2-21-2014

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Recommended Citation

Jiao, L., Wu, Y., Wang, S., Hu, X., Zhang, P., Yu, C., Cong, K., Meng, Q., Hao, E., & Vicente, M. (2014). Accessing near-infrared-absorbing BF_2-azadipyrromethenes via a push-pull effect. *Journal of Organic Chemistry, 79* (4), 1830-1835. https://doi.org/10.1021/jo402160b

HHS Public Access

Author manuscript

J Org Chem. Author manuscript; available in PMC 2015 April 30.

Published in final edited form as:

J Org Chem. 2014 February 21; 79(4): 1830–1835. doi:10.1021/jo402160b.

Accessing Near-Infrared-Absorbing BF_2-Azadipyrromethenes via a Push–Pull Effect

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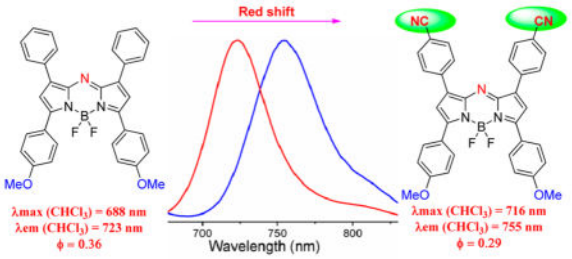
Abstract

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,[1] such as sensing and imaging since NIR light penetrates deeper into most biological tissues than visible light.[2] 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,[3,4] have recently received much attention due to their remarkable photochemical properties[5] and have found various applications in photovoltaics, optoelectronics, bioimaging, sensing, and photodynamic therapy.[5–7]

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Notes

The authors declare no competing financial interest.

Supporting Information

Copies of NMR spectra and high resolution mass spectra for all new compounds, calculated frontier orbitals and absorption spectra, subcellular localization of aza-BODIPY **A6**, and cytotoxicities of aza-BODIPYs **A3**, **A4**, and **A6**. This material is available free of charge via the Internet at http://pubs.acs.org.

The parent 1,3,5,7-tetraphenyl-aza-BODIPY **A1** (Figure 1) absorbs at 650 nm and emits at 682 nm in $CHCl_3$. 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 **B**[8] and **C**,[9] (ii) planarization of the π-system through reducing the torsion angles, as demonstrated by replacing 3,5-phenyls with thiophenes in aza-BODIPY **E**,[10] (iii) extension of the π-conjugation, as demonstrated by the benzene-fused aza-BODIPY **D**[11] and by aza-BODIPY **F**.[12] 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 $CHCl_3$, 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 (σ_p = 0.66) at the *para*-positions of 1,7-phenyls shows the longest λ_{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 μM 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 μM, 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 μM 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 $CHCl_3$ ($\phi = 0.36$) 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). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{17}H_{13}F_3O_2$ $[M + H]^+$: 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). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{17}H_{13}NO_2$ $[M + H]^+$: 264.1019, found 264.1017. Mp 171–172 °C.

1c was synthesized as a light yellow solid in 85% yield (4.8 g) using the above procedure from 4-dimethylaminobenzaldehyde (3.0 mL, 0.02 mol) and 4-methoxyacetophenone (3.0 g, 0.02 mol). ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 8.04 (d, *J* = 6.0 Hz, 2H), 7.80 (d, *J* = 18.0 Hz, 2H), 7.56 (d, *J* = 6.0 Hz, 2H), 7.37 (d, *J* = 15.0 Hz, 2H), 6.98 (d, *J* = 6.0 Hz, 2H), 6.71 (d, *J* = 9.0 Hz, 2H), 3.89 (s, 3H), 3.05 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 188.9, 163.0, 151.9, 145.0, 131.9, 130.6, 130.3, 122.8, 116.6, 113.7, 111.8, 55.5, 40.2. HRMS (APCI) calcd for $C_{17}H_{13}NO_2$ $[M + H]^+$: 282.1489, found 282.1482. Mp 141–143 °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). ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 7.87 (d, *J* = 9.0 Hz, 2H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.43 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 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 NMR ($CDCl_3$, 75 MHz) *δ*: 193.9, 162.9, 142.7, 129.3, 128.7, 128.1, 127.1, 124.8 (q, *J* = 15 Hz), 117.6, 112.4, 78.1, 54.4, 39.8, 38.1. HRMS (APCI) calcd for $C_{18}H_{16}F_3NO_4$ $[M + H]^+$: 368.1140, found 368.1107. Mp 141–142 °C.

