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Phenanthrene-Fused Boron–Dipyrromethenes as Bright Long-Wavelength Fluorophores

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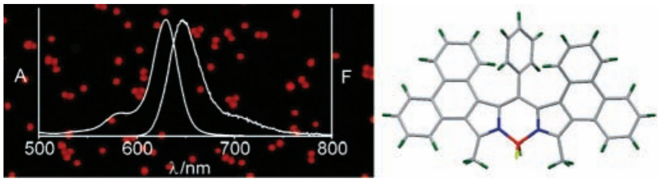
ABSTRACT



A new class of boron–dipyrromethene (BDP or BODIPY) dyes was obtained by phenanthrene fusion to the β-pyrrole positions, absorbing in the wavelength range of important laser sources. Despite a 'propeller-like' distorted structure in the crystalline state, the chromophore absorbs (log $\epsilon \geq 5$) and fluoresces ($\Phi_f \geq 0.8$) strongly and can be easily turned into a fluorescence light-up probe. Incorporation into latex beads produces bright and photostable single-dye and Förster Resonance Energy Transfer (FRET) particles for microscopy applications.

Rapid advances in optical technologies have recently led to significant progress in medical diagnostics, drug discovery, and (bio)chemical analysis, in particular for methods that use fluorescence detection in microscopy, for microarrays or single-molecule tracking.[1] These developments have fueled the search for novel fluorophores (stains, labels, and indicators)[2] with high brightness that can be excited and that emit well within the visible or near-infrared (NIR) region of the spectrum.[3] Research in this field has basically two aims: (i) to create dyes with ever red-shifted spectra[4] and (ii) to develop functional dyes that match well the emission lines or excitation/emission filter ranges of commercial light sources and equipment.[5] In the latter case, besides the advancement of well-known dyes such as cyanines or rhodamines,[5] the design of long-wavelength boron−dipyrromethene (BDP) dyes[6] has especially attracted attention in the past decade. Dyes with the parent BDP chromophore (**2**, Scheme 1) usually carry alkyl substituents in the 1,7-, 3,5-, and/or 2,6-positions of the core and show absorption and emission maxima in the 495−545 nm range.[7,8] Red-shifts of the optical spectra can be realized by (a)symmetric substitution with aryl, vinyl, styryl, or ethynylphenyl groups at the core.[9−11] Alternatively, exchange of C_{meso} for an N

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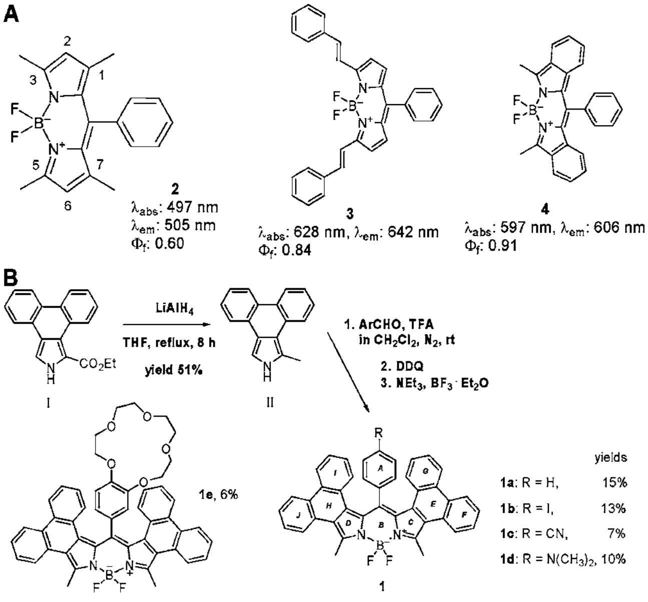
Scheme 1. (A) Chemical Structures of BDPs from the Literature: **2**,[7] **3**,[10] and **4**;[14] Data in MeCN. (B) Preparation Scheme of **1a**−**e**.



atom or fusion of aromatic rings yields more deeply colored dyes.[12−15] In particular, ring fusion is a promising way to long-wavelength BDPs while maintaining all possibilities of functionalization. Up to now, BDP analogues with isoindole,[11,14,15] and 3*H*-benzo[*e*]indole[13] have been realized. On the basis of our previous attempts in this regard[11,14] and the motivation to develop a potent dye platform for widespread commercial equipment, we report here on the use of the 2*H*-dibenzo[*e*,*g*]isoindole (or β-phenanthropyrrole) unit to construct highly annelated BDP derivatives that match the output range (594−635 nm) of the main HeNe or diode laser excitation sources.

