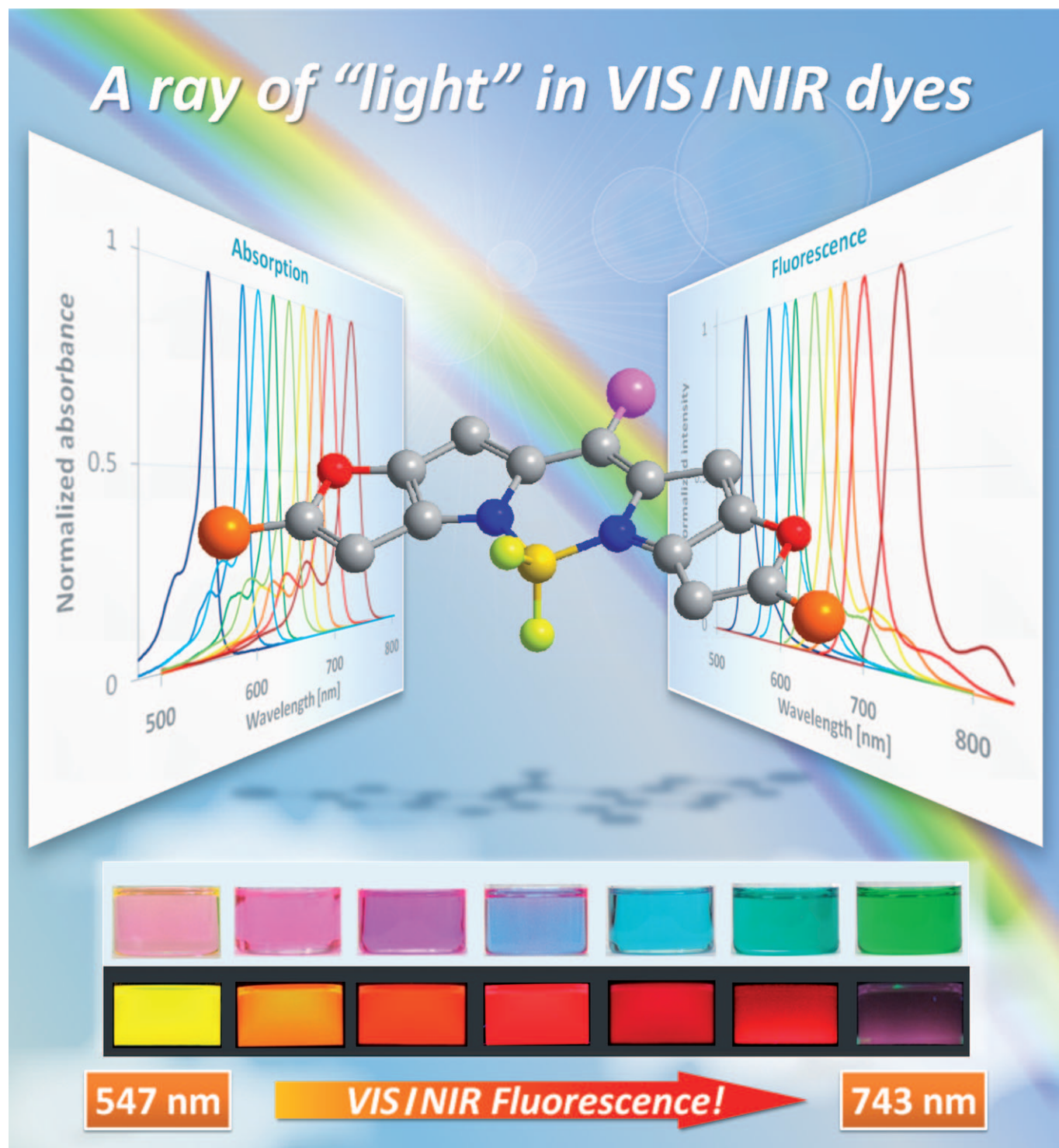


Bright, Color-Tunable Fluorescent Dyes in the Vis/NIR Region: Establishment of New “Tailor-Made” Multicolor Fluorophores Based on Borondipyrromethene

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Abstract: A new series of high-performance fluorophores named Keio Fluors (KFL), which are based on borondipyrromethene (BODIPY), are reported. The KFL dyes cover a wide spectral range from the yellow (547 nm) to the near-infrared (NIR, 738 nm) region, and their emission wavelength could be easily and subtly controlled based on simple molecular modifications only, without losing their optical properties. This “tailor-made”

synthetic strategy for tuning the emission wavelength enabled the creation of fourteen KFL dyes with well-controlled emission colors (yellow, orange, red, far-red, and NIR). Moreover, these KFL dyes also retain their excellent optical properties, such as spectral bands sharper than quantum dots, high

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extinction coefficients ($140\,000$ – $316\,000\text{ M}^{-1}\text{ cm}^{-1}$), and high quantum yields (0.56 – 0.98), without any critical solvent polarity dependent decrease of their brightness. These advantageous characteristics make the KFL dyes potentially useful as new candidates of fluorescent standard dyes to substitute or to complement existing long-wavelength fluorescent dyes, such as cyanines, oxazines, rhodamines, or other BODIPY dyes.

Introduction

Bright and long-wavelength emitting fluorescent dyes with large color variation over a wide spectral region from the visible (Vis) to the near-infrared (NIR), are of interest in many fields such as optical engineering, analytical chemistry, biological chemistry, photochemistry, and others. For example, the development of multicolor fluorescent dye standards is expected to significantly contribute to the progress of fundamental and practical technologies: for example, dye laser series,^[1] library of fluorescent reference dyes for photophysical measurements,^[2] and fluorescent probes for multicolor bioanalysis and bioimaging enabling simultaneous analysis of multiple analytes.^[3–5] Additionally, NIR fluorescent dyes have been often spotlighted as new revolutionary tools for noninvasive and simple in vivo optical imaging,^[6–10] owing to the advantages of NIR light between 650 – 900 nm (often called “optical window”): for example, 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 NIR light, and the possibility to use low-cost excitation light sources.^[11]

Ideal multicolor long-wavelength fluorescent dyes should feature the following characteristics: 1) high fluorescence quantum yields (ϕ near 1.0); 2) sharp fluorescence spectra (full width at half maximum height: $\Delta\lambda_{1/2}$ around 500 nm); 3) large molar extinction coefficients (ϵ over $10^5\text{ M}^{-1}\text{ cm}^{-1}$); and 4) ease of tuning the fluorescence emission over a wide spectral range (Vis/NIR). The brightness, determined by the product of the extinction coefficient and the quantum yield ($\epsilon \times \phi$), is also an important parameter of each fluorescent dye. According to a recently published review article, in which most major fluorophores are introduced, there are

only few dyes which have satisfyingly high brightness ($\epsilon \times \phi > 10^5\text{ M}^{-1}\text{ cm}^{-1}$).^[5] For instance, some cyanine dyes suffer from low fluorescence quantum yields (< 0.3 in most cases), rendering their brightness lower than $10^5\text{ M}^{-1}\text{ cm}^{-1}$.^[12]

Borondipyrromethene (BODIPY) fluorescent dyes have recently been focused on due to their intense fluorescence quantum yields (near 1.0), their sharp absorption and emission spectra, and their high photostability.^[13–17] However, typical BODIPYs have some drawbacks, such as relatively short fluorescence emission maxima (λ_{max} around 500 nm) and low extinction coefficients (ϵ around $80\,000\text{ M}^{-1}\text{ cm}^{-1}$). Different research groups have used various approaches to overcome these disadvantages: 1) introduction of electron-donating substituents,^[18,19] 2) replacement of a carbon atom by a nitrogen atom,^[20–22] 3) rigidification of rotatable moieties,^[23–25] 4) extension of the π -conjugation of the structure,^[26,27] and 5) fusion of benzene moieties into the BODIPY chromophore.^[28–30] However, a significant increase of the extinction coefficients has not been achieved. Instead, it has been reported, that some modifications resulted in an undesired decrease of the fluorescence quantum yields and a hypsochromic shift of the fluorescence maxima.^[18,24–26] For example, the introduction of aryl moieties (phenyl, methoxyphenyl, and others) at the α -position of some BODIPYs has been shown to induce a bathochromic shift, but the optical properties except for the emission wavelength are generally not significantly improved. Moreover, the introduction of *ortho*-methoxyphenyl groups sometimes invokes more negative effects, such as a hypsochromic shift, a broadening of the emission spectra, and a decrease of the extinction coefficients and quantum yields.^[18,24] These ambiguous correlations between chemical modification to BODIPYs and the corresponding change of their optical properties make it difficult to design the desired multicolor fluorescent dyes without losing the advantageous optical properties.

To overcome these drawbacks, we have recently reported four novel types of heteroaryl-fused BODIPYs, named Keio Fluors (KFL), with an introduction site for electron-donating and/or accepting groups.^[31] KFL dyes have advantageous characteristics, such as sharp emission spectra, and high quantum yields and molar extinction coefficients over a

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wide spectral range from the visible into the NIR region (583–738 nm). So far, four basic types of these new dyes have been introduced. To make full use of the features of the KFL fluorophores, we extended our investigation to further derivatives. Ideal multicolor fluorescent dyes should allow the easy and fine control of the emission wavelength over a wide spectral range, with retention of the optical properties. Preferably, it should also be possible to estimate the emission wavelength from the chemical modification applied to the KFL core. In this paper, we report on new strategies enabling the easy control of the emission wavelength: introduction of several types of electron-donating moieties (KFL-5–10) and synthesis of asymmetric KFL dyes (KFL-11–14, shown here).

