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Synthesis and effect of heterocycle modification on the spectroscopic properties of a series of unsymmetrical trimethine cyanine dyes

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ABSTRACT

Carbocyanine dyes are a class of organic compounds that contain two heterocycles that act as electron donors and acceptors connected by a conjugated methine bridge. Herein the synthesis of a series of 16 novel unsymmetrical trimethine cyanine dyes is reported. Their structures were characterized by various spectroscopic techniques, and their optical properties were measured. Absorption maxima of the dyes were calculated using the time-dependent density-functional theory method and the computational absorption maxima are consistent with the experimental data. The addition of electron withdrawing substituents such as halogens on the heterocycle gave more favorable optical properties such as higher quantum yield and molar absorptivity. The aggregation of these cyanine dyes was studied and compared to a similar series of symmetric cyanine dyes. It was determined that the heterocycle has more effect on aggregation than the side chain and a dye with two different heterocycles will aggregate less than a dye with the same heterocycle. The dyes were also investigated for Lipinski Rule violations as their use is becoming more prevalent for *in vivo* applications.

1. Introduction

Cyanine dyes possess two nitrogen containing heterocyclic groups that are connected by a conjugated methine bridge as shown in Fig. 1. The delocalization of electrons across this chain causes them to be highly fluorescent and exhibit long wavelength absorption that span from the visible to the near infrared regions [1–4]. These unique properties as well as their high extinction coefficients have allowed cyanine dyes to be employed in various applications. Beginning in the 1800s, cyanine dyes were used in photographic emulsions and chemotherapy [5], but more recently cyanine dyes have been used in laser printing [6], pH sensors [7], fluorescence *in vivo* imaging [8–11], data storage [12], and as labels for nucleic acid detection [13–15].

Until the mid-1900s, it was widely accepted that an unsymmetrical cyanine dye would absorb halfway between the absorption of the parent symmetrical dyes. Brooker et al. showed that if the basicities of the nitrogen containing heterocycles are not identical, or if the relative stabilities of the two forms are not different, the absorption would not be at the midpoint [16]. If the basicity of the two nuclei is not equal the absorption should be found at a lower wavelength than the intermediate position. As the difference in basicity is continually increased, the absorption maximum gets further from the midpoint, and this deviation increases with an increasing length of the polymethine chain [16]. Although many unsymmetrical cyanine dyes have been made [16], there is a lack in the literature of cyanine dyes with indolenine moieties and those with substitutions on the benzene ring of the indolenine are sporadic.

When designing new dyes for specific applications, it is important to understand how altering the parent structure, such as the substituents on the heterocyclic moieties, affects the optical properties of the dyes. These changes can be made to improve solubility or increase binding and permeability, but structural modifications also cause changes in optical properties. Herein we report the synthesis of a series of unsymmetrical trimethine cyanine dyes prepared with various heterocyclic modifications to determine how these substitutions affect the optical properties of the dyes including molar absorptivity, fluorescence quantum yield, and stokes shift. Absorption maxima are compared to those of the symmetrical dyes previously reported [17].

A popular application of cyanine dyes is for imaging and *in vivo* labeling. Lipinski's Rule of Five is used to evaluate if a chemical

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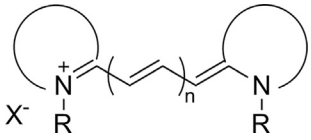

Fig. 1. General structure of cyanine dyes.

compound has properties that would make it orally active in humans [18]. These properties include molecular weight, hydrogen bond donors and acceptors, a partition coffecient, and polar surface area [19]. Partition coefficients, such as log *P*, are ratios of concentrations of a compound in a mixture of two immiscible phases. Log *D* is much like log *P*, but it also takes into account positions that can be fully protonated or deprotonated such as sulfonate or carboxylate groups. Based on log *D*, it can be speculated as to where a compound will distribute in biological tissues. The Lipinski Rule of Five properties of each dye were investigated and the log *D* value of each dye was predicted to give a better idea of how each substituent affects partitioning of the dye and where it will distribute in biological tissues.

Previously, a study comparing the optical properties of six symmetrical and unsymmetrical tricarbocyanine dyes containing benzothiazole and benzoxazole heterocycles was presented [20]. The authors concluded that the replacement of one sulfur atom with one oxygen atom blue shifted the absorption wavelength about 20 nm. Our recent work presented in this paper is similar but takes into account dyes with various alkyl chains as well as substitutions on a number of different heterocycles including benz[*c*,*d*] indole, benzoxazole and benzothiazole.

2. Experimental

2.1. General information

Most reagents were purchased from Sigma–Aldrich and were used without purification. Absorption spectra were recorded on a Cary 3G UV–Visible Spectrophotometer (Santa Clara, CA) in DMSO for dyes and 0.1 M NaOH/water solution for fluorescein standard using VWR disposable two-sided polystyrene cuvettes with a pathlength of 1 cm. Fluorescence (LIF) emission analyses were performed using a K2 Multifrequency Phase Spectrofluorometer (ISS Inc., Champaign, Il) in DMSO for dyes and 0.1 M NaOH/water solution for the fluorescein standard using Sigma–Aldrich disposable polystyrene fluorimeter cuvettes with a pathlength of 1 cm. Excitation was achieved with a 300 W Excelitas Short Arc Xenon Lamp (Fremont, CA) at 645 nm. Slit widths were set to 2 mm and integration time of 3 s. Microsoft Excel 2010 was used for all calculations.

