ORGANIC
LETTERS

2006
Vol. 8, No. 1
111−114

Synthesis of New Cholesterol- and Sugar-Anchored Squaraine Dyes: Further Evidence of How Electronic Factors Influence Dye Formation

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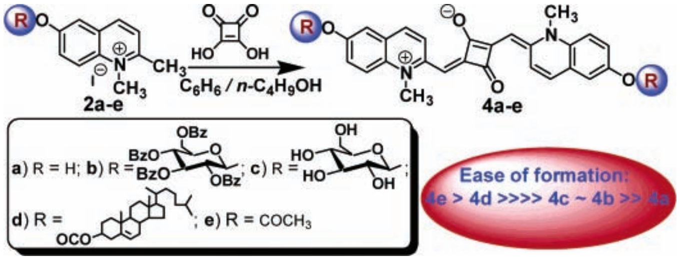
Received October 31, 2005

ABSTRACT



Synthesis of new quinaldine-based squaraine dyes linked to cellular recognition elements that exhibit near-infrared absorption (>740 nm) are described. Both product analysis and theoretical calculations substantiate the interesting electronic effects of various substituents in the dye formation reaction. These results are useful in the synthesis of symmetrical and unsymmetrical squaraine dyes that can have potential biological and photodynamic therapeutical applications.

Squaraine dyes have been the subject of many recent investigations.[1] The current interest in these dyes may be attributed to their interesting photochemical and photophysical properties,[2] which make them attractive for a variety of applications. These include photoconductors in organic solar cells,[3] photoreceptors in copiers and laser printers,[4] IR absorbers in organic optical disks,[5] sensors for metal ions,[1e−g] and as sensitizers for photodynamic therapeutical (PDT) applications.[6] Squaraine dyes in general can be considered as acceptors in conjugation with two donors D−A−D. The donor molecules can be the same or different giving rise to

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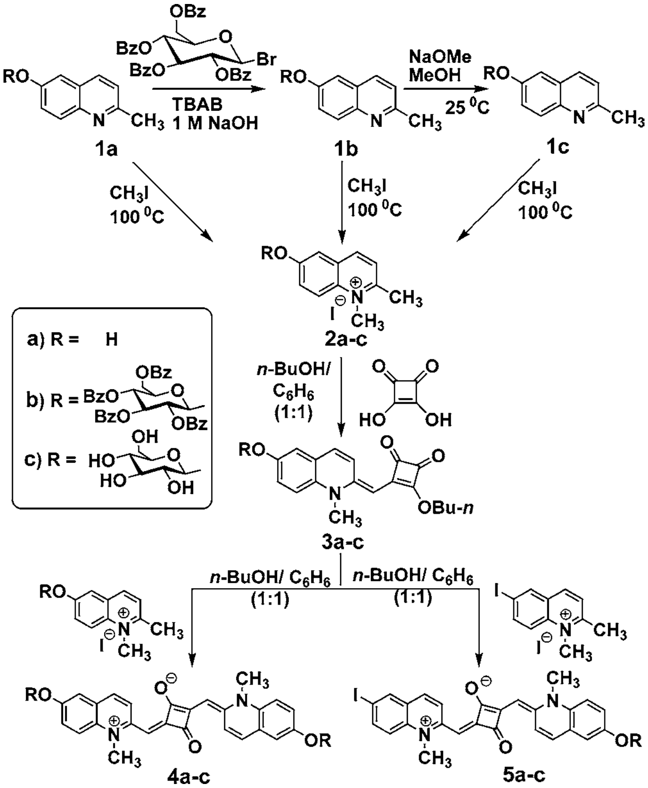
10.1021/ol052639j CCC: $33.50

symmetrical and unsymmetrical dyes. These dyes are usually prepared by the condensation between squaric acid and electron-rich aromatic, heteroaromatic, or olefinic compounds in a one-step reaction.[7] The success of the reaction depends on the nucleophilicity of the aryl species, wherein only the highly nucleophilic species was found to undergo the condensation reaction.[8] Recently,[9] we have demonstrated that quinaldinium salts with neutral and electron withdrawing substituents give the corresponding squaraine dyes in quantitative yields, whereas the salts with electron-donating substituents yield only the semisquaraine intermediates.

