Photochemical & Photobiological Sciences

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c8pp00218e

Introduction of various substitutions to the methine bridge of heptamethine cyanine dyes *Via* substituted dianil linkers†

Andrew Levitz, Fahad Marmarchi and Maged Henary *

Received 29th May 2018,
Accepted 10th September 2018

DOI: 10.1039/c8pp00218e

rsc.li/pps

The unique optical properties of cyanine dyes have prompted their use in numerous applications. Heptamethine cyanines are commonly modified on the methine bridge after synthesis of a *meso*-chlorine containing cyanine. Herein, a series of heptamethine cyanines containing modified methine bridges were synthesized using substituted dianil linkers. Their optical properties including, molar absorptivity, fluorescence, and quantum yield were measured as well as their hydrophobic effects in polar buffer solution. It was shown that dyes containing cyclopentene in the methine bridge or a phenyl ring in the *meso* position display increased molar absorptivity while the increased flexibility of the dye containing a cycloheptene in the methine bridge prevented fluorescence.

Introduction

Over the last decade, research interest in cyanine dyes has heightened due to their use in a wide range of applications spanning analytical, biological and biomedical research fields.[1] This broad class of dyes are distinguished from other dyes in that they possess two nitrogen containing heterocycles connected by a conjugated methine bridge.[2–4] The heterocycles act as both electron donors and acceptors creating an electron deficient system throughout the molecule that gives cyanines a wide range of absorption and fluorescence from the visible to infrared regions. They are characterized as having narrow absorption bands and high extinction coefficients.[5] These unique properties along with the excellent safety profile of indocyanine green (ICG) in humans[6] and the ease of which cyanines can be modified have allowed the dyes to be used in numerous applications.[7,8] Specifically, near-infrared (NIR) absorbing and fluorescing cyanine dyes have gained attention in biomedical imaging[9–12] for the low background signal in this spectral region and in dye-sensitized solar cells (DSSCs) to allow for use of the red/near-IR part of the spectrum of sunlight.[13,14]

Heptamethine cyanine dyes have most commonly been modified with substituents on the heterocycles. Cyclohexene rings are often introduced into a methine bridge to increase rigidity and make the dyes more stable,[15] but there is a lack of literature dealing with modification at the 5 position of this cyclohexene ring. This position is not conjugated into the dye scaffold and therefore allows for modification of the dye structure with little effect on the dyes optical profile. Nucleophilic substitution of the chlorine atom at the *meso*-position of the methine bridge has allowed for conjugation to targeting ligands or biomolecules[3,4] through an $S_{RN}1$ reaction, and most recently, carbon–carbon coupling at the *meso*-position has been explored utilizing an adapted Suzuki–Miyaura method,[1,16,17] but this method uses an expensive palladium catalyst, has generally only been applied to water soluble cyanine dyes, and requires difficult purification of the product and unreacted chloro version by column chromatography.

Herein, the synthesis of a series of substituted heptamethine cyanines has been described using substituted dianil linkers. Substitutions include methyl, phenyl, chloro, and bromo groups at the *meso*-position as well as phenyl and *t*-butyl groups at the 5-position of the commonly used cyclohexene in heptamethine cyanines. Cyclopentene and cycloheptene have also been explored and how these methine bridge substitutions affect the dyes optical properties is studied. The use of these substituted dianil linkers as an alternative to C–C coupling can make for a more facile condensation to the final cyanine dyes.