2.2. Synthesis

2.2.1. 5-Methoxy-2,3,3-trimethyl-indole, (**4b**)

5-Methoxy-2,3,3-trimethyl-indole (**4b**) was synthesized following the procedure published by Zimmerman and Hennig in 92% yield (lit 95% yield [21]).

2.2.2. Sodium 2,3,3-trimethyl-3H-indole-5-sulfonate (**4c**)

Sodium 2,3,3-trimethyl-3H-indole-5-sulfonate (**4c**) was previously synthesized by our group [8].

2.2.3. 5-Bromo-2,3,3-trimethyl-3H-indole (**4d**)

5-Bromo-2,3,3-trimethyl-3H-indole (**4d**) was previously synthesized by our group [22].

2.2.4. 2-Methyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium bromide (**5a**) and 2-methyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate bromide (**5h**)

2-Methyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium bromide (**5a**) and 2-Methyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate bromide (**5h**) were previously synthesized by our group [8].

2.2.5. 3-(2,3,3-Trimethyl-3H-indol-1-ium-1-yl)propane-1-sulfonate (**5b**)

3-(2,3,3-Trimethyl-3H-indol-1-ium-1-yl)propane-1-sulfonate (**5b**) was synthesized following the procedure published by Tang et al. in 82% yield; mp 217–221 °C (lit mp 165–166 °C [23]).

2.2.6. 1-Ethyl-2,3,3-trimethyl-3H-indol-1ium iodide (**5c**), 1-butyl-2,3,3-trimethyl-3H-indol-1ium iodide (**5d**), 3-butyl-2-methyl-1H-benzo[e]indol-3-ium iodide (**5i**) and 1-butyl-2-methylbenzo[cd] indol-1-ium iodide (**7a**)

1-Ethyl-2,3,3-trimethyl-3H-indol-1ium iodide (**5c**), 1-Butyl-2,3,3-trimethyl-3H-indol-1ium iodide (**5d**), 3-Butyl-2-methyl-1H-benzo[e]indol-3-ium iodide (**5i**) and 1-Butyl-2-methylbenzo [cd]indol-1-ium iodide (**7a**) were previously synthesized by our group [24].

2.2.7. 2,3,3-Trimethyl-1-(3-phenylpropyl)-3H-indol-1-ium bromide, (**5e**)

A mixture of 2,3,3-trimethyl-3*H*-indole and 3-(bromopropyl) benzene were refluxed for 72 h. The reaction was cooled to room temperature, poured into cold ether, and the precipitate was filtered; mp 156–158 °C, 86% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.53 (s, 6 H), 2.13–2.20 (m, 2 H), 2.80–2.83 (m, 5 H), 4.51 (t, J = 8.0 Hz, 2 H), 7.19–7.32 (m, 5 H), 7.61–7.63 (m, 2 H), 7.84 (t, J = 5.2 Hz, 1 H), 7.98 (t, J = 5.2 Hz, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.3, 22.0, 28.8, 31.7, 47.4, 54.1, 115.4, 123.5, 126.0, 128.2, 128.3, 128.8, 129.3, 140.6, 141.0, 141.8, 196.6 HRMS: Calcd for $C_{20}H_{24}N^+$ *m*/*z* 278.1904, obsd *m*/*z* 278.1915.

2.2.8. 5-Bromo-1-butyl-2,3,3-trimethyl-3H-indol-1-ium iodide, (**5f**)

Compound **5f** was synthesized by a similar procedure to **5e**; mp 176–178 °C, 78% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.93 (t, J = 7.2 Hz, 3 H), 1.39–1.47 (m, 2 H), 1.55 (s, 6 H), 1.76–1.83 (m, 2 H), 2.85 (s, 3 H), 4.45 (t, J = 7.2 H, 2 H), 4.43 (t, J = 7.6 Hz, 2 H), 7.83 (d, J = 8.4 Hz, 1 H), 7.97 (d, J = 8.4 Hz, 1 H), 8.19 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.08, 14.83, 19.79, 22.31, 29.68, 48.23, 54.92, 117.99, 123.28, 127.39, 132.32, 140,89, 144.65, 197.43; HRMS: Calcd for $C_{15}H_{21}NBr^+$ *m*/*z* 294.0852, obsd *m*/*z* 294.0853.

2.2.9. 1-Butyl-5-methoxy-2,3,3-trimethyl-3H-indol-1-ium iodide, (**5g**)

Compound **5g** was synthesized by a similar procedure to **5e**; mp 145–147 °C, 56% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.93 (t, J = 7.2 Hz, 3 H), 1.37–1.46 (m, 2 H), 1.53 (s, 6 H), 1.76–1.84 (m, 2 H), 2.80 (s, 3 H), 3.86 (s, 3 H), 4.43 (t, J = 7.6 Hz, 2 H), 7.13 (dd, J = 10.8 Hz, 1 H), 7.50 (s, 1 H), 7.89 (d, J = 9.2 Hz, 1 H); ^{13}C NMR (100 MHz, MeOD-d_4): δ 12.44, 19.14, 21.61, 29.09, 47.93, 53.81, 55.78, 108.43, 114.50, 116.04, 133.49, 143.54, 160.71, 192.31; HRMS: Calcd for $C_{16}H_{24}NO^+$ *m*/*z* 246.1852, obsd *m*/*z* 246.1857.

2.2.10. 5-Bromo-2-methyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium bromide (**5j**)

5-Bromo-2-methyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium bromide (**5j**) was previously synthesized by our group [22].