1a−**e** were synthesized from ethylphenanthro[9,10-*c*]-pyrrole-1-carboxylate (**I**)[16] via 1-methylphenanthro[9,10-*c*]-pyrrole (**II**) and subsequent condensation with various aromatic aldehydes according to a published procedure (Scheme 1).[14] Oxidation with DDQ, treatment with triethylamine and $BF_3 \cdot Et_2O$ and purification by column chromatography and recrystallization completed the procedure. ^1H NMR measurements revealed 10 signals between 6.42 and 8.55 ppm (e.g., for **1d**). The two doublets at 8.55 and 8.43 ppm arise from H_{ortho} and H_{meta} of the phenyl ring, since they are directed toward the BDP core and are thus deshielded by the indacene and phenanthrene planes. The phenanthrene protons lying over the phenyl ring are shielded and give upfield-shifted signals at 6.42, 6.72, 7.01, and 7.19 ppm compared with those of **II**. These data indicate that **1d** possesses a distorted structure yet maintains its aromatic character. The ^1H NMR spectra of **1a**−**c**,**e** also confirmed the proposed structures.

Single-crystal X-ray structures were obtained for **1a** and **1d** (Figures 1,S1). As compared to the structures of alkylpyrrole or -isoindole BDPs that possess planar indacene planes and virtually orthogonal meso-phenyl rings,[14,17] **1a** and **1d** display distorted, ‘propeller-like’ conformations. The indacene plane deviates from planarity with an average dihedral angle $\theta^{av} = 12.2°$ between the central six-membered ring B and the two adjacent pyrrole rings C,D.[18] The phenanthrene rings[19] are also distorted from the mean plane of indacene (rings B−D) with $\theta^{av} = 21.4°$. The torsion angle between the two phenanthrene rings[19] is 31.7° for **1a** and 47.3° for **1d**. The highly distorted structure can be ascribed to the steric hindrance between the large phenanthrene units and the C8-appended phenyl group, forcing the molecules into the ‘propeller-like’ conformation. The latter determines the angle between the planes of indacene ring B and phenyl ring A, θ_{AB} amounting to only 57.9°/49.7° for **1a**/**1d**. In contrast, $\theta_{AB} = 90.0°$ for **4**.[14] The more distorted conformation of **1d** compared to **1a** is most likely due to packing effects because of the alkylamino group.

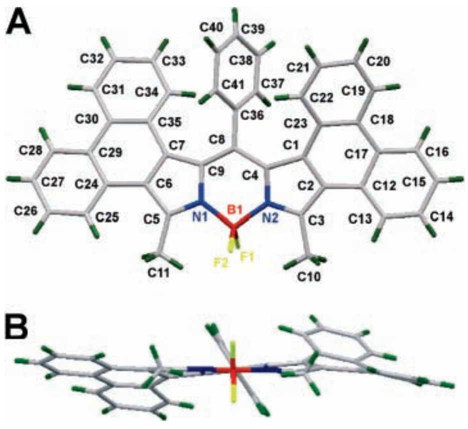


Figure 1. Crystal structure of **1a** viewed from the top (A) and along the B(1)−C(8) axis (B).

Selected spectroscopic data and optical spectra of **1a**−**e** are presented in Figure 2 and Table 1. It is evident that the

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(18) All θ between the adjacent ring planes are listed in Table S1.

(19) As defined by the mean planes of rings G, E, F and rings I, H, J. The mean planes of the phenanthrene units were taken as a reference here because the single phenanthrene rings are not co-planar, Table S1.