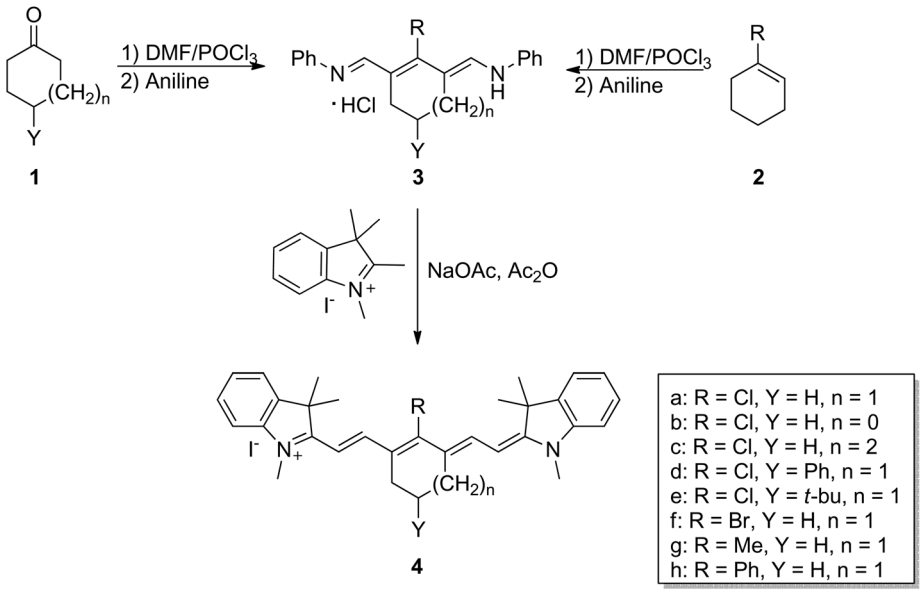
Results and discussion

Synthesis

As described in Scheme 1, a series of substituted heptamethine cyanines has been synthesized by a condensation reaction between two quaternary ammonium salts and a dianil

Department of Chemistry, Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30303, USA. E-mail: mhenary1@gsu.edu; Fax: +1 404-413-5505; Tel: +1 404-413-5566

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c8pp00218e



Scheme 1 Synthesis of *meso*-substituted heptamethine cyanines.

linker. The key step in the synthesis is the formation of the dianil linkers which can individually be used to form large libraries of cyanine compounds with substitutions on the methine chain. These dianil linkers are formed by reaction of a substituted cycloketone **1** or 1-substituted cycloalkene **2** with a Vilsmeier–Haack reagent, generated from phosphorous oxychloride and *N*,*N*-dimethylformamide, which gives a dialdehyde that is later capped with aniline for stability to give dianil linkers **3**. These dianil linkers **3** are then condensed with quaternary ammonium salts in acetic anhydride in the presence of sodium acetate. The dyes are then purified either by washing with methanol, dissolution in a minimal amount of methanol followed by precipitation in ether or column chromatography (1–5% methanol/DCM).

Using this classical method to synthesize heptamethine cyanines, many of these dyes were able to be purified solely by washing with methanol and were produced in good yields. Cyanines have been widely tested for biomedical imaging applications. Our lab has shown that small modifications in the structure of these compounds can cause them to have specific targeting.[9,11,18,19] While modifications to the heterocycles and *N* substituents have been widely explored, changes to the methine bridge have not. The combination of these changes substantially increases the number of structures that can be made, further enhancing the modifiability of cyanine dyes for their desired application.

Heptamethine cyanines undergo rapid photooxidation in solution.[20] Ring systems have been introduced to heptamethine cyanine dyes for added stability,[21] but only cyclohexene rings have been explored in detail. In this work cyclopentene and cylcloheptene have also been introduced as shown in Scheme 1. Column chromatography was needed to purify compound **4b** and it was synthesized in the lowest yield at 53%. The cyclopentene ring is more planar potentially creating a more stable and less reactive dianil linker. The increased rigidity causes the absorption maximum to red shift to 803 nm and could also cause the dye to slightly twist out of cyanine's general planar structure. Correspondingly, the addition of these cyclic rings generally involves the addition of a chlorine substitutent at the *meso*-position, but it has been reported that alkyl or aryl substituents such as the methyl or phenyl substitutions described herein further increase photostability.[21]

Suzuki–Miyaura coupling has become a useful tool for conjugating the dyes to targeting ligands or biomolecules using water soluble cyanines.[1,16] While there has been success synthesizing these dyes through carbon–carbon coupling with a *meso*-chlorine atom, a *meso*-bromine atom should give better success. These reactions generally have lower yields due to the need to separate the unreacted *meso*-halogenated dye from the C–C coupled dye. Typically compounds **4g** and **4h** would be synthesized by first preparing **4a** and then reacting it with methyl- or phenyl-boronic acid with a palladium catalyst,[1,22] but by using this classical method with a substituted dianil linker the Suzuki–Miyaura coupling step can be avoided. In addition to removing a step and the need for an expensive catalyst, this method allows for application to both water soluble and insoluble compounds. Modifying the dianil linkers and synthesizing these compounds in a larger scale allows for the facile synthesis of a large number of substituted cyanines by one step.