The objective of the present investigation has been to design squaraine dyes for PDT applications that exhibit long wavelength absorption and good cell permeability, since the transport of the sensitizer through cell membrane is critical to its effectiveness. The cellular uptake of both natural and synthetic molecules can be enhanced by modification with cationic peptides, proteins, lipids, encapsulation with liposomes, and siderophores.[10,11] Herein, we report the synthesis of new squaraine dyes covalently linked to acetyl and cellular recognition elements such as sugar and cholesterol (**4b**−**e** and **5b**−**e**) by making use of favorable electronic effects of substituents. The presence of sugar and cholesterol units in these dyes would render them amphiphilic and thereby enhance their cell permeability and eventually their use as sensitizers in PDT applications.

As shown in Scheme 1, the reaction of 6-hydroxyquinaldine (**1a**) with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide in the presence of NaOH and tetrabutylammonium bromide (TBAB) gave the corresponding quinaldine **1b**. Subsequent reaction of **1b** with sodium methoxide in methanol yielded the corresponding sugar-linked quinaldine derivative **1c**. The reaction of **1a**−**c** with methyl iodide at 100 °C in a sealed tube gave the corresponding quinaldinium salts **2a**−**c** in 90−95% yields (for details, see the Supporting Information). The dye forming reaction between 2:1 equiv of **2b** and squaric acid was carried out in a mixture (1:1) of *n*-butanol and benzene and was monitored by absorption spectroscopy and product analysis. The absorption spectrum of the reaction mixture after 0.5 h showed the formation of a band at 500 nm corresponding to the semisquaraine **3b**, which increased with time (Figure S1, Supporting Information). After about 7 h, the absorption spectrum showed the formation of a new band at 740 nm, corresponding to the symmetrical squaraine dye **4b**, which, however, insignificantly increased with the time. The reaction mixture following workup and column chromatography after 30 h gave semisquaraine **3b** (80%) as the major product, along with small amounts (10%) of the squaraine dye **4b**. Similar observations were made with the sugar-linked quinaldinium salt **2c**, which upon condensation with squaric acid gave the corresponding semisquaraine **3c** and the symmetrical dye **4c** in 85% and 10% yields, respectively. As reported previously,[9] the reaction of the model quinaldinium salt **2a** with squaric acid, under similar reaction conditions, gave only the semisquaraine intermediate **3a**, in 95% yield. Interestingly, the condensation reaction of the sugar-linked semisquaraine derivatives **3b** and **3c** with 6-iodoquinaldinium salt in a mixture (1:1) of *n*-butanol and benzene gave the corresponding unsymmetrical dyes **5b** and **5c** in good yields (Scheme 1).

Scheme 1



With a view to understand the role of electronic effects in the squaraine dye formation reaction and to improve their biological intake, we synthesized cholesteryl- and acetyl-linked quinaldine derivatives **1d** and **1e** as shown in Scheme 2. Quaternization of these derivatives with methyl iodide gave the corresponding quinaldinium salts **2d** (95%) and **2e** (85%) in quantitative yields (Supporting Information). The condensation between squaric acid and the cholesterol-linked quinaldinium salt **2d** was carried out using 1:2 equiv in a mixture (1:1) of *n*-butanol and benzene. The progress of the reaction was monitored by absorption spectroscopy as in the earlier cases (Figure 1). As is evident from Figure 1, after about 1 h, the absorption spectrum showed two absorption bands at 505 and 740 nm, corresponding to the semisquaraine intermediate **3d** and the symmetrical squaraine dye **4d**, respectively. The intensity of both these bands increased with

