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A microwave-assisted and environmentally benign approach to the synthesis of near-infrared fluorescent pentamethine cyanine dyes

Eric A. Owens [a, b, c], Nicholas Bruschi [a], Joseph G. Tawney [a], Maged Henary [a, b, c, *]

[a] Department of Chemistry, Georgia State University, 100 Piedmont Road SE, Atlanta, GA 30303, USA
[b] The Center for Diagnostics and Therapeutics, Georgia State University, 100 Piedmont Road SE, Atlanta, GA 30303, USA
[c] The Center for Biotechnology and Drug Design, Georgia State University, 100 Piedmont Road SE, Atlanta, GA 30303, USA

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ABSTRACT

A time-efficient and eco-conscious microwave methodology was developed and applied to synthesize a systematic library of pentamethine cyanine dyes and their corresponding precursors. The synthesis outlined herein drastically reduced the reaction pathway for pentamethine carbocyanine dye syntheses from days to min, as well as producing increased yields (89–98%) to the conventional heating method (18–64%). Twelve examples of pentamethine cyanine dyes were synthesized by means of microwave-assisted organic synthesis which provided excellent yield in expedited reaction time and were obtained using facile isolation methods. Furthermore, three cyanines were prepared with a novel methylene dioxy heterocyclic structure which imparted an approximately 40 nm bathochromic shift compared to unsubstituted counterparts; these results were shown to be in agreement with DFT calculations and HOMO-LUMO energy differences.

1. Introduction

Mother Nature provides our atmosphere to breathe, food required to eat and beautiful scenery to enjoy. Protecting and nurturing our environment should be a task shared by all in the research community. There are many methods for helping to preserve our precious natural resources which include the following: (1) using green solvents, (2) reducing heating times to conserve energy, (3) increasing atom efficiency by using higher yielding reaction conditions and (4) eliminating wasteful purification steps by using optimized methods that reduce side-products. These principles have been applied to the synthesis of many classes of compounds that are interesting to a broad population of the scientific community. Correspondingly, we have chosen to develop a completely benign synthetic route for the synthesis of near-infrared fluorescent compounds that have shown excellent promise in biological imaging, solar-cell technology, as chemodosimeters in sensing biologically relevant species and for non-covalent labeling of biomolecules.

Near-infrared (NIR, 640–900 nm) fluorescent chromophores have garnered considerable research interest for biomolecular labeling because of their unique red-shifted optical properties [1–4]. The majority of fluorescent sensors have shown to emit light in the visible region (400–600 nm), which forces unwanted competition with background noise that arises from inherent biomolecular auto-fluorescence. This competition disrupts the meaningful signal and can lead to extreme difficulty in signal delineation often with undesirable results [5]. To avoid the problems associated with visible-light-emitting fluorophores, molecules which absorb and emit light in the near-infrared region have been of significant interest to the scientific community. [1–3,5–7]

Specifically among the NIR emitting dyes, immense interest has been placed in the particular class of chromophores known as cyanine dyes, and they have shown extensive applications in cancer imaging, nucleic acid detection, biomolecular labeling, photographic processes, information storage and dye lasers [7–10]. Possessing relatively high molar absorption coefficients and a broad range of tunable fluorescence wavelengths (600–900 nm) cyanine dyes have been synthesized to emit light in the NIR range while maintaining biological efficacy [4,5,8–11]. Chiefly among these fluorophores, pentamethine cyanine dyes have shown significant promise for image guided surgery using NIR light [1]. Specifically, we reported several pentamethine cyanine dyes that have been

* Corresponding author. Department of Chemistry, Georgia State University, 100 Piedmont Road SE, Atlanta, GA 30303, USA. Tel.: +1 404 413 5566 (office).
E-mail address: mhenary1@gsu.edu (M. Henary).

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shown to specifically locate various tissues of clinical importance during image-guided surgery [1]. Expanding upon the synthesis of pentamethine cyanine dyes as biomolecular imaging agents has been a focus of many bioorganic research labs worldwide [12,13].

The design and synthesis of various substituted pentamethine cyanines has been achieved in a combinatorial manner by changing the *N*-alkyl substituent or functionalizing different positions of the heterocyclic backbone [2,4]. It has been observed that minor structural alterations elicits a drastic biological response which makes synthesis of highly varied chemical structures very important [1]. This provides a significant rationale for developing a fast, facile and effective method for generating a library of pentamethine cyanines with high purity levels.

Synthesis of various pentamethine cyanine dyes with slightly altered connectivity has been shown to take hours to days by the conventional oil bath heating method with lengthy, difficult column chromatography that generates environmentally polluting solvent waste [2,6,14]. Cyanine dyes are sensitive to the acidic nature of silica gel, photodegrade during purification steps and cleave in the basic solutions required for their synthesis (Fig. 1); therefore, expediting the reaction time and increasing purity levels is of highly important. Recently, the implementations of microwave chemistry has helped speed reactions and decrease the environmentally harmful waste common in organic labs; it has received interest throughout the scientific community and it is very desirable to conduct synthetic protocol under microwave irradiation.

In order to implement this technology in cyanine synthesis, we have harnessed the ability of our reaction mixture to absorb microwave energy. Electromagnetic irradiation of molecules results in rapid, volumetric heating caused by the dielectric effect which yields the final compounds without the need for column purification that may jeopardize the chemical integrity of the compounds from photodegradation or decomposition on the silica matrix [9]. The pentamethine class of cyanine dyes has not been optimized using MAOS. Utilizing a green approach, an entire synthetic pathway has been designed to prepare pentamethine cyanines with diverse chemical structures. Specifically, in the final synthetic step, using the CEM Discover LabMate several different substituted pentamethine cyanine dyes have been synthesized within 20 min in analytical purity without column purification. In comparison to the conventional heating method, the syntheses described herein provide drastically decreased reaction times and comparable or increased yields (89–98% in the final step, 80–91% overall).

2. Results and discussion

2.1. Eco-friendly synthesis of pentamethine cyanine fluorophores

The common synthetic method for the preparation of pentamethine cyanines begins with the formation of the terminal heterocyclic moieties. The four heterocycles used to afford the compounds were synthesized according to Scheme 1 and Equation (2). We optimized each step for the synthesis beginning with heterocyclic formation and begins when substituted phenylhydrazines are refluxed in glacial acetic acid with 3-methyl-2-butanone to afford **1** and **2**. This reaction affords the desired indolenine compounds in good yield, but this method requires extended periods of reaction time exceeding 24 h and excess acetic acid as solvent. Our green microwave method employs water as an environmentally conscious solvent and a catalytic (0.1 mol eq.) amount of sulfuric acid to achieve quantitative conversion in 10 min. The second synthetic step is the quaternization of the indolenine nitrogen atom. A classical method for this synthesis is mixing the two reagents in acetonitrile and refluxing until the reaction goes to completion. The volatile nature of short chain

