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Conformationally Restricted Aza-BODIPY: Highly Fluorescent, Stable Near-Infrared Absorbing Dyes

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Abstract: Novel NIR fluorescent, conformational restricted aza-dipyrromethene boron difluoride (aza-BODIPY) dyes were prepared by an efficient process. Such conformational restricted aza-BODIPY dyes possess intense absorption, strong fluorescence, high chemical and photostability. Additionally, the sharp fluorescence of non-amine containing aza-BODIPY dyes is insensitive to solvent polarity.

Keywords: biosensors · dyes · fluorescent probes · heterocycles

Introduction

Near-infrared (NIR) dyes have many applications in the market place such as laser optical recording, laser thermal writing displays, infrared photography, and applications in medicine.[1] In recent years, there has been intense interest on the preparation and study of NIR dyes as safe, non-invasive probes.[2]

The advantages of imaging in the NIR region (700–1100 nm) have been extensively discussed.[2] The most prominent of these are the absence or significant reduction of background absorption, fluorescence, and light scattering.[3,4] The wide availability of low-cost diode lasers as sources of irradiation is also a plus.[2] NIR fluorescent probes enable researchers to detect particular components of complex biomolecular systems with good sensitivity and selectivity. Several NIR absorbing dyes such as cyanines, oxazines, squarines have been developed for imaging purposes.[1,2] However, it is very difficult to design NIR fluorophores to fulfil all the requirements for an ideal NIR probe such as: peak fluorescence at 700–900 nm, high fluorescence quantum yield, narrow excitation/emission, high chemical and photo-stability, low toxicity, and excellent biocompatibility.[2] Problems such as aggregation,[5] photobleaching,[6] and low fluorescence quantum yields[6,7] are frequently encountered for NIR dyes. For example, NIR absorbing cyanine dyes are most frequently used for labeling bioactive molecules, however, all NIR absorbing cyanine dyes have poor photostability, and in most cases possess low fluorescence quantum yields.[4] To the best of our knowledge, the highest fluorescence quantum yield for NIR cyanine dyes was reported to be 0.28 in water.[5] Thus, there is a pressing need for the identification of stable and effective NIR probes. In a recent communication, we reported a highly fluorescent, photostable, conformationally restricted aza-dipyrromethene boron difluoride (aza-difluoroboradiaza-*s*-indacene, aza-BODIPY) dye.[8] Herein we present detailed studies on synthesis and fluorescence of a series of NIR absorbing aza-BODIPY dyes.

Dipyrromethene boron difluoride (difluoroboradiaza-*s*-indacene, BODIPY) dyes are well-known to be highly fluorescent, very stable, and exceptionally insensitive to the polarity of solvents as well as to pH. BODIPY dyes are unusual in that they are relatively non-polar and are electronically neutral. They have found widespread applications as laser dyes, molecular probes and sensors in the visible region.[2e,9] Recent efforts have been focused on tuning the fluorescent emission to the red region and even to the NIR region by modifying the dipyrromethene core. Those modifications include: 1) attaching strongly electron-donating groups;[10] 2) rigidifying the structure of the core of the dye;[11] and 3) extending conjugation of the system.[12,13] A BODIPY dye with extended conjugation can absorb over 600 nm, however, further extension of conjugation only leads to slight red-shift in general.[13] Additionally, such modifications have led to dyes with absorption maxima over 700 nm, however, no fluorescence quantum yield was reported for these, thus it is questionable whether such systems can have practical applications.[14] Moreover, these structural alterations can lead to molecules exhibiting undesired properties; for example, BODIPY dyes substituted with strongly electron-donating groups such as amines display sensitivity to the polarity of

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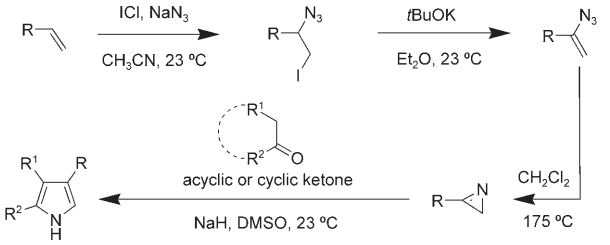
solvents, leading to low Φ in polar solvents as a consequence of intramolecular electron transfer.[12b] A general phenomenon observed in NIR dyes is that decreased fluorescence quantum yield was often observed in a highly extended conjugating system due to increased internal conversion according to the energy gap law that states that the non-radiative deactivation probability of S_0–S_1 increases as the energy gap of S_0–S_1 decreases.[15]

In contrast to the well-known BODIPY dyes, aza-dipyrromethene dyes have not been extensively studied.[16] Recently, O'Shea's group reported that aza-BODIPY dyes carrying heavy atoms absorb up to 688 nm, and act as efficient photodynamic therapy (PDT) agents.[16] We selected the previously unknown structurally rigidified aza-dipyrromethene dyes as our targets for synthesis. We speculated that the rigidified aza-BODIPY core would offer a number of advantages including: 1) inherent bathochromic shift of the absorption maxima in comparison to the carbon analogue; 2) high extinction coefficient, and high fluorescence quantum yield; and 3) better conjugation in the system, which permits absorption of the dye at longer wavelength. However, the required synthesis of aza-BODIPY dyes comprises a nitrosopyrrole intermediate, which requires 2,4-diaryl substituted pyrrole substrate in the reported synthesis due to the inherent problem of nitrosation in pyrroles.[17] To the best of our knowledge, no syntheses of rigidified pyrroles possessing 2,4-diaryl substituents have been reported. The known synthesis of a rigidified pyrrole with 2-aryl-4-methyl substituents is a low yielding process.[11] We took note of a report in the 1960s for the synthesis of 2,4-diphenyl pyrrole in moderate yield, wherein azirine was used as the key intermediate.[18] Herein, we prepare both rigidified and non-rigidified 5-aryl pyrroles in a practical manner and use these in the synthesis of novel symmetric and non-symmetric aza-BODIPY dyes.



Scheme 1. Series 1: R = Ph; series 2: R = *p*-MeOPh; series 3: R = phenylethyl.

Results and Discussion

Synthesis of 2-aryl substituted pyrroles: Our first goal was to develop an efficient way to construct restricted 2,4-diaryl pyrroles. The synthesis of rigidified pyrroles commences with a four-step sequence from alkene and includes addition of iodo azide,[19] dehydro-halogenation,[19] pyrrolysis (azirine formation),[20] and carbanion induced pyrrole formation,[18] as shown in Scheme 1. The examples of pyrroles synthesized and the combined yields over four steps are shown in Figure 1. In the developed four-step procedure, purification was only required at the last step, and the pyrroles synthesized were found to be crystalline.

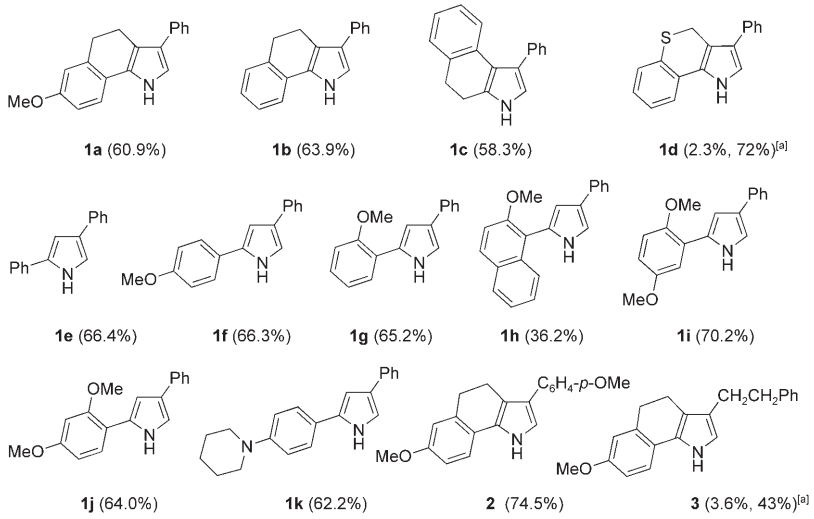


Figure 1. Pyrroles prepared according to Scheme 1 with combined yields over four steps shown. [a] For **1d** and **3** modified conditions were employed in order to obtain good yields of the pyrroles.

Conformationally restricted 2,5-diaryl pyrroles (**1a**/**1b**) and 3,4-diaryl pyrrole (**1c**) were obtained in good combined yields over four steps. However, **1c** is more sensitive to oxidation by oxygen compared with **1b**, and is more difficult to crystallize. The four-step synthetic pathway was also applied to the syntheses of 2,4-diaryl pyrroles. 2,4-Diaryl pyrroles were prepared in good combined yields in most cases. However, when the 2-aryl substituent becomes sterically hindered (**1h**), the yield was diminished. Severe limitations are found for pyrrole **1d** incorporating a heterocyclic ring and pyrrole **3** with a 4-arylalkyl substituent, with which low yields were obtained. These limitations could be overcome by using LDA/THF at −78 °C instead of NaH/DMSO at room temperature. With such modifications, compound **1d** and **3** could be obtained in 72 and 43 % yield, respectively.

