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

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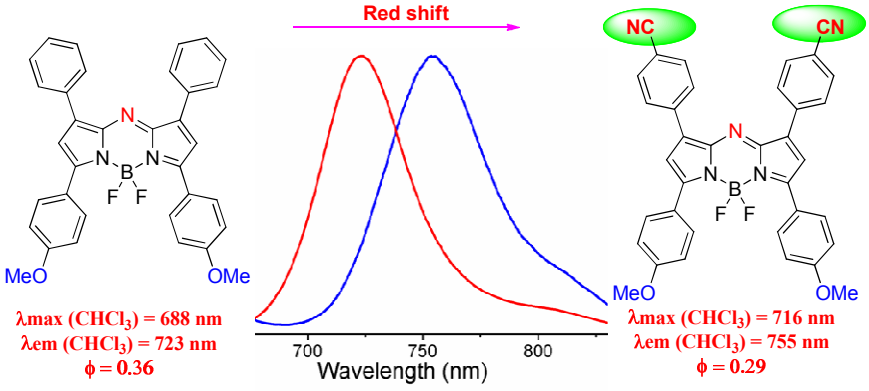



**Abstract**: Novel aza-BODIPYs with significant bathochromic shifts were designed and synthesized by installation of strong electron-withdrawing groups on the *para*-positions of 1,7-phenyls. These dyes show strong NIR fluorescence emissions up to 756 nm, with absorptions up to 720 nm.

Fluorescent dyes with absorptions and emissions in the near-infrared (NIR) region have found important applications in biology and medicine 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 an around 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]

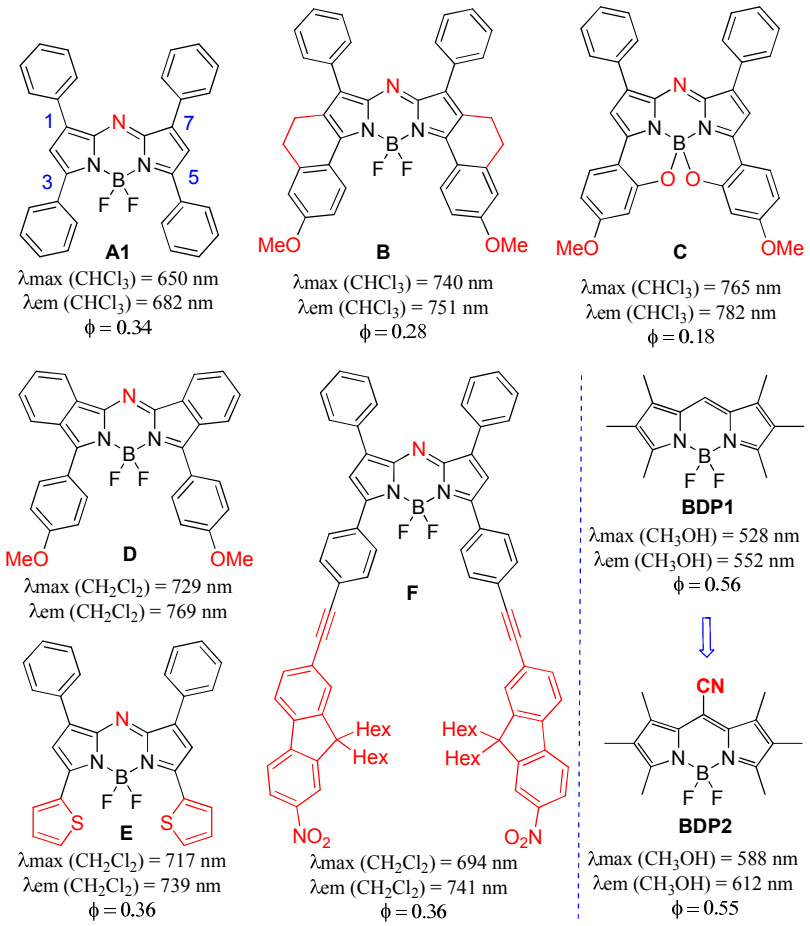



**Figure 1.** Reported strategies aimed at extending the absorption and emissiom of aza-BODIPYs to the NIR spectral range.

