Fluorescence

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# High Stokes Shift Anilido-Pyridine Boron Difluoride Dyes**

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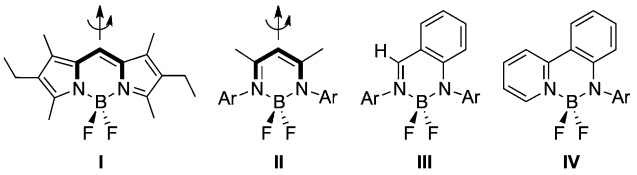
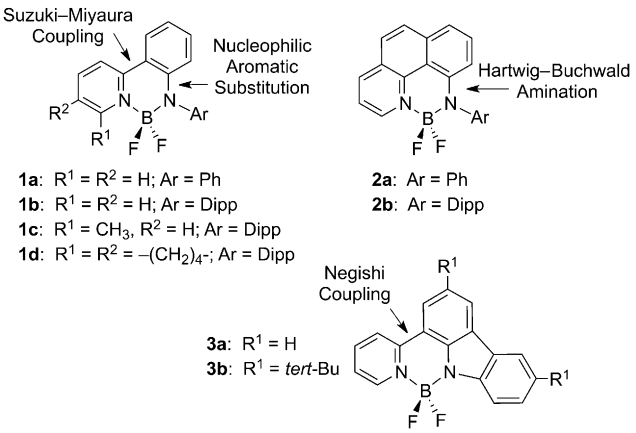
Fluorescent dyes are important tools for a variety of fields, including nanoscience,[1] biological chemistry,[2] and solar energy conversion.[3] Ideally, a dye possesses high absorption coefficients and quantum yields, a large Stokes shift, tunable absorption/emission profiles, and high chemical and photochemical stability. Few compounds, or families of compounds, have optimal attributes in all of these categories and thus, there are many dyes available for researchers to choose from.[2c] Despite this cornucopia of options, new dyes with a specific set of properties are still of considerable interest.

One family of dyes that enjoys widespread use are the dipyrrins rigidified by boron difluoride, or BODIPY dyes,[4] exemplified by **I**. While the dipyrrin ligand[5] itself is non-emissive, when complexed to the $BF_2$ unit, the compounds are high-quantum-yield emitters. The chromophore is largely associated with the rigidified dipyrrin ligand, the $BF_2$ unit serving as a light-atom anchor for the fluorophore. The ligand framework provides a versatile scaffold for chemical modification and there are hundreds of examples of this royal family of dyes.



**I** **II** **III** **IV**

One significant deficit in the properties of BODIPY dyes is their generally low Stokes shift.[4b] Stokes shifts of $>80$ nm are desirable to minimize reabsorption of emitted photons; BODIPY dyes typically exhibit shifts of 7–15 nm. Efforts to address this issue involve the incorporation of the BODIPY dye into an energy transfer cassette[6] in which the energy absorbed by the BODIPY chromophore is transferred to a second fluorophore (by some through-space or through-bond mechanism) that emits at a wavelength well-separated from the $\lambda_{max}$ of absorption. Such assemblies, although effective, are usually structurally complex and require significant synthetic effort.




***Scheme 1.*** Anilido-pyridine boron difluoride dyes.

A second, less explored strategy for improving Stokes shifts in $BF_2$-rigidified dyes relies on principles of ligand design. The dipyrrin core is symmetrical about a $C_2$ axis and desymmetrization of the bidentate nitrogen ligand represents a potential way to render ground and excited states more energetically distinct. Recognizing that the $C_3N_2$ core of the dipyrrin ligand is similar to that of the ubiquitous β-diketiminato ("nacnac") ligand framework **II**,[7] we postulated that desymmetrization of this donor set (synthetically less challenging than modification of the dipyrrin core) may lead to effective boron-based dyes.[8] Indeed, a first-generation desymmetrized nacnac analog, the anilido-imine framework (**III**) introduced by our group in 2003,[9] was employed by Mu et al. in this context,[10] producing dyes with improved Stokes shifts of around 70 nm over comparable BODIPY dyes. Given the sensitivity of the imine moiety in **III** to nucleophiles,[9] we have evolved the ligand design to the anilido-pyridine scaffold generically depicted in **IV**. This design renders the resulting $BF_2$ complexes indefinitely air- and moisture-stable in contrast to dyes **III**. Herein we report the synthesis and properties of several $BF_2$-rigidified dyes based on this novel, unsymmetrical bidentate nitrogen donor ligand motif.[11] Dyes **IV** are exceptionally photostable and show high Stokes shifts of 90–120 nm.

