

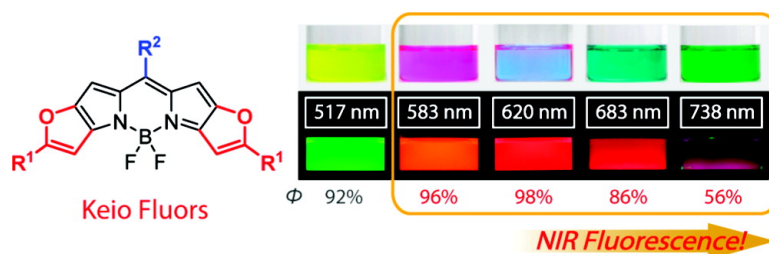
Communication

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## Bright, Color-Tunable Fluorescent Dyes in the Visible–Near-Infrared Region

Keitaro Umezawa, Yuki Nakamura, Hiroshi Makino, Daniel Citterio, and Koji Suzuki\*

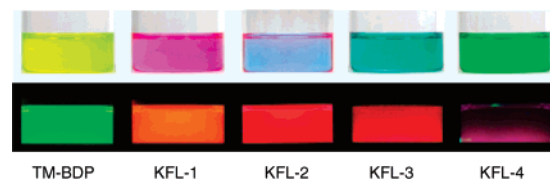
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Bright and long-wavelength fluorescent dyes, which emit light in the red or near-infrared (NIR) region, have been recently required as new standard luminescent dyes, fluorescent probes for biomolecules and further applications. Especially, the demand for NIR fluorescent dyes for noninvasive and simple diagnosis techniques such as *in vivo* imaging and photodynamic therapy (PDT) has been drastically growing.<sup>1</sup> The spectral range between 650–900 nm (often called “optical window”) has many advantages: significant reduction of the background signal due to the lowest autoabsorption and autofluorescence of biomolecules in the NIR region, low light scattering and deep penetration of the NIR light, and the possibility to use low-cost excitation light sources.<sup>2</sup> While various NIR fluorescent dyes, as for example typified by cyanines, have been developed, many of them suffer from certain insufficient optical characteristics such as low fluorescence quantum yields or a lack of photostability.<sup>3</sup> Hence, alternative NIR fluorescent dyes are still required.

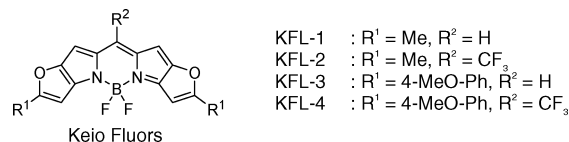
Boron-dipyrromethene (BDP) fluorescent dyes have been recognized as one of the alternatives because of their excellent characteristics such as intense fluorescence quantum yields ( $\phi$ ), sharp absorption and fluorescence emission spectra, and high photo- and chemostability.<sup>4</sup> However, they have relatively short fluorescence emission maxima (around 500 nm) and relatively low extinction coefficients ( $\epsilon$  around  $80\,000\text{ M}^{-1}\text{ cm}^{-1}$ ) compared to other dyes such as cyanines and phthalocyanines (often over  $200\,000\text{ M}^{-1}\text{ cm}^{-1}$ ). To create alternative BDPs exhibiting red or NIR fluorescence and having high extinction coefficients, various approaches have been attempted by different research groups as described in a recent review:<sup>4a</sup> (1) introduction of electron-donating moieties,<sup>5</sup> (2) replacement of a C atom by a N atom,<sup>6</sup> (3) rigidification of rotatable moieties,<sup>7</sup> (4) extension of the  $\pi$ -conjugation by introduction of methylene structures,<sup>8</sup> and (5) the extension of the  $\pi$ -conjugation by the fusion of aryl moieties.<sup>9</sup> In particular, the fusion of aryl moieties is expected to result in an extension of the  $\pi$ -system while retaining the rigidity and to increase the planarity of the BDP chromophore. Therefore, we considered that an increase of the quantum yield and of the extinction coefficient could be achieved by this method. However, relatively few reports have been published concerning this method, so far. Although the positive effect of the fusion of aryl moieties is known, the fusion of  $\pi$ -sufficient heteroaryl moieties has not been investigated well, yet. Hence, we focused on the fusion of  $\pi$ -sufficient heteroaryl moieties, such as furan, into the BDP chromophore. On the basis of this concept, we designed and synthesized the boron–difuropyrromethene fluorescent dyes named Keio Fluors (KFL, Chart 1).