The parent 1,3,5,7-tetraphenyl-aza-BODIPY **A1** (Fig. 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,

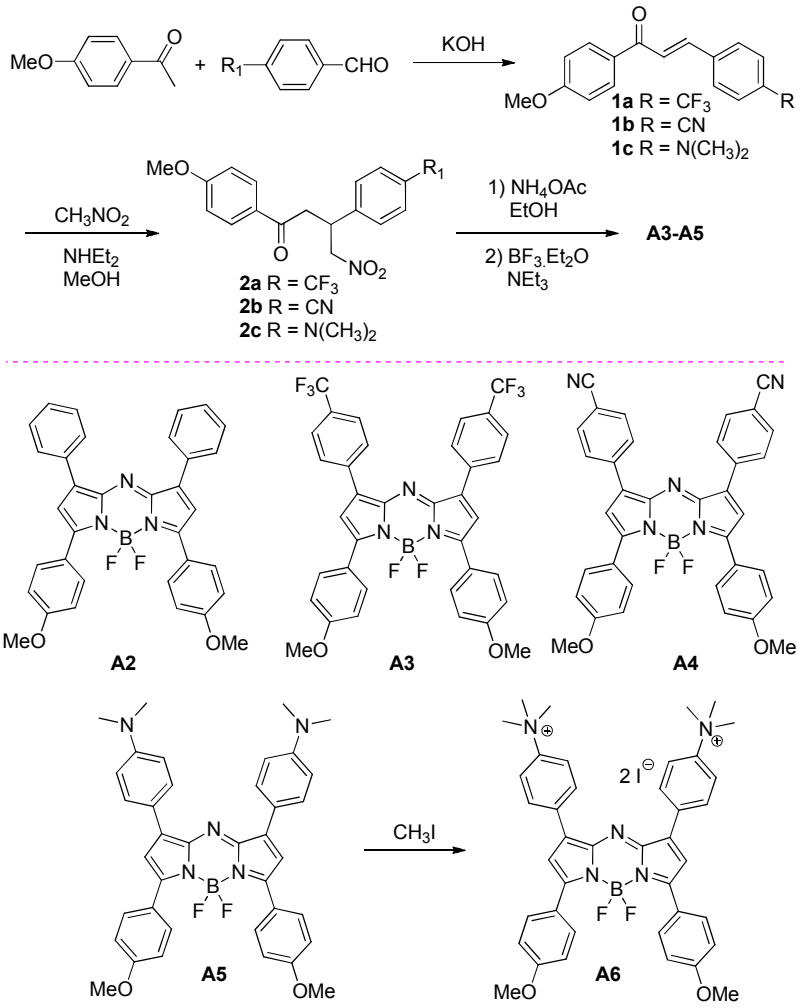
include: (i) rigidification of rotatable moieties, as demonstrated by aza-BODIPYs **B**[8] and **C**,[9] (ii) planarization of the π-system through reducing the torsion angles, as demonstrated by replacing 3,5 -phenyls with thiophenes in aza-BODIPY **E**,[10] (iii) extension of the π-conjugation, as 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 limit 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 a 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** (Fig. 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] Based on 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**.

**Table 1**. DFT Calculation Results

| dyes | excited state | orbital | $\lambda_{abs}$ [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] Stands for calculated absorption peaks. [b] Oscillator strength.




