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Bright, Color-Tunable Fluorescent Dyes Based on π-Expanded Diketopyrrolopyrroles

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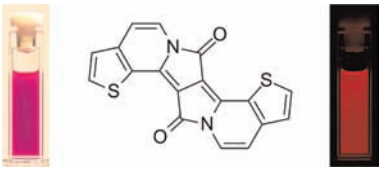
ABSTRACT



A synthetic approach to the structurally diverse family of π-expanded diketopyrrolopyrroles is described. A three-step strategy appears to be very general and starts with the preparation of diketopyrrolopyrroles followed by *N*-alkylation with bromoacetaldehyde diethyl acetal and electrophilic aromatic substitution. The final reaction regioselectively furnishes S-shaped, violet and blue functional dyes of previously unknown structure. New dyes possess sharp absorption and emission peaks, with very high molar absorption coefficients and reasonable fluorescence quantum yields. As a proof of principle, cell uptake of selected dye was demonstrated.

Effective methods for the synthesis of diketopyrrolopyrroles (DPP) have interested scientists since their development in the 1980s.[1] Expiration of the patent protection describing DPP preparation in 2003 and the discovery of efficient methods for their alkylation[2] have allowed researchers to intensify studies on these colorants. Initially DPPs were used mainly as pigments for paint for luxury cars and for coloring polymers. Now there are numerous examples of their successful application as functional dyes in dye sensitized[3] and bulk heterojunction solar cells,[4] organic light-emitting diodes,[5] organic field-effect transistors,[4d,h,6] fluorescence imaging,[7] sensors,[8] solid-state dye lasers,[9] etc. The increasing importance of DPP is due to the superb combination of properties of these compounds,[10] such as large molar absorption coefficients, resistance to fading by light, and high thermal stability. Moreover, *N*-alkylated DPPs are

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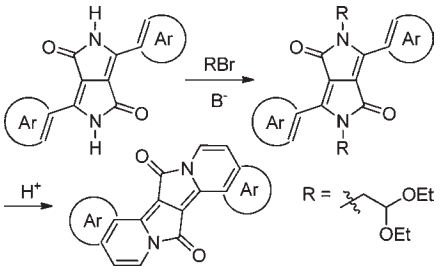
characterized by good solubility in typical solvents, high fluorescence quantum yields, and reasonable Stokes shifts.

The basic DPP structure can be altered in different ways, giving access to derivatives exhibiting desired chemical and physical properties. It is possible to exchange carbonyl oxygen atoms with sulfur, aromatic amines,[10] and various arylacetonitriles.[11] The latter reaction is particularly interesting, because the resulting dyes possess a π-expanded chromophore and display absorption in the near-infrared region.[7,11,12] Bathochromic shifts of absorption can also be realized *via* attachment of multiple five-membered aromatic rings on DPPs.[13]

In searching for alternative ways to expand the DPP-chromophore, we wondered whether we could form additional six-membered rings by introducing two vinylene moieties between the nitrogen atoms and the aromatic substituents. Such a conversion would lead to products containing two isoquinoline-like moieties and displaying different spectroscopic properties relative to the DPP core.

A retrosynthetic analysis allowed us to envision that such compounds could, in principle, be synthesized *via* alkylation of a given DPP with bromoacetaldehyde diethyl acetal followed by an intramolecular Friedel–Crafts-type reaction. In the preparation of simple isoquinolines, if an aromatic substituent is electron rich, the cyclization step can be effectively performed using aqueous hydrochloric acid as a catalyst.[13,14] Electron-poor aromatic substituents require an anhydrous system and a much stronger acid such as TfOH.[15] The above-described, three-step synthetic strategy is presented in Scheme 1.

Scheme 1. General Synthetic Strategy of DPP Chromophore Expansion



We chose bis(3,4-dimethoxyphenyl)DPP (**1a**) as a model system for investigating this concept, because of its favorable electronic structure; i.e., the donating effect of the 4-methoxy substituent should compensate for the electron withdrawing influence of the DPP core, while the 3-methoxy group should activate positions 2 and 6 for electrophilic attack. Pigment **1a** was synthesized from 3,4-dimethoxybenzonitrile (**0a**) using a modified standard procedure of DPP synthesis[16] involving the condensation of the aromatic nitrile with diisopropyl succinate (DIPS) in the presence of sodium *tert*-amylate as a catalyst. The low reactivity of this electron-rich nitrile required that we extend the reaction time to 16 h and increase the temperature. Still, the product was isolated in a rather low 18% yield (Scheme 2).