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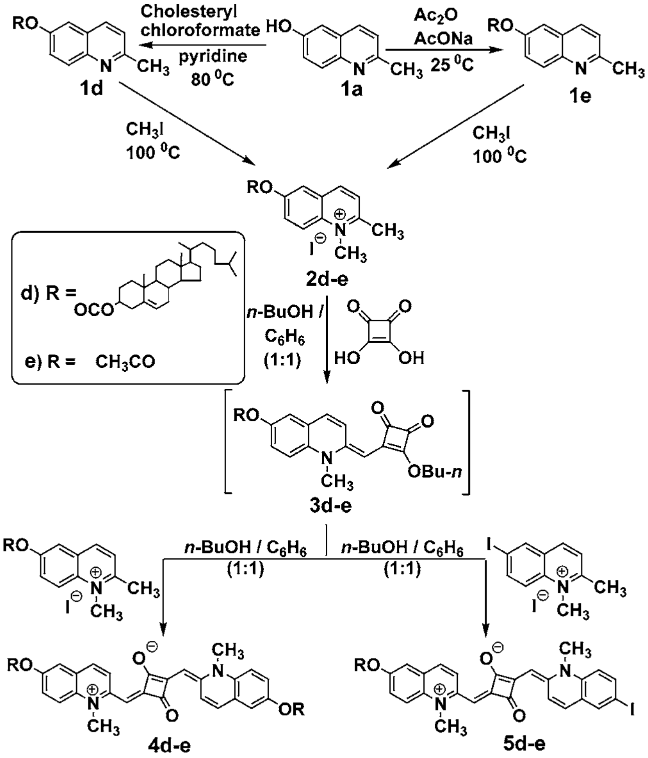
Scheme 2



the increase in reaction time. After about 7.5 h, the absorbance at 505 nm decreased with time, while the absorption band at 740 nm intensified and reached a plateau by about 20 h (inset of Figure 1). The product analysis after column chromatography gave the symmetrical squaraine dye

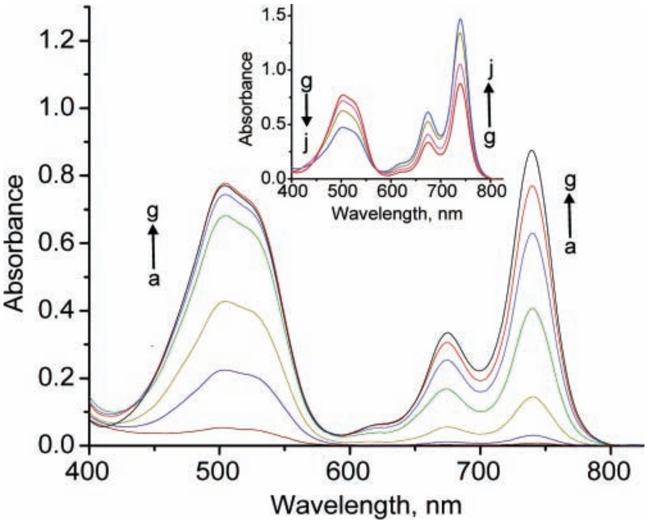


Figure 1. Change in absorption spectra obtained for the reaction between squaric acid (2.5 mM) and the quinaldinium salt **2d** (5.0 mM) at various time intervals. Time: (a) 0.5, (b) 1, (c)1.45, (d) 2.45, (e) 4, (f) 5.5, (g) 7.5 h. Inset shows the change in absorption spectra obtained for the same reaction after long time intervals. Time: (g) 7.5, (h) 10, (i) 15, (j) 20 h.

4d in quantitative yields (85%), while the semisquaraine **3d** (5%) was isolated in minor amounts. Similar observations were made with the salt **2e**. The reaction of **2e** with squaric acid gave the symmetrical squaraine dye **4e** (90%), along with 5% of **3e** (Scheme 2). The synthesis of the unsymmetrical dyes **5d** and **5e** was achieved in quantitative yields from the condensation reaction of the semisquaraine derivatives **3d** and **3e**, respectively. However, these semisquaraine intermediates were prepared separately from the reaction of the stable semisquaraine **3a** with cholesteryl chloroformate and acetic anhydride. Subsequent condensation of semisquaraine intermediates **3d** and **3e** with 6-iodoquinaldinium salt gave the unsymmetrical squaraine dyes **5d** (80%) and **5e** (85%), respectively.