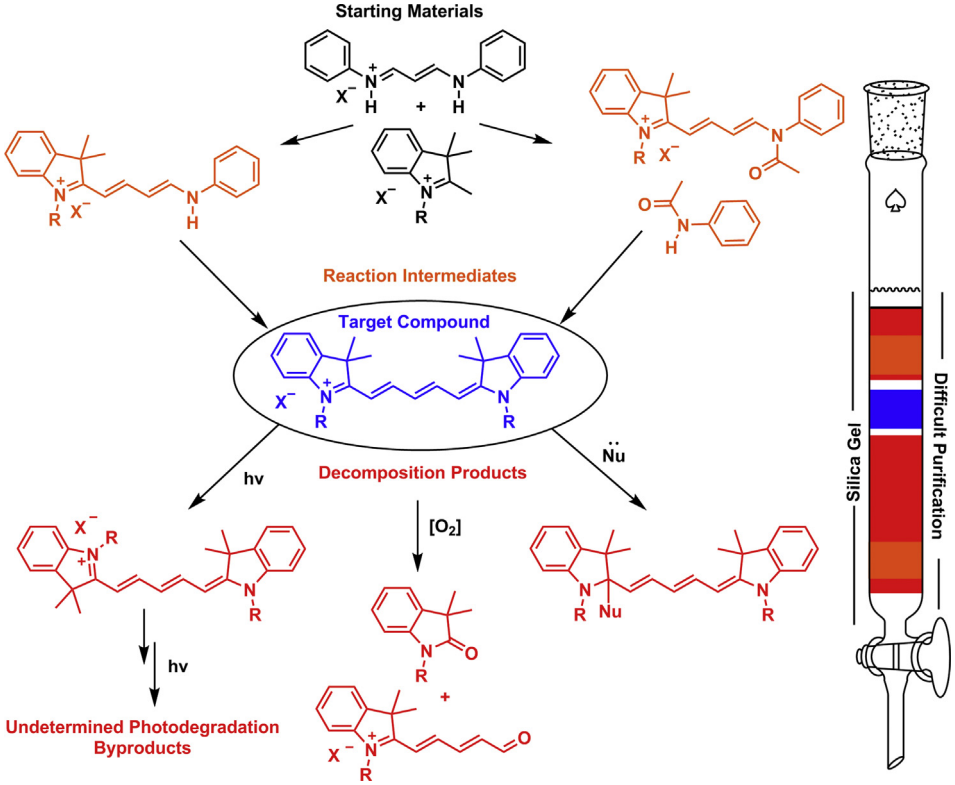
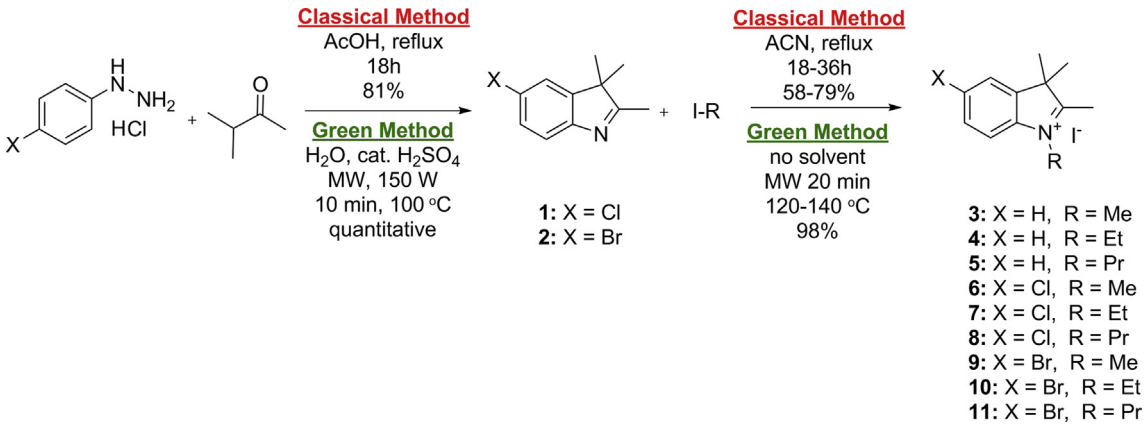


Fig. 1. The reaction intermediates and postulated degradation products that form during either the classical heating method of synthesis or purification of cyanine dyes that lead to difficulty in obtaining analytically pure compounds for biological testing.

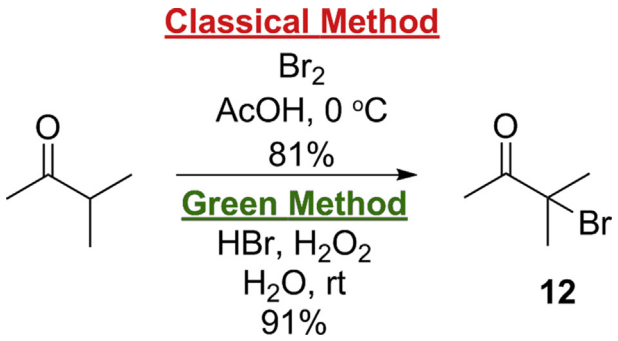


Scheme 1. The new, green microwave assisted method for preparation of alkylated salts **3**–**11** based off of the indolenine heterocyclic structure.

alkyl halides limits the reaction scope and many molar equivalents should be used to completely react with the heterocyclic starting materials. This leads to low atom efficiency and the use of an unnecessarily high quantity of alkylating agent. Our optimized alkylation is effective at a 1.2 molar equivalency of alkyl iodide (Me, iodomethane, Et, iodoethane, Pr, iodopropane) without the use of harmful solvents at a drastically reduced reaction time. After 20 min, the corresponding quaternary salts precipitate in >98% yield as a fine power and can be utilized in the next reaction without purification procedures.

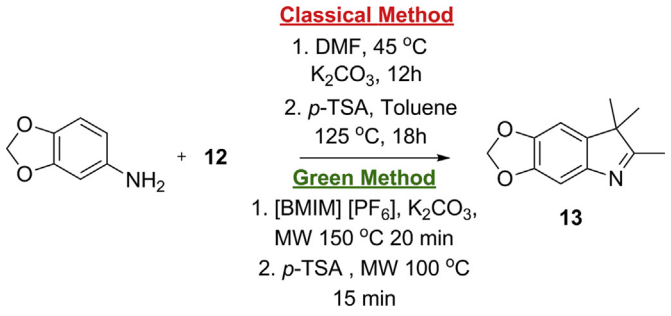
Achieving new heterocyclic building blocks for the synthesis of cyanine dyes has been limited by the number of commercially available hydrazine hydrochlorides. The synthesis of hydrazines with a number of interesting substitutions has remained scarce throughout the literature; however, utilizing a modified Bischler–Möhlau approach, we can achieve the synthesis of an acidic methylene proton with the quaternized nitrogen through a facile microwave-assisted approach (accessing the scope of this reaction using additional heterocycles are explored in another paper, Owens et al. manuscript in preparation). Our model compound was the dioxolane structure.

The initial precursor to the dioxolane heterocyclic unit, 3-bromo-3-methylbutan-2-one (shown in Equation (1)), was classically prepared beginning with bromination of 3-methyl-2-butanone under acidic conditions at 0 °C. The high volatility and dangerous nature of working with molecular bromine requires the use of an alternate and environmentally friendly route. Applying the the bromine "on water" method reported by the Iskra lab [15], we achieved selective and high yielding bromination of 3-methy-2-butanone to afford **12**.



Equation 1. The preparation the brominated ketone **12**.2

The cyclic product **13** is formed through treatment of the commercially available 3,4-(methylenedioxy)aniline with intermediate **12** in DMF with potassium carbonate followed by addition of para-toluenesulfonic acid in toluene at 125 °C (Equation (2)).