Chloro-substituted cyanines are weakly or non-reactive in comparison to iodo- or bromo-derivatives. Suzuki coupling often requires strong base which is not suitable for use with sensitive heptamethine cyanines because they undergo hydrolysis or decomposition in strong base. The addition of a second heteroatom in the quaternary ammonium heterocycle, such as benzothiazolium, further activates the C-2 position by inductive effect making it even more vulnerable to nucleophi-

lic addition. Suzuki coupling has been shown to fail with these compounds.[1] This approach provides a convenient way to prepare a wide array of functionally different heptamethine cyanines by reacting a variety of Vilsmeier reagents such as **3** with different quaternary ammonium salts and produces various dyes in good yields.

Hydrophobicity

Cyanine dyes are known to aggregate, but it was previously reported that the *t*-butyl group in compound **4e** could be used to prevent aggregation and make solar cells more efficient.[13] Cyanines with heterocycles containing dimethylindolenine have a more pronounced H-band that tends to become amplified when the dyes aggregate.[23] Generally this aggregation, caused by polar solvents such as water or PBS buffer, can be prevented using small amounts of organic solvents (*i.e.* 2% DMSO or ethanol) although more hydrophobic dyes have required as much as 30% DMSO to completely break up the aggregation.[24] Hydrophobicity studies of the synthesized dyes shown in Scheme 1, were carried out in ethanol/PBS buffer mixtures. As the polarity of the solvent decreased with increasing ethanol concentration from 1% to 35%, a general amplification of the monomeric band in the absorption spectra was observed (Fig. 4). In dyes that are aggregating as the polarity decreases there is also an increase in the ratio of monomeric band to the aggregate (H-band) until all aggregation is broken up and the ratio becomes steady. The increased absorbance is related to the higher molar absorptivity of these compounds in organic solvents compared to more polar ones and aggregation is only observed if the ratio of the 2 peaks differs as the solvent polarity changes. As explained by our group previously, this aggregation is predominantly due to a plane-to-plane arrangement.[25]

Changes in the hydrophobic nature of the dyes can make them less biocompatible regarding uptake and *in vivo* transportation. Of these newly synthesized heptamethine cyanines only compound **4e** displayed aggregation beyond 2% ethanol as shown in Fig. 4. As shown in Table 1 this compound has a calculated log *D* value above 6. While compounds **4d** and **4h** also have log *D* above 6 the ability of the phenyl ring to rotate into and out of plane with the rest of the dye can act to prevent stacking while the *t*-butyl in compound **4e** is locked in a single conformation. The lack of aggregation observed in the rest of these compounds increases their attractiveness as biological probes.

Optical properties

Because it is important for structural changes to retain the favorable optical properties of cyanine dyes, the absorbance and fluorescence of all compounds in Scheme 1 were measured. The absorption shifts in ethanol caused by the different substitutions from the dianil linkers can be seen in Fig. 1. The λ_{max} varies from 759 nm to 805 nm in the dyes containing a *meso*-phenyl ring and a cyclopentene in the methine chain, respectively. The λ_{max} of heptamethine cyanines with an open chain or with the most common *meso*-chlorine substitution is generally about 780 nm. Absorption shifts in cyanines are generally seen when different heterocycles are used that have more interaction with the conjugated system.[7] When the methine chain substitutions are changed, the most notable absorption shifts come from the alternate conjugation pathway in the *meso*-phenyl compound **4h** and the strain caused by the cyclopentene ring on the methine chain in compound **4b**.

Optical properties of the dyes are shown in Table 1. The molar absorptivity of these compounds ranges from 78 000–256 000. Interestingly, the two compounds **4b** and **4h** that were furthest from the usual 780 nm absorbance maximum measured in heptamethine cyanines also showed the highest molar absorptivities, over 250 000. The *meso*-phenyl group in **4h** and the strain put on the methine chain by the cyclopentene in **4b** can prevent *cis–trans* isomerization increasing the molar absorptivity.

Subsequently, the lowest molar absorptivities were observed in the compounds with the cycloheptene **4c** and the *t*-butyl **4e**. The cycloheptene can have the opposite effect of the cyclopentene allowing for more rotation at the C2–C3 bond (Fig. 2) while the *t*-butyl can cause increased aggregation in polar solvents. Stokes shifts ranged from 12–20 nm, but the most interesting

