Fluorescent Dyes

Conformationally Restricted Aza-Bodipy: A Highly Fluorescent, Stable, Near-Infrared-Absorbing Dye**

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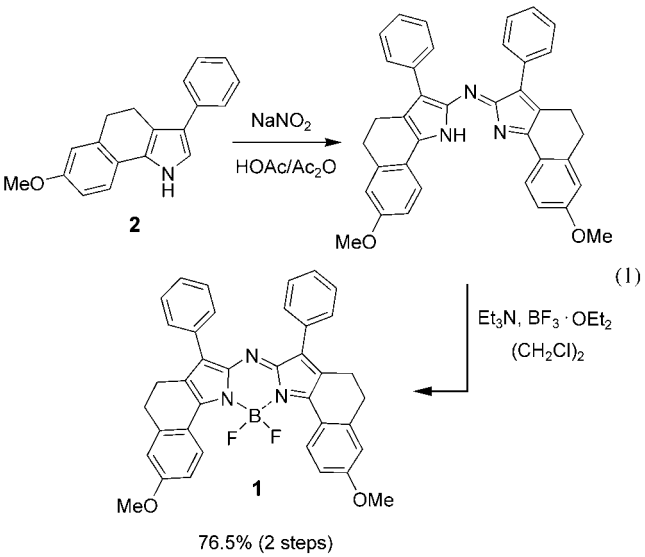
There has been recent intense interest in the preparation and study of near-infrared (NIR)-absorbing dyes as well as their applications as safe, noninvasive imaging/contrasting probes.[1–5] The advantages of imaging in the NIR region (700–1100 nm) are numerous and have been extensively discussed.[2] Prominent among these advantages is the absence or significant reduction of background absorption, fluorescence, and light scattering[6,7] along with the availability of low-cost sources of irradiation. Compared to chromophores absorbing in the visible region, problems have been encountered in the design and synthesis of the red-shifted NIR counterparts, such as aggregation,[3] photobleaching,[4] and low fluorescence quantum yields.[4,5] There is a pressing need for the identification of newer, more effective dyes that absorb and emit in the NIR region. Herein, we report a highly fluorescent ($\Phi = 0.28$), photostable aza-dipyrromethene dye **1** with very sharp and intense absorption (full width at half maximum height, fwhm = 30.4 nm; $\varepsilon = 159000$) in the NIR region ($\lambda_{max} = 740$ nm). Additionally as a NIR-absorbing dye, Φ is not sensitive to solvent polarity, and the absorption band remains sharp throughout a range of concentrations (0.1–10 μM). Thus, **1** offers new opportunities for the use of such a dye in biological probes.

The dipyrromethene boron difluoride (difluoroboradiaza-*s*-indacene, bodipy) fluorescent dyes have found widespread applications.[2,8–17] Recent efforts have been focused on tuning the fluorescence emission to the NIR region of the bodipy core by attaching strongly electron-donating groups,[18] by rigidifying the structure,[19] and by extending the conjugation of the system.[20] Such modifications can lead to dyes with large red-shifted absorption maxima, but it is questionable whether such systems display useful fluorescence quantum yields, and thus their utility is far from clear.[21] Moreover, these structural alterations can lead to molecules that exhibit undesired properties; for example, structures substituted with strongly electron-donating groups display sensitivity to solvents, which leads to low Φ values in polar solvents as a consequence of electron transfer.[20b]

We selected the previously unknown, structurally rigidified aza-dipyrromethene dye as our target for synthesis. Compared to the carbon analogues, this class of dyes has not been extensively studied.[22] We speculated that the aza-bodipy core would offer a number of advantages including ease of synthesis and an inherent bathochromic shift in the absorption maxima in comparison to the carbon analogue. This latter feature would permit the synthesis of chromophores that avoid strongly electron-donating groups such as amines. The synthesis of the NIR-absorbing dye commences with pyrrole **2** (made from phenylazirine)[23] and proceeds through a convenient two-step sequence to **1** in 76.5% overall yield [Eq. (1)].



(1)

The spectral characteristics of **1** were then examined and compared to those of the known dye **3**[22] (Figure 1). The most notable feature of **1** is its intense, sharp absorption band at $\lambda_{max} = 740$ nm with $\varepsilon = 159000\,M^{-1}cm^{-1}$ and a fwhm of 30.4 nm. In comparison, **3** has $\lambda_{max} = 688$ nm with $\varepsilon = 78500\,M^{-1}cm^{-1}$ and a fwhm of 57 nm. Thus, the effect of restricting the methoxyphenyl substituent is dramatic, and results in a 52-nm bathochromic shift (cf **3**) and a concomitant halving of the fwhm. The emission maximum of **1** occurs at $\lambda_{max} = 751$ nm with $\Phi = 0.28$ (compared to $\Phi = 0.36$ for **3** in $CHCl_3$).[24] Importantly, the fluorescence quantum yield for **1** is insensitive to solvent polarity: $\Phi_{toluene} = 0.28$, $\Phi_{EtOAc} = 0.27$, $\Phi_{MeCN} = 0.26$, and $\Phi_{EtOH} = 0.26$.