An attempt to alkylate **1a** with bromoacetaldehyde diethyl acetal was carried out under classical conditions[2] but gave only traces of the desired diacetal. However, the yield was significantly improved when the reaction was performed in the presence of tetrabutylammonium bisulfate (TBAHS) as a phase-transfer catalyst.

Diacetal **2a** was subjected to the cyclization step, which was performed using the procedure described earlier for isoquinoline synthesis;[13] i.e., **2a** was refluxed in a mixture of aqueous hydrochloric acid, dioxane, and ethanol. In this fast reaction, the product precipitated rapidly from the reaction mixture as a black solid and after 1 h of heating all of the substrate was converted, according to TLC. The product was isolated by simple filtration and purified by suspending the crude material in boiling methanol followed by a second filtration. As expected, the resulting dark violet compound was almost insoluble in common solvents due to the presence of a large planar aromatic system which favors π-stacking. Limited solubility in chloroform allowed us to measure the UV–vis and fluorescence spectra (see Table 2 and Figure S-1), but the soluble concentration was too low for NMR spectroscopy. Fortunately, pigment **3a** was more soluble in trifluoroacetic acid which allowed us to record ^{1}H and ^{13}C NMR spectra in TFA-d. The structure of **3a** was also confirmed by high-resolution mass spectrometry.

Scheme 2. Synthesis of Compound **3a**

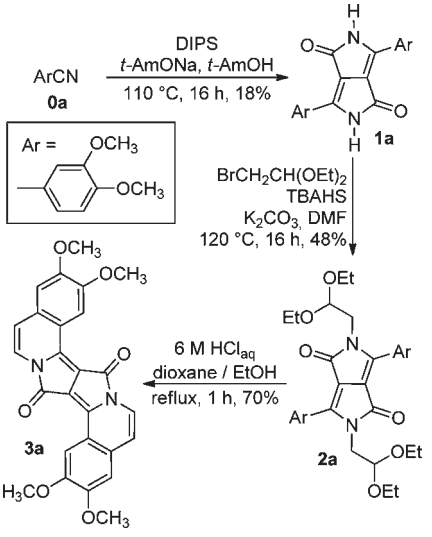


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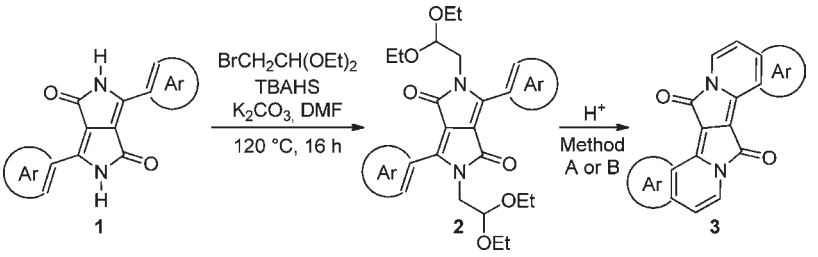
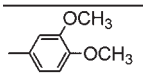
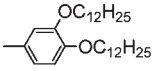
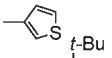
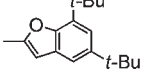
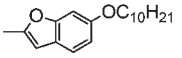
Table 1. Alkylation and Cyclization of DPPs



-Ar	DPP	alkylation time (temperature)	diacetal	yield	cyclization method[a]	product	yield
	**1a**	24 h (120 °C)	**2a**	48%	A	**3a**	70%
	**1b**	16 h (130 °C)[b]	**2b**	56%	B	**3b**	83%
	**1c**	16 h (120 °C), 2 h (140 °C)	**2c**	92%	B	**3c**	75%
	**1d**	16 h (120 °C)	**2d**	52%	A	**3d**	63%
	**1e**	16 h (120 °C)	**2e**	66%	B	**3e**	90%
	**1f**	16 h (120 °C)	**2f**	82%	B	**3f**	94%

[a] Method A: 6 M HCl_{aq}/dioxane/EtOH, reflux, 1 h; Method B: TfOH/CH_2Cl_2, rt, 1 h. [b] Reaction in NMP as solvent without TBAHS catalyst.

