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Design and Synthesis of a Chemiluminescent Solvatochromic Dye

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Chemiluminescent solvatochromic dye has been synthesized by the condensation of a chemiluminescent moiety into a fluorescent solvatochromic dye via the Suzuki–Miyaura cross-coupling. The chemiluminescent wavelength is shifted by solvent polarity, and ratiometric measurement enables the accurate determination of the proportion of water in an aqueous acetone solution.

Chemiluminescence (CL) refers to the emission of light as the result of certain chemical reactions. Unlike fluorescence, CL produces very little background noise as it does not require an excitation source beam. CL detection is advantageous with respect to its sensitivity due to high signal-to-noise ratio.[1] On the other hand, CL intensity is easy to change, not only by the modification of reaction conditions such as temperature, pH, and solvents used, but also by changing the dye concentration. However, these effects of these changes are indistinguishable from each other, and consequently, a number of quantitative problems associated with CL detection remain to be solved.[2,3] In general, ratiometric techniques are preferred for quantitative measurements because the ratio of intensities is independent with respect to changes made in dye concentration.[4] For this purpose, ratiometric indicators based on resonance energy transfer (RET), which exhibit changes in dual emission intensity, were developed. However, these indicators consist of large fluorescent protein molecules, and may inhibit many of the biological reactions to be observed. Meanwhile, it is difficult to control the distance between dyes (1–10 nm) in fluorescent dye-based indicators, and the RET mechanism has been applied almost exclusively to long wavelength emission.[5–8] Therefore, a small and simple dye-based indicator in which CL wavelength is shifted by microenvironmental changes is required for monitoring biological reactions with high sensitivity and quantitativity.

Fluorescent solvatochromic dyes are chromic dyes in which the emission wavelength is changed by the polarity of the solvent molecules surrounding them. These dyes are made up of small and simple molecules, composed of an electron-donating group, an aromatic ring, and an electron-withdrawing group.[9–11] Recently, we succeeded in preparing a series of fluorescent solvatochromic dyes by condensation of the electron-donating, aromatic ring, and electron-withdrawing moieties via Suzuki–Miyaura cross-coupling reactions.[12] Applying the same synthetic strategy, we have tried to design a chemiluminescent solvatochromic dye using CL molecules as the electron-withdrawing moiety (Figure 1). Phthalhydrazide was selected as the electron-withdrawing group for the following three reasons: (1) phthalhydrazide has a partial luminol structure,[13] which is a representative CL dye; (2) the two carbonyl groups exhibit strong electron-withdrawing properties; and (3) the phthalimide group can be easily converted to a phthalhydrazide group. A 4-dihexylaminophenyl group was chosen as the electron-donating moiety, as the two alkyl chains facilitate purification due to their high solubility in organic solvents. Thiophene was used as the aromatic ring as it demonstrates good photophysical properties in fluorescent solvatochromic dyes.[12]

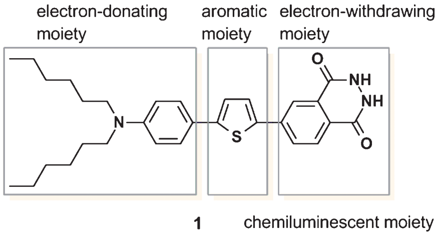
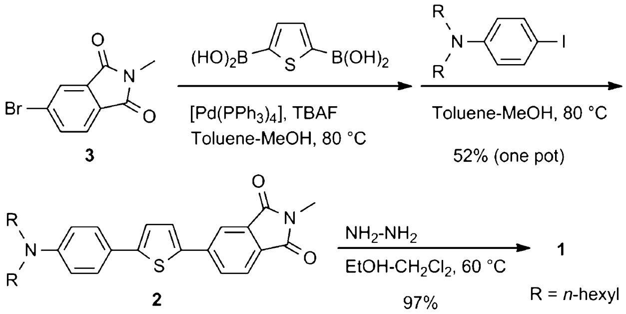


Figure 1. Molecular design of chemiluminescent solvatochromic dye **1**.