Results and Discussion

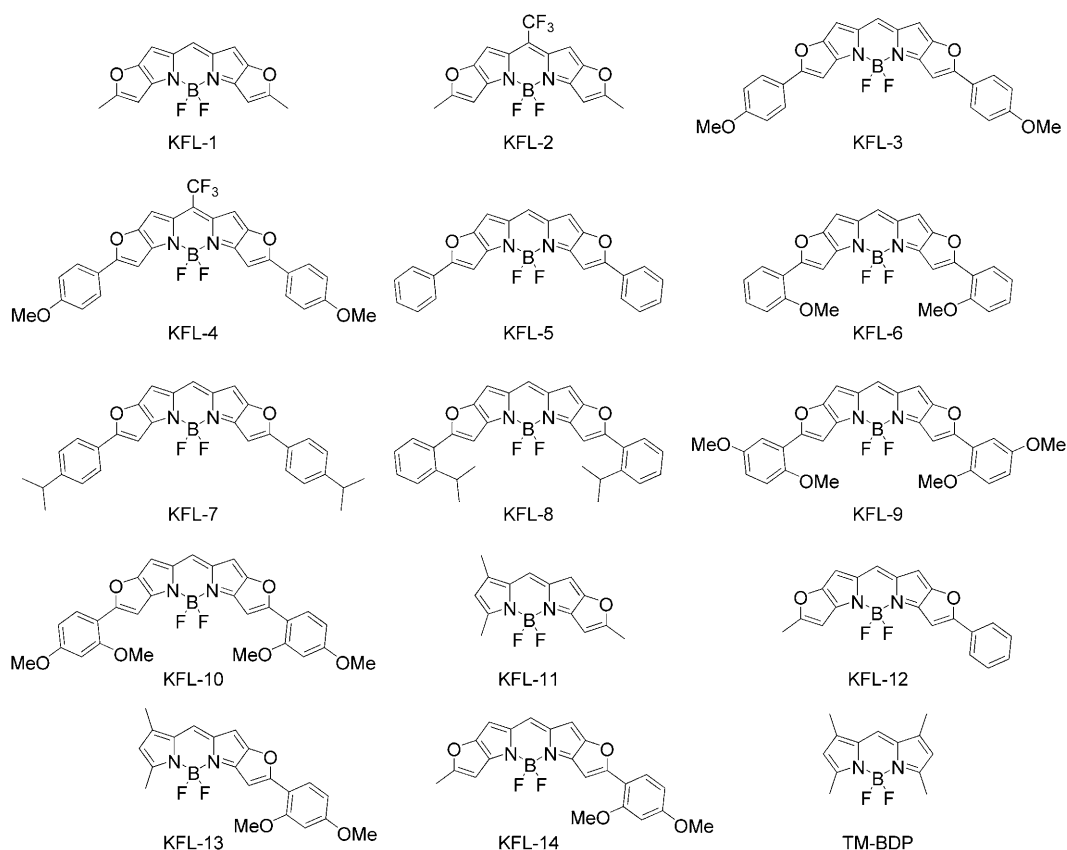
Synthesis: The synthetic scheme is outlined in Scheme 1. Aryl substitutions were performed in the first step by Suzuki–Miyaura coupling with high yields. Fuopyrrole 5-carboxylic acid segments were synthesized by Hemetsberger–Knittel synthesis, and α -formylation of fuopyrroles was performed in the presence of triethyl orthoformate and trifluoroacetic acid. KFL dyes were obtained from α -formylated fuopyrrole and α -carboxylated fuopyrrole in the presence of trifluoroacetic acid and trichlorophosphate. In this way, a simple and efficient synthetic scheme could be estab-

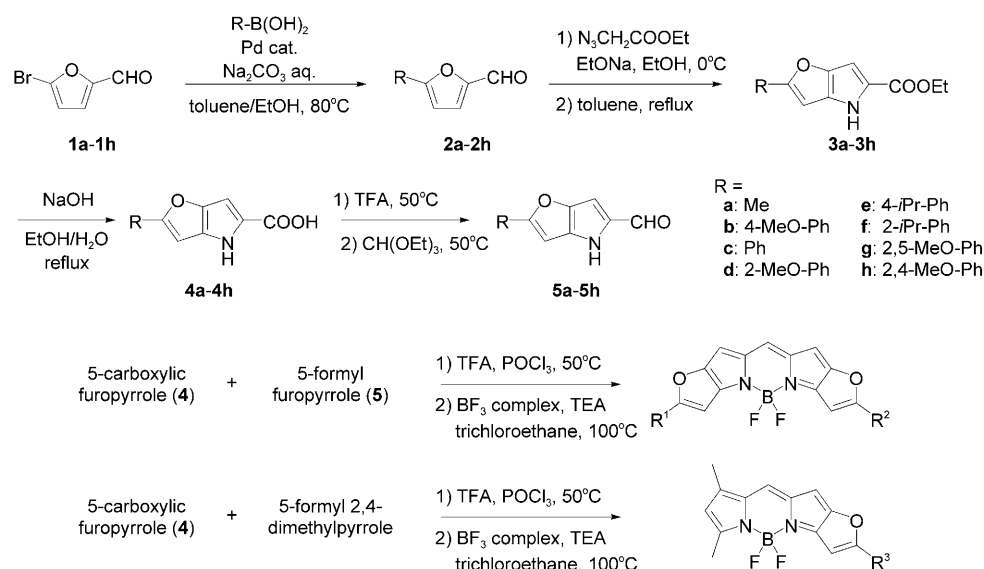
lished. Asymmetric KFLs were easily synthesized by using two different pyrroles or fuopyrroles according to the same synthetic scheme.

Spectral properties of phenyl-substituted KFLs (KFL-5–8):

In our previously reported work, the introduction of phenyl rings into the dyes invoked a drastic bathochromic shift in the fluorophores with retention of quantum yield and spectral sharpness, and with an increase of the extinction coefficient. It could also be confirmed by X-ray single-crystal analysis that almost no torsion between the KFL core and the phenyl rings occurred, resulting in the increase of the extinction coefficients.^[31] We therefore assumed that this planar conformation might be crucial for the observed optical properties. On the basis of this hypothesis, new KFL dyes (KFL-5–8), which have several types of substituted phenyl rings, were synthesized and their optical properties were measured. Specifically, some bulky substituents, such as methoxy (KFL-6) and isopropyl groups (KFL-8), were introduced in the *ortho*-position of the phenyl ring to evaluate the effect of substitution on the conformation and the spectral properties of the fluorophores.

As shown in Table 1 and Figure 1, KFL-5–7 exhibited sharp spectra ($\Delta\lambda_{1/2}$ around 500 nm), high extinction coefficients (313 000–316 000 M^{−1} cm^{−1}) and high quantum yields in chloroform (0.90–0.97) in the far-red and NIR region (around 650 nm), and their brightness was in the range of 282 000–286 000 M^{−1} cm^{−1}. To the best of our knowledge,





Scheme 1. Synthetic scheme for Keio Fluors.

Table 1. Optical properties of TM-BDP and Keio Fluors in chloroform.

	λ_{abs} [nm]	λ_{flu} [nm]	$\Delta\lambda_{1/2}$ ^[a] [nm]	ϵ ^[b] [M ⁻¹ cm ⁻¹]	ϕ ^[b,c]	Brightness ^[d] [M ⁻¹ cm ⁻¹]
TM-BDP ^[31]	509	517	19 (704)	80 000	0.92	73 600
KFL-1 ^[31]	579	583	16 (457)	202 000	0.96	194 000
KFL-2 ^[31]	613	620	22 (574)	185 000	0.98	181 000
KFL-3 ^[31]	673	683	24 (516)	288 000	0.86	248 000
KFL-4 ^[31]	723	738	31 (577)	253 000	0.56	142 000
KFL-5	652	661	18 (405)	314 000	0.90	286 000
KFL-6	671	680	19 (421)	313 000	0.91	282 000
KFL-7	662	671	19 (425)	316 000	0.97	284 000
KFL-8	629	655	30 (700)	142 000	0.87	118 000
KFL-9	679	689	28 (586)	280 000	0.82	230 000
KFL-10	690	701	29 (583)	282 000	0.81	226 000
KFL-11	542	549	16 (543)	140 000	0.96	134 000
KFL-12	614	620	16 (416)	248 000	0.95	236 000
KFL-13	596	605	23 (619)	184 000	0.95	175 000
KFL-14	634	644	24 (568)	192 000	0.87	167 000

[a] The values converted to wavenumbers (cm⁻¹) are shown in parentheses. [b] Error: within 5%. [c] Reference dyes are listed in the Experimental Section. [d] Brightness is defined as $\epsilon \times \phi$.

there are probably no fluorescent dyes with extinction coefficients over 300 000 M⁻¹ cm⁻¹ and quantum yields over 0.90 in this far-red region of the spectrum. Their brightness values (over 200 000 M⁻¹ cm⁻¹) are higher than those of any commonly used fluorescent dyes listed in a recent review article.^[5] Interestingly, only KFL-8 (*ortho*-isopropylphenyl-substituted KFL) showed different spectral characteristics compared to KFL-3 and KFL-5–7. Dye KFL-8 exhibited a high quantum yield (0.87), broad spectral band, and low extinction coefficient at a shorter wavelength (λ_{abs} : 629 nm) compared to KFL-5 (phenyl-substituted KFL). The *ortho*-methoxyphenyl-substituted KFL-6 on the other hand, exhibited similar characteristics to KFL-3, KFL-5, and KFL-7. These results demonstrate a certain substituent effect of *ortho*-substituted phenyl rings on the spectral properties. Similar observations have also been reported previously, dis-