The range of compounds prepared is shown in Scheme 1. The ligands were synthesized as their free anilines and complexed to boron using standard, high-yielding methods that involve treatment with $BF_3$ in the presence of $NEt_3$. The key steps in any synthesis of ligands are indicated in Scheme 1; full details are given in the Supporting Informa-

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tion. The parent anilido-pyridine ligands in compounds **1a–d** were prepared by Suzuki–Miyaura coupling[12] of 2-fluorophenylboronic acid with the appropriate α-bromo pyridine partner. Treatment of the resulting aryl fluoride with two equivalents of LiN(H)Ar (Ar = Ph or 2,6-diisopropylphenyl) gave the ligands in excellent yield. The more rigid bidentate nitrogen donor in compounds **2a–b** was prepared through palladium-catalyzed Hartwig–Buchwald amination[13] of 10-bromobenzo[*h*]quinoline using aniline (**2a**, 76%) or 2,6-diisopropylaniline (**2b**, 46%). Finally, the ligands in compounds **3a–b**, based on the 9*H*-carbazole unit were prepared using a Negishi coupling protocol[14] from the potassium salt of the corresponding carbazole bromide and a 2-pyridylzinc reagent[15] in 67–85% yield.

All eight new compounds were fully characterized by multinuclear NMR spectroscopy and elemental analysis. All except compound **3a** were structurally characterized by X-ray crystallography.[16] The $^{11}$B chemical shifts are in the range of 1 to 3 ppm and appear as well-defined triplets because of coupling with the two $^{19}$F nuclei ($^{1}J_{BF} \approx 27$ Hz). The resonances for the fluorine atoms appear at −129 to −141 ppm in the $^{19}$F[17] NMR spectra, either as broad signals or 1:1:1:1 quartets when the coupling to boron is resolved. Figure 1

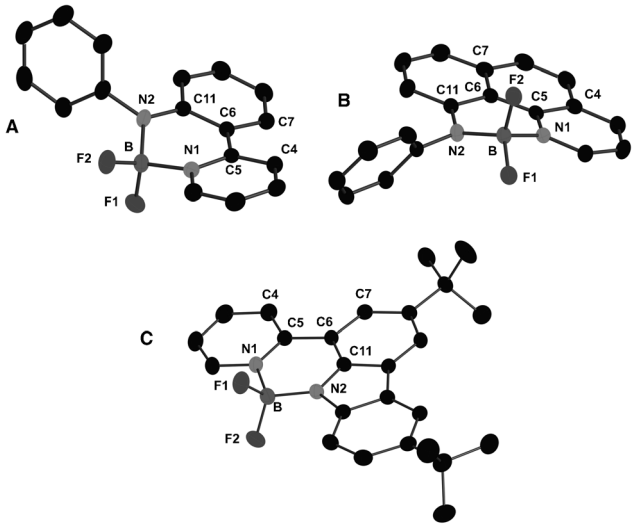


***Figure 1.*** Thermal ellipsoid (50%) diagrams of the molecular structures of **1a** (A), **2a** (B), and **3b** (C). Selected bond lengths [Å] and angles [°] for **1a**: B–N1 1.598(3), B–N2 1.498(3), N1–B–N2 106.5(2), and C4–C5–C6–C7 −14.7(3). Selected bond lengths [Å] and angles [°] for **2a**: B–N1 1.5942(12), B–N2 1.5053(14), N1–B–N2 109.99(8), and C4–C5–C6–C7 3.2(1). Selected bond lengths [Å] and angles [°] for **3b**: B–N1 1.608(5), B–N2 1.508(5), N1–B–N2 107.5(3), and C4–C5–C6–C7 0.0.

shows thermal ellipsoid diagrams for **1a**, **2a** and **3b**, along with selected metrical data. In each family, the B–N1 distance (pyridyl nitrogen) is around 0.1 Å longer than the B–N2 lengths to the anilido nitrogen, emphasizing the metrical asymmetry present in these molecules. In compounds **1a–d**, there is a considerable twist about the C4–C5–C6–C7 dihedral angle in the ligand backbone (10–15°) that flattens dramatically in the more rigid benzo[*h*]quinolido-derived compounds **2**, and the carbazolido compounds **3**, where this dihedral angle is essentially 0°. Full details on these and the structures of **1b–d** and **2b** can be found in the Supporting Information.