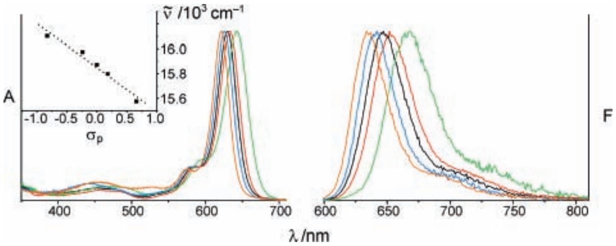

Figure 2. Normalized absorption (left) and emission (right) spectra of **1a** (black), **1b** (red), **1c** (green), **1d** (orange), and **1e** (blue) in hexane at 298 K. Inset: correlation of absorption maximum (in cm^{-1}) and Hammett constant σ_P, $r = -0.982$.

spectra of **1a**−**e** possess similar shapes as those of the classic BDP dyes. Depending on the substituent in the meso position, the absorption maxima are found between ca. 620 nm for **1d** and 640 nm for **1c** (Table 1). Like for pyrrole- or isoindole-BDPs, solvatochromic shifts are not observed. Molar absorption coefficients are favorably high and have been determined exemplarily in acetonitrile to $\log \epsilon_{625}{}^{\mathbf{1a}} = 5.07$, $\log \epsilon_{628}{}^{\mathbf{1b}} = 4.99$, $\log \epsilon_{637}{}^{\mathbf{1c}} = 4.98$, $\log \epsilon_{617}{}^{\mathbf{1d}} = 5.06$, and $\log \epsilon_{622}{}^{\mathbf{1e}} = 5.02$. Excitation of the phenanthro-BDPs yields fluorescence spectra of mirror image shape with maxima in the 630−670 nm region (Figure 2). The emission maxima lack a particular solvatochromic trend. Except for **1d** in polar solvents (see below), the fluorescence quantum yields of all dyes in solvents of any polarity between hexane and 1:1 v/v mixtures of water/methanol are favorably high with $\Phi_f \geq 0.8$.[20] The quenched fluorescence of **1d** (e.g., $\Phi_f = 0.02$ in MeCN) is due to a fast charge-transfer process from the strong donor in the meso position to the BDP core, in agreement with previous reports on *meso*-dialkylanilino-BDPs.[7,10,14] A more detailed account of the solvent-modulated photophysics of **1a**−**e** will be published separately.

Table 1. Spectroscopic Data of **1a**−**e** in Dibutyl Ether at 298 K[a]

	λ_{abs} /nm	λ_{em} /nm	Φ_f (±0.03)	τ_f /ns
1a	630	648	1.01	5.75
1b	633	654	0.92	5.65
1c	642	668	0.95	5.92
1d	621	636	0.96	5.54
1e	626	642	1.02	5.58

[a] $c_{dye} = 2 \times 10^{-6}$ M; $\lambda_{exc} = 580$ nm.

The spectroscopic data reveal two important findings. First, the molar absorption coefficients of the phenanthro-BPDs and the boron−diindomethene (BDI) dyes (**4**, Scheme 1) are equally high despite the fact that the absorption and emission spectra of the BDI dyes are narrower. This suggests that fusion of the larger phenanthrene π-electron systems induces the 'propeller-like' distortion of the title chromophore, yet also accounts for the favorably intense bands of $\log \epsilon \geq 5$.

The second important observation relates to the correlation between Hammett constant of the 4-substituents of the meso-phenyl ring and the absorption maximum (Figure 2, inset). An increase of the substituent's electron accepting nature results in a bathochromic shift of the absorption and emission bands, reflecting the charge redistribution that takes place in indacene chromophores upon excitation: the electron density increases at the meso position.[7,14] Electron-accepting substituents thus stabilize these features: **1c** with the strongest acceptor (CN) shows the longest wavelength and **1d** with the strongest donor [$N(CH_3)_2$] the shortest wavelength absorption. In contrast, for BDPs such as **2** or **4** with a dihedral angle between the indacene and meso ring plane $\theta_{AB} \approx 90°$, identical absorption maxima are found for different *meso*-(4-X)-phenyl groups.[7,14] These results stress the influence of the crowded structure on the electronic properties and enable a fine-tuning of the spectral bands.[21]