Synthesis of aza-BODIPY dyes: We next put our efforts on the development of a convenient synthesis of the desired conformationally restricted aza-BODIPY dyes. The typical

process reported for preparation of aza-BODIPY dyes constitutes reaction of a pyrrole with a nitrosopyrrole, which requires preparation in a separate step.[21] We developed a two-step sequence wherein the dipyrromethene intermediates were prepared from nitrosopyrroles, which were generated in situ. The complexation of dipyrromethene dyes were effected with $BF_3{\cdot}Et_2O$ in the presence of a base in nonpolar solvent (see Scheme 2). Both symmetric and non-symmetric conformationally restricted aza-BODIPY dyes shown in Figure 2 were synthesized using this method. The aza-BODIPY dyes are highly crystalline and could be easily purified. The symmetric aza-BODIPY dyes restricted with carbocyclic rings were prepared in high yields. Substitution at the 4-position in pyrroles has little effect on the yield of aza-BODIPY dyes (see compounds **4**–**6**). The symmetric aza-BODIPY dye restricted with a heterocyclic ring **7** was produced in acceptable yield. The 2-aryl substituent in the pyrrole is essential for the formation of aza-BODIPY dyes. For example, when 2,4-dimethylpyrrole was used to react with nitrosated **1a**, no trace of the expected aza-BODIPY dye was detected. When pyrrole **1c** was used with nitrosated **1a**, only dehydrogenated aza-BODIPY **9** was isolated in low yield. When the 2-aryl substituent becomes more sterically congested, lower yield of the desired dye was obtained (compare compounds **10**–**15**). In the case where pyrrole **1h** carries a very hindered 2-methoxy-1-naphthyl group, low yield of the desired non-symmetric dye **15** was generated. The known reference dye **17** was also prepared in excellent yield with our methodology.

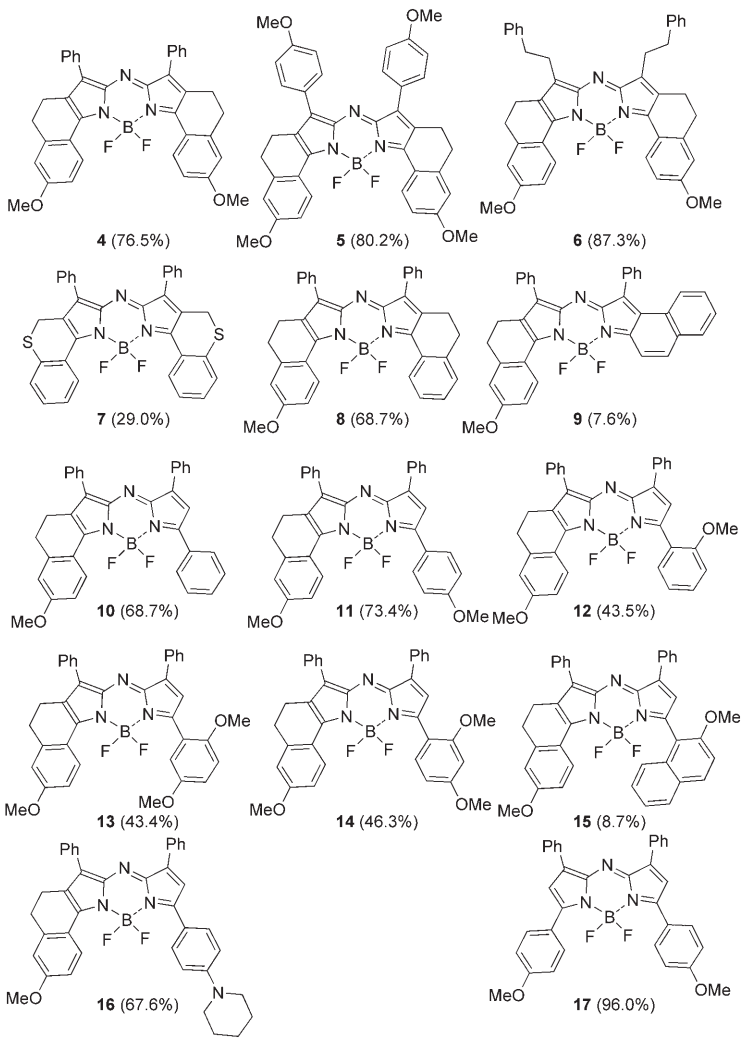
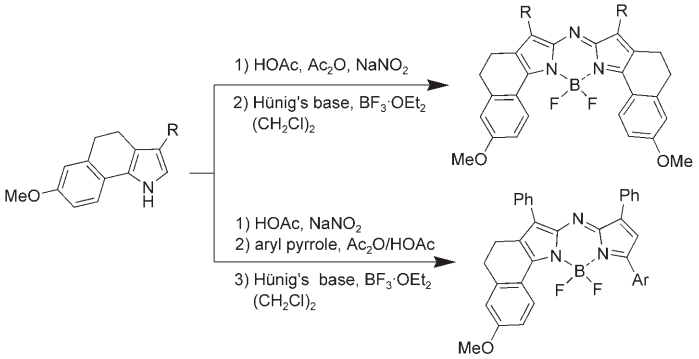


Figure 2. Aza-BODIPY dyes prepared according to Scheme 2 with yields shown.



Scheme 2.

Spectroscopic properties of aza-BODIPY dyes: The spectral characteristics of the conformationally restricted dyes **4**–**16** were then examined and compared with the known dye **17**.[16] Spectroscopic data for **4**–**17** are collected in Table 1. All aza-BODIPY dyes prepared absorb over 650 nm in $CHCl_3$. The most notable features of aza-BODIPY dyes **4**–**6** with both sides restricted with carbocyclic rings are their intense, narrow absorption bands at long absorption maxima and high extinction coefficients (Table 1, entries 1–3). The best example is compound **4**, which has the longest absorption maximum ($\lambda_{abs}=740$ nm) with ε up to $159000\,M^{-1}cm^{-1}$ and full-width at half-maximum (fwhm) of 30.4 nm (Table 1, entry 1). In comparison, non-restricted reference **17** has λ_{abs} 688 nm with $\varepsilon = 78500\,M^{-1}cm^{-1}$ and fwhm of 57 nm (Table 1, entry 14). Thus, restriction of the aromatic ring re-

Table 1. Spectroscopic data of aza-BODIPY dyes in chloroform at room temperature.

Compound	$\lambda_{abs}/\lambda_{em}$ [nm]	ε [$M^{-1}cm^{-1}$]	fwhm [nm]	Φ	Stokes shift [nm]
4	740/752	159000	30.4	0.28	12
5	736/748	157000	29.5	0.29	12
6	721/732	162000	27.1	0.31	11
7	706/730	115000	38.6	0.11	24
8	723/734	157000	27.8	0.32	11
9	715/730	141000	36.1	0.11	15
10	688/710	108000	45.3	0.44	22
11	708/732	96200	51.4	0.38	24
12	678/713	83900	59.2	0.38	35
13	681/728	66500	71.0	0.24	47
14	707/735	78000	74.5	0.18	28
15	668/692	113000	34.3	0.46	24
16	774/815	70300	93.0	0.05	41
17	688/715	78500	52.0	0.36	27

sults in a bathochromic shift of up to 52 nm (cf. **17**) and a concomitant halving of the fwhm.

The emission of **4** occurs in the near infrared with $\lambda_{em}=$ 754 nm. Dye **4** possesses high fluorescence quantum yield ($\Phi=0.28$ relative to **17** with $\Phi=0.36$ in $CHCl_3$), which matches the best value of fluorescence quantum yield reported to date for the widely used NIR cyanine dyes.[5] The Stokes shift of dye **4** is 12 nm which is smaller than reference compound **17** (27 nm).

Substitution at the β position of the pyrrole in aza-BODIPY dyes does not affect the ε value of the dye (compare **4**–**6**, Table 1, entries 1–3); however, electron-rich aryl substituents lead to a slight hypsochromic shift of the absorption maximum, and slightly higher fluorescent quantum yield (compare Table 1, entries 2 and 1). It should be noted that the solubility of dye **5** is much lower than that of **4**. Arylalkyl substituent results in a large hypsochromic shift of the λ_{abs}, and higher fluorescent quantum yield (compare Table 1, entries 3 and 1).

Heterocyclic restriction (see dye **7**, Table 1, entry 4) leads to many deleterious effects such as shorter λ_{abs}, lower ε, broader absorption (larger fwhm), and lower fluorescent quantum yield (compare Table 1, entry 4 and entries 1–3). The low fluorescence quantum yield ($\Phi=0.11$) in **7** may be due to the heavy atom effect. Dye **7** has slightly better solubility than dye **5**, however much lower solubility than **4** and **6**.

