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Synthesis of Novel Fluorescent Benzothiazole Cyanine Dyes as Potential Imaging Agents

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SYNTHESIS OF NOVEL FLUORESCENT BENZOTHIAZOLE CYANINE DYES AS POTENTIAL IMAGING AGENTS

by

SHIRISH PARANJPE

Under the Direction of Prof. Maged Henary

ABSTRACT

Near-infrared (NIR) fluorescence imaging has emerged as an attractive non-invasive approach for direct visualization of diseases which depends on the development of stable, highly specific and sensitive optical probes. The NIR region of the electronic spectrum offers a reduction in the background autofluorescence and an increase in the tissue penetration depth. Cyanine dyes have often been considered promising contrast optic agents owing to their photophysical properties.

Herein the synthesis of various penta- and heptamethine benzothiazole cyanine dyes has been described and their in vivo imaging efficacy was determined. Varying functionalities on the benzothiazole aromatic ring and changing substituents on the benzothiazolium nitrogen atom reflected subsequent changes in the imaging pattern and have resulted in the development of promising brain targeting agents.

INDEX WORDS: Cyanine dyes, Benzothiazole, Heptamethine, Pentamethine, Near-infrared, Imaging, Brain
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SHIRISH PARANJPE

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**Figure 20.** Real-time intraoperative image-guided surgery of compound 85 in orthotopic brain tumor mice (biodistribution and targeting study of the benzothiazole cyanine dyes was conducted by Drs. Hak Soo Choi and John Frangioni at Beth Israel Deaconess Medical Center (BIDMC), Harvard Medical School in Boston, MA) ................................................................. 42
1 INTRODUCTION TO CYANINE DYES

1.1 Various structural characteristics of carbocyanine dyes

Cyanine dyes are unique class of compounds that have a wide range of applications in numerous fields. The first member of this type was reported by C.H.G. Williams in 1856.1 The name cyano was given due to beautiful blue (blue – kyano in Greek) color of the dye.2 This dye was obtained by treatment of quinoline and 4-methylquinoline with amyl iodide followed by reaction with ammonia. H. W. Vogel in 1873 found that cyanine dyes can be used to increase sensitivity of the photographic plate.3 It was the turning point in the history of the cyanine dyes.

The general structure of the polymethine dye is represented as shown in Figure 1. Polymethine dyes consist of two nitrogen centers joined by conjugated chain of odd number of methine carbons or conjugated system of double bonds. This polymethine bridge connects an electron acceptor group at one end and electron donor group at the other. Conjugation between electron donor and acceptor group results in delocalization of π electrons and hence positive charge over the two nitrogen atoms.

![General structure of the polymethine dye](image)

**Figure 1.** General structure of the polymethine dye

Common names of the cyanine dyes are based on the number of methine carbons present in the polymethine chain. In Figure 1, the polymethine cyanine dyes are designated as mono-, tri-, penta- and heptamethine cyanines for n = 0, 1, 2, 3 respectively. The absorption/emission wavelengths of the cyanine dyes depend upon the length of the polymethine chain and the nature of the terminal groups. The monomethine and trimethine cyanine dyes usually absorb in the visible region (500–600 nm) of the elec-
tronic spectrum with each added (CH = CH) methine unit causes bathochromic shift of about 100 nm in
the electronic spectrum resulting in an absorption wavelength of 700-800 nm for penta- and heptamethine
cyanines. The 4-pyridinium, 4-thiopyridinium and benz[c.d]indole heterocyclic end groups extend absorp-
tion/emission wavelength well into the near-infrared (NIR) region whereas presence of benzoxazole end
group results in hypsochromic shift in the electronic spectrum. Polymethine cyanine dyes are generally
classified based on the nature of the end groups present on the polymethine chain. Polymethine dyes with
two heterocyclic terminal groups are referred to as closed chain cyanine dyes or generally referred to as
cyanines. The two heterocycles in the cyanines can either be the same or can be different. Hemicyanines
are characterized by presence of one heterocyclic and another noncyclic end group. Dyes without a termi-
nal heterocyclic moiety are defined as streptocyanines or open chain cyanines as shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Classification of cyanine dyes</th>
</tr>
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<tbody>
<tr>
<td><strong>Structural Formula</strong></td>
</tr>
<tr>
<td><strong>Cyanine</strong></td>
</tr>
<tr>
<td>![Structural Formula for Cyanine]</td>
</tr>
<tr>
<td><strong>Hemicyanine</strong></td>
</tr>
<tr>
<td>![Structural Formula for Hemicyanine]</td>
</tr>
<tr>
<td><strong>Streptocyanine</strong></td>
</tr>
<tr>
<td>![Structural Formula for Streptocyanine]</td>
</tr>
</tbody>
</table>

Cyanine dyes are structurally classified as symmetrical or asymmetrical cyanine dyes as depicted in Fig-
ure 2. These two classes of cyanine dyes show wide variety of differences with respect to spectral charac-
teristics and nucleic acid binding behavior.⁴
Figure 2. Classification of cyanine dyes

In 1926, Koenig identified the chromophoric nature of the polymethine structure of the cyanine dye. The first reported synthesis of chiral polymethine dye was reported by Koenig et al. Since then many different types of cyanine dyes have been synthesized. The first bridged cyanine dye synthesis was published in 1933 where trimethine chain formed a part of cyclopentadiene ring. Some naturally occurring cyanine dyes have been isolated from *beta vulgaris* and *amanita muscaria*. Cyanine dyes possess some characteristic properties which include relatively high photochemical stability, narrow absorption band, high molar absorption coefficients (~10^5 M^-1 cm^-1), ability to form H- or J-aggregates and high fluorescence intensity. A large number of cyanine dyes have been synthesized using several different heterocycles such as indolenine, quinoline, benzoxazole and benzothiazole.

Benzothiazole derivatives have a planar structure which is essential criteria for nucleic acid binding and hence in the past few years benzothiazole dyes are emerging as an effective biological marker. The use of benzothiazole compounds as an *in vivo* imaging agent for Alzheimer’s disease considered as a major breakthrough for benzothiazole studies. This imaging capability of the benzothiazole compounds was the crucial factor for synthesis of novel penta- and heptamethine benzothiazole cyanine dyes which will be discussed later in the thesis.

The next chapter reviews various synthetic routes and applications of benzothiazole cyanine dyes.
1.2 Synthesis of benzothiazole cyanine dyes

The first benzothiazole cyanine dye was reported back in 1887 by Hoffman. It was synthesized by heating amyl iodides of benzothiazole and 2-methylbenzothiazole in presence of ammonia. Since then several symmetric and asymmetric benzothiazole cyanine dyes have been synthesized.

Benzothiazole cyanine dyes are commonly classified into four categories namely mono-, tri-, penta- and heptamethine cyanine dyes. The spectral range for the benzothiazole cyanine dyes extends roughly between 450–750 nm in the electronic spectrum depending upon the different substitutions and modifications of benzothiazole cyanine dyes. In the past few years, many synthetic routes have been discussed for the synthesis of benzothiazole cyanine dyes. Following review covers different synthetic routes and modifications of benzothiazole cyanine dyes.

1.2.1 Monomethine cyanine dyes

Monomethine benzothiazole cyanine dyes typically absorb in the visible region (450–470 nm) of the electronic spectrum depending upon the substituents attached to the benzothiazole core structure. These dyes are characterized by a narrow absorption peak in the electromagnetic spectrum and high fluorescence intensity, and they are best known for their nucleic acid binding properties. The oldest method for the synthesis of monomethine benzothiazole cyanine dye has been suggested by Brooker et al. This method involves condensation of 2-methylthio salt of alkylated benzothiazole with another alkylated heterocycle with an activated methyl group. This method was adopted for the synthesis of β-cyclodextrin functionalized benzothiazole cyanine dye 1. The compound 1 was used to study supramolecular interactions of β-cyclodextrin directly by visible spectroscopy which is otherwise impossible due to insufficient chromophore system.

\[
\begin{align*}
\text{HS} & + \text{N}^+ \\
\text{TsO} & \rightarrow \text{N}^+ \\
& \hspace{1cm} \text{(1)}
\end{align*}
\]
The synthetic procedure shown above involves a major drawback of producing toxic pollutant methyl mercaptan. Researchers overcome this problem by developing a new procedure to use 2-imino benzothiazoline instead of 2-thiomethyl benzothiazolium salt. This method can be used to synthesize symmetric as well as asymmetric cyanine dyes. In this procedure, cyanine dyes are prepared by melting of 2–iminobenzothiazoline with quaternary heterocyclic salt containing 2- or 4-methyl group as depicted in Equation 2.

Gadjev et al. described a novel procedure for the synthesis of benzothiazole monocyanines which involves heating sulfobetaine salt of N-alkylbenzothiazolium compound with the quaternary salt of heterocyclic compound containing reactive methylene group as described in Equation 3. These reactions are usually carried out neat at about 150–200 °C. Alternative route is used for less thermostable compounds where reactions are carried out in presence of boiling polar solvents. This synthetic procedure gives high yields in less reaction time.

Another approach towards synthesis of monomethine cyanine dyes involves condensation of N-alkyl-2-methyl benzothiazolium salt with 2- or 4-chloro heterocycle in a basic media. This synthetic procedure was used to synthesize dicationic and tricationic benzothiazole cyanine dyes (Equation 4). The purpose of increased cationic charge helps to increase water solubility of the dye.
It is also been observed that increasing cationic charges in the dye molecule results in improved binding to nucleic acid and leads to higher fluorescence intensity. Several homodimeric benzothiazole cyanine dyes have been published with tetracationic charge on the overall cyanine dye (Equation 5).\textsuperscript{19}

A novel method for the synthesis of monomeric asymmetric benzothiazole derivatives containing mercapto and thioacetyl substituents was developed by Deligeorgiev et al.\textsuperscript{20} The synthetic methodology is described in Scheme 1. The preparation of the dye intermediate 6 was carried out by refluxing 2-methylmercaptobenzothiazole with 1,3-dibromopropane for 2 hours. Compound 6 was then treated with 1,4-dimethylquinolinium iodide in solvent acetic anhydride and a base triethylamine yields dye 7 with 95% overall yield.

![Scheme 1](image-url)
A new method for the synthesis of benzothiazole monocyanines was developed making use of solid phase resin support. The schematic representation of the solid phase synthesis is described in the Scheme 2. The first step of this synthetic method involves the attachment of 2-mercaptobenzothiazole to the merrifield resin. This merrifield resin attached benzothiazole was then reacted in the next step with 4-methylbenzenesulfonate to yield N-methyl benzothiazolium salt 8. In the final step the condensation of the N-methylbenzothiazolium salt 8 with carboxylic acid derivative of lepidine afforded cyanine dye 9. Traceless cleavage of merrifield resin was carried out during the course of the synthesis of the cyanine dye. Presence of Carboxylic acid side chain attached to the quinoline ring makes dye available for post synthetic modification. Attaching amino acid to the carboxylic end group and modifying it with folic acid can be used to identify cancer cells that have extra folacin acceptor on the cellular surface. The most important feature of the solid phase synthesis is the elimination of the lengthy purification step involved with the conventional liquid phase method which allows the use of synthesized dyes directly for the biological screening and elimination of the use of toxic catalyst provides greener approach.

\[ \text{Scheme 2} \]
1.2.2 Trimethine cyanine dyes

Benzothiazole trimethine cyanine dyes absorb in the visible region (550–570 nm) of the electronic spectrum. The classical orthoester method discovered by Koenig is the oldest known method for the synthesis of symmetric trimethine cyanine dyes. The general synthetic procedure involves condensation of the quaternary benzothiazolium salt with activated methyl group with orthoesters under basic conditions. The orthoester approach has been exemplified in Scheme 3 by describing synthesis of a dumbbell-type [60] fullerene dimer containing a benzothiazole trimethine cyanine dye. Fullerene chemistry is one of the rapidly growing divisions of material science. C₆₀ dyads have received a lot of attention in recent years due to their applicability in artificial photosynthesis and molecular electronic devices. The first step of the synthesis involves attachment of [60] fullerene to the benzothiazole ring which is achieved by refluxing a solution of [60] fullerene, sarcosine and 5-formyl-2-methylbenzothiazole under nitrogen atmosphere. The resulting compound is then treated with dimethyl sulfate to generate quaternary salt which undergoes coupling reaction with triethyl orthoformate in the next step to yield cyanine dye.
A new class of phosphonate labeled trimethine benzothiazole cyanine dyes has been published using orthoester approach. Synthetic methodology for the phosphonate labeled cyanine dye starts with the synthesis of compound 12 which is obtained by refluxing 2-methylbenzothiazole with diethyl 3-bromopropylphosphonate in acetonitrile. Phosphonate salt 12 was then converted to cyanine dye 14 using triethyl orthoformate under basic conditions. Conversion of phosphonate salt to phosphonic acid salt was achieved by acidic hydrolysis which is used in the further step to synthesize dye 13. The presence of phosphonic acid side chain improves water solubility and helps to increase complexing ability of the benzothiazole cyanine dye.

