Synthesis, Characterization, Optical Properties and DNA Cleavage Study of Symmetrical Pentamethine Cyanine Dyes Containing Quinoline Moieties

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SYNTHESIS, CHARACTERIZATION, OPTICAL PROPERTIES AND DNA CLEAVAGE STUDY OF SYMMETRICAL PENTAMETHINE CYANINE DYES CONTAINING QUINOLINE MOIETIES

by

KRISTINA ILINA

Under the Direction of Professor Maged Henary, Ph.D.

ABSTRACT

This thesis outlines a review of the history, synthetic procedures, optical properties, and applications of quinoline-containing cyanine dyes in the first chapter. The second chapter introduces the synthesis, characterization, and optical properties of new symmetrical pentamethine cyanine dyes and their potential application in photodynamic therapy (PDT). The structure identification of final compounds was done by $^1$H and $^{13}$C nuclear magnetic resonance (NMR) and mass spectrometry. The studies were performed after full characterization and determination of optical and physicochemical properties. Several representative dyes were selected for analysis of their DNA photocleavage activity.

INDEX WORDS: Synthesis, Pentamethine cyanine, Quinoline, Quinaldine, Lepidine, DNA cleavage
SYNTHESIS, CHARACTERIZATION, OPTICAL PROPERTIES AND DNA CLEAVAGE
STUDY OF SYMMETRICAL PENTAMETHINE CYANINE DYES CONTAINING
QUINOLINE MOIETIES

by

KRISTINA ILINA

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
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SYNTHESIS, CHARACTERIZATION, OPTICAL PROPERTIES AND DNA CLEAVAGE STUDY OF SYMMETRICAL PENTAMETHINE CYANINE DYES CONTAINING QUINOLINE MOIETIES

by

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College of Arts and Sciences
Georgia State University
August 2020
DEDICATION

I would like to dedicate this achievement to my mom, dad, grandmother and husband.
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First, I greatly appreciate patient encouragement, support and advice received from my advisor and mentor Dr. Maged Henary. Second, I wish to thank Dr. Grant for her help and discussion with my thesis. Third, I want to thank Matthew Laramie for helping me in the beginning of my research.

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1 CYANINE DYSES CONTAINING QUINOLINE MOIETIES: HISTORY, SYNTHESIS, OPTICAL PROPERTIES AND APPLICATIONS

Cyanine dyes carrying quinoline moieties are an important class of organic molecules having a great interest and applications in many fields like medicine, pharmacology, and engineering. Despite their exceptional properties, such as stability, high molar extinction coefficients, and high pH-sensitivity, this class of dyes has been less analyzed and reviewed for the last decades. This chapter is describing history, various synthetic routes to prepare symmetrical and asymmetrical mono-, di-, tri-, penta- and heptamethine cyanine dyes, containing quinoline moieties, together with their optical properties and applications as photosensitizers in photodynamic therapy, probes in biomolecules labeling of nucleic acids and DNA, as well as imaging agents.

1.1 Introduction to Cyanine Dyes

Cyanine dyes are a class of organic functional dyes, distinguished by two nitrogen centers. The general structure of dyes is shown in Figure 1.1. Cyanine dyes have conjugated systems represented by alternating single and double bonds in a chain. The chain is constituted of \( sp^2 \)-hybridized carbon atoms forming a conjugated bond. Conjugation between groups end up in the displacement of \( \pi \) electrons and positive charge on the nitrogen atom.\(^1\)

![Figure 1.1. General structure of cyanine dyes.](image)

The carbon chain makes cyanine dyes unique, giving a more extensive absorption wavelength than the other known dyes. By the addition of each covalent bond in a chain, the absorption of a dye is increasing by about 100 nm. As a result, cyanine dyes can display absorption
and fluorescence in the range of the electromagnetic spectrum from the visible to infrared. The strong absorption of the light at various wavelengths causes solutions of the cyanine molecules to become brightly colored. The stronger the absorption, the higher influence the molecule will have towards applications in various research studies. These dyes also tend to aggregate what gives rise to narrower absorption of light.

Figure 1.2. Electromagnetic spectrum.

The name of the dye becomes specific to every compound from the length of the chain between the two nitrogen atoms, and the methine groups involved. If one methine group is present in the chain, the dye is called a monomethine cyanine. The dyes with 2, 3, 5 and 7 methine groups are called dimethine, trimethine, pentamethine and heptamethine cyanine dyes accordingly.

Cyanine dyes can be structurally classified based on the nature of the end groups. Compounds with two heterocycles are called closed chain cyanines, with one terminal heterocyclic group and non-cyclic end group – hemicyanines and without heterocyclic groups are defined as streptocyanines. Three classes of cyanine dyes are presented in Figure 1.3.
Each nitrogen atom is an independent part of heterocyclic moieties, such as indole, benzoxazole, benzothiazole, and quinoline. A variety of commonly used heterocyclic groups is illustrated in Figure 1.4.

If the heterocyclic moieties on both sides of the chain have the same structure, then the dye is called symmetrical. The electron density distribution of such dyes is also symmetrical. If the heterocyclic terminal groups differ from one another, then the dye is called asymmetrical.

There is one more type of cyanines in which two nitrogen groups are directly linked to and have no methine groups called apocyanine dyes. The only known compounds of this type of dyes are red Erythroapocyanine and Xanthoapocyanine as shown in Figure 1.5.

---

**Figure 1.3. Classification of cyanine dyes.**

**Figure 1.4. Examples of heterocyclic moieties used to synthesize cyanine dyes.**

**Figure 1.5. Structures of apocyanine dyes.**
Charles Hanson Greville Williams synthesized the first cyanine dye in 1856. Williams obtained quinoline by distillation of cinchonine and heated the distillate with amyl iodide and excess of ammonia, which resulted in “a magnificent blue color” compound, called Quinoline Blue and illustrated in Figure 1.6.\textsuperscript{9,10}

\begin{center}
\includegraphics[width=0.5\textwidth]{quinoline_blue.png}
\end{center}

\textit{Figure 1.6. The structure of the first synthesized cyanine dye.}

Williams noted that quinolinium salts tend to give intense colors on heating with silver oxide. But it was useless for dyeing fabrics because of its photo instability. Later in 1873, H.W. Vogel started to use them as photosensitizers in photography.\textsuperscript{11} It led to the synthesis of the series of photographic sensitizers, like Ethyl Red and Sensitol Red (Pynacyanole), shown in Figure 1.7. Since then, the class of cyanine dyes has grown exponentially and have been used in a wide range of applications. Another important dye, pseudoisocyanine (PIC) (Figure 1.7), synthesized in 1936 by Jelley and Sheibe, was the first dye, possessed unique J-aggregation properties, showing two absorptions maxima.\textsuperscript{12}

\begin{center}
\includegraphics[width=0.5\textwidth]{ethyl_red_sensitol_red_pic.png}
\end{center}

\textit{Figure 1.7. Dyes used as photosensitizers in photography.}

In this review article we focus on closed-chain cyanine dyes, where at least one heterocyclic moiety contains quinoline. The history, synthesis, optical properties, and application
of symmetrical and asymmetrical mono-, di-, tri-, penta- and heptamethine cyanines with quinoline moieties are discussed in details.

1.2 Introduction to Cyanine Dyes Containing Quinoline Moieties

In 1940s acridine compounds showed antibacterial results. Some simple aminoacridines had a wide range of applications before penicillin became easily accessible to armed forces in World War II. Moreover, in 1981, it was found that aminoacridines’ the affinity for DNA caused the poisonous effect on yeasts in culture.\textsuperscript{13} To understand the reasons for the acridine compounds performance, the activity of recognizable moieties was examined. The quinoline and pyridine fractions were identified by the inspection of the acridine molecule. The cyanine fragment from the acriflavine molecule was also explored as an antibacterial agent and looked promising for \textit{in vivo} studies.\textsuperscript{14}

\begin{center}
\textbf{Figure 1.8. Historic cyanine development from Acriflavine.}
\end{center}

The quinoline ring system has been widely used in medicinal and industrial chemistry.\textsuperscript{15-18} Quinoline has become an attractive scaffold for the development of new fluorescent reagents due to its small molecular size and nitrogen’s ability to form hydrogen bonds. A lot of drugs and natural
compounds have had this moiety in their structures. For these reasons, quinoline-containing dyes are valuable and show great potential for various studies.\textsuperscript{19}

Quinolines (also known as benzo[\(b\)]pyridines, 1-azanaphthalenes and benzazines) are heterocyclic aromatic compounds with one nitrogen atom. It consists of a benzene ring condensed with a pyridine ring at 2,3-positions. First quinoline was synthesized from coal tar in 1834 by a German chemist Friedlieb Ferdinand Runge. Coal tar remains the main source of commercial quinoline.\textsuperscript{20} The prime sources of quinoline are petroleum, coal processing, wood preservation, and shale oil.

In 1881, Skraup presented the classic quinoline synthesis by heating aniline with glycerol and with concentrated sulfuric acid with the subsequent addition of nitrobenzene. The use of inexpensive starting materials in the synthesis of quinolines makes this method important despite the low yields and other practical shortcomings.\textsuperscript{21}

\begin{center}
\textit{Scheme 1.1. Skraup synthesis of quinolines.}
\end{center}

Alkylquinolines can be obtained from petroleum, coal tar, shale oil, soya bean, and green coffee.\textsuperscript{22} The quinoline derivatives can be found in various natural products like tecleabine, tecleoxine, quinine, ciprofloxacin which are regarding to medicinal chemistry, and biomedical use, are obtained by the thermal degradation of natural organic compounds. For instance, furoquinoline alkaloids tecleoxine and tecleabine can be isolated from the aerial parts of Teclea nobitis plant which is used in traditional medicine in Africa as an analgesic and antipyretic and to treat gonorrhea.\textsuperscript{23} Toddaquinoline can be separated from the root bark of Formosan Todalia asiatica which is used as a folk medicine in Asia.\textsuperscript{24} The bark of the cinchona tree was used to isolate quinine in 1820 to treat malaria and babesiosis.\textsuperscript{25} Quinoline-containing drugs have been
used to treat malaria for a long time, and some of them are presented in Figure 1.9. The highly cost-effective chloroquine was widely used in antimalarial therapy during World War II.\(^{26}\)

![Chemical structures of Tecteabine, Tecteoxine, Toddaquinoline, Ciprofloxacin, Quinine, and Chloroquine.](image)

**Figure 1.9. Natural products containing quinoline nucleus.**

The most frequently used quinoline compounds are 2- and 4- methyl derivatives, called quinaldine and lepidine respectively.

Lepidine (4-methyl quinoline) is used in medicine, synthesis of dyes, and as a food additive. Lepidine can be obtained from the highest-boiling fraction of the coal-tar quinoline bases through its addition of o-cresol compound. It can also be formed by reaction of aniline with methyl vinyl ketone in the presence of hydrochloric acid in ethanol or ethanolic ferric chloride or with 3-oxobutanal with catalytic amount concentrated sulfuric acid (Scheme 1.2).\(^{27}\)

![Chemical reactions for the synthesis of Lepidine.](image)

**Scheme 1.2. Lepidine (4-methylquinoline) synthetic routes.**
Quinaldine (2-methyl quinoline) is a useful ingredient in anti-malarial drugs, in manufacturing dyes, food colorants, pharmaceuticals and pH indicators. Quinaldine can be produced from aniline by the various synthetic methods:

a) The condensation of aniline with acetaldehyde under the influence of hydrochloric acid (Doebner -Miller synthesis). This reaction may also be completed in the microwave within several mins.\(^ {27}\)
b) The reaction of aniline with an aldehyde and pyruvic acid (Doebner synthesis).\(^ {28}\)
c) The optimized method – an aerobic oxidative aromatization of simple aliphatic alcohols and anilines using a catalytic system containing 2,4,6-Colidine, Pd(OAc)\(_2\) and Trifluoroacetic acid (TFA) with 93% yield.\(^ {29}\)

\[
\begin{array}{ccc}
\text{a, b, c} & \text{a} & \text{b} \\
\text{NH}_2 & \text{H} & \text{O} \\
\text{HCl / Al}_2\text{O}_3 & \text{HOOC} & \text{EtOH / Pd(OAc)}_2 \\
\text{c} & \text{c} & \text{c} \\
\end{array}
\]

Scheme 1.3. Quinaldine (2-methylquinoline) synthetic routes.

Quinoline is a weak basic compound, since the lone pair of electrons on nitrogen does not participate in the formation of delocalized \(\pi\) molecular orbital. As a result, quinolines can undergo nucleophilic substitution reactions and form various salts. These salts are an essential step in the synthesis of cyanine dyes. A lot of different types of salts has been synthesized and various methods have been optimised.\(^ {30-34}\) There are over 180 published salts synthesized by various methods from quinaldine and almost 450 salts from lepidine.\(^ {28}\)

The common methods of the synthesis of some lepidine and quinaldine based salts available in the literature are presented in Scheme 1.4 and Scheme 1.5. The identical salts are shown to compare the conditions used for the preparation of quinoline-containing salts. Most of
them are synthesized from lepidine and quinaldine directly, as salts 1-6 (Scheme 1.4) and 8-13 (Scheme 1.5), while some of them require a different initial reagent, such as diphenylamine for the synthesis of salts 7 (Scheme 1.4) and 14 (Scheme 1.5).30, 31, 33-48

Scheme 1.4. Synthetic routes of various lepidine salts.
Scheme 1.5. Synthetic routes of various quinaldine salts.

Winstead’s group presented the alternate synthetic route of salts 1a and 2 in 2013. This technique uses microwave and allows to decrease reaction time and to produce the target quinolinium salts with no purification necessary and with higher yields.42

Further, there is presented the detailed discussion of each class of cyanine dyes containing quinoline moieties with the following description of their optical properties and proposed applications in biomedicine.

1.3 Synthesis of Monomethine Cyanine Dyes Containing Quinoline Moieties

Monomethine dyes have only one methine unit in the conjugated chain between heterocycles. They absorb in the visible region at 450-520 nm. In common, monomethine cyanine dyes are synthesized by the condensation of two heterocyclic quaternary salts with a basic reagent.49, 50
One of the methods to synthesize symmetrical monomethine cyanines is carried out by the condensation of sulfobetaines from \( N \)-alkylheterocyclic compounds with quaternary salts of heterocyclic 2- or 4-methyl compounds in the presence of triethylamine. Another method is performed upon heating of 2-methylthioquinolinium salt and quinaldine salts in refluxing pyridine. Both routes are shown in Equation 1.\(^{51}\)

\[
\begin{align*}
\text{Equation 1} \\
\text{Alk} = \text{CH}_3, \text{(CH}_2\text{)}_n\text{CH}_3 \\
n = 1 \text{ to } 17
\end{align*}
\]

The majority of synthesized monomethine dyes are found asymmetrical, so heterocyclic moieties are usually different. One of them should have a good leaving group, and the reaction should be run in the presence of a base, such as triethylamine or pyridine. Asymmetrical monomethine cyanines have shown very high binding constants, high molar absorptivities and quantum yields, and the generation of high fluorescence signals upon binding. General synthesis of monomethine cyanine dyes containing quinoline, where \( X \) is a leaving group, and \( Y \) is an anion is shown in Equation 2.

\[
\begin{align*}
\text{Equation 2} \\
\text{Base} \\
\text{X} = \text{S, SCH}_3, \text{SO}_3^-, \text{NH, Cl} \\
\text{Y} = \text{anion} \\
\text{R, R}^\prime = \text{Alk, Ar}
\end{align*}
\]

Most approaches towards the preparation of these monomethine dyes function under the principle where quaternary salts of heterocyclic compounds are catalyzed by heat in the presence of a basic compound. Two well-known asymmetrical dyes Thiazole Orange (TO) and Oxazole Yellow (YO) are synthesized by the reaction of 2-methylthiobenzothiazole (TO) or 2-
methylthiobenzoxazole (YO) salts with quaternized heterocyclic salts containing active methyl group in the presence of triethylamine.\textsuperscript{37,49} The various TO and YO related monomethine dyes containing different side chains and anions 15-18 are shown in Equation 3. The alternate method of synthesis of TO monomethine dyes uses microwave-assisted synthesis, decreasing reaction time up to 10 min and improving yield up to 90%.\textsuperscript{37} But both methods have some disadvantages due to the toxicity of released methyl thiol.

\[
\begin{align*}
\text{15 TO} & \quad \text{16 TO-PRO-1} & \quad \text{17 YO} & \quad \text{18 YO-PRO-1} \\
X & S & S & O & O \\
Y & I & 2I & I & 2I \\
R & CH_3 & CH_3 & CH_3 & CH_3 \\
R' & CH_3 & (CH_2)_3N^+(CH_3)_3 & CH_3 & (CH_2)_3N^+(CH_3)_3 \\
\end{align*}
\]

In 1995, Deligeorgiev offered another method of TO and YO monomethine dyes synthesis, which avoided the evolution of methyl thiol.\textsuperscript{52} The new synthetic route is based on the condensation of 2-iminobenzothiazolines with 1-alkyl-4(or 2)-methylquinolinium salts with no solvents and presented in Scheme 1.6.\textsuperscript{51} In 1999, he also offered an option to heat together an \(N\)-alkylheterocyclic compound and the quaternary salt of a heterocyclic 4-methyquinoline either in solvents or by melting of the starting compounds under vacuum at 150-220 °C. Dyes 19 and 20 are the examples of compounds synthesized by these methods with good yields above 60%.\textsuperscript{52}
Another pathway of the production of a monomethine dye involves the reaction of N-alkyl-2-methylbenzothiazolium salt with 4-chloro-quinolinium salt. This method also allows to avoid the formation of toxic volatile by-products. This synthetic route can be used for the preparation of dicationic benzothiazole cyanine dyes 21 and 22. The goal of increased cationic charge is to increase the water solubility of the dyes.\textsuperscript{52}

Fei \textit{et al.} offered a new method of synthesis TO and its derivatives by using solid-phase resin support. The schematic representation of TO and its derivatives preparation is shown in Scheme 1.7. The first step is the attachment of 2-mercaptobenzothiazole to the Merrifield resin, and then the formed intermediate 23 reacts with 4-methylbenzenesulfonate to form N-methyl-benzothiazole salt 24. The final step involves the condensation of the N-methyl-benzothiazole salt with lepidine derivatives forming cyanine dyes 15, 25-27 (Scheme 1.7) in high yields above 95\%.\textsuperscript{53}
Scheme 1.7. Solid-phase synthesis of monomethine cyanines.

