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Rational Design of Fluorophores Using MO Theory: Our Journey from BODIPYs to BOIMPYs

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Thieme

Rational Design of Fluorophores Using MO Theory: Our Journey from BODIPYs to BOIMPYs

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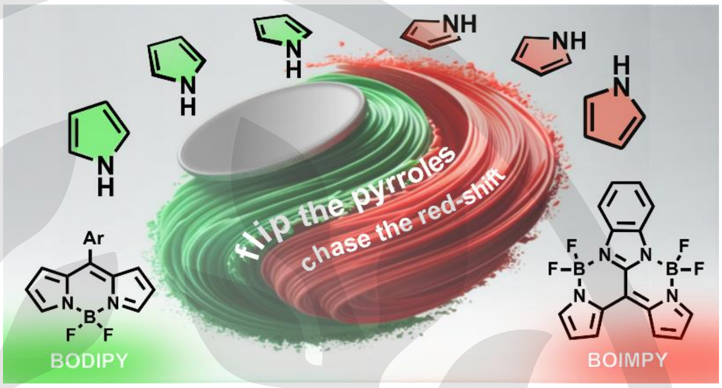

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Key words Fluorophores; Dyes; BODIPY; BOIMPY; MO considerations

Introduction and General Considerations

Fluorescent dyes have become indispensable in today's biological, biophysical, and medical research. They are routinely employed to specifically stain cell organelles and biomolecules, allowing for the visualization and localization of targets down to the nanoscopic or even single-molecule level. Beyond their use in imaging, fluorescence also serves as a key output signal for a wide range of analytical approaches due to its excellent and emitter-specific signal-to-noise ratio.[1] In addition to their relevance in biology, dyes and fluorophores have been exploited to convert photon energy into charge separations, as seen in solar energy devices, or into redox potentials in the growing field of photocatalysis. Two different systems can be distinguished: photoredox systems that enable the oxidation and/or reduction of substrates through single electron-transfer processes (as in photosynthesis), and photosensitizers that either use their excited state reactivity for chemical transformations or transfer long-lived triplet energies for the generation of triplet oxygen (as in photodynamic therapy).[2] A prerequisite for most of these applications, however, is the chemist's ability to foresee the fate of structural manipulations in order to create tailor-made dyes. This allows for the tuning of absorption or emission energies, increasing quantum yields, modifying the lipo-/hydrophilicity of these systems, or changing oxidative and reductive potentials.[3]

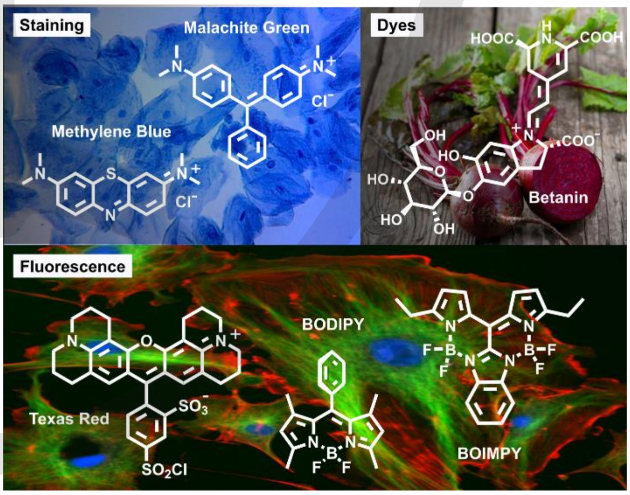

Figure 1. Example of dyes and fluorophores with cyanine characteristics.

This short review aims to trace the development of a new class of cyanine-inspired fluorophores that began in our labs in 2016.[4] These fluorophores, known as BOIMPYs[5,4], were structurally

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derived from their BODIPY ancestors (Figure 1) using simple principles from molecular orbital (MO) and excited state theory.[4] However, before delving into our considerations, we will briefly summarize the profile of other dye classes, formally derived from canonical cyanines.[6]

