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ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 1617–1623

Design and synthesis of squaraine based near infrared fluorescent probes

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Received 22 August 2006; revised 22 November 2006; accepted 7 December 2006
Available online 8 January 2007

Abstract—A novel class of dialkylanthracene containing squaraine dyes (**Sq1–3**) possessing intense absorption and emission in the NIR region has been synthesized. Structural and electronic features investigated using DFT methods suggest that the significant bathochromic shifts observed on replacing dialkylaniline by dialkylanthracene in this class of molecules can be attributed to a reduction in the HOMO–LUMO gap mainly due to enhanced hydrogen bonding between the carbonyl group of the cyclobutane ring and the neighboring aromatic hydrogen in the dyes containing the anthracene moiety. The absence of fluorescence in aqueous media and high fluorescence when encapsulated into hydrophobic domains make this class of dyes especially useful as probes for mapping such domains in biological systems.

1. Introduction

The development of new dyes with strong emission in the near infrared (NIR) region (700–900 nm) plays a crucial role in advancing techniques for noninvasive monitoring of diseased tissues.[1] Cells excited at wavelengths below 500 nm produce considerable autofluorescence mainly from flavins, flavoproteins, and NADH, which can very often swamp the probe fluorescence. This aspect combined with greater tissue penetration and reduced photochemical damage of cells makes fluorescence microscopy in the NIR region especially attractive for biomedical applications.[2] The limited number of photostable fluorochromes with strong emission in the NIR region on the other hand, forms a major bottleneck in the use of fluorescence microscopy.

Squaraines form a class of dyes with intense absorption in the red to near infrared region.[3] Their unique photochemical and photophysical properties[4] make them useful in a variety of applications such as in copiers,[5] solar cells,[6] optical discs,[7] and sensors.[8] Squarylium dyes with tertiary arylamine end groups are known to possess better stability and solubility than those with heterocyclic end groups.[3b,9] However, dyes in this class with absorbance maxima beyond 700 nm are limited.[9a,10] Our continued efforts to develop strongly luminescent NIR dyes led us to the synthesis of a novel class of tertiary arylamine derivatives (**Sq1–3**, Fig. 1).

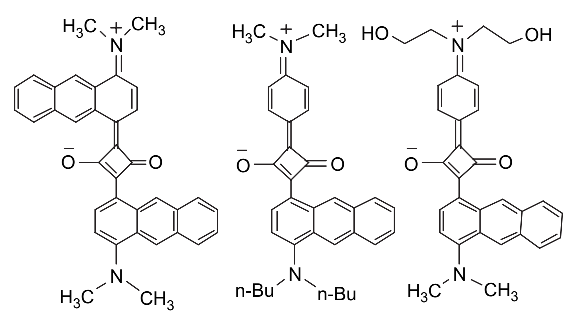

Figure 1.

2. Results and discussion

2.1. Synthesis

Several modes of addition of squaric acid to the dimethyl aminoanthracene ring are possible (see Supplementary data). On heating *N*,*N*-dimethyl-1-aminoanthracene (**DMA**) and squaric acid at 120 °C for 12 h in *n*-butanol/toluene, however, only a single product with an absorption maximum centered around 800 nm was formed (Scheme 1).[11]

Keywords: Near infrared dyes; Squaraine; Fluorescent probes; Micelles; Modeling.
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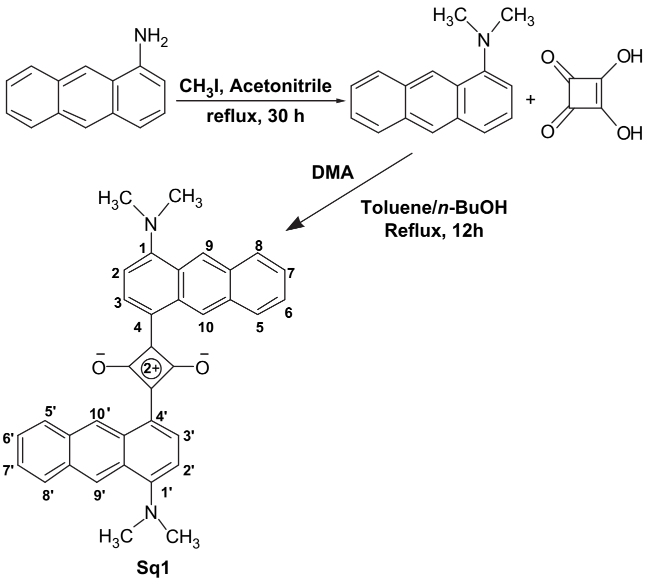
doi:10.1016/j.tet.2006.12.007



Scheme 1.