![Scheme 4](image_url)

**Scheme 4**

Another approach for the synthesis of benzothiazole trimethine cyanine dye involves usage of \(N,N\)-diphenylformamidine or iodoform as a condensing agent. This synthetic method allows synthesis of asymmetrical cyanine dyes as well. Scheme 5 describes the use of \(N,N\)-diphenylformamidine for the synthesis of benzothiazole trimethine cyanine dye. Alkylated benzoazole derivative is first treated with \(N,N\)-diphenylformamidine in presence of acetic anhydride to obtain half dye 15. Compound 15 in the next step is condensed with 2, 3-dimethylbenzothiazolium iodide to yield final asymmetric dye 16. Different
solvents systems can be used for the synthesis of asymmetric cyanine dyes using N,N-diphenylformamidine approach. The reactions are usually carried out in acetic anhydride but can also be conducted in n-propanol or DMSO at high temperatures for several hours.

**Scheme 5**

Park *et al.* developed novel triazacarbocyanine intramolecular charge transfer dye sensor as described in Equation 6.\(^{27}\) This dye sensor showed reversible pH induced color switching effects. Here methoxy group acts as a donor group and nitro group acts as an acceptor group.

\[
\text{Equation 7 depicts the synthesis of trimethine cyanine dye with meso substitution in the trimethine chain. The meso methyl group undergoes condensation reaction with 9-julolidinecarboxaldehyde to furnish benzothiazole derivative 18.}^{28}
\]
1.2.3 Pentamethine cyanine dyes

Pentamethine cyanine dyes absorb generally in the visible region (650–670 nm) but fluoresce in the near-infrared region (690–710 nm) of the electronic spectrum. The general synthetic procedure involves use of malonaldehyde bis(phenylimine)monohydrochloride under basic conditions as exemplified in Equation 8.\textsuperscript{29, 30}

\[
\text{PhHN} \xrightarrow{\text{AC}_2\text{O or ACN}} \text{NHPh} \xrightarrow{\text{Et}_3\text{N or NaOAc}} \text{19}
\]

Mehranpour \textit{et al.} modified malonaldehyde linker to form γ-substituted pentamethine cyanine dyes as shown in Scheme 6.\textsuperscript{31} The new γ-substituted pentamethine cyanine dyes were synthesized using three step procedure. First step involves synthesis of \(N\)-pyridinium acetic acid salt \(20\) by reaction between 3, 5-dimethylpyridine with bromoacetic acid. In the second step compound \(20\) was formylated in presence of \(N,N\)-dimethylformamide and phosphorus oxychloride to obtain compound \(21\). The synthesis of cyanine dye \(22\) was carried out by condensation reaction between compound \(21\) and 2,3-dimethyl benzothiazole tetrafluoroborate in presence of sterically hindered hunig base. The presence of the pyridine ring in the pentamethine chain makes the dye more rigid and the cationic charge makes it water soluble.

\[
\text{Scheme 6}
\]
Yagupolskii et al. developed a benzothiazole cyanines with fluorine substituted pentamethine chain as described in Scheme 7. Stille coupling reaction was employed in the first step for coupling of 2-iodobenzothiazole with fluorinated polymethine chain in presence of copper iodide and tetrakis(triphenylphosphine) palladium (0) which afforded compound 23. Compound 23 was alkylated by reaction with methyl iodide in presence of silver tetrafluoroborate to obtain compound 24 which on reaction with 2-fluoromethylbenzothiazole methylene base yields cyclic compound 25. Compound 25 was subjected to alkylation reaction which on further reaction with p-dimethylaminotoluidine provides pentamethine cyanine dye 26.

The synthesis of asymmetrical pentamethine benzothiazole cyanine dyes can be furnished utilizing aldehyde analogue of the benzothiazole moiety. Meguellati et al. adapted aldehyde analogue strategy to synthesize imino pentamethine cyanine dye as depicted in Scheme 8. These dyes remain stable in solid state where as in solution within an hour regenerate aldehyde and imine. Compound 29 was synthesized by reacting malonaldehyde bis(phenylimine)monohydrochloride with 1,2,3,3-tetramethylindolenine iodide salt. Basic hydrolysis of the activated hemicyanine derivative 27 in sodium hydroxide solution afforded compound 28 in excellent yield. In the last step compound 28 was treated
with N-methyl-2-amino benzothiazolium salt to yield cyanine dye 29. The introduction of the imine bond in the polymethine chain makes dye formation reversible owing to the external reaction conditions.

Scheme 8

Losytskyy et al. synthesized a series of benzothiazole fluorophores with cyclohexene and cyclopentene ring substitution in the pentamethine chain. These synthesized compounds were studied as fluorescent probes for nucleic acid and proteins and hypothesized to act as nucleic acid groove binders. Synthesis of pentamethine cyanine dyes 34, 35 containing cyclopentene group in the pentamethine chain was furnished by condensing 2-methylcyclopentane-1,3-diones 30, 31 with quaternary salts of 2-methylbenzothiazolium 32, 33 at 210 °C under basic conditions of triethylamine (Equation 9).

Synthesis of pentamethine dyes with cyclohexene ring in the pentamethine chain was achieved by condensation of quaternary salts of benzothiazole containing active methylene group with the 1,5-dimethoxy-1,4-cyclohexadienes or with 1,3-diethoxy-5,5-dimethyl- or 1,3-diethoxy-2,5,5-trimethyl-1,3-
cyclohexadienes either by melting the two compounds or by heating in benzonitrile at 120-130°C as shown in Equation 10.

\[ \text{[Diagram of reaction]} \]

\[ 210^\circ C, 30 \text{ min} \]

\[ \text{Et}_3\text{N} \]

1.2.4 Heptamethine cyanine dyes

Heptamethine cyanine dyes typically absorb and fluoresce in the near-infrared region (750 – 1100 nm) of the electronic spectrum. The general synthetic method for the synthesis of benzothiazole heptacyanines with flexible polymethine chain involves use of glutaconaldehyde dianil monohydrochloride as a heptamethine linker. As described in Equation 11, various benzothiazole heptamethine derivatives have been synthesized varying the substituent attached to the benzothiazole nitrogen. (Equation 11)

\[ \text{[Diagram of reaction]} \]

\[ \text{R} = \text{CH}_3, \text{CH}_2\text{CH}_3, \text{CH}_2\text{Ph}, \text{CH}_2\text{CH}_2\text{OCOCH}_3 \]

Another class of benzothiazole heptamethine dyes has been published containing five- or six-membered cyclic system as a part of heptamethine linker making use of Vilsmeier-Haack reagent. The presence of carbocyclic ring in the heptamethine chain makes dye more rigid which helps to increase fluorescence quantum yield and decrease the aggregation of the dye. Vilsmeier-Haack reagents are derived from cyclopentanone or cyclohexanone in two step procedure as described in Equation 12.
General methodology for the synthesis of heptamethine cyanines with rigid polymethine chain involves condensation of a Vilsmeier-Haack reagents 38 or 39 and a heterocycle with activated methyl group in ethanol in presence of sodium acetate catalyst.\textsuperscript{41} Another approach for the synthesis of heptamethine cyanine dyes involves refluxing a quaternary salt of benzothiazole and bis aldehyde in butanol:benzene solvent mixture with continuous removal of the water from the reaction mixture using Dean–Stark apparatus.\textsuperscript{42} The major advantage of using this method of synthesis is it eliminates usage of catalyst and provides final dye 40 in high yields (Equation 13).

1.3 Applications of benzothiazole cyanine dyes

The field of cyanine dyes is ever expanding field of science. Since the discovery of cyanine dyes, their application was limited to photographic sensitizers.\textsuperscript{43} Other than photography, cyanine dyes also finds applications in several other fields such as recording media\textsuperscript{44}, laser materials\textsuperscript{45}, solar cells\textsuperscript{46} \textsuperscript{47} \textsuperscript{48} semiconductors.\textsuperscript{49} \textsuperscript{50}

Cyanine dyes have always been regarded as good silver halide photography sensitizers. A silver halide solution possesses limited sensitivity up to 550 nm but when it is doped with dye its sensitivity extends up to 650 nm and sometimes even into near-infrared region of the electronic spectrum. Sensitizer dyes show bathochromic shift when added to the silver halide solution by up to 20-80 nm. Benzothiazole dyes 41 and 42 are used as photographic sensitizers.\textsuperscript{51}
Another important application of benzothiazole dyes is its use in recording media. Most of the CD-R uses indolenine based cyanine dyes as recording dye. With the discovery of the DVD-R which requires shorter wavelength laser beam, benzothiazole trimethine cyanine dye was considered as better dye to get appropriate reflection and modulation of the DVD-R. Another advantage of using trimethine cyanine dye is it shows good chemical and photostability. Typically benzothiazole cyanine dyes such as compound 43 are used for the purpose of recording media.

Dye sensitized solar cells have attracted tremendous attention since last decade. Cyanine dyes substituted heavy metal based complexes as sensitizers in these solar cells. Advantages of using cyanine dyes as solar cell sensitizers include cost efficiency and easy recycling of solar cells. Several benzothiazole cyanine dyes such as compound 44 have been synthesized and studied for their efficiency towards sensitization of solar cell.
Optical imaging is one of the widely recognized and emerging areas of research which focuses mainly on studying molecular and metabolic functions *in vivo*. In the past few years, cyanine dyes have emerged as optical contrast agents for image guided surgery owing to their appealing photophysical properties. Compared to alternate imaging methods such as positron emission tomography (PET), this optical imaging technique using fluorescent probe is relatively harmless, highly sensitive and non-invasive. Different imaging modalities such as magnetic resonance imaging (MRI), computed tomography (CT) make use of exogenously applied contrast agents and mainly produce signals particular to the disease tissue thus allowing to monitor physiological and molecular conditions distinctive of specific disease state. The major obstacles to optical imaging involves strong absorption by hemoglobin, deoxyhemoglobin, water, and lipids in the mid-visible region of the electronic spectrum which eventually affects the penetration of the light inside tissue and hence the obtained information reflects only the superficial characterization. Cyanine dyes used for optical imaging particularly absorb and fluoresce in the near-infrared region of the electronic spectrum. Indocyanine green (ICG) was the first cyanine dye approved by FDA for imaging techniques contains the benz[e]indolium heterocyclic moiety as a part of the dye structure.

![Figure 3. Structure of Indocyanine Green (ICG)](image)

There are certain drawbacks associated with ICG as a contrast agent such as lack of target specificity, low renal clearance of the dye (high tissue retention) and instability in the aqueous media. In the past few years, more and more research has focused on correcting the problems associated with ICG. Researchers chemically modified the indolenine moiety to increase hydrophilicity. In another approach, ICG was bound to either peptides or antibodies to make the dye target specific. As most of the research
is associated with already developed contrast optic agent ICG, very few publications concerning \textit{in vivo} imaging are associated with other cyanine dye structures. Frangioni and Henary recently developed a zwitterionic near-infrared fluorophore to be used as a probe for image-guided surgery.\textsuperscript{54}

In this thesis we focus on developing hepta- and pentamethine benzothiazole cyanine dyes as imaging agents. The bio-distribution study was conducted in collaboration with Drs. Frangioni and Choi, Beth Israel Deaconess Medical Center, Boston, MA, USA.