None of the free ligands described above shows visible fluorescence upon irradiation with a UV lamp, but all of the difluoroboryl complexes are strongly emissive in both solution ($CH_2Cl_2$) and—qualitatively—in solid state. The solution photophysical properties of these compounds are summarized in Table 1, which also includes data for the BODIPY dye **I** for

***Table 1:*** Absorption, fluorescence, and photophysical data for new dyes and a typical BODIPY dye (**I**) for comparison.[a]

| | $\lambda_{abs}$[b] [nm] | $\varepsilon_{max}$ [$M^{-1}cm^{-1}$] | $\lambda_{em}$[c] [nm] | $\phi_f$[d] | SS [nm] | SS [$cm^{-1}$] | $\tau_f$ [ns] |
|---|---|---|---|---|---|---|---|
| **1a** | 417 | 9529 | 531 | 0.33 | 114 | 5148 | 2.0 |
| **1b** | 416 | 9640 | 511 | 0.29 | 95 | 4469 | 6.9 |
| **1c** | 419 | 10065 | 515 | 0.27 | 96 | 4449 | 6.1 |
| **1d** | 418 | 10622 | 518 | 0.31 | 100 | 4618 | 5.4 |
| **2a** | 465 | 9114 | 584 | 0.60 | 119 | 4382 | 6.2 |
| **2b** | 466 | 5844 | 569 | 0.66 | 103 | 3885 | 11.1 |
| **3a** | 416 | 11252 | 496 | 0.75 | 80 | 3877 | 5.5 |
| **3b** | 431 | 9772 | 526 | 0.62 | 95 | 4190 | 5.8 |
| **I** | 517 | 64000 | 538 | 0.83 | 21 | 755 | 6.2 |

[a] Recorded in deoxygenated anhydrous dichloromethane. [b] Longest absorption maximum. [c] Emission maximum upon excitation at the longest absorption maximum. [d] Absolute quantum yield determined by calibrated integrating sphere systems.

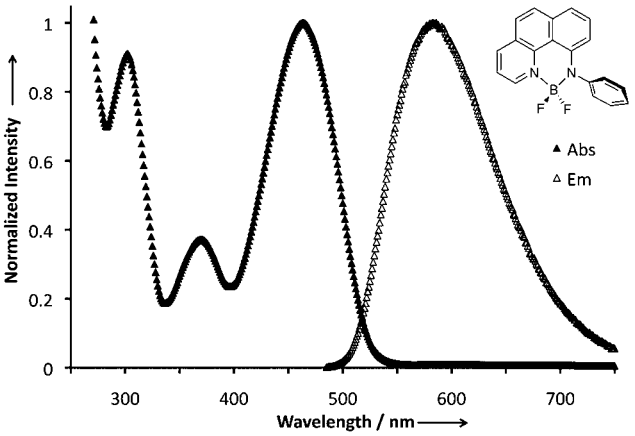


***Figure 2.*** Normalized absorption and emission profiles of dye **2a**, showing the 119 nm Stokes shift.

comparison. Figure 2 shows the UV/Vis absorption and emission profiles for compound **2a**; the absorption profiles for each dye match closely the excitation profiles obtained by monitoring the wavelength of maximum emission. The parent anilido-pyridine compounds **1a–d** show absorption maxima around 100 nm shifted to shorter wavelengths relative to **I**, but emit with significantly improved Stokes shifts in the range of 95–114 nm, albeit with moderate absolute quantum yields of around 0.30. Surmising that the flexibility about the C5–C6 vector in these compounds may be providing nonradiative pathways for excited-state relaxation, the more rigidified

  

ligands found in compounds **2** and **3** were designed to minimize this effect. Indeed, these families of dyes show a doubled emission quantum yield relative to that of family **1**, with compound **3a** giving a quantum yield comparable to that of the BODIPY dye **I**. Significantly, dyes **2** and **3** retain the high Stokes shifts found in compounds **1**; as the representative absorption/emission profile for dye **2a** (Figure 2) shows, there is very little overlap between the absorption and emission profiles for these dyes. Fluorescence lifetime values are competitive with that found for **I**; the lifetime of 11.1 ns for **2b** suggests that the benzo[*h*]quinolido-based dyes may be useful in applications requiring longer fluorescence lifetimes. One drawback of all of these dyes is the low molar absorption coefficients; efforts towards increasing the absorptivity of these dyes are underway.

Another significant advantage of the dye families reported here in comparison to BODIPY and other commonly employed dyes is their exceptional photostability. Dye samples ($10^{-5}$ M in $CH_2Cl_2$) were irradiated at 420 nm at room temperature and absorption spectra were recorded periodically. Under these conditions, BODIPY dye **I** was $>90\%$ photodegraded after 3 h, whereas rhodamine 101 bleached in less than 2 h. In contrast, the absorption profile of **1a** was essentially unchanged after 10 h of irradiation. Dyes **2a,b** were photostable for at least 16 h, whereas the carbazole-based dyes **3a,b** retained around 80% of the intensity of absorbance after this time period. Thus, in addition to air and moisture stability, the anilido-pyridyl-based dyes do not exhibit significant photodegradation over the course of a day of continuous irradiation.