Detailed 2D NMR studies have been carried out to characterize the structure of the product formed. The [1]H NMR spectra (500 MHz) showed four doublets, two triplets, two singlets in the aromatic region (6.5–10.7 ppm), and a singlet at 3.39 ppm corresponding to $N(CH_3)_2$ protons indicating a symmetrical structure. Formation of a symmetrical dye through coupling of squaric acid at C-9 or C-10 position was ruled out due to the presence of two singlet peaks in the aromatic region. All the protons in the aromatic region were further established by ^{1}H–^{1}H COSY and ^{1}H–^{1}H ROESY experiments. It was observed that the doublets at 7.00 and 9.57 ppm were mutually coupled in ^{1}H–^{1}H COSY experiments (Fig. 2). The spatial interaction was observed between the $N(CH_3)_2$ protons and the doublet at 7.00 ppm as well as the singlet at 8.60 ppm in ^{1}H–^{1}H ROESY experiments (see Supplementary data). By comparing the 2D NMR spectra the doublet at 7.00 ppm is attributed to C-2 proton, the doublet at 9.57 ppm to C-3 proton, and the singlet at 8.60 ppm to the C-9 proton. Similarly, it was observed from ^{1}H–^{1}H COSY experiment that the triplet at 7.53 ppm (C-7) was coupled with the doublet at 7.99 ppm (C-8) and the triplet at 7.59 ppm (C-6) was coupled with the doublet at 8.34 ppm (C-5) (Fig. 2). These studies further confirm that the addition of squaric acid occurs through 4,4′-positions of **DMA**, resulting in the formation of the dye shown in Scheme 1.

The unsymmetrical squaraine dyes, **Sq2** and **Sq3**, were synthesized by the condensation of 3-[4-(*N*,*N*-dialkylamino)anthracene]-4-hydroxy-cyclobutene-1,2-dione with the appropriate dialkylanilines. The dihydroxyethanolamine moiety at the terminal position of **Sq3**, made this dye water compatible, which is an essential requirement for biological applications.

2.2. Absorption and fluorescence properties

Figure 3 shows the absorption and emission spectra of **Sq1** and **Sq2** along with that of bis[4-(*N*,*N*-dibutylamino)phenyl]squaraine (**DBAS**).[12] The photophysical properties of these dyes in toluene and ethanol are summarized in Table 1.

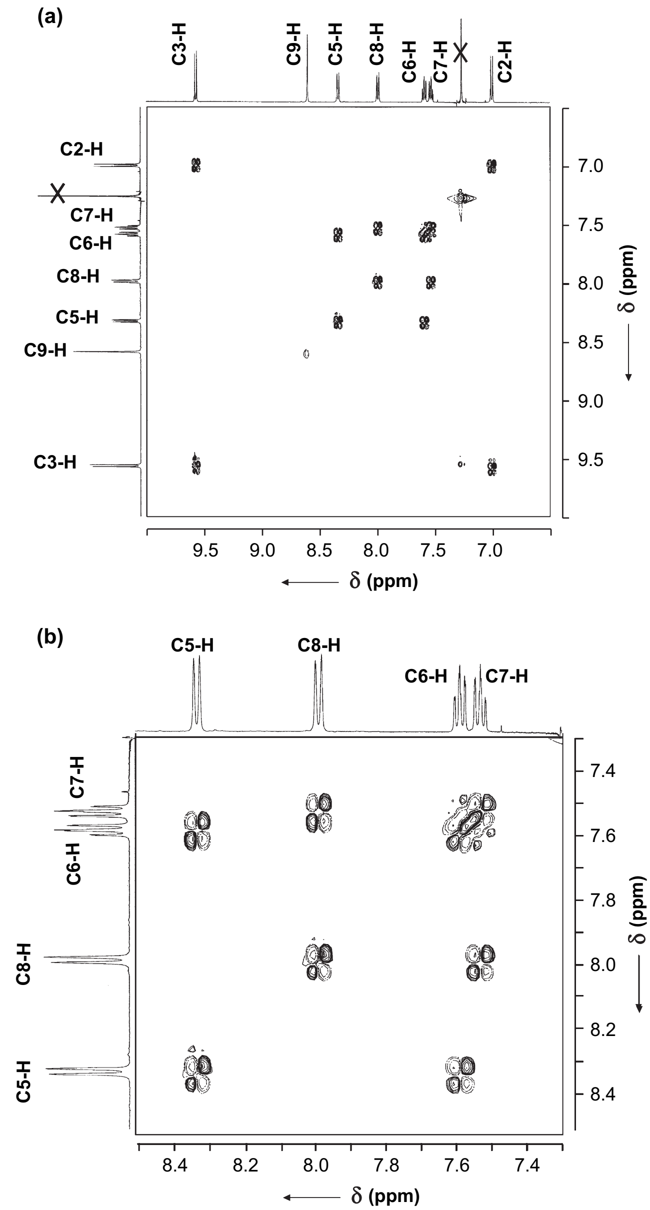


Figure 2. ^{1}H–^{1}H COSY spectrum of **Sq1** (a) 6.5–10 ppm, (b) 7.3–8.5 ppm.

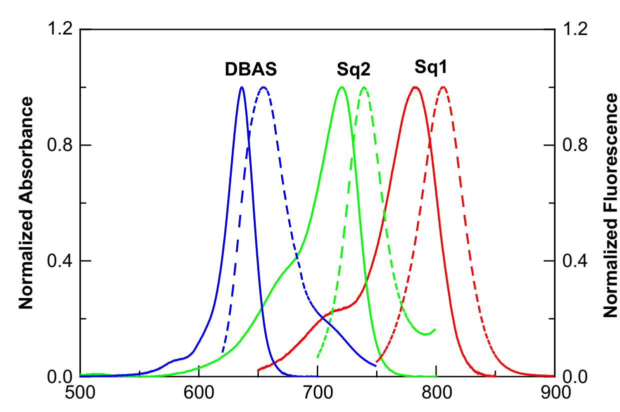


Figure 3. Normalized absorption (solid line) and emission spectra (dashed line) of **Sq1**, **Sq2**, and **DBAS** in toluene.