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Accessing Near-Infrared-Absorbing BF₂-Azadipyrromethenes via a Push–Pull Effect

Lijuan Jiao†,†*, Yayang Wu†,†, Sufan Wang†,†, Xiaoke Hu‡, Ping Zhang†, Changjiang Yu†, Kebing Cong†,†, Qianli Meng‡, Erhong Hao†,†, and M. Graça H. Vicente‡,‡

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Abstract

Novel aza-BODIPYs with significant bathochromic shifts were designed and synthesized by installation of strong electron-withdrawing groups on the para-positions of 1,7-phenyls and electron-donating groups on the para-positions of 3,4-phenyls. These dyes show strong NIR fluorescence emissions up to 756 nm, and absorptions up to 720 nm.

Fluorescent dyes with absorptions and emissions in the near-infrared (NIR) region have found important applications in biological and medicinal sciences, such as sensing and imaging since NIR light penetrates deeper into most biological tissues than visible light. Azadipyrromethenes boron difluoride (known as aza-BODIPYs), which typically show a ~90 nm red-shift of the main absorption band with respect to that of their BODIPY analogues, have recently received much attention due to their remarkable photochemical properties and have found various applications in photovoltaics, optoelectronics, bioimaging, sensing, and photodynamic therapy.
The parent 1,3,5,7-tetraphenyl-aza-BODIPY A1 (Figure 1) absorbs at 650 nm and emits at 682 nm in CHCl₃. Several studies have focused on the development of new aza-BODIPY dyes with red-shifted absorptions and emissions further to the NIR range. Various elegant approaches have been reported, including (i) rigidification of rotatable moieties, as demonstrated by aza-BODIPYs B⁸ and C⁹ (ii) planarization of the π-system through reducing the torsion angles, as demonstrated by replacing 3,5-phenyls with thiophenes in aza-BODIPY E¹⁰ (iii) extension of the π-conjugation, as demonstrated by the benzene-fused aza-BODIPY D¹¹ and by aza-BODIPY F¹² However, the syntheses of these compounds generally require multiple steps with lower yields in comparison to that of the parent compound A₁, which limits their practical applications. On the other hand, the simple introduction of electron-donating groups on the para-positions of 3,5-phenyls results in significant red-shifts of both absorption and emission bands. For example, aza-BODIPY A₂ (Scheme 1) bearing electron-donating methoxy groups (Hammett parameter \( \sigma_p = -0.27 \)) absorbs at 680 nm and emits at 723 nm in CHCl₃, giving 38 and 41 nm red-shifts in absorption and emission, respectively, in comparison to aza-BODIPY A₁. The reducing of the band gap by increasing the HOMO and/or decreasing the LUMO energy is crucial for the development of longer wavelength aza-BODIPYs.¹¹ The attachment of an electron-withdrawing group may reduce the LUMO energy. For example, BDP₂ bearing a strong electron-withdrawing cyano group at the meso-position shows a 60 nm red-shift of absorption with respect to BDP₁ (Figure 1).³ᵃ,¹³ By contrast, BODIPYs with electron-donating methylamino and methoxy groups on the meso-position exhibit about 51 and 85 nm blue-shifts of the absorption bands due to the increase of the LUMO energy.¹⁴ On the basis of these considerations, aza-BODIPYs A₃, A₄, and A₆ bearing strong electron-withdrawing groups at the para-positions of 1,7-phenyls were designed, synthesized, characterized, and compared with their reference compounds: aza-BODIPYs A₁ and A₂.