\section{RESULTS AND DISCUSSIONS}

\subsection{Aim of the current study}

Most of the published papers report radio labeled benzothiazole compounds as contrast agents which are used in conjunction with Positron Emission Tomography (PET) imaging technology.\textsuperscript{57, 58} PET provides good sensitivity for deep tissue imaging but it is bound by several limitations such as low spatial resolution, patient exposure to radioactive substances, high instrumentation cost and data acquisition rate is relatively low.\textsuperscript{59} Optical imaging is emerging as a quick, non-radioactive, cost efficient and high resolution imaging technique. In the standard imaging technique, the contrast signal is generated by irradiating light on the tissue and collection of the emitted fluorescent light at a higher wavelength with optical separation of the excitation wavelength. The near-infrared region of the electromagnetic spectrum furnishes two basic advantages which include reduction in the background fluorescence arising from biological tissues and an increase in the light penetration depth. As tissues generate very little near- infrared signal, external near-infrared absorbing agent is administered for \textit{in vivo} studies. The fundamental problems associated with this optical imaging technique include non-specificity of the contrast agents towards body tissues and several physiological issues regarding tissue retention causing increased background signal.

Designing near-infrared absorbing optical contrast agents possessing organ selectivity and total body clearance has always remained a challenge to scientists. There is not much literature available for the applicability of the benzothiazole dyes towards near-infrared \textit{in vivo} imaging studies; therefore, we
decided to design and synthesize various near-infrared dyes having the benzothiazole core structure. In the first chapter, the synthesis of heptamethine dyes is discussed as a part of an ongoing project. The second chapter concerns structural optimization of the pentamethine cyanine fluorophores for brain targeting application.

2.1.1 Synthesis of novel heptamethine benzothiazole cyanine dyes

Benzothiazole heptamethine cyanine dyes generally show absorption and fluorescence in the 750–850 nm region of the electromagnetic spectrum. Previously, our group synthesized a series of heptamethine cyanine dyes utilizing indolenine heterocyclic moiety with a rigid cyclohexene ring as a part of the heptamethine linker. This class of compound demonstrated promising in vivo near-infrared imaging performance.

Currently, our laboratory is investigating the synthesis of various heptamethine cyanine dyes with flexible polymethine linker for in vivo imaging applicability, and additional analogs were prepared using the benzothiazole heterocyclic structure. Commercially available 3-bromopropyltrimethyl ammonium bromide has been extensively employed for the synthesis of various heptamethine cyanine dyes with various heterocycles in our lab for the ongoing project. Additional bromopropyl salts with quaternized nitrogen were synthesized with pyridinium, methylpyrrolidinium and methylmorpholinium moieties with slight modification to published procedure as described in Scheme 9.

![Scheme 9](image-url)
Commercially available 1,3-dibromopropane was treated with pyridine at 45 °C in acetonitrile for eight hours to afford compound 45. Synthesis of compound 46 was furnished by reacting 1,3-dibromopropane with N-methylpyrrolidinone for two hours in ice cold conditions. N-methylmorpholine on stirring with 1,3-dibromopropane at room temperature for eight hours yielded compound 47. Compounds 45–47 were synthesized in 45-55 % overall yield. Bromopropyl quaternary salts 45–47 and commercially available 3-bromopropyl trimethylammonium bromide were then melted with 2-methylbenzothiazole in sealed tube to furnish compounds 48–51 as described in Scheme 10 with slight modification of the previously published procedure.²³ The quaternized benzothiazole compounds 48–51 were condensed with glutaraldehyde dianil monohydrochloride under basic conditions to yield tricationic heptamethine cyanine dyes 52–55 which were purified on reversed phase column using acetone:water solvent system. The overall yields of the purified dyes 52–55 ranged between 60 - 65%.

Scheme 10

The basic principle behind synthesizing cyanine dyes with multiple cationic charges was focused on increasing the solubility of the cyanine dye in water which is expected to result in a more appealing
distribution of the heptamethine cyanine dyes *in vivo* and should help in obtaining increased renal clearance. The predicted physiochemical properties for the compounds 52–55 are listed in Figure 4.

![Distribution coefficient data of heptamethine benzothiazole cyanine dyes predicted using ChemAxon software](image)

**Figure 4.** Distribution coefficient data of heptamethine benzothiazole cyanine dyes predicted using ChemAxon software

In past few years, total polar surface area has been considered as a major factor for target-oriented imaging studies. Modification of the end group on the propyl chain attached to the benzothiazole nitrogen showed simultaneous changes in the total polar surface area as well as in distribution coefficient (Log D) values as shown in Figure 4. The Log D values of the synthesized cyanine dyes 52–55 were predicted to be less than zero suggesting compounds possess high hydrophilicity. The imaging studies of the synthesized heptamethine cyanine dyes are still in progress. Following is representation of the absorption/emission spectrum for the heptamethine cyanine dye 52. This dye absorbs at 760 nm in the electromagnetic spectrum and fluoresces at 791 nm in the emission spectrum (excitation wavelength 660 nm), as shown below in Figure 5.
Figure 5. Absorption and emission spectrum of compound 52

The rest of the synthesized compounds 53–55 showed absorption/emission wavelength maxima within the same range as that of the compound 52 in the electromagnetic spectrum. Compounds 52–55 were characterized by $^1$H and $^{13}$C NMR, liquid chromatography and mass spectrometry analysis. Compound 54 was chosen for complete characterization and analysis.

$^1$H NMR spectrum of the compound 54 shows two doublets at 7.99 ppm and 7.88 ppm corresponding to the benzothiazole aromatic protons nearer to the sulfur and nitrogen atoms $H_b$. A multiplet formed between 7.58 ppm ~ 7.37 ppm corresponds to the protons from the heptamethine chain $H_d$, $H_f$ and the benzothiazole ring protons $H_a$. The protons $H_c$ and $H_e$ in the cationic polymethine chain produce a characteristic doublet and a triplet at 6.92 ppm and 6.56 ppm respectively. $J$ coupling constant of 12.8 Hz for these protons suggest the all trans character of the heptamethine chain. Broad doublets at 4.39 ppm and 3.73 ppm correspond to the four methylene protons from the propyl chain attached to the nitrogen atom. The multiplets at 3.55 ppm and 2.12 ppm represent remaining protons from the propyl chain and the protons from the pyrrolidine ring. Finally, a sharp singlet at 3.05 ppm corresponds to the $N$ – methyl group attached to the pyrrolidine ring.
Figure 6. $^1$H NMR spectrum of compound 54 in DMSO-$d_6$ at 25 °C

$^{13}$C NMR of the compound 54 shows as expected well resolved 11 peaks in the aromatic region and 6 peaks in the aliphatic region of the spectrum.
Figure 7. $^{13}$C NMR spectrum of compound 54 in DMSO-$d_6$ at 25°C

The liquid chromatography analysis of compound 54 confirmed analytical purity of the compound.

Figure 8. Liquid chromatography analysis of compound 54

High resolution mass spectrometry analysis confirmed the molecular weight of the compound.
2.1.2 Design and synthesis of novel pentamethine benzothiazole cyanine dyes

Currently, there are not many references associated with cyanine dyes targeting brain tumors. One of the major challenges associated with tumor identification is developing an agent that differentiates between tumor and normal cells.\textsuperscript{61} Introduction of a benzothiazole cyanine dye showing brain tumor imaging capability will lay the foundation for synthesis of a new class of compound active towards targeting the brain \textit{in vivo}. The aim of the presented work was to synthesize various pentamethine benzothiazole cyanine dyes and to study their \textit{in vivo} imaging specificity towards targeting brain tumor.

Thioflavin-T is the benzothiazole containing imaging agent which shows fluorescence enhancement upon binding to aggregates of protein “amyloids”.\textsuperscript{62,63}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{thioflavin_t}
\caption{Structure of Thioflavin-T}
\end{figure}

It has been observed that patients with Alzheimer’s disease have shown accumulation of β-amyloid fibrils in the brain.\textsuperscript{64,65} These fragments are then converted to the hard plaques. In recent studies it has been suggested that by imaging these plaques, the severity of the Alzheimer’s disease can be estimated.\textsuperscript{9} Thioflavin-T is structurally composed of 6-methylbenzothiazole heterocyclic ring quaternized at the nitrogen atom by methyl substituent linked to a hydrophobic phenyl ring possessing a dimethylamino end group. For the current research project, the benzothiazole structure of the Thioflavin-T was kept as an integral part of the cyanine dye replacing the hydrophobic phenyl group with the pentamethine chain. This feature allowed benzothiazole cyanine dyes to fluoresce in the near-infrared region of the electronic spectrum. It was hypothesized that the structural similarity of the synthesized dyes towards Thioflavin-T might promote benzothiazole dyes towards targeting the brain.

The blood brain barrier is the physical and biochemical barrier which prevents entry of specific substances from blood into the cerebrospinal fluid and consequently into central nervous system. There
exists ambiguous factors regarding the passage of a substance through the blood brain barrier though it is believed that compounds furnishing modified Lipinski’s rule of five have highest chances to surpass the blood brain barrier. The Lipinski’s rule suggests that compounds should fulfill the following basic requirements:

1. Partition coefficient should not be more than 5 (Log D < 5)
2. Molecular mass should be less than 500 g/mol
3. hydrogen bond donors ≤ 5
4. hydrogen bond acceptors ≤ 10
5. Total polar surface area should be less than 90 Å²

Considering Thioflavin-T as a lead compound, initial attempts were focused onto the synthesis of cyanine dyes with methyl substituted benzothiazole aromatic ring in accordance with Lipinski’s rule of five. Physiochemical predictions are a powerful tool to help expect in vivo efficacy without synthesizing an extensive library of compounds; using ChemAxon software we designed benzothiazole pentamethine cyanines and excluded those compounds which significantly deviate from Lipinski’s rule. Commercially available 2,5-dimethylbenzothiazole was heated at 80 °C with methyl and ethyl iodides. The resulting crude compounds were crystallized from methanol to afford quaternary benzothiazolium salts 56 and 57 in 76% and 65% yield, respectively. Various alkyl tosylates were heated at 120 °C with 2,6-dimethylbenzothiazole to furnish benzothiazolium quaternary salts 58–60 in greater than 60% yield. The quaternized salts 56–60 in the next step were condensed with malonaldehyde bisphenylimine hydrochloride in the presence of triethylamine in acetonitrile yielding benzothiazole cyanine dyes 61–65, as depicted in Scheme 11.
The purification of the cyanine dyes 61–65 was performed using solvent precipitation. The crude dye was dissolved in \(N,N\)-dimethylformamide (5 mL) and the pure dye was precipitated out of the solution by the dropwise addition of diethyl ether (15 mL) leaving all the soluble impurities in solution. The yields obtained for the synthesized benzothiazole cyanine dyes 61–65 were more than 60% with the remaining material being starting material, half-converted products and other non-target products. The synthesized benzothiazole cyanine dyes 61–65 followed the desired parameters of the Lipinski’s rule of five. The predicted total polar surface area of 7.12 Å², hydrogen donor/acceptor capability of 0/1 and the molecular weight less than 500 g/mol are well within range of the Lipinski’s rule. The only parameter that differed in all these discussed compounds was distribution coefficient (Log D) value, as shown in Figure 10.

Scheme 11

\[ \text{R1} \quad \text{R2} \quad \text{R3} \]

\[ \text{Yield} \]

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<td>56:</td>
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<td>60:</td>
<td>R₁ = CH₃</td>
<td>R₂ = H</td>
<td>R₃ = CH₂CH₂CH₃</td>
</tr>
</tbody>
</table>

b Shi et al., *Dyes and Pigments*, 2012, 93, 1506

c Eibergen et al., *ChemBiochem*, 2012, 13, 574
Although the predicted Log D values for the cyanine dyes 61-65 fall well within the Lipinski’s range, results obtained from bio-distribution studies indicated that none of the dyes exhibited contrast signal in the brain. It has also been noted that as the length of the side chain attached to the benzothiazole nitrogen increases, a marked increase in the accumulation of the dye in the liver was observed which can be attributed to the increasing lipophilic nature of the dye referring to their elevated Log D values.