A solution of KFL-1 in chloroform exhibits a vivid pink absorption color and bright orange fluorescence emission (Figure 1). The optical properties of KFL-1 in chloroform (shown in Table 1 and Figure 2) indicate a significant spectral red-shift with retention of extremely sharp absorption and fluorescence spectral bands (full width at half-maximum height:  $\text{fwhm} = 457\text{ cm}^{-1}$ ), and outstand-



**Figure 1.** Absorption (upper row) and fluorescence emission (bottom row) colors of TM-BDP and Keio Fluors in chloroform.

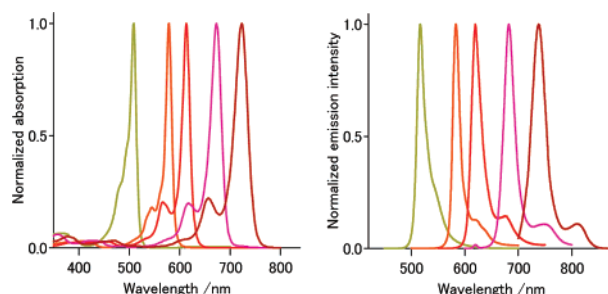
### Chart 1. Chemical Structures of Keio Fluors



**Table 1.** Optical Properties of TM-BDP and Keio Fluors<sup>a</sup>

dye	$\lambda_{\text{abs}}$ (nm)	$\lambda_{\text{flu}}$ (nm)	fwhm <sup>b</sup> (nm)	$\epsilon^c$ ( $\text{M}^{-1}\text{ cm}^{-1}$ )	$\phi^{c,d}$
TM-BDP	509	517	19 (704)	80000 <sup>e</sup>	0.92 <sup>f</sup>
KFL-1	579	583	16 (457)	202000	0.96
KFL-2	613	620	22 (574)	185000	0.98
KFL-3	673	683	24 (516)	288000	0.86
KFL-4	723	738	31 (577)	253000	0.56

<sup>a</sup> Measured in chloroform. <sup>b</sup> The values converted to wavenumbers ( $\text{cm}^{-1}$ ) are shown in parentheses. <sup>c</sup> Error: within 5% for KFL-1 and -2; within 10% for KFL-3 and -4. <sup>d</sup> Reference dyes are shown in Supporting Information. <sup>e</sup> Reference 5a. <sup>f</sup> Reference 13.



**Figure 2.** Normalized absorption (left) and fluorescence emission (right) spectra of TM-BDP and Keio Fluors in chloroform (lime, TM-BDP; orange, KFL-1; red, KFL-2; purple, KFL-3; and brown, KFL-4, respectively).

ingly bright fluorescence emission ( $\phi = 0.96$ ) at 583 nm. These excellent optical properties, especially the high quantum yield, can be observed in any solvent ( $\phi_{\text{toluene}} = 0.94$ ,  $\phi_{\text{CHCl}_3} = 0.96$ ,  $\phi_{\text{THF}} = 0.93$ ,  $\phi_{\text{MeCN}} = 0.95$ ,  $\phi_{\text{EtOH}} = 0.97$ , and  $\phi_{\text{MeOH}} = 0.96$ ). Furthermore, the extinction coefficient increased up to  $202\,000\text{ M}^{-1}\text{ cm}^{-1}$ , which is more than 2.5-fold higher compared to TM-BDP (1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene). Hence, not only a spectral red shift while retaining the attractive optical characteristics of the BDP chromophore, but also additional new

characteristics such as a high extinction coefficient could be accomplished. Compared to sulforhodamine 101 ( $\lambda_{\text{abs}} = 575$  nm,  $\lambda_{\text{flu}} = 590$  nm,  $\phi = 0.95$ , fwhm = 930  $\text{cm}^{-1}$ ,  $\epsilon = 139\,000$   $\text{M}^{-1}$   $\text{cm}^{-1}$  in EtOH), which is a commercially available fluorescent standard dye for laser applications or bioconjugate probes, the optical properties of KFL-1 are similar or even superior. Therefore, KFL-1 is a suitable candidate for becoming a fluorescent standard dye. The photostability of KFL-1 in EtOH solution was measured by continuous irradiation with a Xe lamp (150 W) at 10 nm slit width. The result showed that over 98.5% of the initial fluorescence intensity was retained after 1 h. Moreover, KFL-1 dissolved in EtOH retained a strong fluorescence emission after a 1-month storage under ambient conditions with daylight exposure. This remarkable photostability indicates the usefulness of KFL-1 for various applications requiring sufficient photostability, which is difficult to achieve with less photostable dyes.