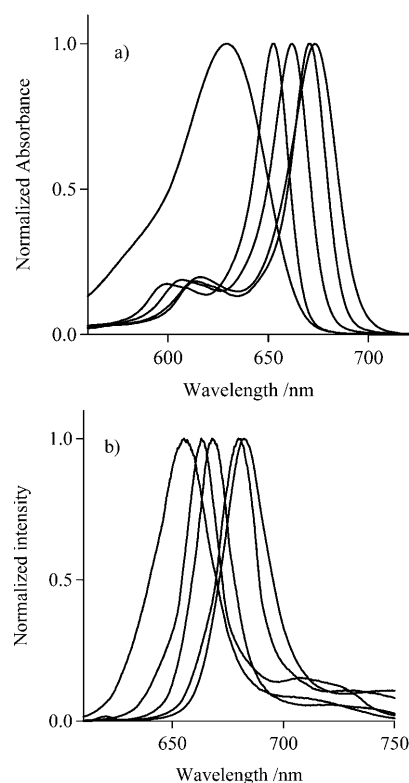


Figure 1. a) Normalized absorption and b) fluorescence spectra of (from left to right) KFL-3 and KFL-5–8 in chloroform.

cussing the effect of *ortho*-substituents of phenyl rings on the optical characteristics of BODIPY derivatives.^[18,24] According to these reports, the introduction of *ortho*-methoxyphenyl rings into BODIPY or aza-BODIPY at the α -positions of pyrroles tends to negatively influence their optical extinction coefficients and quantum yields, shorten the wavelengths of their absorption and emission maxima, and

broaden their spectral bands. It is stated that this is due to the twisted conformation between the *ortho*-methoxyphenyl rings and the BODIPY core arising from steric hindrance induced by the *ortho*-methoxy groups.^[24] The twisted form may allow flexible rotation of the phenyl rings and hence, have an effect on the extinction coefficients, quantum yields and spectral sharpness. Therefore, the twisting angle between the phenyl rings and the BODIPY core is considered to relate to the trend of the optical properties.

In the case of KFL-4, a planar conformation between the KFL core and the phenyl rings was confirmed by X-ray single-crystal analysis. Also in the case of KFL-3 and KFL-6, ring coplanarity is assumed based on the results of AM1 quantum calculation (Figure 2). For KFL-6, this is further supported by NMR measurements. As shown in Figure 3, the chemical shifts of the H_c protons (3- and 7-positions of

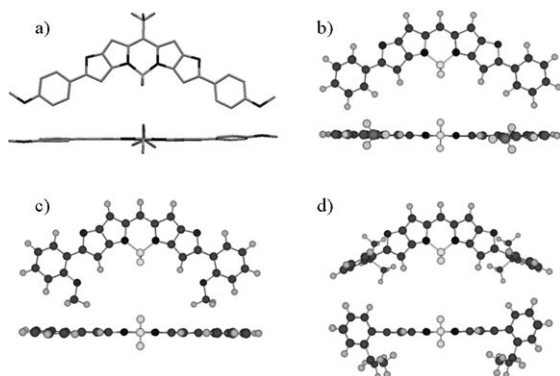


Figure 2. a) X-ray molecular structure of KFL-4^[31] and AM1 calculated molecular structures of b) KFL-5, c) KFL-6, and d) KFL-8. Top figures and bottom figures in each dye represent front view and side view, respectively.

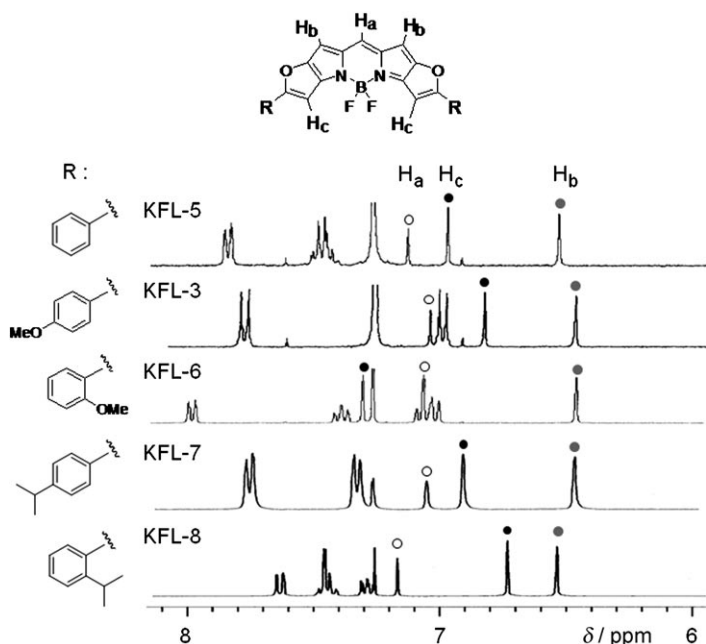


Figure 3. NMR spectra of KFL-3 and KFL-5-8 in [D₁]chloroform at room temperature.

the KFL core) are most strongly influenced by the substitution pattern of the phenyl rings. Especially in the case of KFL-6, the chemical shift of H_c shifted downfield ($\delta = 7.3$ ppm) compared to the other KFL-3, KFL-5, KFL-7, and KFL-8 ($\delta = 6.7$ – 6.9 ppm). This is assumed to be due to the presence of the O atoms (at the *ortho* position of the phenyl rings) near the H_c protons, indicating a certain interaction through space.

In contrast, AM1 calculation for KFL-8 indicated an almost orthogonal orientation between the phenyl rings and the KFL core, owing to the presence of the bulky isopropyl units at the *ortho* position of the phenyl rings (Figure 2). This resulted in a decrease of the extinction coefficient, a hypsochromic shift of the spectral maxima, and reduced spectral sharpness. The quantum yield remains almost unaffected (0.87), implying that the phenyl rings are hindered in rotation by the bulky isopropyl units. Previous research into BODIPY derivatives (with phenyl rings at the α -position forced into an orthogonal orientation by bulky methyl groups) showed quite similar results,^[32] which further supports our considerations. Consequently, it is suggested that coplanarity of the KFL core and the phenyl substituents is a prerequisite for the desired advantageous spectral properties, and that a twisted conformation results in a reduction of certain properties.

Spectral properties of dimethoxyphenyl-substituted KFL dyes (KFL-9 and KFL-10): As discussed above, we discovered a positive effect of *ortho*-methoxy substitution of phenyl rings on the optical properties of the KFL fluorophores (red-shift of absorption and fluorescence emission with sharp spectra, high extinction coefficient and fluorescence quantum yield), although it had been reported to evoke a negative effect in the case of BODIPYs. It also implies that further spectral shift can be expected by increasing the number of electron donors at the phenyl ring. On the basis of this consideration, dimethoxyphenyl-substituted KFL dyes (KFL-9 and KFL-10) were designed, synthesized, and characterized regarding the degree of spectral shift.

As shown in Figure 4, KFL-9 and KFL-10 exhibited significantly long-wavelength-shifted fluorescence reaching up to 700 nm, while retaining high extinction coefficients ($\approx 282\,000\text{ M}^{-1}\text{ cm}^{-1}$), high quantum yields (≈ 0.82), and sharp spectra relative to KFL-5 (benzene-substituted KFL) in chloroform. These results are noteworthy, because the previously reported dimethoxyphenyl-substituted BODIPY dyes exhibited less positive properties: broader spectra, low extinction coefficient, and relatively short or identical wavelength.^[24] In the case of KFL, fine tuning of absorption and fluorescence emission peak maxima could be achieved by simply attaching the methoxy units at various positions of the phenyl rings.

Spectral properties of asymmetric KFL dyes (KFL-11-14): Asymmetric KFL dyes were expected to expand the fluorescence wavelength variations. Therefore, four asymmetric dyes (KFL-11-14) were designed and synthesized. For ex-

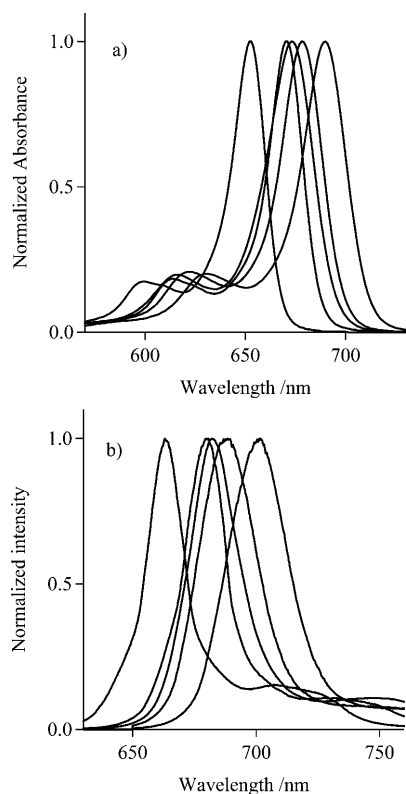


Figure 4. a) Normalized absorption and b) fluorescence spectra of (from left to right) KFL-3, KFL-5, KFL-6, KFL-9, and KFL-10 in chloroform.