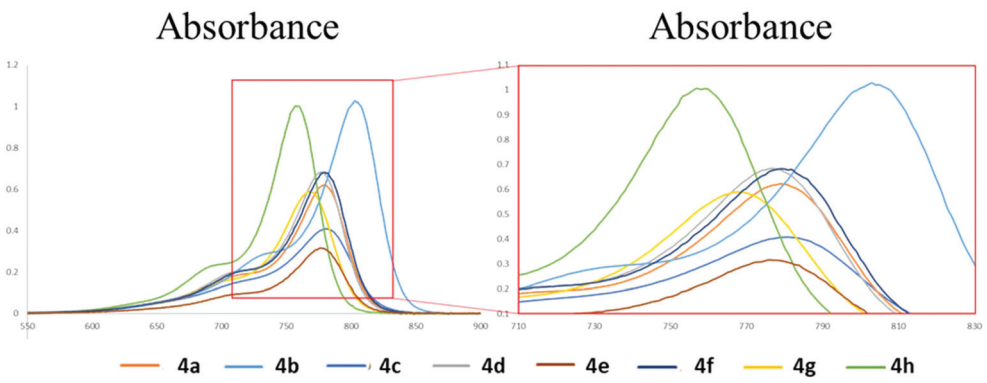


Fig. 1 Absorption spectra of 4 μM heptamethine cyanines in ethanol (Right side is magnified).

Table 1 Summary of optical properties of dyes **4a–h** in ethanol and calculated log *D* values

Dye	Absorbance λ_{max} (nm)	Fluorescence λ_{max} (nm)	Molar absorptivity (M^{-1} cm^{-1})	Stokes shift (nm)	Fluorescence quantum yield (%)	log *D*
4a	780	798	230 500	18	0.21	4.79
4b	803	815	256 762	12	0.14	4.34
4c	781	[a]	102 014	—	[a]	5.23
4d	777	793	171 369	16	0.24	6.21
4e	776	795	78 827	19	0.22	6.11
4f	779	797	170 611	18	0.18	4.96
4g	768	788	147 331	20	0.08	4.80
4h	759	774	264 657	15	0.31	6.07

[a] Not fluorescent.

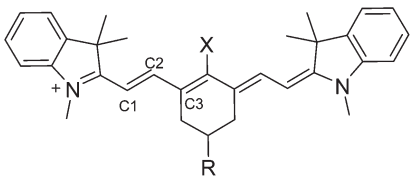


Fig. 2 Numbering of methine bridge carbons.

find from fluorescence studies was that compound **4c**, containing a cycloheptene in the methine chain, was not fluorescent (Fig. 3). The increased flexibility due to the seventh carbon in the cyloheptene ring must allow for the excited energy to be lost through motion in the ring rather than fluorescence.

Quantum yields of the synthesized dyes range from 8% to 31%. The dyes with halogens at the *meso*-position show similar quantum yields ranging from 18% in the bromine containing dye **4f** to 21–24% in the 3 dyes with chlorine in the *meso*-position **4a,d,e**. The *t*-butyl and phenyl substitutions at the 5 position of the cyclohexene have little effect on quantum yield as they are not conjugated into the system. The dye with a *meso*-methyl group **4g** had the lowest quantum yield at 8% due to the its ability to relax through rotation of the methyl position while the dye with the *meso*-phenyl **4h** had the highest quantum yield at 31%. The long wavelengths exhibited by cyanine dyes are caused by the delocalization of electrons throughout the molecule. Cyclic structures within the dyes lead to rigidity enhancing the stability of the molecule in the excited state, ultimately increasing the quantum yield. A trend is observed with decreasing quantum yield as the rigidity of the cyclic system increases with the addition of carbons. The cycloheptene containing dye shows no fluorescence.

Conclusion

Several heptamethine cyanine derivatives were synthesized from modified dianil linkers. The simple general procedure is advantageous for carbon–carbon coupling over previous Suzuki–Miyaura methods in that a large number of structurally diverse fluorophores can be synthesized after a single step without the use of palladium catalysts. This one-pot method can also eliminate the cumbersome steps of protecting/deprotecting amino or hydroxy groups usually applied to reactions that required conjugation to *meso*-chlorine substituted dyes. In addition, the procedure is applicable to hydrophobic heterocyclic salts well as water soluble ones. It was shown that dyes containing cyclopentene in the methine bridge or a *meso*-phenyl display increased molar absorptivity. The dye containing a cycloheptene in the methine bridge did not fluoresce. The compound containing a *t*-butyl showed lower molar absorptivity and large amounts of aggregation in PBS buffer. These structural substitutions can be useful in the many applications of cyanines.