The next approach towards synthesizing benzothiazole dyes was focused on removing the methyl substituent attached to the benzothiazole aromatic ring taking into account the lipophilicity factor associated with the earlier discussed benzothiazole dyes. It was hypothesized that removal of the methyl substituent from benzothiazole aromatic ring will help in decreasing the distribution coefficient (Log D) of the cyanine dye and this might result in directing the cyanine dye towards brain. It has already been published that radio labeled benzothiazole compounds without substituent(s) on the benzothiazole aromatic ring can serve as brain targeting agents. Scheme 12 represents the synthetic route used for the synthesis of the benzothiazole cyanine dyes 71–75.
Scheme 12

Synthesis of quaternary salts 66–70 was furnished in more than 50% yield after precipitation from methanol by heating different alkyl halides with 2-methylbenzothiazole under neat conditions. The utilization of the neat conditions for the synthesis of benzothiazolium salts 66–70 helped to obtain excellent yields in less reaction time than conventional solvent based chemistry.67, 68 Though compound 70 showed a single spot on the TLC analysis, it had displayed multiple peaks in aromatic as well as in aliphatic region of the 1H NMR spectrum. This compound was then used as crude in the dye synthesis step. In the next step, quaternary benzothiazolium salts 66–70 were treated with malonaldehyde bisphenylimine hydrochloride under basic conditions to furnish benzothiazole dyes 71–75. The crude dyes were purified using the aforementioned precipitation method from N,N-dimethylformamide by addition of diethyl ether (20 mL, 1:4) affording pure cyanine dyes in more than 60% yield. The predicted Log D values for the cyanine dyes 71-75 are as shown in Figure 11.

Figure 11. Distribution coefficient data of pentamethine benzothiazole cyanine dyes 71-73 predicted using ChemAxon software
By comparing the Log D values for the benzothiazole dyes from Figures 10 and 11, it can be observed that the removal of the methyl substituent from the benzothiazole aromatic ring lowered the Log D value substantially. Bio-distribution data revealed that compound 71 displayed no signal in the brain tumor though a signal in the pituitary gland was remarkably bright. It should be noted that cyanine dye 71 has the lowest Log D value as per the Figures 10 and 11. Benzothiazole cyanine dyes 72 and 73 displayed no contrast signal in the brain. This bio-distribution pattern for compounds 72 and 73 can be attributed to their higher Log D values as the predicted total polar surface area and hydrogen donor/acceptor capability remained the same as that of the earlier discussed benzothiazole dyes 61–65. Bio-distribution results obtained for benzothiazole cyanine dyes 61–65 and 71–75 suggested that lowering the Log D value would definitely help to direct the benzothiazole dyes towards the brain.

Klunk et al. developed a radio labeled 6-hydroxybenzothiazole derivative of Thioflavin-T which acted as better contrast agent that more effectively crossed the blood brain barrier. According to study reported by Chopra, 6-hydroxy derivatives of benzothiazole proved to be superior imaging agents over 4–5– and 7–hydroxy derivatives. Further approach towards lowering the Log D value of benzothiazole cyanine dyes was achieved by substituting hydroxy group in the benzothiazole aromatic ring. Scheme 13 describes the synthetic procedure used for the synthesis of various hydroxy substituted benzothiazole cyanine dyes 81–85. In order to achieve the synthesis of hydroxy substituted benzothiazole cyanine dyes 81–83, 6-hydroxy-2-methylbenzothiazole was first heated with various alkyl tosylates in neat conditions to furnish quaternary salts 76–78. This synthetic approach provided more than 60% yields of the quaternary salts. These salts were then reacted with malonaldehyde bisphenylimine hydrochloride under basic conditions to yield final hydroxyl-substituted benzothiazole dyes 81–83. The pure compounds were isolated by using precipitation technique making use of the DMF:diethyl ether solvent system (20...
mL, 1:4). Generally, hydroxy substituted cyanine dyes show limited solubility and the tosylate counter anion was utilized to increase the overall solubility of the cyanine dye.

![Scheme 13](image)

| 76: R = CH₃ | X = OTs | Yield: 65% |
| 77: R = CH₂CH₃ | X = OTs | Yield: 64% |
| 78: R = CH₂CH₂CH₃ | X = OTs | Yield: 68% |
| 79: R = CH₂CH₂CH₂NHMe₃ | X = 2Br | Yield: 52% |
| 80: R = CH₂CH₂NHMe₂ | X = 2Br | Yield: 47% |
| 81: R = CH₃ | X = OTs | 61% |
| 82: R = CH₂CH₃ | X = OTs | 56% |
| 83: R = CH₂CH₂CH₃ | X = OTs | 62% |
| 84: R = CH₂CH₂CH₂NHMe₃ | X = 2Br | 54% |
| 85: R = CH₂CH₂NHMe₂ | X = Br | 53% |

**Scheme 13**

It is evident from Figures 10, 11 and 13 that different substitutions on the benzothiazole ring affected the Log D values significantly. The highest Log D values were observed for methyl substituted benzothiazole cyanine dyes ranging between 2.8 to 4.5. The predicted Log D values of hydroxy substituted benzothiazole cyanine dyes 81–83 however ranged in 1 to 3.

![Figure 13](image)

**Figure 13.** Distribution coefficient data of pentamethine benzothiazole cyanine dyes 81–83 predicted using ChemAxon software.

Bio-distribution data obtained for compounds 81–83 revealed that compound 81 displayed increased contrast signal in the brain. This improved imaging capability of the cyanine dye 81 can be attributed to lowering the Log D value of all of the earlier discussed benzothiazole compounds. The total polar surface area and hydrogen donor/acceptor values for dyes 81-83 predicted as 47.58 Å² and 2/3 respectively. To determine if lowering the Log D value further could increase the brain targeting efficacy of the dye, we introduced the quaternary ammonium side chain on the benzothiazole cyanine dye 84 to increase the ap-
pealing imaging characteristics by synthesizing a more hydrophilic dye. The cyanine dye 84 was synthesized in 2 steps beginning with fusing 6-hydroxy-2-methylbenzothiazole with 3-bromopropyltrimethylammonium bromide at 140 °C for 4 hours. In the subsequent step, the benzothiazolium salt was subjected for the dye synthesis as shown in Scheme 13. The yields obtained for compounds 79 and 84 were 52% and 54%, respectively. The Log D value of compound 84 was predicted to be -6.28 and confirms its highly hydrophilic nature. This dye failed to target the brain though hydrophilicity of the dye aided in better renal clearance. The total polar surface area and hydrogen donor/acceptor capability remained the same for this dye as of compounds 81–83. It was then believed that the better brain targeting benzothiazole cyanine dye should possess Log D value less than +1.00 but it should not be as low as that for compound 84 keeping 6-hydroxybenzothiazole as a base structure. As described earlier, Thioflavin-T possesses a tertiary amine group in the structure. It was then assumed that incorporating the tertiary amine substituent in the existing benzothiazole model structure would definitely help to attain improved contrast signal in the brain. This task was achieved by modifying the side chain attached to compound 85 to a tertiary amine group. The quaternary salt 80 was synthesized by fusing 6-hydroxy-2-methylbenzothiazole with 2-bromo-N,N-dimethylethanamine hydrobromide at 150 °C for 4 hours. The crude solid on precipitation furnished compound 80 in a 47% yield as the hydrobromide salt. In the next step, compound 80 was treated with malonaldehyde bisphenylimine hydrochloride and triethylamine to afford final dye 85. Cy-anine dye 85 was purified by reversed phase column chromatography using acetone:water solvent system in 53% yield. The final dye was obtained in the tertiary amine form which was confirmed by various spectroscopic methods.
Replacing the quaternary ammonium group to a tertiary amine and removing one carbon atom from the propyl side chain resulted in lowering the Log D value to -0.35 as seen in compound 85 (Figure 14). The total polar surface area and hydrogen donor/acceptor capability for compound 85 predicted to be highest when compared to all of the earlier discussed pentamethine benzothiazole cyanine dyes as 54.06 Å² and 2/5 respectively. As hypothesized earlier, lowering the Log D value for compound 85 helped to observe a significant change towards the brain targeting ability of the dye. The in vivo imaging behavior of the most promising compounds (71, 81 and 85) out of the whole set of synthesized benzothiazole pentamethine cyanine dyes has been summarized in the proceeding chapter concerning the bio-distribution and targeting study.

The comprehensive synthetic procedure for all the synthesized compounds 45–85 has been described in the experimental section and respective spectral data has been reported in the appendix. Benzothiazole cyanine dye 73 was chosen for complete characterization analysis.

The ¹H NMR of compound 73 depicts a total of 10 signals, seven in the aromatic region and three in the aliphatic region. This suggests that compound 73 has a symmetric chromophoric structure. Doublets at 7.99 ppm and 7.74 ppm correspond to the protons H₆ of the benzothiazole aromatic ring. Two triplets at 7.54 ppm and 7.39 ppm are related to benzothiazole aromatic ring protons H₅. The doublets of the benzothiazole aromatic ring are more downfield than triplets due to the deshielding effect caused by nitrogen and sulfur atoms next to them. Three peaks were expected from the pentamethine chain. The signal
for protons H₄ should be a triplet which is merged with the doublet formed by benzothiazole proton H₆ at 7.79 ppm. The next multiplet formed between 6.58 ppm ~ 6.47 ppm can be attributed to the protons Hₑ and Hₑ from pentamethine bridge. Although proton peaks in the above discussed multiplet region are not well resolved, it still can be seen that J coupling constant for the doublet can be calculated as 12.8 Hz. This high value of J coupling constant suggests that the pentamethine bridge adopts an entirely trans conformation. A triplet formed at 4.32 ppm corresponds to methylene proton of the propyl chain nearer to the nitrogen atom of the benzothiazole ring. The next quartet at 1.76 ppm corresponds to other methylene protons of the propyl chain. Finally triplet at 0.98 ppm corresponds to the methyl protons of the propyl chain which are highly shielded protons in the molecule and hence shows the most upfield signal for the compound 73.
Figure 15. $^1$H NMR spectrum of compound 73 in DMSO-$d_6$ at 23°C

$^{13}$C spectrum for the same compound showed well resolved 13 peaks as expected. Three peaks at 47.26 ppm, 20.76 ppm and 10.83 ppm correspond to the propyl side chain attached to the benzothiazole nitrogen atom. All other remaining signals between 163.54 to 100.43 correspond to the carbon atoms from the benzothiazole ring and the pentamethine bridge.
Finally, compound purity was determined performing liquid chromatography-mass spectrometry analysis using a reversed phase C$_{18}$ column with water/acetonitrile gradient elution. The detectors used for liquid chromatography were Sedex 75 ELSD and absorbance detector at wavelength 650 nm. The well resolved single peaks at 10.40 min and 10.43 min on the either of the spectrum ensures that the sample is analytically pure as shown in Figure 17. High resolution mass spectrometry analysis confirmed the molecular weight of the compound.

Figure 16. $^{13}$C NMR spectrum of compound 73 in DMSO-$d_6$ at 23 $^\circ$C
2.2 Bio-distribution and targeting study

The biodistribution and targeting study of the benzothiazole cyanine dyes was conducted by Drs. Hak Soo Choi and John Frangioni at Beth Israel Deaconess Medical Center (BIDMC), Harvard Medical School in Boston, MA.

**Brain Tumor Animal Models:**

Neurosphere cells from S100β-verbB;p53−/− or S100β-verbB;p53+/− mouse brain tumors were obtained from Dr. Kyuson Yun (Jackson Laboratory, Bar Harbor, ME) and cultured as tumorspheres in modified DME/F-12 supplemented with 1% B27 (Invitrogen) and 1% Penicillin/Streptomycin. Animals were housed in an AAALAC-certified facility, and all animal studies were performed under the supervision of Beth Israel Deaconess Medical Center’s Institutional Animal Care and Use Committee (IACUC) in accordance with approved institutional protocol #155-2008. For the tumor targeting study, 6-week-old (20-30 g) male C57BL/6 mice were purchased from Taconic Farms (Germantown, NY), and the mice were anesthetized using ketamine/xylazine in a 100:10 mg/kg ratio. 3 × 10⁵ neurosphere cells resuspended in 3 μL of fresh medium were delivered 3 mm deep into the right side of midline slowly (0.2 μL/min) via guidance from a stereotactic frame (Harvard Apparatus, Holliston, MA). Control mice were implanted...
with 3 µL of medium without cells to allow similar surgical procedures to be performed on the tumor-bearing and non-tumor-bearing mice. Mice formed reasonable sized tumors in 1-2 weeks post-inoculation.