Encouraged by this initial success, we investigated whether this general synthetic route was applicable for other DPP pigments. Several DPPs with various aromatic substituents were synthesized, including derivatives containing thiophene and benzofuran moieties (see SI and Scheme S-1). Table 1 summarizes the results of alkylation and cyclization steps performed with DPPs **1a**–**f**.

The alkylation of DPP **1b**–**f** proceeded with moderate to very good yields. Among dyes **2b**–**f** only **2d** underwent the intramolecular electrophilic cyclization giving the desired product under the same conditions as those for **2a** (6 M HCl_{aq}/dioxane/EtOH, Method A). Diacetal **2c** did not react at all in this system. Probably in compound **2d** the substitution can occur at the more active 2-position of the thiophene ring, whereas, in **2c**, this position is not available and the reaction would have to occur at the less electron-rich 3-position. Compounds **2b**, **2e**, and **2f** reacted using Method A, but due to low solubility, they gave complex mixtures of insoluble products which could not be separated. Fortunately, we found that dyes **2b**, **2c**, **2e**, and **2f** were easily cyclized at rt in methylene chloride containing triflic acid (Method B). This method gave the desired products in very good yields and high purity.

Products **3c** and **3d** showed very low solubility in typical solvents and were readily soluble only in TFA and in hot DMSO. The solubility of **3b** and **3f** was much higher, but still unsatisfactory. On the other hand, compound **3e**, consisting of eight fused rings, exhibited very good solubility in chlorinated solvents and in toluene, which indicated that the solubility of planar pigments can be efficiently increased by introducing branched and bulky substituents rather than via long *n*-alkyl chains. It should be emphasized that the whole three-step synthesis of compound **3f** from nitrile **0f** was performed using only simple purification methods such as filtration and recrystallization and without the need for column chromatography at any stage. The overall yield of this transformation (**0f** → **3f**) is 60%.

Table 2 contains the spectroscopic data of compounds **2a**–**f** and **3a**–**f** in $CHCl_3$ and in DMF, and absorption and emission spectra of compounds **3a**, **3c**, **3d**, and **3e** are presented in Figure S-1. Absorption maxima of the expanded DPPs **3a**–**f** are strongly bathochromically shifted by 60 to 100 nm in comparison with their precursors **2a**–**f**, and the molar absorption coefficients are much higher relative to the respective diacetals.

The additional ring closures caused the dramatic decrease of Stokes shifts of the newly synthesized chromophores which can be explained by the very low flexibility of the six or eight ring-fused structures of **3a**–**f**.

Fluorescence quantum yields of these compounds, were also lower than the corresponding values of their substrates (Table 2).

As a general trend, strong bathochromic shifts of the absoprtion maxima were observed with increasing numbers of aromatic units, confirming full conjugation of the

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Table 2. Spectroscopic Data of Diacetals **2a**–**f** and Products of Their Electrophilic Cyclizations **3a**–**f**

compd	solvent	$^{max}\lambda_{abs}$ [nm]	$^{max}\lambda_{em}$ [nm]	Stokes shift [cm^{-1}]	ε_{max} [$M^{-1}\,cm^{-1}$]	Φ_{fl}[a]
2a	$CHCl_3$	493	537	1660	23 000	0.70
2b	$CHCl_3$	497	541	1640	31 000	0.67
2c	$CHCl_3$	533	560	900	24 000	0.41
2d	$CHCl_3$	493	524	1200	24 000	0.82
2e	$CHCl_3$	581	593	350	75 000	0.23
2f	$CHCl_3$	595	609	390	83 000	0.15
3a	$CHCl_3$	593	600	200	110 000	0.19
	DMF	593	601	220	91 000	0.22
3b	$CHCl_3$	590	598	230	97 000	0.23
	DMF	611	630	490	135 000	0.06
3c	$CHCl_3$	604	613	240	64 000	0.13
	DMF	605	616	300	62 000	0.12
3d	$CHCl_3$	574	582	240	73 000	0.40
	DMF	575	585	300	68 000	0.32
3e	$CHCl_3$	643	652	210	120 000	0.05
	DMF	645	655	240	107 000	0.02
3f	$CHCl_3$	648	656	190	89 000	0.03
	DMF	649	657	190	58 000	0.04

[a] Rhodamine B in EtOH as reference.