Owing to the promising results obtained with KFL-1, we aimed to further extend the emission maximum into the NIR range. Hence, we focused on one of the basic phenomena of color chemistry: the wavelength relies on the strength of  $\pi$ -electron donors (D) and acceptors (A).<sup>10</sup> On the basis of this concept, the new Keio Fluors (KFL-2, A chromophore; KFL-3, D–D chromophore; and KFL-4, D–A–D chromophore), which have  $\pi$ -electron donors (at R<sup>1</sup>) or/and acceptors (at R<sup>2</sup>), were designed and synthesized. As a  $\pi$ -electron donor and acceptor, a 4-methoxyphenyl and a trifluoromethyl substituent were selected, respectively.

KFL-2, -3, and -4 in chloroform show vivid blue, blue-green, and green absorption colors and bright red and purplish red fluorescence emission (Figure 1). All three dyes exhibit fluorescence emissions in the red or NIR region (620, 683, and 738 nm) with high fluorescence quantum yields ( $\phi = 0.98, 0.86,$  and  $0.56$ , for KFL-2, -3, and -4 in chloroform, respectively). Overall, the fluorescence emission of KFL-4 is red-shifted by 221 nm compared to the TM-BDP chromophore. Therefore, a drastic spectral red shift and a fine-tuning of the emission wavelength could be achieved by relatively simple modifications of the KFL chromophore. In comparison to the CyDye series (one of the most widely used set of fluorescent dyes), the wavelengths of fluorescence emission of KFL-1, -3, and -4 are nearly identical to those of Cy3 (570 nm)/Cy3.5 (596 nm), Cy5 (670 nm)/Cy5.5 (694 nm), and Cy7 (767 nm), respectively. Furthermore, the fluorescence spectral bands of the Keio Fluors are extremely sharp (fwhm: 574, 516, and 577  $\text{cm}^{-1}$ ) and almost no spectral overlap between their emission spectra is observed (Figure 2). Interestingly, these fluorescent spectra are even much sharper than those of available quantum dots, which are known to show narrow spectral bands.<sup>11</sup> These characteristics enable easy spectral separation, and therefore, render Keio Fluors potentially useful for multicolor assays and imaging. The extinction coefficients of KFL-3 (288 000  $\text{M}^{-1}$   $\text{cm}^{-1}$ ) and KFL-4 (253 000  $\text{M}^{-1}$   $\text{cm}^{-1}$ ) were further increased relative to KFL-1. This drastic increase is quite remarkable, because the increase of extinction coefficients by introduction of electron-donating moieties is not usually expected to such an extent, especially not in the case of aryl-substituted BDP.<sup>5a,7c</sup> This increase is supposed to be due to the planarity of the KFL, which has been confirmed by X-ray single-crystal structure analysis in the case of KFL-4 (refer to Supporting Information). The photostabilities of KFL-2, -3, and -4 in ethanol were also measured under the same condition as for KFL-1. The results showed that 97.3–98.8% of the initial fluorescence intensities were retained after 1 h.

As far as we know, NIR fluorescent dyes including other BDP-based types, which have higher extinction coefficients and quantum yields than KFL-3 and -4 have not been reported, yet. So far, a

rigidified aza-dipyromethene NIR dye reported in the literature<sup>7b</sup> has been regarded to have the best optical characteristics among BDP-derivatives ( $\lambda_{\text{flu}} = 751$  nm,  $\epsilon = 159\,000$   $\text{M}^{-1}$   $\text{cm}^{-1}$ ,  $\phi = 0.28$ , in chloroform). The very recently reported diketopyrrolopyrrole-based NIR fluorescent dyes are the only ones to show comparable optical properties ( $\lambda_{\text{flu}} = 831$  nm,  $\epsilon = 256\,000$   $\text{M}^{-1}$   $\text{cm}^{-1}$ ,  $\phi = 0.53$ , in chloroform).<sup>12</sup>

In conclusion, we successfully designed and synthesized a series of novel fluorescent dyes with particularly useful optical performance such as sharper fluorescence spectral bands compared to quantum dots, vivid absorption and emission colors, high quantum yields (up to 0.98), high extinction coefficients (up to 288 000  $\text{M}^{-1}$   $\text{cm}^{-1}$ ), and high photostability. Also, the wavelength is finely tunable over a wide range including the NIR region, by the introduction of electron donors and acceptors. These results indicate that Keio Fluors have the potential to substitute or to complement existing commercially available fluorescent dyes and to be used as new standard dyes in the vis-NIR region.

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**Supporting Information Available:** Synthetic details, characterization data for each compound, measurement details of optical properties, X-ray single-crystal structure of KFL-4, and X-ray crystallographic file for KFL-4 (CIF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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