**Intraoperative NIR Fluorescence Imaging System:**

The dual-NIR channel FLARE™ imaging system has been described in detail previously.\(^70,71\) The FLARE™ imaging system is capable of visualizing surgical anatomy along with two independent channels of NIR fluorescence, where the first NIR fluorescence channel can be used for highlighting brain tumors, while the second NIR channel can be used for visualizing blood vessels and/or normal nervous tissues. In this study, 670 nm excitation and 760 nm excitation fluence rates used were 4.0 and 11.0 mW/cm\(^2\), respectively, with white light (400 to 650 nm) at 40,000 lx. Color video and 2 independent channels (700 nm and 800 nm) of NIR fluorescence images were acquired simultaneously with custom FLARE software at rates up to 15 Hz over a 15 cm diameter field of view (FOV). The imaging system was positioned at a distance of 18 inches from the surgical field. A custom filter set (Chroma Technology Corporation, Brattleboro, VT) composed of a 650 ± 22 nm excitation filter, a 680 nm dichroic mirror, and a 710 ± 25 nm emission filter were used.

**Biodistribution and Targeting using Optical Fluorescence Imaging:**

The *in vivo* behavior of the benzothiazole pentamethine cyanine dyes was significantly different depending upon the physiochemical properties. Compound 71 showed high affinity for the pituitary gland (arrowhead in Figure 18) 4 hr after a single IV injection, but almost no signal was detected in the glioma (brain tumor) as shown resected in Figure 18 (bottom left). Starting with this lipophilic and cationic fluorophore (Log D at pH 7.4 = 1.8), we modified the alkyl chain length and functional moieties to have higher hydrophilicity and lowering lipophilicity and better blood-brain barrier (BBB) penetration. Although compound 81 has lower Log D value (1.18) compared to compound 71, tumor targeting efficiency was not remarkably improved (Figure 19). Finally compound 85 was synthesized with two hydroxyl groups to improve hydrophilicity and lowering Log D value at pH 7.4 and two tertiary amine moieties to improve blood brain barrier (BBB) penetration.
As shown in Figure 20, compound 85 efficiently penetrated BBB and targeted to the glioma with high affinity. However, strong background signals were found in choroid plexus and lateral ventricles that decrease the tumor specificity of this molecule. Table 2 summarizes the physiochemical properties of compounds 71, 81 and 85.

Table 2. *In silico* physicochemical properties of NIR fluorophores calculated using Marvin and JChem calculator plugins (ChemAxon, Budapest, Hungary). Log D = partition coefficient at pH 7.4, TPSA = total polar surface area.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol Weight</th>
<th>Formula</th>
<th>Log D</th>
<th>TPSA</th>
<th>H bond acceptors</th>
<th>H bond donors</th>
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<td>71</td>
<td>363.52</td>
<td>C_{21}H_{19}S_{2}N_{2}</td>
<td>1.80</td>
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<td>81</td>
<td>395.52</td>
<td>C_{21}H_{19}S_{2}N_{2}O_{2}</td>
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</tr>
<tr>
<td>85</td>
<td>509.71</td>
<td>C_{27}H_{33}S_{2}N_{4}O_{2}</td>
<td>-0.35</td>
<td>54.06</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

A 25 nM sample of targeted fluorophore (compounds 71, 81, 85) was injected intravenously 4 hours prior to imaging. Shown in Figures 18, 19 and 20 are representative images of *in situ* biodistribution (top) and resected consecutive brain tissue after targeting (bottom). Abbreviations used are: Bl, bladder; Du, duodenum; In, intestine; Ki, kidneys; Li, liver; Pa, pancreas; and Sp, spleen. Arrowhead = pituitary gland; arrows = tumor. All NIR fluorescence images have identical exposure times and normalization. Scale bars = 1 cm.
Figure 18. Real-time intraoperative image-guided surgery of compound 71 in orthotopic brain tumor mice (biodistribution and targeting study of the benzothiazole cyanine dyes was conducted by Drs. Hak Soo Choi and John Frangioni at Beth Israel Deaconess Medical Center (BIDMC), Harvard Medical School in Boston, MA)
Figure 19. Real-time intraoperative image-guided surgery of compound 81 in orthotopic brain tumor mice (biodistribution and targeting study of the benzothiazole cyanine dyes was conducted by Drs. Hak Soo Choi and John Frangioni at Beth Israel Deaconess Medical Center (BIDMC), Harvard Medical School in Boston, MA)
**Figure 20.** Real-time intraoperative image-guided surgery of compound 85 in orthotopic brain tumor mice (biodistribution and targeting study of the benzothiazole cyanine dyes was conducted by Drs. Hak Soo Choi and John Frangioni at Beth Israel Deaconess Medical Center (BIDMC), Harvard Medical School in Boston, MA)
3 CONCLUSION

In past few years, cyanine dyes have attracted interest as potential in vivo imaging agents for biomedical applications.\textsuperscript{72,73} The development of cyanine dyes containing heterocycles other than indolenine and benz[e]indolenine would definitely facilitate a new approach towards development of new class of imaging agents.

In summary, tricationic heptamethine benzothiazole cyanine dyes with various quaternary ammonium side chains and three different sets of pentamethine benzothiazole cyanine dyes which fluoresce between 690 nm and 790 nm were synthesized in high yield and characterized using various spectroscopic techniques for their optical properties, structure confirmation and analytical purity. The synthesized cyanine dyes were purified by either precipitation or by reversed phase column chromatography technique. Furthermore, benzothiazole pentamethine cyanine dyes showed promising in vivo imaging capability towards targeting the brain tumor after successfully incorporating the tertiary amine side chain on the benzothiazole nitrogen. These results might provide a lead for the further development of ideal in vivo brain tumor targeting agent.
All the chemicals were purchased from commercial sources and were used without further purification. $^1$H and $^{13}$C NMR data was collected on a Bruker DPX-400 spectrometer at ambient temperature in DMSO-$d_6$ or Methanol-$d_4$ with tetramethylsilane as internal standard. Absorption spectra were obtained on a Cary 5 vis-NIR spectrophotometer. Fluorescence data was collected on Schimadzu RF 5301 PC spectrofluorometer. Excitation wavelength was set at 660 nm for heptamethine cyanine dyes and 620 nm for pentamethine cyanine dyes. Slit width was kept at 5 nm for excitation and emission mode. Light source xenon arc lamp was utilized in the fluorometer. All absorption and emission measurements were performed in spectrometric grade methanol. Liquid chromatography was operated using Waters 2487 single wavelength absorption detector with wavelength set to either 650 or 750 nm. Sedex 75 ELSD was used as alternate detector in liquid chromatography. Waters Delta-Pak 5 uM 100A 3.9 x 150 mm reversed phase C$^{18}$ column was used for the liquid chromatography. High resolution mass spectra were obtained on either Waters Micromass LCT TOF ES$^+$ Premier Mass Spectrometer or Georgia state mass spectrometry facility using Waters Q-TOF micro (ESI-Q-TOF) mass spectrometer.

**Physiochemical predictions of cyanine dyes:**

Hydrogen bond donors and acceptors, total polar surface area and Log D were predicted using MarvinSketch 5.9 (ChemAxon, Budapest, Hungary). The Log D predictions derived from the calculations by Viswanadhan, Klopman, and the PHYSPROP© database at pH 7.4 were averaged. The total polar surface area estimation is based on the method given by Ertl.

**Procedure for preparation of compound 45:**

This compound was synthesized as per the previously published procedure. A mixture of pyridine (1 eq.) and 1, 3-dibromopropane (3 eq.) was stirred in acetonitrile at 45 °C for 8 hours. The resulting salt was then filtered and washed with diethyl ether.

**1-(3-bromopropyl)pyridin-1-iium bromide (45):** Yield 45%; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.23 (d, $J = 15.6$ Hz, 2H), 8.66 (t, $J = 7.6$ Hz, 1H), 8.21 (t, $J = 6.4$ Hz, 2H), 4.81 (t, $J = 7.2$ Hz, 2H), 3.61 (t, $J =$...
6.8 Hz, 2H), 2.55 (q, J = 6.8 Hz, 2H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 145.72, 145.01, 128.10, 59.37, 33.13, 30.16.

**Procedure for preparation of compound 46:**

This compound was synthesized with slight modification of the previously published procedure. A mixture of N-methylpyrrolidine (1 eq.) and 1, 3-dibromopropane (3 eq.) was stirred at 0 °C for 2 hours. The precipitate thus formed is filtered and washed with diethyl ether.

**1-(3-bromopropyl)-1-methylpyrroldin-1-i um bromide (46): Yield 55%;** $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 3.62~3.48 (m, 8H), 3.05 (s, 3H), 2.35~2.29 (m, 2H), 2.09 (brs, 4H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 63.62, 61.57, 47.62, 30.75, 26.40, 21.01.

**Procedure for preparation of compound 47:**

This compound was synthesized with slight modification of the previously published procedure. A mixture of N-methylmorpholine (1 eq.) and 1, 3-dibromopropane (3 eq.) was stirred at room temperature for 24 hours. The resulting precipitate was filtered and washed with diethyl ether.

**4-(3-bromopropyl)-4-methylmorpholin-4-i um bromide (47): Yield 50%;** $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 3.95 (s, 4H), 3.68 ~ 3.60 (m, 4H), 3.51~3.45 (m, 4H), 3.21 (s, 3H), 2.35~2.28 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 62.16, 59.78, 59.15, 46.42, 30.51, 24.37.

**General procedure for preparation of compounds 48–51:**

These compounds were synthesized with slight modification of the previously published procedure. A mixture of 2-methylbenzothiazole and either compounds 45–47 or 3-bromopropyltrimethyl ammonium bromide was heated in a sealed tube at 130 °C for 18 hours. The resulting sticky solid was washed with diethyl ether and crude compound was used as is in the further step.

**General procedure for synthesis of compounds 52–55:**

A mixture of benzothiazolium salt 48–51 (1eq.) and glutacanaldehyde dianil monohydrochloride (0.5 eq.) was heated in acetonitrile at 45 °C for 4 hours in presence of triethylamine (1eq.) under nitrogen atmosphere. The precipitate obtained was filtered and washed with diethyl ether. The target compounds were isolated by purification using reverse phase column chromatography (acetone:water 5:1).
3-(3-(trimethylammonio)propyl)-2-((1E,3E,5E,7Z)-7-(3-(trimethylammonio)propyl)benzo[d]thiazol-2(3H)-ylidene)hepta-1,3,5-trien-yl)benzo[d]thiazol-3-ium bromide (52): Yield 62% (0.18 g); m.p. 205–207 °C; 1H NMR (DMSO-d6, 400 MHz): δ 8.00 (d, J = 8 Hz, 2H), 7.86 (d, J = 8 Hz, 2H), 7.57–7.37 (m, 7H), 6.88 (d, J = 13.2 Hz, 2H), 6.56 (t, J = 12.8 Hz, 2H), 4.39 (brs, 4H), 3.75 (brt, J1 = 6.8 Hz, J2 = 8 Hz, 4H), 3.13 (s, 18H), 2.17 (brs, 4H). 13C NMR (DMSO-d6, 100 MHz): δ 162.05, 152.01, 148.16, 141.28, 128.09, 125.34, 125.00, 123.56, 123.10, 113.25, 102.00, 62.07, 52.55, 43.00, 21.25. ESI-HRMS m/z: calc. for (C33H45S2N4Br)2+ 640.2269, found 640.2264. λabs = 760 nm λfluo = 791 nm