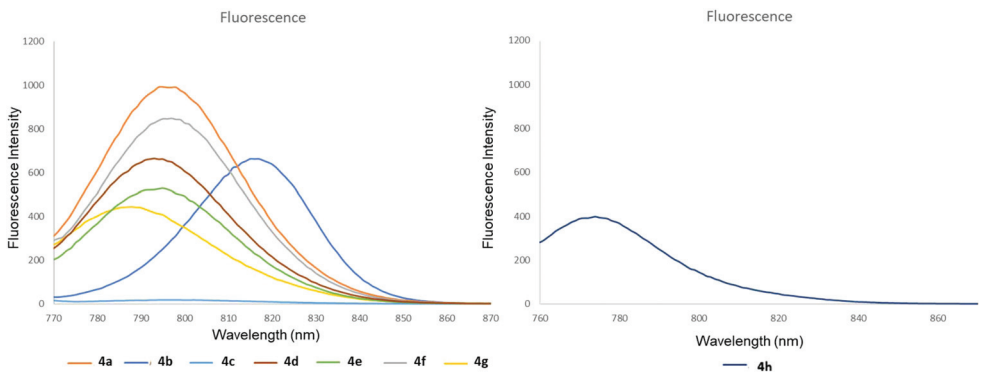


Fig. 3 Fluorescence spectra of 0.4 μM heptamethine cyanines in ethanol (Left side: dyes were excited at 760 nm. Right side: dye **4h** was excited at 750 nm due to blueshifted fluorescence).

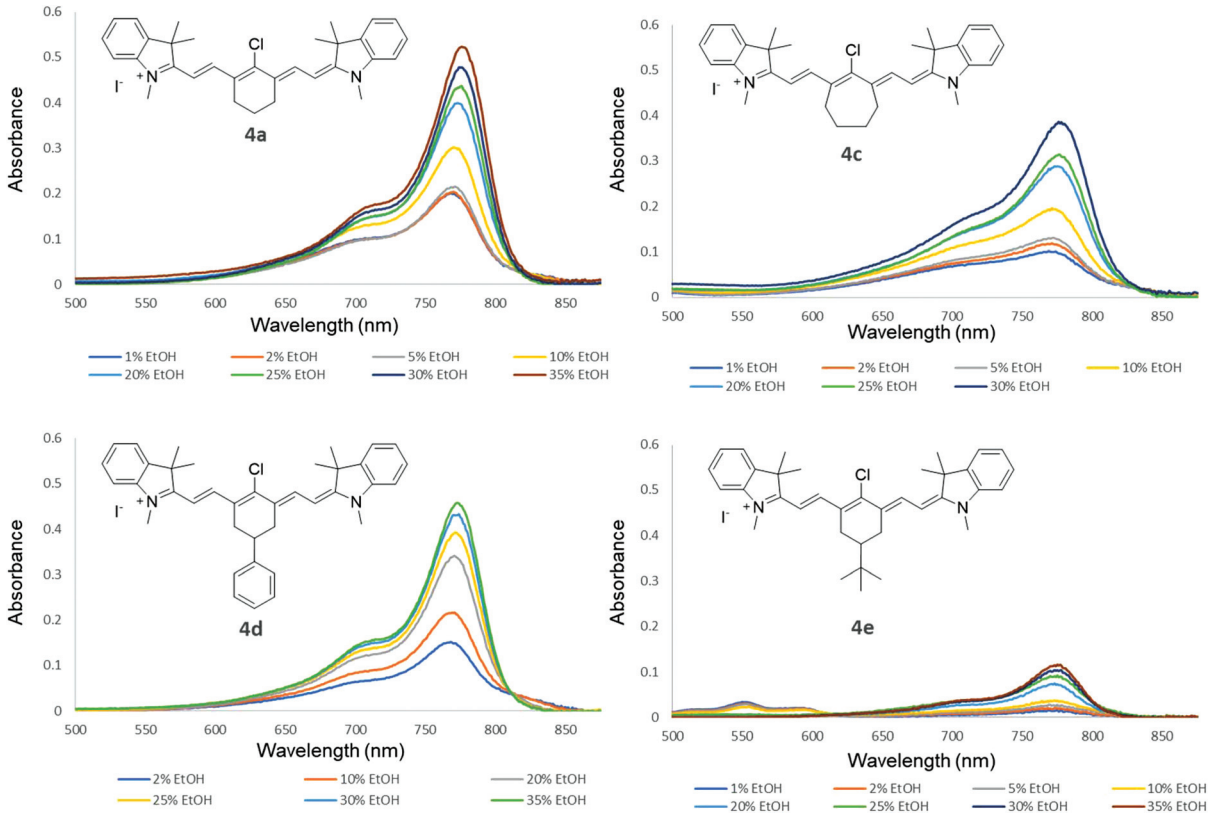


Fig. 4 Absorption spectra of **4a**, **4c**, **4d**, and **4e** as a function of solvent hydrophobicity (% v/v ethanol to PBS buffer) at constant dye concentration of 4 μM.

