Optimising the synthesis and red-green-blue emission of a simple organic dye

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Synthetic dyes have been widely used in supramolecular chemistry not only to probe fundamental chemical interactions but also as components of functional materials. Most current efforts in this regard are directed at designing new host systems for the dyes. Herein we report on the study of a versatile new organic fluorophore. We describe a synthesis which affords improved yields in a convenient one pot procedure. Moreover, a simple method for predicting and controlling the dye's responses to external stimuli affords a potentially practical method for achieving red–green–blue and concomitant white light generation.

Keywords: xanthene; fluorescence; white light emission; multicolor emission; red–green–blue emission

Introduction

Supramolecular chemistry and organic fluorophores

Supramolecular chemistry and organic dyes embody respective research areas that have enjoyed great synergy for several decades. Currently there is resurgent interest in the encapsulation chemistry of organic fluorophores. In addition to classical studies of dyes as direct reporters of supramolecular interactions, several key investigations describe significant enhancements to fluorophore properties via host–dye complex formation. An excellent review summarises a variety of promising approaches in this latter regard (*1*).

One focus of our present work is the design and study of organic fluorophores that can serve as a series of unique substrates for molecular encapsulation and supramolecular chemistry. Herein we report the optimised synthesis and properties of a representative example. Chemical and photophysical properties are shown to be tunable via the controlled equilibration of the various dye ionisation states. These studies are intended to serve as a basis for developing analogous functional supramolecular systems.

Discovery of a red–green–blue emitting material and white-light emission

Fluorophores with tunable emissive properties are of current interest for multiplexing applications (*2*) as well as applications requiring white light (*3*). Two simple synthetic organic fluorophores were recently shown to emit white light (*4*). One of these (Figure 1, compound **1**)

can allow fluorescence excitation at any wavelength between 260 and 650 nm under proper conditions. It exhibits a unique emission spectrum corresponding to each excitation wavelength in various solvents. It is pH sensitive, soluble in cell culture media, stains cells and exhibits no cytotoxicity in studies carried out to date.

When excited in the ultraviolet region, white light is emitted and observed as three lambda maxima of approximately equal intensities within the red, green and blue (RGB) spectral regions (Figure 2). Fluorophores that emit white light may potentially simplify the fabrication of devices such as white organic light-emitting diodes (*5*), flat panel displays (*6*) and electronic paper displays (*7*) currently relying on blends or layers of respective red, green and (often unstable) blue emitters (*8*).

A solution containing dye **1** (30 μM, 0.25% phosphate buffer, pH 7, 50 mM) in DMSO excited anywhere between 260 and 340 nm produced three emission maxima at 390 nm (covering violet and blue), 561 nm (green) and 670 nm (red, Figure 2). Interestingly, the triple-band emission covers almost the entire visible region from 380 to 740 nm except cyan (485–500 nm). The dye shows white or near-white-light emission depending on the specific excitation. The 340 nm excitation leads to the most evenly distributed RGB emission. However, the 280 and 300 nm excitations lead to the whitest emission (Figure 2). Organic fluorophores have at times been criticised for wide band emission. However, broad visible region emission bands improve the colour rendering of white light (*9*).

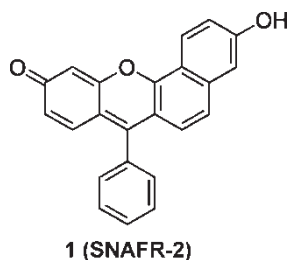*Corresponding author. Email: strongin@pdx.edu

Figure 1. Compound **1**, dubbed seminaphthofluorone-2 (**SNAFR-2**).

Unique compatibility with several common laser lines and live cell imaging filter sets

While excitation at the specific wavelengths between 280 and 340 nm result in optimal visible spectral region coverage and white-light emission, excitation at longer wavelengths shifts the emission to longer wavelengths. Examination of Figure 2 reveals that the first excitation band stretches from approximately 250 to 350 nm, while another excitation band stretches from approximately 400 to 550 nm and is centred at approximately 492 nm, an ideal wavelength for the 488 line of an Ar ion laser. Excitation at wavelengths between 425 and 525 nm leads to predominantly green fluorescence centred at *c.* 560 nm and a small amount of red fluorescence at 670 nm. Increasing the excitation to wavelengths beyond the green emission begins to excite a band stretching from approximately 560 to 670 nm. It is centred at *c.* 620 nm making excitation with a HeNe laser at 633 nm possible. Excitation of this latter band results in a very strong red fluorescence that tails into the near-infrared region.