ample, asymmetric KFL-11, which is a combination of the 1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (TM-BDP) and the KFL-1 backbone, was expected to exhibit its absorption/fluorescence peak maxima centered between those of TM-BDP and KFL-1 (probably at around 540/550 nm). The experimental result showed that KFL-11 exhibited a vivid pink absorption color (542 nm) and intense yellow emission color (549 nm, $\phi=0.96$), very close to the expected peak wavelengths (Figures 5 and 6). Based on these results, several types of asymmetric KFL (KFL-12–14) were synthesized to obtain new fluorescent dyes the emission peaks of which can be flexibly controlled. The dye KFL-12, which is a combination of KFL-1 and KFL-5, also exhibited its absorption/emission peak at 614/620 nm, which is very close to the arithmetic mean of the combination of KFL-1 (579/583 nm) and KFL-5 (652/661 nm). The absorption and fluorescence maxima of KFL-2 and KFL-12 are almost identical, with the spectral broadness and the extinction coefficient of KFL-12 being improved. The absorption/emission maxima of KFL-13 and KFL-14, which have one 2,4-dimethoxyphenyl moiety, are also centered between the corresponding values of TM-BDP/KFL-10, and KFL-1/KFL-10, respectively. The absorption maxima of KFL-11 and KFL-14 are just identical to the excitation wavelengths of the green He–Ne laser (543 nm) and the red He–Ne laser (633 nm), respectively, which are widely used as standard lasers for confocal laser fluorescence microscopy and flow cytometry. This indicates that very efficient excitation of

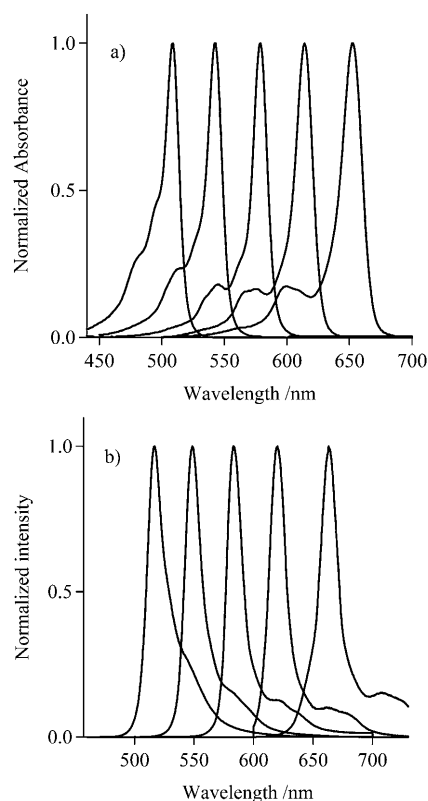


Figure 5. a) Normalized absorption and b) fluorescence spectra of (from left to right) TM-BDP, KFL-11, KFL-1, KFL-12, and KFL-5 in chloroform.

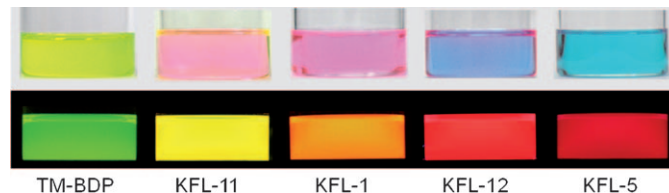


Figure 6. Absorption (upper row) and fluorescence (bottom row) colors of TM-BDP and KFLs in chloroform.

KFL-11 and KFL-14 using existing standard lasers could be realized. Therefore, with asymmetric KFL dyes, a fine-tuning of absorption/emission wavelengths to a desired region could be achieved relying on a very simple combination of KFL dyes without losing their optical properties.

Effect of solvent polarity: The effect of solvent polarity on the optical properties of the KFL dyes was investigated, because many fluorescent dyes are influenced by the solvent polarity, and their optical characteristics (especially quantum yields) are changed. All KFL dyes exhibited small blue shifts (about 10 nm) with increasing solvent polarity, as typical BODIPY dyes exhibit the same trend.^[33]

In terms of quantum yields, KFL-1 and KFL-5 showed almost no fluorescence decrease, and retained extremely high quantum yields even in polar solvents, such as ethanol and methanol (Table 2). KFL-3 and KFL-10 also exhibited

Table 2. Fluorescence quantum yields of selected KFLs in various solvents.^[a]

	Toluene	CHCl ₃	THF	MeCN	EtOH	MeOH
KFL-1	0.94	0.96	0.93	0.95	0.97	0.96
KFL-2	0.97	0.98	0.92	0.84	0.77	0.70
KFL-3	0.87	0.86	0.83	0.85	0.77	0.77
KFL-4	0.58	0.56	0.49	0.45	0.37	0.32
KFL-5	0.89	0.90	0.89	0.88	0.86	0.87
KFL-10	0.83	0.81	0.75	0.76	0.68	0.63
KFL-12	0.98	0.96	0.97	0.95	0.93	0.96

[a] Reference dyes are listed in the Experimental Section.

quantum yields high enough for bioimaging applications, although a slight fluorescence decrease was observed with increasing solvent polarity. This slight fluorescence decrease of KFL-10 in polar solvents is assumed to be due to the presence of the electron-donating moieties, which cause intramolecular charge transfer (ICT). ICT is known to influence the rate of nonradiative relaxation of fluorophores, resulting in modified fluorescence quantum yields. Similar tendencies are found in the case of KFL-2 and KFL-4, which have an electron-accepting moiety (-CF₃). On the other hand, KFL-12, which has almost the same absorption/emission maxima as KFL-2, but lacks electron-donating/accepting moieties, showed no fluorescence quenching and exhibited high fluorescence in any solvent ($\phi_{\text{EtOH}}=0.93$, $\phi_{\text{MeOH}}=0.96$). Thus, a significant improvement in terms of fluorescence quantum yields of red fluorescent dyes emitting around 620 nm could be achieved. These high quantum yields in any solvent are promising for high-sensitive and high-resolution analysis even in aqueous solution, although the KFL dyes in their present state are not sufficiently soluble in water. To evaluate the photostability of KFLs in aqueous solution (DMSO/water=50:50 and 70:30 for KFL-1 and KFL-5, respectively), the decrease of the fluorescence intensity of KFL-1 and KFL-5 was monitored upon continuous irradiation with white light from a Xe lamp (150 W, without passing the monochromator) at 25 °C for one hour, after which over 96% of fluorescence intensity was retained in both cases.

Comparison of KFL spectral properties to commercially available fluorescent dyes: Optical properties of some commercially available long-wavelength emitting dyes (e.g., rhodamine, oxazine, BODIPY, cyanine) are summarized in Table 3. It can be concluded that the KFL dyes are at least equivalent and often superior to most of the commercially available fluorescent dyes in terms of extinction coefficient, quantum yield, spectral sharpness, and brightness (Tables 1 and 3). The most remarkable fact is that KFL dyes exhibit extremely high brightness ($118\,000\text{--}286\,000\text{ M}^{-1}\text{ cm}^{-1}$), since only few fluorescent dyes with brightness values over $100\,000\text{ M}^{-1}\text{ cm}^{-1}$ are reported.^[5] Rhodamine dyes and Cy3B, which are among the most widely used yellow-red fluorescent dye standards, show high optical properties with brightness around $100\,000\text{ M}^{-1}\text{ cm}^{-1}$. The emission maxima of KFL-1 and KFL-11 are identical to some rhodamine dyes, but the

Table 3. Optical properties of typical long-wavelength fluorescent dyes.

	$\lambda_{\text{abs}}/\lambda_{\text{flu}}$ [nm]	ϵ [M ⁻¹ cm ⁻¹]	ϕ	Brightness [M ⁻¹ cm ⁻¹]
rhodamine dyes				
R6G ^[34]	530/556	116 000 ^[37]	0.95 ^[a]	110 200
TMR ^[38]	540/565	95 000	0.68 ^[b]	64 600
ShR101 ^[39]	575/590	139 000 ^[37]	0.95 ^[a]	132 000
oxazine dyes				
cresyl violet ^[35]	598/620	83 000 ^[37]	0.54 ^[b]	44 800
nile blue ^[40]	625/649	76 800 ^[37]	0.27 ^[a]	20 700
BODIPY dyes				
restricted BODIPY ^[25]	619/629	145 750	0.72 ^[c]	105 000
aza-BODIPY ^[20]	688/714	83 000	0.36 ^[c]	29 900
restricted aza-BODIPY ^[23]	740/751	159 000	0.28 ^[c]	44 500
cyanine dyes ^[41]				
Cy3B	558/572	130 000	0.67 ^[d]	87 100
Cy5	649/670	250 000	0.28 ^[d]	70 000
Cy5.5	675/694	250 000	0.28 ^[d]	70 000
Cy7	743/767	200 000	0.28 ^[d]	56 000

[a] In ethanol. [b] In methanol. [c] In chloroform. [d] In aqueous buffer.

brightness of KFL-1 and KFL-11 is still higher than that of rhodamines. Also, the optical properties of KFL-2, KFL-5, and KFL-12–14 are generally superior to those of oxazines (e.g., Cresyl violet, Nile blue), which are widely used as red fluorescent dye standards. The emission maxima of KFL-7, KFL-9, and KFL-4 are almost identical to Cy5, Cy5.5, and Cy7, respectively, but the brightness of these three KFL dyes is higher than those of cyanine dyes. Considering long-wavelength emitting BODIPYs, the dyes of the KFL series are among the most high-performance BODIPY derivatives.