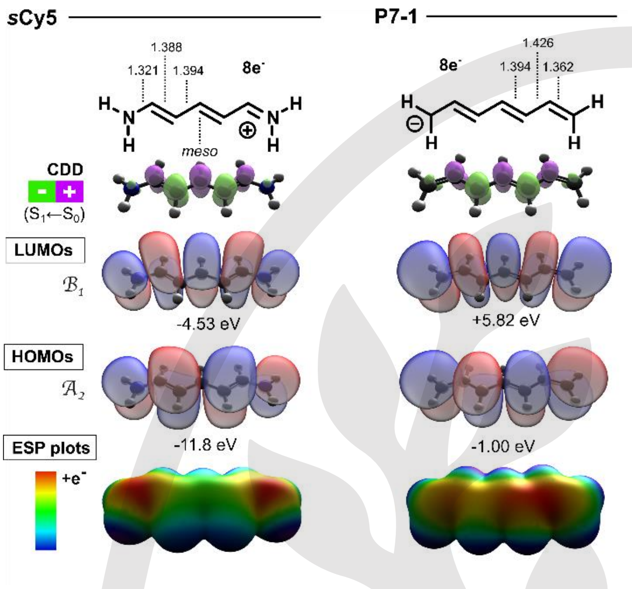

Figure 2. Comparison of cyanine sCy5 with an isoelectronic all-carbon analog P7-1. Bond lengths are given in Å. Frontier orbitals depicted with respective orbital energies (DFT: wB97xD/Def2SVP/in vacuo) and symmetry groups given in italics. CDD: Charge Density Difference of $S1 \leftarrow S0$ excitation. Regions in green highlight electron density depletion, areas marked in purple denote up-concentrated negative charge. Electrostatic potentials (ESP) plotted along total densities (Gasteiger).

Dyes such as malachite green,[7] methylene blue,[8] rhodamines (e.g. Texas Red),[9] or BODIPYs might seem quite unique at first glance, but upon closer examination, their fundamental structures reveal significant similarities and a common electronic signature. Also dyes such as Betanine found in beetroot (*Beta vulgaris*) and Muscaaurin I found in the red cap of fly amanita (*Amanita muscavia*) are based on this structural motif.[10]

Linear streptocyanines were introduced and investigated more than 100 years ago by the chemist Walter König in Dresden.[6] These conjugated oligoene systems formally incorporate an amine and iminium terminus, respectively, capping an odd number of sp^2-carbons in between. This arrangement leads to a delocalized positive charge at the π-system providing cyanines with enhanced polarity and a beneficial water-solubility for life science applications. While a polyene structure integrates $2n$ electrons at n double bonds, an analogous cyanine system is characterized by a surplus of two electrons due to the participating nitrogen lone pair, namely $2n+2$ electrons at n double bonds. This electronic "overload" is counterbalanced by a positive charge maintaining overall chemical stability. Figure 2 contrasts exemplarily a streptocyanine ***sCy5*** with an isoelectronic all-carbon analog **P7-1**. Interestingly, despite electronic equivalence and mesomerism, the cyanine system of ***sCy5*** lacks the balanced C-C bond length propagation of streptocyanines (ca. 1.40 Å). This uniformity creates a scenario akin to a 'particle in a box,' contributing to the electronic singularity of cyanines, alongside their low excitation energies. The key excitation event of cyanines is mostly characterized by a $S_0 \rightarrow S_1$ transition involving primarily the HOMO-LUMO orbital pair. Due to their complementary symmetries (A_2 and B_1 or *vice versa*) this process becomes highly symmetry-allowed, resulting in high oscillator strengths and extinction coefficients, ultimately producing the vibrant and brilliant colors of cyanine-derived chromophores.[11]

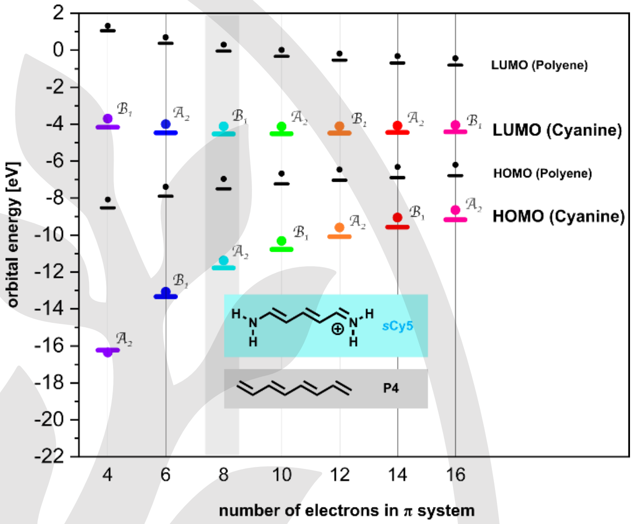

Figure 3. HOMO and LUMO energies of prototypical polyenes and cyanines, computed at two different levels of theory in vacuo (bars: DFT/wB97xD/Def2SVP; dots: Hybrid/DSD-BLYP-D3/Def2TZVPP). Corresponding orbital symmetries are given in italics.

Unlike polyenes, where the π-system extension does not correlate proportionally with the excitation energy, the incorporation of extra double bonds in the cyanine system consistently induces experimental red-shifts of approximately 100 nm.[11b] Figure 3 contrasts the shrinking HOMO-LUMO gaps of polyenes and cyanines along growing chain lengths. Due to the inherent positive charge of the cyanine π-system, both frontier orbitals have lower energies than the corresponding polyene structures.