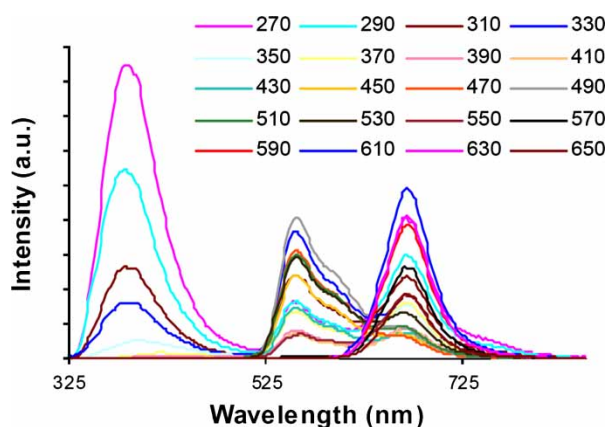Since DMSO decomposes photolytically to strong acid (*10*), we studied photostability in MeOH. It exhibits excellent stability against bleaching compared with fluorescein. The dye shows strong fluorescence in various solvents such as 0.1 M NaOH, MeOH ($\phi = 0.41$) and DMSO ($\phi = 0.33$). Cellular imaging studies reveal that the fluorophore readily enters HEp2 cells. At the concentrations tested, **1** exhibits no toxic effects. Either red, green or blue emission could be observed emanating from the regions stained by **1**, simply by varying the commercial filter set selected.
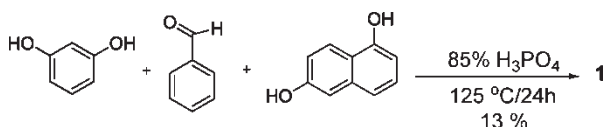
Results and discussion

Optimising the synthesis of a RGB emitting substrate

Compound **1** has been synthesised previously via the acid-catalysed condensation of 2,4-dihydroxybenzophenone and 1,6-dihydroxynaphthalene with MeSO$_3$H (*4b*). However, **1** was isolated in extremely low yield (less than 1%) due to multiple side products formed which hindered purification. As an alternative, the acid-catalysed condensation using H$_3$PO$_4$ was tested. It had been used in the synthesis of other asymmetric xanthene dyes (*11*). A convenient three component, one-pot condensation reaction between resorcinol, 1,6-dihydroxynaphthalene and benzaldehyde promoted by 85% H$_3$PO$_4$(aq) at 125°C for 24 h (Scheme 1) furnishes **1** in a 13% isolated yield. The reaction is concentration dependent. A 0.18 M concentration of each substrate in the reaction mixture is found optimal in suppressing by-product formation. The ^{1}H NMR, UV–vis and fluorescence spectra were all consistent with the previously published data for **1**. Considering the often prohibitive cost of long-wavelength emitting dyes, and our prior unoptimised synthesis, this is an attractive protocol for attaining useful amounts of **1**.

Facile tuning of multicolour emission

RGB are the primary colours of light. They can be mixed to attain any other colour. The relative populations of the various emissive states of **1** are sensitive to a number of environmental factors. Excitation wavelength (Figure 2) plays a major role in this regard as do choice of solvent and pH. Additionally, it has been determined previously that the blue region emission arose as a result of a controllable solvolysis reaction at the methine carbon of **1** since this gives rise to an isolated naphthol moiety (Scheme 2). The green and red emissions were found to arise via the neutral and anionic states of **1**, respectively.

We now report that in order to control the relative intensities of the RGB emitting states, one can add small amounts of neutral buffer solution to a DMSO solution of **1** and form its solvate in one pot. To **1** (30 μM in DMSO), 7 μl of 0.1 M HCl, 50 μl of MeOH and 60 μl of 50 mM phosphate buffer (pH 7.5) are added. The HCl promotes solvolysis (*4b*). Subsequent addition of buffer changes the red and green emission intensities. Figure 3 shows how titration of a 30 μM DMSO solution of compound **1** and its

Figure 2. Excitation of a solution of 30 μM **1** in DMSO with 0.25% phosphate buffer [50 mM (pH 7.4) at 320 and 340 nm] yields RGB peaks of approximately equal intensity. Excitation at longer wavelengths shifts the emission to longer wavelengths.

Scheme 1. One-pot synthesis of **1**.

MeOH adduct with microlitre aliquots of 50 mM phosphate (pH 7.5) buffer solution results in significant final buffer concentration-dependent variations in the relative intensities of the emission peaks. This is a simple way to tune the relative intensities of the primary colours.

Experimental section

Materials and methods

Unless otherwise indicated, all commercially available starting materials were used directly without further purification. 1,6-Dihydroxynaphthalene was recrystallised from toluene. Silica gel Sorbent Technologies 32–63 μm was used for flash column chromatography. ^{1}H-NMR spectra were obtained on a ARX-400 Advance Bruker spectrometer. Chemical shifts (δ) are given in ppm relative to DMSO-d_6 (2.50 ppm, 1H). MS (LRMS) and ESI spectra were obtained at the Mass Spectrometry Facility of Georgia State University, Atlanta, GA.

Fluorescence spectroscopy

Fluorescence spectra were collected on a Cary Eclipse Fluorescence Spectrophotometer using a 1 cm path length quartz cell. Emission spectra were collected following excitation with a Xenon pulse lamp, pulsed at 80 Hz. Emission wavelengths were scanned with 2 nm step size, emission bandwidth 5 nm, excitation bandwidth 2.5 nm and the averaging time per point was 0.1 s, and 600 V was applied to a R928 PMT.