The majority of published monomethine dyes are synthesized from lepidine salts. However, various asymmetrical monomethine dyes have been synthesized from quinaldine and thiazole salts or two quinolinium salts connected at 2- and 4-positions. Asymmetrical compounds 28-34 in Figure 1.10 are synthesized by the condensation of two salts in the presence of methyl p-toluenesulfonate and triethylamine as shown in Equation 5.\cite{54}

\[ 
\text{Scheme 1.7. Solid-phase synthesis of monomethine cyanines.} 
\]

Figure 1.10. Monomethine quinaldine-containing cyanine dyes.\cite{54,55}

1.4 Synthesis of Dimethine Cyanine Dyes Containing Quinoline Moieties

Dimethine cyanine dyes have two methine carbons in a chain and absorb in the visible range at 450-620 nm. As well as monomethine cyanines, dimethine cyanines have been found mostly asymmetrical and don’t differ much by synthetic methods. Compared to monomethine
cyanine dyes, dimethine and other polymethine cyanine dyes are environmentally friendly synthesized, avoiding the formation of thiol molecules.\textsuperscript{15}

Only two available symmetrical dimethine cyanine dyes were found, which were synthesized by Kuz’mina \textit{et al.} in 2006.\textsuperscript{56-57} Dyes 36 and 37 contain several cations and anions and are prepared in two steps with the formation of intermediate 35, which is subsequently quaternized by ethyl 4-methylbenzenesulfonate or 3-bromopropylamine hydrobromide and treated by concentrated perchloric acid. The synthesis is presented in Scheme 1.8.

\textit{Scheme 1.8. Synthesis of symmetrical dimethine cyanine dyes.}

The commonly used synthesis of asymmetrical dimethine dyes includes the reaction of a heterocyclic quaternary salt having an active methyl group at 2- or 4-position with an aromatic heterocyclic aldehyde. Ethanol with piperidine has become the most often used condition for making dimethine cyanine dyes (Equation 6), showing the highest yield results.

The method, shown in Equation 6, has been improved by microwave irradiation with no solvent used. This approach results in shorter reaction times and simple product isolation.
procedures with no toxicity or harm.\textsuperscript{32} There are some examples of dimethine cyanine dyes \textsuperscript{38-48} available in the literature and synthesized in ethanol under piperidine (Figure 1.11).\textsuperscript{15, 32, 58-61}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Structures of some known dimethine cyanine dyes, synthesized from Lepidine and Quinaldine.\textsuperscript{15, 32, 58-61}}
\end{figure}

1.5 Synthesis of Trimethine Cyanine Dyes Containing Quinoline Moieties

Trimethine cyanine dyes have three methine carbons in bridge and absorb in the visible range at 450-620 nm. The first trimethine quinoline-containing cyanine dye was symmetrical and described in 1920 by Mills and Pope and by Wise, Adams, Stewart and Lund. Symmetrical dye 1,1-Diethyl-2,2-carbocyanine iodide, also known as Pinacyanol or Sensitol Red (Figure 1.12), was prepared from two molecules of quinaldine ethyl iodide and formaldehyde. The same method was used to synthesize another symmetrical 1,1-Diethyl-4,4-carbocyanine iodide, called kryptocyanine. Another synthesis method offers to use ethyl orthofomate in the presence of acetic anhydride instead of formaldehyde.\textsuperscript{9}
One more synthetic route to synthesize symmetrical trimethine cyanines includes the reaction of lepidine salts with triethoxymethane. The resulted cyanine iodide 51 and its tosylate 52 are treated with lithium bis(perfluorobutylsulfonyl)imide forming new trimethine cyanine dyes 53 and 54 (Scheme 1.9). \(^\text{62}\)

**Scheme 1.9. Synthesis of symmetrical trimethine cyanine dyes.**

Usually, for the synthesis of asymmetrical trimethine cyanine dyes, one molecule of quaternary salt is used to form hemicyanine intermediate, which then reacts with another salt molecule to gain the asymmetrical dye. Triethylamine and pyridine are the most used bases. Two the most famous trimethine asymmetrical cyanines are TO-3 (55) based on Thiazole Orange (TO) and YO-3 (56) based on Oxazole Yellow (YO) with three carbons in a chain, and their dicationic derivatives TO-PRO-3 (57) and YO-PRO-3 (58) are presented in Figure 1.13.
Figure 1.13. Chemical structures of TO-3, YO-3, TO-PRO-3 and YO-PRO-3.

TO analogues have attracted researchers’ attention and different modifications and incorporations of TO-3 have been synthesized. Firstly, either quinoline or thiazole salt is connected to the linker. The formed hemicyanine 59 condenses with another quaternary salt and gains various alternatives of TO-3. Sun et al. synthesized the dye 61 with two positive charges, forming firstly the intermediate bromo-version 60. Then bromine atom in dye 60 is converted into quaternary ammonium in dye 61, as shown in Scheme 1.10.63

Scheme 1.10. Synthesis of trimethine cyanine TEAB-TO-3.

Indole nucleus is often used instead of thiazole in asymmetrical cyanine dyes and may form Dimethyl Indole Red (DIR) 63. The first step of 63 synthesis includes quaternary salt preparation by alkylation of lepidine with propanesultone, which is subsequently condensed with N,N-diphenylformamidine and forms hemicyanine dye 62. The last step includes the formation of
trimethine dye 63 by condensation of hemi-dye with methyl indoline bromide under the base, as illustrated in Scheme 1.11.64

\[ \text{Scheme 1.11. Synthesis of Dimethyl indole Red (DIR).} \]

Another synthetic approach of asymmetrical trimethine cyanine dyes with indole moiety in a multistep was accomplished by Fei Group (Scheme 1.12). Herein the hemicyanine 64 is formed from methyl indoline derivative, which then reacts with lepidine salt under basic conditions to form the asymmetrical cyanine dyes 65-68.65

\[ \text{Scheme 1.12. Synthesis of trimethine indoline quinoline dyes.} \]

The synthetic approach, presented in Scheme 1.12, has some disadvantages, such as the formation of intermediates and difficulties of purification of dyes. In order to avoid these drawbacks in 2005, Mason et al. offered some new approaches to synthesize quinoline-containing trimethine cyanine dyes, as 72-76.66 The synthesis of carbocyanine dye 72 is presented in Scheme 1.13 and involves two synthetic routes. The first approach includes the formation of hemicyanine 69, which then is activated by sulfonylation with sulfonyl chloride polystyrene forming 70 which then reacts with quinolinium salt 2. Unreacted hemicyanine remains on the resin, and the pure
trimethine dye can be isolated. The second method involves the synthesis of polystyrene-bound immediate 71 from immobilized aniline, which is subsequently used to react with quinolinium salt 2 to form the dye 72. Figure 1.15 shows various dyes synthesized by this method, including both quinaldine (73 and 75) and lepidine (74 and 76) based fluorophores.

Scheme 1.13. Solid-phase synthesis of trimethine cyanine dyes.

Figure 1.14. Trimethine cyanine dyes synthesized by solid-phase method.

1.6 Synthesis of Pentamethine Cyanine Dyes Containing Quinoline Moieties

Pentamethine cyanine dyes commonly absorb in the visible and near-infrared (NIR) region at 650–850 nm. The general synthetic procedure of symmetrical pentamethine dyes involves the use of malonaldehyde bis(phenylimine) derivatives under basic conditions and 2 moles of quinoline-containing salts as exemplified in Equation 7. Pentamethine fluorophores often have a
precursor at the meso-position in order to improve their photophysical properties. Mesosubstituents in the pentamethine chain usually belong to proton or halogen atoms.

The presented method in Equation 7 can be used to synthesize symmetrical pentamethine dyes 77-83 in good yields, which are illustrated in Figure 1.15.\textsuperscript{16, 67, 68}

\begin{equation}
\begin{array}{c}
\text{N}^- \quad \text{X}^- \\
\text{Ph} \quad \text{N} \quad \text{Ph} \\
\text{Ac}_2\text{O}, \text{EtOH or ACN} \\
\text{Et}_3\text{N} \text{ or NaOAc}
\end{array}
\quad \begin{array}{c}
\text{N}^- \quad \text{X}^- \\
\text{Ph} \quad \text{N} \quad \text{Ph} \\
X = \text{H, Cl, Br}
\end{array}
\end{equation}

\textbf{Figure 1.15. Examples of symmetrical pentamethine cyanine dyes.}\textsuperscript{16, 67, 68}

In order to synthesize asymmetrical pentamethine cyanine dyes the first step is to prepare hemicyanine intermediate, which then reacts with quinolinium salts under basic conditions. The same method as described earlier for trimethine synthesis was offered by Mason \textit{et al.} for the synthesis of pentamethine cyanines 86-90, based on the use of solid-phase resin support (Scheme 1.14). The first step involves the reaction of malonaldehyde diacetal forming the intermediate precursor 84. The second step is the reaction of received precursor with methyl indolinium salt in order to form hemicyanine 85, which generates the pentamethine cyanine dye 86 by the subsequent reaction with quinolinium salts.\textsuperscript{66} The described method was used for the synthesis of dyes 87-90, which are illustrated in Figure 1.16.
Figure 1.16. Pentamethine cyanines synthesized by solid-phase method.

1.7 Synthesis of Heptamethine Cyanine Dyes Containing Quinoline Moieties

Heptamethine cyanine dyes usually absorb in the near-infrared (NIR) region (750-900 nm) and contain seven carbons in a chain. Heptamethine dyes usually have substituents at different positions of a chain, as they can help to improve the photophysical and photochemical properties of the dyes. Strekowski found that the introduction of a chlorocyclohexenyl ring in the middle of the polymethine chain may enhance dyes’ photostability. That’s why the majority of synthesized heptamethine cyanines are with a six-membered ring in the chain.

Chloro-heptamethine cyanine dyes have traditionally been synthesized by the condensation of the quaternary ammonium salt and bisaldehyde or its equivalent with the subsequent addition of the second molar equivalent of the salt. The process is usually going in
acetic anhydride or ethanol under sodium acetate, triethylamine, piperidine or pyridine. But sometimes benzene-butanol mixture may be also used as a solvent (Scheme 1.15).70

Scheme 1.15. Synthesis of symmetrical heptamethine cyanine dye.

The cyclohexene moiety in the polymethine core can be replaced or modified with substituents instead of the chlorine atom with phenyls, or at any other position of the chain, as in dyes 93-97. The synthesis of symmetrical heptamethine dyes 93-97 implies the synthesis of 2 moles of quinolinium salts and 1 mole of the linker, which allows to form a dye within one step.71-73 The resulted iodides 94 and 95 may be treated with sodium tetrakisylborate in order to form new fluorophores 94 and 97.

Scheme 1.16. Synthesis of symmetrical heptamethine cyanine dyes with substitutions at the center of the bridge.

The same synthetic route is used to synthesize asymmetrical cyanine dyes, but instead of 2 moles of salts, one salt differs from another one as illustrated in Scheme 1.17 and 18. 17,74 For the
synthesis of fluorophores 99 and 101, the first step is the preparation of hemicyanines 98 and 100 accordingly, which then undergo the reflux with other salts under basic conditions.


The methine bridge may be modified and have substituents at different positions in the chain. Štacková et al. offered a new approach of the generation of heptamethine cyanine dyes with the introduction of substituents at C3 position of the chain. The synthesis includes the condensation
of 3-phenylpyridine and 2,4-dinitrophenyl p-toluenesulfonate. The subsequent reaction of the resulted semi-product 102 with 3 eq of salt 9 in the presence of sodium acetate and 4-bromoaniline forms the final product 103 in 47% yield.\textsuperscript{72}

\begin{center}
\includegraphics[width=0.8\textwidth]{synthesis.png}
\end{center}

Scheme 1.19. Synthesis of heptamethine cyanine dye 103 with the substituent at C\textsubscript{3} position.

The synthetic routes of cyanine dyes containing quinoline moieties that are published in the literature are summarized and presented in Schemes and Equations as outlined above. The optical properties and recent applications of some selected fluorophores are further discussed in the next section of this review article.

1.8 Optical Properties and Applications of Cyanine Dyes Containing Quinoline Moieties

The first synthesized cyanine dyes were mostly used as photosensitizers in photography. In 1936, Scheibe and Jelley discovered pseudoisocyanine (PIC) (Figure 1.7), which showed an absolutely new type of aggregation with two absorption maxima at 490 and 525 nm and the formation of the third peak in aqueous solutions with DNA at 580 nm (Figure 1.17). This type of dyes was named J-aggregates, which may be formed through dispersion forces, induced by the high molecular polarizability. The J-aggregates have a highly emissive property, while a monomeric PIC indicates no emission.\textsuperscript{75} Pseudoisocyanine has become one of the most investigated organic compound due to its unique properties and has been used in photographic processes as a component of signal processing light-harvesting materials.\textsuperscript{76} For many years J-aggregates of cyanines have been used in silver halide photography and as photodetectors. A lot
of fluorophores, forming J-aggregates have been designed to bind to biological membranes and to stain the membranes of living cells.\textsuperscript{77}

![Figure 1.17. The absorption spectra of PIC in mixture water and methanol in the absence (dash) and the presence (solid) of DNA at different concentrations.\textsuperscript{78}](image)

The major application of monomethine cyanine dyes is as fluorescent labels for DNA and imaging probes for macromolecules, because of their fluorescence enhancement and outstanding photophysical properties. Thiazole Orange (TO) \textsuperscript{15}, Oxazole Yellow (YO) \textsuperscript{17} (Equation 3), and their derivatives are used as fluorescent polymer base substitutions, imaging probes in biology, and DNA and RNA detection.\textsuperscript{64,79-80} TO is an example of a dye that has favorable physical, optical, and biological staining characteristics with absorption wavelength at 502-510 nm both in methanol and with the addition of DNA, RNA, and protein. In the absence of DNA or RNA, TO has quite low fluorescence because of the absence of a binding site. TO is very sensitive, whether it is free or bound to the nucleic acid. In general, if dyes are sensitive to the environment with high quantum yield are useful for imaging of biological and other systems.\textsuperscript{81} This property of the dyes has led to the development of “light-up probes” where TO is attached to the end of the probe strand.\textsuperscript{82}

YO is pretty like TO, having a high enhancement of quantum yield after binding to DNA. The molecular structures of TO and YO often undergo modifications in order to achieve higher
sensitivity, photostability, or imaging of bioactivity in living cells. Both TO and YO derivatives usually have quite low fluorescence, but become strongly fluorescent with high quantum yield after binding to biomolecule.

TO-PRO-1 and YO-PRO-1 can form the dimers TOTO and YOYO (Figure 1.18), which excite at 490 and 460 nm and emit at 530 and 510 nm, respectively, bounded to dsDNA in solution. The length of the linker between two TO monomers affects the quantum yield. As a result, TOTO exhibits a nucleic acid binding affinity 100 times greater than TO monomeric form. TOTO causes a single-strand cleavage approximately five times less efficiently than YOYO. The binding of YO and YOYO to Bacteriophage T5 has been analyzed and has proved the usefulness of dyes as spectroscopic probes for T5, despite the YOYO association to the T5 phages is much slower than to DNA, whereas YO association is quite similar. YO shows very fast association to T5 within just 1 min.

Table 1, represents the spectral properties of two similar dimethine cyanine dyes 38 and 48 (Figure 1.11), differing only by the position the linker is attaching to quinoline moiety (4-position for 38 and 2-position for 48). According to the spectral data both absorption and fluorescence wavelengths for dye 38 are higher than for dye 48 in four different solvents. Stokes shift values
increase with the increase of solvent polarity, whereas the molar extinction coefficient of dye 38 is decreasing. The analysis of spectral characteristics of dyes shows that dye 38 has very low cytotoxicity which helps to be applicable in biomedicine as fluorescent probes and in cellular imaging. The results of cell staining of dye 38 by incubation with A172 cells demonstrate that 38 may stain cytoplasm (Figure 1.19).

Table 1.1. The spectral characteristics of dyes 38 and 48 in different solvents.\textsuperscript{15}

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Characteristics</th>
<th>Dye 38</th>
<th>Dye 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>H\textsubscript{2}O</td>
<td>(\lambda_{ex}, \text{nm})</td>
<td>473</td>
<td>458</td>
</tr>
<tr>
<td></td>
<td>(\lambda_{em}, \text{nm})</td>
<td>574</td>
<td>554.2</td>
</tr>
<tr>
<td></td>
<td>Stokes shift, nm</td>
<td>101</td>
<td>96.2</td>
</tr>
<tr>
<td></td>
<td>(\varepsilon\cdot10^{-4}, \text{L mol}^{-1}\text{cm}^{-1})</td>
<td>1.91</td>
<td>3.52</td>
</tr>
<tr>
<td>DMSO</td>
<td>(\lambda_{ex}, \text{nm})</td>
<td>503</td>
<td>481</td>
</tr>
<tr>
<td></td>
<td>(\lambda_{em}, \text{nm})</td>
<td>596.6</td>
<td>565</td>
</tr>
<tr>
<td></td>
<td>Stokes shift, nm</td>
<td>93.6</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>(\varepsilon\cdot10^{-4}, \text{L mol}^{-1}\text{cm}^{-1})</td>
<td>2.09</td>
<td>3.21</td>
</tr>
<tr>
<td>MeOH</td>
<td>(\lambda_{ex}, \text{nm})</td>
<td>503</td>
<td>479</td>
</tr>
<tr>
<td></td>
<td>(\lambda_{em}, \text{nm})</td>
<td>581.6</td>
<td>558.4</td>
</tr>
<tr>
<td></td>
<td>Stokes shift, nm</td>
<td>78.6</td>
<td>79.4</td>
</tr>
<tr>
<td></td>
<td>(\varepsilon\cdot10^{-4}, \text{L mol}^{-1}\text{cm}^{-1})</td>
<td>2.28</td>
<td>4.13</td>
</tr>
<tr>
<td>CHCl\textsubscript{3}</td>
<td>(\lambda_{ex}, \text{nm})</td>
<td>513</td>
<td>503</td>
</tr>
<tr>
<td></td>
<td>(\lambda_{em}, \text{nm})</td>
<td>570</td>
<td>568</td>
</tr>
<tr>
<td></td>
<td>Stokes shift, nm</td>
<td>57</td>
<td>65</td>
</tr>
</tbody>
</table>
Another dimethine cyanine SLM 40 (Figure 1.11) is investigated as an excellent theranostic agent for diagnosis and therapy of Alzheimer’s disease (AD). This compound has been successfully applied to perform near-infrared in vivo imaging of Aβ species in transgenic (Tg) and wild-type (WT) mice in vivo (Figure 1.20). Dye 40 strongly binds to the Aβ species in the brain of Tg mouse, and as a result, the mouse can recover from Alzheimer’s disease. Moreover, SLM-treated mice have shown a considerable reduction in each of oligomeric Aβ contents and τ proteins in their brain. These findings prove that dye 40 is a good theranostic agent with in vivo effectualness for diagnosing and treatment of AD in mouse models. Dye SLOH 41 (Figure 1.11) also shows potential as a therapeutic agent for Alzheimer’s disease, inhibiting the aggregation of Aβ peptides and oligomers.
Figure 1.20. Fluorescence images of Tg (9 months old) and wild-type mice at different time points before (Blank) and after intravenous injection of SLM 40 (dosage of 5 mg/kg) with recordings at 10-20-30-60-90 min.\textsuperscript{18}

Intensity properties for labeling living cells with low cell cytotoxicity and little photobleaching are shown by dyes 42 and 43 (Figure 1.11).\textsuperscript{58} The report says that these dyes can be applied as fluorescent probes in flow cytometry and as double staining agents for measuring sperm cell viability.\textsuperscript{58} The Figure 1.21 illustrates the fluorescence intensity profile of the sperm cells stained with dyes 42 and 43.