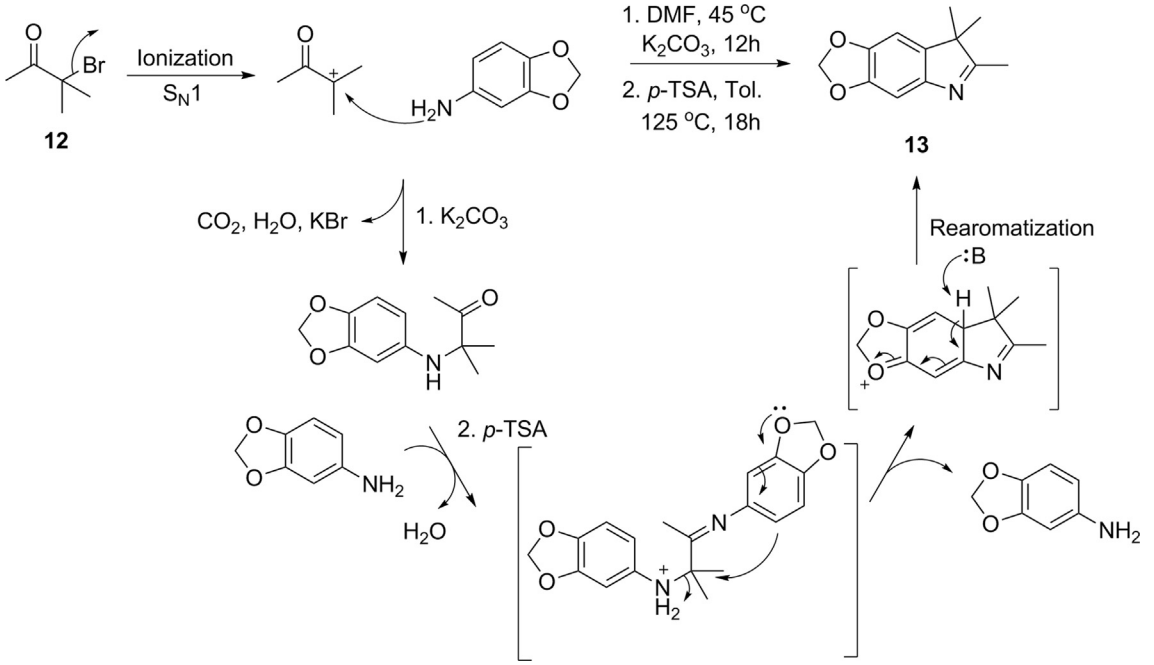


Equation 2. The preparation of the fused dioxolane heterocycle **13**.3

In order to develop a green-method for the formation of this dioxolane, we began by examining the mechanism of this reaction. From a mechanistic point-of-view, this reaction is very interesting (Scheme 2). In basic conditions, the anilino-amine is highly nucleophilic substituting the tertiary bromine alpha to the carbonyl of **12** through an S_N^1 pathway. Monoalkylation of the arylamine is successful due to high steric hindrance from the dimethyl groups. Treatment of this intermediate with *para*-toluenesulfonic acid in the presence of excess catalytic aniline yields the dehydrated arylimine intermediate that undergoes loss of aniline through electrophilic aromatic substitution and re-aromatization yields the heterocyclic precursor **13**. We postulated that this may work satisfactorily using eco-friendly conditions through microwave irradiation and successfully replaced the organic solvents with the ionic liquid [BMIM][PF6] and we maintained the use of potassium carbonate as the base. The first part of the reaction was completed using microwave irradiation (150 W) at 150 °C followed by treatment with para-toluene sulphonic acid as the catalytic activation of the carbonyl and cyclization of the ring. The alkylation of **13** was performed using the methodology previously employed in Scheme 1 and the salts **14**–**16** were obtained in moderate yield with classical reflux methods and excellent yield using MAOS (Equation (1)).

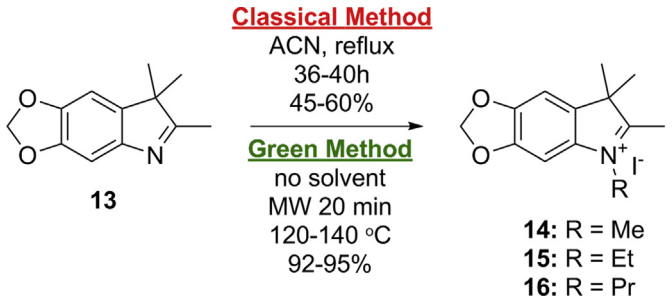
2.2. The final synthetic Step—Optimization

In the final synthetic step, shown in Scheme 3, two equivalents of individual monocationic salt were allowed to react with malonaldehyde bis(phenylimine)monohydrochloride in the presence of sodium acetate and acetic anhydride open vessel classical oil-heating causes a wide variety of decomposition and incomplete reaction products and results in a substantial net loss of product, yielding between 18% and 64%. In order to optimize the



Scheme 2. The proposed mechanism based off of an adaptation of the Bischler–Möhlau method for indole formation.

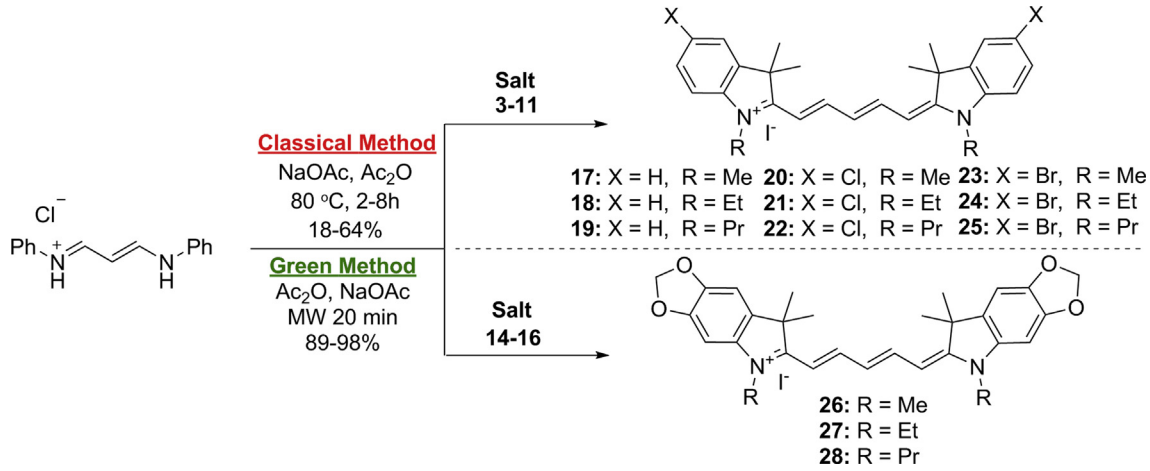
microwave-assisted reaction temperature, we first anticipated that the optimum reaction temperatures would be most heavily dependent on the length of the *N*-indolenyl substituent. We performed the synthesis of a representative fluorophore with various alkyl lengths on the indolenyl nitrogen to obtain the correct temperature to employ for all of the pentamethine cyanines Equation (3).



Equation 3. The preparation of the alkylated fused dioxolane heterocycles **14**–**16**.4

As the alkyl length increases, the insulation properties of the compound also increase. In order to determine the optimal temperature, the systematic set of dyes **17**–**19** were tested at a range of temperatures. The isolated yield for each of the reactions are shown as a function of temperature in Fig. 2 and shows a correlation as the alkyl group becomes longer, the most desirable reaction temperature also increases.

The medium length N-substituted ethyl dye **18** was chosen as a model for discussing optimal reaction temperatures. As shown in Table 1, multiple trials were conducted varying temperature by 10 °C increments starting with 90 °C while alternating reaction time for 10 and 20 min. Conducting the reaction at temperatures between 50 °C and 90 °C showed comparatively low yields. The optimum temperature was observed as a maximum and heating above this temperature resulted in undesirable byproducts and decomposition of the product, which decreased yield shown in Fig. 2 this method was applied to the synthesis of compounds **17** and **19**. Methyl substituted dye, **17** was shown to reach a maximum yield at 80 °C, ethyl **18** at 130 °C and propyl **19** at 140 °C. Continuing



Scheme 3. The microwave assisted preparation of a systematic set of pentamethine cyanines with variance in heterocyclic and *N*-alkyl groups.