Scheme 1. Synthesis of phthalhydrazide **1**.

The target compound **1** was synthesized as shown in Scheme 1. A phthalimide intermediate **2** was prepared through one-pot synthesis using a Suzuki–Miyaura cross-coupling reaction, and subsequent treatment of **2** with hydrazine afforded **1** in 50% overall yield. Compounds **1** and **2** were readily purified by silica gel column chromatography due to the presence of alkyl chains in the electron-donating moiety. Their structures were fully characterized by ^{1}H and ^{13}C NMR spectra, and ESI-HRMS data.[15]

Dye **1** has very low solubility in water due to its hydrophobicity. However, an aqueous acetone solution of **1** exhibits strong chemiluminescence under similar reaction conditions as for luminol. Furthermore, the CL color changes from blue-green to greenish yellow, depending on the concentration of water (Figure 2). To evaluate the effect of solvents on the CL properties of **1**, we measured the CL spectra of **1** in pure organic solvents of different polarities when added to an aqueous solution of NaOH, $K_3[Fe(CN)_6]$, and H_2O_2. The CL maximum progressively shifted toward longer wavelengths with increases in solvent polarity (Figure 3a). The $E_T(30)$ value is

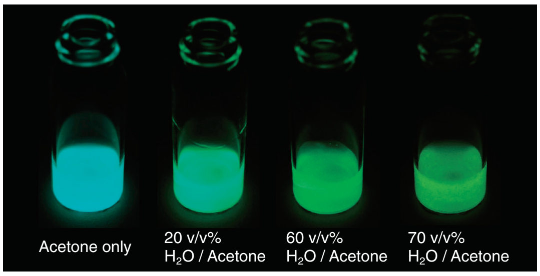


Figure 2. Photographs of the CL color (Figure S4[15]).

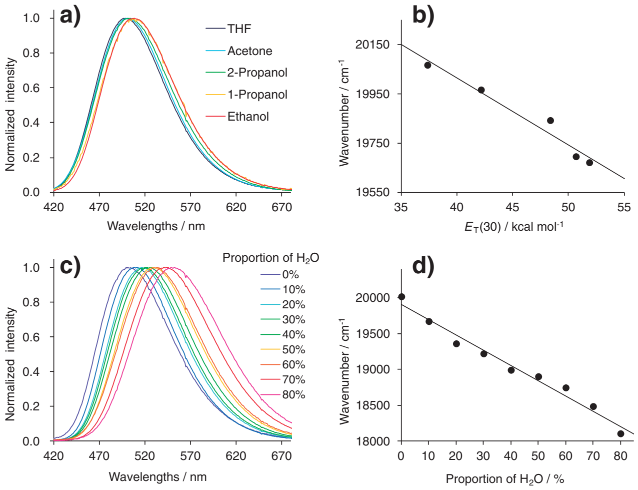


Figure 3. a) Normalized CL spectra of **1** in the pure solvents (THF, acetone, 2-propanol, 1-propanol, and ethanol). b) Correlation of the emission maxima with the solvent polarity parameter $E_T(30)$. c) Normalized CL spectra of **1** in the mixed solutions. d) Correlation of the emission maxima with the proportion of H_2O.

commonly used as an empirical indicator of solvent polarity.[14] Figure 3b shows a plot of $E_T(30)$ versus the CL maximum in wavenumbers. This plot gave an almost linear correlation ($r^2 = 0.9586$). This tendency was similar to those of the emission maximum of the fluorescent solvatochromic dyes. This result suggests that dye **1** clearly exhibits chemiluminescent solvatochromism. In order to effect a significant change in the solvent polarity and the following emission wavelength, CL spectra were also measured in the aqueous acetone solution with different concentrations of water. The CL maximum can be measured up to 80% water concentration, and shifted to longer wavelengths (499 to 552 nm) with increasing water concentration (Figure 3c). Figure 3d shows a plot of the proportion of water against the CL maximum in wavenumbers. This plot also gave an excellent linear correlation between the water concentration and the CL wavenumber ($r^2 = 0.9766$). Dye **1** showed similar chromism in the mixed solutions.