Initially, DFT calculations on aza-BODIPYs A₁, A₂, and A₄ were carried out for the evaluation of our hypothesis, and the results are summarized in Table 1 and in Figures S1 and S2 in the Supporting Information. A general trend of red-shift of the absorption band maximum was observed from aza-BODIPYs A₁, A₂ to A₄ in the calculated absorption spectra (Supplementary Figure S2). For example, aza-BODIPY A₂ shows a 49 nm red-shift in absorption with respect to that of aza-BODIPY A₁, which is close to the experimental result. More importantly, aza-BODIPY A₄ bearing electron-withdrawing cyano groups exhibits a 40 nm further red-shift of the absorption with respect to that of aza-BODIPY A₂. The calculation of aza-BODIPY A₄ also gives an oscillator strength of 0.7946 for the \( S_0 \rightarrow S_1 \) transition. The comparison of the HOMO and LUMO orbital energies clearly demonstrated that the methoxy and cyano groups have different impact on the absorptions of aza-BODIPYs. For aza-BODIPY A₂ containing only methoxy groups at para-positions of 3,5-phenyls, more increase of HOMO than LUMO energy was observed, which leads to the decrease of excitation energy and a red-shift of the absorption band in aza-BODIPY A₂, whereas in aza-BODIPY A₄ with further installation of cyano groups on the para-positions of 1,7-phenyls with respect to A₂, not only the increase of HOMO energy but also the decrease of LUMO energy were observed. In other words, the installation of both the methoxy on the para-positions of 3,5-phenyls and the cyano groups on the para-positions of 1,7-phenyls further reduces the excitation energy in aza-BODIPY A₄.
Encouraged by these DFT calculations, we synthesized aza-BODIPYS A1–A5 in three steps from the aldol condensation of aldehydes and ketones, Michael addition of nitromethane, followed by condensation with ammonium acetate and subsequent BF$_3$ complexation using reported methods$^{5a}$ (Scheme 1). A6 was synthesized in 81% yield from the reaction of A5 with methyl iodide. All of these new compounds were characterized by NMR and HRMS.

Photophysical properties of aza-BODIPYS A3–A6 in solvents with different polarities were studied as shown in Figure 2 and are summarized in Table 2. For comparison purposes, the reference compounds A1 and A2 were also evaluated in three different solvents. In comparison with those of A2, our aza-BODIPYS A3, A4, and A6 generally exhibit 15–28 nm and 17–30 nm bathochromic shifts for absorption and emission, respectively. In the various solvents studied, aza-BODIPY A4 bearing two cyano groups ($\sigma_p = 0.66$) at the para-positions of 1,7-phenyls shows the longest $\lambda_{\text{max}}$ for both absorption (720 nm) and emission (756 nm). Interestingly, in agreement with the DFT calculations, these dyes all show a much enhanced absorption at about 470 nm in comparison with those of A1 and A2. This band can be assigned to the $S_0\rightarrow S_3$ transition according to the DFT calculations and can be used to excite these dyes to achieve larger Stokes shifts. This type of transition has previously been reported in several distyryl-BODIPY dyes and indicates the existence of a push–pull effect in those dyes.$^{13,15}$

Aza-BODIPYS A3, A4, and A6 exhibit moderate fluorescent quantum yields (0.14–0.38) in various solvents studied, with respect to that of A2. These dyes show a relative longer wavelength absorption and emission with higher fluorescence quantum yields in nonpolar solvents, similar to those of the reference compound A2. The solvent dependence of photophysical properties is also in good agreement with classic BODIPYs containing electron-donating groups as discussed in detail by Boens.$^{16}$

To demonstrate the possible utility of our aza-BODIPY dyes, compounds A3, A4, and A6 were applied for cell imaging. They were incubated at the concentration of 10 $\mu$M for 6 h with human carcinoma HEp2 cells at 37 °C. All aza-BODIPYS were found to rapidly accumulate within the cells and gave bright red fluorescence, as shown in Figure 3b and Figure S3b in the Supporting Information. To investigate the main intracellular sites of the localization, the HEp2 cells were co-incubated with each aza-BODIPY and ER Tracker Blue/White (ER) at 100 nM for 30 min, MitoTracker Green (mitochondria) at 250 nM for 30 min, BODIPY FL C5-ceramide (Golgi) at 50 nM for 30 min, and LysoSensor Green (lysosomes) at 50 nM for 30 min. The corresponding overlay images are shown in Figure 3d, f, h and j. These results indicate that aza-BODIPY A4 localizes in all the subcellular sites tested, whereas A3 and A6 localize preferentially in the cell mitochondria, lysosomes and Golgi apparatus. We also studied the phototoxicity of this series of aza-BODIPYS, since high cytotoxicity would limit their potential applicability in cellular imaging. The cytotoxicity of A3, A4, and A6 was evaluated using the Cell Titer Blue viability assay, at concentrations up to 100 $\mu$M, upon exposure to 1 J/cm$^2$ light dose (see Figure S4 in the Supporting Information). None of the dyes showed any phototoxicity up to 100 $\mu$M concentrations. These results are in agreement with previous studies$^{17,18}$ and warrant further investigation of these dyes as bioimaging probes.
In summary, the installation of strong electron-withdrawing groups on the para-positions of 1,7-phenyls and electron-donating groups on the para-positions of 3,4-phenyls in aza-BODIPYs resulted in novel long wavelength NIR aza-BODIPYs that emit above 740 nm, are highly cell-permeable, and show very low cytotoxicity. This strategy provides a simple approach to the development of red-shifted aza-BODIPY dyes, through a push–pull effect.