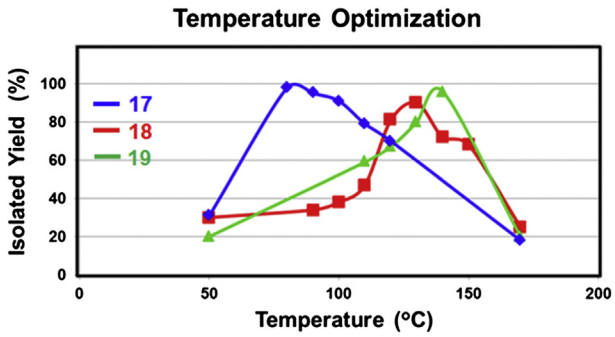


Fig. 2. Isolated yield (%) versus reaction temperature (°C) versus the three pentamethine carbocyanine dyes with varied lengths of the *N*-alkyl groups **17** (methyl, blue), **18** (ethyl, red), and **19** (propyl, green). Reactions were held at 20 min for the isolated yields appearing in this table. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the reaction time past 20 min—25 min and 30 min also resulted in reduced percentage isolated yield.

We suspected that the increasing *N*-alkyl chain length was corresponded to a decrease in overall dipole moment of the corresponding indolinium salt, causing a reduced microwave energy absorption which required an increase in thermal energy to attain a comparable reaction yield [9]. We became intrigued with this observation and performed DFT calculations at the BLYP 6-31G* level beginning with the conformer of the lowest energy. The electrostatic maps qualitatively show less polarization and the decrease in dipole moment corresponds directly to the decrease in microwave energy absorbed by the compound. In order for the molecule to react in the microwave, a higher amount of energy is required. The salt containing a methyl group has a dipole moment of 3.39 debye which is more than double the dipole moment of the salt with a propyl substituent which was calculated to be only 1.69 debye (Fig. 3). This is in agreement with the thermal energy required to form the final product with methyl and propyl; the methyl salt required only 80 °C to form the final compound in optimized yield whereas the propyl compound was optimized at 140 °C.

Table 1
Optimization data for pentamethine carbocyanine dye **18** with an ethyl group.

Trial	Temp (°C)	Reaction time (min)	Yield (%)
1	50	10	25
2	50	20	31
3	90	10	32
4	90	20	36
5	100	10	38
6	100	20	38
7	110	10	40
8	110	20	47
9	120	10	73
10	120	20	81
11	130	10	89
12	**130**	**20**	**95**
13	140	10	76
14	140	20	72
15	150	10	70
16	150	20	68
17	170	10	35
18	170	20	25
19	130	25	91
20	130	30	84

2.3. Microwave assisted organic synthesis application to a systematic library of pentamethine cyanines

After optimizing the reaction conditions for each of the three sets of pentamethine cyanines (Me, Et and Pr), we subjected the other structures with heterocyclic variation to the same reaction conditions and isolated the compounds using precipitation and facile recrystallization techniques. Notably, the reaction time, shown in Table 2, is drastically reduced in some cases more than 13-fold. Additionally, the purified yield increased, especially for compound **23** which showed an increase from 32% to 98%; correspondingly, compound **28**, which was difficult to purify and obtain an 18% overall yield using classical methods, precipitated as a nearly analytically pure solid after initial workup in 89% yield. The conventional synthetic method affords numerous byproducts which are not efficiently removed from the desired compound; however, the microwave methods developed herein have avoided the undesirable side reactions which have led to much reduced solvent waste with substantially less time and hazardous solvent waste spent on purification.

2.4. Heterocyclic variation alters the optical properties of the cyanine fluorophores

The effect of changing the *N*-alkyl chain length of cyanine dyes has been shown by our laboratory to minimally alter optical properties [2]; therefore, we will not concentrate on re-evaluating this phenomenon. However, while keeping the alkyl groups constant, differences in absorbance, emission, molar absorptivity (extinction coefficient) and relative quantum yield were observed for dyes containing heterocyclic variations, including chlorine, bromine and methylene dioxy groups (Table 3). The effect of chlorine **20** and bromine **23** was almost negligible in methanol along with except for an increased Stoke's Shift from 22 nm to 32/34 nm (Table 3). The addition of the methylene dioxy group **26** was shown to red shift the dye 35 nm (Fig. 4), where the Stokes' Shift was observed to increase to 43 nm. Extinction coefficients for all dyes were shown to be favorably large, (85,100 to 222,800 M^{-1} cm^{-1}) while quantum yields ranged from 30.5 to 9.5% (Table 3). The quantum yield of the dioxy compound **26** is lower due to the possibility for non-radiative energy decay associated with the non-aromatic substituents on the heterocyclic ring.

In order to confirm the observed red-shift of the methylenedioxy substituted compounds, and knowing the *N*-alkyl substitution does not significantly alter the optical properties of the chromophore, we performed density functional theory calculations on the synthesized compounds **17** and **26** to look at the HOMO and LUMO levels to determine energetic differences between the compounds. The calculations were performed at the BLYP 6-31G* level starting from the most energetically stable state. The DFT calculations reveal a larger energy gap for compound **17** (E_{gap} (eV) = 1.41 eV) predicting a blue-shifted absorption spectrum compared to the small energy barrier for compound **26** (E_{gap} (eV) = 1.11 eV) which displays a 41 nm bathochromic shift. These results corroborate the observed bathochromic shift of compound **26** and we can use similar computational methods for predicting a red or blue shift before synthesizing additional compounds (Fig. 5).

3. Conclusions

The complete synthetic route for various substituted pentamethine carbocyanine dyes was completed using microwave-based green eco-conscious methods including the preparation of a novel methylene dioxy heterocyclic structure. Optimal reaction conditions were found at a constant reaction time of 20 min for all dyes

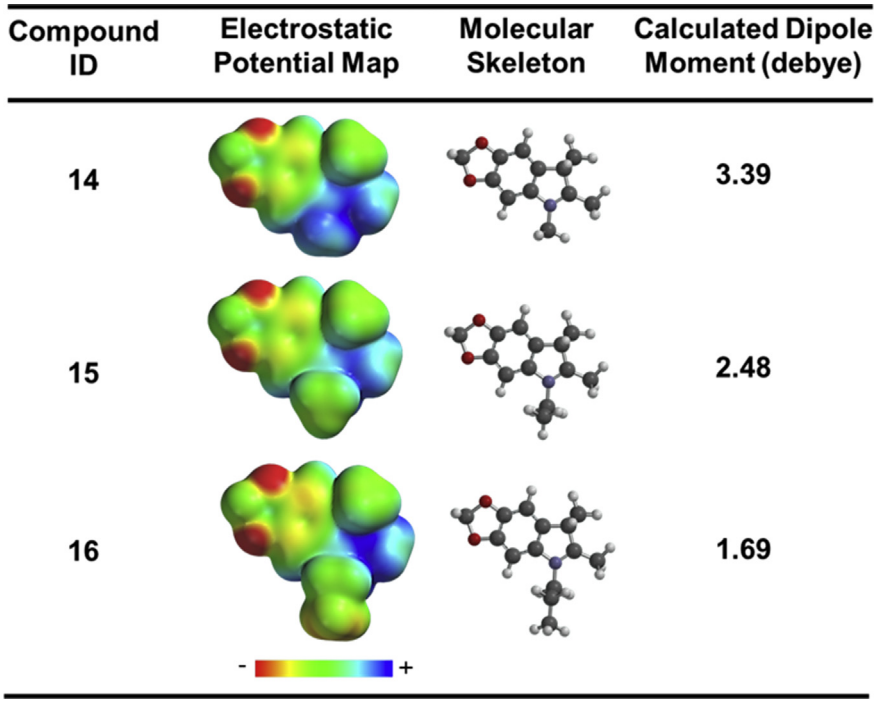


Fig. 3. DFT calculation results showing a decrease in the dipole moment as alkyl length increases.

Table 2
Microwave synthesis versus conventional synthesis.