3-(3-(pyridin-1-ium-1-yl)propyl)-2-((1E,3E,5E,7Z)-7-(3-(pyridin-1-ium-1-yl)propyl)benzo[d]thiazol-2(3H)-ylidene)hepta-1,3,5-trien-1-yl)benzo[d]thiazol-3-ium bromide (53): Yield 61% (0.15 g); m.p. 189 – 191 °C; 1H NMR (DMSO-d6, 400 MHz): δ 9.38 (d, J = 5.6 Hz, 4H), 8.65 (t, J = 7.6 Hz, 2H), 8.21 (t, J = 6.8 Hz, 5H), 7.97 (d, J = 8 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.53–7.33 (m, 6H), 6.97 (d, J = 12.8 Hz, 2H), 6.54 (t, J = 12.4 Hz, 2H), 5.07 (brt, J = 7.2 Hz, 4H), 4.53 (brs, 4H), 2.42 (brs, 4H). 13C NMR (DMSO-d6, 100 MHz): δ 161.88, 151.69, 147.85, 145.52, 144.75, 141.17, 127.98, 127.81, 125.23, 124.77, 123.31, 122.91, 113.20, 102.06, 57.35, 43.03, 29.24. ESI-HRMS m/z: calc. for (C37H37S2N4Br2)2+ 759.0826, found 759.0848. λabs = 760 nm λfluo = 795 nm
3-(3-(1-methylpyrrolidin-1-yl)propyl)-2-((1E,3E,5E,7Z)-7-(3-(1-methylpyrrolidin-1-yl)propyl)benzo[d]thiazol-2(3H)-ylidene)hepta-1,3,5-trien-1-yl)benzo[d]thiazol-3-im bromide (54):

Yield 65% (0.17 g); m.p. 168 – 170 °C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 7.99 (d, \(J = 8\) Hz, 2H), 7.88 (d, \(J = 8.4\) Hz, 2H), 7.58–7.58 (m, 7H), 6.92 (d, \(J = 12.8\) Hz, 2H), 6.56 (t, \(J = 12.8\) Hz, 2H), 4.39 (brd, \(J = 7.2\) Hz, 4H), 3.73 (brd, \(J = 7.6\) Hz, 4H), 3.55 (brd, \(J = 5.2\) Hz, 8H), 3.05 (s, 6H), 2.13 (m, 6H). \(^13\)C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 162.04, 151.95, 148.08, 141.29, 128.04, 125.30, 124.95, 123.46, 123.06, 113.28, 102.03, 63.86, 59.63, 47.86, 43.06, 21.98, 21.23. ESI-HRMS m/z: calc. for \((C_{37}H_{10}S_2N_3Br_2)^+\) 771.1765, found 771.1734. \(\lambda_{\text{abs}} = 760\) nm \(\lambda_{\text{max}} = 795\) nm

4-methyl-4-(3-(2-((1E,3E,5E,7Z)-7-(3-(4-methylmorpholino-4-im)propyl)benzo[d]thiazol-2(3H)-ylidene)hepta-1,3,5-trien-1-yl)benzo[d]thiazol-3-im-3-yl)propyl)morpholino-4-im bromide (55):

Yield 63% (0.18 g); m.p. 222 – 224 °C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 8.01 (d, \(J = 7.6\) Hz, 2H), 7.87 (d, \(J = 8.4\) Hz, 2H), 7.59–7.38 (m, 7H), 6.91 (d, \(J = 13.2\) Hz, 2H), 6.57 (t, \(J = 12.4\) Hz, 2H), 4.41 (brs,
4H), 4.04-3.88 (m, 12H), 3.48 (s, 8H), 3.19 (s, 6H), 2.17 (brs, 4H). $^1$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 161.95, 151.85, 148.02, 141.18, 127.95, 125.20, 124.87, 123.42, 122.97, 113.19, 101.96, 59.72, 59.27, 47.00, 42.94, 19.85. ESI-HRMS m/z: calc. for (C$_{37}$H$_{49}$S$_2$N$_4$O$_2$Br$_2$)$^+$ 803.1664, found 803.1663. Anal. Calcd for C$_{37}$H$_{49}$S$_2$N$_4$O$_2$Br$_2$·7H$_2$O: C, 48.40; H, 5.90; N, 6.19; Br, 23.28; Found: C, 48.53; H, 6.05; N, 6.12; Br, 23.56. $\lambda_{\text{abs}}$ = 760 nm $\lambda_{\text{fluo}}$ = 790 nm

General procedure for preparation of compounds 56 and 57:

These compounds were synthesized with slight modification of the previously published procedure. A mixture of 2, 5-dimethylbenzothiazole (1 eq.) and alkyl halide (2 eq.) were heated at 80°C for 6 hours. After cooling the mixture to room temperature resulting crude solid was purified by precipitation from methanol and diethyl ether. The precipitated solid was then filtered and dried in vacuo.

2, 3, 5-trimethylbenzo[d]thiazol-3-ium iodide (56): Yield 76%; $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 8.31 (d, $J$ = 8.4 Hz, 1H), 8.15 (s, 1H), 7.62 (d, $J$ = 8.4 Hz, 1H), 4.18 (s, 3H), 3.17 (s, 3H), 2.56 (s, 3H). $^1$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 176.73, 141.79, 139.75, 129.45, 125.75, 124.00, 123.90, 116.51, 116.37, 36.29, 21.14, 17.32.

3-ethyl-2,5-dimethylbenzo[d]thiazol-3-ium iodide (57): Yield 65%; $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 8.32 (d, $J$ = 8.4 Hz, 1H), 8.20 (s, 1H), 7.64 (d, $J$ = 8.4 Hz, 1H), 4.73 (q, $J$ = 7.2 Hz, 2H), 3.21 (s, 3H), 2.58 (s., 3H), 1.46 (t, $J$ = 7.2 Hz, 3H). $^1$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 176.45, 140.76, 140.01, 129.52, 126.25, 124.15, 116.33, 44.60, 21.17, 16.73, 13.21.
General procedure for synthesis of compounds 58–60:
A mixture of 2,6-dimethylbenzothiazole (1 eq.) and alkyl tosylate (2 eq.) were heated at 120°C for 2 hours. After cooling the mixture to room temperature acetone was added to the thick slurry. Resulting sticky solid was purified by precipitation from methanol and diethyl ether and dried in vacuo.

2,3,6-trimethylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (58): Yield 72%; **1**H NMR (DMSO-d$_6$, 400 MHz): δ 8.18–8.13 (m, 2H), 7.67 (d, J = 8 Hz, 1H), 7.45 (d, J = 7.2 Hz, 2H), 7.07 (d, J = 7.2 Hz, 2H), 4.15 (s, 3H), 3.12 (s, 3H), 2.51 (s, 3H), 2.25 (s, 3H). **13**C NMR (DMSO-d$_6$, 100 MHz): δ 176.06, 145.55, 139.77, 138.30, 137.68, 130.51, 128.77, 128.05, 125.42, 123.68, 116.35, 36.08, 20.97, 20.77, 16.85.

3-ethyl-2,6-dimethylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (59): Yield 70%; **1**H NMR (DMSO-d$_6$, 400 MHz): δ 8.22–8.20 (m, 2H), 7.68 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 8 Hz, 2H), 4.72 (q, J = 7.2 Hz, 2H), 3.17 (s, 3H), 2.51 (s, 3H), 2.26 (s, 3H), 1.42 (t, J = 7.6 Hz, 3H). **13**C NMR (DMSO-d$_6$, 100 MHz): δ 175.72, 145.56, 138.66, 138.36, 137.71, 130.72, 129.26, 128.06, 125.48, 123.95, 123.86, 116.27, 44.68, 20.97, 20.78, 16.47, 13.23.

2,6-dimethyl-3-propylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (60): Yield 60%; **1**H NMR (Methanol-d$_4$, 400 MHz): δ 7.94–7.86 (m, 2H), 7.60 ~ 7.50 (m, 4H), 7.01 (d, J = 7.6 Hz, 3H), 4.52 (t, J = 8 Hz, 2H), 3.02 (s, 3H), 2.30 (s, 3H), 2.17 (s, 3H), 1.82 (p, J = 7.6 Hz, 2H), 0.93 (t, J = 7.6 Hz, 3H). **13**C NMR (Methanol-d$_4$, 100 MHz): δ 176.68, 143.74, 141.65, 140.91, 140.72, 132.42, 130.81, 129.86, 126.93, 124.70, 117.57, 52.20, 22.95, 21.57, 21.44, 17.12, 11.33.

General procedure for synthesis of compounds 61–65:
A mixture of benzothiazole quaternary salt 56–60 (2 eq.) and malonaldehyde bis(phenylimine) monohydrochloride (1 eq.) were heated to 45°C in presence of triethylamine (2 eq.) in acetonitrile for 2 hours. The solution was cooled down to room temperature and resulting solid was filtered. The resulting crude solid was purified by precipitation from DMF–diethyl ether (20 mL, 1:4).

2-(1E,3E,5Z)-5-(3,5-dimethylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-3,5-dimethylbenzo[d]thiazol-3-ium iodide (61): Yield 63% (0.15 g); m.p. >260°C; **1**H NMR (DMSO-d$_6$, 400 MHz): δ 8.32–8.30 (m, 2H), 7.67 (d, J = 8 Hz, 1H), 7.45 (d, J = 7.2 Hz, 2H), 7.07 (d, J = 7.2 Hz, 2H), 4.15 (s, 3H), 3.12 (s, 3H), 2.51 (s, 3H), 2.25 (s, 3H). **13**C NMR (DMSO-d$_6$, 100 MHz): δ 176.06, 145.55, 139.77, 138.30, 137.68, 130.51, 128.77, 128.05, 125.42, 123.68, 116.35, 36.08, 20.97, 20.77, 16.85.
400 MHz): δ 7.83 (d, J = 8 Hz, 2H), 7.70 (t, J = 12 Hz, 2H), 7.52 (s, 2H), 7.21 (d, J = 8 Hz, 2H), 6.50–6.42 (m, 3H), 3.77 (s, 6H), 2.43 (s, 6H). 13C NMR (DMSO-d6, 100 MHz): δ 164.05, 149.90, 142.14, 138.03, 125.94, 122.47, 122.02, 113.53, 100.54, 33.6, 21.11. ESI-HRMS m/z: calc. for $(C_{23}H_{23}N_2S_2)^+$ 391.1297, found 391.1308. λ\text{abs} = 654 nm λ\text{fluo} = 678 nm

3-ethyl-2-((1E,3E,5Z)-5-(3-ethyl-5-methylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-5-methylbenzo[d]thiazol-3-ium iodide (62): Yield 56% (0.16 g); m.p. >260 °C; 1H NMR (DMSO-d6, 400 MHz): δ 7.85 (d, J = 8 Hz, 2H), 7.73 (t, J = 12.4 Hz, 2H), 7.57 (s, 2H), 7.22 (d, J = 7.6 Hz, 2H), 6.54–6.42 (m, 3H), 4.36 (brd, J = 6.4 Hz, 4H), 2.44 (s, 6H), 1.31 (t, J = 6.8 Hz, 6H). 13C NMR (DMSO-d6, 100 MHz): δ 163.36, 150.14, 141.05, 138.14, 125.94, 125.53, 122.20, 113.22, 99.91, 41.10, 21.02, 12.56. ESI-HRMS m/z: calc. for $(C_{25}H_{27}N_2S_2)^+$ 419.1610, found 419.1602. λ\text{abs} = 660 nm λ\text{fluo} = 679 nm
2-((1E,3E,5Z)-5-(3,6-dimethylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-3,6-dimethylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (63): Yield 70% (0.18 g); m.p. 210 - 212 ⁰C; ¹H NMR (DMSO-\(d_6\), 400 MHz) : \(\delta\) 7.75 (s, 2H), 7.63 (t, \(J = 12.8\) Hz, 2H), 7.54 (d, \(J = 8.8\) Hz, 2H), 7.47 (d, \(J = 7.2\) Hz, 2H), 7.32 (d, \(J = 8\) Hz, 2H), 7.11 (d, \(J = 7.6\) Hz, 3H), 6.43~6.34 (m, 3H), 3.75 (s, 6H), 2.35 (s, 6H), 2.28 (s, 3H). ¹³C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 163.15, 149.59, 145.77, 139.84, 137.40, 134.61, 128.60, 127.91, 125.39, 125.01, 122.53, 112.76, 100.16, 33.28, 20.67, 20.51. ESI-HRMS m/z: calc. for (C\(_{23}\)H\(_{23}\)N\(_2\)S\(_2\))\(^+\) 391.1297, found 391.1298. \(\lambda_{\text{abs}}\) = 655 nm \(\lambda_{\text{fluoro}}\) = 676 nm