π-system (**3a**–**d**→**3e**–**f**). Furthermore, the molar absorption coefficients increased in the same manner. Naked-eye observations revealed that in solution compounds **3a**–**d** are violet while compounds **3e** and **3f** were distinctly blue. As expected, the absorption of dyes bearing benzofurane moieties (**3e**, **3f**) were strongly bathochromically shifted versus the remaining products **3a**–**d** (Figure 1). At the same time, their molar absorption coefficients were higher and fluorescence quantum yields are lower than those for dyes with six fused rings (Table 2). Except for **3b** the optical properties in polar and nonpolar solvents are very similar. The alteration of the position of the thiophene ring in the final structures **3c** and **3d** had a surprisingly high effect on the optical properties. Both the absorption and emission spectra of **3c** were red-shifted as compared to **3d** and the fluorescence quantum yield of **3d** exceeded that of **3c** by a factor of 3 (Table 1). Fluorescence quantum yields varied, reaching a maximum for dye **3d** derived from thiophene.

To clarify whether these new dyes can be used in fluorescence imaging, compound **3d** was studied in aqueous formulated solutions of Cremophor EL. Efficient cellular uptake and cytoplasmic localization were observed with fluorecence microscopy, when both fixed (Figure 1) and alive HeLa cells were incubated with formulated **3d**.

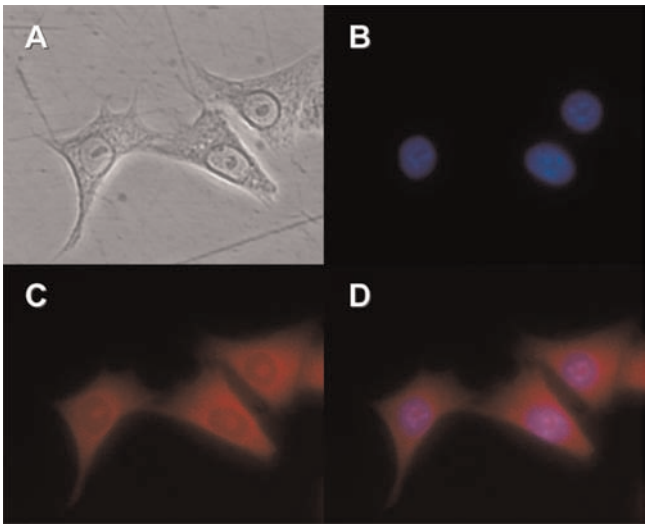

Figure 1. Fluorescent microscopy of fixed HeLa cells incubated with 10 μM of **3d** for 30 min (A), unstained cells (B), cells stained with DAPI (only blue nuclei visible) (C), and **3d** staining of cytoplasm (D) overlay image, showing almost exclusive cytoplasmic localization of **3d** (red) and cell nucleus (blue).

This paper establishes a foundation for a short synthetic pathway leading to S-shaped, stable, π-expanded DPP-analogues.[17] In a process involving just 3–4 steps from commercially available and inexpensive materials, the final products were obtained in a 6–60% overall yield. The methodology appears to tolerate structurally diverse substrates, facilitating the introduction of electron-rich or -neutral, aryl, and heteroaryl rings into the DPP scaffold, thus allowing for fine-tuning of properties for various applications. In spite of low solubility, mammalian cells can be easily labeled with selected π-expanded DPP.

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Supporting Information Available. Full experimental procedures for the synthesis of compounds **0a**–**f**, **1a**–**f**, **2a**–**f**, and **3a**–**f**, NMR spectra for these compounds, and description of cell studies. This material is available free of charge via Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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