Figure 1.21. Flow cytometry histograms of dyes 42 and 43. The X-axis presents the fluorescence intensity of stained sperm cells, and the Y-axis shows the number of the stained sperm cells.\textsuperscript{58}

As it was already mentioned before, Sensitol Red (Figure 1.7), a symmetrical trimethine cyanine dye, was widely used as a photosensitizer in photography and against staphylococci and
B. coli. Another symmetrical trimethine cyanine dye Kryptocyanine (Figure 1.12) after study in red blood cell (RBC) membrane and isolation of mitochondria, shows the ability to be useful for photochemotherapy in tumors, as light-activated cytotoxic agents and for photodestruction of leukemic cells.\(^8^9\)

Dyes 55 (TO-3) and 57 (TO-PRO-3) (Figure 1.13) are trimethine analogs of TO dyes 15 (TO) and 16 (TO-PRO-1) (Equation 3). Compared to TO-1, the TO-3 and TO-PRO-3 contain an extended carbomethine bridge that considerably shifts the absorption and emission to the red at 642 and 661 nm, respectively. Like TO, TO-PRO-3 strongly binds to nucleic acids with a high fluorescence enhancement. Moreover, the dye can bind to human cytoplasmic, and mitochondrial A-site RNAs.\(^9^0\) TO-PRO-3 is also used in the pretreatment of samples for gel or capillary electrophoresis, determination of viability and resistance to staining in experiments with several labels.\(^9^0\)

TO-PRO-3 (57 – Figure 13), the same as TO-PRO-1 (16 – Equation 3), has a dimeric analog, TOTO-3 (Figure 1.22). TOTO-3 has the same absorption and emission wavelengths, strongly stains the cytoplasmic and nucleolar RNAs but weakly stains the nuclear DNA.\(^9^1\) YO-PRO-3 (58 – Figure 13) has a dimeric analog YOYO-3 (Figure 1.22) which has the same optical properties and applications for DNA staining.
Figure 1.22. Structure of the dimers TOTO-3 and YOYO-3.

Figure 1.23. Absorption and emission wavelengths of TO and YO monomers and dimers

According to the collected properties of TO and YO dicaticonic monomers and tetracaticonic dimers (Figure 1.23), monomers’ absorption and emission wavelengths correspond to wavelengths of their dimers. TO derivatives’ wavelengths differ from YO derivatives by 22-30 nm. Stokes shifts of all considered dyes (Table 1.2) lie in the range of 16-19 nm. Molar extinction coefficients increase with the extension of chain length and number of cations. The dimers have bright fluorescence signals and may be used as nuclear and chromosome counterstains for multicolor fluorescence labeling experiments and for staining nucleic acids on solid supports, such as microarrays.92
Table 1.2. The spectral characteristics of monomers YO-PRO-1 (18 – Equation 3), TO-PRO-1 (16 – Equation 3), YO-PRO-3 (58 – Figure 1.13), TO-PRO-3 (57 – Figure 1.13), and their dimers YOYO and TOTO (Figure 1.18), and YOYO-3 and TOTO-3 (Figure 1.22).92

<table>
<thead>
<tr>
<th>Dye</th>
<th>(\lambda_{\text{abs}}, \text{nm})</th>
<th>(\lambda_{\text{em}}, \text{nm})</th>
<th>Stokes shift, nm</th>
<th>(\varepsilon \cdot 10^{-4}, \text{L mol}^{-1} \text{cm}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>YO-PRO-1</td>
<td>491</td>
<td>509</td>
<td>18</td>
<td>5.2</td>
</tr>
<tr>
<td>YOYO-1</td>
<td>491</td>
<td>509</td>
<td>18</td>
<td>9.9</td>
</tr>
<tr>
<td>TO-PRO-1</td>
<td>515</td>
<td>531</td>
<td>16</td>
<td>6.3</td>
</tr>
<tr>
<td>TOTO-1</td>
<td>514</td>
<td>533</td>
<td>19</td>
<td>11.7</td>
</tr>
<tr>
<td>YO-PRO-3</td>
<td>612</td>
<td>631</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>YOYO-3</td>
<td>612</td>
<td>631</td>
<td>19</td>
<td>16.7</td>
</tr>
<tr>
<td>TO-PRO-3</td>
<td>642</td>
<td>661</td>
<td>19</td>
<td>10.2</td>
</tr>
<tr>
<td>TOTO-3</td>
<td>642</td>
<td>660</td>
<td>18</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Another trimethine dye 63 dimethyl indole red (DIR) (Scheme 1.11), exhibits a weak visible fluorescence when an excess quantity of calf thymus DNA and artificial dsDNA is present, thereby making it potential for specific light-up probe of G-quadruplex emitting long wavelength.59 The study of the conformational switch of G-quadruplex structure within the absence and presence of 63 has been explored by circular pleochroism (CD) spectroscopic analysis. The CD spectra of the parallel G-quadruplex exhibits a specific positive peak at 265 nm and a negative peak at 240 nm. And no elicited CD signal peaks within the wavelength longer than 350 nm can be detected. The probe DIR (63) has a negligible impact on the production of G-quadruplex structures. Such a G-quadruplex probe can disclose the distribution of intracellular shaped G-quadruplexes and avoid the controversy regardless of whether the detectable G-quadruplexes are present.
Figure 1.24. CD spectra of 4 μm G-quadruplex-forming oligonucleotides c-myc (A), HT 22 (B) and Hras (C) in the absence and presence of DIR 63 (8 μm) in 20 mm Tris–HCl buffer with 100 mm KCl, pH 7.4.93

For fluorophores 65-68 shown in Scheme 1.12, the effect of various substituent groups on the absorption and emission maxima can be seen in Figure 1.25. Absorption maxima of dyes 65-68 (Figure 1.25 A) lies between 599 and 603 nm, while absorption of dye 68 with R = CH₃ shifts red by 9-12 nm. Also, intensities of Cl-substituted dyes 66 and 67 are higher than of 65 (R = H) and 68 (R = CH₃).
According to Figure 1.25 B, it indicates that each curve has 2 peaks with the utmost emission wavelengths of indole quinoline dyes at regarding 650 nm. The fluorescence intensity decreases in order $67 > 66 > 65 > 68$. This means that the utmost peaks of dyes with electron-withdrawing groups shift red compared to those dyes with electron-donating groups. The second peaks were completely different, and the fluorescence intensity of 65 was stronger than others.

The fluorescence spectra of compounds excite at 550 nm are illustrated in Figure 1.25 C. All fluorophores show similar fluorescent characteristics. There is only one peak in each line, and the fluorescence intensities of all compounds are higher than those excited at 480 nm. The most emission wavelengths are set between 647 nm and 657 nm. The Stokes shifts of dyes 65-68 are all
regarding one hundred nm. The longer wavelength signs and larger Stokes shifts indicate low UV interference when labeling the organism.\(^{65}\)

Three identical symmetrical dyes, differing only by the number of carbons in a chain, such as monomethine Ethyl Red (Figure 1.7), trimethine Kryptocyanine (Figure 1.12) and pentamethine Dye 79 (Figure 1.15), have the absorption wavelengths at around 600 nm, 700 nm, and 800 nm respectively (Figure 1.26). Emission wavelengths also lie in a range 600-830 nm (Figure 1.26). Meanwhile, absorption and emission wavelengths for identical mono-, tri- and pentamethine cyanines, PIC (Figure 1.7), Sensitol Red (Figure 1.12) and Dye 83 (Figure 1.15), connected to the linker at 2-position (instead of 4-position) are 100 nm less for each one comparing to the same ones at 4-position and lie in ranges 500-700 nm and 550-750 nm respectively (Figure 1.27). It means that lepidine-containing cyanine dyes have a higher excitation and emission wavelengths due to the presence of extra double bond between the nitrogen atoms of heterocyclic rings.

*Figure 1.26. Absorption (main graph) and emission (inset) spectra of three symmetrical dyes: Ethyl Red (black), Kryptocyanine (blue) and 79 (red).*\(^{68}\)
Figure 1.27. Absorption (main graph) and emission (inset) spectra of three symmetrical dyes: Pseudoisocyanine (black), Sensitol Red (blue) and 83 (red).68

Four other dyes differ by the position of chain connection to the moiety (at 2- and 4-position) and substitution at the meso carbon. Comparison of two symmetrical 4-methylquinoline-based pentamethine dyes with meso-substitution H (77) and Cl (78) in the polymethine bridge illustrated in Figure 1.15, results in the 32-90 nm difference of the UV-visible wavelength in DMSO, buffer and buffer/DNA (Figure 1.28). In the presence of DNA dye 78 shows high stabilization, in contrast to dye 77, due to the presence of electron-withdrawing chlorine atom. After the addition of CT DNA to dye 78, the absorption maxima of the dye increases to 805 nm. As a result, dye 78 shows excellent DNA photo-cleaving properties. Likewise, this dye was used to treat ES2 cancer cells and exposed to an 808 nm laser diode. The results showed the increase of intracellular ROS levels in the cells and the decrease of their viability (Figure 1.29).16,94

Fluorophores connected to the linker at 2-position with meso-substitution Br (81) and H (82) show the similar results with stability in DMSO. With the addition of CT DNA, the absorbance of dye 81, the same as 78 stays constant, while of dye 82 noticeably decreases even before DNA inclusion (Figure 1.30). The electron-withdrawing bromine atom allows the dye to
stay stable with no autooxidation in aqueous solutions. After showing promising photo-cleaving properties, dye 81 has been incubated with ES 2 ovarian carcinoma cells and shows ready uptake by cells and quick localization in the intracellular cytosolic and perinuclear regions (Figure 1.31). Also, dye 81 shows high phototoxicity, decreasing ES 2 cells viability under 694 nm light irradiation.

Figure 1.28. UV-visible spectra of 10 μm of dyes 77 (left) and 78 (right): in DMSO, 10 mm sodium phosphate buffer pH 7.0 and in buffer with 150 μm bp CT DNA.

Figure 1.29. (A) Superimposed fluorescence microscopy image of revealing intracellular localization of dye 78 (red) in ES2 cancer cells after 24h incubation with the subsequent staining of nuclei Hoechst 33342 (blue). (B) ES2 cancer cell viability: with no treatment, under 808 nm laser expose, after dye 78 incubation in dark, after dye 78 incubation under 808 nm laser expose.
Figure 1.30. UV-visible spectra of dyes 81 (top) and 82 (bottom) in DMSO (A, B), buffer pH 7.0 (C, D) and buffer pH 7.0 with 150 µM CT DNA (E, F).\textsuperscript{94}

Figure 1.31. (A) Superimposed fluorescence microscopy image of revealing intracellular localization of dye 81 (red) in ES2 ovarian cells after 24h incubation with the subsequent staining of nuclei Hoechst 33342 (blue). (B) ES2 cancer cell viability: with no treatment, under 694 nm laser expose, after dye 81 incubation in dark, after dye 81 incubation under 694 nm laser expose.\textsuperscript{16}

As it was mentioned earlier, heptamethine cyanine dyes are near-infrared (NIR) absorbing and emitting. It allows them to be applicable in photovoltaics and nonlinear optics and \textit{in vivo} cell
imaging. Synthesized heptamethine dyes 96 and 97 (Scheme 1.16) with chlorine atom and the phenyl at the meso carbon, respectively. The neat thin-film absorption spectra of dyes and in DCM solutions are presented in Figure 1.32. Interestingly, that phenyl substitution leads to the blue shift in DCM compared to chloride substitution, due to the inductive effect, while the neat thin film leads to the red shift.

![Absorption spectra](image)

*Figure 1.32. The absorption spectra for dyes 96 (black) and 97 (red) in DCM (left) and as neat thin film (right).*

Heptamethine cyanine dye 99 (Scheme 1.17) has been investigated for application in dye-sensitized solar cells (DSCs). Figure 1.33, represents the absorption spectra of dye 99 and AN50 electrolyte as well as the transmission spectra of DSC. Transmittance is in the visible range 400-700 nm is achievable. Dye 99 has the high potential for application in DSCs sensitization.
Fluorophore 101 (Scheme 1.18) was analyzed by Shimizu after encapsulation in a lactosome and evaluation as NIR fluorescent probes for in vivo tumor imaging.\textsuperscript{17} The optical properties of dye 101, and its lactosome are shown in Table 1.3. The dye has a maximum emission wavelength in methanol at 932 nm, while its lactosome shows emission maxima at 905 nm. Interestingly, that absorption maxima of dye lactosome in water is higher than of the dye itself in organic solvents. While excitation and emission wavelengths are higher for dye 101 in methanol. As for photostability, the absorbance of lactosome is stable within 1 h under tungsten lamp irradiation.

\textit{Table 1.3. Optical properties of dye 101 and its lactosome.}\textsuperscript{17}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Abs max</th>
<th>Ex max</th>
<th>Em max</th>
<th>$\epsilon \cdot 10^{-4}$, L mol$^{-1}$cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye 101</td>
<td>Chloroform</td>
<td>781</td>
<td>805</td>
<td>897</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>790</td>
<td>883</td>
<td>932</td>
<td>2.6</td>
</tr>
<tr>
<td>Dye 101 lactosome</td>
<td>Water</td>
<td>830</td>
<td>844</td>
<td>905</td>
<td>6.0</td>
</tr>
</tbody>
</table>
After injection of dye 101 lactosome into the mouse, fluorescence intensity in the tumor region becomes visible and gradually grows up after 6 and 24 h after injection. The developed dye 101 lactosome probes, possessing low toxicity, good photostability, high fluorescence in the tumor and low background signals in liver and muscle tissue, have been offered for application in noninvasive in vivo imaging techniques to detect tumors.  

Figure 1.34. Back (top) and front (bottom) sides of mouse fluorescence images of FM3A cell xenografted mouse after injection of dye 101 lactosome, recorded at 0, 3, 6, 9, 24 and 48 h.  

1.9 Conclusion

The cyanine fluorophores containing quinoline moieties are worthy of attention because of their optical properties such as stability, high molar extinction coefficients, high sensitivity, and low aggregation. Therefore, they are useful for biomedicine, as possess antiseptic and photo-cleaving properties.

They have always had widespread applications in various fields such as pigmenting and photosensitizing, biomedical and pharmaceutical, and as probes in biomolecules labeling, detection of nucleic acids and tumors, and potential imaging agents.
This review summarizes the synthetic procedures with different initial compounds with no solvent, using solid-phase resin support or microwave irradiation. Even though, the synthesis of these dyes has a traditional methodology, the synthetic routes of these dyes are efficient to produce them in high yields.

Based on the valuable optical properties and various applications of these fluorophores, as presented and discussed in this review, it is evident that these dyes have a great potential for many useful applications. With further development of cyanines containing quinoline moieties, the forthcoming improvements and refinements of their structures, it would be possible to widen the practical applications of these dyes based on their excellent properties.
2 SYMMETRICAL PENTAMETHINE CYANINE DYES: SYNTHESIS, CHARACTERIZATION, OPTICAL PROPERTIES AND OXIDATIVE CLEAVAGE OF DNA STUDY

This chapter reports the synthesis of a series of symmetrical pentamethine cyanine dyes containing quinoline moieties with the subsequent characterization of their physical and optical properties and oxidative cleavage of DNA with the goal of developing a photosensitizer for photodynamic therapy (PDT).

2.1 Summary and the Goal of Our Study

After reviewing various cyanine dyes containing quinoline moieties, it was observed that there are not many publications discussing synthesis and optical properties of symmetrical pentamethine cyanine dyes containing quinoline moiety. Literature reports have demonstrated that the already synthesized fluorophores have been widely used in many applications, especially in biomedical and pharmaceutical fields. Therefore, we decided to synthesize new symmetrical pentamethine cyanine dyes containing quinoline moieties.

When designing new dyes, it is crucial to understand how the moieties and substituents on the heterocyclic rings can affect the properties of the dyes. Structural modifications can alter absorption and fluorescence results, solubility, and photostability. It is important to synthesize and investigate various dye analogs for comparison in order to define the ones with the best properties for biomedical applications. The positive charge of each fluorophore allows them to interact with the negatively charged phosphate backbone of DNA, producing an electrostatic attraction between DNA and the dye.

We started the synthesis of novel pentamethine quinoline-containing cyanine dyes with the preparation of dyes with benzyl-N-substituent. Deligeorgiev et al. reported that pentamethine...
benzothiazole-containing cyanine dye with a benzyl ring exhibited a very high aggregation tendency and transition into the monomeric state after binding to CL67 lipid vesicles.\textsuperscript{95} That’s why we decided to synthesize the first set of cyanine dyes containing quinaldine and lepidine moieties with benzyl-\textit{N}-substituent.

Literature analysis showed that lepidine-based dyes which have an extra double bond between nitrogen atoms of heterocycles, absorbed light at higher wavelength than quinaldine-based dyes.\textsuperscript{8} Therefore, we decided to focus our efforts on the synthesis of lepidine-based symmetrical pentamethine cyanine dyes by altering the substituent on the nitrogen atom of heterocycle and meso carbon.

The next series of dyes were prepared with carboxybenzyl, (methoxycarbonyl)benzyl, phenylpropyl, and triphenylphosphonium substituents. Zhang \textit{et al.} synthesized a heptamethine 3H-indocyanine dye with carboxybenzyl group at \textit{N}-position, showed high photostability required for fluorescent imaging technology.\textsuperscript{96} Moreover, carboxylic acid moieties exhibited the improvement of chromophores’ water-solubility.\textsuperscript{97}

(Methoxycarbonyl)benzyl substituents were developed and synthesized because no any reported synthesis of these dyes with the chosen substituents was found in the literature, and it was a new modification to add to our pentamethine scaffold. We want to investigate the effect of these substituents on the chromophores’ properties.