The non-symmetric aza-BODIPY **8** with both sides restricted displays slightly higher Φ than the symmetric dye **4**, however, with shorter λ_{abs} because of the lack of an electron-donating methoxy substituent on one side (compare Table 1, entries 5 and 1). In non-symmetric dye **9**, lower quantum yield was observed (compare Table 1, entries 6 and 5), which is consistent with the observation in the parent BODIPY system that oxidation of the carbocyclic restricted ring decreases the rigidity of the molecule, leading to a diminished fluorescence quantum yield.[11c]

Aza-BODIPY dyes with only one side restricted have much lower extinction coefficients than **4** (compare Table 1, entries 7–13 and entry 1). The λ_{abs} of such dyes is also shorter except in dye **16** with a very electron-rich amine substituent. The quantum yields of non-symmetric aza-BODIPY dyes are highly dependent on the substituents on the aromatic ring. In the absence of substitution or with a single methoxy substituent at the *para*-position of the phenyl ring (dye **10** and **11**), higher quantum yield is observed, however with shorter λ_{abs}, which indicates that the phenyl ring is twisted (compare Table 1, entries 7, 8 and entries 1, 5). When a single methoxy is present in the *ortho*-position of the phenyl ring (dye **12**), short λ_{abs}, low ε, and broad absorption peak (low fwhm) were observed (compare Table 1, entries 9 and 8). The quantum yield of **12** is much lower than that of dye **10** without a substituent on the phenyl ring (compare Table 1, entries 9 and 7). These features indicate that the phenyl ring in **12** is twisted and electron transfer might take place. When two methoxy substituents are present in the phenyl ring (dye **13** and **14**), the λ_{abs} was unexpectedly shorter than that of the mono-methoxy substituted dye (compare Table 1, entries 10, 11 and 8). The absorption peaks become even broader (low ε, low fwhm) than those of the monosubstituted species, and quantum yields are lower. Such facts suggest electron transfer does take place in both **13** and **14**. Electron transfer is more obvious in dye **16** with the very electron rich *para*-piperidino substituent. Dye **16** displays very long λ_{abs}, very broad absorption peak, and possesses much lower fluorescence quantum yield (compare Table 1, entries 13 and 7). In a special case (dye **15**) wherein a 2-methoxy-1-naphthyl substituent is present, the λ_{abs} is the shortest among all the synthesized dyes (compare Table 1, entries 12 and 7–11). The sharp absorption (surprisingly higher ε than other mono-restricted dyes, and smaller fwhm), and the high quantum yield of **15** suggest that naphthyl ring is distorted so that electron transfer is suppressed. It was reported that in a BODIPY dye substituted with naphthalene at the *para*-position of a phenyl group, the torsion angle between the naphthyl and the BODIPY core is as great as 55°.[11a]

Thus dye **4** is the best concerning solubility, extinction coefficient, absorption maximum, and fluorescence quantum yield. Both the absorption and emission behavior of **4** are insensitive to solvent polarity. The spectroscopic data of **4** in various solvents are given in Table 2. Both the maxima of absorption and emission are slightly blue-shifted with increased polarity of solvents. The fluorescence quantum yield is also slightly decreased in polar solvents compared to nonpolar solvents.

The novel aza-BODIPY dyes synthesized have excellent stability. Solutions of **4**–**17** in $CHCl_3$ remain unchanged over

Table 2. Absorption maxima and fluorescence quantum yields of compound **4** in various solvent at 298 K.

	$CHCl_3$	Toluene	EtOAc	CH_3CN	EtOH
λ_{abs} [nm]	740	740	732	732	731
λ_{em} [nm]	754	752	745	746	747
Φ[a]	0.28	0.28	0.23	0.23	0.22

[a] Relative to reference compound **17**.

months in the dark, indicating excellent chemical stability. The photostabilities for dye **4**–**17** are also very good. For example, compound **4** was compared with **17** in toluene as well as against the well-known indocyanine green dye (ICG, 2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2*H*-benz[*e*]indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-1*H*-benz[*e*]indolium inner salt) which is widely used and was approved by the FDA as a NIR fluorochrome ($\Phi = 0.11$ in DMSO).[2b] Compound **4** retains 97.7% of the initial intensity of the fluorescence after 1 h strong excitation, which is similar to that observed for **17** (98.0%); by comparison ICG loses 75% of the initial intensity after 1 h exposure to light.

The excellent stabilities of **4**–**17** are also reflected in the changes of absorbance (Figure 3). The normalized absorbance of **4** versus ICG is shown in Figure 3a. After 1 h of strong illumination with visible light, 99.7% of dye **4** remained. By contrast, only 39.9% of ICG remained under same condition.

Even amine-containing dye **16** displays excellent photostability, the absorbance remained almost constant over the period of 1 h under strong irradiation as shown in Figure 3b. Dye **16** possessed 99.8% of the initial absorbance after 1 h of strong illumination with visible light.

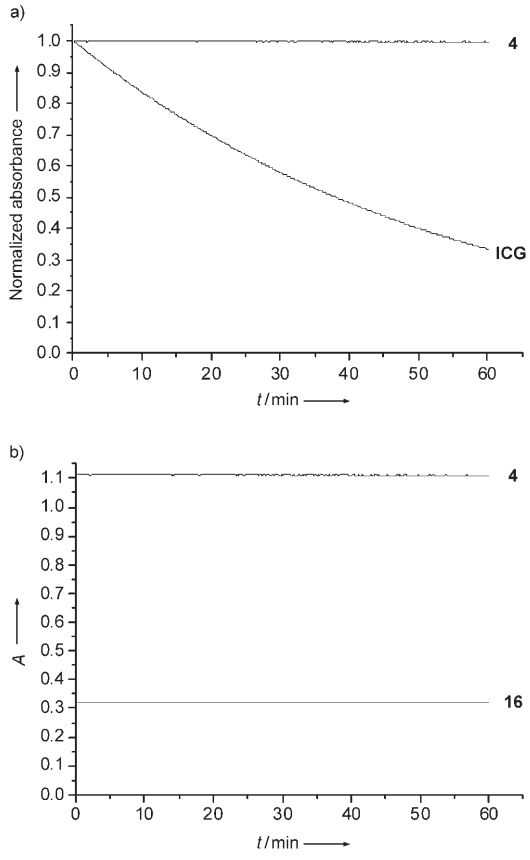


Figure 3. Absorbance change of aza-BODIPY dyes (5.0×10^{-6} mol L^{-1}) under continuous irradiation with light >540 nm (the intensity of the light was 70.5 mW cm^{-2}). a) Normalized absorbance change of compound **4** in non-degassed toluene and ICG in aerated water. b) Dye **4** and **16** in non-degassed toluene.

In summary, we have developed novel fluorescent aza-dipyrromethene dyes with exceptional stabilities. With appropriate substitution and restriction, the novel aza-BODIPY dyes possess: 1) peak fluorescence at 700–900 nm; 2) high quantum yield; 3) narrow excitation/emission; and 4) high chemical and photostability. Moreover, the convenient syntheses allow the generation of useful quantities. Additionally, the sharp fluorescent peaks are insensitive to the polarity of solvents. Efforts are currently underway to develop nonsymmetrically substituted, water soluble versions to allow conjugation for biosensing experiments.

Experimental Section

General: All solvents used for UV and fluorescence measurements were either spectroscopic grade or HPLC grade, and were purchased from Acros, Aldrich, or Fluka. 1-Acetyl-2-methoxynaphthalene, 2,5-dimethoxyacetophenone, 2,4-dimethoxyacetophenone were prepared by literature procedures.[22] Other ketones were obtained from commercial sources.

Indocyanine green dye (ICG) was purchased from Aldrich. CH_2Cl_2 and THF were dried by passing through two 4×36 inch columns of anhydrous neutral A-2 alumina (8×14 mesh; Macherey und Nagel; activated under a flow of N_2 at 300 °C over night) to remove water. 1,2-Dichloroethane was dried over molecular sieves 3 Å. Other commercial available chemicals were purchased from Aldrich and Fluka, and used as such. Melting points were not corrected.

Chromatographic purification was performed as flash chromatography using Brunschwig silica (32–63, 60 Å), or MN-aluminiumoxide (neutral, deactivated with water to activity III prior to use) with 0.3–0.5 bar pressure, and technical grade solvents were used, which were distilled prior to use.

^{1}H NMR spectra were recorded on a VARIAN Mercury 300 MHz spectrometer in $CDCl_3$, all signals are reported in ppm with the internal chloroform signal at δ 7.26 ppm as standard. The data are being reported as (s = singlet, d = doublet, t = triplet, m = multiplet or unresolved, coupling constant(s) in Hz, and integration).

^{13}C NMR spectra were recorded with ^{1}H decoupling on a VARIAN Mercury 75 MHz spectrometer in $CDCl_3$, all signals are reported in ppm with the internal chloroform signal at δ 77.0 ppm as standard.

Infrared spectra were recorded on a Perkin-Elmer Spectrum RX-I FT-IR spectrophotometer and are reported as cm^{-1}.