In terms of spectral sharpness, the spectral bands of KFL dyes are sharper than those of almost all existing fluorescent dyes. Moreover, KFL dyes exhibit sharper emission spectra than quantum dots, which are known as fluorescent materials with sharp emission spectra (e.g., Qdot 605: $\lambda_{\text{flu}}=655\text{ nm}$, $\Delta\lambda_{1/2}=26\text{ nm}$ in methanol, Qdot 705: $\lambda_{\text{flu}}=705\text{ nm}$, $\Delta\lambda_{1/2}=74\text{ nm}$ in methanol). These sharp spectra enable easy spectral separation, and therefore, allow high-resolution multicolor bioanalysis and bioimaging.

Conclusion

In summary, we could establish a new series of long-wavelength fluorescent dyes, named Keio Fluors (KFLs), based on BODIPY with sophisticated optical properties, such as vivid colors in the Vis/NIR region (yellow, orange, red, far-red, and NIR), high extinction coefficients ($140\,000\text{--}316\,000\text{ M}^{-1}\text{ cm}^{-1}$), high quantum yields (0.56–0.98), high brightness ($118\,000\text{--}286\,000\text{ M}^{-1}\text{ cm}^{-1}$), and emission bands even sharper than quantum dots. In addition, chemical modification of the KFL dyes allows the easy and fine tuning of absorption/emission peaks without negatively influencing their optical properties. Simple and accurate estimation of the absorption/emission wavelength is possible based on the substituents attached to the BODIPY cores: for example, fusion of one furan part (ca. +30 nm), introduction of one phenyl ring (ca. +40 nm), and attachment of

one methoxy moiety at *ortho*- or *para*-position of a phenyl ring (ca. +10 nm). Therefore, various tailor-made fluorescent dyes with desired absorption/emission wavelength could be easily designed and synthesized solely relying on the simple estimation of these factors. To the best of our knowledge, KFL dyes show higher optical performance than any other known fluorescent dyes in the long-wavelength region (around 550–750 nm). These properties make the KFL series dyes very promising for new fluorescent standard dyes.

Experimental Section

Materials: All chemical reagents and solvents for synthesis were purchased from commercial suppliers (Wako Pure Chemical, Tokyo Kasei Industry, and Aldrich Chemical), and used without further purification. All moisture-sensitive reactions were carried out under an atmosphere of argon. The composition of mixed solvents is given by the volume ratio (v/v). Synthetic procedures for KFL-1–4 are described in the literature.^[31]

Instruments: ¹H NMR and ¹³C NMR spectra were recorded on a JEOL JNM-LA 300 (JEOL, Tokyo, Japan) or Varian MVX-300 (Varian, Palo Alto, CA) spectrometer at room temperature (if no specific temperature is indicated). The measurements were performed at 300 MHz (for ¹H) and 75 MHz (for ¹³C), respectively. All chemical shifts are relative to an internal standard of tetramethylsilane (δ =0.0 ppm), and coupling constants are given in Hz. Flash chromatography separation was undertaken using a YFLC-AI-560 chromatograph (Yamazen, Osaka, Japan). MALDI-TOF (matrix-assisted laser desorption/ionization—time-of-flight) mass spectra were recorded on an Ultraflex TOF/TOF spectrometer (Bruker) with α -cyano-4-hydroxycinnamic acid (CHCA) as matrix.

General procedure for the synthesis of 5-substituted furan-2-carbaldehydes 2c–2h: Aryl boronic acid (1.0 equiv) and 5-bromo-2-furaldehyde (1.0 equiv) were dissolved in a mixture of toluene (100 mL), ethanol (20 mL), and an aqueous solution of Na₂CO₃ (2 M, 20 mL), and degassed in vacuo. [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloromethane complex (1:1; 0.02 equiv) was added to the solution, and the mixture was heated at 80 °C while monitoring the reaction progress with TLC. After the reaction was completed (30 min–overnight), the mixture was cooled to room temperature and the organic phase was washed with water and brine, dried over Na₂SO₄, and evaporated. The resulting residue was purified by flash chromatography (silica gel) to obtain the arylfuran-2-carbaldehyde.

5-Phenyl-furan-2-carbaldehyde (2c): Phenylboronic acid (2.50 g, 20.5 mmol) was used as the starting material and **2c** was obtained as a yellow liquid (3.15 g, 89.2%). Eluent for chromatography: *n*-hexane/ethyl acetate 80:20→50:50. ¹H NMR (CDCl₃): δ =9.65 (s, 1H), 7.83 (dd, *J*=8.4, 1.3 Hz, 2H), 7.48–7.39 (m, 3H), 7.32 (d, *J*=3.9 Hz, 1H), 6.84 ppm (d, *J*=3.6 Hz, 1H).

5-(2-Methoxyphenyl)-furan-2-carbaldehyde (2d): 2-Methoxyphenylboronic acid (2.60 g, 17.1 mmol) was used as the starting material, and **2d** was obtained as a yellow liquid (2.86 g, 82.7%). Eluent for chromatography: *n*-hexane/ethyl acetate 80:20→50:50. ¹H NMR (CDCl₃): δ =9.64 (s, 1H), 8.04 (dd, *J*=7.8, 1.8 Hz, 1H), 7.36 (dt, *J*=7.8, 1.5 Hz, 1H), 7.33 (d, *J*=3.6 Hz, 1H), 7.13 (d, *J*=3.6 Hz, 1H), 7.06 (dt, *J*=7.8, 0.6 Hz, 1H), 6.99 (d, *J*=8.1 Hz, 1H), 3.96 ppm (s, 1H).

5-(4-Isopropylphenyl)-furan-2-carbaldehyde (2e): 4-Isopropylphenylboronic acid (968 mg, 5.90 mmol) was used as the starting material, and **2e** was obtained as a yellow liquid (885 mg, 70.0%). Eluent for chromatography: *n*-hexane/chloroform 50:50→20:80. ¹H NMR (CDCl₃): δ =9.63 (s, 1H), 7.76 (d, *J*=8.5 Hz, 2H), 7.32 (d, *J*=3.7 Hz, 1H), 7.31 (d, *J*=8.3 Hz, 2H), 6.80 (d, *J*=3.7 Hz, 1H), 3.00–2.90 (m, 1H), 1.29 (s, 3H), 1.27 ppm (s, 3H).

5-(2-Isopropylphenyl)-furan-2-carbaldehyde (2f): 2-Isopropylphenylboronic acid (950 mg, 5.79 mmol) was used as the starting material, and **2f**

was obtained as a yellow liquid (915 mg, 73.8%). Eluent for chromatography: *n*-hexane/chloroform: 80:20→60:40. ¹H NMR (CDCl₃): δ =9.67 (s, 1H), 7.57 (d, *J*=6.9 Hz, 1H), 7.44–7.41 (m, 2H), 7.34 (d, *J*=3.9 Hz, 1H), 7.29–7.24 (m, 1H), 6.66 (d, *J*=3.6 Hz, 1H), 3.44–4.40 (m, 1H), 1.29 (s, 3H), 1.26 ppm (s, 3H).

5-(2,5-Dimethoxyphenyl)-furan-2-carbaldehyde (2g): 2,5-Dimethoxyphenylboronic acid (3.64 g, 20.0 mmol) was used as the starting material, and **2g** was obtained as a yellow liquid (3.82 g, 82.2%). Eluent for chromatography: *n*-hexane/chloroform 50:50→20:80. ¹H NMR (CDCl₃): δ =9.65 (s, 1H), 7.56 (t, *J*=1.7 Hz, 1H), 7.33 (d, *J*=3.7 Hz, 1H), 7.17 (d, *J*=3.7 Hz, 1H), 6.93 (d, *J*=1.7 Hz, 2H), 6.72 (d, *J*=3.6 Hz, 1H), 3.86 ppm (s, 3H).

5-(2,4-Dimethoxyphenyl)-furan-2-carbaldehyde (2h): 2,4-Dimethoxyphenylboronic acid (2.50 g, 13.7 mmol) was used as the starting material, and **2h** was obtained as a yellow liquid (2.47 g, 78.3%). Eluent for chromatography: *n*-hexane/chloroform 50:50→20:80. ¹H NMR (CDCl₃): δ =9.58 (s, 1H), 7.97 (d, *J*=8.8 Hz, 1H), 7.31 (d, *J*=3.6 Hz, 1H), 7.00 (d, *J*=3.6 Hz, 1H), 6.60 (d, *J*=8.8 Hz, 1H), 6.53 (s, 1H), 3.94 (s, 3H), 3.87 ppm (s, 3H).

General procedure for synthesis of 2-substituted 4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl esters 3c–3h: 5-Substituted furan-2-carbaldehyde **2c–2h** (1.0 equiv) and ethyl azidoacetate (2.0 equiv) were dissolved in anhydrous ethanol (100 mL) and stirred at 0 °C. A solution of sodium ethoxide (20 wt % in ethanol, 2.0 equiv) was added dropwise into the mixture, and stirring was continued for 2–4 h until the reaction was over. Excess saturated aqueous NH₄Cl solution was added to form a precipitate, which was collected by filtration. The precipitate was washed with water and dried in vacuo. In the cases in which no precipitate was formed, the water phase was extracted with ethyl acetate, and the organic phase then washed with water and brine, dried over Na₂SO₄, and evaporated. The resulting residue was dissolved in toluene (30 mL) and heated to reflux for 1 h. After cooling, the solvent was evaporated. The residue was purified by recrystallization from *n*-hexane/ethyl acetate mixture, or by chromatography (silica gel) to obtain the 2-substituted 4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl ester.