Dye structure	M.W. time (min)	Conventional time (min)	Microwave yield[a]	Conventional yield[a]
17	20	120	98%	64%
18	20	180	95%	62%
19	20	220	96%	61%
20	20	190	95%	36%
21	20	180	96%	58%
22[b]	20	250	94%	64%
23	20	240	98%	32%
24	20	270	98%	60%
25[b]	20	340	96%	55%
26[b]	20	480	90%	27%
27[b]	20	480	92%	21%
28[b]	20	480	89%	18%

[a] The percentage value refers to the purified yield.
[b] These compounds are novel.

with increasing alkyl lengths resulting in increased optimal reaction temperatures. The benefits associated with our methodology include decreased reaction time with significantly improved yield and outstanding purity (based on ^{1}H NMR spectra) without the need for lengthy purification methods. Additionally, the red-shifted compounds dioxymethylene compounds were shown, through DFT calculations, to have small HOMO-LUMO energy differences resulting in the red-shifted optical profile. As NIR fluorophores continue to garner increasing interest in the scientific community, the optimized synthetic protocol will help to increase efficiency while simultaneously reducing the environmental impact.

Table 3
The optical properties of propyl substituted dyes **17**, **20**, **23** and **26** with respect to different substituents on the heterocyclic backbone. The data presented was obtained in methanol from 1 mM stock solutions made in DMSO.

Dye	λ_{abs} (nm)	λ_{em}(nm)	ε(M^{-1} cm^{-1})	Φ (%)
17	644	666	212, 500	30.5
20	640	674	222, 800	29.4
23	640	672	210, 100	16.9
26	679	722	85, 100	9.5

4. Experimental

4.1. Synthesis

The chemical reagents used in the synthesis of these compounds were obtained from Acros Organics (Geel, Belgium), Alfa Aesar and Matrix Scientific. Microwave irradiation was completed using a Discover LabMate apparatus interfaced to a PC using the provided Synergy software to monitor reaction temperature, pressure, wattage and simultaneous cooling. Scale-up reactions of indolenine salts were performed using the open-vessel accessories and the

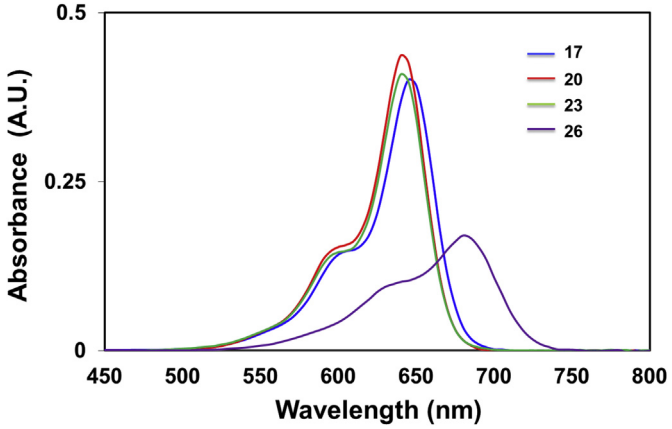


Fig. 4. Near infrared (NIR) absorbance of fluorohores in methanol at a 2.0 μM concentration.

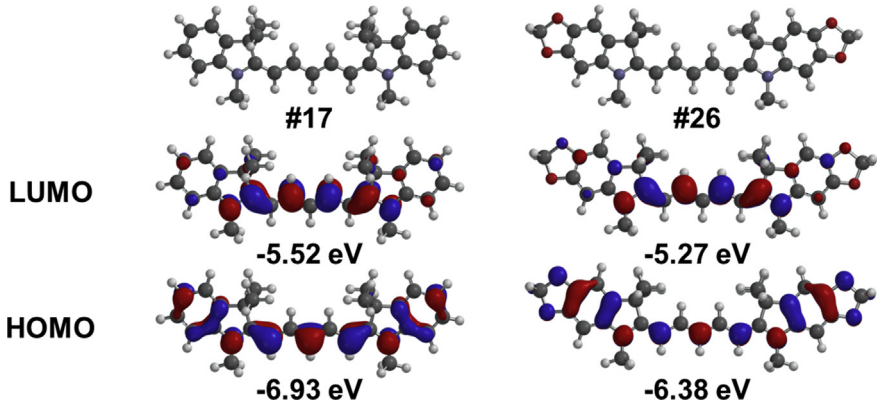


Fig. 5. *In silico* DFT calculations depicting the HOMO and LUMO for each compound **17** and **26** with corresponding energy levels (eV) shown below the structures.

same protocol as detailed in the experimental with similar yield. All classical method reactions were kept under a positive pressure of nitrogen during the entirety of the reaction. Microwave-assisted reaction vessels were purged with nitrogen before the reaction was initially subjected to irradiation. The reactions were followed using silica gel 60 F_{254} thin layer chromatography plates (Merck EMD Millipore, Darmstadt, Germany) with 5% methanol in DCM as the mobile phase. Open column chromatography was utilized for the purification of the final compounds needing additional purification using 60–200 u, 60 A classic column silica gel (Dynamic Adsorbents, Norcross, GA). The ^{1}H NMR and ^{13}C NMR spectra were obtained using high quality Kontes NMR tubes (Kimble Chase, Vineland, NJ) rated to 500 MHz and were recorded on a 400 MHz Bruker Avance (at either 400 MHz or 100 MHz for ^{1}H NMR and ^{13}C NMR, respectively) spectrometer using DMSO-d_6 or $CDCl_3$ containing tetramethylsilane (TMS) as an internal calibration standard. High-resolution accurate mass spectra (HRMS) were obtained either at the Georgia State University Mass Spectrometry Facility using a Waters Q-TOF micro (ESI-Q-TOF) mass spectrometer or utilizing a Waters Micromass LCT TOF ES + Premier Mass Spectrometer. Liquid chromatography utilized a Waters 2487 single wavelength absorption detector with wavelengths set between 640 and 700 nm depending on the dye's photophysical properties. The column used in LC was a Waters Delta-Pak 5 μm 100 Å 3.9 × 150 mm reversed phase C18 column. Evaporative light scattering detection analyzes trace impurities that cannot be observed by alternate methods; a SEDEX 75 ELSD (Olivet, France) was utilized in tandem with liquid chromatography to confirm purity.

2,3,3-trimethylindolenine was commercially obtained and used in the alkylation reaction without purification.

4.2. Classical synthesis of indolenine compounds 1 and 2

Substituted phenylhydrazine hydrochloride heterocyclic starting materials (1 g) were added to an oven dried and nitrogen flushed round bottom flask. Acetic acid (15 mL) was added to the flask and the reaction mixture was heated using an oil bath to reflux. Ketone, 3-methyl-2-butanone (1.5 mol eq.), was added to the reaction mixture. The mixture was allowed to heat for 12–48 h until TLC indicated a consumption of starting materials. After the reaction was completed, the acetic acid and excess ketone was removed under high vacuum rotary evaporation at elevated temperature. The resulting reddish oil was dissolved in dichloromethane and a saturated aqueous solution of sodium bicarbonate was added carefully to the organic layer. The organic layer was washed with sodium bicarbonate (3 × 10 mL), the organic layer was extracted, dried over magnesium sulfate and concentrated to afford the final compounds in 76% and 81% yield, respectively.

4.3. Microwave synthesis of indolenine compounds 1 and 2

Heterocyclic phenylhydrazine hydrochloride (1 g) was added to a 10-mL microwave vessel equipped with a micro magnetic stir bar along with 3-methyl-2-butanone (1.2 mol eq.). Deionized water (3 mL) and sulfuric acid (0.1 mol eq.) was added to the reaction mixture. The reaction vessel was securely capped and then subjected to microwave irradiation for 10 min at 100 °C allowing for the complete transformation of the starting material. The water was decanted off and a saturated solution of sodium bicarbonate (3 mL) was added. The flask was sonicated for 10 min to quench the reactive acid. The liquid was poured off and the resulting oil was dissolved in minimum dichloromethane dried over sodium sulfate for 30 min, gravity filtered and concentrated to yield the corresponding products in quantitative yield.