Although the experimentally reflected quick narrowing of HOMO-LUMO gaps can be reproduced computationally for cyanines, their exceptional low excitation energies, surpassing isoelectronic polyene analogs by far, fail to be correctly predicted even with MP2 corrections (e.g. DSD/BLYP). Figure 3 illustrates the systematic overestimation of HOMO-LUMO gaps derived with two different computational methods. This phenomenon is a special feature of cyanines and known in literature as the "cyanine challenge".[12] The central or *meso* carbon atom plays a crucial role during the $S_1 \leftarrow S_0$ excitation process, being the position that typically undergoes the highest charge relocalization as shown in the CDD plots (Figure 2). Streptocyanines are chemically labile chromophores that require structural decoration to achieve both stability and functionality. In particular, spectral modifications were key challenges for chemists over the last decades. MO theory has provided a solid rationale for many synthetic endeavors in this field. If the objective is a red-shift, then the HOMO-LUMO gap needs to be closed, which can be achieved through either energetic

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destabilization of the HOMO or stabilization of the LUMO. This simple picture seems to be valid, especially when the main excitation is characterized by the HOMO-LUMO pair, which is widely true for cyanines. Nevertheless, to enhance predictability, all orbitals involved in a respective excitation event need to be considered. Mapping the derived charge density difference (CDD) between the excited state and S_0 then provides a solid basis for structural modifications at native chromophore scaffolds. Figure 4 shows two structures that represent a further step along the evolution to modern cyanine fluorophores. The linear geometry of streptocyanines was cyclized into aromatic moieties, at which the nitrogens are either external functionalities (aniline, **DiAr**) or integrated into heterocyclic moieties (pyrrole, **DiPy**). The frontier orbitals, however, still follow the same symmetry rules as in the canonical case maintaining the singularity of the *meso* position. Synthetic issues as well as the urge for a beneficial structural planarity prompted chemists to achieve rigidified variants of both structures. In case of **DiAr** a range of bridging units (X) has been implemented with contrasting consequences for the optical properties: In case of a π electronically innocent isopropyl group the excitation energy is roughly centered at 615 nm, leading to red C-pyronin (R^m = H) or C-rhodamine (R^m = Ar) dyes, along with silicon analogs.[13]

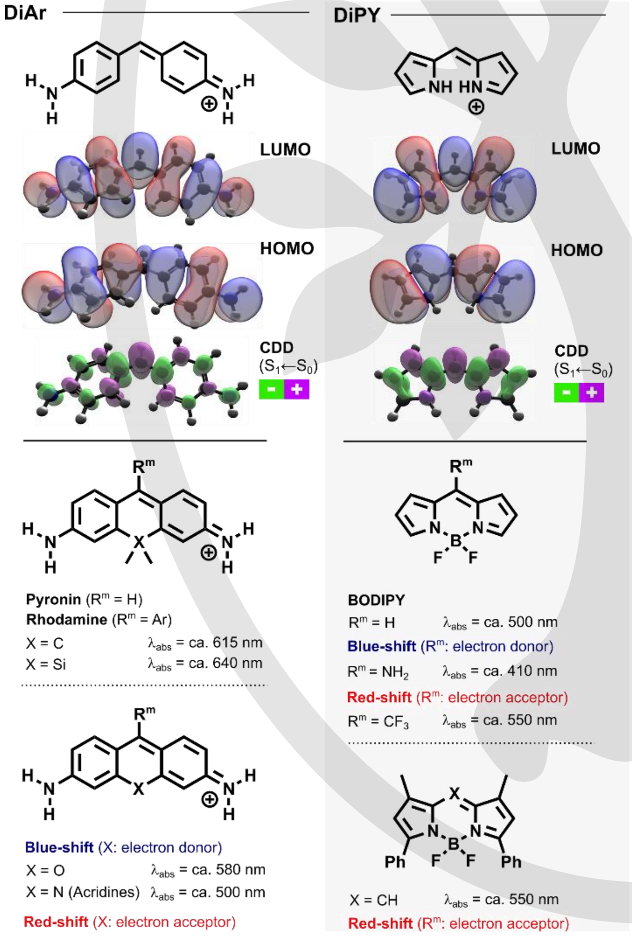

Figure 4. Evolution of modern dyes and fluorophores based on two cyclization strategies **DiAr** and **DiPy** starting from streptocyanines (DFT: wB97xD/Def2SVP/in vacuo). CDD: Charge Density Difference of $S_1 \leftarrow S_0$ excitation. Regions in green highlight electron density depletion, areas marked in purple denote up-concentrated negative charge.

Using electron donors such as oxygen or nitrogen for cyclization, however, destabilizes a position of negative charge concentration after transition (see CDD plot, positions in purple) triggering a higher excitation energy and ultimately a blue-shift. The opposite is true for stabilizing units such as sulfones or phosphinates, which can induce strong red-shifts reaching the end of the optical spectrum (700 nm).[14]