2-Phenyl-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl ester (3c): Compound **2c** (2.85 g, 16.6 mmol) was used as the starting material, and **3c** was obtained as a yellow solid (1.77 g, 41.9%). Purification: recrystallization. ¹H NMR (CDCl₃): δ =8.69 (s, 1H), 7.73 (d, *J*=7.8 Hz, 2H), 7.41 (t, *J*=7.2 Hz, 2H), 7.29 (t, *J*=7.5 Hz, 1H), 6.82 (s, 1H), 6.72 (s, 1H), 4.36 (q, *J*=7.2 Hz, 2H), 1.39 ppm (t, *J*=7.2 Hz, 3H).

2-(2-Methoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl ester (3d): Compound **2d** (2.66 g, 13.2 mmol) was used as the starting material, and **3d** was obtained as a yellow solid (1.53 g, 40.8%). Purification: recrystallization. ¹H NMR (CDCl₃): δ =8.69 (brs, 1H), 7.97 (dd, *J*=7.7, 1.7 Hz, 1H), 7.28 (dt, *J*=7.8, 1.8 Hz, 1H), 7.05 (t, *J*=7.5 Hz, 1H), 7.07 (s, 1H), 6.81 (s, 1H), 4.36 (q, *J*=7.2 Hz, 2H), 3.98 (s, 3H), 1.39 ppm (t, *J*=7.2 Hz, 3H).

2-(4-Isopropylphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl ester (3e): Compound **2e** (885 mg, 4.13 mmol) was used as the starting material, and **3e** was obtained as a brown solid (465 mg, 37.9%). Purification: recrystallization. ¹H NMR (CDCl₃): δ =8.69 (brs, 1H), 7.67 (d, *J*=6.6 Hz, 2H), 7.28 (d, *J*=6.6 Hz, 2H), 6.82 (s, 1H), 6.67 (s, 1H), 4.36 (q, *J*=7.2 Hz, 2H), 2.98–2.89 (m, 1H), 1.39 (t, *J*=7.2 Hz, 3H), 1.29 (s, 3H), 1.27 ppm (s, 3H).

2-(2-Isopropylphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl ester (3f): Compound **2f** (900 mg, 4.20 mmol) was used as the starting material, and **3f** was obtained as a brown solid (390 mg, 31.2%). Purification: chromatography (eluent: *n*-hexane/chloroform 50:50→20:80). ¹H NMR (CDCl₃): δ =8.68 (brs, 1H), 7.53 (d, *J*=7.1 Hz, 1H), 7.44–7.34 (m, 2H), 7.27–7.22 (m, 1H), 6.84 (dd, *J*=1.0, 1.7 Hz, 1H), 6.50 (d, *J*=0.8 Hz, 1H), 4.36 (q, *J*=7.1 Hz, 2H), 3.48–3.39 (m, 1H), 1.39 (t, *J*=7.3 Hz, 3H), 1.28 (s, 3H), 1.26 ppm (s, 3H).

2-(2,5-Dimethoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl ester (3g): Compound **2g** (3.82 g, 16.4 mmol) was used as the starting material, and **3g** was obtained as a yellow solid (2.70 g, 52.1%). Purification: recrystallization. ¹H NMR (CDCl₃): δ =8.71 (brs, 1H), 7.51 (d, *J*=

3.3 Hz, 1H), 7.09 (s, 1H), 6.90 (d, $J=9.0$ Hz, 1H), 6.84–6.80 (m, 2H), 4.36 (q, $J=7.2$ Hz, 2H), 3.92 (s, 3H), 3.86 (s, 3H), 1.39 ppm (t, $J=7.2$ Hz, 3H).

2-(2,4-Dimethoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid ethyl ester (3h): Compound **2h** (2.33 g, 10.0 mmol) was used as the starting material, and **2h** was obtained as a yellow solid (932 mg, 27.0%). Purification: chromatography (eluent: chloroform/ethyl acetate 95:5). ^1H NMR (CDCl_3): $\delta=8.65$ (brs, 1H), 7.87 (d, $J=8.5$ Hz, 1H), 6.91 (s, 1H), 6.78 (s, 1H), 6.59 (d, $J=8.6$ Hz, 1H), 6.54 (s, 1H), 4.35 (q, $J=7.2$ Hz, 2H), 3.94 (s, 3H), 3.86 (s, 3H), 1.38 ppm (t, $J=7.1$ Hz, 3H).

General procedure for synthesis of 2-substituted 4H-furo[3,2-*b*]pyrrole-5-carboxylic acids 4c–4h: NaOH (ca. 15 equiv) in water (5 mL) was added to a solution of 2-substituted acid ethyl esters **3c–3h** (1.0 equiv) in ethanol (10 mL) and the mixture was refluxed for 30 min. After cooling, concentrated aqueous HCl was added until a precipitate formed, which was filtered. The resulting precipitate was washed with water and dried in vacuo to obtain the 2-substituted 4H-furo[3,2-*b*]pyrrole-5-carboxylic acid **4c–4h**.

2-Phenyl-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid (4c): Compound **3c** (1.57 g, 6.15 mmol) was used as the starting material, and **4c** was obtained as a gray solid (1.34 g, 95.5%). ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.41$ (s, 1H), 11.62 (s, 1H), 7.80 (d, $J=7.5$ Hz, 2H), 7.43 (t, $J=7.5$ Hz, 2H), 7.31 (t, $J=7.8$ Hz, 1H), 7.14 (s, 1H), 6.73 ppm (s, 1H).

2-(2-Methoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid (4d): Compound **3d** (1.38 g, 4.84 mmol) was used as the starting material, and **4d** was obtained as a green solid (1.15 g, 92.4%). ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.41$ (s, 1H), 11.56 (s, 1H), 7.83 (d, $J=7.8$ Hz, 1H), 7.32 (t, $J=7.5$ Hz, 1H), 7.15 (d, $J=8.4$ Hz, 1H), 7.05 (t, $J=8.7$ Hz, 1H), 7.01 (s, 1H), 6.71 (s, 1H), 3.96 ppm (s, 3H).

2-(4-Isopropylphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid (4e): Compound **3e** (345 mg, 1.16 mmol) was used as the starting material, and **4e** was obtained as a gray solid (284 mg, 91.0%). ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.40$ (s, 1H), 11.57 (s, 1H), 7.72 (d, $J=8.1$ Hz, 1H), 7.31 (d, $J=8.4$ Hz, 1H), 7.05 (s, 1H), 6.70 (s, 1H), 2.95–2.86 (m, 1H), 1.23 (s, 3H), 1.21 ppm (s, 3H).

2-(2-Isopropylphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid (4f): Compound **3f** (300 mg, 1.01 mmol) was used as the starting material, and **4f** was obtained as a gray solid (252 mg, 92.7%). ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.40$ (s, 1H), 11.59 (s, 1H), 7.51 (d, $J=7.8$ Hz, 1H), 7.47 (d, $J=7.8$ Hz, 1H), 7.40 (t, $J=7.2$ Hz, 1H), 7.28 (t, $J=7.5$ Hz, 1H), 6.74 (s, 1H), 6.66 (s, 1H), 3.43–3.34 (m, 1H), 1.23 (s, 3H), 1.21 ppm (s, 3H).

2-(2,5-Dimethoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid (4g): Compound **3g** (2.67 g, 8.47 mmol) was used as the starting material, and **4g** was obtained as a green solid (1.90 g, 78.1%). ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.42$ (s, 1H), 11.57 (s, 1H), 7.35 (d, $J=3.0$ Hz, 1H), 7.08 (d, $J=9.0$ Hz, 1H), 7.04 (s, 1H), 6.89 (dd, $J=9.3$, 3.3 Hz, 1H), 6.72 (s, 1H), 3.91 (s, 3H), 3.78 ppm (s, 3H).

2-(2,4-Dimethoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carboxylic acid (4h): Compound **3h** (0.91 g, 2.89 mmol) was used as the starting material, and **4h** was obtained as a green solid (0.78 g, 94%). ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.34$ (s, 1H), 11.49 (s, 1H), 7.74 (d, $J=8.4$ Hz, 1H), 6.84 (s, 1H), 6.70–6.68 (m, 2H), 6.66 (dd, $J=8.4$, 2.1 Hz, 1H), 3.95 (s, 3H), 3.82 ppm (s, 3H).

General procedure for synthesis of 2-substituted 4H-furo[3,2-*b*]pyrrole-5-carbaldehydes 5c–5h: Compounds **4c–4h** were dissolved in trifluoroacetic acid (1 mL) and heated at 50°C for 10 min. Triethylorthoformate (0.5 mL) was added into the reaction mixtures, and stirred for further 10 min. After cooling, the reaction mixture was poured into saturated aqueous NaHCO_3 solution to neutralize. The formed precipitate was filtered, washed with water and dried in vacuo. The precipitate was purified with flash chromatography (silica gel) to obtain the 2-substituted 4H-furo[3,2-*b*]pyrrole-5-carbaldehyde **5c–5h**.

