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# A Systematic Analysis of Aromatic Heterocyclic Rings in Solvatochromic Fluorophores

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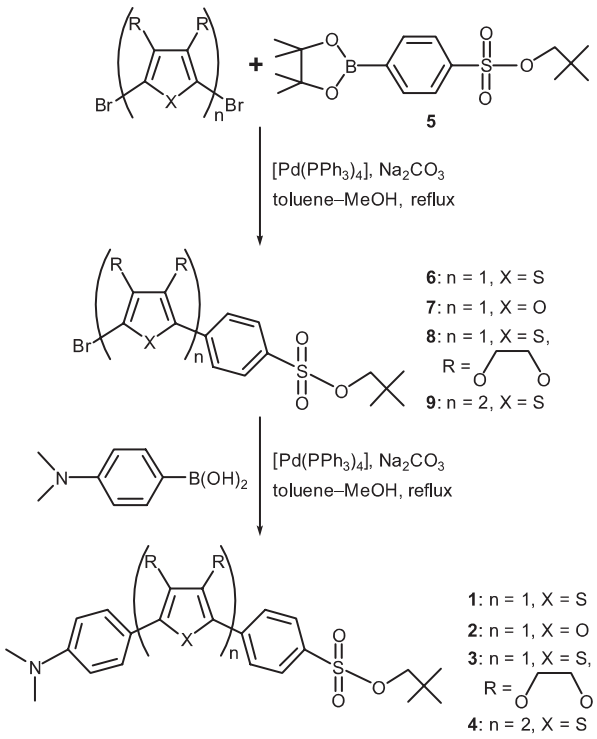
The Suzuki–Miyaura cross-coupling was found to be effective for the modification of aromatic heterocyclic rings in solvatochromic fluorophores, thereby providing quantitative evaluation of the ring effects on photophysical properties. The effect of heteroatom, a $\beta$-substituent, and the number of rings in the aromatic moiety were investigated systematically.

A fluorescent indicator is a powerful tool for the imaging of various living biological systems due to its high sensitivity, good spatial and time resolution, and low invasiveness.[1] Among these indicators, solvatochromic fluorophores, which exhibit modest changes in their absorption spectra but large changes in their emission spectra through their electronic interaction with solvents or solute molecules, provide a range of information on the surrounding microenvironment.[2] For this reason, fluorophores have been used in the monitoring of various biological processes that demonstrate polarity changes, such as antigen–antibody reactions, denaturation and renaturation of DNA,[3] and cell membrane dynamics,[4] through the measurement of two different emission wavelengths. To date, several solvatochromic fluorophores, such as *N*,*N*-dimethyl-6-propionyl-2-naphthylamine (PRODAN),[5] 5-(dimethylamino)naphthalene-1-sulfonyl ethylenediamine (dansyl EDA),[6] 4-fluoro-7-nitrobenzofurazan (NBD),[7] and *N*-(2-aminoethyl)-4-[5-[4-(dimethylamino)phenyl]-2-oxazolyl]benzenesulfonamide (Dapoxyl SEDA),[8] have been reported. All of these compounds are composed of three moieties: electron-donating, aromatic, and electron-withdrawing. However, the chemical structures of these compounds are not consistent and, therefore it is difficult to evaluate the effects of each moiety. Furthermore, these compounds are excited by near UV light, which limits their applications in biological fields.[9] Thus, to broaden their biological applications, it remains necessary to develop new fluorophores that can be excited by visible light at relatively longer wavelengths.

In order to further improve their photophysical properties, various combinations of aromatic heterocyclic rings with both electron-donating and -withdrawing aromatic groups need systematic investigation. However, solvatochromic fluorophores are usually synthesized in a stepwise manner and electron-donating and -withdrawing moieties cannot be freely substituted, making it difficult for systematic studies on the effects of molecular structures.

The Suzuki–Miyaura cross-coupling reaction provides a feasible approach to the condensation of aromatic ring systems from stable and easily manipulated boronic acid derivatives.[10] This reaction is widely used, not only in electronics and pharmacology, but also for the synthesis of photofunctional molecules.[11] To develop solvatochromic fluorophores, it is advantageous to replace the aromatic moiety, regardless of the electron-donating and -withdrawing moieties. In this paper, we have investigated new synthetic approaches to the synthesis of solvatochromic fluorophores: the three starting moieties were synthesized separately, before being connected directly by the hetero-coupling reactions.