**EXPERIMENTAL SECTION**

**General**

The NMR experiments were performed on a 300 MHz NMR spectrometer at room temperature. Chemical shifts (δ) are given in ppm relative to TMS. High-resolution mass spectra were obtained using APCI-TOF in positive mode. UV–vis absorption spectra and fluorescence emission spectra were recorded on a commercial spectrophotometer (190–900 nm scan range). The slit width was set at 2.5 nm for excitation and 5.0 nm for emission. Relative fluorescence quantum yields were calculated using A2 in CHCl3 (φ = 0.36) as the standard. All φ values are corrected for changes in refractive index using a previous reported method.19

**Computational Details**

The ground state geometries of molecules A1, A2, and A4 were fully optimized by the DFT method at the B3LYP/6–31g level. The stationary structures have been verified with no imaginary vibration in the frequency calculations. The vertical excitation properties have been estimated by taking TD-DFT single-point calculations under the same level with the optimized ground state geometries. The solvation by chloroform has been estimated in the calculations under the PCM scheme. All of the calculations were carried out by the methods implemented in the Gaussian 09 package.20

**Synthesis of 1a**

To 4-(trifluoromethyl)benzaldehyde (3.0 mL, 0.02 mol) and 4-methoxyacetophenone (3.3 g, 0.02 mol) in anhydrous methanol (20 mL) was added 3 g of KOH. The mixture was stirred at room temperature for 1 h. The precipitate was filtered, washed with methanol, and dried under reduced pressure to give 1a as a light yellow solid in 86% yield (5.5 g).1H NMR (CDCl3, 300 MHz) δ: 8.07 (d, J = 9.0 Hz, 2H), 7.59–7.84 (m, 6H), 7.01 (d, J = 6 Hz, 2H), 3.91 (s, 3H). 13C NMR (CDCl3, 75 MHz) δ: 188.2, 163.7, 141.9, 138.5, 131.5, 130.9, 130.7, 128.4, 125.9 (q, J = 15 Hz), 124.0, 122.1, 114.0, 55.6. HRMS (APCI) calcd for C17H13F3O2 [M + H]+: 307.0940, found 307.0940. Mp 161–163 °C.

1b was synthesized as a light yellow solid in 92% yield (4.8 g) using the above procedure from 4-cyanobenzaldehyde (2.6 g, 0.02 mol) and 4-methoxyacetophenone (3.0 g, 0.02 mol).1H NMR (CDCl3, 300 MHz) δ: 8.03 (d, J = 9.0 Hz, 2H), 7.57–7.69 (m, 6H), 6.98 (d, J = 9.0 Hz, 2H), 3.88 (s, 3H). 13C NMR (CDCl3, 75 MHz) δ: 187.9, 163.8, 141.3, 139.4, 132.7, 131.0, 130.5, 128.6, 125.0, 118.5, 114.0, 113.2, 55.6. HRMS (APCI) calcd for C17H13NO2 [M + H]+: 264.1019, found 264.1017. Mp 171–172 °C.
**Synthesis of 2a**

To compound 1a (6.1 g, 20 mmol) in anhydrous methanol (30 mL) were added diethylamine (10 mL) and nitromethane (10 mL). The mixture was refluxed for 10 h and concentrated under vacuum to afford 2a in 97% yield (7.1 g). 