While aniline residues require a covalently bound bridging unit for planarization, the **DiPY** scaffold offers a more elegant way to inhibit rotational freedom. Since both nitrogen atoms of the pyrrole units point inwards to a central cavity, e.g. metalloids and cations can be used for chelation after a deprotonation step.[15] The introduction of a BF_2 unit through $BF_3{\cdot}OEt_2$ is the most prominent bridging pattern, resulting in the well-known BODIPY motif (borondifluoride dipyrromethene), first synthesized by Treibs and Kreuzer in 1968.[16] Although the molecule becomes electronically neutral by release of HF, the cyanine signature of the π-system is still present as illustrated in Figures 2 and 4. The impact of the *meso* position was exploited extensively at BODIPYs and led to blue-shifted (e.g. $R^m = NH_2$) and red-shifted (e.g. R^m = CN, CF_3) congeners.[17] Replacing the *meso* methene moiety itself with an electronegative nitrogen atom is an atom-economic alternative to induce a strong red-shift, revealing the realm of so-called Aza-BODIPYs. Although their synthesis is relatively simple these compounds commonly show rather low solubility. In addition, their stability can be hampered when steric shielding of this highly electron-depleted π-system is insufficient.[18]

The BOIMPY design

The unsubstituted BODIPY motif absorbs at around 500 nm and emits at ca. 510 nm with quantum yields close to unity.[1a] The relatively small Stokes shift reflects the rigidity of the unsubstituted scaffold in the excited state. However, rhodamine fluorophores exhibit similar photophysical features with an optical center red-shifted by approximately 100 nm (at ca. 600 nm), a more favorable spectral range for modern imaging techniques.[19] Confronted with that drawback, we became interested in strategies to transform the parent BODIPY scaffold into a red-shifted, fluorescent analog.

The derivatization of a standard BODIPY with electron-withdrawing aryls at the *meso* position faces challenges, especially as they fail in achieving complete co-planarity with the BODIPY core structure due to steric hindrance with the hydrogen atoms. A maximum electron pull, which would result in a significant red-shift in absorption, is thus usually compromised. Since an analysis of the frontier orbitals of a BODIPY reveals that the BF_2 unit has practically no coefficients in either the HOMO or the LUMO, we considered the following design for more red-shifted BODIPY derivatives (Figure 5): In a thought experiment, we first removed the BF_2 unit from a *meso* imidazole substituted BODIPY of type **1**. A 180° rotation of the pyrrolic subunits in **2a** causes only slight changes in the energy levels of the corresponding orbitals but opens up the possibility of utilizing the nitrogen atoms of the pyrrole units, along with those of the imidazole unit, as coordinating elements (**2b**). As a result, two pockets suitable for coordinating BF_2 units are formed. This approach compels the molecule into a planar arrangement, hindering the rotation of its three π-systems. Simultaneously, the imidazolium character of the introduced bridging unit induces a

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strong electron pull, thereby significantly stabilizing the electron density shifted to the *meso* position upon excitation. In turn, the rigidity and planarity of **3** established by twofold BF_2 chelation ensures little geometrical reorganization in the excited state and should promote high fluorescence quantum yields.[4]

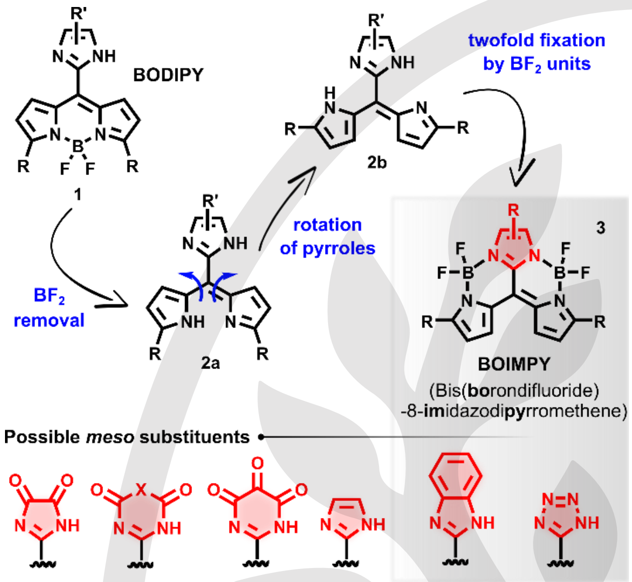


Figure 5. Design of the BOIMPY scaffold **3** and possible bridging units (units in box at the right have been realized so far).

In theory, a series of bridging ligands with two nitrogen atoms in the 1- and 3-positions should realize the idea of twofold BF_2 incorporation fulfilling the generic BOIMPY design principle (Figure 5). In order to facilitate synthesis and enhance stability by steric shielding, our initial focus became a benzimidazole moiety rather than an imidazole. However, we also explored the feasibility of introducing a tetrazole unit in order to challenge the maximization of electron-withdrawing strength.[20] For ease of reference, we termed the latter species Aza-BOIMPYs since the number of nitrogens at the *meso*-unit is virtually maximized. Figure 6 illustrates the electronic signatures of the BOIMPY motif and its aza-analog. It is evident that the orbital shapes and symmetries still adhere to their cyanine ancestors. The CDD plots underscore that both *meso*-units, benzimidazole, and tetrazole, play only a minor role in the charge redistribution process upon $S_1 \leftarrow S_0$ excitation. This is an intriguing fact since the introduction of these coordinating bridging units introduces a pseudo 3-fold rotational axis perpendicular to the molecular plane allowing (Aza-)BOIMPYs to be considered as fusion structures of two BODIPY analogs with a common hemisphere (benzimidazole or tetrazole). Accordingly, despite of rotated pyrroles, two BF_2 units, and a different symmetry this new class of chromophores still meets the electronic criteria of cyanines.[4,20]