Figure 2 shows the relative formation of symmetrical dyes **4a**, **4b**, and **4d** from the reaction between the squaric acid

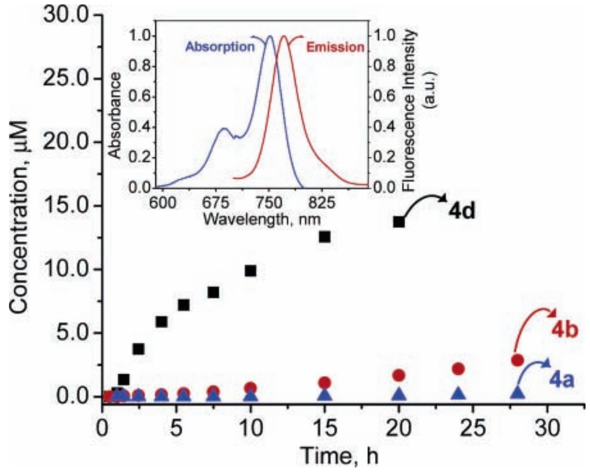


Figure 2. Change in concentration of the dye **4a** (monitored at 745 nm), **4b** (746 nm), and **4d** (740 nm) during the reaction between squaric acid and the corresponding quinaldinium salts **2a**, **2b**, and **2d**, at various time intervals. Inset shows the absorption and fluorescence emission spectra of **4d** in CHCl$_3$.

and the respective quinaldinium salts **2a**, **2b**, and **2d**, under similar conditions. The salts **2d** and **2e** linked through comparatively less electron-donating groups react with squaric acid efficiently, leading to the formation of the symmetrical dyes **4d** and **4e**, whereas the salts such as **2b** and **2c** gave the semisquaraine intermediates **3b** and **3c** as the major products, along with small amounts of the squaraine dyes **4b** and **4c**. The formation of sugar-linked squaraine dyes **4b** and **4c**, albeit in low yields from **2b** and **2c**, is quite interesting, which can be attributed to the lower electron-donating effect of the acetal linkage. The feasibility of the symmetrical dye formation from various salts was found to vary with the electron-donating power of the linked groups, acetyl > cholesteryl formate ⋙ benzoylated sugar ∼ sugar ≫ hydroxy. These results clearly indicate that the ester and acetal groups decrease the electron-donating effect of the hydroxyl group and thereby favor the squaraine dye formation, whereas electro-donating groups result only in the formation of the semisquaraine intermediates. Table 1 summarizes the absorption properties of the representative squaraine dyes. These dyes show sharp and intense absorption ($\epsilon = 0.85-2.1 \times 10^5$ M^{-1} cm^{-1}) in the long wavelength region (720−800 nm) (inset of Figure 2) and exhibit structurally similar fluorescence emission >765 nm.

Table 1. Absorption Properties of the Representative Symmetrical and Unsymmetrical Squaraine Dyes[a]

squaraine dye	**4b**[b]	**4d**[b]	**5c**[c]	**5d**[b]	**5e**[c]
λ_{max}, nm	746	740	751	743	747
ϵ, 10^5 M^{-1} cm^{-1}	1.7	2.1	0.85	2.8	1.1

[a] Average of more than two experiments. [b] $CHCl_3$. [c] DMSO.