**Scheme 1.** Synthesis of fluorophores **1**–**4**.

Herein, we describe the synthesis of a series of fluorophores **1**–**4** with different aromatic rings via the Suzuki–Miyaura cross-coupling reaction (Scheme 1). Four different five-membered heterocycles, thiophene,[12] furan,[13] bithiophene,[12] and 3,4-ethylenedioxythiophene (EDOT),[14] were selected as the aromatic heterocyclic rings. A 4-dimethylaminophenyl group was used as the electron-donating moiety in the same manner as dansyl amide and Dapoxyl SEDA. Furthermore, a 4-(neopentyloxysulfonyl)phenyl group was chosen as the electron-withdrawing group for the following three reasons: (1) the sulfonyl group is strongly electron-withdrawing; (2) the neopentyl group improves solubility in organic solvents thereby facilitating purification; and (3) a 4-(neopentylsulfonyloxy)phenyl group can be easily cleaved to form a sulfonic acid group[15] thereby improving

**Table 1.** Spectral properties fluorophores **1**–**4** in different solvents[a]

| Fluorophore | Toluene | | | | Dichloromethane | | | | DMF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\lambda_{abs}$ /nm | $\varepsilon$ /$M^{-1}$ $cm^{-1}$ | $\lambda_{em}$ /nm | $\Phi_{fl}$ | $\lambda_{abs}$ /nm | $\varepsilon$ /$M^{-1}$ $cm^{-1}$ | $\lambda_{em}$ /nm | $\Phi_{fl}$ | $\lambda_{abs}$ /nm | $\varepsilon$ /$M^{-1}$ $cm^{-1}$ | $\lambda_{em}$ /nm | $\Phi_{fl}$ |
| **1** | 394 | 27000 | 482 | 0.60 | 394 | 27000 | 517 | 0.44 | 397 | 27000 | 551 | 0.24 |
| **2** | 388 | 30000 | 468 | 0.67 | 391 | 30000 | 512 | 0.48 | 392 | 30000 | 541 | 0.28 |
| **3** | 404 | 34000 | 482 | 0.58 | 405 | 35000 | 518 | 0.41 | 407 | 34000 | 546 | 0.26 |
| **4** | 419 | 32000 | 515 | 0.37 | 421 | 33000 | 563 | 0.22 | 424 | 33000 | 590 | 0.06 |

[a]Quantum yields were measured using quinine sulfate ($\Phi_{fl} = 0.546$ in 0.5 M $H_2SO_4$) as the standard.

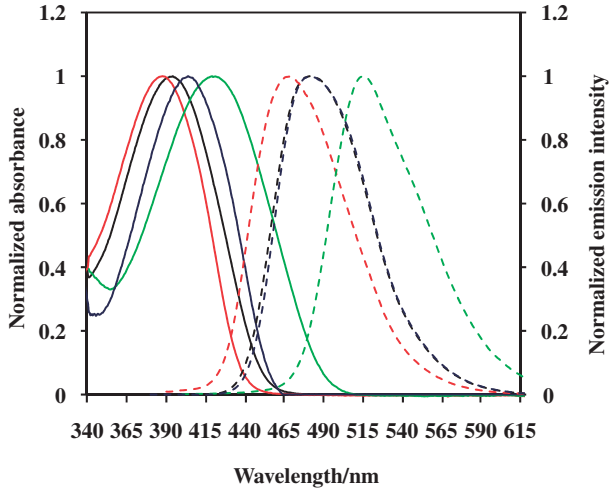



**Figure 1.** Normalized absorption (solid lines) and emission spectra (dashed lines) for fluorophores **1**–**4**. —: fluorophore **1**, —: fluorophore **2**, —: fluorophore **3**, and —: fluorophore **4**.