One-pot synthesis of 1

1,3-Dihydroxybenzene (0.5 g, 4.54 mmol) and 1,6-dihydroxynaphthalene (0.727 g, 4.54 mmol) were ground to a fine powder and transferred to a 100 ml round bottom flask. About 25 ml of 85% Phosphoric acid was added. The mixture was stirred to obtain a uniform suspension. Benzaldehyde (0.482 g, 4.54 mmol) was added in one portion. A condenser was secured on the flask. The mixture was stirred and heated at 125°C, 24 h. The mixture was allowed to cool to rt and poured into 200 ml of water. The dark red solid was filtered and washed with water until a neutral pH was attained. The solid residue was dissolved in MeOH, dried over Na$_2$SO$_4$, filtered and the solvent evaporated under vacuum, to leave a dark red residue. The target compound was isolated by flash chromatography on silica gel using EtOAc:MeOH 95:5 for elution. Yield: 0.205 g, 13%. ^{1}H NMR, LRMS (ESI), UV–vis and fluorescence spectra were consistent with the previously published data (*4b*).

Fluorescence titration to obtain RGB 1

A 1 mM stock solution of **1** was prepared in MeOH. An aliquot of the stock solution was transferred to a vial to prepare 1 ml of a 30 μM solution of the fluorophore.
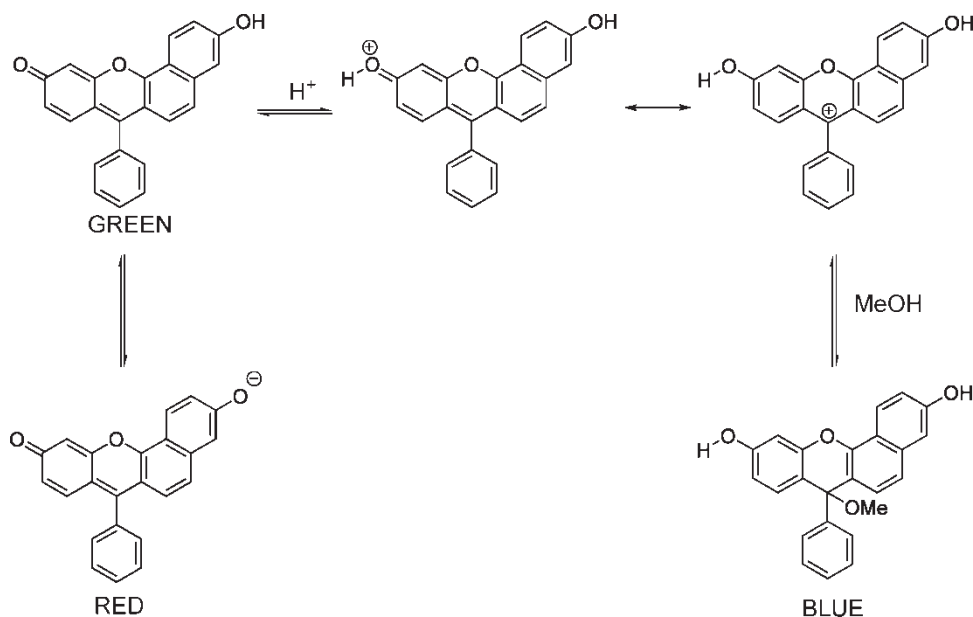
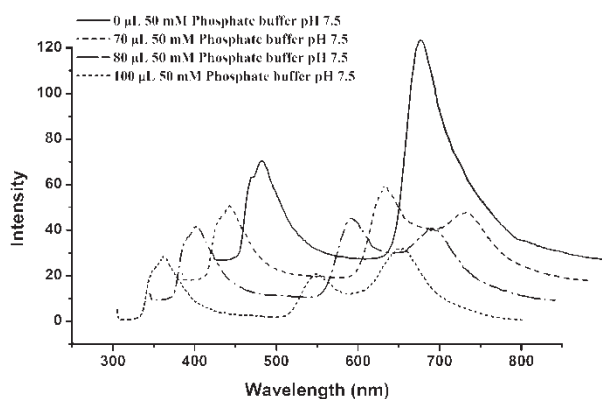Scheme 2. Equilibrium and molecular origin of the RGB states of **1**.

Figure 3. Tunability of the emission intensities of **1** via successive addition of sub-ml amounts of neutral buffer solution.

The MeOH was removed by evaporation under vacuum. To the solid residue were added 1 ml of DMSO, 7 µl of 0.1 M HCl, 50 µl of MeOH and 60 µl of 50 mM phosphate buffer (pH 7.5). The emission spectrum of the solution was obtained using an excitation wavelength at 300 nm. A spectrum with two emission bands was obtained [361 nm (water adduct) and 556 nm (neutral form)]. To obtain the red emission (652 nm, anionic form), additional 50 mM phosphate buffer (pH 7.5) was added in increments. The red and green band intensities were varied upon buffer addition as shown in Figure 3. The emission fluorescence spectrum was collected after each buffer addition.

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Note

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