Experimental

General information

All chemicals and solvents were of American Chemical Society grade or HPLC purity and were used as received. All chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA), Sigma-Aldrich (Saint Louis, MO) and Acros Organics. Melting points (mp, open Pyrex capillary) were measured on a Thomas Hoover apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on BrukerAvance (400 MHz) spectrometer. Absorption spectra were recorded on a Varian Cary 50 UV-Visible Spectrophotometer (Santa Clara, CA) in ethanol using VWR disposable two-sided polystyrene cuvettes path length 1 cm. Fluorescence emission analyses were performed using a Shimadzu RF-5301PC Spectrofluorophotometer (Kyoto, Japan) in ethanol using Sigma-Aldrich disposable polystyrene fluorimeter cuvettes of pathlength 1 cm. Excitation was achieved at 760 nm (750 nm for **4h**) with slit widths of 5 mm. Microsoft Excel 2010 was used for all calculations.

Synthesis

General procedure for synthesis of dianil linkers 3a–h. Under nitrogen atmosphere, DMF was added dropwise to phosphorous oxychloride keeping the reaction temperature at or below 5 °C. Cycloketones or 1-substitutedcyclohexenes in dry dichloromethane were added to this solution, respectively, while stirring still at 0 °C. After the solution becomes yellow, the slurry is heated at 100 °C for 2 h. The reaction mixture is then cooled to room temperature and aniline in ethanol is added slowly in an ice bath. The reaction mixture is then poured into ice and concentrated HCl is added to give a burgundy precipitate. The powder is then filtered and washed with ether and hexanes and used without further purification.

General procedure for synthesis of heptamethine dyes 4a–h. The quaternary ammonium salt (200 mg, 2 mol eq.) was stirred in acetic anhydride (3 mL) followed by addition of sodium acetate (2 mol eq.) and dianil linker **3** (1 mol eq.). The reaction was heated at 80 °C for 2 h. The reactions were monitored closely using regular phase thin layer chromatography with a mobile phase of DCM/MeOH (99 : 1) as well as UV-Vis-NIR spectrophotometer with methanol as the solvent to visualize the absorption band at ~780 nm. Upon completion of the reaction the mixtures were allowed to cool to room temperature before the dye was precipitated in diethyl ether (50 mL). The pure products were obtained after washing with methanol, dissolving the dyes in acetonitrile (1 mL) and precipitating with ether (50 mL), or column chromatography with eluent from 1–5% methanol/DCM.

2-((*E*)-2-((*E*)-2-Chloro-3-(2-((*E*)-1,3,3-trimethylindolin-2-ylidene) ethylidene)cyclohex-1-en-1-yl)vinyl)-1,3,3-trimethyl-3*H*-indol-1-ium iodide, **4a**: was synthesized as previously described.[26]

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67 (s, 12 H), 1.85 (m, 2 H), 2.72 (t, J = 6.0 Hz, 4 H), 3.68 (s, 6 H), 6.30 (t, J = 14.4 Hz, 2 H), 7.28 (m, 2 H), 7.44 (m, 4 H), 7.62 (d, J = 7.2 Hz, 2 H), 8.25 (d, J = 14.4 Hz, 2 H).

2-((E)-2-((E)-2-Chloro-3-(2-((E)-1,3,3-trimethylindolin-2-ylidene) ethylidene)cyclopent-1-en-1-yl)vinyl)-1,3,3-trimethyl-3H-indol-1-ium iodide, ***4b****.* Yield 53%; mp >260 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 (s, 12 H), 3.10 (m, 4 H), 3.77 (s, 6 H), 6.13 (d, J = 14.0 Hz, 2 H), 7.16 (d, J = 8.0 Hz, 2 H), 7.24 (t, J = 8.0 Hz, 2 H), 7.40 (m, 4 H), 7.83 (d, J = 14.0 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.1, 28.0, 32.6, 49.0, 102.9, 110.6, 122.0, 125.1, 128.8, 136.8, 138.3, 140.9 142.9, 151.8, 171.4. HRMS m/z: calc for $C_{31}H_{34}N_2Cl^+$ 469.2405, obsd 469.2387.