**Scheme 1**. Syntheses of aza-BODIPYs **A1-A7**.

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 Figs. 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 (Fig. 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 increases 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**. While in aza-BODIPY **A4** with a 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 decreases of LUMO energy were observed. In another word, 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 facilitates the reduce of 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 the 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 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 Fig. 2 and 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 $\lambda_{max}$ 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]

**Table 2**. Photophysical properties of aza-BODIPYs in different solvents**.**

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

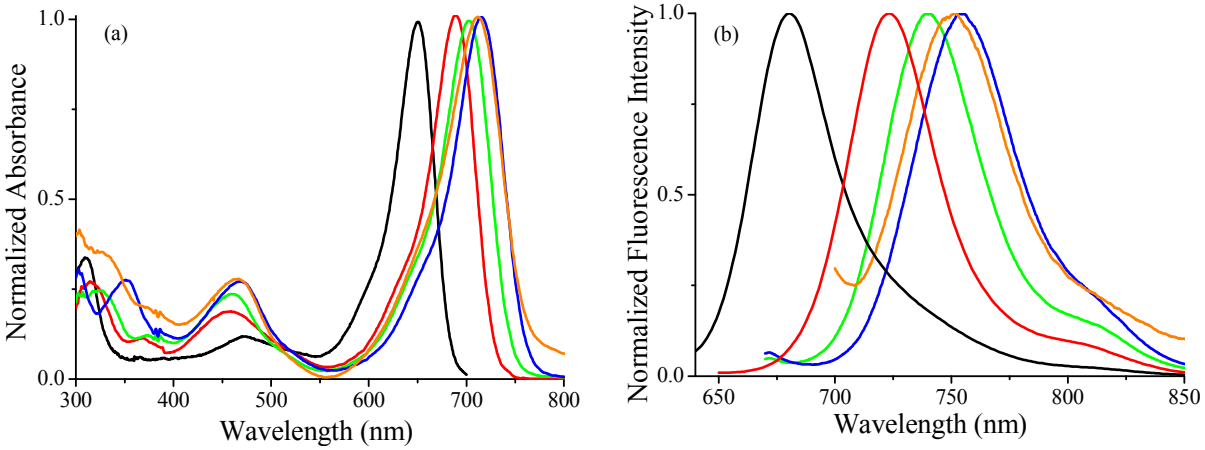



**Figure 2.** Normalized absorption (a) and fluorescence spectra (b) of aza-BODIPYs **A1** (black), **A2** (red), **A3** (green), **A4** (blue) and **A6** (orange) in $CHCl_3$.

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 are also in good agreement with classic BODIPYs containing electron-donating groups as disclosed 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 Fig. 3b and Fig. 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 were shown in Figs. 3d, 3f, 3h and 3j. These results indicate that aza-BODIPY **A4** localizes in all the subcellular sites tested, while **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 Fig. 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.