2-Phenyl-4H-furo[3,2-*b*]pyrrole-5-carbaldehyde (5c): Compound **4c** (99.7 mg, 0.439 mmol) was used as the starting material, and **5c** was obtained as a gray solid (75.9 mg, 81.9%). Eluent for chromatography: *n*-hexane/ethyl acetate 75:25→60:40. ^1H NMR (CDCl_3): $\delta=9.46$ (s, 1H),

9.32 (brs, 1H), 7.76 (d, $J=7.2$ Hz, 2H), 7.43 (t, $J=7.5$ Hz, 2H), 7.34 (t, $J=7.2$ Hz, 1H), 6.79 (s, 1H), 6.76 ppm (s, 1H).

2-(2-Methoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carbaldehyde (5d): Compound **4d** (50.0 mg, 0.194 mmol) was used as the starting material, and **5d** was obtained as a yellow solid (41.0 mg, 87.4%). Eluent for chromatography: *n*-hexane/ethyl acetate: 75:25→60:40. ^1H NMR (CDCl_3): $\delta=9.49$ (brs, 1H), 9.44 (s, 1H), (dd, $J=7.7$, 1.7 Hz, 1H), 7.32 (dt, $J=7.5$, 1.8 Hz, 1H), 7.11 (s, 1H), 7.06 (dt, $J=7.8$, 0.9 Hz, 1H), 6.77 (s, 1H), 3.98 ppm (s, 1H).

2-(4-Isopropylphenyl)-4H-furo[3,2-*b*]pyrrole-5-carbaldehyde (5e): Compound **4e** (32.2 mg, 0.120 mmol) was used as the starting material, and **5e** was obtained as a gray solid (25.1 mg, 82.8%). Eluent for chromatography: chloroform/ethyl acetate 98:2. ^1H NMR (CDCl_3): $\delta=9.52$ (brs, 1H), 9.44 (s, 1H), 7.70 (d, $J=8.1$ Hz, 2H), 7.29 (d, $J=8.4$ Hz, 2H), 6.78 (s, 1H), 6.71 (s, 1H), 2.99–2.90 (m, 1H), 1.29 (s, 3H), 1.27 ppm (s, 3H).

2-(2-Isopropylphenyl)-4H-furo[3,2-*b*]pyrrole-5-carbaldehyde (5f): Compound **4f** (27.8 mg, 0.103 mmol) was used as the starting material, and **5f** was obtained as a gray solid (25.0 mg, 95.8%). Eluent for chromatography: chloroform/ethyl acetate 98:2. ^1H NMR (CDCl_3): $\delta=9.88$ (brs, 1H), 9.47 (s, 1H), 7.54 (d, $J=7.8$ Hz, 1H), 7.46–7.37 (m, 2H), 7.26 (t, $J=7.2$ Hz, 2H), 6.81 (s, 1H), 6.56 (s, 1H) 3.48–3.39 (m, 1H), 1.29 (s, 3H), 1.27 ppm (s, 3H).

2-(2,5-Dimethoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carbaldehyde (5g): Compound **4g** (36.6 mg, 0.127 mmol) was used as the starting material, and **5g** was obtained as a gray solid (30.2 mg, 87.4%). Eluent for chromatography: chloroform/ethyl acetate 95:5. ^1H NMR (CDCl_3): $\delta=9.57$ (brs, 1H), 9.44 (s, 1H), 7.52 (d, $J=3.0$ Hz, 1H), 7.14 (s, 1H), 6.92 (d, $J=9.3$ Hz, 1H), 6.87 (dd, $J=9.0$ Hz, 3.0 Hz, 1H), 6.77 (s, 1H), 3.93 (s, 1H), 3.86 ppm (s, 1H).

2-(2,4-Dimethoxyphenyl)-4H-furo[3,2-*b*]pyrrole-5-carbaldehyde (5h): Compound **4h** (28.1 mg, 0.098 mmol) was used as the starting material, and **5h** was obtained as a gray solid (22.4 mg, 84.9%). Eluent for chromatography: chloroform/ethyl acetate 95:5. ^1H NMR (CDCl_3): $\delta=9.41$ (s, 1H), 9.07 (brs, 1H), 7.89 (d, $J=8.8$ Hz, 1H), 6.95 (s, 1H), 6.74 (s, 1H), 6.61 (dd, $J=8.8$, 2.2 Hz, 1H), 6.55 (d, $J=2.2$ Hz, 1H), 3.96 (s, 3H), 3.87 ppm (s, 3H).

General procedure for synthesis of symmetric KFL dyes (KFL-5–10): Compounds **4c–4h** (1 equiv) were dissolved in trifluoroacetic acid (1 mL) and heated at 50°C for 10 min. Compounds **5c–5h** (1 equiv) and phosphoryl chloride (0.8 mL) were added into the reaction mixture, and stirred for further 10 min until an intense color was formed. After cooling, the reaction mixture was poured into saturated aqueous NaHCO_3 solution to neutralize. The formed precipitate was filtered, washed with water, and dried in vacuo. The resulting compound was dissolved in 1,1,2-trichloroethane (10 mL), before $\text{BF}_3\cdot\text{Et}_2\text{O}$ (0.3 mL) and triethylamine (0.2 mL) were added and the mixture was stirred at 100°C for 15 min. After cooling, the reaction mixture was diluted with chloroform and washed with saturated aqueous NaHCO_3 solution and brine, dried over Na_2SO_4 , and evaporated. The residue was purified by chromatography (silica gel) to obtain the KFL dyes (KFL-5–10).

2,8-Diphenyl-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-5): Compounds **4c** (24.0 mg, 0.106 mmol) and **5c** (22.2 mg, 0.106 mmol) were used as the starting materials, and KFL-5 was obtained as a green metallic solid (37.1 mg, 82.8%). Eluent for chromatography: chloroform. ^1H NMR (CDCl_3): $\delta=7.83$ (dd, $J=8.2$, 1.3 Hz, 4H), 7.50–7.42 (m, 6H), 7.12 (s, 1H), 6.96 (s, 2H), 6.53 ppm (s, 2H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$, 80°C): $\delta=167.6$, 152.5, 149.2, 139.3, 130.1, 128.8, 127.9, 125.3, 125.2, 103.9, 95.3 ppm; MALDI-TOF: m/z calcd: 424.1; found: 424.0 [M^+].

2,8-Bis(2-methoxyphenyl)-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-6): Compounds **4d** (44.7 mg, 0.174 mmol) and **5d** (41.0 mg, 0.170 mmol) were used as the starting materials, and KFL-6 was obtained as a green metallic solid (56.7 mg, 67.4%). Eluent for chromatography: chloroform. ^1H NMR (CDCl_3): $\delta=7.98$ (d, $J=7.8$ Hz, 2H), 7.39 (t, $J=7.8$ Hz, 2H), 7.30 (s, 2H), 7.09–7.00 (m, 5H), 6.46 (s, 2H), 4.01 ppm (s, 6H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$, 80°C): $\delta=164.2$, 163.6, 156.8,

139.4, 131.5, 126.5, 120.6, 117.4, 112.0, 103.0, 98.7, 55.6 ppm; MALDI-TOF: m/z calcd: 484.1; found: 484.0 [M^+].

2,8-Bis(4-isopropylphenyl)-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-7): Compounds **4e** (26.8 mg, 0.100 mmol) and **5e** (25.1 mg, 0.100 mmol) were used as the starting materials, and KFL-7 was obtained as a green metallic solid (22.5 mg, 44.5%). Eluent for chromatography: *n*-hexane/chloroform 40:60→10:90. ^1H NMR (CDCl_3): δ = 7.75 (d, J = 6.8 Hz, 4H), (d, J = 7.1 Hz, 4H), 7.05 (s, 1H), 6.91 (s, 2H), 6.46 (s, 2H), 3.01–2.92 (m, 2H), 1.31 (s, 6H), 1.28 ppm (s, 6H); ^{13}C NMR (CDCl_3): δ = 168.6, 153.5, 151.7, 150.3, 127.4, 127.2, 125.9, 125.7, 102.4, 94.9, 34.2, 29.7, 23.7 ppm; MALDI-TOF: m/z calcd: 508.2; found: 508.1 [M^+].

2,8-Bis(2-isopropylphenyl)-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-8): Compounds **4f** (27.5 mg, 0.102 mmol) and **5f** (26.1 mg, 0.103 mmol) were used as the starting materials, and KFL-8 was obtained as a green metallic solid (24.7 mg, 47.6%). Eluent for chromatography: *n*-hexane/chloroform 40:60→20:80. ^1H NMR (CDCl_3): δ = 7.63 (d, J = 7.3 Hz, 2H), 7.48–7.41 (m, 4H), 7.29 (t, J = 7.8 Hz, 2H), 7.17 (s, 1H), 6.73 (s, 2H), 6.54 (s, 2H), 3.56–3.49 (m, 2H), 1.30 (s, 6H), 1.26 ppm (s, 6H); ^{13}C NMR (CDCl_3): δ = 169.4, 153.3, 150.5, 148.1, 139.4, 130.5, 129.5, 128.6, 127.5, 126.4, 126.0, 103.2, 99.2, 30.3, 24.1 ppm; MALDI-TOF: m/z calcd: 508.2; found: 508.1 [M^+].