First experiments on the suitability of the phenanthro-BDPs as efficient analyte-responsive fluorescent switches and labels also yielded promising results. For instance, Figure 3 demonstrates the potential of **1d** as a pH light-up probe.[22] In the polar protic solvent mixture, **1d** is quenched. Upon addition of acid, the typical BDP fluorescence is recovered with an enhancement factor of 150 in the 650 nm region. The spectral red-shifts reflect the conversion of **1d** into **1d**−H^+, i.e., toward **1c** (cf. Table 1). Analysis of several titration curves yields a pK_a of 1.92 ± 0.05, indicating that the dimethylamino group is rather acidic.[23]

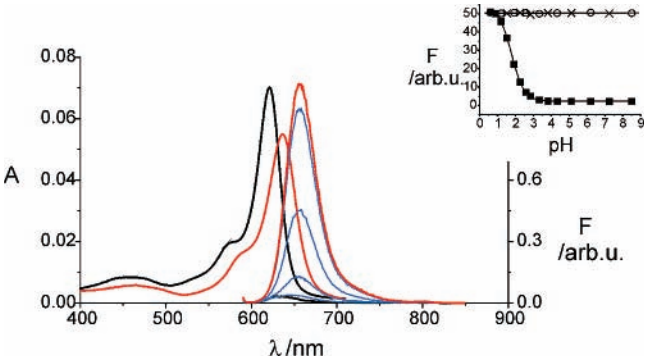

Figure 3. Absorption and emission spectra of **1d** (black) and **1d**−H^+ (red) in ethanol/water 1:1. Blue emission spectra correspond to intermediate steps of a pH titration with $HClO_4$. Inset: representative titration curve (■) and fit (—) for **1d**; the emission of **1a**−**c**,**e** is pH independent (**1a**, ○; **1c**, ×; **1b**,**e** not shown).

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[20] (20) A heavy atom substituent such as the iodo atom in **1b** has obviously no influence on the fluorescence of such chromophores when attached through the 4-position of the meso group.

[21] (21) Because $\theta_{AB} \approx 50-60°$, the molecular orbitals involved in the $S_1 \leftarrow S_0$ transition are presumably also localized in part on the meso unit. Current theoretical studies address these aspects in detail.

[22] (22) All the dyes **1a**−**e** are readily soluble in 1:1 (v/v) H_2O/alcohol or H_2O/MeCN mixtures. For **1a**−**c**,**e**, the absorption and emission spectra are similar as in organic solvents, independent of pH and Φ_f are equally high as in MeOH. The 'propeller-like' distortion of the chromophore's structure thus prevents aggregation of the dyes in polar aqueous media.