4.4. Classical synthesis of indolenine salts 3–11

Individual indolenine derivative **1**–**3** was added to a 50 mL round bottom flask with a magnetic stir bar along with appropriate alkyl iodide (3 mol eq) and acetonitrile (10 mL). The low boiling point of the alkyl iodide limits the reaction temperature; also, some of the alkylating agent will evaporate during the reactions, higher equivalents are needed in the classical methods. The reaction mixtures were heated to reflux for 48–72 h. After TLC indicated a consumption of the starting materials, the reaction was allowed to cool and the mixture was concentrated under reduced pressure. Diethyl ether (25 mL) was added to the flask resulting in an oily residue. Acetone (5 mL) was added to the residue and the reaction mixture was sonicated for 10 min resulting in a free flowing solid which was filtered, washed with diethyl ether and hexanes and dried under vacuum. The compounds were obtained in 58–79% yield.

4.5. Microwave synthesis of indolenine salts 3–11

Individual indolenine derivative, 2,3,3-trimethlyindolenine, **1**, or **2** was added to a 10-mL microwave vessel equipped with a micro magnetic stir bar along with appropriate alkyl iodide (1.2 mol eq.). The reaction vessel was securely capped and then subjected to microwave irradiation for 20 min allowing for the complete

transformation of the heterocyclic starting material to the corresponding salt. The resulting residue was filtered, dried overnight on vacuum and the corresponding salts were obtained in 98% + yield in excellent purity as determined by NMR (^{1}H and ^{13}C) spectroscopy.

Salts **3**, **4**, **6**, **7**, **9** and **10** are previously published compounds and the data agrees with the literature values. [16]

4.5.1. 2,3,3-Trimethyl-1-propyl-3H-indol-1-ium iodide (*5*)

^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.00 (t, J = 7.20 Hz, 3H), 1.56 (s, 6H) 1.80–1.98 (m, 2H), 2.88 (s, 3H), 4.47 (t, J = 7.45 Hz, 2H) 7.63 (d, J = 5.05, J = 3.28 Hz, 2H), 7.82–7.92 (m, 1H), 7.96–8.07 (m, 1H), ^{13}C NMR (100 MHz, DMSO-d_6) **δ**: 10.76, 14.22, 20.78, 22.06, 48.83, 54.14, 115.53, 123.51, 128.90, 129.37, 141.06, 141.82, 196.54.

4.5.2. 5-Chloro-2,3,3-trimethyl-1-propyl-3H-indol-1-ium iodide (*8*)

^{1}H NMR (400 MHz, $CDCl_3$) **δ**: 1.05 (t, J = 8.00 Hz, 3H) 1.65 (s, 6H) 1.93–2.10 (m, 2H) 3.09 (s, 3H) 4.62 (t, J = 8.0 Hz, 2H) 7.48–7.60 (m, 2H) 7.79 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) **δ**: 11.22, 14.77, 21.22, 22.35, 49.52, 54.92, 118.01, 123.29, 127.41, 132.32, 140.94, 144.64, 197.61.

4.5.3. 5-Bromo-2,3,3-trimethyl-1-propyl-3H-indol-1-ium iodide (*11*)

^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 0.99 (t, J = 7.20 Hz, 3H) 1.57 (s, 6H) 1.86 (d, J = 7.33 Hz, 2H) 2.81–2.91 (m, 3H) 4.37–4.55 (m, 2H) 7.86 (s, 1H) 8.00 (d, J = 8.59 Hz, 1H) 8.21 (s, 1H), ^{13}C NMR (100 MHz, DMSO-d_6) **δ**: 11.24, 14.86, 21.24, 22.17, 22.36, 49.56, 54.92, 118.03, 123.29, 127.41, 132.32, 140.94, 144.64, 182.07, 188.62, 197.60.

4.6. Classical bromination

3-bromo-3-methyl-2-butanone (**12**): A mixture of 3-methylbutan-2-one (25.00 mL, 233.65 mmol) and acetic acid (38.00 mL) was added to a three-neck oven-dried round bottom flask and maintained at a temperature of 5 °C. Next, both molecular bromine (1.2 mL, 23.4 mmol) and acetic acid (5.0 mL) was combined in an addition funnel and added dropwise to the round bottom flask. When all of the bromine was added the reaction mixture was allowed to stir overnight at room temperature. To the resulting mixture, water (10 mL) was added, and then transferred to a separatory funnel where another 10 mL of water was added and the organic layer was extracted with diethyl ether (3 × 15 mL). The compound was then washed with cold, saturated sodium bicarbonate (4 × 10 mL), dried over anhydrous sodium sulfate, and the reaction mixture was concentrated *in vacuo* to leave yellow oil. The crude product was further purified via vacuum distillation (52 °C, ~25 mmHg) to yield a colorless oil, 3.14 g (81%). ^{1}H NMR (400 MHz, CDCl3) **δ**: 1.824 (s, 6H), 2.401 (s, 3H).

4.7. Eco-friendly bromination

4.7.1. 3-bromo-3-methyl-2-butanone (*12*)

3-methylbutan-2-one (20.1 mmol) was added to water (10 mL) a round bottom flask at room temperature. The round bottom flask was enveloped in aluminum foil to prevent light-induced interactions. A 48% aqueous solution of HBr (30.2 mmol) was added to the stirring mixture. After 10 min of stirring at room temperature, a solution of H_2O_2 (30% in water, 40.2 mmol) was added to the reaction mixture. The clear solution was allowed to stir for 18 h. After full consumption of the starting materials, the reaction was quenched by adding excess saturated sodium bicarbonate solution and the final brominated compound was extracted in diethyl ether. After concentration under reduced pressure, the resulting oil was vacuum distilled (52 °C, ~25 mmHg) to afford the final compound in 91% yield.

4.8. Classical cyclization

4.8.1. 6,7,7-trimethyl-7H-[1,3]dioxolo[4,5-f]indole (*13*)

A solution of DMF, (8 mL), aniline (1.56 g, 11.38 mmol), and potassium carbonate (1.05 g, 6.8 mmol) was brought to 45 °C in a two-neck round bottom flask with small magnetic stir-bar. 3-Bromo-3-methylbutan-2-one (**12**, 1.86 g, 1 mL) was added to the solution overnight with a syringe pump (Kd Scientific, Model 100). After addition was completed, solution was stirred for 24 h at 45 °C and monitored by TLC. After TLC showed that the starting material was consumed, dimethylformamide was evaporated *in vacuo*, and concentrate was extracted with toluene (5 × 20 mL) and was washed with deionized water. Toluene was concentrated to an amount of 10–20 mL, and *p*-methyltoluenesulfonic acid (0.110 g, 0.1 mol eq.) was added to solution. Solution was allowed to reflux for 24 h, until TLC showed an absence of starting material. Toluene was evaporated *in vacuo*, and dichloromethane (20 mL) was added to the reaction mixture. Solution was washed with a saturated solution of sodium carbonate (5 × 50 mL) until the organic layer was a red/brown color. DCM was removed under reduced pressure and the resulting red/brown oil was recovered and the crude **13** (obtained in 57% yield) was used without purification.