In our lab we reported that dyes containing a phenylpropyl substituent on the heterocyclic nitrogen exhibited functional inhibition, particularly on protein arginine methyltransferase 1 (PRMT1).\textsuperscript{98, 99} Also phenylpropyl substituent was chosen for making hydrophobic dyes for optoacoustic imaging in collaboration with Dr. Lacey McNally at Oklahoma University.
Compounds containing triphenylphosphonium (TPP) moieties have been known for their excellent solubility, DNA stabilization, antimicrobial properties, and ability to stain bacterial cells and target mitochondria for many years.\textsuperscript{100-102} Furthermore, TPP-containing compounds showed the ability to produce mitochondrial ROS, prevent mitochondrial oxidative damage, and selectively target antioxidants to mitochondria both \textit{in vitro} and \textit{in vivo}.\textsuperscript{103}

Our synthetic project included the preparation of six sets of symmetrical pentamethine cyanine dyes differing by substituent at \textit{N}-position: benzyl, carboxybenzyl, (methoxycarbonyl)benzyl, phenylpropyl, and triphenylphosphonium substituents. Each set included three dyes with bromine, chlorine, and hydrogen substituent at meso carbon in the methine chain. We also synthesized dyes with two modifications of the quinoline moieties, differing by the linker’s position attaching to the heterocycle, at 2 and 4-positions. Six different precursor salts were prepared to further reaction with polymethine linkers to yield eighteen new symmetrical cyanine dyes. These dyes were synthesized by both conventional and microwave-assisted methods. The resulted compounds were purified by recrystallization and column chromatography methods. The purity of dyes was confirmed by \textit{^1}H and \textit{^{13}}C NMRs, and mass spectrometry analyses. Final products were analyzed \textit{via} both computational and experimental methods. Their photophysical properties, including \textit{log D}, polarizability, and dipole moment were calculated. Their optical properties in different solvents with the determination of absorbance wavelength, molar extinction coefficient and their thermal stability were studied. The DNA interactions of dyes were conducted in collaboration with Dr. Grant’s lab to define their potential in PDT application.
2.2 Introduction to Photodynamic Therapy

Photodynamic therapy (PDT) is a type of treatment where a photosensitizing agent (PS) is used along with the light for their activation to kill cancer cells precisely without affecting healthy ones. PDT possesses some advantages over other therapies, such as precise targeting only diseased cells, the absence of long-term side effects, the ability to be repeated, short time, and low-cost. Mostly, PDT is used in cases for which surgery is not an option, such as treating Barrett’s esophagus and tracheobronchial carcinoma, and in dermatology, offering minimum phototoxicity to healthy cells of skin.\textsuperscript{104-105}

The technique of PDT is straightforward. A photosensitizing agent is injected into the bloodstream, travels around the body, and locates preferentially in cancer cells, or it can be delivered straight into the target tissue or topically.\textsuperscript{106} Then a laser with a specific wavelength, which excites the molecule, is turned on. The tumor is destroyed by activated PS, which releases energy that contributes to oxidative cleavage of DNA or other macromolecules in diseased cells. The generated reactive oxygen species (ROS) play essential roles in supporting healthy cellular life and protect our body from invading organisms by cellular immunity and microbiocidal activity.\textsuperscript{107} Enough excited state of oxygen formation leads to the irreversible destruction of the diseased cells without the involvement of surrounding healthy ones.\textsuperscript{106, 108}
The Jablonski diagram illustrating the production of reactive oxygen species (ROS) from the excited triplet state of a photosensitizing agent (PS).

PDT mechanism is based on the Jablonski diagram, performed in Figure 2.1. It includes the activation of photosensitizer under the light of a specific wavelength to an excited singlet state ($^1\text{PS}^*$), which undergoes ‘intersystem crossing’ to form a more stable triplet state ($^3\text{PS}^*$). The triplet state PS reacts with the surrounding oxygen molecules and produces reactive oxygen species (ROS) by electron and energy transfers, which can occur via two different pathways, Type 1 and Type 2. Type 1 process includes the reaction of $^3\text{PS}^*$ with triplet oxygen ($^3\text{O}_2$) by electron transfer to form a superoxide radical anion (\(\cdot\text{O}_2^\cdot\)), which can produce hydrogen peroxide (H$_2$O$_2$) and highly reactive hydroxyl radicals (\(\cdot\text{OH}\)). The generation of free radical \(\cdot\text{OH}\) leads to the formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) that damages single and double-strand breaks in DNA. Type 2 process involves the formation of singlet oxygen ($^1\text{O}_2$) upon the direct reaction of $^3\text{PS}^*$ with $^3\text{O}_2$. The mechanism of these two pathways is presented below.

Type I Mechanism – Electron Transfer:

$$\text{PS} + h\nu \rightarrow ^1\text{PS}^* \rightarrow ^3\text{PS}^*$$

$$^3\text{PS}^* + ^3\text{O}_2 \rightarrow \text{PS}^{**} + \text{O}_2^-$$

Figure 2.1. The Jablonski diagram illustrating the production of reactive oxygen species (ROS) from the excited triplet state of a photosensitizing agent (PS).
\[ PS^{++} + O_2^- \rightarrow PS^* + HO_2^* \]
\[ HO_2^* + O_2^- + H^+ \rightarrow O_2 + H_2O_2 \]
\[ H_2O_2 + O_2^- \rightarrow \cdot OH + O_2 + OH^- \]
\[ H_2O_2 + HO_2^* \rightarrow \cdot OH + O_2 + H_2O \]

Type II Mechanism – Energy Transfer:
\[ PS + hv \rightarrow ^1PS^* \rightarrow ^3PS^* \]
\[ ^3PS^* + ^3O_2 \rightarrow PS^{++} + ^1O_2 \]

Photosensitizers can differ by their chemical and biological characteristics and should possess some specific properties. Photosensitizers must contribute damage to DNA only when irradiated by light to provide localized treatment. Also, PSs should possess low dark toxicity, ensuring that only the tumor cells are damaged; high solubility in aqueous solutions for rapid elimination from the body after the treatment, ensuring that no mutations are formed; high lifetime and yield of ROS with efficient ROS generation, and long wavelength of light absorption for the maximum light penetration in the tissues. Commonly, red light (600-650 nm) can penetrate a depth of 2-3 mm. In comparison, the longer wavelengths in the near-infrared (NIR) window (700-900 nm) can penetrate up to several centimeters in tissue, allowing to reach the desired tumor (Figure 2.2).\textsuperscript{112-113} NIR irradiation, comparing to ultraviolet, performs the lower photodamage effects and lower levels of the auto-fluorescence of the non-target tissue.\textsuperscript{114} Penetration depth also depends on photon energy, polarization, coherence, power density, time of illumination, and the tissue physiology (pigmentation, hydration, structure).\textsuperscript{108, 115} Also, it is important to remember that PSs should be easy-purified in high yields for low costs. A compound with a higher molar extinction coefficient will absorb more light at lower concentrations, which allows to reduce the expense.
Here are some examples of photosensitizing agents absorbing the light in the wavelength region above 700 nm, where the light has a deeper tissue penetration. Bacteriochlorins (BCA) are tetrapyrrole macromolecules with pyrrole and pyrroline rings. BCA absorb the light at 760 nm and is used for head and neck cancer\textsuperscript{116}, and malignant melanoma\textsuperscript{117} treatment. Texaphyrins (LUTRIN, Lutex) are metal-coordinating expanded porphyrins, possessing high water solubility, low toxicity, and light absorption at 730 nm. Texaphyrins are used for the treatment of colon carcinoma\textsuperscript{118}, recurrent breast cancer\textsuperscript{119}, brain, and prostate cancers\textsuperscript{120}. Iron(III) catecholate complex showed absorption at 805 nm and high yield DNA photocleavage\textsuperscript{106}. 
Cyanine dyes have shown great potential for PDT application, indicating the absorption band in the desired NIR range, low cytotoxicity, and high molar extinction coefficient. One example of FDA approved photosensitizer is Indocyanine Green (ICG), absorbing the light at 820 nm. ICG was first developed in the 1950’s to determine cardiac output and hepatic function. Its fabulous properties led to its application to a variety of medical diagnostics. Now ICG is used for visualization of liver segments and subsegments, \textit{in vitro} laser-assisted fat cell destruction,\textsuperscript{122} detection of hepatocellular carcinoma during a laparoscopic hepatectomy,\textsuperscript{123} stain caries lesions for the further removal of lesion by a laser by the help of the high light absorption of ICG at the excitation wavelength,\textsuperscript{124} as NIR imaging probe of hepatocellular carcinoma (HCC) cells\textsuperscript{125} and as a photosensitizer in PDT for the treatment of various types of cancer, such as gallbladder, lung, and triple-negative breast cancer (TNBC).\textsuperscript{126} PDT combined with ICG, a near-infrared photosensitizer, has advantages compared with PD combined with an ultraviolet photosensitizer.
As was discussed in Chapter 1, quinoline-based cyanine dyes have attracted researchers’ attention by their anti-bacterial activity and ability to detect and bind to DNA. Some TO and YO derivatives showed DNA cleavage properties, but their short wavelength absorption band limit their therapeutic PDT application. Recently, our lab reported the synthesis of a symmetrical pentamethine dye 78, which was illustrated in Figure 15, and showed an excellent DNA photo-cleaving property, absorbing red light at higher wavelength at 805 nm. Therefore, we decided to expand on this finding to develop eighteen novel symmetrical cyanine dyes containing quinoline moieties as summarized above in Section 2.1 and will discuss below in details.

2.3 Synthesis of Symmetrical Pentamethine Cyanine Dyes

As mentioned, this work included the synthesis of eighteen symmetrical pentamethine cyanine dyes. For the synthesis of these NIR compounds, it was necessary to prepare linkers and salts. Halogenated polymethine linkers 104a and 104b, required for pentamethine dyes synthesis, were developed by Dr. Henary’s lab earlier by heating aniline with enoic acids in dry ethanol, as shown in Scheme 2.1.

\[ \text{Scheme 2.1. Synthesis of polymethine linkers.}^{127} \]

The salts with benzyl substituents 105 and 107 were synthesized by the alkylation of commercially available quinaldine and lepidine with bromomethyl benzene in ratio 1:1, as shown in Scheme 2.2. The reaction mixture was monitored via thin-layer chromatography (TLC), which is used to identify compounds until the starting materials were gone. Upon completion of the
reaction, the mixture was cooled down, and acetone was added to precipitate out the salt. The crude product was suction filtered, washed with acetone and diethyl ether, and then, dried under vacuum.

Symmetrical pentamethine cyanine dyes with benzyl substituents 106a-c (quinaldine-based) and 108a-c (lepidine-based) were synthesized with altering a substituent at meso carbon in the methine chain by reacting of prepared salts 105 and 107 with polymethine linkers 104a-c in acetic anhydride in the presence of triethylamine, as presented in Scheme 2.2. The synthesis of pentamethine dyes 106a-c and 108a-c was performed by both conventional and microwave-assisted methods. Compared to using a hotplate to heat a reaction mixture, microwave irradiation was more efficient and dramatically reduced the reaction time. Microwave energy interacted directly with the reaction mixture molecules, heating the reactants much faster than conventional methods. Conventional method was performed by heating the reaction up to 70 °C for 1h, while microwave irradiation was performed at 100-110 °C and took only 2 min. The formation of dyes was monitored with a UV-Vis spectrophotometer until peaks at 700-720 nm for 106a-c and 800-820 nm for 108a-c were obtained and a peak at ~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing dye precipitation. The mixture was filtered under vacuum and purified via recrystallization by solvation in a minimal amount of methanol and dilution with diethyl ether causing precipitation. And then, the filtered crystals were dissolved in dichloromethane and precipitated by hexanes. The solid product was collected and dried under vacuum. All dyes 106a-c and 108a-c were synthesized in high yields above 60%.
Scheme 2.2. Synthesis of salts 105 and 107, and symmetrical pentamethine cyanine dyes 106a-c and 108a-c with benzyl substituents.

Based on our collected results, lepidine-based dyes showed absorption peak at higher wavelength of light in comparison to quinaldine-based dyes, due to the presence of an extra double bond between nitrogen atoms of heterocycles. That’s why we decided to focus our efforts on the synthesis of lepidine-based symmetrical pentamethine cyanine dyes altering the substituent on the nitrogen atom and at meso carbon in the methine chain.

Then, we synthesized another set of symmetrical pentamethine cyanine dyes 110a-c had carboxybenzyl substituents. The quinoline salt with carboxybenzyl substituent 109 was synthesized by the alkylation of commercially available lepidine with 4-(bromomethyl)benzoic acid in ratio 1 : 0.83, as shown in Scheme 2.3. The reaction mixture was monitored via thin-layer chromatography (TLC), until the starting materials were gone. Upon completion of the reaction, the product was dissolved in a minimal amount of methanol and diluted with diethyl ether causing precipitation. The crude product was suction filtered and dried under vacuum to yield the final desired compounds.
Symmetrical pentamethine cyanine dyes with carboxybenzyl substituents 110a-c were synthesized with altering a substituent at meso carbon in the methine chain by reacting of prepared salt 109 with polymethine linkers 104a-c in acetic anhydride in the presence of triethylamine, as presented in Scheme 2.3. The synthesis of pentamethine dyes 110a-c was performed by both conventional and microwave-assisted methods. Conventional method was performed by heating the reaction up to 70 °C for 1h, while microwave irradiation was held at 100-110 °C for 10 min. The formation of dyes was monitored via UV-Vis until a peak at 800-820 nm was obtained, and peak at ~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing dye precipitation. After filtration, the collected crystals were dissolved in water with TEA and extracted using DCM (3 x 20 mL). Hydrochloric acid (10%) was added to the resulted organic layer to cause precipitation. The green solid was obtained after filtration and dissolved in a minimal amount of methanol and diluted with diethyl ether causing precipitation. And then, the filtered crystals were dissolved in dichloromethane and precipitated by hexanes. The solid product was collected and dried under vacuum. The overall yields of the isolated purified dyes 110a-c ranged between 22 – 53%.

![Scheme 2.3. Synthesis of salt 109, and symmetrical pentamethine cyanine dyes 110a-c with carboxybenzyl substituent.](image)

Another set of symmetrical pentamethine cyanine dyes 112a-c with (methoxycarbonyl)benzyl substituents was synthesized. The salt 111 used for the synthesis of NIR dyes 112a-c was prepared from 4-methyl quinoline salt with carboxybenzyl substituent 109 in
methanol and a catalytic amount of concentrated sulfuric acid in the reaction mixture at 60 °C for around 2 h. The reaction mixture was monitored via thin-layer chromatography (TLC), until the starting materials were gone. Upon completion of the reaction, the solvent was evaporated off, and the product was dissolved in dichloromethane and extracted with a saturated solution of sodium bicarbonate in order to get rid of unreacted sulfuric acid. Calcium chloride, a drying agent, was added to the extracted organic layer for 10 min. After the solution was filtered and evaporated, the oily product 111 was formed and used for the next step without further purification.

Salt 111 and polymethine linkers 104a-c were refluxed in acetic anhydride in the presence of triethylamine at 70 °C for 2 h to furnish the symmetrical dyes 112a-c with carboxybenzyl substituents, as presented in Scheme 2.4. The reaction mixture was monitored via UV-Vis until a peak at 800-820 nm was obtained, and peaks at ~450 nm and ~650 nm disappeared. Upon cooling to room temperature, diethyl ether was added to the reaction mixture, causing dye precipitation. The mixture was filtered under vacuum and the final dyes purified via recrystallization by solvation in a minimal amount of dichloromethane and dilution with hexanes causing precipitation. Then the filtered crystals were dissolved in warm methanol, filtered, and the resulted filtrate was concentrated under reduced pressure, washed with diethyl ether, and sonicated for several hours. The solid product was collected and dried under vacuum. Dyes 112a-c were produced in 39 – 64% yields.

Scheme 2.4. Synthesis of salt 111, and symmetrical pentamethine cyanine dyes 112a-c with (methoxycarbonyl)benzyl substituent.
For another set of hydrophobic NIR dyes, the salt 113 with phenylpropyl substituent was synthesized from lepidine and (3-bromopropyl)benzene in acetonitrile by heating at 120 °C for around 3 h. The reaction mixture was monitored via TLC until the starting materials were gone. Upon completion of the reaction, the product was dissolved in dichloromethane and diluted with diethyl ether causing precipitation. The salt 113 was then suction filtered and dried under vacuum.

The synthesized salt 113 and polymethine linkers 104a-c were refluxed in acetic anhydride in the presence of triethylamine for 1 h at 70 °C under nitrogen atmosphere. The synthesis of pentamethine dyes 114a-c was performed by both conventional and microwave-assisted methods. Conventional method was performed by heating at 70 °C for 1 h, while microwave irradiation was held at 80-90 °C for 8 min. The formation of dyes was monitored via UV-Vis until a peak at 800-820 nm was obtained, and peak at ~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing dye precipitation. Then, it was filtered under vacuum and the collected crystals were dissolved in a minimal amount of dichloromethane and diluted with diethyl ether causing precipitation. The final product was collected and dried under vacuum to furnish symmetrical dyes 114a-c with phenylpropyl substituents in 35 – 68% yields, as presented in Scheme 2.5.

Scheme 2.5. Synthesis of salt 113, and symmetrical pentamethine cyanine dyes 114a-c with phenylpropyl substituent.

The synthesis of last set of symmetrical pentamethine cyanine dyes 116a-c containing triphenylphosphonium substituents was shown in Scheme 2.6. For the synthesis of salt 115, it was
necessary to prepare the precursor (3-bromopropyl)triphenylphosphonium bromide 115a by a condensation reaction of 1,3-dibromopropane and triphenylphosphine at 80 °C under nitrogen atmosphere for around 3 h. Then lepidine and 115a in acetonitrile were refluxed at 110 °C under nitrogen atmosphere for 18 h. The reaction mixture was monitored via TLC until the starting materials were gone. After the reaction mixture was completed and cooled down, the product was dissolved in a minimal amount of acetone and diluted with diethyl ether causing precipitation. The further purification was performed by column chromatography with a gradient solvent system of methanol in dichloromethane (1:20). The salt 115 was suction filtered and dried under vacuum.

The salt 115 and polymethine linkers 104a-c were refluxed in acetic anhydride in the presence of triethylamine for 1 h at 70 °C under nitrogen atmosphere. An additional 2 eq. of TEA or DIPEA were added and stirred for another 1 h. The reaction mixture was monitored via UV-Vis until a peak at 800-820 nm was obtained and peak at ~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing the dye to precipitate. The mixture was filtered under vacuum and purified by column chromatography with a gradient solvent system of methanol in dichloromethane (1 : 10). The dark green fractions were mixed together and concentrated under reduced pressure. The final product was collected after being washed with diethyl ether and sonicated for several hours and dried under vacuum to furnish symmetrical dyes 116a-c with triphenylphosphonium substituents in 11 – 15% yields, as shown in Scheme 2.6.
Scheme 2.6. Synthesis of salt 115 and symmetrical pentamethine cyanine dyes 116a-c with triphenylphosphonium substituent.