2-((E)-2-((E)-2-Chloro-3-(2-((E)-1,3,3-trimethylindolin-2-ylidene) ethylidene)cyclohept-1-en-1-yl)vinyl)-1,3,3-trimethyl-3H-indol-1-ium iodide, ***4c****.* Yield 79%; mp 139–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (s, 12 H), 1.91 (m, 4 H), 2.83 (m, 4 H), 3.79 (s, 6 H), 6.31 (d, J = 14.4 Hz, 2 H), 7.19 (d, J = 7.2 Hz, 2 H), 7.26 (t, J = 7.2 Hz, 2 H), 7.38 (d, J = 7.2 Hz, 2 H), 7.44 (t, J = 7.2 Hz, 2 H), 8.37 (d, J = 14.4 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.9, 26.9, 28.0, 32.8, 49.2, 101.4, 110.7, 122.0, 125.2, 128.8, 131.2, 142.8, 147.2, 154.7, 173.1. HRMS m/z: calc for $C_{33}H_{38}N_2Cl^+$ 497.2718, obsd 497.2698.

2-((E)-2-((E)-4-Chloro-5-(2-((E)-1,3,3-trimethylindolin-2-ylidene) ethylidene)-1,2,5,6-tetrahydro-[1,1'-biphenyl]-3-yl)vinyl)-1,3,3-trimethyl-3H-indol-1-ium iodide, ***4d****.* Yield 72%; mp 171–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (s, 12 H), 2.70 (m, 2 H), 3.04 (m, 1 H), 3.15 (m, 2 H), 3.65 (s, 6 H), 6.32 (d, J = 14.0 Hz, 2 H), 7.29 (m, 3 H), 7.41 (m, 6 H), 7.48 (d, J = 7.2 Hz, 2 H), 7.63 (d, J = 7.6 Hz, 2 H), 8.31 (d, J = 14.0 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8, 32.0, 33.6, 38.7, 49.3, 102.4, 111.9, 122.8, 125.6, 126.3, 127.1, 127.9, 129.0, 141.5, 143.3, 145.4, 147.7, 173.3; HRMS m/z: calc. for $C_{38}H_{40}N_2Cl^+$ 559.2875, obsd 559.2855.

2-((E)-2-((E)-5-(tert-Butyl)-2-chloro-3-(2-((E)-1,3,3-trimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-1,3,3-trimethyl-3H-indol-1-ium iodide, ***4e****.* Yield 76%, mp 171–173; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (s, 9 H), 1.54 (m, 2 H), 1.67 (s, 12 H), 2.18 (m, 2 H), 2.97 (m, 2 H), 3.71 (s, 6 H), 6.32 (d, J = 13.6 Hz, 2 H), 7.29 (m, 2 H), 7.44 (m, 4 H), 7.62 (d, J = 7.2 Hz, 2 H), 8.25 (d, J = 13.6 Hz, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.7, 32.1, 32.7, 49.3, 102.0, 111.8, 122.8, 125.6, 126.8, 129.0, 141.4, 143.2, 173.1. HRMS m/z: calc. for $C_{38}H_{40}N_2Cl^+$ 539.3188, obsd 539.3187.

2-((E)-2-((E)-2-Bromo-3-(2-((E)-1,3,3-trimethylindolin-2-ylidene) ethylidene)cyclohex-1-en-1-yl)vinyl)-1,3,3-trimethyl-3H-indol-1-ium iodide, ***4f****.* Yield 74%; mp >260 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.69 (s, 12 H), 1.85 (m, 2 H), 3.91 (m, 4 H), 3.69 (s, 6 H), 6.23 (d, J = 14.4 Hz, 2 H), 7.29 (m, 2 H), 7.44 (m, 4 H), 7.63 (d, J = 7.2 Hz, 2 H), 8.26 (d, J = 14.0, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.5, 31.9, 49.3, 102.6, 111.9, 122.8, 125.6, 128.4, 129.0, 141.4, 143.3, 144.9, 146.7, 173.1. HRMS m/z: calc for $C_{32}H_{36}N_2Br^+$ 527.2056, obsd 527.2040.