3-ethyl-2-((1E,3E,5Z)-5-(3-ethyl-6-methylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-6-methylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (64): Yield 64% (0.14 g); m.p. 194 - 196 ⁰C; ¹H NMR (DMSO-\(d_6\), 400 MHz) : \(\delta\) 7.76 (s, 2H), 7.69~7.57 (m, 4H), 7.48 (d, \(J = 12.8\) Hz, 2H), 7.11 (d, \(J = 8\) Hz, 2H), 7.07 (d, \(J = 8.4\) Hz, 2H), 7.33 (d, \(J = 8.4\) Hz, 2H), 7.11 (d, \(J = 8\) Hz, 3H), 4.33 (brd, \(J = 7.2\) Hz, 4H), 2.36 (s, 6H), 2.28 (s, 3H), 1.28 (t, \(J = 6.8\) Hz, 6H). ¹³C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 162.63, 150.13, 145.89, 138.93, 137.48, 134.61, 128.60, 127.91, 125.39, 125.01, 122.53, 120.23, 112.76, 100.16, 33.28, 20.67, 20.51.
134.81, 128.88, 128.01, 125.49, 125.42, 125.49, 122.82, 120.37, 112.74, 99.74, 41.24, 20.77, 20.62, 12.67. ESI-HRMS m/z: calc. for (C_{25}H_{27}N_{2}S_{2})^{+} 419.1610, found 419.1615. \lambda_{\text{abs}} = 660 \text{ nm } \lambda_{\text{fluo}} = 679 \text{ nm}

6-methyl-2-((1E,3E,5Z)-5-(6-methyl-3-propylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-3-propylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (65): Yield 65\% (0.13 g); m.p. 195 – 197 0\text{ C}; \text{H NMR (DMSO-\text{d}_6, 400 MHz)}: \delta 7.74 (s, 2H), 7.66–7.58 (m, 4H), 7.49 (d, \text{J} = 7.6 \text{ Hz, 2H}), 7.31 (d, \text{J} = 8.4 \text{ Hz, 2H}), 7.11 (d, \text{J} = 7.6 \text{ Hz, 3H}), 6.46–6.38 (m, 3H), 4.24 (brt, \text{J} = 7.2 \text{ Hz, 4H}), 2.35 (s, 6H), 2.28 (s, 3H), 1.71 (q, \text{J} = 7.2\text{Hz, 4H}), 0.95 (t, \text{J} = 7.2\text{Hz, 6H}). \text{C NMR (DMSO-\text{d}_6, 100 MHz)}: \delta 162.96, 149.92, 145.90, 139.40, 137.46, 134.76, 128.79, 127.99, 125.49, 125.23, 122.71, 120.45, 113.01, 100.08, 47.21, 20.76, 20.59, 10.80. ESI-HRMS m/z: calc. for (C_{27}H_{31}N_{2}S_{2})^{+} 447.1923, found 447.1927. \lambda_{\text{abs}} = 660 \text{ nm } \lambda_{\text{fluo}} = 680 \text{ nm}
General Procedure for preparation of compounds 66–70:

These compounds were synthesized with slight modification of the previously published procedure. A mixture of 2-methylbenzothiazole (1 eq.) and alkyl halide (3 eq.) was heated at 80°C for 6 to 18 hours. The mixture was cooled to room temperature and resulting crude solid was subjected for purification by precipitation from methanol and diethyl ether (10 mL, 1:4). The precipitated solid was then filtered and dried in vacuo. Compound 70 was used as crude in the next step.

2,3-dimethylbenzo[d]thiazol-3-ium iodide (66): Yield 81%; 1H NMR (DMSO-d6, 400 MHz): δ 8.49 (d, J = 8 Hz, 1H), 8.31 (d, J = 8.4 Hz, 1H), 7.89 (q, J1 = 7.2 Hz, J2 = 0.8 Hz, 1H), 7.80 (t, J1 = 8.4 Hz, J2 = 7.2 Hz, 1H), 4.24 (s, 3H), 3.22 (s, 3H). 13C NMR (DMSO-d6, 100 MHz): δ 177.04, 141.47, 129.15, 128.58, 127.93, 124.47, 116.70, 36.44, 17.48.

3-ethyl-2-methylenzo[d]thiazol-3-ium iodide (67): Yield 72%; 1H NMR (DMSO-d6, 400 MHz): δ 8.47 (d, J = 8 Hz, 1H), 8.35 (d, J = 8.4 Hz, 1H), 7.92 ~ 7.79 (m, 2H), 4.78 (q, J = 7.2 Hz, 2H), 3.23 (s, 3H), 1.46 (t, J = 7.2 Hz, 3H). 13C NMR (DMSO-d6, 100 MHz): δ 176.87, 140.46, 129.37, 129.14, 128.05, 124.69, 116.68, 44.74, 16.79, 13.22.

2-methyl-3-propylbenzo[d]thiazol-3-ium iodide (68): Yield 60%; 1H NMR (DMSO-d6, 400 MHz): δ 8.47 (d, J = 8 Hz, 1H), 8.38 (d, J = 8.4 Hz, 1H), 7.91 ~ 7.79 (m, 2H), 4.70 (t, J = 7.6 Hz, 2H), 3.23 (s, 3H), 1.90 (p, J = 7.6 Hz, 2H), 1.01 (t, J = 7.6 Hz, 3H). 13C NMR (DMSO-d6, 100 MHz): δ 177.09, 140.08, 129.33, 129.04, 128.06, 124.63, 116.93, 50.44, 21.32, 17.01, 10.76.

3-butyl-2-methylbenzo[d]thiazol-3-ium iodide (69): Yield 50%; 1H NMR (DMSO-d6, 400 MHz): δ 8.47 (d, J = 8 Hz, 1H), 8.35 (d, J = 8.8 Hz, 1H), 7.88 ~ 7.78 (m, 2H), 4.72 (t, J = 7.6 Hz, 2H), 3.23 (s, 3H), 1.89 ~ 1.78 (m, 2H), 1.50 ~ 1.41 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H). 13C NMR (DMSO-d6, 100 MHz): δ 176.98, 140.81, 129.33, 129.05, 128.05, 124.64, 116.88, 49.06, 29.77, 19.27, 16.99, 13.52.

General Procedure for synthesis of compounds 71–75:

A mixture of benzothiazole quaternary salt 66–70 (2 eq.) and malonaldehyde bis(phenylimine) monohydrochloride (1 eq.) were heated to 45°C in presence of triethylamine (2 eq.) in acetonitrile for 2
hours. The solution was cooled to room temperature and the resulting solid was filtered. It was then purified by precipitation from DMF–diethyl ether (20 mL, 1:4).

3-methyl-2-((1E,3E,5Z)-5-(3-methylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)benzo[d]thiazol-3-ium iodide (71): Yield 68% (0.21 g); m.p. >260 °C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 7.98 (d, \(J = 8\) Hz, 2H), 7.75–7.67 (m, 4H), 7.55 (t, \(J = 7.2\) Hz, 2H), 7.38 (t, \(J = 7.6\) Hz, 2H), 6.53–6.50 (m, 3H), 3.80 (s, 6H). \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 164.01, 150.28, 142.02, 127.95, 125.14, 124.94, 122.93, 113.35, 100.62, 33.48. ESI-HRMS m/z: calc. for (C\(_{21}\)H\(_{19}\)N\(_2\)S\(_2\))\(^+\) 363.0990, found 363.1004. \(\lambda_{abs} = 650\) nm \(\lambda_{fluo} = 670\) nm

3-ethyl-2-((1E,3E,5Z)-5-(3-ethylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)benzo[d]thiazol-3-ium iodide (72): Yield 65% (0.19 g); m.p. >260 °C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 7.98 (d, \(J = 7.6\) Hz, 2H), 7.77–7.69 (m, 4H), 7.53 (t, \(J = 7.6\) Hz, 2H), 7.37 (t, \(J = 7.6\) Hz, 2H), 6.55–6.46 (m, 3H), 4.38 (q, \(J = 6.8\) Hz, 4H), 1.30 (t, \(J = 7.2\) Hz, 3H). \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 163.22, 150.57, 140.93, 128.00, 125.39, 124.91, 123.05, 120.74, 113.09, 100.06, 41.28, 12.65. ESI-HRMS m/z: calc. for (C\(_{23}\)H\(_{23}\)N\(_2\)S\(_2\))\(^+\) 391.1297, found 391.1298. \(\lambda_{abs} = 650\) nm \(\lambda_{fluo} = 673\) nm
3-propyl-2-((1E,3E,5Z)-5-(3-propylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)benzo[d]thiazol-3-ium iodide (73): Yield 61% (0.16 g); m.p. 250 - 252 °C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 7.99 (d, \(J = 8\) Hz, 2H), 7.79 ~ 7.73 (m, 4H), 7.54 (t, \(J = 7.6\) Hz, 2H), 7.39 (t, \(J = 7.6\) Hz, 2H), 6.58 ~ 6.47 (m, 3H), 4.32 (brt, \(J = 6.8\) Hz, 4H), 1.76 (q, \(J = 7.2\) Hz, 4H), 0.98 (t, \(J = 7.6\) Hz, 6H). \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 163.54, 150.38, 141.39, 127.92, 125.22, 124.88, 123.04, 120.82, 113.38, 100.43, 47.26, 20.76, 10.83. ESI-HRMS m/z: calc. for (C\(_{25}\)H\(_{27}\)N\(_2\)S\(_2\))\(^+\) 419.1616, found 419.1626. \(\lambda_{abs} = 654\) nm \(\lambda_{flu} = 675\) nm
3-butyl-2-((1E,3E,5Z)-5-(3-butylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)benzo[d]thiazol-3-ium iodide (74): Yield 62% (0.14 g); m.p. 240 - 242 °C; ¹H NMR (DMSO-d₆, 400 MHz): δ 7.99 (d, J = 8 Hz, 2H), 7.79–7.71 (m, 4H), 7.55 (t, J = 7.6 Hz, 2H), 7.39 (t, J = 7.6 Hz, 2H), 6.55–6.48 (m, 3H), 4.34 (brt, J = 7.2 Hz, 4H), 1.71 (m, 4H), 1.41 (m, 4H), 0.94 (t, J = 7.2 Hz, 6H). ¹³C NMR (DMSO-d₆, 100 MHz): δ 163.34, 150.26, 141.23, 127.89, 125.21, 124.82, 122.95, 122.95, 120.91, 113.26, 100.36, 45.82, 29.38, 19.29, 13.63. ESI-HRMS m/z: calc. for (C₂₇H₃₁N₂S₂)⁺ 447.1923, found 447.1933. λᵦᵦ = 655 nm λᵦᵧ = 673 nm

![Graph](image_url)

3-(3-phenylpropyl)-2-((1E,3E,5Z)-5-(3-phenylpropyl)benzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)benzo[d]thiazol-3-ium bromide (75): Yield 63% (0.17 g); m.p. 130 - 132 °C; ¹H NMR (DMSO-d₆, 400 MHz): δ 7.97 (d, J = 8 Hz, 2H), 7.73 ~ 7.52 (m, 6H), 7.39 ~ 7.21 (m, 10H), 6.42 ~ 6.39 (m, 3H), 4.35 (brt, J = 6.8 Hz, 4H), 2.76 (t, J = 7.6 Hz, 4H), 2.03 (m, 4H). ¹³C NMR (DMSO-d₆, 100 MHz): δ 163.56, 150.40, 141.31, 140.77, 128.40, 128.21, 127.94, 126.09, 125.31, 124.93, 123.01, 120.77, 113.21, 100.26, 45.65, 31.81, 28.79. ESI-HRMS m/z: calc. for (C₃₇H₃₅N₂S₂)⁺ 571.2236, found 571.2240. λᵦᵦ = 655 nm λᵦᵧ = 674 nm
General Procedure for synthesis of compounds 76–78:

A mixture of 6-hydroxy-2-methylbenzothiazole (1 eq.) and alkyl tosylate (2 eq.) was heated at 120°C for 2 hours. After cooling the mixture to room temperature, acetone was added where hydroxy salts precipitated out. Crude compounds were further purified by precipitation from methanol and diethyl ether (5 mL, 1:9).