As discussed above, all synthesized the salts and dyes were purified, isolated and obtained in 14 – 68% and 11 – 70% overall yields, respectively. Each compound was characterized and analyzed by $^1$H and $^{13}$C NMR, and MS spectrometry for dyes individually and consistently one after another one, as discussed below in the Experimental Section. The photophysical and optical properties of synthesized pentamethine cyanine dyes containing quinoline moieties were studied, as presented below.

2.4 Results and Discussion

After a successful synthesis of the eighteen symmetrical pentamethine cyanine dyes with different substituents, purified, characterized and analyzed, as outlined above, their photophysical properties and optical properties in different solvents were studied. The DNA interactions of dyes were studied in collaboration with Dr. Grant’s lab to define if they are applicable for PDT.

2.4.1 NMR and MS Characterization

A symmetrical pentamethine cyanine dye 108c (Scheme 2.2) with benzyl moiety at the nitrogen atom of the quinoline ring, was chosen as a representative example for characterization and analysis of all sets of synthesized symmetrical pentamethine dyes. The compound had 37 carbons and 31 protons. $^1$H NMR spectra of compound 108c is presented in Figure 2.4. The peaks
at 2.5 and 3.3 ppm corresponded to DMSO and water accordingly. The dye structure could be visualized through the quinoline, benzyl substituent and the polymethine bridge. There were five protons in the methine chain, twelve protons in the quinoline ring system, four protons in the methylene group of \(N\)-substituent, and ten protons in phenyl rings. As the structure is symmetrical and two sides of the dye are identical, then the structure could be divided into two identical parts. But we kept the original integration as we have one proton at the meso carbon in the methine chain.

The spectra appeared with four protons in the aliphatic region, five in the vinyl region, and eleven in the aromatic region. The structure of the pentamethine dye 108c was colored accordingly with four different colors. The blue color presented 4 protons which belonged to the methylene group of \(N\)-substituent and performed one singlet at 6.78 ppm. The green color presented four protons of the methine bridge and exhibited two doublets in the spectra. The yellow color presented the proton at meso position and showed one triplet peak. All protons in the aromatic region were colored in red showed five triplets and six doublets in the range of 7-9 ppm.

![Figure 2.4. \(^1\)H NMR of dye 108c in DMSO-d6.](image-url)
The $^{13}$C spectra of the symmetrical pentamethine cyanine dye 108c had thirty-seven carbons, but the spectra showed seventeen peaks, as indicated in Figure 2.5. The structure of the pentamethine dye 108c was colored accordingly with four different colors. The blue color presented carbon, which belonged to the methylene group of $N$-substituent and appeared at 56.69 ppm in the spectra. The green color presented five vinyl carbons of the methine bridge appeared at 110-120 ppm. All carbons in the aromatic region were colored in red and presented in the range 120-150 ppm. But two highest peaks (colored with aquamarine and fuchsia colors) corresponded to two sets of two identical carbons from phenyl ring of the benzyl substituent. Two other carbons from substituent appeared as two different peaks, and nine remained peaks belonged to quinoline moiety.

![Figure 2.5. $^{13}$C NMR of dye 108c in DMSO-d6.](image)

All eighteen new NIR pentamethine dyes were also characterized by using high resolution accurate mass spectrometry in methanol with 0.1% of formic acid. Mass spectrometry of symmetrical pentamethine dye 108c was also taken as a representative, and the data was shown in Figure 2.6. The spectra displayed a molecular ion peak at 503.2479 within the acceptable range from the calculated value of 503.25, confirming the molecular weight of the compound. The obtained composition of $C_{37}H_{31}N_2$ corresponded to the synthesized structure of 108c.
2.4.2 Physicochemical Properties

Physicochemical properties (log $D$, polarizability and dipole moment) of symmetrical pentamethine cyanine dyes 106a-c, 108a-c, 110a-c, 112a-c, 114a-c and 116a-c were calculated and summarized in Table 2.1.

Both log $P$ and log $D$ are distribution coefficients used for measuring the hydrophobicity of a compound at a given pH value. But log $D$ is mostly used for ionized compounds and can also help to predict whether the compound will distribute in biological tissues. Polarizability describes the tendency of a nonpolar molecule to experience a shift in charge distribution across its electron cloud. It helps to determine if an ionized site is stable or not. The dipole moment determines the polarity of the molecule; therefore, it provides a direct assessment of its potential to participate in
van der Waals interactions. The cyanine dyes having low dipole and high $\log D$ tend to be hydrophobic.\textsuperscript{128} Dyes with high dipole moments can interact with highly polar tissue elements through Keesom (dipole-dipole) attractions, especially if ionic forces bring the two partners together. These dyes also can bond \textit{via} Debye (dipole-induced dipole) forces if the tissue component is polarizable.\textsuperscript{129} Dyes with low dipole moment and high polarizability are prone to bond to hydrophobic tissue \textit{via} London (induced dipole-induced dipole) forces and suitable for Debye interactions with polar tissue elements.\textsuperscript{129}

\textbf{Table 2.1. Physicochemical properties of dyes calculated using MarvinSketch (ChemAxon).}

<table>
<thead>
<tr>
<th>Dye</th>
<th>log D at pH=7.4</th>
<th>Polarizability</th>
<th>Dipole moment (Debye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICG</td>
<td>4.91</td>
<td>86.00</td>
<td>24.92</td>
</tr>
<tr>
<td>106a</td>
<td>4.80</td>
<td>66.52</td>
<td>4.30</td>
</tr>
<tr>
<td>106b</td>
<td>4.63</td>
<td>65.93</td>
<td>3.68</td>
</tr>
<tr>
<td>106c</td>
<td>4.39</td>
<td>64.22</td>
<td>2.20</td>
</tr>
<tr>
<td>108a</td>
<td>5.02</td>
<td>66.51</td>
<td>3.17</td>
</tr>
<tr>
<td>108b</td>
<td>4.86</td>
<td>65.92</td>
<td>2.55</td>
</tr>
<tr>
<td>108c</td>
<td>4.62</td>
<td>64.22</td>
<td>1.65</td>
</tr>
<tr>
<td>110a</td>
<td>1.09</td>
<td>71.09</td>
<td>12.48</td>
</tr>
<tr>
<td>110b</td>
<td>1.00</td>
<td>70.37</td>
<td>12.43</td>
</tr>
<tr>
<td>110c</td>
<td>0.76</td>
<td>68.59</td>
<td>13.14</td>
</tr>
<tr>
<td>112a</td>
<td>5.02</td>
<td>75.28</td>
<td>12.52</td>
</tr>
<tr>
<td>112b</td>
<td>4.88</td>
<td>74.59</td>
<td>12.97</td>
</tr>
<tr>
<td>112c</td>
<td>4.60</td>
<td>72.81</td>
<td>13.61</td>
</tr>
<tr>
<td>114a</td>
<td>6.50</td>
<td>73.76</td>
<td>6.82</td>
</tr>
</tbody>
</table>
Log $D$ values of dyes lied in the range from 0.7 to 10.20. In comparison, log $D$ of heptamethine cyanine ICG was equal to 4.91. Dyes 110a-c with carboxybenzyl substituents showed the highest hydrophilicity, while dyes 116a-c with triphenylphosphonium substituents were found to be more hydrophobic according to calculated properties. It was also determined that in each set of dyes log $D$ values depended on meso carbon substituent and increased in order H < Cl < Br.

Cyanine dyes with triphenylphosphonium substituents 116a-c showed the highest polarizability above 100, while all other compounds had values in the range from 64 to 75, which a little bit lower than for ICG. Polarizability values were also affected by meso carbon substituent and increase in the same way as log $D$ in order H < Cl < Br.

The calculated dipole moment of all considered compounds was much less than ICG’s value. However, cyanine dyes 110a-c with carboxybenzyl substituents, 112a-c with (methoxycarbonyl)benzyl substituents and 116a-c with triphenylphosphonium substituents investigated sufficiently high dipole moments above 12 Debye. Dyes 108a-c with benzyl substituents showed the lowest values of dipole moment.
2.4.3 Optical properties

The optical properties of the synthesized dyes were studied and compared in four different organic solvents, such as methanol (MeOH), dimethylsulfoxide (DMSO), acetonitrile (ACN) and N,N–dimethylformamide (DMF). As plastic cuvette is not compatible with some solvents such as DMF, the absorbance spectra in DMF did not show the absorbance curve starting at zero. These studies helped to determine how different solvents and substituents affected on the fluorophores’ properties. Molar absorptivity was calculated for each compound in each solvent by Beer-Lambert Law. The solvatochromism of each dye was investigated in four solvents.

The symmetrical pentamethine cyanine dye with benzyl substituent 108c, shown in Scheme 2.2, was chosen as a representative example. Figure 2.7 illustrated the obtained optical properties of compound 108c. The absorbance was measured at various concentrations in four solvents. Molar absorptivity was determined after plotting the maximum absorbance of each sample as a function of dye concentration. The slope of each plotted graph corresponded to the molar absorptivity of the sample in the given solvent multiplied by $10^5$.

Compound 108c showed the absorbance wavelength at 818 nm in methanol, 829 nm in DMSO, 817 nm in acetonitrile, and 826 nm in DMF. The increase of wavelength corresponded to the increase of solvent polarity and enhanced in order acetonitrile < methanol < DMF < DMSO. The obtained molar absorptivity was 218 030 in methanol, 180 038 in DMSO, 140 012 in acetonitrile, and 137 630 in DMF. The molar absorptivity of dye 108c increased in order DMF < acetonitrile < DMSO < methanol.
Figure 2.7. Absorption spectra of dye 108c in methanol, DMSO, acetonitrile and DMF.
The effect of solvents was recorded at a constant concentration of 0.3 µM and performed in Figure 2.8. Dye 108c exhibited the highest absorption intensity in methanol and the lowest one in DMF. The longest wavelength was achieved in DMSO and the shortest one in acetonitrile. A positive solvatochromism was observed as the solvatochromic band was hypsochromically shifted with the decrease in the polarity of the solvent in order acetonitrile < methanol < DMF < DMSO.

![Solvatochromism of Dye 108c at 0.3 µM](image)

**Figure 2.8. Solvatochromism spectra of dye 108c in methanol, DMSO, acetonitrile and DMF.**

The optical properties studies for all synthesized dyes were combined in Table 2.2. The shortest wavelength, as expected, was achieved by 2-quinolinium dyes 106a-c. All 4-quinolinium dyes absorbed the light at a longer wavelength than ICG. The longest wavelength was achieved by dyes with hydrogen substituent at meso-carbon of the methine chain in DMSO solvent. With the increase of solvent polarity in order acetonitrile < methanol < DMF < DMSO, the red shifting occurred. Absorption maxima for all dyes depended on the meso-substituent and increased in order Br < Cl < H, but not on N-substituent. As a result, all 4-quinolinium-based cyanine dyes 108a-c, 110a-c, 112a-c, 114a-c, 116a-c showed absorption maxima wavelength at 791-830 nm.
In each set of dyes, the lowest molar absorptivity was achieved by fluorophores with hydrogen in the methine chain in DMF, except pentamethine cyanine dyes 116a-c with TPP. Dye 116a with bromine substituent showed the lowest molar absorptivity in the set 116a-c.

Green colored values of molar absorptivity in Table 2.2 correspond to the highest values above 200 000 M\(^{-1}\)cm\(^{-1}\), and red colored values corresponded to the lowest values below 100 000 M\(^{-1}\)cm\(^{-1}\). Only three dyes showed molar absorptivity below 100 000 M\(^{-1}\)cm\(^{-1}\): 110b (X = Cl) and 110c (X = H) with carboxybenzyl substituents, and 112c (X = H) with (methoxycarbonyl)benzyl substituents. All other dyes exhibited high molar absorptivity coefficient above 100 000 M\(^{-1}\)cm\(^{-1}\), which is very important for the nucleic acid binding applications.

**Table 2.2. Optical properties of synthesized dyes.**

<table>
<thead>
<tr>
<th>Dye</th>
<th>λ.max, nm</th>
<th>ε, M(^{-1})cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MeOH</td>
<td>DMSO</td>
</tr>
<tr>
<td>ICG</td>
<td>785 nm</td>
<td>795 nm</td>
</tr>
<tr>
<td>106a</td>
<td>702 nm</td>
<td>706 nm</td>
</tr>
<tr>
<td>106b</td>
<td>707 nm</td>
<td>710 nm</td>
</tr>
<tr>
<td>106c</td>
<td>715 nm</td>
<td>726 nm</td>
</tr>
<tr>
<td>108a</td>
<td>799 nm</td>
<td>802 nm</td>
</tr>
<tr>
<td>108b</td>
<td>805 nm</td>
<td>808 nm</td>
</tr>
<tr>
<td>108c</td>
<td>818 nm</td>
<td>829 nm</td>
</tr>
<tr>
<td>110a</td>
<td>800 nm</td>
<td>804 nm</td>
</tr>
<tr>
<td>110b</td>
<td>806 nm</td>
<td>810 nm</td>
</tr>
<tr>
<td>110c</td>
<td>819 nm</td>
<td>829 nm</td>
</tr>
<tr>
<td>112a</td>
<td>801 nm</td>
<td>804 nm</td>
</tr>
</tbody>
</table>
The fluorescence quantum yield of these synthesized compounds couldn’t be observed because of the very weak fluorescence of these dyes due to their nonplanar conjugated structure and free rotation. The nonplanar chromophoric system experiences motions to consume the excitation energy. But despite the structure of quinoline-base compounds is not planar and shows weak fluorescence, usually after binding to DNA, the structure’s correction happens, and the structure becomes planar and fluorescent.

Thermal stability studies of all synthesized symmetrical pentamethine cyanine dyes were performed in ethanol under dark and light conditions and can be seen in Figure 2.9 and Figure 2.10. ICG was selected as a reference. Only dye 110a (X = Br) with carboxybenzyl substituent showed decomposition in dark for more than 40% in 30 h. All other dyes were relatively stable for 48 h, showing degradation not more than 20%.

<table>
<thead>
<tr>
<th></th>
<th>807 nm</th>
<th>810 nm</th>
<th>803 nm</th>
<th>808 nm</th>
<th>211 990</th>
<th>225 950</th>
<th>182 720</th>
</tr>
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<tbody>
<tr>
<td>112b</td>
<td>820 nm</td>
<td>830 nm</td>
<td>818 nm</td>
<td>827 nm</td>
<td>148 910</td>
<td>186 260</td>
<td>89 710</td>
</tr>
<tr>
<td>114a</td>
<td>797 nm</td>
<td>799 nm</td>
<td>791 nm</td>
<td>797 nm</td>
<td>259 140</td>
<td>199 210</td>
<td>200 810</td>
</tr>
<tr>
<td>114b</td>
<td>803 nm</td>
<td>805 nm</td>
<td>797 nm</td>
<td>803 nm</td>
<td>265 690</td>
<td>173 770</td>
<td>163 090</td>
</tr>
<tr>
<td>114c</td>
<td>817 nm</td>
<td>826 nm</td>
<td>815 nm</td>
<td>823 nm</td>
<td>163 100</td>
<td>143 700</td>
<td>137 170</td>
</tr>
<tr>
<td>116a</td>
<td>800 nm</td>
<td>802 nm</td>
<td>796 nm</td>
<td>800 nm</td>
<td>240 040</td>
<td>171 670</td>
<td>183 810</td>
</tr>
<tr>
<td>116b</td>
<td>807 nm</td>
<td>808 nm</td>
<td>802 nm</td>
<td>807 nm</td>
<td>251 390</td>
<td>189 780</td>
<td>312 990</td>
</tr>
<tr>
<td>116c</td>
<td>821 nm</td>
<td>829 nm</td>
<td>819 nm</td>
<td>827 nm</td>
<td>240 200</td>
<td>164 820</td>
<td>258 190</td>
</tr>
</tbody>
</table>

Thermal stability studies of all synthesized symmetrical pentamethine cyanine dyes were performed in ethanol under dark and light conditions and can be seen in Figure 2.9 and Figure 2.10. ICG was selected as a reference. Only dye 110a (X = Br) with carboxybenzyl substituent showed decomposition in dark for more than 40% in 30 h. All other dyes were relatively stable for 48 h, showing degradation not more than 20%.
Figure 2.9. Thermal degradation profile of synthesized pentamethine cyanine dyes 106a-c, 108a-c, 110a-c, 112a-c, 114a-c, and 116a-c in dark for 48 h.

Dyes 110b (Cl), 110c (H), 112a-c, 116c (H) decomposed after being exposed to light for 48 h. Dyes 108c (H) and 114c (H) almost decomposed, showing the degradation of more than 50% after 20 h. The only apparent trend seems to be that mostly dyes with hydrogen at meso-position of the methine chain showed degradation of more than 90% of the original absorbance after 48 h of irradiation. Also, the whole set of dyes 112a-c with (methoxycarbonyl)benzyl at N-position absolutely decomposed in 48 h. Two dyes 112c and 116c showed absolute decomposition in 20 and 30 h, respectively. The most stable dyes that lost less than 45% of absorbance after 48 h of light irradiation and showed better results than ICG were 106a, 106b, 106c, 108a, 108b, 114a, 114b, 116a and 116b. A comparative analysis of these dyes and ICG is presented in Figure 2.10. Based on this comparison, it can be concluded that dyes 106a-c, 108a, 108b and 114b exhibited a remarkably higher stability than ICG with intensity decrease less than 15%. Also, it was noticed that electron withdrawing atoms of Br and Cl slowed down absorption loss over time in most cases.
After the optical properties’ analyses of all synthesized symmetrical pentamethine cyanine dyes, it could be concluded that dyes 108a-b, 114a-b, and 116a-b exhibited the best properties, having the highest molar absorptivity coefficient and thermal stability. In comparison to ICG, the only dye approved by the United States Food and Drug Administration, the new synthesized dyes showed enhanced absorption wavelength and stability.

2.4.4 UV-Visible Screening of Symmetrical Pentamethine Cyanine Dyes for DNA Interactions

In collaboration with Dr. Kathryn Grant’s lab, two sets of synthesized symmetrical pentamethine cyanine dyes were evaluated for their photocleavage properties with pUC19 DNA by student Effibe Ahoulou as shown in Figures 2.11 – 2.19 and Appendix A.4. As they showed absorption in the NIR range and good solubility, thereby satisfying two requirements for an ideal PDT photosensitizer.
UV-visible analysis of spectrometry in DMSO and aqueous buffered solutions over time was performed in order to define the stability of the symmetrical pentamethine cyanine dyes $106a$-$c$ (2-quinolinium) and $108a$-$c$ (4-quinolinium) and the interaction of dyes with DNA. Spectra of aqueous solutions at pH = 7.0 indicated the behavior in simulated biological conditions in which these dyes could be used as photosensitizers. It is important to know whether the absorbance intensity of each dye changes or wavelength peaks position shifts in different solvents over time. The first set of experiments showed that both $106a$-$c$ and $108a$-$c$ remained stable in DMSO over 30 min, displaying no change in absorption maxima and no wavelength shifting. The spectra can be seen in Figure 2.11.
Next, pentamethine cyanine dyes 106a-c and 108a-c were transferred to aqueous buffered solutions. Regarding the set of 2-quinolinium dyes 106a-c, both halogen-substituted dyes showed stability with a low drop over time in aqueous solutions with and without addition of DNA, but at low wavelengths at 550 nm without DNA and 557 nm with DNA for 106a (X = Br) and at 562 nm
without DNA 567 nm with DNA for 106b (X = Cl), comparing with resulted 705 nm for 106a and 710 nm for 106b in DMSO. Hydrogen substituted dye 106c, exhibited the absorption loss in aqueous solution without DNA at 642 nm and 708 nm and the stability in the presence of DNA. All dyes in set 106a-c didn’t exhibit noticeable dye-DNA interactions, as shown in Figure 2.14.