1,3,3-Trimethyl-2-((E)-2-((E)-2-methyl-3-(2-((E)-1,3,3-trimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-3H-indol-1-ium iodide, ***4g****.* Yield 71%; mp 217–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.73 (s, 12 H), 1.92 (t, J = 5.6 Hz, 2 H), 2.43 (s, 3 H), 2.62 (t, J = 5.6 Hz, 4 H), 3.72 (s, 6 H), 6.16 (d, J = 13.6 Hz, 2 H), 7.16 (d, J = 8.0 Hz, 2 H), 7.23 (d, J = 7.6 Hz, 2 H), 7.38 (m, 4 H), 8.05 (d, J = 13.2 Hz, 2 H). ^{13}C NMR (100 MHz, CDCl3) δ ppm 15.1, 20.9, 25.7, 28.3, 32.2, 100.6, 110.3, 122.1, 124.8, 128.7, 132.1, 140.5, 142.4, 156.0, 171.5. HRMS m/z: calc. for $C_{33}H_{39}N_2^+$ 463.3108, obsd 463.3091.

1,3,3-Trimethyl-2-((E)-2-((E)-6-(2-((E)-1,3,3-trimethylindolin-2-ylidene)ethylidene)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl)-3H-indol-1-ium iodide, ***4h****.* Yield 77%; mp >260 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11 (s, 12 H), 1.95 (m, 2 H), 2.68 (t, J = 6.0 Hz, 4 H), 3.58 (s, 6 H), 6.17 (d, J = 14.0 Hz, 4 H), 7.16 (m, 4 H), 7.25 (d, J = 8.0 Hz, 2 H), 7.34 (m, 4 H), 7.46 (d, J = 8.0 Hz, 2 H), 7.62 (m, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 24.5, 27.3, 31.5, 48.5, 100.7, 111.3, 122.7, 124.9, 128.5, 128.8, 129.0, 129.6, 131.0, 139.1, 140.9, 143.3, 147.3, 161.5, 172.1. HRMS m/z: calc. for $C_{38}H_{41}N_2^+$ 525.3264, obsd 525.3241.

Stock solutions

Stock solutions of the dyes and standard were prepared by weighing the solid on a 5-digit analytical balance in an amber vial and adding solvent *via* a class A volumetric pipette to a final concentration of 1.0 mM. The vials were vortexed for 20 s and then sonicated for 15 min to ensure complete dissolution. The stock solutions were stored in a dark freezer at 4 °C when not in use. Working solutions were prepared just prior to use by dilution of the stock to final concentrations.

Method of determining molar absorptivity and fluorescence quantum yield

Stock solutions were used to prepare six dilutions of dyes in ethanol with concentrations ranging from 1 μM to 4 μM using a class A volumetric pipette in order to maintain absorption between 0.1 and 1.0. The dye solutions were diluted ten-fold for fluorescence in order to minimize inner filter effect. The absorbance spectra of each sample was measured in triplicate from 550–900 nm. The emission spectrum of each sample was measured in triplicate with a 760 nm (750 nm for **4h**) excitation wavelength.

For molar absorptivity, the absorbance at the wavelength of maximum absorbance (λ_{max}) was determined and the absorbance of each sample at λ_{max} was plotted as a function of dye concentration. The linear regression equation was computed using Microsoft Excel.

The fluorescence quantum yields were determined relative to the indocyanine green standard utilizing the gradient from the plot of integrated fluorescence intensity *vs.* absorbance (Grad) and the published quantum yield of the standard (φ_S, 13.2%[27]) as per eqn (1).

$$\varphi_D = \varphi_S \times \mathrm{Grad}_D/\mathrm{Grad}_S \times {\eta_S}^2/{\eta_D}^2. \quad (1)$$

Hydrophobicity studies

Hydrophobic characteristics of the dyes were assessed by acquiring absorbance spectra of the dyes in varying ratios of ethanol-buffer mixture (in the range of 1–35% ethanol) using

PBS buffer pH 7.4. The concentration of ethanol was slowly increased until there were no apparent changes in the ratio between the monomeric peak and H-band.

Log *D* calculations

Log *D* values were predicted using MarvinSketch 5.9 (ChemAxon, Budapest, Hungary). Values were derived from calculations by Viswanadhan,[28] Klopman[29] and the PHYSPROP database at pH 7.4 and the three were averaged.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank the Department of Chemistry at Georgia State University for the support of the Ph.D. dissertation of AL and the MSc. thesis of FM. This study was supported by grants to MH from the Georgia State University Brains and Behavior Seed Grant, the Atlanta Clinical and Translational Science Institute Healthcare Innovation Seed Grant, and the Georgia Research Alliance Ventures Phase 1 Grant. MH would like thank the U.S. Department of Health and Human Services, National Institutes of Health National Institute of Biomedical Imaging and Bioengineering (Grant Number: RO1EB022230).

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