6-hydroxy-2,3-dimethylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (76): Yield 65%; ¹H NMR (DMSO-­⁶, 400 MHz): δ 10.62 (s, 1H), 8.06 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 2.4 Hz, 1H), 7.46 (d, J = 8.4 Hz, 2H), 7.27 (dd, J₁ = 2 Hz, J₂ = 7.2 Hz, 1H), 7.08 (d, J = 7.6 Hz, 2H), 4.10 (s, 3H), 3.06 (s, 3H), 2.27 (s, 3H). ¹³C NMR (DMSO-­⁶, 100 MHz): δ 173.24, 157.57, 145.68, 137.61, 134.81, 130.31, 128.02, 125.45, 118.54, 117.68, 117.54, 108.65, 36.05, 20.78, 16.59.

3-ethyl-6-hydroxy-2-methylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (77): Yield 64%; ¹H NMR (DMSO-­⁶, 400 MHz): δ 10.67 (s, 1H), 8.09 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 1.2 Hz, 1H), 7.49 (d, J = 7.6 Hz, 2H), 7.27 (d, J = 7.6 Hz, 1H), 7.06 (d, J = 7.6 Hz, 2H), 4.64 (d, J = 6.8 Hz, 2H), 3.09 (s, 3H), 2.23 (s, 3H), 1.38 (t, J = 7.2 Hz, 3H). ¹³C NMR (DMSO-­⁶, 100 MHz): δ 173.76, 157.60, 145.39, 137.88, 133.58, 130.78, 128.22, 128.07, 125.35, 118.72, 117.65, 117.47, 108.86, 44.61, 20.80, 16.25, 13.30.

6-hydroxy-2-methyl-3-propylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (78): Yield 68%; ¹H NMR (DMSO-­⁶, 400 MHz): δ 10.63 (s, 1H), 8.14 (d, J = 9.2 Hz, 1H), 7.71 (s, 1H), 7.47 (d, J = 7.6 Hz,
2H), 7.27 (d, J = 9.2 Hz, 1H), 7.10 (d, J = 7.6 Hz, 2H), 4.59 (t, J = 7.2 Hz, 2H), 3.17 (s, 3H), 2.27 (s, 3H), 1.84 (q, J = 7.2 Hz, 2H), 0.98 (t, J = 7.2 Hz, 3H).$^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 173.07, 157.56, 145.71, 137.58, 134.04, 130.70, 128.02, 125.46, 118.68, 117.80, 108.66, 50.30, 21.38, 20.76, 16.42, 10.70.

Procedure for synthesis of compound 79:

A mixture of 6-hydroxy-2-methylbenzothiazole (1 eq.) and 3-bromopropyltrimethyl ammonium bromide (2 eq.) was heated at 120°C for 4 hours. After cooling the mixture to room temperature methanol was added and insoluble solid was filtered. The crude solid was purified by precipitation from DMSO-ethyl acetate.

6-hydroxy-2-methyl-3-(trimethylammonio)propylbenzo[d]thiazol-3-ium bromide (79): Yield 52%; $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 10.58 (s, 1H), 8.35 (d, J = 8.8 Hz, 1H), 7.81 (s, 1H), 7.29 (d, J = 8 Hz, 1H), 4.73 (brs, 2H), 3.72 (brs, 2H), 3.24 (s, 3H), 3.13 (s, 9H), 2.34 (brs, 2H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 174.03, 157.54, 133.92, 130.71, 118.67, 117.86, 108.89, 61.75, 52.54, 45.91, 21.84, 17.07.

Procedure for synthesis of compound 80:

A mixture of 6-hydroxy-2-methylbenzothiazole (1 eq.) and 2-bromo-$N,N$-dimethylethanamine hydrobromide (2 eq.) was heated at 150°C for 4 hours. After cooling the mixture to room temperature methanol was added and insoluble solid was filtered. The crude solid was purified by precipitation from DMSO-ethyl acetate.

3-(dimethylammonio)propyl-6-hydroxy-2-methylbenzo[d]thiazol-3-ium bromide (80): Yield 47%; $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 10.65 (brs, 1H), 10.33 (brs, 1H), 8.27 (d, J = 9.2 Hz, 1H), 7.81 (d, J = 2.4 Hz, 1H), 7.32 (q, J_1 = 2 Hz, J_2 = 7.2 Hz, 1H), 5.10 (brt, J = 7.6 Hz, 2H), 3.71 (brs, 2H), 3.25 (s, 3H), 2.98 (s, 6H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 175.52, 157.60, 133.72, 130.76, 118.66, 117.64, 108.95, 51.85, 43.29, 42.59, 17.15.

General Procedure for synthesis of compounds 81–85:

A mixture of benzothiazole quaternary salt 76–80 (2 eq.) and malonaldehyde bis(phenylimine) monohydrochloride (1 eq.) were heated to 45°C in presence of triethylamine (3 eq.) in acetonitrile for 2
hours. The solution was cooled down to room temperature and resulting solid was filtered. Compounds 81–83 were purified by precipitation from DMF-diethyl ether (20 mL, 1:4). Compounds 84 and 85 were purified using reverse phase column chromatography (acetone:water 5:1).

6-hydroxy-2-((1E,3E,5Z)-5-(6-hydroxy-3-methylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-3-methylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (81): Yield 61% (0.16 g); m.p. >260 °C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) : \(\delta\) 10.06 (s, 2H), 7.61–7.46 (m, 5H), 7.35 (s, 2H), 7.10 (d, \(J = 7.6\) Hz, 1H), 6.96 (d, \(J = 6.96\) Hz, 2H), 6.40–6.34 (m, 3H), 3.73 (m, 6H), 2.28 (s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 162.04, 155.25, 148.70, 134.80, 127.90, 126.32, 125.37, 115.69, 113.86, 108.63, 99.62, 33.28, 20.73. ESI-HRMS m/z: calc. for (C\(_{21}\)H\(_{19}\)N\(_{2}\)O\(_2\)S\(_2\))\(^+\) 395.0888, found 395.0893. \(\lambda_{abs} = 665\) nm \(\lambda_{flu} = 688\) nm

3-ethyl-2-((1E,3E,5Z)-5-(3-ethyl-6-hydroxybenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-6-hydroxybenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (82): Yield 56% (0.17 g); m.p. 234–236 \(^0\)C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) : \(\delta\) 10.45 (s, 2H), 7.58–7.48 (m, 5H), 7.31 (s, 2H), 7.11 (d, \(J = 6.8\) Hz, 1H), 6.93 (d, \(J = 8\) Hz, 2H), 6.41–6.34 (m, 3H), 4.30 (brs, 4H), 2.28 (s, 2H), 1.28 (s, 6H). \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz): \(\delta\) 161.07, 156.14, 148.69, 145.81, 137.55, 133.43, 128.03, 126.75, 125.49, 119.53, 116.13, 113.76, 108.86, 99.18, 41.18, 20.77, 12.71. ESI-HRMS m/z: calc. for (C\(_{23}\)H\(_{21}\)N\(_{2}\)O\(_2\)S\(_2\))\(^+\) 423.1195, found 423.1193. \(\lambda_{abs} = 665\) nm \(\lambda_{flu} = 689\) nm
6-hydroxy-2-((1E,3E,5Z)-5-(6-hydroxy-3-propylbenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-3-propylbenzo[d]thiazol-3-ium 4-methylbenzenesulfonate (83): Yield 62% (0.15 g); m.p. >260 °C; \( ^1H \) NMR (DMSO-\( d_6 \), 400 MHz): \( \delta \) 10.13 (s, 2H), 7.62 – 7.52 (m, 4H), 7.36 (s, 2H), 6.96 (d, \( J = 8.8 \) Hz, 2H), 6.44 – 6.35 (m, 3H), 4.23 (brs, 4H), 1.72 (brd, \( J = 6.8 \) Hz, 4H), 0.95 (t, \( J = 7.2 \) Hz, 6H). \( ^{13}C \) NMR (DMSO-\( d_6 \), 100 MHz): \( \delta \) 161.83, 155.42, 148.87, 134.27, 126.55, 115.91, 114.11, 108.77, 95.54, 47.26, 20.82, 10.84. ESI-HRMS m/z: calc. for (C\(_{25}\)H\(_{27}\)N\(_2\)O\(_2\)S\(_2\))\(^+\) 451.1508, found 451.1514. \( \lambda_{\text{abs}} \) = 670 nm \( \lambda_{\text{fluor}} \) = 689 nm.

6-hydroxy-2-((1E,3E,5Z)-5-(6-hydroxy-3-(2-(trimethylammonio)ethyl)benzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-3-(2-(trimethylammonio)ethyl)benzo[d]thiazol-3-ium bromide (84): Yield 54% (0.16 g); m.p. 242 - 244 °C; \( ^1H \) NMR (DMSO-\( d_6 \), 400 MHz): \( \delta \) 10.12 (s, 2H), 7.67 – 7.62 (m, 4H), 7.39 (d, \( J = 2 \) Hz, 2H), 6.99 (dd, \( J_1 = 2 \) Hz, \( J_2 = 7.2 \) Hz, 2H), 6.68 (d, \( J = 13.2 \) Hz, 2H), 6.50 (t, \( J = 12 \) Hz, 1H), 4.34 (brs, 4H), 3.65 (brs, 4H), 3.10 (s, 18H), 2.13 (brs, 2H). \( ^{13}C \) NMR (DMSO-\( d_6 \), 100 MHz):
δ 162.01, 155.43, 149.34, 133.87, 126.52, 115.81, 113.93, 108.81, 99.77, 61.99, 52.40, 42.87, 21.17. ESI-HRMS m/z: calc. for (C_{31}H_{43}Br_N_{4}O_{2}S_{2})^{+} 725.1194, found 725.1180. λ_{abs} = 670 nm λ_{flu} = 691 nm

3-(2-(dimethylamino)ethyl)-2-((1E,3E,5Z)-5-(3-(2-(dimethylamino)ethyl)-6-hydroxybenzo[d]thiazol-2(3H)-ylidene)penta-1,3-dien-1-yl)-6-hydroxybenzo[d]thiazol-3-ium bromide (85): Yield 53% (0.22 g); m.p. 229 - 231 °C; ¹H NMR (DMSO-d$_6$, 400 MHz): δ 10.05 (s, 2H), 7.61–7.48 (m, 4H), 7.34 (d, , J = 2 Hz, 2H), 6.96 (dd, J$_1$ = 2 Hz, J$_2$ = 6.8 Hz, 2H), 6.42-6.35 (m, 3H), 4.36 (brd, J = 5.6 Hz, 4H), 2.62 (brs, 4H), 2.25 (s, 12H). ¹³C NMR (DMSO-d$_6$, 100 MHz): δ 161.95, 155.33, 148.66, 134.34, 126.44, 120.04, 115.88, 114.12, 108.72, 99.82, 56.12, 45.57, 44.43. ESI-HRMS m/z: calc. for (C_{27}H_{35}N_{4}O_{2}S_{2})^{+} 509.2045, found 509.2048. Anal. Calcd for C$_{27}$H$_{35}$N$_{4}$O$_{2}$S$_{2}$.1.5H$_{2}$O: C, 52.72; H, 5.65; N, 9.13; Br, 12.57; Found C, 52.59; H, 5.88; N, 9.09; Br, 12.96. λ_{abs} = 670 nm λ_{flu} = 693 nm
SP-66b

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REFERENCES


37. SMITH, G. S. L. B. A. L. 1,1’-diaryl-4,4’-carbocyanine salts 1941.


56. Licha, K., Contrast Agents for Optical Imaging


APPENDIX

$^1\text{H}$ and $^{13}\text{C}$ NMR