Mass spectrometric measurements were performed by the mass spectrometry service of the LOC at the ETHZ on an IONSPEC Ultima ESI-FT-ICR spectrometer.

Combustion analysis was performed by the Mikroelementaranalytisches Laboratorium at ETHZ.

UV/Vis spectra were recorded on Varian Cary 50 UV/Vis spectrometer at room temperature. Florescence measurements were performed on Fluorolog-3 fluorometer (Model FL-3-22, equipped with a R928P photomultiplier tube which is sensitive up to $\approx$850 nm). To obtain accurate excitation spectra, excitation monochromator was calibrated by a Xenon lamp scan. The emission monochromator was calibrated using a mercury lamp. The response of the detector was calibrated with a standard tungsten-halogen lamp. Fluorescence quantum yield determination was performed following the method recommended by Varian, see: (www.jobinyvon.com/usadivisions/Fluorescence/applications/quantumyieldstrad.pdf), and was compared with the method reported by Fery-Forgues and Lavabre.[23] Compound **17** was used as a standard, and the measurements were performed under identical conditions with **4**–**16**. Non-degassed, spectroscopic grade chloroform and a 10 mm quartz cuvette were used for fluorescence measurements, and very dilute solutions ($A = 0.010$) were used to minimize reabsorption effect. The optical densities of solutions of aza-BODIPY dyes and **17** were adjusted to 0.200 at 650 nm or 670 nm, and

these solutions were diluted by factors 20, 40, 60, 80, and 100. Excitation wavelength was 650 nm or 670 nm, and a 510 nm cut-off optical filter was placed between the excitation monochromator and sample cuvette to eliminate UV (335 nm) excitation.

Photostability measurements were conducted in a thermostated cuvette in toluene at 298 K under continuous illumination from xenon lamp in fluorometer with 16 nm slit width at the corresponding absorption maxima with identical optical density (0.10). Temperature was controlled with RM6 LAUDA thermo-cryostat. After 5 min of continuous illumination, emission intensity data were collected with 2 nm slit width at the emission maxima of individual samples.

Photostability evaluation was also conducted in aerated toluene in a thermostated cuvette connected to Cary 50 UV/Vis spectrometer at 298 K under magnetic stirring. The temperature was controlled with RM6 LAUDA thermo-cryostat. A cut-off OC 13 optical filter was used to obtain light $>$ 540 nm from a 200 W Xenon lamp as irradiation source. The intensity of the light was measured with EPM-1000 powermeter to be 70.5 mW cm^{-2}. The beam of illumination was at 90° angle to the monitoring beam of Cary 50.

General procedure for preparation of pyrroles

i) 1-Azido-2-iodoethylbenzene:[19] Iodine monochloride (5.02 g, 30.3 mmol) was added dropwise to a stirred suspension of sodium azide (2.26 g, 33.0 mmol) in dry MeCN (55 mL) over 4 min at 0 °C and then stirred for 20 min. Styrene (3.16 g, 30 mmol) was added dropwise over 15 min, the reaction mixture was stirred for 0.5 h and was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was poured into water (20 mL) and extracted with Et_2O (3×20 mL). The combined extracts were washed with 5% aqueous $Na_2S_2O_3$ (20 mL) followed by water (20 mL), then dried over anhydrous Na_2SO_4. Solvent was removed in vacuo, The residue was evacuated at 40 mbar/23 °C to afford a mixture of 1-chloro-2-iodoethylbenzene and the desired 1-azido-2-iodoethylbenzene (about 1:10). This mixture was direct used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46–7.36 (m, 3H), 7.35–7.30 (m, 2H), 4.72 (t, J = 7.2 Hz, 1H), 3.39 ppm (d, J = 7.2 Hz, 2H).

ii) (1-Azidovinyl)benzene:[19] The mixture from step i) was dissolved in dry Et_2O (55 mL). *t*BuOK (4.04 g, 36 mmol) was added in portions at 0 °C with vigorous stirring, then the mixture was stirred for 40 min, quenched with cold water (50 mL), and was extracted with Et_2O. The solution was washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo to give a brown oil, which was dissolved in CH_2Cl_2, filtered through a pad of silica gel and was washed with CH_2Cl_2. Removal of solvent gave a brown oil which was evacuated at 40 mbar/23 °C. The oil containing (1-chloro-vinyl)benzene and desired (1-azido-vinyl)benzene (1:10) was not purified further and was used directly in the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.60–7.52 (m, 2H), 7.39–7.32 (m, 3H), 5.43 (d, J = 2.5 Hz, 1H), 4.96 ppm (d, J = 2.5 Hz, 1H).

iii) 3-Phenyl-2*H*-azirine:[20] Crude (1-azidovinyl)benzene prepared above was divided into 3 portions. Each portion was dissolved in CH_2Cl_2 (100 mL) and heated in an autoclave (300 mL) at 175 °C for 25 min. Removal of the solvent under slight vacuum followed by evacuation at 50 mbar/23 °C for 1.5 h in rotavap gave a brown oil (3.77 g) containing (1-chloro-vinyl)benzene and desired 3-phenyl-2*H*-azirine (about 1:8.5 estimated by ^{1}H NMR) which was used directly in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95–7.88 (m, 2H), 7.62–7.55 (m, 3H), 3.90 (s, 3H), 1.77 ppm (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2, 163.1, 131.4, 118.0, 114.5, 55.6, 19.5 ppm.

iv) Pyrrole:[18] Ketone (2.0 mmol) was added in portions at room temperature to a stirred suspension of NaH (purity 95%, 50.2 mg, 2.0 mmol) in DMSO (2 mL) over 8 min, stirred for 0.5 h. Crude azirine from step iii) (251.3 mg, about 2.0 mmol) was added dropwise under water cooling (10 °C) with vigorous stirring over 5 min. The mixture was stirred at room temperature for 1.5 h, quenched with crushed ice (15 g). The solid collected by filtration was dissolved in CH_2Cl_2/hexane, filtered through a short silica gel column, washed with CH_2Cl_2/hexane. Solvent was removed, the residue was recrystallized from CH_2Cl_2/hexane, and the resulted solid was collected by filtration. The mother liquid was concentrated, purified by chromatography on silica gel (CH_2Cl_2/hexane as eluent) and recrystallization gave more solid product.

4,5-Dihydro-7-methoxy-3-phenylbenzo[g]indole (1a): The general procedure was followed and 6-methoxy-1-tetralone was used as starting material. Yield: 336 mg, 61.0% (4 steps). M.p. 147–148 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.30 (br s, 1H), 7.49–7.34 (m, 4H), 7.26–7.20 (m, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 2.5 Hz, 1H), 6.83 (d, J = 2.5 Hz, 1H), 6.76 (dd, J = 8.1, 2.5 Hz, 1H), 3.82 (s, 3H), 2.98–2.90 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.4, 136.7, 135.8, 128.5, 128.4, 127.0, 125.5, 123.8, 122.5, 119.3, 116.1, 115.1, 114.5, 111.3, 55.3, 30.6, 21.3 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3473, 3003, 2939, 2837, 1603, 1590, 1510, 1480, 1462, 1440, 1302, 1280, 1246, 1150, 1039, 935 cm^{-1}; MS (EI): m/z: 275.1307 [M^+].

4,5-Dihydro-3-phenylbenzo[g]indole (1b): The general procedure was followed and 1-tetralone was used as starting material. Yield: 314 mg, 63.9% (4 steps). M.p. 150–151 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.39 (br s, 1H), 7.50–7.34 (m, 4H), 7.28–7.17 (m, 4H), 7.15–7.05 (m, 1H), 6.98 (d, J = 2.8 Hz, 1H), 3.04–2.88 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 135.7, 134.8, 129.0, 128.4, 127.1, 126.4, 125.5, 125.2, 123.9, 118.2, 117.8, 116.0, 30.1, 21.2 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3472, 3062, 3009, 2938, 2840, 1604, 1506, 1481, 1455, 1446, 1396, 1107, 1076, 934 cm^{-1}; MS (EI): m/z: 245.1201 [M^+]; elemental analysis calcd (%) for $C_{18}H_{15}N$: C 88.13, H 6.16, N 5.71; found: C 87.91, H 6.25, N 5.67.

4,5-Dihydro-3-phenyl-3*H*-benzo[e]indole (1c): The general procedure was followed and 2-tetralone was used as starting material. Yield: 286 mg, 58.3% (4 steps). M.p. 110–111 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.02 (br s, 1H), 7.53–7.46 (m, 2H), 7.41–7.33 (m, 2H), 7.32–7.27 (m, 1H), 7.21–7.12 (m, 2H), 7.02–6.94 (m, 2H), 6.68 (d, J = 2.5 Hz, 1H), 3.07–2.98 (m, 2H), 2.84–2.76 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 136.4, 133.7, 133.2, 131.2, 128.8, 128.1, 127.8, 126.1, 126.0, 124.1, 122.8, 122.7, 115.9, 30.3, 22.4 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3468, 3062, 3008, 2944, 2837, 1604, 1530, 1496, 1222, 1208, 1006, 947, 702 cm^{-1}; MS (EI): m/z: 245.1199 [M^+].