2b was synthesized as a yellow oily product in 96% yield (3.1 g) using the above procedure from **1b** (2.6 g, 10 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 7.85 (d, *J* = 6.0 Hz, 2H), 7.59 (d, *J* = 9.0 Hz, 2H), 741 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 6.0 Hz, 2H), 4.80–4.87 (m, 1H), 4.64–4.71 (m, 1H), 4.23–4.28 (m, 1H), 3.83 (s, 3H), 3.37 (d, *J* = 6.0 Hz, 2H). ^{13}C NMR($CDCl_3$, 75 MHz) *δ*: 194.6, 164.0, 144.9, 132.7, 130.3, 129.0, 128.6, 118.5, 114.0, 111.6, 78.9, 55.6, 40.6, 39.3. HRMS (APCI) calcd For $C_{18}H_{16}N_2O_4$ $[M + H]^+$: 325.1183, found 325.1181. Mp 93–94 °C.

2c was synthesized as a yellow oily product in 89% yield (6.1 g) using the above procedure from **1c** (5.6 g, 20 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 7.91 (d, *J* = 6.0 Hz, 2H), 7.14 (d, *J* = 9.0 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 2H), 6.68 (d, *J* = 9.0 Hz, 2H), 4.76–4.82 (m, 1H), 4.59–4.66 (m, 1H), 4.11 (m, 1H), 3.87 (s, 3H), 3.35 (d, *J* = 6.0 Hz, 2H), 2.92 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 194.8, 162.7, 148.9, 129.3, 128.5, 127.0, 125.5, 112.8, 111.7, 79.0, 54.5, 41.3, 40.4, 39.4. HRMS (APCI) calcd for $C_{19}H_{22}N_2O_4$ $[M + H]^+$: 343.1652, found 343.1643. Mp 113–114 °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 aza-dipyrromethene as a metallic blue-black powder. ^{1}H NMR ($CDCl_3$/CF_3COOH, 300 MHz) *δ*: 11.87 (brs, 2H), 7.95 (d, *J* = 8.1 Hz, 4H), 7.62 (d, *J* = 7.8 Hz, 4H), 7.49 (d, *J* = 7.8 Hz, 4H), 7.30 (s, 2H), 7.06 (d, *J* = 8.4 Hz, 4H), 3.94 (s, 6H). This aza-dipyrromethene was directly used for the subsequent BF_3 complexation reaction without further purification: To aza-dipyrromethene (0.15 g, 0.23 mmol) in toluene (100 mL) were added triethylamine (4 mL) and $BF_3{\cdot}OEt_2$ (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 dichoromethane/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) *δ*: 8.09 (d, *J* = 10.0 Hz, 4H), 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). ^{13}C NMR ($CDCl_3$, 125 MHz) *δ*: 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 $C_{36}H_{24}BF_8N_3O_2$ $[M + H]^+$: 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_3COOH, 300 MHz) *δ*: 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) *δ*: 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). ^{13}C NMR was not available due to poor solubility. HRMS (APCI) calcd for $C_{36}H_{25}BF_2N_5O_2$ $[M + H]^+$: 608.2064, found 608.2035. Elemental analysis calcd (%) for $C_{36}H_{24}BF_2N_5O_2$: 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) *δ*: 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). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 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) *δ*: 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). ^{13}C NMR ($CDCl_3$, 125 MHz) *δ*: 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 $C_{38}H_{36}BF_2N_5O_2$ $[M + H]^+$: 644.3003, found 644.2976. 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) *δ*: 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). ^{13}C NMR (DMSO-d_6, 75 MHz) *δ*: 162.8, 158.2, 147.9, 145.2, 140.2, 133.7, 132.5, 130.9, 123.3, 121.5, 121.4, 115.1, 57.0, 56.2. HRMS (APCI) calcd for $C_{38}H_{36}BF_2N_5O_2$ $[M – 2CH_3 + H]^+$: 644.3003, found 644.2980. Elemental analysis calcd (%) for $C_{40}H_{42}BF_2I_2N_5O_2$: 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% CO_2 incubator at 37 °C. The cells were subcultured biweekly to maintain subconfluent stocks. The compounds were dissolved in DMSO and 1% of Cremophor 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% CO_2 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/cm^2. 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

This work is supported by the National Nature Science Foundation of China (Grant Nos. 21072005, 21272007, and 21372011), National Science Foundation of Anhui Province (1208085MB29), and the United States National Science Foundation (CHE-0911629).