4.9. Microwave assisted cyclization

4.9.1. 6,7,7-trimethyl-7H-[1,3]dioxolo[4,5-f]indole (*13*)

A solution of [BMIM][PF6] (4 mL), potassium carbonate (1.2 mol eq.) and aniline (1.2 mol eq.) was added to a microwave vessel (10 mL). 3-Bromo-3-methylbutan-2-one (1.0 mol eq.) was added to the microwave vessel. The contents were subjected to microwave irradiation at 150 W, 150 °C for 20 min. Approximately 90% of the reactive aniline (100% of the butanone was consumed) was allowed to go to completion leaving an additional amount to participate in the imine formation seen in the mechanism. Para-toluene sulphonic acid was added to the vessel (0.2 mol eq.) and the reaction was heated to 100 °C for 20 min. The brown solution was dissolved in DCM (10 mL) and washed with saturated solution of sodium bicarbonate (3 × 15 mL). The organic layer was dried over magnesium sulfate and was evaporated under reduced pressure to afford the final heterocyclic dioxolane. The compound was obtained in 92% yield.

4.10. Classical alkylation

The classical alkylation of *6,7,7-trimethyl-7H-[1,3]dioxolo[4,5-f] indole* was achieved using identical procedures found above and the respective yields are found below.

4.11. Microwave assisted alkylation

Dioxyindolenine was added to a 10-mL microwave vessel equipped with a micro magnetic stir bar along with appropriate alkyl iodide (1.2 mol eq.). The reaction vessel was securely capped and then subjected to microwave irradiation for 20 min allowing for the complete transformation of the heterocyclic starting material to the corresponding salt. The resulting residue was filtered, dried overnight on vacuum and the corresponding salts were obtained in 98% yield in excellent purity as determined by NMR (^{1}H and ^{13}C) spectroscopy.

*4.11.1. 5,6,7,7-Tetramethyl-7H-[1,3]dioxolo[4,5-f]indol-5-ium iodide (**14**)*

Classical Yield 60%, Microwave Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.48 (s, 6H) 2.70 (br. s., 3H) 3.90 (s, 3H) 6.19 (s, 2H) 7.48 (s, 1H) 7.64 (s, 1H), ^{13}C NMR (100 MHz, DMSO-d_6) **δ**: 15.43, 21.48, 23.05, 53.61, 55.34, 101.44, 103.34, 125.76, 128.64, 129.34, 139.90, 145.50, 146.91, 147.91, 187.11.

*4.11.2. 5-Ethyl-6,7,7-trimethyl-7H-[1,3]dioxolo[4,5-f]indol-5-ium (**15**)*

Classical Yield 53%, Microwave Yield 94%. ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 1.48 (s, 6H) 2.70 (br. s., 3H) 3.90 (s, 3H) 6.19 (s, 2H) 7.48 (s, 1H) 7.64 (s, 1H), ^{13}C NMR (100 MHz, DMSO-d_6) **δ**: 13.28, 14.33, 22.37, 43.71, 54.33, 97.77, 103.21, 104.52, 134.88, 137.31, 148.54, 149.29, 194.54.

*4.11.3. 6,7,7-Trimethyl-5-propyl-7H-[1,3]dioxolo[4,5-f]indol-5-ium (**16**)*

Classical Yield 45%, Microwave Yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) **δ**: 0.85–1.06 (m, 3H) 2.79 (s, 6H) 4.38 (br. s., 1H) 6.20 (s, 4H) 7.51 (s, 1H) 7.75 (s, 1H), ^{13}C NMR (100 MHz, DMSO-d_6) **δ**: 11.14, 14.50, 21.35, 22.55, 49.39, 54.36, 97.65, 103.23, 104.44, 135.27, 137.24, 148.55, 149.34, 195.03.

4.12. General classical synthesis for NIR dyes 17–25 and 26–28

A mixture of indolium salt (40 mg, 0.154 mmol), bis-iminium salt (20 mg, 0.077 mmol), NaOAc (17 mg, 0.23 mmol) and acetic anhydride (1 mL) was added to an oven dried round bottom flask with stirring bar. The reaction mixture was heated using a standard oil bath for a particular reaction time (refer to Table 1). After starting materials were consumed, the reaction was allowed to cool to room temperature. Diethyl ether was added to the round bottom flask resulting in an oily metallic blue residue. The diethyl ether was decanted and the oil was dissolved in a minimal amount of methanol followed by the addition of diethyl ether (50 mL) resulting in the formation of light blue crystals, which were filtered. The crystals were dissolved in dichloromethane leaving unreacted sodium acetate on the funnel. The dichloromethane was removed *in vacuo*. Many compounds needed additional purification methods. Silica-gel column chromatography eluting with 2–5% methanol in dichloromethane afforded the various pentamethine cyanines in their respective yield.

4.13. General microwave assisted synthesis for NIR dyes

A mixture of indolium salt (40 mg, 0.154 mmol), bis-iminium salt (20 mg, 0.077 mmol), NaOAc (17 mg, 0.23 mmol) and acetic anhydride (1 mL) was placed in sealed microwave vessel with stirring bar. Sealed vessel was placed in single-mode microwave (CEM Discover) on standard power setting, at indicated temperature, see Table 1 for 20 min resulting in increased pressure (40–100 psi). Reaction mixture was diluted with diethyl ether (10–20 mL) and filtered *in vacuo*. Solid was washed twice with diethyl ether (5 mL). A clean filter flask was attached to the funnel and the resulting solid was dissolved with dichloromethane (10–15 mL) leaving unreacted sodium acetate crystals on the filter funnel. The filtrate was transferred to a clean round bottom flask and dichloromethane was removed with a rotary evaporator. Metallic blue/green crystals were formed after solvent removal resulting in the yield of individual compounds reported in Table 2. Compounds **17**–**21, 23** and **24** have been previously reported by conventional method [16].

*4.13.1. 1,3,3-Trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium iodide (**17**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.71 (s, 12H), 3.63 (s, 6H), 6.28 (d, J = 16.0 Hz, 2H), 6.65 (t, J = 12.0 Hz, 1H), 7.24 (t, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 7.39 (t, J = 8.0 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 8.25 (t, J = 12.0 Hz, 2H).

*4.13.2. 1-Ethyl-3,3-trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium iodide (**18**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.27 (t, J = 4.0, 6H), 1.68 (s, 12H), 4.15 (d, J = 8.0, 4H), 6.57 (t, J = 12.0, 1H), 7.26 (t, J = 4.0, 2H), 7.39 (s, 4H), 7.63 (d, J = 8.0, 2H), 8.34 (t, J = 12.0, 2H).

*4.13.3. 1-Propyl-3,3-dimethyl-2-((1E,3E,5E)-5-(1-propyl,3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium iodide (**19**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 0.95 (t, J = 4.0 Hz, 6H), 1.69 (s, 12H), 4.08 (m, J = 4.0 Hz, 4H), 6.35 (d, J = 16.0 Hz, 2H), 6.60 (t, J = 12.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 2H), 7.42 (s, 4H), 7.64 (d, J = 8.0 Hz, 2H), 8.34 (t, J = 12.0 Hz, 2H). HR-MS calculated for $[C_{31}H_{39}N_2]^+$ 439.3108 found 439.3093.

*4.13.4. 2-((1E,3Z,5E)-3-Chloro-5-(1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (**20**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.69, (s, 12H), 3.58 (s, 6H), 6.29 (d, J = 12.0 Hz, 2H), 6.55 (t, J = 12.0 Hz, 1H), 7.39 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.81 (s, 2H), 8.33 (t, J = 12.0 Hz, 2H).

*4.13.5. 2-((1E,3Z,5E)-3-Chloro-5-(1-ethyl-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-ethyl-3,3-dimethyl-3H-indol-1-ium iodide (**21**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.25 (s, 6H), 1.69 (s, 12H), 4.13 (s, 4H), 6.33 (d, J = 12.0 Hz, 2H), 6.58 (t, J = 12.0 Hz, 1H), 7.46 (m, J = 12.0 Hz, 2H), 7.89 (s, 2H), 8.35 (t, J = 12.0 Hz, 2H).