2,8-Bis(2,5-dimethoxyphenyl)-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-9): Compounds **4g** (32.0 mg, 0.111 mmol) and **5g** (30.2 mg, 0.111 mmol) were used as the starting materials, and KFL-9 was obtained as a green metallic solid (23.6 mg, 39.3%). Eluent for chromatography: chloroform/ethyl acetate 98:2. ^1H NMR (CDCl_3): δ = 7.51 (t, J = 1.5 Hz, 2H), 7.32 (s, 2H), 7.07 (s, 1H), 6.94 (d, J = 1.8 Hz, 4H), 6.47 (s, 2H), 3.96 (s, 6H), 3.86 ppm (s, 6H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$, 80°C): δ = 168.1, 163.9, 152.5, 138.0, 131.0, 124.1, 117.6, 113.4, 112.7, 111.0, 103.1, 99.7, 98.9, 56.0, 55.5 ppm; MALDI-TOF: m/z calcd: 544.2; found: 544.1 [M^+].

2,8-Bis(2,4-dimethoxyphenyl)-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-10): Compounds **4h** (23.0 mg, 0.080 mmol) and **5h** (21.5 mg, 0.079 mmol) were used as the starting materials, and KFL-10 was obtained as a green metallic solid (31.2 mg, 71.6%). Eluent for chromatography: chloroform/ethyl acetate 98:2. ^1H NMR (CDCl_3): δ = 7.90 (d, J = 9.0 Hz, 2H), 7.15 (s, 2H), 6.98 (s, 1H), 6.61 (dd, J = 9.0, 2.4 Hz, 2H), 6.54 (d, J = 2.1 Hz, 2H), 6.40 (s, 2H), 3.98 (s, 6H), 3.89 ppm (s, 6H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$, 80°C): δ = 164.4, 162.5, 158.4, 151.1, 149.4, 139.0, 127.7, 125.7, 110.7, 106.5, 102.1, 98.8, 96.7, 55.7, 55.3 ppm; MALDI-TOF: m/z calcd: 544.2; found: 544.1 [M^+].

General procedure for synthesis of asymmetric KFL dyes (KFL-11–14): A 2-substituted 4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acid (1 equiv) was dissolved in trifluoroacetic acid (1 mL) and heated at 50°C for 10 min. 2,4-Dimethylpyrrole-5-carbaldehyde (1 equiv) or 2-substituted 4*H*-furo[3,2-*b*]pyrrole-5-carbaldehyde (1 equiv) and phosphoryl chloride (0.8 mL) was added into the reaction mixture, and stirred for further 10 min until an intense color was formed. After cooling, the reaction mixture was poured into saturated aqueous NaHCO_3 solution to neutralize. The formed precipitate was filtered, washed with water, and dried in vacuo. The resulting compound was dissolved in 1,1,2-trichloroethane (10 mL), before $\text{BF}_3\cdot\text{Et}_2\text{O}$ (0.3 mL) and triethylamine (0.2 mL) were added and the mixture was stirred at 100°C for 15 min. After cooling, the reaction mixture was diluted with chloroform and washed with saturated aqueous NaHCO_3 solution and brine, dried over Na_2SO_4 and evaporated. The residue was purified by chromatography (silica gel) to obtain the KFL-11–14.

2,7,9-Trimethylfuro[2,3-*b*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-11): Compound **4a** (39.0 mg, 0.236 mmol) and 2,4-dimethylpyrrole-5-carbaldehyde (29.7 mg, 0.224 mmol) were used as starting materials, and KFL-11 was obtained as an orange metallic solid (43.2 mg, 66.8%). Eluent for chromatography: *n*-hexane/chloroform 20:80→0:100. ^1H NMR (CDCl_3): δ = 7.05 (s, 1H), 6.40 (s, 1H), 6.29 (s, 1H), 6.05 (s, 1H), 2.54 (s, 3H), 2.45 (s, 3H), 2.22 ppm (s, 3H); ^{13}C NMR (CDCl_3): δ = 168.8, 158.0, 152.4, 150.0, 141.8, 136.1, 135.3, 124.1, 119.3, 102.5, 100.6, 97.7, 15.7, 15.6, 11.3 ppm; MALDI-TOF: m/z calcd: 274.1; found: 273.9 [M^+].

2-Methyl-8-phenyl-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-12): Compounds **4a** (16.0 mg, 0.097 mmol) and **5c** (20.4 mg, 0.094 mmol) were used as starting materials, and KFL-12 was obtained as a golden metallic solid (15.6 mg, 44.4%). Eluent for chromatography: chloroform. ^1H NMR (CDCl_3): δ = 7.80 (d, J = 6.9 Hz, 2H), 7.48–7.40 (m, 3H), 7.08 (s, 1H), 6.93 (s, 1H), 6.48 (s, 1H), 6.41 (s, 1H), 6.35 (s, 1H), 2.49 ppm (s, 3H); ^{13}C NMR (CDCl_3): δ = 170.6, 167.6, 153.2, 151.7, 149.4, 139.0, 130.0, 129.9, 129.0, 127.3, 125.4, 102.9, 102.5, 98.0, 95.4, 15.9 ppm; MALDI-TOF: m/z calcd: 362.1; found: 362.0 [M^+].

2-(2,4-Dimethoxyphenyl)-7,9-dimethyl-furo[2,3-*b*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-13): Compound **4h** (29.0 mg, 0.101 mmol) and 2,4-dimethylpyrrole-5-carbaldehyde (12.5 mg, 0.102 mmol) were used as starting materials, and KFL-13 was obtained as a golden metallic solid (16.1 mg, 40.3%). Eluent for chromatography: *n*-hexane/chloroform 15:85→0:100. ^1H NMR (CDCl_3): δ = 7.88 (d, J = 9.0 Hz, 1H), 7.12 (s, 1H), 7.02 (s, 1H), 6.59 (dd, J = 8.7, 2.4 Hz, 1H), 6.53 (d, J = 2.1 Hz, 1H), 6.42 (s, 1H), 6.05 (s, 1H), 3.96 (s, 3H), 3.87 (s, 3H), 2.57 (s, 3H), 2.23 ppm (s, 3H); ^{13}C NMR (CDCl_3): δ = 165.1, 162.5, 158.8, 156.6, 151.1, 140.5, 137.7, 135.2, 128.5, 122.5, 118.8, 112.3, 105.5, 101.8, 98.6, 98.3, 55.6, 55.5, 14.7, 11.3 ppm; MALDI-TOF: m/z calcd: 396.1; found: 396.0 [M^+].

2-(2,4-Dimethoxyphenyl)-8-methyl-difuro[2,3-*b*][3,2-*g*]-5,5-difluoro-5-bora-3a,4a-diaza-s-indacene (KFL-14): Compounds **4h** (17.1 mg, 0.060 mmol) and **5a** (8.8 mg, 0.059 mmol) were used as starting materials, and KFL-14 was obtained as a green metallic solid (10.7 mg, 42.6%). Eluent for chromatography: *n*-hexane/chloroform 10:90. ^1H NMR (CDCl_3): δ = 7.90 (d, J = 8.7 Hz, 1H), 7.14 (s, 1H), 7.02 (s, 1H), 6.61 (dd, J = 8.7, 2.4 Hz, 1H), 6.53 (d, J = 2.4 Hz, 1H), 6.42 (s, 1H), 6.38 (s, 1H), 6.32 (s, 1H), 3.98 (s, 3H), 3.89 (s, 3H), 2.48 ppm (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$, 80°C): δ = 171.7, 169.7, 163.6, 158.4, 152.1, 149.7, 147.8, 137.7, 133.4, 127.9, 107.1, 102.7, 99.7, 97.0, 95.0, 56.0, 55.4, 15.0 ppm; MALDI-TOF: m/z calcd: 422.1; found: 422.0 [M^+].

Measurements: All solvents for spectrometry were purchased from Kanto Chemical. Absorption spectra were recorded on a Hitachi U-2001 double beam spectrophotometer (Hitachi, Tokyo, Japan). Fluorescence emission spectra and photostabilities were recorded on a F-4500 fluorophotometer (Hitachi, Tokyo, Japan) at 25°C. Quantum yields were recorded on a SREX Fluorolog-3 (Model FL-3-11, Horiba Jobin Yvon, Kyoto, Japan) at 25°C. The instrument was equipped with a R2658P photomultiplier tube (Hamamatsu Photonics, Shizuoka, Japan) as a fluorescence detector. Measurements of quantum yields were performed by following the method recommended by Horiba Jobin Yvon (see: http://www.jp.jobinyvon.horiba.com/product_j/spex/quantum_yield/img/quantum_yields.pdf). A number of diluted solutions of different dye concentrations ($A < 0.10$, to prevent reabsorption) were prepared, and the absorbance (A) and the integrated fluorescence intensity (F) at each concentration were recorded. Then a graph of F versus A was plotted to determine the gradient (G). Quantum yields ϕ were calculated by using Equation (1):

$$\phi_s = \phi_R \left(\frac{G_R}{G_s} \right) \left(\frac{n_R}{n_s} \right) \quad (1)$$

The subscripts R and S denote the reference dye and the sample, respectively, and n is the refractive index of the solvent. The following reference dyes were used: rhodamine 6G (ϕ = 0.95 in ethanol)^[34] for KFL-11, cresyl violet (ϕ = 0.54 in methanol)^[35] for KFL-1, KFL-2, KFL-12 and KFL-13, 3,3'-diethyl-thiadiazocarbocyanine (ϕ = 0.35 in ethanol)^[36] for KFL-5, KFL-8 and KFL-14, boronazadipyrromethene compound aza-BODIPY (ϕ = 0.36 in chloroform)^[22] for KFL-3, KFL-4, KFL-6, KFL-7, KFL-9, and KFL-10.

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