Syntheses, Derivatisations and Photophysical Investigations

The synthesis proceeds in an analogous manner to that of common BODIPY derivatives (Scheme 1).[4,20] The starting point is a benzimidazole carbaldehyde **4**. Under Friedel-Crafts-like conditions, two pyrroles condense with the aldehyde at room

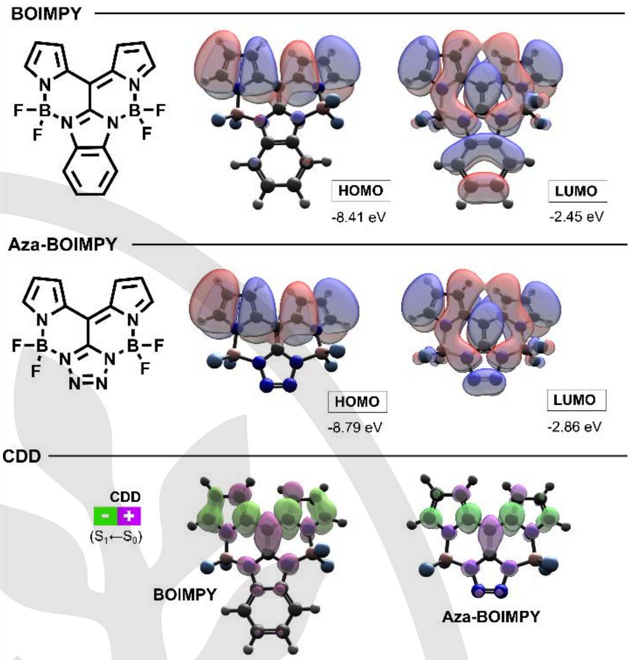
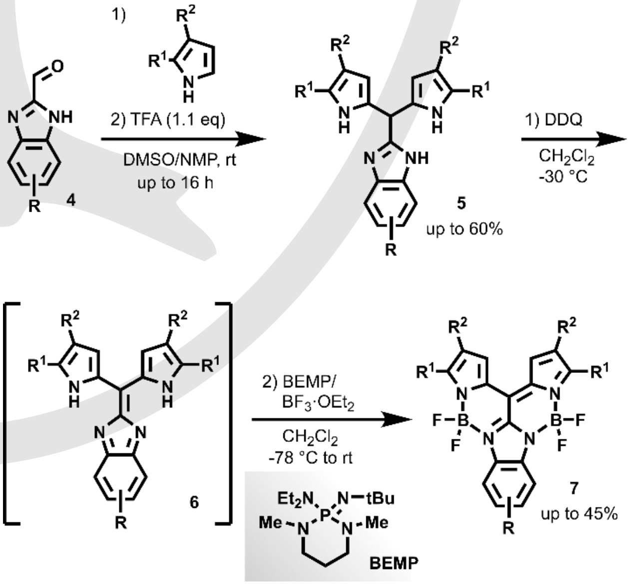


Figure 6 Frontier orbitals and CDD plots of native BOIMPY and Aza-BOIMPY structures (DFT: wB97xD/Def2SVP/in vacuo). The HOMO and LUMO energies of a corresponding BODIPY core structure, compare Fig. 4, $R^m = H$, are HOMO: -8.00 eV, LUMO: -1.55 eV.

temperature. Stoichiometric amounts of TFA in a mixture of DMSO and *N*-methylpyrrolidone (NMP) are used for activation. The dipyrromethane **5** can be obtained in yields of up to 60% and consists of three isolated π-systems which are brought into conjugation by a subsequent oxidation step with DDQ at -30 °C, formally removing one molecule of H_2. Dipyrrin **6** is not isolated, but used for the final step as a crude reaction intermediate. In order to enable the complexation of the BF_2 units, $BF_3{\cdot}OEt_2$ is added to the reaction mixture in the presence of a sterically hindered Schwesinger base (BEMP). Although alternative bases such as DBU also enable the loading of both chelation pockets, yields tend to be significantly lower.



Scheme 1. Two-step synthesis of BOIMPYs 7.