1,4-Dihydro-3-phenyl-[1]-benzothiopyran[4,3-*b*]pyrrole (1d)

i) NaH/DMSO process: The general procedure was followed and thiochroman-4-one was used as starting material. Yield: 12.2 mg, 2.3% (4 steps).

ii) LDA/THF process: Thiochroman-4-one (2.0 mmol) in THF (6 mL) was cooled to −78 °C, LDA (Acros 2.0 M, 1.01 mL, 2.02 mmol) was added dropwise with stirring over 5 min under nitrogen. Crude phenyl azirine (2.05 mmol) was added dropwise with stirring over 5 min. The mixture was stirred at −78 °C for 2 h, and was allowed to warm up to room temperature slowly. It was quenched with water, neutralized with dilute HCl to a pH about 7. THF was removed in vacuo, the residue was extracted with Et_2O. The solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2, filtered through a short alumina column (neutral, activity III) and washed with CH_2Cl_2. After removal of solvent, the residue was recrystallized from Et_2O/hexane. The resulting beige solid was collected by filtration. The mother liquid was concentrated and was purified by chromatography (silica gel, CH_2Cl_2/hexane 2:3) and recrystallization; yield: 389 mg, 72% (4 steps). M.p. 150.8–151.3 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.43 (br s, 1H), 7.45–7.32 (m, 5H), 7.31–7.23 (m, 2H), 7.15 (td, J = 7.5, 1.3 Hz, 1H), 7.07 (td, J = 7.8, 1.6 Hz, 1H), 6.97 (d, J = 2.8 Hz, 1H), 4.12 ppm (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 134.8, 130.6, 128.5, 128.1, 127.8, 127.6, 126.0, 125.7, 123.7, 119.9, 116.8, 113.2, 24.5 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3470, 3064, 3008, 2894, 2833, 1604, 1580, 1559, 1527, 1481, 1448, 1441, 1430, 1392, 1287, 1172, 1112, 1073, 1059, 1033, 936 cm^{-1}; MS (EI): m/z: 263.0768 [M^+]; elemental analysis calcd (%) for $C_{17}H_{17}NS$: C 77.53, H 4.98, N 5.32; found: C 77.32, H 5.01, N 5.27.

2,4-Diphenylpyrrole (1e): The general procedure was followed and acetophenone was used as starting material. Yield: 291 mg, 66.4% (4 steps). M.p. 180.4–181.4 °C (lit. 179.5–180.5 °C[24]); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.44 (br s, 1H), 7.64–7.47 (m, 4H), 7.45–7.32 (m, 4H), 7.29–7.17 (m, 2H), 7.15 (dd, J = 3.8, 1.7 Hz, 1H), 6.84 (dd, J = 3.8, 1.7 Hz. 1H) ppm; IR ($CHCl_3$): ν = 3472, 3078, 3009, 1607, 1490, 1454, 1414, 1122, 1037, 932, 810 cm^{-1}; MS (EI): m/z: 219.1044 [M^+]; elemental analysis calcd (%) for $C_{16}H_{13}N$: C 87.64, H 5.98, N 6.39; found: C 87.42, H 6.03, N 6.33.

2-(*p*-Methoxyphenyl)-4-phenylpyrrole (1f): The general procedure was followed and 4-methoxyacetophenone was used as starting material. Yield: 331 mg, 66.3% (4 steps). M.p. 208.8–209.6°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.34 (brs, 1H), 7.60–7.53 (m, 2H), 7.40–7.32 (m, 2H), 7.20 (tt, J=7.5, 1.2 Hz, 1H), 7.11 (dd, J=3.5, 1.9 Hz. 1H), 6.98–6.90 (m, 2H), 6.72 (dd, J=2.5, 1.9 Hz, 1H), 3.84 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=158.3, 135.5, 133.0, 128.5, 125.5, 125.2, 125.0, 114.7, 114.3, 102.9, 95.4, 55.4, 25.9 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3472, 3035, 3007, 2942, 2837, 1604, 1530, 1249, 1180, 1119, 1038, 926, 832 cm^{-1}; MS (EI): m/z: 249.1150 [M^+]; elemental analysis calcd (%) for $C_{17}H_{15}NO$: C 81.90, H 6.06, N 5.62; found: C 81.65, H 6.10, N 5.54.

2-(*o*-Methoxyphenyl)-4-phenylpyrrole (1g): The general procedure was followed and 2-methoxyacetophenone was used as starting material. Yield: 325 mg, 65.2% (4 steps). M.p. 110.7–111.3°C (lit. 105–106°C[24]); ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.85 (brs, 1H), 7.73 (dd, J=7.8, 1.6 Hz, 1H), 7.63–7.55 (m, 2H), 7.40–7.32 (m, 2H), 7.23–7.13 (m, 3H), 7.04 (dd, J=7.5, 1.3 Hz, 1H), 7.01–6.96 (m, 1H), 6.92 (dd, J=2.5, 1.6 Hz, 1H), 3.99 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=154.7, 135.7, 130.7, 128.5, 126.8, 126.5, 125.3, 125.2, 125.0, 121.4, 120.6, 114.6, 111.5, 103.9, 55.7 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3459, 3065, 3008, 2943, 2838, 1604, 1587, 1578, 1525, 1485, 1461, 1406, 1300, 1251, 1237, 1182, 1122, 1027, 936, 807, 696 cm^{-1}; MS (EI): m/z: 249.1153 [M^+]; elemental analysis calcd (%) for $C_{17}H_{15}NO$: C 81.90, H 6.06, N, 5.62; found: C 81.67, H 6.16, N 5.57.

2-(2-Methoxynaphthalene-1-yl)-4-phenylpyrrole (1h): The general procedure was followed and 1-acetyl-2-methoxynaphthalene was used as starting material. Yield: 217 mg, 36.2% (4 steps). M.p. 129.8–130.5°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.80 (brs, 1H), 8.30 (d, J=8.4 Hz, 1H), 7.85 (d, J=8.7 Hz, 1H), 7.83 (d, J=8.1 Hz, 1H), 7.68–7.60 (m, 2H), 7.50–7.29 (m, 6H), 7.24–7.16 (m, 1H), 6.82 (dd, J=2.5, 1.6 Hz, 1H), 3.93 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=154.0, 135.8, 133.1, 129.3, 129.2, 128.5, 127.9, 126.6, 126.3, 125.5, 125.3, 125.0, 123.8, 116.1, 114.5, 113.3, 108.9, 56.7 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3471, 3061, 3008, 2940, 2840, 1622, 1604, 1594, 1511, 1463, 1413, 1367, 1335, 1269, 1252, 1127, 1077, 928, 809 cm^{-1}; MS (EI): m/z: 299.1306 [M^+]; elemental analysis calcd (%) for $C_{21}H_{17}NO$: C 84.25, H 5.72, N 4.68; found: C 84.01, H 5.83, N 4.62.

2-(2,5-Dimethoxyphenyl)-4-phenylpyrrole (1i): The general procedure was followed and 2,5-dimethoxyacetophenone was used as starting material. 415 mg, Yield: 74.2% (4 steps). M.p. 113.5–114.2°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.93 (brs, 1H), 7.64–7.57 (m, 2H), 7.42–7.33 (m, 2H), 7.28 (d, J=2.8 Hz, 1H), 7.24–7.13 (m, 2H), 6.95–6.90 (m, 1H), 6.92 (d, J=9.0 Hz, 1H), 6.74 (dd, J=2.5, 1.6 Hz. 1H), 3.93 (s, 3H), 3.85 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=154.0, 149.2, 135.6, 130.6, 128.5, 125.4, 125.2, 125.0, 121.4, 114.8, 112.8, 111.8, 111.7, 104.1, 56.3, 55.8 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3454, 3007, 2941, 2836, 1606, 1526, 1491, 1467, 1226, 1212, 1179, 1127, 1048, 1024, 928, 808 cm^{-1}; MS (EI): m/z: 279.1254 [M^+]; elemental analysis calcd (%) for $C_{18}H_{17}NO_2$: C 77.40, H 6.13, N 5.01; found: C 77.27, H 6.07, N 4.96.