*4.13.6. 2-((1E,3Z,5E)-3-Chloro-5-(1-propyl-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-propyl-3,3-dimethyl-3H-indol-1-ium iodide (**22**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 0.94 (t, J = 8.0 Hz, 6H), 1.70 (s, 16H), 4.07 (t, J = 8.0 Hz, 4H), 6.35 (d, J = 12.0 Hz, 2H), 6.65 (t, J = 12.0 Hz, 1H), 7.45 (s, 4H), 7.83 (s, 2H), 8.35 (t, J = 12.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) **δ:** 0.57, 11.39, 20.80, 27.43, 45.33, 49.57, 104.08, 113.08, 123.50, 128.75, 129.48, 141.53, 143.65, 154.93, 173.20. HR-MS calculated for $[C_{31}H_{37}N_2Cl_2]^+$ 507.2328 found 507.2315.

*4.13.7. 2-((1E,3Z,5E)-3-Bromo-5-(1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (**23**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.91 (s, 12H), 3.82 (s, 6H), 6.36 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H, 7.26–7.31 (m, 4H), 7.39–7.44 (m, 4H), 8.93 (d, J = 12.0 Hz, 2H).

*4.13.8. 2-((1E,3Z,5E)-3-Bromo-5-(1-ethyl-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-ethyl-3,3-dimethyl-3H-indol-1-ium iodide (**24**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.25 (m, J = 4.0 Hz, 6H), 1.69 (s, 12H), 4.12 (m, J = 8.0 Hz, 4H), 6.30 (d, J = 16.0 Hz, 2H), 6.58 (t, J = 8.0 Hz, 1H), 7.37 (t, J = 8.0 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.94 (s, 2H), 8.35 (d, J = 12.0 Hz, 2H).

*4.13.9. 2-((1E,3Z,5E)-3-Bromo-5-(1-propyl-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-propyl-3,3-dimethyl-3H-indol-1-ium iodide (**25**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 0.94 (t, J = 4.0 Hz, 6H), 1.70 (m, J = 12.0 Hz, 16H), 4.06 (t, J = 4.0 Hz, 4H), 6.35 (d, J = 12.0 Hz, 2H),

6.65 (t, J = 12.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.95 (s, 2H), 8.35, (t, J = 12.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) **δ:** 11.39, 20.79, 27.43, 45.29, 49.56, 104.09, 113.53, 117.49, 126.26, 126.60, 131.59, 141.95, 143.97, 154.97, 173.06. HR-MS calculated for $[C_{31}H_{37}N_2Br_2]^+$ 595.1318 found 595.1311.

*4.13.10. 5,7,7-Trimethyl-6-((1E,3E,5Z)-5-(5,7,7-trimethyl-5H-[1,3]dioxolo[4,5-f]indol-6(7H)-ylidene)penta-1,3-dien-1-yl)-7H-[1,3]dioxolo[4,5-f]indol-5-ium iodide (**26**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.63 (s, 12H), 3.54 (s, 6H), 6.06 (s, 4H), 6.18 (d, J = 16.0 Hz, 2H), 6.46 (t, J = 16.0 Hz, 1H), 7.13 (s, 2H), 7.28 (s, 2H), 8.16 (t, J = 15 Hz, 2H). HR-MS calculated for $[C_{29}H_{31}O_4N_2]^+$ 471.2278 found 471.2268.

*4.13.11. 5-Ethyl-7,7-dimethyl-6-((1E,3E,5Z)-5-(5-ethyl-7,7-dimethyl-5H-[1,3]dioxolo[4,5-f]indol-6(7H)-ylidene)penta-1,3-dien-1-yl)-7H-[1,3]dioxolo[4,5-f]indol-5-ium iodide (**27**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 1.24 (t, J = 4.0 Hz, 6H), 1.63 (s, 12H), 4.08 (m, J = 4.0 Hz, 4H), 6.07 (s, 4H), 6.22 (d, J = 16.0 Hz, 2H), 6.48 (t, J = 12.0 Hz, 1H), 7.18 (s, 2H), 7.32 (s, 2H), 8.20 (t, J = 12.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) **δ** 12.68, 27.55, 49.34, 94.24, 102.17, 102.95, 104.30, 135.15, 136.19, 145.50, 148.05, 152.99, 172.22. HR-MS calculated for $[C_{31}H_{35}O_4N_2]^+$ 499.2591 found 499.2577.

*4.13.12. 5-Propyl-7,7-dimethyl-6-((1E,3E,5Z)-5-(5-propyl-7,7-dimethyl-5H-[1,3]dioxolo[4,5-f]indol-6(7H)-ylidene)penta-1,3-dien-1-yl)-7H-[1,3]dioxolo[4,5-f]indol-5-ium iodide (**28**)*

^{1}H NMR (400 MHz, DMSO-d_6) **δ** 0.93 (t, J = 8.0 Hz, 6H), 1.63 (m, 16H), 4.01 (s, 4H), 6.07 (s, 4H), 6.24 (d, J = 16.0 Hz, 2H), 6.50 (t, J = 12.0 Hz, 1H), 7.21 (s, 2H), 7.32 (s, 2H), 8.20 (t, J = 16.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) **δ:** 11.35, 20.89, 27.69, 49.06, 49.32, 94.49, 102.19, 103.42, 104.20, 135.01, 136.77, 145.50, 148.04, 152.81, 172.78. HR-MS calculated for $[C_{33}H_{39}O_4N_2]^+$ 527.2904 found 527.2883.

4.14. Computational experimental

Spartan Computational Modeling Software was utilized to draw the chemical structures and calculations were performed using Density Functional Theory at the B3LYP level. Chemical structures were minimized to the conformer of the lowest energy and calculations were performed using appropriately applied charges in a vacuum.

4.15. Analytical instrumentation

Absorbance spectra were measured using a Varian Cary 50 UV–Visible Spectrophotometer interfaced to a PC, with a spectral bandwidth of 2 nm. Fluorescence spectra for the pentamethine cyanine dyes were obtained using a Shimadzu RF-1501 Spectrofluorophotometer (Shimadzu Scientific Instruments, Colombia, MD) interfaced to a PC, with spectral bandwidths for both excitation and emission set to 10 nm and the sensitivity set to “high”. Disposable absorbance and fluorescence cuvettes were used with a pathlength of 1.00 cm. All calculations were performed on Microsoft Excel 2010, (Microsoft Corporation, Redmond, WA).

4.16. Stock solutions

Stock solutions of the dyes in 1 mM concentration were prepared by weighing the dye on a 5-digit analytical balance directly into a brown glass vial and adding DMSO, (98.5% Spectroscopy grade, Sigma Aldrich) with an auto pipette (100–1000 uL, Eppendorf). The stock solutions were vortexed for 30 s, and subsequently sonicated for 20 min to ensure complete dissolution.

4.17. Method of determining molar absorptivity

Stock solutions were used to prepare six to seven samples in methanol with various concentrations such that absorbance values remained less than 1. Samples were prepared in 5.00 mL of DMSO having used 5–50 uL, 10–100 uL, and 100–1000 uL Micropipettes, (Eppendorf). Samples were prepared in disposable glass test tubes, (10 mL, Fischer Scientific) and vortexed for 20 s to ensure dissolution. Appropriate measurements were chosen to ensure the absorbance values were less than 1.0 but greater than 0.1. Five measurements were taken and the recorded absorbance values versus concentration were plotted and the linear regression was determined to obtain the molar absorptivity according to Beer's law.

4.18. Method of determining quantum yield

Rhodamine 800, (99.9% Fluorescence grade, Sigma Aldrich) was used as a standard within the given range of absorbance maxima, (648–682 nm). Samples of the dyes and their respective standards were prepared from stock solutions such that their absorbance maxima were less than 0.1. The absorbance and fluorescence spectra of each sample were obtained concurrently to minimize experimental error from photobleaching. Each dye was diluted accordingly, to ensure fluorescence was below 1000 units.

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