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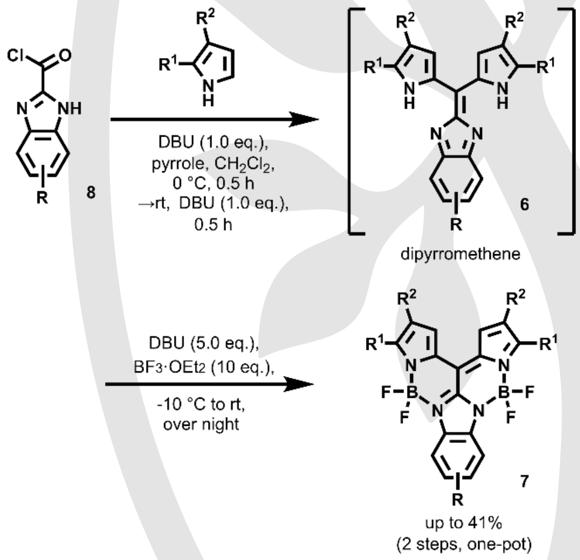
Different solvents of higher polarity (e.g. THF) were found to be incompatible. Employing this synthetic protocol, BOIMPYs of type **7** were furnished in yields of up to 45% (over two steps).[4] Our original synthesis described above was inspired by the well-known condensation between pyrroles and aldehydes, characterizing also the standard protocol of traditional BODIPY syntheses. In order to reduce the amount of intermediates and avoid the necessity of isolating dipyrromethanes **5** (Scheme 2) a higher oxidation state of the benzimidazole coupling partner was considered, avoiding the use of DDQ. This simplified the synthesis of BOIMPYs into a one-pot route over two steps.[21] The corresponding starting materials thus became acyl chlorides of type **8**, which can be obtained in high yields from carboxylic acids. Under the influence of DBU, which neutralizes the HCl formation, dipyrrins are obtained directly. The addition of $BF_3 \cdot OEt_2$ in the presence of DBU finally leads to chelation of the BF_2 units furnishing BOIMPYs **7** in yields up to 41% in a one-pot process. When using highly substituted pyrroles, such as kryptopyrrole (R^1 = Et, R^2 = Me), their steric demand establishes twisted BOIMPY species with a helical axis at the *meso* position (**7c**, Figure 7). The methyls at C2 and C6 lead to interplanar angles of about 16° between the pyrrolic units and the benzimidazole moiety.



Scheme 2. One-pot synthesis of BOIMPYs **7**.

The new method also allows the preparation of unsymmetrical BOIMPYs (Scheme 3). Even though a one-pot sequence to such unsymmetrical congeners is feasible, a separation can be challenging; thus, we employed a modified two-step strategy. Combining a freshly prepared acyl chloride derived from benzimidazole-2-carboxylic acid (**9**) with a pyrrolic Grignard reagent yields methanones **11**. This step can be easily conducted in gram-scale still providing high yields. The target compounds **12** are obtained after Friedel-Crafts-like reaction with another pyrrole moiety and insertion of the BF_2 units.[21] In addition to several synthetic strategies, BOIMPYs also offer similar post-functionalizations as their BODIPY counterparts (Scheme 4).

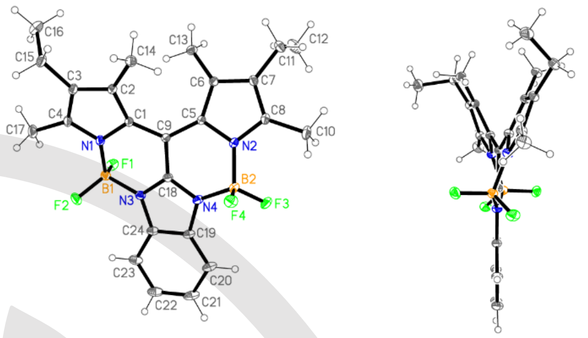
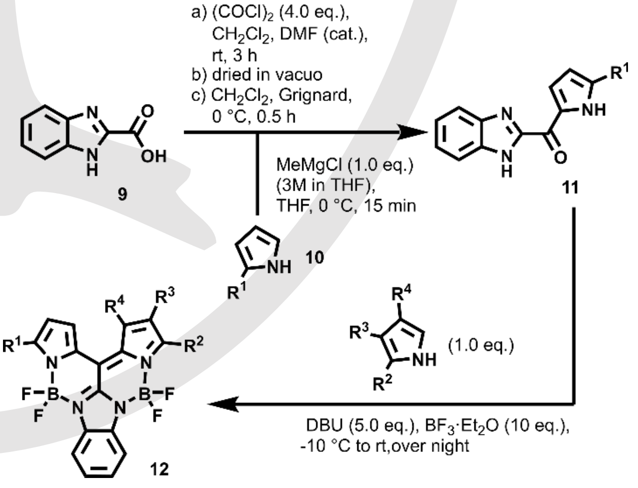


Figure 7. X-ray structure of twisted BOIMPY **7c** (R = H; R^1 = Me; R^2 = Et): (left) top view and (right) side view.)