Compound **1** has excellent stability, and a solution of **1** in $CHCl_3$ remains unchanged over months. The photostability of **1** was compared with that of **3** in toluene as well as against the well-known indocyanine green dye (ICG), which enjoys both wide use and FDA approval as a NIR fluorochrome ($\Phi = 0.11$ in dimethyl sulfoxide).[1b,25] Thus, **1** retains 97.7% of the fluorescence intensity after strong excitation for 1 hour, which is similar to that observed for **3** (98.0%); by comparison ICG loses 75% of its initial intensity after 1 hour.

In summary, we have developed a novel NIR fluorescent aza-dipyrromethene dye with exceptionally intense absorp-

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[**] This research is supported by ETH-Zürich. bodipy = difluoroboradiaza-*s*-indacene.

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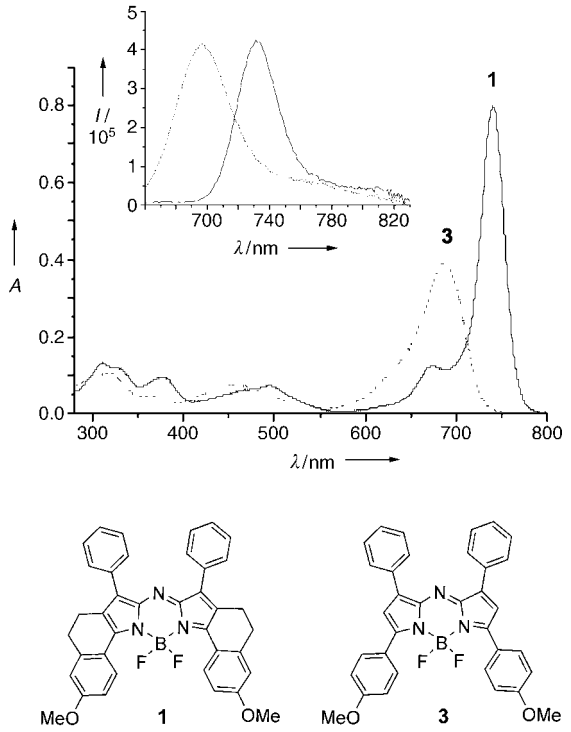

Figure 1. Absorption spectra of **1** and reference **3** (5.0×10^{-6} M $CHCl_3$). The inset shows corrected fluorescence spectra of **1** and **3** (in $CHCl_3$) at 298 K upon illumination at 670 nm.

tion ($\varepsilon = 159\,000$; $\Phi = 0.28$). The dye meets the necessary requirements of a NIR chromophore: 1) peak fluorescence at 700–900 nm; 2) high quantum yield; 3) narrow excitation/emission spectrum; and 4) high chemical stability and photostability, as well as a convenient commercially viable synthesis for the generation of useful quantities. Additionally, the sharp fluorescence of the dye is insensitive to solvent polarity. Efforts are currently under way to develop nonsymmetrically substituted, water-soluble versions to allow conjugation for biosensing experiments.

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[21] There have been reports of structurally rigidified bodipy dyes absorbing up to 766 nm. However, the efficiency of these systems is unclear as no fluorescence quantum yield has been documented. Notably, fusion and 3,5-diaryl substitution in these systems can result in a decreased fluorescence quantum yield (see ref. [19]); a) M. Wada, S. Ito, H. Uno, T. Murashima, N. Ono, T. Urano, Y. Urano, *Tetrahedron Lett.* **2001**, *42*, 6711; b) K. Tan, L. Jaquinod, R. Paolesse, S. Nardis, C. D. Natale, A. D. Carlo, L. Prodi, M. Montalti, K. M. Smith, *Tetrahedron* **2004**, *60*, 1099; c) Y. Wu, D. H. Klaubert, H. C. Kang, Y. Z. Zhang, US patent 6005113, **1999**.

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[24] Fluorescence quantum yield measurements were performed on a Fluorolog-3 instrument (Model FL-3-22) equipped with an R928P photomultiplier tube which is sensitive up to ≈850 nm. To obtain accurate excitation spectra, the excitation monochromator was calibrated by a xenon-lamp scan. The emission monochromator was calibrated using a water Raman scan. The response of the detector was calibrated with a standard

tungsten–halogen lamp. Fluorescence quantum yield determination was performed following the method recommended by the manufacturer of the fluorometer (see: www.jobinyvon.com/usadivisions/Fluorescence/applications/quantumyieldstrad.pdf), and was compared with other reported methods (S. Fery-Forgues, D. Lavabre, *J. Chem. Educ.* **1999**, *76*, 1260). Compound **3** was used as standard, and the measurements were performed under identical conditions to those with **1**. Nondegassed, spectroscopic-grade chloroform and a 10-mm quartz cuvette were used. Very dilute solutions ($A \leq 0.010$) were used to minimize reabsorption effects. The optical densities of solutions of **1** and **3** were adjusted to 0.200 at 670 nm, and these solutions were diluted by factors of 20, 40, 60, 80, and 100. The excitation wavelength was 670 nm for both compound **1** and reference **3**, and a 510-nm cutoff optical filter was placed between the excitation monochromator and sample cuvette to eliminate UV (335 nm) excitation. The fluorescence quantum yield of compound **1** was calculated to be 0.278 relative to the reference (0.36), which is comparable to the data obtained by the traditional method (0.284 ± 0.006).

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