\[ ^1H \text{NMR (CDCl}_3, 300 \text{ MHz}) \delta: 7.87 (d, J = 9.0 \text{ Hz}, 2H), 7.53 (d, J = 9.0 \text{ Hz}, 2H), 7.43 (d, J = 9.0 \text{ Hz}, 2H), 6.89 (d, J = 9.0 \text{ Hz}, 2H), 4.83–4.89 (m, 1H), 4.66–4.73 (m, 1H), 4.27–4.31 (m, 1H), 3.79 (s, 3H), 3.39–3.41 (m, 2H). \]

\[ ^{13}C \text{NMR (CDCl}_3, 75 \text{ MHz}) \delta: 193.9, 162.9, 142.7, 129.3, 128.7, 128.1, 127.1, 124.8 (q, J = 15 \text{ Hz}), 117.6, 112.4, 78.1, 54.4, 39.8, 38.1. \]

HRMS (APCI) calcd for C\textsubscript{18}H\textsubscript{16}F\textsubscript{3}NO\textsubscript{4} [M + H]\^+: 368.1140, found 368.1107. Mp 141–142 °C.

**Synthesis of A3**

A mixture of 2a (0.37 g, 1.0 mmol) and ammonium acetate (1.5 g, 20 mmol) in ethanol (20 mL) was refluxed for 9 h, cooled to room temperature, and concentrated to about 5 mL. The precipitate was filtered and washed with water and ethanol to give the intermediate azadiipyromethene as a metallic blue-black powder. 

\[ ^1H \text{NMR (CDCl}_3/CF}_3\text{COOH, 300 MHz}) \delta: 11.87 (brs, 2H), 7.95 (d, J = 8.1 \text{ Hz}, 4H), 7.62 (d, J = 7.8 \text{ Hz}, 4H), 7.49 (d, J = 7.8 \text{ Hz}, 4H), 7.30 (s, 2H), 7.06 (d, J = 8.4 \text{ Hz}, 4H), 3.94 (s, 6H). \]

This azadiipyromethene was directly used for the subsequent BF\textsubscript{3} complexation reaction without further purification: To azadiipyromethene (0.15 g, 0.23 mmol) in toluene (100 mL) were added triethylamine (4 mL) and BF\textsubscript{3}·OEt\textsubscript{2} (6 mL). The mixture was stirred at 60 °C for 2 h, and solvents were removed under vacuum. The residue was washed with ethanol and further purified by recrystallization.
from dichloromethane/methanol or by passing through a small plug of silica gel using dichloromethane/hexane as eluent to give the product as a red copper-colored solid in 46% yield over two steps (0.14 g). \(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\) 8.09 (d, \(J = 10.0\) Hz, 8H), 8.08 (d, \(J = 10.0\) Hz, 4H), 7.68 (d, \(J = 10.0\) Hz, 4H), 7.09 (s, 2H), 7.00 (d, \(J = 10.0\) Hz, 4H), 3.88 (s, 6H). \(^1\)C NMR (CDCl\(_3\), 125 MHz) \(\delta\) 162.3, 159.0, 145.2, 141.4, 135.6, 131.8, 130.8 (q, \(J_{F-C} = 31.3\) Hz), 129.1, 125.5 (q, \(J_{F-C} = 2.5\) Hz), 124.1 (q, \(J_{F-C} = 270\) Hz), 123.9, 119.8, 114.5, 55.4. HRMS (APCI) calcd for C\(_{36}\)H\(_{24}\)BF\(_8\)N\(_3\)O\(_2\) [M + H]\(^+\): 694.1907, found 694.1896. Mp >260 °C.