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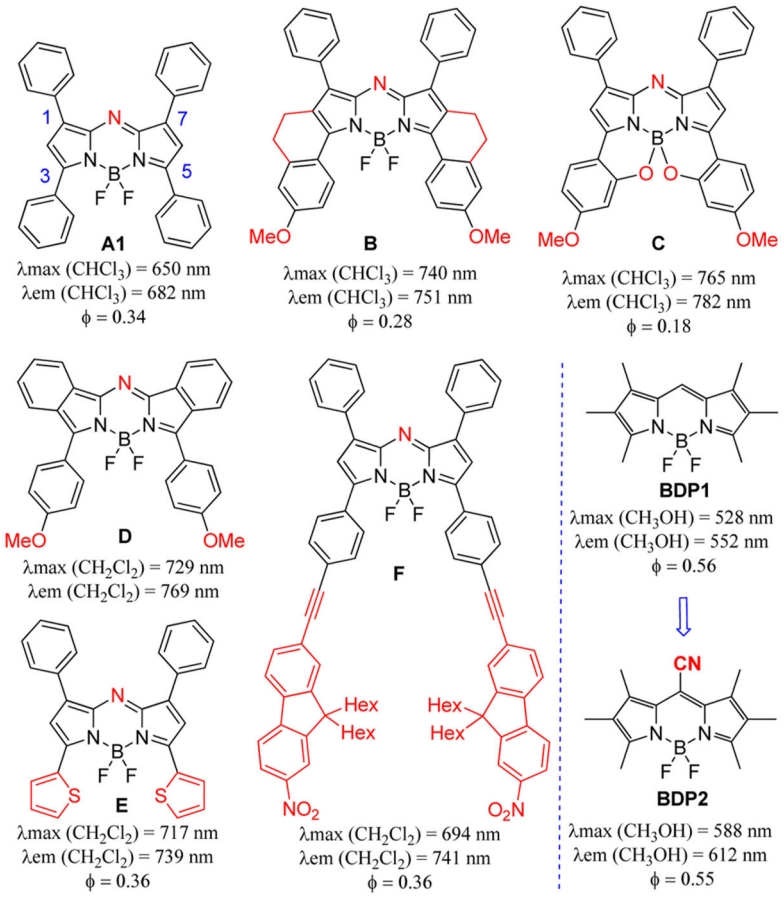


Figure 1.
Reported strategies aimed at extending the absorption and emission of aza-BODIPYs to the NIR spectral range.

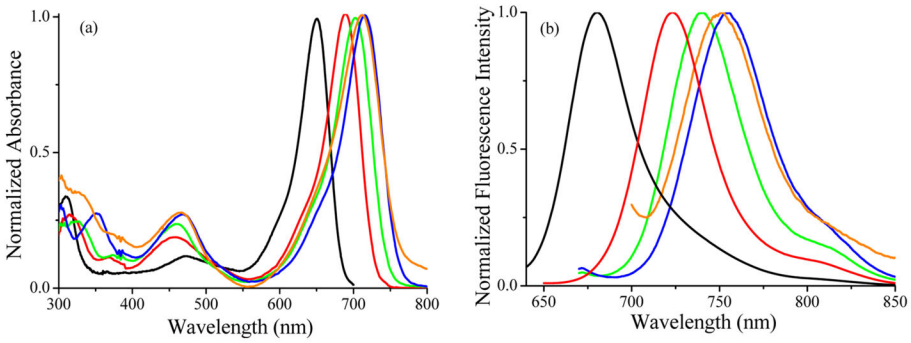


Figure 2.

Normalized absorption (a) and fluorescence (b) spectra of aza-BODIPYs **A1** (black), **A2** (red), **A3** (green), **A4** (blue), and **A6** (orange) in $CHCl_3$.