Using *N*-halosuccinimides, two or three halogen atoms such as Br or I can be easily attached to the main core structure at the most electron-rich parts of the π-system.[4] About 2 eq. of NBS lead to double bromination (**13a**), a large excess of NBS generates the tri-Br-substituted derivative **13b**, whereas only double iodination was observed with NIS, presumably due to steric reasons (**14b**). Similarly to BODIPYs, the fluorides of the BF_2 groups can be substituted by Grignard reagents. Interestingly, the fluorides of one BF_2 unit are substituted first (**15a**) followed by the second BF_2 unit being attacked. A 12-fold excess of Grignard reagent was required for complete substitution of all four fluoride atoms affording **15b**.[4] Engaging tailor-made Grignard reagents that allow deprotection into hydrophilic moieties also enabled the preparation of water-soluble BOIMPYs.[4] Owing to the electron pull of two BF_2 units, α-methyl groups at the pyrrole termini (**7b**) are more acidic than at a BODIPY motif. Under carefully controlled reaction conditions BOIMPYs are highly qualified for extension of their π-systems by Knoevenagel-type condensations with respective aldehydes. Depending on the conditions, styryl moieties can be installed on one (**17**) or both sides of the BOIMPY π-system (**16**).[4] The scope of the aldehyde reaction partner is large and also allowed access to species suitable for biolabeling purposes (**17a**).



Scheme 3. Approach to unsymmetrical BOIMPYs.

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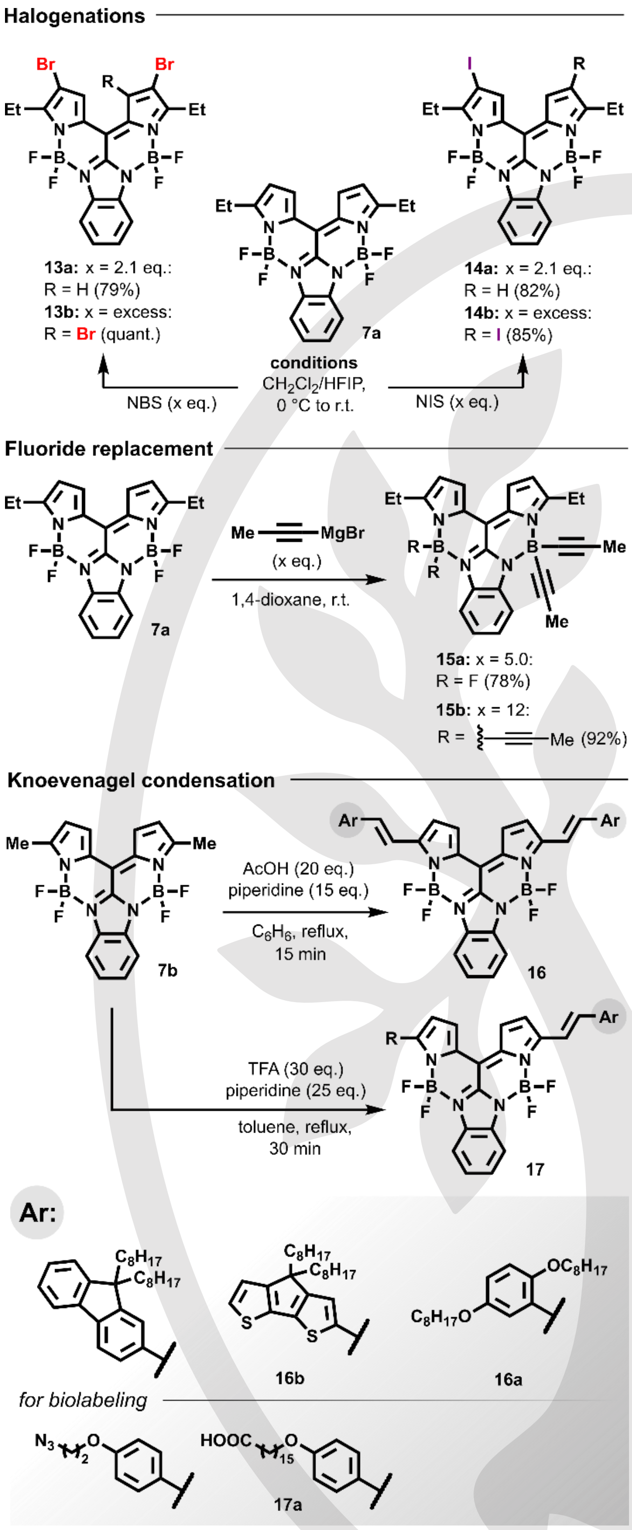


Scheme 4. Different derivatizations of the BOIMPY scaffold.