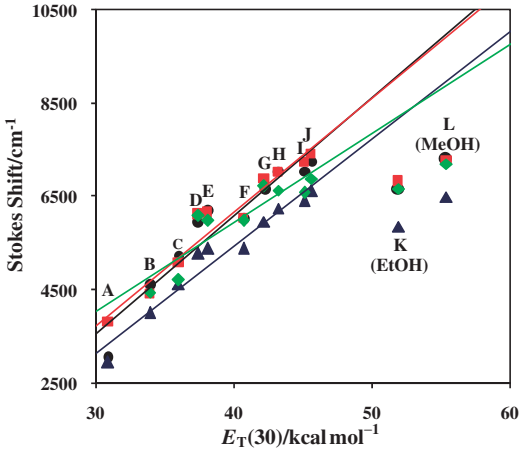



**Figure 2.** The plot of the Stokes shift of fluorophores **1**–**4** versus solvent polarity parameter $E_T$ (30). A: cyclohexane; B: toluene; C: 1,4-dioxane; D: tetrahydrofuran; E: ethyl acetate; F: dichloromethane; G: acetone; H: *N*,*N*-dimethylformamide; I: dimethyl sulfonate; J: acetonitrile; ●: fluorophore **1**; ■: fluorophore **2**; ▲: fluorophore **3**; ◆: fluorophore **4**. The slopes for the fits to aprotic solvents are 253 ($R^2 = 0.906$), 244 ($R^2 = 0.939$), 230 ($R^2 = 0.947$), and 191 $cm^{-1}$ (0.805), respectively.

water solubility and the ability of the resultant probes to attach to amino group in proteins.

With the above in mind, we investigated the synthesis of novel fluorophores **1**–**4**.[16] We found that they were readily synthesized in four steps from commercially available substances by Suzuki–Miyaura cross-coupling reactions of dibromo derivatives, with the boronic ester of the electron-withdrawing and the boronic acid of the electron-donating components (Supporting Information; SI[18]). Their structures were fully characterized by $^1$H, $^{13}$C NMR spectra, and FAB-HRMS data. The synthetic pathways to fluorophores **1**–**4** are given in the SI.[18]

The photophysical properties of fluorophores **1**–**4** measured in solvents with different polarities are summarized in Table 1 and SI,[18] while Figure 1 shows the normalized absorption and emission spectra in toluene. The excitation wavelengths of the absorption spectra were found to be more favorable for biological samples compared with Dapoxyl dye, because these fluorophores would be excited efficiently using a common 405 nm blue diode laser. The absorption maxima $\lambda_{abs}$ of **1**–**4** changed little, allowing improved ratiometric determination using only a single beam. On the other hand, the emission maxima $\lambda_{em}$ of **1**–**4** exhibited large bathochromic shifts, increasing solvent polarity. All fluorophores showed fluorescent solvatochromism.

We also evaluated the effect of the aromatic heterocyclic ring moiety on fluorescent solvatochromic properties.[17] The plot for **1** shows a good linear correlation with aprotic solvents. In polar protic solvents, such as methanol and ethanol, however, the plots for **1** deviate from linearity. A similar phenomenon was observed for Dapoxyl dye. The absorption maximum of fluorophore **1** was shifted further to the red end of the spectrum than was that of Dapoxyl dye. Fluorophore **2**, in which the hetero atom in the aromatic ring was replaced, has almost the same plot pattern. The absorption maximum of **2** is slightly more blue-shifted than that of **1**. Moreover, the synthetic yield of fluorophore **2** was low, probably due to the relative instability of the furan intermediate. The furan ring appears to be useless as the aromatic ring. Fluorophore **3**, in which substituent groups at the $\beta$-positions of thiophene were introduced, showed the same slope as, but has a lower intercept than **1**. The absorption maximum of **3** is red-shifted slightly. Fluorophore **4**, having bithiophene as the aromatic heterocyclic ring, exhibits a similar extinction coefficient and both the absorption and emission maxima of **4** were more strongly red-shifted. However, the slope of the plot was the lowest value (Figure 2) and the fluorescent quantum yield ($\Phi_{fl}$) was lower than those of fluorophore **1**–**3**. These results suggest that bithiophene was an unfavorable aromatic ring for the solvatochromic fluorophores. As mentioned above, the strategy for the improvement of solvatochro-

mic fluorescent probes could be identified through a comparison of fluorophores **1**–**4**, containing different heterocyclic aromatic rings.

In conclusion, we have efficiently synthesized a series of solvatochromic fluorophores **1**–**4** with different aromatic heterocyclic rings via Suzuki–Miyaura cross-coupling reactions. The effect of heteroatom, a $\beta$-substituent, and the number of rings in the aromatic moiety were investigated systematically. Our future research will focus on the modification of the structure of these fluorophore, employing alternative electron-donating and -withdrawing moieties, to obtain various photophysical properties.

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