Considering the set of 4-quinolinium dyes 108a-c, upon the transferring to aqueous buffered solutions, all dyes’ peaks appeared with the lower intensity at the lower wavelength except one dye 108c (X = H). Absorption maxima of compound 108a (X = Br) in DMSO at 800 nm was replaced by new blue-shifted peak at 674 nm, which was shifted to 680 nm overtime in buffer solution and at 608 nm which was shifted to 601 nm in the presence of DNA. As well as a peak of compound 108b (X = Cl) at 808 nm in DMSO was replaced by a new peak at 672 nm, which was shifted to 675 nm overtime in buffer solution and formed one more peak at 589 nm with the noticeable intensity decrease over time in the presence of DNA. The peak was replaced by new absorption maxima at 670 nm in 30 min. Meanwhile, peak maxima of 108c (X = H) at 829 nm in DMSO was replaced by a new red-shifted peak at 988 nm in buffer which showed the extensive absorption loss overtime and shifted to 983 nm forming the extra peak at 832 nm which also showed the absorption intensity decrease upon the addition of DNA.
Figure 2.12. UV-Vis of dyes 108a-c in buffer with absence and presence of CT-DNA at 10 µM concentration recorded every 5 min over 30 min.
As a result, cyanine dye 108a \((X = Br)\) showed stability over time in both organic and aqueous solutions with and without DNA. In contrast, fluorophore 108b \((X = Cl)\) produced 3 peaks upon the addition of DNA, where only the most blue-shifted peak indicated the absorption loss over 30 min. Dye 108c \((X = H)\) exhibited an absorption loss both in the presence and absence of DNA.

Figure 2.13. UV-Vis of dyes 108a-c in buffer with absence and presence of CT-DNA at 10 
\(\mu\text{M}\) concentration recorded every 5 min over 30 min.
Figure 2.14. UV-Vis of 10µM of dyes in 10 mM of sodium phosphate buffer solutions with and without addition of 150 µM of bp CT DNA

It can be concluded that electron withdrawing atoms, especially bromine substituents, stabilized the dyes in aqueous solutions, thereby preventing the dye autooxidation process. Furthermore, upon the addition of DNA 2-quinolinium dyes didn’t show any changes of peak
wavelengths, as can be seen in Figure 2.14. Meanwhile, the interaction of DNA in 4-quinolinium
dyes solutions led to more noticeable changes in the absorption spectra showing hypochromic shift
and decrease in absorbance.

Additional spectroscopic experiment for cyanine dyes with 4-quinolinium moieties 108a-c was performed for better understanding of dye-DNA interactions and can be seen in Figure 2.15. The boost of DNA concentration leads to the destruction of cyanine aggregation for subsequent formation of a monomeric dye. In the case of compound 108a (X = Br) with the increase of DNA concentration, one peak was blue-shifting, and another one stayed constant. The intensity of both peaks significantly decreased. Dye 108b (X = Cl) was characterized by the decrease of absorption values of all three peaks upon the sequential addition of DNA. All three peaks remained at constant wavelength with no shifting. Dye 108c (X = H) showed one bathochromic and one hypochromic shift with the DNA intensity increase. Both peaks illustrated substantial loss of absorption intensity.

As a result, saturating of pentamethine cyanine dyes with 4-quinolinium moiety 108a-c with DNA induced hypochromicity effect and all peaks showed the intensity decrease, proving that competitive binding interaction between dyes and DNA occurred. But at the same time, no aggregate monomer changes were seen.
2.4.5 DNA cleavage investigation

Next goal was to determine if cyanine dyes with 4-quinolinium moiety 108a-c could act as phototherapeutic agents. Compounds 106a-c and 108a-c were tested for photocleavage with and without light irradiation at 850 nm for 30 min and presented in Figures 2.16 – 2.18. All cleavage experiments were conducted with 50 μM of dye, 40 μM bp pUC19 DNA and 10 mM sodium phosphate buffer pH 7.0.

Figure 2.15. UV-Vis of dyes 108a-c upon the subsequent addition of DNA at concentration 34 to 1495 μM.
Figure 2.16. Agarose gels depicting cyanine dyes 108a and 106a photocleavage of pUC19 plasmid DNA. The reactions were irradiated for 30 min with laser light power of 100 mW 850 nm and in dark at 10°C. Reactions contained 10 mM sodium phosphate buffer pH 7.0 and 40 μM bp DNA in the presence of 50 μM of dyes.

Figure 2.17. Agarose gels depicting cyanine dyes 108b and 106b photocleavage of pUC19 plasmid DNA. The reactions were irradiated for 30 min with laser light power of 100 mW 850 nm and in dark at 10°C. Reactions contained 10 mM sodium phosphate buffer pH 7.0 and 40 μM bp DNA in the presence of 50 μM of dyes.
81

850 nm; hv 10

<table>
<thead>
<tr>
<th>Dy</th>
<th>-</th>
<th>108c</th>
<th>108c</th>
<th>106c</th>
<th>106c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (850)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Plasmid DNA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure 2.18. Agarose gel depicting cyanine dyes 108c and 106c photo cleavage of pUC19 plasmid DNA.** The reactions were irradiated for 30 min with laser light power of 100 mW 850 nm and in dark at 10°C. Reactions contained 10 mM sodium phosphate buffer pH 7.0 and 40 μM bp DNA in the presence of 50 μM of dyes.

As expected, dyes 106a-c didn’t show good results, cleaving in dark at 850 nm, while they absorbed the light at around 700-720 nm. It could happen because of dyes decomposition or heating up by the light.

Dye 108a performed the highest production of nicked DNA from supercoiled under NIR light irradiation. Therefore, dye 108a was tested using several lasers lights at specific wavelengths (830, 850, 905 and 980 nm) in order to determine the best wavelengths to use for DNA cleavage. It was found, that the lower concentrations of 108a helped to stabilize the dye solution and showed better aggregation and DNA cleavage.
Figure 2.19. Agarose gel depicting cyanine dye 108a photo cleavage of pUC19 plasmid DNA. The reactions were irradiated for 30 min with various laser light wavelengths and in the dark. Temperature was kept at 10 °C. Reactions contained 10 mM sodium phosphate buffer pH 7.0 and 38 μM bp DNA in the presence and absence of 20 μM dye.

Figure 2.19 showed that the experiments held with dye and without, in dark and with light irradiation at 830, 850, 905 and 980 nm at low temperatures at 10 °C. According to received results, the best photocleavage of plasmid DNA by dye 108a (X = Br) was achieved under light irradiation at 830 and 850 nm. It displayed no cleavage activity in the dark and under laser irradiation at 905 and 980 nm as it does not absorb as much light at these wavelengths. Furthermore, it is not necessary as the water starts absorbing light above 1000 nm, and the ideal photosensitizer should absorb light in the NIR window not exceeding 900 nm.

2.5 Experimental

2.5.1 General

All used reagents and solvents were purchased from Sigma Aldrich, Fisher Scientific and Combi-Blocks. The reactions were monitored using silica and alumina gel thin layer chromatography plates (Sorbtech Silica XHL TLC plates w/UV 254 and Universal Scientific Incorporated Alumina G Neutral). Column chromatography for the purification of the final compounds was organized by using alumina gel Sorbtech, particle size 32-63 μM. 1H and 13C NMR data was collected on a Bruker Avance (400 MHz) spectrometer using the software TopSpin
3.6.2. Absorption data was collected on Perkin Elmer LS55 spectrometer using the software PerkinElmer Lambda 35 in range 400-1000 nm. Melting point was measured on MEL-TEMP instrument. Physicochemical measurements were performed in ChemAxon MarvinSketch software. All calculations were made in Microsoft Excel. High resolution mass spectra (HRMS) were obtained at the Georgia State University Mass Spectrometry Facility using electrospray ionization (ESI) positive mode in methanol with 0.1 % of formic acid by Thermo Scientific Dionex Ultimate 3000 instrument.

2.5.2 Optical Measurements

Stock solutions of compounds with the concentration of 1.0 mM were prepared by weighing the appropriate mass of each individual dye on analytical balance accordingly with their molecular weight and adding the appropriate volume of dimethyl sulfoxide (DMSO). The amber vials were vortexed and sonicated for complete dissolution and stored at 4 ℃ when not in use. Stock solutions were used to make three to five dilutions of dyes with concentrations at 1-20 µM keeping the absorption intensity up to 1 a.u. in the following solvents: methanol, dimethyl sulfoxide (DMSO), acetonitrile and dimethylformamide (DMF). The spectra of dyes at different concentrations and in different solvents were recorded and plotted with the wavelength (nm) on the x-axis and the absorption intensity (a.u.) on the y-axis.

For molar absorptivity calculations, the absorbance intensity at the maximum wavelength was recorded for each sample and plotted with the concentration (mol/L) on the x-axis and the absorbance intensity (a.u.) on the y-axis. A linear slope of the graph was generated, and the resulted value of the slope is equal to the molar absorptivity.

Thermal stability experiment was performed by the preparation of samples of stock solution dissolved in ethanol. Each sample was diluted to give absorbance intensity close to 0.6
and were separated into two portions: one was stored in dark, and another was placed 6 inches away from 15 W F15T8 UV lamp. The absorbance of each sample was recorded every 6-8 h for 48 h. The absorbance values were plotted versus time to obtain the thermal stability graphs in light and dark. ICG (>99%) was taken as a reference.

2.5.3 Dye-DNA Interactions Measurements

UV-visible spectrometry experiments were performed in cuvettes containing 10 µM of dyes in DMSO and in 10mM sodium phosphate buffer (pH = 7.0) with absence and presence of 150 µM of CT DNA. Absorbance spectra were recorded every 5 min up to 30 min. DNA titration experiments were done by adding small volumes (up to 50 µL = 1493 µM) of concentrated CT DNA in already prepared solutions containing 10 µM of dyes in 10mM sodium phosphate buffer (pH = 7.0).

DNA photocleavage experiments included reactions containing 10 mM of sodium phosphate buffer pH 7.0 and 38 µM of pUC19 plasmid DNA in the presence and absence of 20 µM dye. The reactions were irradiated for 30 min with various laser lights and in dark at 10°C.

2.5.4 Synthesis of Salts 105, 107, 109, 111, 113 and 115

Synthesis of salts with benzyl substituent 105 and 107. A mixture of commercially available lepidine/quinaldine and bromomethyl benzene in ratio 1 : 1 was heated to reflux at 90 ℃ for around 3 h. The reaction mixture was monitored via thin layer chromatography (TLC) using dichloromethane/methanol (1 : 25) as eluent until the starting material were gone. Upon completion of the reaction, the mixture was cooled down, and acetone was added to precipitate out the salt. The crude product was suction filtered, washed with acetone and diethyl ether, and then, dried under vacuum.
1-Benzyl-4-methylquinolin-1-ium bromide (105). Yield 46%. MP 194-196 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.04 (s, 3 H), 6.37 (s, 2 H), 7.35 (m, 5 H), 8.01 (t, $J = 7.7$ Hz, 1 H), 8.19 (m, 2 H), 8.49 (d, $J = 8.9$ Hz, 1 H), 8.55 (d, $J = 8.4$ Hz, 1 H), 9.7 (d, $J = 6$ Hz, 1 H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 20.36, 59.91, 120.20, 123.44, 127.61, 127.78, 129.14, 129.53, 129.65, 130.13, 134.62, 135.64, 137.30, 149.67, 160.08.

1-Benzyl-2-methylquinolin-1-ium bromide (107). Yield 44%. MP 205-207 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.09 (s, 3 H), 6.36 (s, 2 H), 7.14 (d, $J = 6.6$ Hz, 2 H), 7.38 (d, $J = 7.5$ Hz, 3 H), 7.99 (d, $J = 7.6$ Hz, 1 H), 8.16 (t, $J = 7.6$ Hz, 1 H), 8.27 (d, $J = 8.5$ Hz, 1 H), 8.41 (d, $J = 8.9$ Hz, 1 H), 8.48 (d, $J = 7.9$ Hz, 1 H), 9.27 (d, $J = 8.5$ Hz, 1 H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 23.31, 54.89, 119.75, 126.26, 126.36, 128.65, 128.90, 129.68, 131.19, 133.73, 135.99, 139.41, 147.22, 162.22.

Synthesis of salt with carboxybenzyl substituent 109. A mixture of commercially available lepidine (10.0 mL, 75.4 mmol) and 4-(bromomethyl)benzoic acid (13.46 g, 62.6 mmol) in ratio 1 : 0.83 in acetonitrile (10.0 mL) was heated to reflux at 110 °C under nitrogen atmosphere for around 6 h. The reaction mixture was monitored via thin layer chromatography (TLC) using dichloromethane/methanol (1 : 20) as eluent until the starting materials were gone. Upon completion of the reaction, the product was dissolved in a minimal amount of methanol and diluted with diethyl ether causing precipitation. The crude product was suction filtered and dried under vacuum to yield the final desired compounds.

1-(4-Carboxybenzyl)-4-methylquinolin-1-ium bromide (109). Yield 68%. MP 206-208 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.07 (s, 3 H), 6.42 (s, 2 H), 7.43 (d, $J = 8.2$ Hz, 2 H), 7.93 (d, $J = 8.2$ Hz, 2 H), 8.04 (t, $J = 7.7$ Hz, 1 H), 8.2 (m, 2 H), 8.39 (d, $J = 8.9$ Hz, 1 H), 8.58 (d, $J = 8.2$ Hz, 1 H), 9.62 (d, $J = 6$ Hz, 1 H), 13.1 (s, 1 H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.37, 59.67, 120.06,
Synthesis of salt with (methoxycarbonyl)benzyl substituent 111. A previously synthesized salt 109 (1.0 g, 2.8 mmol) was dissolved in methanol (10 mL), and catalytic amount of concentrated sulfuric acid was added in the mixture and refluxed at 60 °C for around 2 h. The reaction mixture was monitored via thin layer chromatography (TLC) using dichloromethane/methanol (1 : 20) as eluent until the starting materials were gone. Upon completion of the reaction, the solvent was evaporated off, and the product was dissolved in dichloromethane and extracted with a saturated solution of sodium bicarbonate in order to get rid of unreacted sulfuric acid. Calcium chloride, a drying agent, was added to the extracted organic layer for 10 min. After the solution was filtered and evaporated, the oily product of 111 was formed and used for the next step without further purification.

1-(4-(Methoxycarbonyl)benzyl)-4-methylquinolin-1-ium bromide (111). Yield 15%. 1H NMR (400 MHz, DMSO-d6) δ 3.06 (s, 3 H), 3.83 (s, 3 H), 6.41 (s, 2 H), 7.46 (t, J = 8.3 Hz, 2 H), 7.96 (m, 3 H), 8.21 (m, 2 H), 8.36 (d, J = 8.9 Hz, 1 H), 8.58 (d, J = 8.2 Hz, 1 H), 9.6 (d, J = 5.9 Hz, 1 H).

Synthesis of salt with phenylpropyl substituent 113. A mixture of lepidine and (3-bromopropyl)benzene in ratio 1 : 1 was heated in acetonitrile to reflux at 120 °C for around 3 h. The reaction mixture was monitored via thin layer chromatography (TLC) using dichloromethane/methanol (1 : 20) as eluent until the starting materials were gone. Upon completion of the reaction, the product was dissolved in dichloromethane and diluted with diethyl ether causing precipitation. The salt 113 was then suction filtered and dried under vacuum.
4-Methyl-1-(3-phenylpropyl)quinolin-1-ium bromide (113). Yield 47%. MP 143-144 °C. 1H NMR (400 MHz, DMSO-d$_6$) δ 2.27 (t, $J = 7.2$ Hz, 2 H), 2.77 (t, $J = 7.7$ Hz, 2 H), 2.98 (s, 3 H), 5.06 (t, $J = 7.3$ Hz, 2 H), 7.16 (m, 3 H), 7.24 (t, $J = 7.2$ Hz, 2 H), 8 (m, 2 H), 8.22 (t, $J = 7.8$ Hz, 1 H), 8.5 (d, $J = 8.4$ Hz, 1 H), 8.54 (d, $J = 8.9$ Hz, 1 H), 9.38 (d, $J = 5.9$ Hz, 1 H). $^{13}$C NMR (DMSO-d$_6$) δ 20.17, 31.31, 32.21, 57.19, 119.81, 123.14, 126.53, 127.62, 128.64, 128.83, 129.41, 130.02, 135.54, 137.24, 140.86, 149.01, 158.99.

Synthesis of precursor (3-bromopropyl)triphenylphosphonium bromide 115a. A mixture of 1,3-dibromopropane (5.0 mL, 49.0 mmol) and triphenylphosphine (5.1 g, 19.6 mmol) in ratio 3 : 1 was heated to reflux at 80 °C under nitrogen atmosphere for around 3 h. Upon completion of the reaction, the product was washed with toluene, suction filtered and dried under a reduced pressure.

(3-Bromopropyl)triphenylphosphonium bromide (115a). Yield 97%. MP 215-216 °C. 1H NMR (400 MHz, DMSO-d$_6$) δ 2.08 (m, 2 H), 3.69 (m, 4 H), 7.81 (m, 12 H), 7.92 (m, 3 H).

Synthesis of salt with triphenylphosphonium substituent 115. A mixture of synthesized 115a (5.00 g, 10.8 mmol) and lepidine (2.86 mL, 21.5 mmol) in acetonitrile was refluxed at 110 °C under nitrogen atmosphere for 18 h. The reaction mixture was monitored via thin layer chromatography (TLC) using dichloromethane/methanol (1 : 25) as eluent until the starting materials were gone. After the reaction mixture was completed and cooled down, the product was dissolved in a minimal amount of acetone and diluted with diethyl ether causing precipitation. The further purification was performed by column chromatography with a gradient solvent system of methanol in dichloromethane (1 : 20). The crude product was suction filtered and dried under vacuum.
4-Methyl-1-(3-(triphenylphosphonio)propyl)quinolin-1-i um bromide (115). Yield 53%. MP 201-202 °C. 1H NMR (400 MHz, DMSO- $d_6$) $\delta$ 2.28 (m, 2 H), 3.01 (s, 3 H), 3.95 (m, 2 H), 5.26 (t, $J = 7.1$ Hz, 2 H), 7.78 (m, 8 H), 7.9 (t, $J = 6.8$ Hz, 4 H), 8.05 (t, $J = 7.5$ Hz, 3 H), 8.1 (d, $J = 5.9$ Hz, 2 H), 8.22 (t, $J = 7.7$ Hz, 1 H), 8.57 (m, 2 H), 9.51 (d, $J = 6.1$ Hz, 1 H).