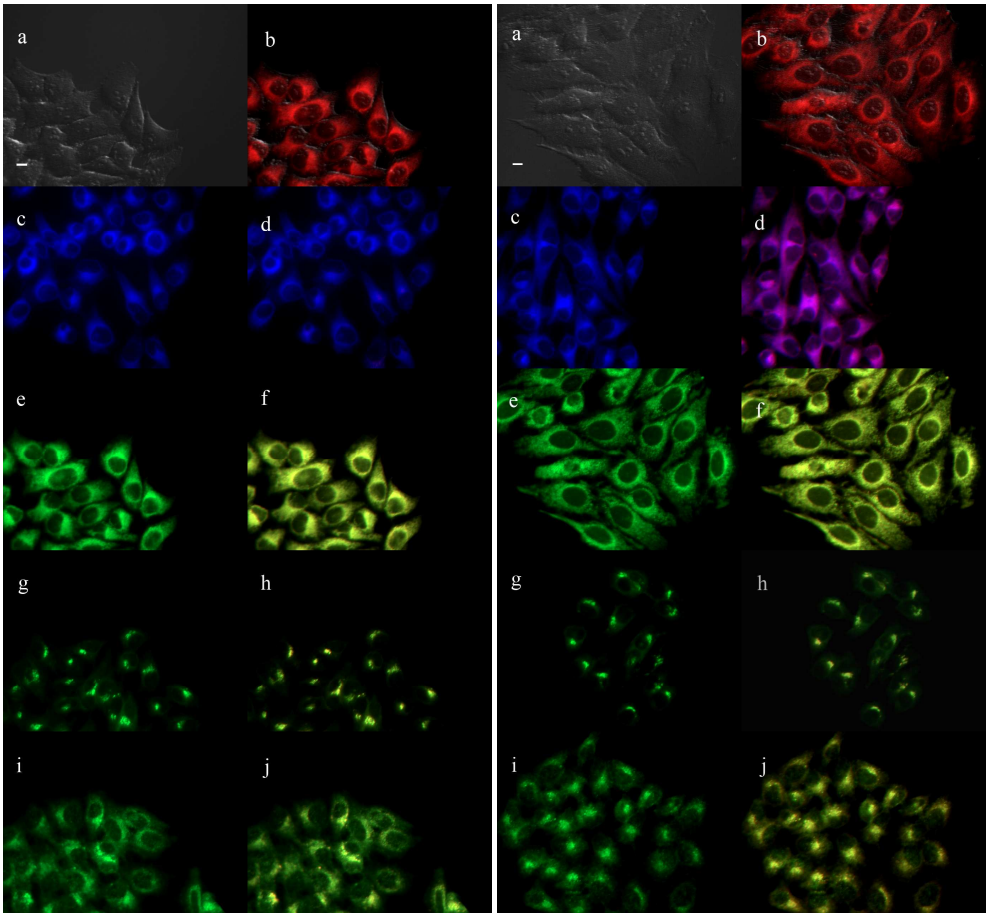



**Figure 3.** Subcellular localization of aza-BODIPYs **A3** (left) and **A4** (right) in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) overlay of **A3**/**A4** fluorescence and phase contrast, (c) ER tracker Blue/White fluorescence, (e) MitoTracker Green fluorescence, (g) BODIPY Ceramide fluorescence, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with **A3**/**A4** fluorescence. Scale bar: 10 μm.

In summary, the installation of strong electron-withdrawing groups on the *para*-positions of 1,7-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 obtained 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-visible 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 $\phi$ 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 with DFT method under 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 as well. All of the calculations were carried out by the methods implemented in Gaussian 09 package.[20]

**Synthesis of 1a**. To 4-(trifluoromethyl)benzaldehyde (3.0 mL, 0.02 mol) and 4-methoxyacetophone (3.3 g, 0.02 mol) in anhydrous methanol (20 mL) were 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 $^o$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-methoxyacetophone (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-methoxyacetophone (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, 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 $^oC$.

**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) were refluxed for 9 h, cooled down to room temperature and concentrated to about 5 mL. The precipitate was filtered, washed with water and ethanol to give the intermediate aza-dipyrromethene as a metallic blue-black powder. $^1H$ 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) was added triethylamine (4 mL) and $BF_3{\cdot}OEt_2$ (6 mL). The mixture was stirred at 60 $^oC$ for 2hand removed solvents under vacuum. The residue was washed with ethanol, and further purified by recrystallization from dichoromethane/methanol or by passing 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). $^1H$ 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, $J_{F\text{-}C}$ = 31.3 HZ), 129.1, 125.5 (q, $J_{F\text{-}C}$ = 2.5 HZ), 124.1 (q, $J_{F\text{-}C}$ = 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 $^oC$.

**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 black powder. $^1H$ 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). It 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). $^1H$ 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, $J$ = 9.0Hz, 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 $^oC$.

**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. $^1H$ 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. It 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). $^1H$ 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 $^oC$.

**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 $^oC$ in 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). $^1H$ NMR (DMSO-$d_6$**,** 300 MHz) δ: 8.37 (d, $J$ = 9.0Hz, 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 $^oC$.

**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 incubation in the 37 °C, 5% $CO_2$ incubator, both the media and the organelle tracers were removed and washed with PBS buffer for 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 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 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.

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**Supporting Information Available:** 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** are available free of charge via the Internet at http://pubs.acs.org.

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