2-(2,4-Dimethoxyphenyl)-4-phenylpyrrole (1j): The general procedure was followed and 2,4-dimethoxyacetophenone was used as starting material. Yield: 370 mg, 66.2% (4 steps). M.p. 143–144°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.66 (brs, 1H), 7.62 (d, J=8.4 Hz, 1H), 7.61–7.55 (m, 2H), 7.39–7.31 (m, 2H), 7.18 (tt, J=7.5, 1.3 Hz, 1H), 7.11 (dd, J=2.5, 1.8 Hz, 1H), 6.79 (dd, J=2.5, 1.8 Hz, 1H), 6.61–6.6.54 (m, 2H), 3.96 (s, 3H), 3.85 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=158.9, 155.8, 135.8, 130.7, 128.4, 127.3, 125.3, 125.0, 114.1, 113.8, 105.5, 102.8, 99.1, 55.7, 55.5 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3464, 3064, 3006, 2941, 2839, 1613, 1588, 1578, 1529, 1493, 1462, 1439, 1299, 1243, 1159, 1120, 1048, 1030, 943, 838 cm^{-1}; MS (EI): m/z: 279.1256 [M^+]; elemental analysis calcd (%) for $C_{18}H_{17}NO_2$: C 77.40, H 6.13, N 5.01; found: C 77.16, H 6.00, N 4.99.

2-(*p*-Piperidinophenyl)-4-phenylpyrrole (1k): The general procedure was followed and 4-piperidinoacetophenone was used as starting material. Yield: 376 mg, 62.2% (4 steps). M.p. 143–144°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.34 (brs, 1H), 7.60–7.53 (m, 2H), 7.45–7.30 (m, 4H), 7.18 (tt, J=7.4, 1.3 Hz, 1H), 7.09 (dd, J=2.5, 1.8 Hz, 1H), 7.00–6.92 (m, 2H), 6.69 (dd, J=2.5, 1.8 Hz, 1H), 3.22–3.14 (m, 4H), 1.80–1.60 ppm (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=150.5, 135.6, 133.4, 128.5, 126.2, 125.5, 124.7, 116.6, 114.5, 102.4, 50.6, 25.8, 24.3 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3471, 3061, 3009, 2940, 2841, 1622, 1604, 1594, 1511, 1463, 1269, 1252, 1127, 1077, 928, 809 cm^{-1}; MS (EI): m/z: 302.1779 [M^+]; elemental analysis calcd (%) for $C_{21}H_{22}N_2$: C 83.40, H 7.33, N 9.26; found: C 83.53, H 7.34, N 9.20.

4,5-Dihydro-7-methoxy-3-(*p*-methoxyphenyl)-benzo[g]indole (2): The general procedure of step i)–iv) was followed except that *p*-methoxy styrene was used instead of styrene. Yield: 458 mg, 74.5% (4 steps).

i) 1-Azido-2-iodoethyl-(*p*-methoxy)benzene: ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.28–7.20 (m, 2H), 6.96–6.89 (m, 2H), 4.68 (t, J=7.2 Hz, 1H), 3.83 (s, 3H), 3.37 ppm (d, J=7.2 Hz, 2H).

ii) 1-Azidovinyl(*p*-methoxy)benzene: ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.52–7.46 (m, 2H), 6.90–6.84 (m, 2H), 5.32 (d, J=2.5 Hz, 1H), 4.86 (d, J=2.5 Hz, 1H), 3.83 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 160.3, 144.6, 126.9, 113.7, 96.1, 55.3 ppm.

iii) 3-(*p*-Methoxy)phenyl-2*H*-azirine: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.88–7.82 (m, 2H), 7.09–7.03 (m, 2H), 3.90 (s, 3H), 1.74 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2, 163.1, 131.4, 118.0, 114.5, 55.6, 19.5 ppm; IR (KBr film): $\tilde{\nu}$ = 3039, 2969, 2840, 1744, 1605, 1577, 1510, 1463, 1442, 1422, 1324, 1305, 1289, 1258, 1172, 1125, 1107, 1028, 988, 836 cm^{-1}.

iv) 4,5-Dihydro-7-methoxy-3-(*p*-methoxyphenyl)-benzo[g]-indole: M.p. 186–187.6°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.23 (brs, 1H), 7.41–7.34 (m, 2H), 7.12 (d, J=8.4 Hz, 1H), 6.97–6.91 (m, 2H), 6.86 (d, J=2.5 Hz, 1H), 6.82 (d, J=2.5 Hz, 1H), 6.85 (dd, J=8.4, 2.5 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.00–2.84 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=157.6, 157.4, 136.7, 128.5, 128.3, 128.1, 123.4, 122.5, 119.2, 115.9, 114.5, 113.9, 111.2, 55.3, 55.3, 30.6, 21.2 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3474, 3006, 2939, 2837, 1614, 1590, 1563, 1532, 1511, 1490, 1463, 1442, 1302, 1280, 1247, 1176, 1037, 936, 835 cm^{-1}.

4,5-Dihydro-7-methoxy-3-phenylethylbenzo[g]indole(3)

i) NaH/DMSO process: The general procedure of step i)–iv) was followed except that 4-phenyl-1-butene was used instead of styrene and in step (ii) the product was isolated by chromatography on silica gel (hexane/CH_2Cl_2 1:6) to afford of desired intermediate in 69% combined yield (2 steps), and final product 22 mg in 3.6% combined yield (4 steps).

(1-Azidovinyl)ethylbenzene: Yield: 69% (2 steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.38–7.16 (m, 5H), 4.67–4.63 (m, 2H), 2.84–2.76 (m, 2H), 2.42–2.33 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=145.8, 140.6, 128.3, 128.2, 126.0, 98.5, 35.7, 33.8 ppm; IR (KBr film): $\tilde{\nu}$ =3064, 3028, 2928, 2861, 2104, 1628, 1604, 1496, 1454, 1280, 1076, 846, 748, 699 cm^{-1}.

3-Phenylethyl-2*H*-azirine: ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.35–7.28 (m, 2H), 7.26–7.15 (m, 3H), 3.14–3.06 (m, 4H), 1.40 ppm (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=169.3, 139.8, 128.5, 128.2, 126.4, 30.4, 30.4, 19.4 ppm; IR (KBr film): $\tilde{\nu}$ = 3062, 3029, 2977, 2927, 2861, 1763, 1604, 1496, 1454, 1418, 1076, 1030, 987, 750, 700 cm^{-1}.

4,5-Dihydro-7-methoxy-3-phenylethylbenzo[g]indole: M.p. 104.3–106.3°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.30 (brs, 1H), 7.49–7.34 (m, 4H), 7.26–7.20 (m, 1H), 7.13 (d, J=8.4 Hz, 1H), 6.93 (d, J=2.5 Hz, 1H), 6.83 (d, J=2.5 Hz, 1H), 6.76 (dd, J=8.1, 2.5 Hz, 1H), 3.82 (s, 3H), 2.98–2.90 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=157.4, 136.9, 135.8, 128.5, 128.4, 127.0, 125.5, 123.8, 122.5, 119.3, 116.1, 115.1, 114.5, 111.3, 55.3, 30.6, 21.3 ppm; IR ($CHCl_3$): $\tilde{\nu}$ = 3477, 3064, 3029, 3006, 2936, 2837, 1615, 1590, 1566, 1517, 1496, 1462, 1442, 1426, 1300, 1281, 1247, 1221, 1209, 1151, 1062, 1037, 700 cm^{-1}; MS (EI): m/z: 303.1617 [M^+]; elemental analysis calcd (%) for $C_{21}H_{21}NO$: C 83.13, H 6.98, N 4.62; found: C 83.07, H 7.02, N 4.56.

ii) LDA/THF process: The process of **1d** was followed except 6-methoxy-1-tetralone and 3-phenylethyl-2*H*-azirine were used instead of thio-chroman-4-one and phenyl azirine. The desired product (269 mg, 43%) was obtained.

General procedure for preparation of symmetric aza-BODIPY dyes: Sodium nitrite (6.9 mg, 0.1 mmol) was added at 5°C with stirring to a solution of pyrrole derivative (0.2 mmol) in a mixture of acetic acid/acetic anhydride (1 mL/0.4 mL). The mixture was stirred for 0.5 h, followed by heating at 80°C for 0.5 h. Crushed ice was added to the cold reaction mixture, the resulted blue dye was filtered and washed with water. The blue dye was dissolved in CH_2Cl_2, filtered through a pad of alumina (ac-

tivity III) and washed with CH_2Cl_2. Solvent was removed in vacuo, the residue was dissolved in dry 1,2-dichloroethane, triethylamine (0.24 mL) was added, followed by slow addition of $BF_3{\cdot}Et_2O$ (0.24 mL) with stirring at room temperature. The mixture was stirred for 0.5 h, then heated in a 80 °C oil bath for 0.5 h, and was cooled down. The reaction was quenched with crushed ice, extracted with CH_2Cl_2, filtered through a pad of alumina (activity III). Solvent was removed, the residue was recrystallized from CH_2Cl_2/hexane. Coppery solid was obtained. Mother liquid was purified by chromatography on alumina (activity III) or silica gel (CH_2Cl_2/hexane 1:1) and recrystallization from CH_2Cl_2/hexane.