### 2.5.5 Synthesis of Symmetrical Pentamethine Cyanine Dyes 106a-c, 108a-c, 110a-c, 112a-c, 114a-c and 116a-c.

Synthesis of symmetrical pentamethine cyanine dyes with benzyl substituents 106a-c and 108a-c. 2 eq of synthesized salt with benzyl substituent 105 or 107 and 1 eq of polymethine linkers 104a-c were dissolved in 5 mL of acetic anhydride in the presence of catalytic amount of triethylamine (TEA). The synthesis was performed by both conventional and microwave-assisted methods. Conventional method was performed by heating the reaction up to 70 °C for 1h, while microwave irradiation was performed at 100-110 °C and took only 2 min. The formation of dyes was monitored with a UV-Vis spectrophotometer until peaks at 700-720 nm for 106a-c and 800-820 nm for 108a-c were obtained and a peak at ~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing dye precipitation. The mixture was filtered under vacuum and purified via recrystallization by solvation in a minimal amount of methanol and dilution with diethyl ether causing precipitation. And then, the filtered crystals were dissolved in dichloromethane and precipitated by hexanes. The solid product was collected and dried under vacuum.

1-Benzyl-2-((1E,3Z)-5-((E)-1-benzylquinolin-2(1H)-ylidene)-3-bromopenta-1,3-dien-1-yl)quinolin-1-i um bromide (106a). Yield 66%. MP 190-193 °C. 1H NMR (400 MHz, DMSO-$d_6$) $\delta$ 5.77 (s, 4 H), 6.15 (d, $J = 12.5$ Hz, 2 H), 7.2 (d, $J = 7.4$ Hz, 4 H), 7.33 (t, $J = 7.2$ Hz, 2 H), 7.38 (t, $J = 7.6$ Hz, 4 H), 7.55 (t, $J = 7.3$ Hz, 2 H), 7.77 (t, $J = 7.9$ Hz, 2 H), 7.87 (d, $J = 7.8$ Hz, 2
H), 7.98 (d, J = 8.2 Hz, 4 H), 8.28 (m, 4 H). $^{13}$C NMR (DMSO-$d_6$) δ 52.58, 106.06, 113.87, 117.33, 120.40, 125.56, 126.27, 126.66, 128.28, 129.87, 133.64, 134.89, 138.30, 140.21, 147.23, 153.46. ESI-MS (positive mode) calculated for C$_{37}$H$_{30}$BrN$_2$: m/z 581.16, found: m/z 581.1587.

1-Benzyl-2-((1E,3E)-5-((E)-1-benzylquinolin-2(1H)-ylidene)-3-chloropenta-1,3-dien-1-yl)quinolin-1-ium bromide (106b). Yield 69%. MP 215-217 °C. 1H NMR (400 MHz, DMSO-$d_6$) δ 5.79 (s, 4 H), 6.15 (d, J = 12.5 Hz, 2 H), 7.19 (d, J = 6.9 Hz, 4 H), 7.33 (m, 2 H), 7.41 (t, J = 8.4 Hz, 4 H), 7.55 (t, J = 7.4 Hz, 2 H), 7.77 (t, J = 8.2 Hz, 2 H), 7.85 (d, J = 7.2 Hz, 2 H), 7.96 (d, J = 8.9 Hz, 4 H), 8.2 (d, J = 13.2 Hz, 2 H), 8.29 (d, J = 9.24 Hz, 2 H). $^{13}$C NMR (DMSO-$d_6$) δ 52.47, 103.98, 117.31, 120.25, 121.16, 125.55, 126.24, 126.61, 128.26, 129.56, 129.85, 133.61, 134.96, 138.20, 140.19, 145.09, 153.28. ESI-MS (positive mode) calculated for C$_{37}$H$_{30}$ClN$_2$: m/z 537.21, found: m/z 537.2092.

1-Benzyl-2-((1E,3E)-5-((E)-1-benzylquinolin-2(1H)-ylidene)penta-1,3-dien-1-yl)quinolin-1-ium bromide (106c). Yield 70%. MP 213-214 °C. 1H NMR (400 MHz, DMSO-$d_6$) δ 5.7 (s, 4 H), 6.17 (d, J = 12.9 Hz, 2 H), 6.34 (t, J = 11.8 Hz, 1 H), 7.16 (d, J = 7.5 Hz, 4 H), 7.3 (t, J = 8.1 Hz, 2 H), 7.37 (t, J = 7.9 Hz, 4 H), 7.45 (t, J = 6.8 Hz, 2 H), 7.66 (m, 4 H), 7.87 (d, J = 9.2 Hz, 2 H), 7.93 (d, J = 9.6 Hz, 2 H), 8.01 (t, J = 12.2 Hz, 2 H), 8.08 (d, J = 9.5 Hz, 2 H). $^{13}$C NMR (DMSO-$d_6$) δ 51.62, 107.12, 116.86, 120.13, 124.25, 125.27, 125.59, 126.52, 128.05, 129.47, 129.59, 133.10, 135.29, 136.70, 140.16, 150.33, 152.51. ESI-MS (positive mode) calculated for C$_{37}$H$_{31}$N$_2$: m/z 503.25, found: m/z 503.2479.

1-Benzyl-4-((1E,3Z)-5-((Z)-1-benzylquinolin-4(1H)-ylidene)-3-bromopenta-1,3-dien-1-yl)quinolin-1-ium bromide (108a). Yield 60%. MP 193-195 °C. 1H NMR (400 MHz, DMSO-$d_6$) δ 5.58 (s, 4 H), 6.98 (d, J = 12.7 Hz, 2 H), 7.3 (m, 6 H), 7.35 (t, J = 7.0 Hz, 4 H), 7.55 (d, J = 6.7 Hz, 2 H), 7.62 (t, J = 7.4 Hz, 2 H), 7.82 (m, 4 H), 8.31 (d, J = 12.7 Hz, 2 H), 8.38 (d, J = 8.5
Hz, 2 H), 8.54 (d, J = 7.1 Hz, 2 H). 13C NMR (DMSO-d$_6$) δ 57.18, 109.53, 109.98, 118.56, 125.25, 125.42, 127.11, 127.20, 128.57, 129.42, 131.66, 133.50, 136.06, 138.62, 143.09, 144.03, 149.23. ESI-MS (positive mode) calculated for C$_{37}$H$_{30}$BrN$_2$: m/z 581.16, found: m/z 581.1585.

1-Benzyl-4-(((1E,3Z)-5-((Z)-1-benzylquinolin-4(1H)-ylidene)-3-chloropenta-1,3-dien-1-yl)quinolin-1-ium bromide (108b). Yield 64%. MP 202-203 °C. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 5.77 (s, 4 H), 7.04 (d, J = 12.6 Hz, 2 H), 7.31 (m, 6 H), 7.37 (t, J = 8.2 Hz, 4 H), 7.55 (d, J = 7 Hz, 2 H), 7.62 (t, J = 7.3 Hz, 2 H), 7.81 (t, J = 9.2 Hz, 2 H), 7.86 (d, J = 7.6 Hz, 2 H), 8.22 (d, J = 12.9 Hz, 2 H), 8.43 (d, J = 7.9 Hz, 2 H), 8.52 (d, J = 7.6 Hz, 2 H). 13C NMR (DMSO-d$_6$) δ 57.15, 107.64, 109.75, 118.52, 125.29, 125.56, 126.11, 127.11, 127.98, 128.55, 129.43, 133.47, 136.12, 138.63, 141.93, 143.04, 148.97. ESI-MS (positive mode) calculated for C$_{37}$H$_{30}$ClN$_2$: m/z 537.21, found: m/z 537.2090.

1-Benzyl-4-(((1E,3E)-5-((Z)-1-benzylquinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)quinolin-1-ium bromide (108c). Yield 65%. MP 184-186 °C. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 5.69 (s, 4 H), 6.72 (t, J = 12.4 Hz, 1 H), 7.05 (d, J = 12.3 Hz, 2 H), 7.30 (t, J = 12.4 Hz, 6 H), 7.37 (t, J = 12.4 Hz, 4 H), 7.44 (d, J = 7.3 Hz, 2 H), 7.5 (t, J = 8.6 Hz, 2 H), 7.72 (m, 4 H), 8.04 (t, J = 12.7 Hz, 2 H), 8.33 (d, J = 7.3 Hz, 2 H), 8.43 (d, J = 8.4 Hz, 2 H). 13C NMR (DMSO-d$_6$) δ 56.69, 108.84, 111.86, 118.13, 125.18, 125.60, 126.13, 126.43, 127.08, 128.44, 129.39, 132.98, 136.38, 138.69, 141.94, 146.91, 147.77. ESI-MS (positive mode) calculated for C$_{37}$H$_{31}$N$_2$: m/z 503.25, found: m/z 503.2479.

Synthesis of symmetrical pentamethine cyanine dyes with carboxybenzyl substituents 110a-c. 2 eq of synthesized salt with carboxybenzyl substituent 109 and 1 eq of the polymethine linkers 104a-c were mixed together and stirred in 5 mL of acetic anhydride in the presence of 2 eq of triethylamine (TEA). The synthesis of pentamethine dyes 110a-c was performed by both
conventional and microwave-assisted methods. Conventional method was performed by heating the reaction up to 70 °C for 1h, while microwave irradiation was held at 100-110 °C for 10 min. The formation of dyes was monitored via UV-Vis until a peak at 800-820 nm was obtained, and peak at ~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing dye precipitation. After filtration, the collected crystals were dissolved in water with TEA and extracted using DCM (3 x 20 mL). Hydrochloric acid (10%) was added to the resulted organic layer to cause precipitation. The green solid was obtained after filtration and dissolved in a minimal amount of methanol and diluted with diethyl ether causing precipitation. And then, the filtered crystals were dissolved in dichloromethane and precipitated by hexanes. The solid product was collected and dried under vacuum.

4-((1E,3Z)-3-bromo-5-((Z)-1-(4-carboxybenzyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)-1-(4-carboxybenzyl)quinolin-1-ium bromide (110a). Yield 22%. MP 203-205 °C. $^1$H NMR (400 MHz, DMSO-d6) δ 5.88 (s, 4 H), 7.03 (d, $J = 13.2$ Hz, 2 H), 7.4 (d, $J = 8.1$ Hz, 4 H), 7.62 (m, 4 H), 7.8 (s, 4 H), 7.94 (d, $J = 7.8$ Hz, 4 H), 8.41 (t, $J = 7.4$ Hz, 4 H), 8.59 (d, $J = 6.8$ Hz, 2 H), 13.08 (s, 2 H). $^{13}$C NMR (DMSO-d6) δ 56.78, 109.46, 110.02, 118.49, 125.13, 125.47, 127.16, 128.04, 129.99, 130.39, 130.91, 133.62, 138.42, 141.14, 143.25, 143.35, 144.35, 149.25, 167.31. ESI-MS (positive mode) calculated for C$_{39}$H$_{30}$BrN$_2$O$_4$: m/z 669.14, found: m/z 669.1379.

4-((1E,3Z)-3-chloro-5-((E)-1-(4-(methoxycarbonyl)benzyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)-1-(4-(methoxycarbonyl)benzyl)quinolin-1-ium bromide (110b). Yield 53%. MP 213-214 °C. $^1$H NMR (400 MHz, DMSO-d6) δ 5.86 (s, 4 H), 7.07 (d, $J = 8.2$ Hz, 2 H), 7.4 (d, $J = 6.2$ Hz, 4 H), 7.57 (m, 4 H), 7.8 (s, 4 H), 7.94 (d, $J = 7$ Hz, 4 H), 8.25 (d, $J = 8$ Hz, 2 H), 8.43 (d, $J = 6.8$ Hz, 2 H), 8.52 (d, $J = 6.5$ Hz, 2 H), 13.01 (s, 2 H). $^{13}$C NMR (DMSO-d6) δ 56.84, 107.74, 109.85, 118.43, 123.69, 125.25, 125.65, 127.18, 128.03, 129.96, 130.37, 131.03,
ESI-MS (positive mode) calculated for C_{39}H_{30}ClN_{2}O_{4}: m/z 625.19, found: m/z 625.1883.

1-(4-(methoxycarbonyl)benzyl)-4-((1E,3E)-5-((E)-1-(4-(methoxycarbonyl)benzyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)quinolin-1-ium bromide (110c). Yield 40%. MP 194-195 °C. \(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\) 5.78 (s, 4 H), 6.74 (t, \(J = 12.22\) Hz, 1 H), 7.1 (d, \(J = 13.3\) Hz, 2 H), 7.39 (d, \(J = 8.3\) Hz, 4 H), 7.47 (d, \(J = 7.3\) Hz, 2 H), 7.54 (t, \(J = 7.9\) Hz, 2 H), 7.67 (d, \(J = 7.4\) Hz, 2 H), 7.7 (t, \(J = 7.2\) Hz, 2 H), 7.94 (d, \(J = 8.2\) Hz, 4 H), 8.1 (t, \(J = 12.6\) Hz, 2 H), 8.34 (d, \(J = 7.2\) Hz, 2 H), 8.45 (d, \(J = 8.4\) Hz, 2 H), 13.01 (s, 2 H). \(^{13}\)C NMR (DMSO-d6) \(\delta\): 57.06, 107.41, 109.63, 118.59, 123.38, 125.20, 125.60, 127.07, 127.17, 128.56, 129.45, 129.52, 133.52, 136.17, 138.50, 141.97, 143.15, 148.94, 163.38. ESI-MS (positive mode) calculated for C_{39}H_{30}N_{2}O_{4}: m/z 591.23, found: m/z 559.2274.

Synthesis of symmetrical pentamethine cyanine dyes with (methoxycarbonyl)benzyl substituents 112a-c. 2 eq of synthesized salt with (methoxycarbonyl)benzyl substituent 111 and 1 eq of the polymethine linkers 104a-c were mixed together and stirred in 5 mL of acetic anhydride in the presence of 2 eq of triethylamine (TEA) at 70 °C for 2h. The reaction mixture was monitored via UV-Vis until a peak at 800-820 nm was obtained, and peaks at ~450 nm and ~650 nm disappeared. Upon cooling to room temperature, diethyl ether was added to the reaction mixture, causing dye precipitation. The mixture was filtered under vacuum and the final dyes purified via recrystallization by solvation in a minimal amount of dichloromethane and dilution with hexanes causing precipitation. Then the filtered crystals were dissolved in warm methanol, filtered, and the resulted filtrate was concentrated under reduced pressure, washed with diethyl ether, and sonicated for several hours. The solid product was collected and dried under vacuum.
4-((1E,3Z)-3-bromo-5-((E)-1-(4-(methoxycarbonyl)benzyl)quinolin-4(1H)-
ylidene)penta-1,3-dien-1-yl)-1-(4-(methoxycarbonyl)benzyl)quinolin-1-ium bromide (112a). Yield 39%. MP 196-198 °C. \( ^1 H \) NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 3.84 (s, 6 H), 5.89 (s, 4 H), 7.03 (d, \( J = 12.8 \) Hz, 2 H), 7.42 (d, \( J = 8.1 \) Hz, 4 H), 7.62 (m, 4 H), 7.79 (d, \( J = 6.9 \) Hz, 4 H), 7.96 (d, \( J = 7.9 \) Hz, 4 H), 8.41 (m, 4 H), 8.59 (d, \( J = 6.7 \) Hz, 2 H). \( ^{13}C \) NMR (DMSO-\( d_6 \)) \( \delta \) 52.28, 52.57, 56.86, 109.76, 110.12, 118.43, 125.27, 125.50, 127.24, 127.36, 127.82, 128.16, 129.79, 130.22, 133.61, 138.56, 141.50, 143.18, 144.31, 149.31, 166.27. ESI-MS (positive mode) calculated for \( C_{41}H_{34}BrN_2O_4 \): m/z 697.17, found: m/z 697.1690.

4-((1E,3Z)-3-chloro-5-((E)-1-(4-(methoxycarbonyl)benzyl)quinolin-4(1H)-
ylidene)penta-1,3-dien-1-yl)-1-(4-(methoxycarbonyl)benzyl)quinolin-1-ium bromide (112b). Yield 64%. MP 188-189 °C. \( ^1 H \) NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 3.84 (s, 6 H), 5.88 (s, 4 H), 7.06 (d, \( J = 13.1 \) Hz, 2 H), 7.43 (d, \( J = 8.4 \) Hz, 4 H), 7.6 (m, 4 H), 7.78 (d, \( J = 7.3 \) Hz, 4 H), 7.97 (d, \( J = 8.3 \) Hz, 4 H), 8.27 (d, \( J = 13.1 \) Hz, 2 H), 8.46 (d, \( J = 8.3 \) Hz, 2 H), 8.57 (d, \( J = 7.2 \) Hz, 2 H). \( ^{13}C \) NMR (DMSO-\( d_6 \)) \( \delta \) 52.55, 52.60, 56.80, 107.87, 109.92, 118.38, 125.30, 125.64, 127.18, 127.37, 128.16, 129.81, 129.91, 130.23, 133.55, 138.58, 141.56, 142.22, 143.11, 149.08, 166.26. ESI-MS (positive mode) calculated for \( C_{41}H_{34}ClN_2O_4 \): m/z 653.22, found: m/z 653.2206.

1-(4-(Methoxycarbonyl)benzyl)-4-((1E,3E)-5-((E)-1-(4-(methoxycarbonyl)benzyl)quinolin-4(1H)-
ylidene)penta-1,3-dien-1-yl)quinolin-1-ium bromide (112c). Yield 48%. MP 192-193 °C. \( ^1 H \) NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 3.85 (s, 6 H), 5.78 (s, 4 H), 6.76 (t, \( J = 12.3 \) Hz, 1 H), 7.08 (d, \( J = 13.6 \) Hz, 2 H), 7.42 (d, \( J = 8.1 \) Hz, 4 H), 7.46 (d, \( J = 7.5 \) Hz, 2 H), 7.54 (t, \( J = 7.3 \) Hz, 2 H), 7.68 (m, 4 H), 7.96 (d, \( J = 8.1 \) Hz, 4 H), 8.04 (t, \( J = 12.6 \) Hz, 2 H), 8.3 (d, \( J = 7.4 \) Hz, 2 H), 8.43 (d, \( J = 8.1 \) Hz, 2 H). \( ^{13}C \) NMR (DMSO-\( d_6 \)) \( \delta \) 52.57, 53.18, 56.45, 109.03, 112.18, 117.99, 125.22, 125.67, 126.43, 126.52, 127.33, 129.86, 130.21, 130.43, 133.11, 138.69, 141.81, 141.98,
147.03, 147.85, 166.27. ESI-MS (positive mode) calculated for C$_{41}$H$_{35}$N$_2$O$_4$: m/z 619.26, found: m/z 619.2586.