In contrast to the majority of BODIPY dyes,[18] the photophysical properties of which are relatively insensitive to solvent polarity, the anilido-pyridine supported dyes show moderate hypsochromic shifts of the $S_0 \rightarrow S_1$ transition in more polar solvents. For example, **3b** gives a $\lambda_{max}$ of 452 nm in hexane, but 426 nm in DMSO, suggesting that the excited state is less polar than the ground state in these molecules. The behavior of **3b** was modeled using the Lippert–Mataga equation,[19] supporting the notion that general solvent effects are primarily responsible for the observed spectral shifts (see the Supporting Information). The treatment indicates that the dipole moment of the ground state is around 13.9 D greater than that of the excited state, an observation consistent with the desymmetrization of the molecule and a key feature of the dyes that result in greater Stokes shifts.

Because of their planarity, hydrophobicity, and charge neutrality, BODIPY dyes have been used as probes for biological membrane function and dynamics.[20] The efficacy of the anilido-pyridine dyes as probes for investigating membrane properties was assessed in unilamellar liposomes prepared from pure dimyristoylphosphatidylcholine (DMPC)[21] using dye **3a** through a spectroscopic titration technique. The efficient partitioning of the dye into the lipid bilayer is demonstrated by a shift of the maximum emission from 536 nm in water to 482 nm in the presence of the liposomes, along with a significant increase in the relative fluorescence intensity of around 70 fold. The very weak intensity and large red shift in the emission band for **3a** in aqueous solution is likely due to the formation of H aggregates formed through parallel stacking of molecules with a "face-to-face" orientation in this medium. According to Kasha's exciton model, such aggregates are essentially non-fluorescent. The large increase in emission intensity in the presence of the membrane allows for an estimation of the binding constant of $1.4 \times 10^3\ M^{-1}$. Thus, this dye is competitively membrane-specific, but with a large Stokes shift and high photostability in comparison to BODIPY dyes.

In conclusion, a new family of $BF_2$-rigidified dyes with large Stokes shifts and high photostability has been prepared. Their efficacy as probes for biological membranes has been demonstrated using a liposome model. Modular ligand syntheses will allow for attachment of a variety of groups amenable to conjugation with proteins, lipids, and other biological molecules[22] for applications in real biological systems; these modifications are currently underway.

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[16] Crystal data for **1a**: $C_{17}H_{13}BF_2N_2$, MW = 294.10, monoclinic, $P2_1/c$, $a = 11.2101(4)$, $b = 7.3817(4)$, $c = 17.1219(9)$ Å, $\alpha = 90$, $\beta = 102.339(3)$, $\gamma = 90°$, $V = 1384.10(11)$ Å$^3$, $Z = 4$, $\rho = 1.411$ g cm$^{-3}$, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, $T = 173(2)$ K, 4290 measured reflection, 3143 unique, min/max transmission = 0.9839 and 0.9939, $R1$ $(I > 2\sigma) = 0.0586$, $wR2 = 0.1152$, GoF = 1.095, No. of parameters = 195, final difference map within +0.323 and −0.258 e Å$^3$. Crystal data for **2a**: $C_{19}H_{13}BF_2N_2$, MW = 318.12, monoclinic, $P2_1/c$, $a = 7.4793(2)$, $b = 20.1482(6)$, $c = 10.2383(3)$ Å, $\alpha = 90$, $\beta = 111.0840(3)$, $\gamma = 90°$, $V = 1439.57(7)$ Å$^3$, $Z = 4$, $\rho = 1.468$ g cm$^{-3}$, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, $T = 173(2)$ K, 12783 measured reflection, 3313 unique, min/max transmission = 0.9534 and 0.9823, $R1$ $(I > 2\sigma) = 0.0338$, $wR2 = 0.0948$, GoF = 1.048, No. of parameters = 217, final difference map within +0.283 and −0.206 e Å$^3$. Crystal data for **3b**: $C_{26}H_{29}BCl_2F_2N_2$, MW = 489.22, monoclinic, $P2_1/m$, $a = 11.4796(5)$, $b = 6.9227(4)$, $c = 15.4393(5)$ Å, $\alpha = 90$, $\beta = 97.296(3)$, $\gamma = 90°$, $V = 1217.02(10)$ Å$^3$, $Z = 2$, $\rho = 1.335$ g cm$^{-3}$, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, $T = 173(2)$ K, 4108 measured reflection, 2965 unique, min/max transmission = 0. 9650 and 0.9209 $R1$ $(I > 2\sigma) = 0.0746$, $wR2 = 0.1721$, GoF = 1.057, No. of parameters = 208, final difference map within +0.726 and −0.767 e Å$^3$. CCDC 836295, 836296, 836297, 836298, 836299, 836290, 836291 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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