A4 was synthesized using the above procedure from 2b (0.37 g, 1.0 mmol) and ammonium acetate (1.5 g, 20 mmol). The intermediate aza-dipyrromethene was collected as a black powder. \(^1\)H NMR (CDCl\(_3\)/CF\(_3\)COOH, 300 MHz) \(\delta\): 11.94 (brs, 2H), 8.03 (d, \(J = 8.1\) Hz, 4H), 7.80 (d, \(J = 7.8\) Hz, 4H), 7.67 (d, \(J = 7.8\) Hz, 4H), 7.38 (s, 2H), 7.11 (d, \(J = 8.1\) Hz, 4H), 3.96 (s, 6H). The aza-dipyrromethene was directly used for the subsequent BF\(_3\) complexation without further purification to afford aza-BODIPY A4 as a greenish solid in 37% yields over two steps (0.13 g). \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\): 8.09 (m, 8H), 7.74 (d, \(J = 6.0\) Hz, 4H), 7.13 (s, 2H), 7.04 (d, \(J = 9.0\) Hz, 4H), 3.91 (s, 6H). \(^1\)C NMR was not available due to poor solubility. HRMS (APCI) calcd for C\(_{36}\)H\(_{25}\)BF\(_2\)N\(_5\)O\(_2\) [M + H]\(^+\): 608.2064, found 608.2035. Elemental analysis calcd (%) for C\(_{36}\)H\(_{24}\)BF\(_2\)N\(_5\)O\(_2\): C 71.18, H 3.98, N 11.53. Found: C 70.89, H 3.77, N 11.27. Mp >260 °C.

A5 was synthesized using the above procedure from 2c (0.34 g, 1.0 mmol) and ammonium acetate (1.5 g, 20 mmol). The intermediate aza-dipyrromethene was collected as a black powder. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\): 8.00 (d, \(J = 8.1\) Hz, 4H), 7.84 (d, \(J = 8.1\) Hz, 4H), 7.00–6.95 (m, 6H), 7.38 (s, 2H), 6.74 (d, \(J = 8.1\) Hz, 4H), 3.86 (s, 6H), 2.99 (s, 12H). \(^1\)C NMR (CDCl\(_3\), 75 MHz) \(\delta\): 160.6, 153.7, 150.1, 149.1, 142.0, 129.9, 127.9, 125.6, 122.7, 114.4, 112.0, 111.2, 55.4, 40.4. The aza-dipyrromethene was directly used for the BF\(_3\) complexation without further purification to afford Aza-BODIPY A5 as a greenish solid in 44% yield over two steps (0.14 g). \(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\): 8.08 (d, \(J = 5.0\) Hz, 4H), 8.05 (d, \(J = 10.0\) Hz, 4H), 6.98 (d, \(J = 10.0\) Hz, 4H), 6.83 (brs, 6H), 3.87 (s, 6H), 3.09 (s, 12H). \(^1\)C NMR (CDCl\(_3\), 125 MHz) \(\delta\): 161.3, 156.9, 150.8, 145.3, 142.9, 131.2, 130.8, 125.0, 121.3, 115.1, 114.0, 112.2, 55.6, 40.4. HRMS (APCI) calcd for C\(_{38}\)H\(_{36}\)BF\(_2\)N\(_5\)O\(_2\) [M + H]\(^+\): 644.3003, found 644.2976. Elemental analysis calcd (%) for C\(_{40}\)H\(_{42}\)BF\(_2\)I\(_2\)N\(_5\)O\(_2\): C 51.80, H 4.56, N 7.55. Found: C 51.44, H 4.19, N 7.20. Mp >260 °C.

**Synthesis of A6**

Aza-BODIPY A5 (0.13 g, 0.2 mmol) and methyl iodide (3.7 mL, 60 mmol) were stirred in dry chloroform (20 mL) at 50 °C in the dark for 48 h. The precipitate was filtered, washed with chloroform, and dried under vacuum to give aza-BODIPY A6 as a brown solid in 81% yield (0.15 g). \(^1\)H NMR (DMSO-\(d_6\), 300 MHz) \(\delta\): 8.37 (d, \(J = 9.0\) Hz, 4H), 8.21 (m, 8H), 7.76 (s, 2H), 7.14 (d, \(J = 9.0\) Hz, 4H), 3.90 (s, 6H), 3.24 (s, 18H). \(^1\)C NMR (DMSO-\(d_6\), 75 MHz) \(\delta\): 162.8, 158.2, 147.9, 145.2, 140.2, 133.7, 132.5, 130.9, 123.3, 121.5, 114.1, 57.0, 56.2. HRMS (APCI) calcd for C\(_{36}\)H\(_{24}\)BF\(_2\)N\(_3\)O\(_2\) [M – 2CH\(_3\) + H]\(^+\): 644.3003, found 644.2980. Elemental analysis calcd (%) for C\(_{40}\)H\(_{42}\)BF\(_2\)I\(_2\)N\(_5\)O\(_2\): C 51.80, H 4.56, N 7.55. Found: C 51.44, H 4.19, N 7.20. Mp >260 °C.
Cell Culture