**Synthesis of symmetrical pentamethine cyanine dyes with phenylpropyl substituents**

114a-c. 2 eq of synthesized salt with phenylpropyl substituent 113 and 1 eq of polymethine linkers 104a-c were dissolved in 5 mL of acetic anhydride in the presence of catalytic amount of triethylamine (TEA). The synthesis was performed by both conventional and microwave-assisted methods. Conventional method was performed by heating at 70 °C for 1 h, while microwave irradiation was held at 80-90 °C for 8 min. The formation of dyes was monitored via UV-Vis until a peak at 800-820 nm was obtained, and peak at ~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing dye precipitation. Then, it was filtered under vacuum and the collected crystals were dissolved in a minimal amount of dichloromethane and diluted with diethyl ether causing precipitation. The final solid product was collected and dried under vacuum.

4-((1E,3Z)-3-bromo-5-((Z)-1-(3-phenylpropyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)-1-(3-phenylpropyl)quinolin-1-ium bromide (114a) Yield 40%. MP 168-169 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.18 (t, $J = 6.2$ Hz, 4 H), 2.75 (t, $J = 7.3$ Hz, 4 H), 4.52 (t, $J = 6.3$ Hz, 4 H), 6.97 (d, $J = 13.2$ Hz, 2 H), 7.24 (m, 6 H), 7.29 (t, $J = 7$ Hz, 4 H), 7.43 (d, $J = 7$ Hz, 2 H), 7.66 (t, $J = 7.8$ Hz, 2 H), 7.89 (t, $J = 6.5$ Hz, 2 H), 7.94 (d, $J = 6.8$ Hz, 2 H), 8.14 (d, $J = 12.9$ Hz, 2 H), 8.3 (d, $J = 7$ Hz, 2 H), 8.41 (d, $J = 7.9$ Hz, 2 H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 30.72, 32.49, 54.02, 107.09, 109.57, 117.93, 123.04, 125.16, 125.55, 126.47, 126.99, 128.66, 128.88, 133.46, 138.44, 141.12, 141.56, 142.39, 148.71. ESI-MS (positive mode) calculated for C$_{41}$H$_{38}$BrN$_2$: m/z 637.22, found: m/z 637.2213.
4-((1E,3Z)-3-chloro-5-((Z)-1-(3-phenylpropyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)-1-(3-phenylpropyl)quinolin-1-i um bromide (114b) Yield 35%. MP 183-185 °C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 2.18 (t, 4 H), 2.75 (t, \(J = 7.2\) Hz, 4 H), 4.52 (t, 4 H), 6.95 (d, \(J = 12.5\) Hz, 2 H), 7.24 (m, 6 H), 7.28 (d, \(J = 7\) Hz, 4 H), 7.45 (d, \(J = 6.7\) Hz, 2 H), 7.67 (t, \(J = 7.4\) Hz, 2 H), 7.9 (t, \(J = 5.6\) Hz, 2 H), 7.95 (d, \(J = 6.9\) Hz, 2 H), 8.23 (d, \(J = 12.6\) Hz, 2 H), 8.31 (d, \(J = 6.5\) Hz, 2 H), 8.38 (d, \(J = 8.3\) Hz, 2 H). \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 30.69, 32.46, 53.98, 107.07, 109.55, 117.93, 123.02, 125.14, 125.53, 126.49, 126.97, 128.64, 128.84, 133.44, 138.44, 141.12, 141.52, 142.36, 148.67. ESI-MS (positive mode) calculated for C\(_{41}\)H\(_{38}\)ClN\(_2\): m/z 593.27, found: m/z 593.2713.

1-(3-Phenylpropyl)-4-((1E,3E)-5-((Z)-1-(3-phenylpropyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)quinolin-1-i um bromide (114c) Yield 68%. MP 175-176 °C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 2.15 (t, \(J = 7.3\) Hz, 4 H), 2.74 (t, \(J = 7.5\) Hz, 4 H), 4.43 (t, \(J = 6.9\) Hz, 4 H), 6.67 (t, \(J = 12.7\) Hz, 1 H), 6.98 (d, \(J = 13.2\) Hz, 2 H), 7.23 (m, 6 H), 7.29 (m, 6 H), 7.57 (t, \(J = 6.8\) Hz, 2 H), 7.81 (s, 4 H), 7.94 (t, \(J = 12.8\) Hz, 2 H), 8.06 (d, \(J = 7.2\) Hz, 2 H), 8.39 (d, \(J = 8.6\) Hz, 2 H). \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 30.70, 32.42, 53.50, 108.52, 111.16, 117.58, 124.93, 125.46, 125.61, 126.29, 126.50, 128.87, 133.03, 138.39, 141.23, 141.33, 146.54, 147.45. ESI-MS (positive mode) calculated for C\(_{41}\)H\(_{39}\)N\(_2\): m/z 559.31, found: m/z 559.3102.

**Synthesis of symmetrical pentamethine cyanine dyes with triphenylphosphonium substituents 116a-c.** 2 eq of synthesized salt with triphenylphosphonium substituent 115 and 1 eq of polymethine linkers 104a-c in 5 mL of acetic anhydride in the presence of 2 eq. of triethylamine (TEA) or N, N-Diisopropylethylamine (DIPEA) were refluxed for 1 h at 70 °C under nitrogen atmosphere. Additional 2eq. of TEA or DIPEA were added after 1 h and stirred for 1 h more. The reaction mixture was monitored via UV-Vis until a peak at 800-820 nm was obtained and peak at
~450 nm disappeared. Upon cooling the reaction mixture to room temperature, ethyl acetate was added to it, causing the dye to precipitate. The mixture was filtered under vacuum and purified by column chromatography with a gradient solvent system of methanol in dichloromethane (1 : 10). The dark green fractions were mixed together and concentrated under reduced pressure. The final product was collected after being washed with diethyl ether and sonicated for several hours and dried under vacuum.

4-((1E,3Z)-3-bromo-5-((Z)-1-(3-(triphenylphosphonio)propyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)-1-(3-(triphenylphosphonio)propyl)quinolin-4(1H)-ylium bromide (116a) Yield 14%. MP 195-197 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.14 (t, $J = 6$ Hz, 4 H), 3.83 (t, $J = 7.5$ Hz, 4 H), 4.69 (t, 4 H), 6.94 (d, $J = 12.8$ Hz, 2 H), 7.52 (d, $J = 7.3$ Hz, 2 H), 7.66 (t, $J = 7.6$ Hz, 2 H), 7.79 (m, 25 H), 7.9 (m, 10 H), 8.39 (m, 5 H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 18.62, 19.17, 22.47, 109.22, 110.11, 116.21, 117.80, 118.21, 119.07, 125.16, 125.47, 127.07, 130.69, 130.82, 133.43, 134.07, 134.17, 135.52, 138.32, 142.56, 144.11, 149.08. ESI-MS (positive mode) calculated for C$_{65}$H$_{58}$BrN$_2$P$_2$: m/z 1007.32 / 3 = 335.77, found: m/z 335.7752.

4-((1E,3Z)-3-chloro-5-((Z)-1-(3-(triphenylphosphonio)propyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)-1-(3-(triphenylphosphonio)propyl)quinolin-4(1H)-ylium bromide (116b) Yield 15%. MP 193-194 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.13 (t, $J = 6.7$ Hz, 4 H), 3.83 (t, $J = 7.9$ Hz, 4 H), 4.69 (t, $J = 6.3$ Hz, 4 H), 6.94 (d, $J = 12.9$ Hz, 2 H), 7.52 (d, $J = 7.5$ Hz, 2 H), 7.66 (t, $J = 7.6$ Hz, 2 H), 7.79 (m, 25 H), 7.91 (m, 10 H), 8.39 (m, 5 H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 18.22, 18.74, 22.30, 107.07, 109.71, 117.78, 118.16, 119.01, 123.14, 125.10, 125.64, 127.03, 130.71, 130.83, 133.44, 134.09, 134.19, 135.55, 138.23, 141.94, 142.59, 148.79. ESI-MS (positive mode) calculated for C$_{65}$H$_{58}$ClN$_2$P$_2$: m/z 963.37 / 3 = 321.12, found: m/z 321.1254.
1-(3-(Triphenylphosphonio)propyl)-4-((1E,3E)-5-((Z)-1-(3-(triphenylphosphonio)propyl)quinolin-4(1H)-ylidene)penta-1,3-dien-1-yl)quinolin-1-ium bromide (116c) Yield 11%. MP 190-192 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 2.09 (t, J = 7.2 Hz, 4 H), 3.83 (t, J = 7.6 Hz, 4 H), 4.6 (t, 4 H), 6.67 (t, J = 7.6 Hz, 1 H), 7.01 (d, J = 13.4 Hz, 2 H), 7.34 (d, J = 7.5 Hz, 2 H), 7.56 (t, J = 7.2 Hz, 2 H), 7.78 (m, 27 H), 7.91 (m, 9 H), 8.12 (d, J = 7.1 Hz, 2 H), 8.41 (d, J = 8.5 Hz, 2 H). ¹³C NMR (DMSO-d₆) δ 18.17, 18.70, 22.16, 108.78, 111.58, 117.30, 118.15, 119.01, 125.05, 125.71, 126.39, 128.79, 130.71, 130.84, 133.03, 134.06, 134.16, 135.55, 138.30, 141.42, 146.64, 147.55. ESI-MS (positive mode) calculated for C₆₅H₅₈N₂P₂: m/z 929.41 / 3 = 309.80, found: m/z 309.8051.

2.6 Conclusion

A series of eighteen novel symmetrical pentamethine cyanine dyes were synthesized in good yield, purified and characterized by ¹H NMR, ¹³C NMR and ESI-MS. Their optical and physicochemical properties were studied. According to photophysical properties, compounds 110a-c, 112a-c and 116a-c, having the highest polarizability and dipole moment, showed the most promising results for being useful for biomedical applications. Analysis of optical properties showed that all synthesized fluorophores with 4-quinolinium moieties absorb NIR light higher than ICG in 791 – 830 nm range. Dyes with electron withdrawing atoms such as Br and Cl in 108a, 108b, 114a 114b, 116a and 116b, exhibited both high stability over 48 h under dark and light conditions and increased molar absorptivity. In comparison to ICG, the only dye approved by the United States Food and Drug Administration, these dyes exhibited enhanced absorption wavelength and stability. The sets of dyes 106a-c and 108a-c were chosen for their analyses with dye-DNA interaction. Compounds with 4-quinolinium moieties 108a-c showed hypochromicity effect after the saturation of DNA. After DNA photocleavage experiments, symmetrical
pentamethine cyanine dye with benzyl substituent 108a (X = Br) was chosen as the best representative displaying the best photocleavage of plasmid DNA under light irradiation at 830 and 850 nm. It could be the first cyanine dye causing DNA damage at such a high wavelength. The symmetrical pentamethine cyanine dye 108a could be used as a photosensitizer in PDT, as possess high stability, long wavelength of light absorption and cleavage of DNA, which allows to penetrate deeper in tissue. We are confident that this work will lay the foundation for the development of new photosensitizing agents. The in vivo biodistribution data of the synthesized dyes is being studied and will be reported in the future.
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APPENDICES

Appendix A. Optical properties

Appendix A.1. Absorbance and Molar Absorptivity Spectra
**Dye 106a in Methanol**

![Absorbance graph for Dye 106a in Methanol]

**Dye 106a in DMSO**

![Absorbance graph for Dye 106a in DMSO]

**Dye 106a in Acetonitrile**

![Absorbance graph for Dye 106a in Acetonitrile]

**Dye 106a in DMF**

![Absorbance graph for Dye 106a in DMF]
Dye 106c in Methanol

Dye 106c in DMSO

Dye 106c in Acetonitrile

Dye 106c in DMF

Molar absorptivity

\[ y = 1.5205x + 0.0774 \]
\[ R^2 = 0.9906 \]

\[ y = 1.2618x + 0.0696 \]
\[ R^2 = 0.9922 \]

\[ y = 1.6803x + 0.0458 \]
\[ R^2 = 0.9967 \]

\[ y = 1.3207x - 0.0158 \]
\[ R^2 = 0.9915 \]
**Dye 108a in Methanol**

Molar absorptivity

- $y = 2.2429x + 0.0137$
- $R^2 = 0.9961$

**Dye 108a in DMSO**

Molar absorptivity

- $y = 1.7702x + 0.0081$
- $R^2 = 0.995$

**Dye 108a in Acetonitrile**

Molar absorptivity

- $y = 2.3755x - 0.0405$
- $R^2 = 0.9996$

**Dye 108a in DMF**

Molar absorptivity

- $y = 1.7139x - 0.1098$
- $R^2 = 0.9959$
Dye 108c in Methanol

Absorbance vs. Concentration

Molar absorptivity

Dye 108c in DMSO

Absorbance vs. Concentration

Molar absorptivity

Dye 108c in Acetonitrile

Absorbance vs. Concentration

Molar absorptivity

Dye 108c in DMF

Absorbance vs. Concentration

Molar absorptivity
Dye 110a in Methanol

Absorbance vs. Concentration

Molar absorptivity

Dye 110a in DMSO

Absorbance vs. Concentration

Molar absorptivity

Dye 110a in Acetonitrile

Absorbance vs. Concentration

Molar absorptivity

Dye 110a in DMF

Absorbance vs. Concentration

Molar absorptivity
Dye 110b in Methanol

Dye 110b in DMSO

Dye 110b in Acetonitrile

Dye 110b in DMF
Dye 110c in Methanol

\[ y = 0.4594x + 0.0134 \]

\[ R^2 = 0.9978 \]

Dye 110c in DMSO

\[ y = 0.6136x + 0.0096 \]

\[ R^2 = 0.9971 \]

Dye 110c in Acetonitrile

\[ y = 0.5751x + 0.0109 \]

\[ R^2 = 0.994 \]

Dye 110c in DMF

\[ y = 0.2902x + 0.0312 \]

\[ R^2 = 0.9861 \]
Dye 112a in Methanol

Molar absorptivity

Dye 112a in DMSO

Molar absorptivity

Dye 112a in Acetonitrile

Molar absorptivity

Dye 112a in DMF

Molar absorptivity

y = 1.6071x + 0.0599
R² = 0.9832

y = 1.6734x - 0.0186
R² = 0.9936

y = 1.4835x + 0.0631
R² = 0.9937

y = 1.1922x - 0.0161
R² = 0.9957
Dye 112b in Methanol

Dye 112b in DMSO

Dye 112b in Acetonitrile

Dye 112b in DMF

Molar absorptivity
Dye 114a in Methanol

Molar absorptivity

Dye 114a in DMSO

Molar absorptivity

Dye 114a in Acetonitrile

Molar absorptivity

Dye 114a in DMF

Molar absorptivity
Dye 114c in Methanol

Molar absorptivity

Dye 114c in DMSO

Molar absorptivity

Dye 114c in Acetonitrile

Molar absorptivity

Dye 114c in DMF

Molar absorptivity

\[ y = 1.437x - 0.0606 \]

\[ R^2 = 0.9944 \]

\[ y = 1.631x + 0.0159 \]

\[ R^2 = 0.9914 \]

\[ y = 1.3717x - 0.0279 \]

\[ R^2 = 0.9986 \]

\[ y = 1.3092x - 0.1628 \]

\[ R^2 = 0.992 \]
Dye 116a in Methanol

\[ y = 1.7167x - 0.0314 \]

\[ R^2 = 0.986 \]

Absorbance vs Concentration, μM

Dye 116a in DMSO

\[ y = 2.4004x + 0.0057 \]

\[ R^2 = 0.9989 \]

Absorbance vs Concentration, μM

Dye 116a in Acetonitrile

\[ y = 1.8381x + 0.0293 \]

\[ R^2 = 0.9959 \]

Absorbance vs Concentration, μM

Dye 116a in DMF

\[ y = 1.2191x + 0.0123 \]

\[ R^2 = 0.9964 \]

Absorbance vs Concentration, μM
Dye **116b** in Methanol

- Linear equation: $y = 2.5139x + 0.1139$
- $R^2 = 0.994$

Dye **116b** in DMSO

- Linear equation: $y = 1.8978x - 0.0438$
- $R^2 = 0.9991$

Dye **116b** in Acetonitrile

- Linear equation: $y = 3.1299x - 0.0312$
- $R^2 = 0.9943$

Dye **116b** in DMF

- Linear equation: $y = 1.6493x - 0.0613$
- $R^2 = 0.9961$
Appendix A.2. Solvatochromism Graphs

Solvatochromism of Dye 106a at 0.3 μM

Absorbance, a.u.

Wavelength, nm

Solvatochromism of Dye 106b at 0.3 μM

Absorbance, a.u.

Wavelength, nm

Solvatochromism of Dye 106c at 0.3 μM

Absorbance, a.u.

Wavelength, nm
Solvatochromism of Dye 108a at 0.3 μM

Absorbance, a.u.

Wavelength, nm

MeOH
DMSO
ACN
DMF

Solvatochromism of Dye 108b at 0.3 μM

Absorbance, a.u.

Wavelength, nm

MeOH
DMSO
ACN
DMF

Solvatochromism of Dye 108c at 0.3 μM

Absorbance, a.u.

Wavelength, nm

MeOH
DMSO
ACN
DMF
Solvatochromism of Dye 110a at 0.3 μM

Solvatochromism of Dye 110b at 0.3 μM

Solvatochromism of Dye 110c at 0.3 μM
Solvatochromism of Dye 112a at 0.3 μM

Solvatochromism of Dye 112b at 0.3 μM

Solvatochromism of Dye 112c at 0.3 μM
Solvatochromism of Dye 114a at 0.3 μM

Solvatochromism of Dye 114b at 0.3 μM

Solvatochromism of Dye 114c at 0.3 μM
Appendix A.3. Thermal degradation Profile of synthesized pentamethine cyanine dyes 106a-c, 108a-c, 110a-c, 112a-c, 114a-c, and 116a-c irradiated by 15 W F15T8 UV lamp for 48 h.
Appendix A.4. DNA Titration of symmetrical pentamethine cyanine dyes with 2-quinolinium moieties 106a-c.
Appendix B.1. $^1$H and $^{13}$C NMR Spectra of Salts
Appendix B.2. $^1H$ NMR and $^{13}C$ Spectra of Dyes
Appendix B.3. Mass Spectra of Dyes