Aza-BODIPY dye 4: The general procedure for symmetric aza-BODIPY was followed using **1a** as starting material, and coppery solid (46.7 mg, 76.5 %) was obtained. M.p. 297–298 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.76 (d, J = 9.0 Hz, 2H), 7.76–7.66 (m, 4H), 7.49–7.32 (m, 6H), 7.02 (dd, J = 9.0, 2.5 Hz, 2H), 6.84 (d, J = 2.5 Hz, 2H), 3.91 (s, 6H), 2.94 ppm (s, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.6, 151.6, 145.6, 143.9, 136.6, 132.2, 131.0, 130.8, 130.7, 130.2, 127.9, 120.6, 114.4, 112.9, 55.5, 30.8, 21.9 ppm; IR (KBr): $\tilde{\nu}$ = 2932, 2835, 1762, 1601, 1514, 1448, 1394, 1279, 1256, 1235, 1142, 1054, 772, 698 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{38}H_{30}BF_2N_3O_2$: 609.2394; found: 609.2383 (100) [M^+].

Aza-BODIPY dye 5: The general procedure for symmetric aza-BODIPY was followed using **2** as starting material, and coppery solid was obtained (53.8 mg, 80.2 %). M.p. 324–325 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (d, J = 9.3 Hz, 2H), 7.73–7.64 (m, 4H), 7.05–6.93 (m, 6H), 6.83 (d, J = 2.7 Hz, 2H), 3.91 (s, 6H), 3.87 (s, 6H), 2.92 ppm (s, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.4, 159.5, 151.4, 148.2, 148.2, 143.7, 136.4, 133.5, 131.5, 130.8, 129.6, 125.1, 120.8, 114.4, 113.5, 112.8, 55.5, 55.4, 30.8, 22.0 ppm; IR (KBr): $\tilde{\nu}$ = 2935, 2834, 1608, 1514, 1495, 1451, 1430, 1396, 1279, 1256, 1236, 1181, 1164, 1136, 1061, 1032, 839, 762 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{40}H_{34}BF_2N_3O_4$: 670.2683; found: 670.2682 (41) [M+H$^+$].

Aza-BODIPY dye 6: The general procedure for symmetric aza-BODIPY was followed using **3** as starting material, and coppery solid was obtained (58.1 mg, 87.1 %). M.p. 229.5–230.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.66 (d, J = 9.0 Hz, 2H), 7.32–7.15 (m, 10H), 6.96 (dd, J = 8.7, 2.5 Hz, 2H), 6.78 (d, J = 2.5 Hz, 2H), 3.88 (s, 6H), 2.99 (s, 8H), 2.80 (dd, J = 7.5, 6.5 Hz, 4H), 2.44 ppm (t, J = 7.5, 6.5 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.3, 151.2, 145.7, 143.8, 141.7, 138.0, 131.4, 130.6, 128.5, 128.2, 125.9, 120.7, 114.4, 112.7, 55.4, 36.9, 30.5, 26.8, 20.4 ppm; IR (KBr): $\tilde{\nu}$ = 2929, 2833, 1603, 1513, 1493, 1446, 1412, 1391, 1278, 1252, 1191, 1088, 1071, 1061, 1052, 1035, 991, 963, 698 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{42}H_{38}BF_2N_3O_2$: 666.3098; found: 666.3086 (100) [M+H$^+$].

Aza-BODIPY dye 7: The general procedure for symmetric aza-BODIPY was followed except that **1d** was used as starting material and chromatographic purification followed by recrystallization was adopted, dark brown solid (17.0 mg, 29.0 %) was obtained. M.p. 250 °C (decomp); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.74 (d, J = 7.8 Hz, 2H), 7.66–7.56 (m, 4H), 7.51–7.29 (m, 12H), 4.00 ppm (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.8, 146.0, 138.5, 138.3, 131.1, 131.0, 130.7, 130.6, 130.4, 129.0, 128.9, 128.6, 128.3, 127.1, 126.9, 24.4 ppm; IR (KBr): $\tilde{\nu}$ = 3050, 2887, 1583, 1553, 1527, 1449, 1386, 1316, 1266, 1213, 1135, 1124, 1090, 1071, 1055, 1039, 1004, 993, 976, 768, 732, 696 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{34}H_{22}BF_2N_3S_2$: 586.1389; found: 586.1381 (100) [M+H$^+$].

General procedure for preparation of non-symmetric aza-BODIPY dyes: Sodium nitrite (6.9 mg, 0.1 mmol) was added at 5 °C with stirring to a suspension of **1a** (27.6 mg, 0.1 mmol) in acetic acid (1 mL), and was stirred for 10 min. The color changed from colorless to brown, then green, and finally brown was observed. The second pyrrole moiety was added, followed by addition of acetic anhydride (0.4 mL). The mixture turned blue immediately. After 0.5 h stirring, the mixture was heated at 80 °C for 0.5 h. Crushed ice was added to the cold reaction mixture, the resulted blue dye was filtered, washed with water. The blue dye was dissolved in CH_2Cl_2, filtered through a pad of alumina (activity III) and washed with CH_2Cl_2. Solvent was removed in vacuo, the residue was dissolved in dry 1,2-dichloroethane, triethylamine (0.24 mL) was added, followed by dropwise addition of $BF_3{\cdot}Et_2O$ (0.24 mL) with stirring at room temperature. The mixture was stirred for 0.5 h, then heated in 80 °C oil bath for 0.5 h, and was cooled down. The reaction was quenched with crushed ice, extracted with CH_2Cl_2, and was purified by chromatography on silica gel followed by recrystallization from CH_2Cl_2/hexane.

Aza-BODIPY dye 8: The general procedure for non-symmetric aza-BODIPY was followed using **1b** as the second pyrrole moiety, and coppery solid was obtained (39.9 mg, 68.7 %). M.p. 298–299 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.82 (d, J = 8.7 Hz, 1H), 8.74 (d, J = 8.1 Hz, 2H), 7.76–7.66 (m, 4H), 7.50–7.26 (m, 9H), 7.04 (dd, J = 9.0, 2.5 Hz, 1H), 6.85 (d, J = 2.5 Hz, 1H), 3.92 (s, 3H), 2.95 (s, 4H), 2.94 ppm (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.3, 154.0, 150.2, 146.5, 145.1, 144.6, 140.9, 137.8, 136.2, 132.3, 131.8, 131.7, 130.6, 130.2, 128.2, 128.0, 127.9, 127.8, 127.5, 120.1, 114.5, 113.1, 55.5, 30.6, 30.4, 21.9, 21.8 ppm; IR (KBr): $\tilde{\nu}$ = 3043, 2937, 2837, 1599, 1512, 1464, 1450, 1391, 1304, 1292, 1275, 1252, 1236, 1187, 1142, 1122, 1081, 1057, 1029, 1010, 762, 698 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{37}H_{28}BF_2N_3O$: 580.2366; found: 580.2357 (100) [M+H$^+$].

Aza-BODIPY dye 9: The general procedure for non-symmetric aza-BODIPY was followed using **1c** as the second pyrrole moiety, and coppery solid was obtained (4.4 mg, 7.6 %). M.p. 304–305 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.97 (d, J = 9.0 Hz, 1H), 8.19 (d, J = 8.1 Hz, 1H), 7.86–7.64 (m, 7H), 7.56–7.26 (m, 8H), 7.09 (dd, J = 8.7, 2.8 Hz, 1H), 6.89 (d, J = 2.2 Hz, 1H), 3.96 (s, 3H), 3.08–2.92 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1, 146.5, 134.8, 133.8, 133.8, 133.0, 131.1, 130.9, 130.4, 130.2, 129.6, 129.1, 128.9, 128.1, 128.0, 127.7, 127.0, 124.9, 124.0, 119.0, 115.7, 114.6, 113.8, 55.8, 30.4, 22.3 ppm; IR (KBr): $\tilde{\nu}$ = 3046, 3009, 2963, 2921, 2833, 1598, 1544, 1478, 1451, 1386, 1356, 1309, 1284, 1250, 1146, 1118, 1098, 1057, 1027, 818, 749, 692 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{37}H_{26}BF_2N_3O$: 578.2210; found: 578.2201 (100) [M+H$^+$].

Aza-BODIPY dye 10: The general procedure for non-symmetric aza-BODIPY was followed using **1e** as the second pyrrole moiety, and coppery solid was obtained (38.1 mg, 68.7 %). M.p. 231–232 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.73 (d, J = 9.0 Hz, 1H), 8.10–8.00 (m, 3H), 7.78–7.70 (m, 2H), 7.55–7.30 (m, 9H), 6.97 (dd, J = 9.0, 2.8 Hz, 1H), 6.93 (s, 1H), 6.84 (d, J = 2.5 Hz, 1H), 3.90 (s, 3H), 3.04–2.90 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.2, 156.8, 153.8, 147.2, 145.5, 143.9, 139.6, 139.1, 133.0, 132.7, 131.6, 130.4, 129.6, 129.3, 128.8, 128.7, 128.4, 128.4, 128.2, 119.6, 116.9, 114.6, 113.3, 55.5, 30.4, 21.8 ppm; IR (KBr): $\tilde{\nu}$ = 3058, 2920, 2833, 1601, 1533, 1512, 1500, 1485, 1468, 1451, 1384, 1285, 1251, 1229, 1191, 1131, 1092, 1049, 1028, 999, 964, 763, 691 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{35}H_{26}BF_2N_3O$: 554.2210; found: 554.2201 (100) [M+H$^+$].

