: Calcd for C_{29}H_{25}N_{2}S_{2} m/z 465.1454, obsd m/z 465.1455.

3-butyl-2-(3-(3-butylnaphtho[2,1-d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)naphtho[2,1-d]thiazol-3-ium iodide, 91b: was synthesized by a similar procedure to 88a. No purification was needed. This compound
was obtained in 60% yield; mp >260°C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 0.96 (t, J = 7.2 Hz, 6 H), 1.47 (m, 4 H), 1.78 (m, 4 H), 4.45 (t, J = 7.2 Hz, 4 H), 6.69 (d, J = 12.8 Hz, 2 H), 7.61 (t, J = 8.0 Hz, 2 H), 7.74 (t, J = 8.0 Hz, 2 H), 7.93 (m, 5 H), 8.10 (d, J = 8.0 Hz, 2 H), 8.16 (d, J = 9.2 Hz, 2 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 14.22, 19.88, 30.28, 47.00, 99.44, 113.48, 121.30, 123.59, 126.71, 127.11, 129.15, 129.65, 129.88, 130.65, 139.86, 145.96, 163.86. HRMS: Calcd for C$_{33}$H$_{33}$N$_2$S$_2$ $^+$ m/z 521.2080, obsd m/z 521.2075.

3-(3-phenylpropyl)-2-(3-(3-(3-phenylpropyl)naphtho[2,1-d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)naphtho[2,1-d]thiazol-3-ium bromide, 91c: was synthesized by a similar procedure to 88a. No purification was needed. This compound was obtained in 50% yield; mp 231-233°C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.15 (m, 4 H), 2.82 (m, 4 H), 4.515 (m, 4 H), 6.52 (d, J = 12.4 Hz, 2 H), 7.21 (m, 2 H), 7.28 (m, 7 H), 7.64 (t, J = 7.6 Hz, 2 H), 7.76 (t, J = 7.6 Hz, 2 H), 7.92 (m, 3 H), 8.00 (d, J = 8.0 Hz, 2 H), 8.15 (t, J = 9.6 Hz, 4 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 29.60, 32.34, 46.82, 113.39, 121.42, 123.60, 126.59, 126.74, 127.16, 128.70, 128.90, 129.19, 129.69, 129.88, 130.69, 139.86, 141.18, 145.94. HRMS: Calcd for C$_{43}$H$_{37}$N$_2$S$_2$ $^+$ m/z 645.2393, obsd m/z 645.2375.
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APPENDIX

$^1$H NMR, $^{13}$C NMR, and HRMS Spectra.
DY_AL-35_HENARY-ACCU_11-12-2012_ESI-POS 36 (0.377) AM (Cen, 2. 80.00, Ar, 5000 0.556.28, 0.70; Sm (SG, 3x3.00); Cm (34.4) 1.45
ACN+0.1% HCOOH

JY_AL-49_HENARY-ACC_11-14-2012_ESI-POS 125 (1.322) AM (Cen, 2, 80.00, Ar, 5000 0.556 28.0 70); Cm (124:134) TOF MS E: 1.18

Chemical structure and mass spectrum analysis.
diluted in 80% MeOH

ANDY_AL-63-74J_HENARY-ACCU_04022013_ESI-POS01 141 (2.811) Cm (133.146)

TOF MS ES+ 1.38e3
110
diluted in 80% MeOH

ANDY_AL-66-88A_HENARY-ACCU_04022013_ESI-POS01 69 (1.376) AM (Cen, 2, 80.00, Ar, 5000, 0, 556.28, 0.70); Cm (51.75)

3.11e+04
diluted in 80%MeOH

ANDY_AL-70-91B_HENARY-ACCU_04022013_ESI-POS01 87 (1.735) AM (Cen, 2, 80.00, Ar, 5000, 0.556.28, 0.70); Sm (SG, 3x): 6.65e3

91b