In order to assess the quantitative reliability of the ratiometric techniques, we measured CL spectra of dye **1** in the mixed solutions with various dye quantities (from 0.10 to 10.0 µg) and proportions of water (from 0 to 80 v/v%) (Figures S1 and S2).[15] Representative spectra with various amounts of dye in 0 and 60 v/v% solutions are shown in

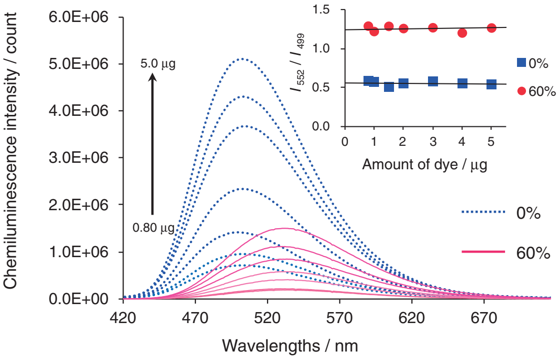


Figure 4. Representative CL spectra of **1** corresponding to different amounts of dye (0.80 to 5.0 µg) in 150 µL of 0 v/v% H_2O/acetone (blue dash line) or 60 v/v% H_2O/acetone (red solid line). Inset: the plots of ratio I_{552}/I_{499} versus amount of dye in 0 v/v% H_2O/acetone (blue square) or 60 v/v% H_2O/acetone (red circle).

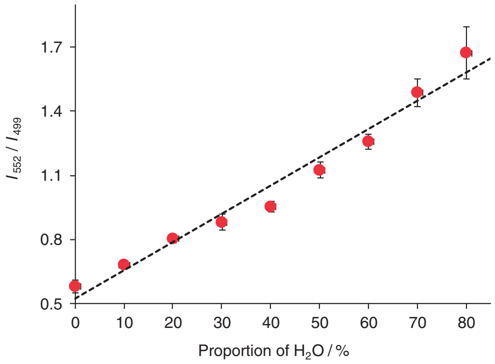


Figure 5. The plots of ratio (I_{552}/I_{499}) as a function of H_2O concentration. Error bars represent average ± standard deviations of $N = 10$ or 20 independent experiments.

Figure 4. The spectra show a maximum at 499 nm in 0 v/v% solutions, and at 533 nm in 60 v/v% solutions, respectively. The CL intensities varied with respect to the proportion of water used. For instance, the CL intensities in 0 v/v% solutions are 3.9-fold higher than those in 60 v/v% solutions on average. However, the ratios of the intensity at 499 nm (I_{499}) to that at 533 nm (I_{533}) remained relatively constant both in 0 and 60 v/v% solutions. In other words, it is possible to distinguish clearly between the dye concentration and the solvent polarity. Figure 5 shows comparisons between the proportion of water and the ratio (I_{552}/I_{499}) in all samples. An excellent linear relationship was obtained from the titration curve ($r^2 = 0.9699$), and thus the proportion of water can be determined accurately regardless of the dye amount.

In summary, we have succeeded in creating a chemiluminescent solvatochromic dye **1** by replacing the electron-withdrawing moiety of the thiophene-based solvatochromic fluorophore with phthalhydrazide, which possesses both electron-withdrawing and CL properties. Dye **1** enables the quantitative measurement of the proportion of water in an aqueous acetone solution using the ratio of CL intensities. The synthetic strategy via the Suzuki–Miyaura cross-coupling reaction is also applicable to the adjustment of CL wavelength and solubility by replacing the electron-donating and aromatic moieties. Our new focus will be to develop a ratiometric CL analytical technique using CL solvatochromic dye derivatives, which would have the added advantages of high sensitivity and quantitativity.

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