Aza-BODIPY dye 11: The general procedure for non-symmetric aza-BODIPY was followed using **1f** as the second pyrrole moiety, and coppery solid was obtained (40.2 mg, 73.4 %). M.p. 246.5–248 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.72 (d, J = 9.0 Hz, 1H), 8.12–8.03 (m, 4H), 7.77–7.70 (m, 2H), 7.54–7.30 (m, 6H), 7.08–7.01 (m, 2H), 6.97 (dd, J = 9.0, 2.8 Hz, 1H), 6.96 (s, 1H), 6.84 (d, J = 2.5 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.00–2.91 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.7, 161.2, 155.2, 154.7, 146.6, 145.0, 144.4, 140.3, 138.4, 132.9, 132.3, 132.1, 131.9, 131.1, 130.4, 128.9, 128.6, 128.5, 128.4, 128.1, 124.9, 120.0, 117.0, 114.5, 114.1, 113.2, 55.5, 55.4, 30.5, 21.8 ppm; IR (KBr): $\tilde{\nu}$ = 2967, 2835, 1603, 1526, 1503, 1474, 1428, 1393, 1303, 1260, 1181, 1124, 1110, 1024, 768, 682 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{36}H_{28}BF_2N_3O_2$: 584.2315; found: 584.2305 (42) [M+H$^+$].

Aza-BODIPY dye 12: The general procedure for non-symmetric aza-BODIPY was followed using **1g** as the second pyrrole moiety, and coppery solid was obtained (20.5 mg, 43.5 %). M.p. 239–240 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.60 (d, J = 9.0 Hz, 1H), 8.10–8.03 (m, 2H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.76–7.70 (m, 2H), 7.55–7.30 (m, 7H), 7.11 (td, J = 7.5, 1.9 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.92 (s, 1H), 6.89 (dd, J = 9.0, 2.8 Hz, 1H), 6.81 (d, J = 2.8 Hz, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.00–2.89 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.7, 157.8, 156.2, 151.0, 145.0, 139.0, 138.8, 133.1, 132.4, 131.8, 131.7, 130.7, 130.3, 128.7, 128.5, 128.2, 128.2, 128.0, 121.9, 120.2, 119.6, 119.0, 114.4, 113.2, 111.1, 56.0, 55.5, 30.5, 21.9 ppm; IR (KBr): $\tilde{\nu}$ = 2935, 2834, 1602, 1540, 1486, 1470, 1451, 1384, 1284, 1252, 1223, 1188, 1142, 1123, 1096, 1068, 1051, 1026, 1006, 972, 768, 731, 698, 680 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{36}H_{28}BF_2N_3O_2$: 584.2315; found: 584.2306 (100) [M+H$^+$].

Aza-BODIPY dye 13: The general procedure for non-symmetric aza-BODIPY was followed using **1i** as the second pyrrole moiety, and coppery solid was obtained (26.6 mg, 43.4%). M.p. 140–142°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.64 (d, J = 9.0 Hz, 1H), 8.10–8.04 (m, 2H), 7.77–7.70 (m, 2H), 7.57 (d, J = 2.2 Hz, 1H), 7.54–7.29 (m, 6H), 6.99–6.95 (m, 3H), 6.91 (dd, J = 9.0, 2.8 Hz, 1H), 6.82 (d, J = 2.5 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.01–2.88 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.8, 156.3, 152.9, 152.2, 150.5, 147.0, 145.1, 143.2, 138.9, 138.8, 133.1, 132.6, 132.4, 131.6, 130.3, 128.7, 128.5, 128.2, 128.0, 122.5, 119.6, 119.0, 116.9, 116.3, 114.4, 113.2, 112.9, 56.9, 55.8, 55.5, 30.5, 21.9 ppm; IR (KBr): $\tilde{\nu}$ = 2931, 2833, 1600, 1538, 1508, 1486, 1476, 1450, 1432, 1382, 1284, 1252, 1227, 1186, 1139, 1093, 1068, 1049, 1025, 1007, 972, 770, 748, 690 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{37}H_{30}BF_2N_3O_3$: 614.2421; found: 614.2411 (100) [M+H$^+$].

Aza-BODIPY dye 14: The general procedure for non-symmetric aza-BODIPY was followed using **1j** as the second pyrrole moiety, and coppery solid was obtained (28.4 mg, 46.3%). M.p. 226–227°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.70 (d, J = 9.0 Hz, 1H), 8.10–8.04 (m, 2H), 7.99 (d, J = 8.5 Hz, 1H), 7.76–7.70 (m, 2H), 7.54–7.29 (m, 6H), 7.00 (s, 1H), 6.91 (dd, J = 9.0, 2.8 Hz, 1H), 6.82 (d, J = 2.8 Hz, 1H), 6.66 (dd, J = 8.5, 2.5 Hz, 1H), 6.57 (d, J = 2.5 Hz, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.00–2.88 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.3, 162.1, 159.3, 154.7, 152.0, 146.4, 144.6, 143.7, 139.4, 138.1, 133.0, 132.8, 131.9, 130.3, 128.8, 128.3, 128.2, 128.0, 119.9, 119.8, 114.5, 114.3, 113.1, 104.5, 98.9, 55.9, 55.5, 55.5, 30.6, 21.9 ppm; IR (KBr): $\tilde{\nu}$ = 2934, 2835, 1611, 1533, 1515, 1499, 1473, 1452, 1389, 1300, 1279, 1257, 1224, 1184, 1129, 1092, 1047, 1022, 824, 768 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{37}H_{30}BF_2N_3O_3$: 614.2421; found: 614.2410 (100) [M+H$^+$].

Aza-BODIPY dye 15: The general procedure for non-symmetric aza-BODIPY was followed using **1h** as the second pyrrole moiety, and coppery solid was obtained (5.5 mg, 8.7%). M.p. 277.5–278.5°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.44 (d, J = 9.6 Hz, 1H), 8.17–8.09 (m, 2H), 7.98 (d, J = 9.0 Hz, 1H), 7.86–7.72 (m, 3H), 7.66 (d, J = 8.1 Hz, 1H), 7.58–7.30 (m, 9H), 6.80–6.71 (m, 3H), 3.96 (s, 3H), 3.80 (s, 3H), 3.00–2.84 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.1, 157.7, 155.7, 149.1, 147.8, 145.4, 142.9, 139.6, 138.9, 134.0, 133.4, 133.0, 131.7, 131.0, 130.5, 128.8, 128.4, 128.3, 128.2, 127.7, 126.6, 125.6, 123.7, 119.4, 117.7, 116.4, 114.5, 113.6, 113.2, 57.0, 55.4, 30.4, 21.8 ppm; IR (KBr): ν = 3047, 2934, 2834, 1600, 1540, 1526, 1482, 1450, 1388, 1352, 1284, 1251, 1218, 1184, 1139, 1081, 1059, 1045, 1003, 972, 810, 768, 688 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{40}H_{30}BF_2N_3O_2$: 634.2472; found: 634.2462 (100) [M+H$^+$].

Aza-BODIPY dye 16: The general procedure for non-symmetric aza-BODIPY was followed using **1k** as the second pyrrole moiety, and coppery solid was obtained (43.0 mg, 67.6%). M.p. 186–188°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.71 (d, J = 9.0 Hz, 1H), 8.19–8.05 (m, 4H), 7.78–7.70 (m, 2H), 7.53–7.30 (m, 6H), 7.10 (s, 1H), 7.02–6.93 (m, 3H), 6.83 (d, J = 2.5 Hz, 1H), 3.89 (s, 3H), 3.48–3.36 (m, 4H), 2.94 (s, 4H), 1.80–1.62 ppm (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.5, 156.1, 152.4, 151.5, 145.3, 145.2, 143.8, 140.8, 136.3, 132.7, 132.3, 131.4, 130.7, 130.3, 128.8, 128.5, 128.2, 127.9, 120.6, 120.2, 117.7, 114.2, 114.0, 112.9, 55.4, 48.6, 30.7, 25.6, 24.5, 21.8 ppm; IR (KBr): $\tilde{\nu}$ = 2933, 2833, 1598, 1503, 1478, 1445, 1418, 1390, 1313, 1281, 1252, 1240, 1211, 1141, 1119, 1094, 1043, 1023, 1010, 763, 679 cm^{-1}; HRMS (MALDI): m/z (%): calcd for $C_{40}H_{35}BF_2N_4O$: 637.2945; found: 637.2934 (61) [M+H$^+$].

Aza-BODIPY dye 17: The general procedure for symmetric aza-BODIPY was followed using **1f** as starting material, and coppery solid was obtained (53.5 mg, 96.0%).

The characterization data are consistent with the data reported.[16b]

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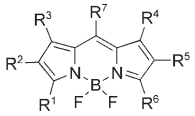

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