All tissue culture media and reagents were obtained from Invitrogen. Human HEp2 cells were obtained from ATCC and maintained in a 50:50 mixture of DMEM/Advanced MEM containing 5% FBS, 1% Primocin antibiotic in a humidified, 5% CO₂ incubator at 37 °C. The cells were subcultured biweekly to maintain subconfluent stocks. The compounds were dissolved in DMSO and 1% of Cremaphor to make a 400 μM stock solution.

Microscopy

The cells were incubated in a glass-bottom 6-well plate (MatTek) and allowed to grow for 48 h. The cells were then exposed to 10 μM of each compound for 6 h. Organelle tracers were obtained from Invitrogen and used at the following concentrations: LysoSensor Green 50 nM, MitoTracker Green 250 nM, ER Tracker Blue/white 100 nM, and BODIPY FL C5 ceramide 1 mM. After 30 min of incubation at 37 °C in 5% CO₂ incubator, both the media and the organelle tracers were removed and the cells washed with PBS 3 times. Images were acquired using a Leica DMRXA microscope with 40× NA 0.8 dip objective lens and DAPI, GFP, and Texas Red filter cubes (Chroma Technologies).

Phototoxicity

HEp2 cells were plated at 10,000 per well in a Costar 96-well plate and allowed 48 h to attach. The cells were exposed to increasing concentrations of aza-BODIPYs up to 100 μM. After compound loading overnight, the medium was removed and replaced with medium containing 50 mM HEPES pH 7.2. The cells were then placed on ice and exposed to light from a 100 W halogen lamp filtered through a 610 nm long pass filter (Chroma) for 20 min. An inverted plate lid filled with water to a depth of 5 mm acted as an IR filter. The total light dose was approximately 1 J/cm². The cells were returned to the incubator for 24 h. The loading medium was then removed, and the cells were fed medium containing Cell Titer Blue (Promega) as per manufacturer’s instructions. Cell toxicity was then measured by reading the fluorescence at 520/584 nm using a BMG FLUOstar plate reader. The signal was normalized to 100% viable (untreated) cells and 0% viable (treated with 0.2% saponin from Sigma) cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References


Figure 1.
Reported strategies aimed at extending the absorption and emission of aza-BODIPYs to the NIR spectral range.
Figure 2.
Normalized absorption (a) and fluorescence (b) spectra of aza-BODIPYs A1 (black), A2 (red), A3 (green), A4 (blue), and A6 (orange) in CHCl$_3$. 
Figure 3.
Subcellular localization of aza-BODIPYs A3 (left) and A4 (right) in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) overlay of A3/A4 fluorescence and phase contrast, (c) ER tracker Blue/White fluorescence, (e) MitoTracker Green fluorescence, (g) BODIPY Ceramide fluorescence, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with A3/A4 fluorescence. Scale bar: 10 μm.
Scheme 1.
Syntheses of aza-BODIPYs A1–A7
Table 1

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<th>dyes</th>
<th>excited state</th>
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<th>$\lambda_{abs}$ (nm)</th>
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$^a$Calculated absorption peaks.

$^b$Oscillator strength.
### Table 2

Photophysical Properties of aza-BODIPYs in Different Solvents

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<th>λ&lt;sub&gt;abs&lt;/sub&gt; (nm)</th>
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<th>SS (cm&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>φ&lt;sub&gt;f&lt;/sub&gt;&lt;sup&gt;a,c&lt;/sup&gt;</th>
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<sup>a</sup>A1 excited at 610 nm, A2–A6 excited at 670 nm.

<sup>b</sup>SS: Stokes shift.

<sup>c</sup>The fluorescence quantum yields of A2–A6 were calculated using A2 in CHCl<sub>3</sub> (φ = 0.36) as the standard.