To test the performance of phenanthro-BDPs in bead-based applications, **1a** was incorporated into commercially available polystyrene nano- and microparticles (mean particle sizes of 300 nm and 1.2 μm). This allows one to use the dyes in aqueous media and would also permit the delivery of such dyes into cells.[24] The dye was loaded into the beads simply by swelling in an organic solvent (cf. Supporting Information). Both the resulting **1a**-loaded nano- and microparticles (red particles, **RP**) show virtually identical spectroscopic properties in aqueous suspensions as the free dye in organic solvents, i.e., the typical spectra (cf. Figures 2 and S2), Φ_f of ca. 0.98, and $\tau_f \approx 4.3$ ns. To demonstrate the versatility and complementarity of ring-fused BDPs for fluorescent beads applications, we prepared FRET-active nano- and microparticles (**FP**) with **4** as the FRET donor and **1a** as the FRET acceptor. On one hand, such FRET particles are very interesting for single wavelength excitation multicolor FRET applications.[25] On the other hand, FRET-based sensing systems can easily be obtained by adequate substitution of dyes such as **1** or **4**. Figure 4 shows the efficiency of the energy transfer as recorded by confocal laser scanning microscopy (CLSM). Excitation of the control particles with **4** (yellow particles, **YP**) and **RP** with the common 543 nm line yields a bright fluorescence image only for **YP** in a 555−625 nm detection window with the other three images remaining dim (Figure 4A,B); **YP** show only residual emission of the long-wavelength tail in the red channel (detection at >650 nm) and **RP** absorb only negligibly at 543 nm. However, employing **FP**, brightly fluorescing beads are only observed in the red channel (Figure 4C). Measurements on a steady-state fluorometer revealed similar spectroscopic and FRET features for nano- and micro-**FP**, suggesting that size-tailored particles can easily be prepared. Analysis of the spectra of **YP** and **RP** according to the FRET formalism[26] yields an overlap integral $J = 7.5 \times 10^{15}$ M^{-1} cm^{-1} nm^4 that is more than 10 times higher than that of a common FRET pair such as FITC-TRITC[27] with 4.9×10^{14} M^{-1} cm^{-1} nm^4.[27] That the emission of the title dyes is not quenched by oxygen and is pH-independent and that the particles do not photobleach (data not shown) stress their potential.

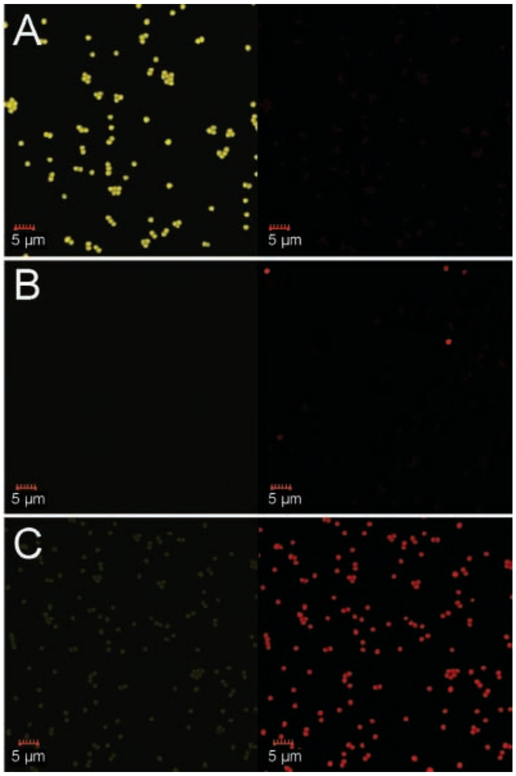

Figure 4. Confocal laser scanning microscope (CLSM) images of control [**YP** (A); **RP** (B)] and FRET polystyrene microparticles **FP** (C). $\lambda_{exc} = 543$ nm; yellow (filter combination for 555−625 nm detection) and red channels (filter for >650 nm detection) shown in the left and right panels, respectively; identical parameters (laser power, PMT voltage, gain) used for all acquisitions.

In conclusion, a novel series of highly annelated phenanthro-BDPs is presented that show intense absorption and fluorescence bands in the long-wavelength region with Φ_f close to 1. The distorted structure facilitates a stronger electronic influence of the meso group while retaining the advantages of the virtually decoupled architecture of meso-substituted BDPs. The particle experiments revealed that the title dyes are a suitable alternative for red beads in fluorescence imaging and flow cytometry. The results of **1d** as a pH indicator for the acidic range suggest that phenanthro-BDPs are a promising platform for designing more elaborate red/NIR labels and probes. The versatility of the chromophore is obvious when considering that it offers more flexibility in functionalization than azaBDPs, especially through the meso position, and that it allows to obtain novel NIR dyes; in accordance with known BDP tuning procedures, disubstitution with dimethylamino-styryl groups at the C3,-C5-positions should for instance lead to spectral red-shifts of 200 nm.[11] Attempts to improve the dyes' water solubility are currently also under way.

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Supporting Information Available: Experimental details, additional X-ray data, and bead spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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