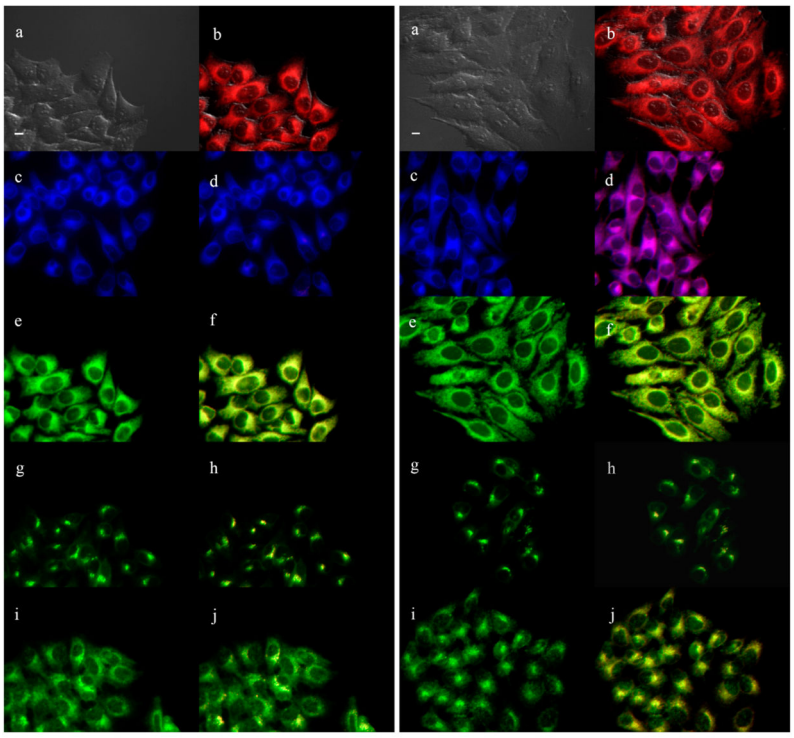
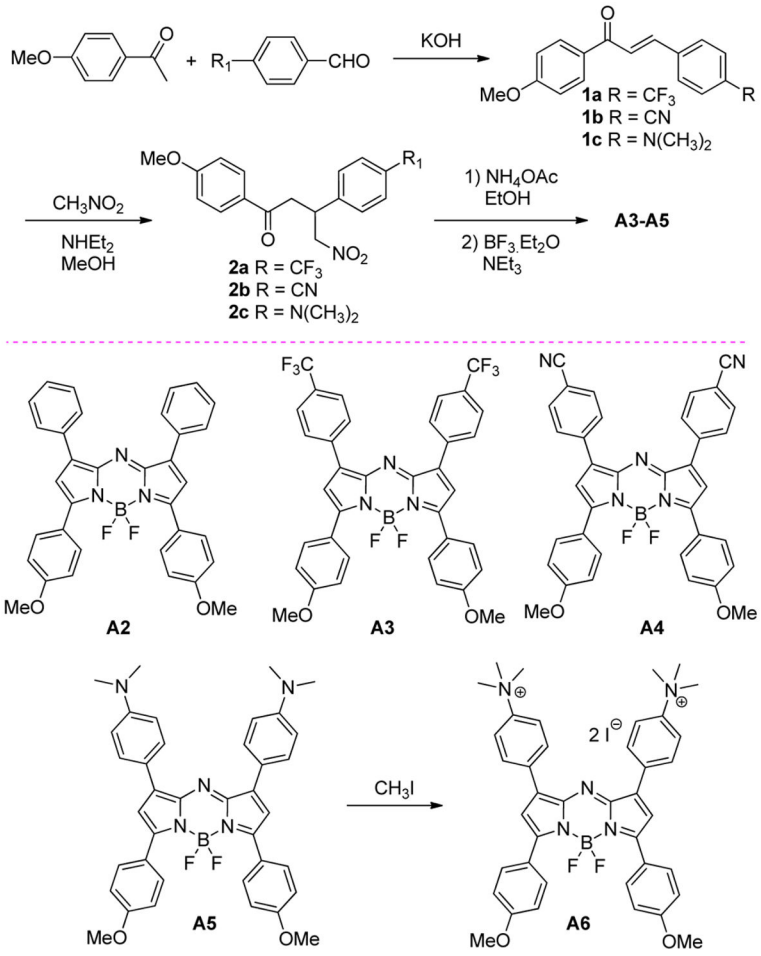


Figure 3.

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

DFT Calculation Results

dyes	excited state	orbital	λ_{abs} [a] (nm)	HOMO/LUMO (eV)	f [b]
A1	S1	H→L	598	−5.665/−3.465	0.8490
	S3	H-2→L	482		0.3882
A2	S1	H→L	647	−5.380/−3.337	0.8575
	S3	H-1→L	485		0.6610
A4	S1	H→L	687	−5.574/−3.645	0.7946
	S3	H-1→L	495		0.4533

[a] Calculated absorption peaks.

[b] Oscillator strength.

Table 2

Photophysical Properties of aza-BODIPYs in Different Solvents

dyes	solvent	λ_{abs} (nm)	λ_{ems} [a] (nm)	SS (cm^{-1})[b]	ϕ_f[a,c]
A1	toluene	654	684	672	0.44
	chloroform	650	682	722	0.34
	methanol	645	673	645	0.17
A2	toluene	693	723	599	0.39
	chloroform	688	723	704	0.36
	methanol	685	720	710	0.26
A3	toluene	709	740	591	0.38
	chloroform	704	741	709	0.34
	THF	709	744	664	0.28
	methanol	700	739	754	0.21
	acetonitrile	697	740	834	0.26
A4	toluene	720	754	626	0.36
	chloroform	716	755	721	0.29
	THF	718	756	700	0.22
	methanol	710	750	751	0.16
	acetonitrile	706	750	831	0.21
A6	toluene	not soluble			
	chloroform	712	752	747	0.14
	THF	714	750	672	0.16
	methanol	706	743	705	0.14
	acetonitrile	702	744	804	0.18

[a] **A1** excited at 610 nm, **A2–A6** excited at 670 nm.

[b] SS: Stokes shift.

[c] The fluorescence quantum yields of **A2–A6** were calculated using **A2** in $CHCl_3$ ($\phi = 0.36$) as the standard.