To confirm the role of electron-donating and electron-withdrawing substituents in the squaraine dye formation, molecular modeling studies have been carried out using TITAN, for the representative quinaldinium salts **2a** (less reactive salt with electron-donating group) and **2e** (highly reactive salt with less electron-donating group) and for the corresponding semisquaraines **3a** and **3e** (Figure 3).[12,13] Starting from a fully optimized geometry, the electrostatic potential was calculated, and it was found that the electron density is significantly higher in the case of **2a**. The area around the 2-methyl group in **2a** is greenish in color, whereas it is quite bluish in the case of **2e**. This is consistent with the fact that the acidity of the 2-methyl protons increases as the electron-donating power of the protecting group decreases, which favors the formation of the squaraine dye, as observed in the case of **2e**. Similarly, in the case of the semisquaraine intermediate **3a**, the squaryl ring is yellowish green in color, whereas it is strongly bluish in the case of **3e** (Figure 3). These results confirm the fact that the presence of electron-donating substituents increases the electron density in the squaryl ring and thereby stabilizes the semisquaraine intermediate. Therefore, a further condensation reaction between the semisquaraine having weak electrophilic termini and a weak nucleophilic quinaldinium salt becomes extremely difficult, as observed in **3a**.

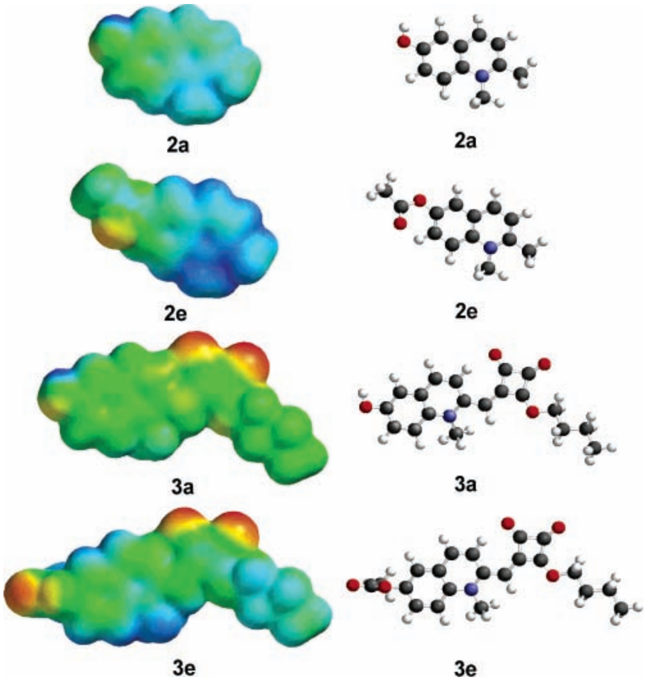


Figure 3. Electrostatic potential maps of quinaldinium salts **2a** and **2e** and semisquaraine intermediates **3a** and **3e**.

In conclusion, we have demonstrated the synthesis of new symmetrical and unsymmetrical squaraine dye conjugates with cellular recognition elements. These dyes show sharp and intense absorption in the long wavelength region, and exhibit structurally similar fluorescence emission. Their intense absorption in the photodynamic window and the presence of cellular recognition elements such as sugar, cholesterol, and heavy atoms such as iodine would increase their cellular kinetics and singlet oxygen efficiency; hence, these dyes can have potential PDT applications.

Acknowledgment. We thank the Council of Scientific and Industrial Research (COR-0003) and DST-DAAD for financial support of this work. This is contribution no. RRLT-PPD-204 from the Regional Research Laboratory (CSIR), Trivandrum, India.

Supporting Information Available: Synthetic details of the quinaldines, quinaldinium salts, semisquaraines, and squaraine dyes; ^{1}H NMR spectra of the representative semisquaraines and the squaraine dyes; absorption spectra showing the progress of the reaction; and theoretical calculation details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) All geometries were optimized using semiemperical AM1 calculations. DFT B3LYP/6-31G* single-point energy calculations were used to obtain the electrostatic potential surfaces using Titan (Wavefunction, Inc.).

(13) The color at each point on these surfaces reflects the interaction energy between a positive test charge at that point. Red indicates an attractive potential while blue represents a repulsive potential. The areas of red therefore indicate a "negative" region; yellow/green indicates a more neutral or "positive" region, depending on how bluish is the color.