An UV/Vis analysis of the new chromophore underlines that a dominant $S_1 \leftarrow S_0$ excitation is responsible for the red color of the BOIMPY scaffold (Figure 8). The main band shape (**7a**) clearly reveals the BODIPY origin, however, the vibronic shoulder at ca. 550 nm is significantly more pronounced. Jacquemin et al. found an explanation in the stronger geometrical reorganization of BOIMPYs in the excited state due to the highly electron depleted *meso* bond between the benzimidazole and the dipyrrole subunit.[22] The strong shift of electron density to that bond which is characterized by a full π-overlap between the two subunits in contrast to traditional BODIPYs triggers an efficient excitation of vibrational stretching modes. Figure 8 contrasts a selection of absorption spectra of four different BOIMPYs and a common BODIPY species for comparison. Compared to the latter the native BOIMPY **7a** shows a red-shift of about 100 nm while maintaining a maximized quantum yield of about 90% with fluorescence lifetimes of about 6-7 ns. The rigidity of the scaffold is in line with the very small Stokes shift of about 250 cm^{-1}.[4] For twisted derivatives such as **7c** the Stokes shift is increased (~800 cm^{-1}).[21] As expected, the extension of the π-system by terminal styryl groups promotes a drastic red-shift that is accompanied by a stronger main absorption band. While a single phenolic unit shifts the absorption maximum to 695 nm (**17a**) the twofold substitution at both termini result in an additional red-shift of 100 nm (**16a**). Exchanging the phenolic subunits by more electron-rich dithiophene motifs enables a main absorption at ca. 950 nm (**16b**). These red-shifts are associated with a strong loss of fluorescence as dictated by the *energy gap law*.[23]

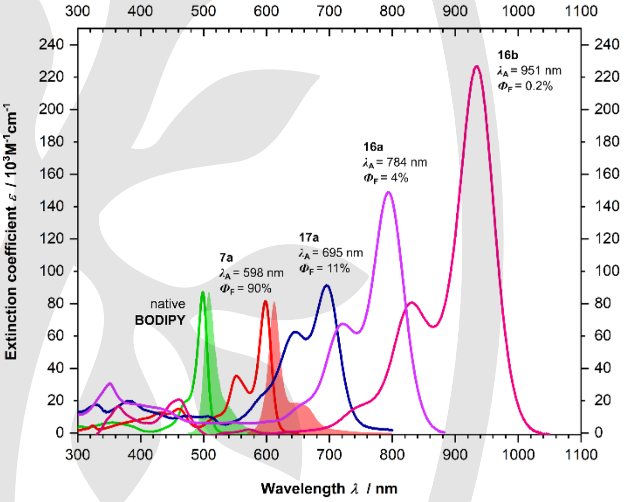
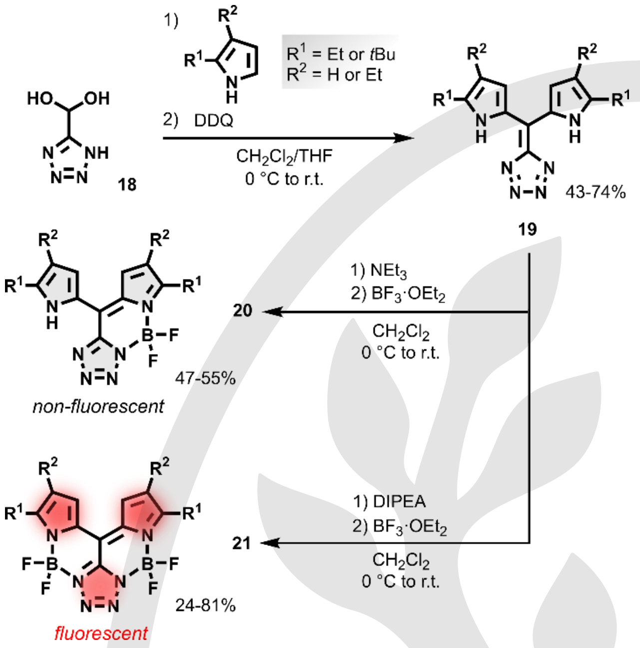


Figure 8. UV/Vis-Spectroscopy of four BOIMPY species in CH_2Cl_2 (r.t.) with a typical BODIPY example. Shaded spectra represent corresponding emission spectra (only shown for BODIPY and BOIMPY **7a**).

As outlined in the introduction the BOIMPY design principle is compatible with a range of *meso*-substituents bearing two *ortho* nitrogens. Aza-BOIMPYs, which incorporate a tetrazole instead of the benzimidazole unit, exemplify maximized electron-depletion and compactness in this respect (Figure 5 and Scheme 5).[20] At the start of our synthetic efforts, a tetrazole carbaldehyde was not preparable, and instead, the stable hydrate **18** was used as starting material for pyrrole condensation. This initial step proceeded very smoothly and appears to even spare the addition of an acidic catalyst. The DDQ oxidation furnished tetraazafulvene derivatives **19** in up to 70% yield (over two steps). In principle, a tautomer with an NH bond at the tetrazole unit would be a valid structural alternative; however, theoretical calculations proved that two symmetrical pyrrole hemispheres of a tetraazafulvene structure are more favorable by ca. 8-9 kcal/mol. Depending on the involved base, products **20** with one BF$_2$ unit (triethylamine) or the Aza-BOIMPY as symmetrical product **21** with two BF$_2$ units (Hünig base) can be accessed.



Scheme 5. Synthesis of the Aza-BOIMPY scaffold **21**.

Figure 9 illustrates the impact of introducing BF$_2$ units into the chelating pockets. The boron-free tetraazafulvene derivative **19a** exhibits a main absorption band at 536 nm with vanishing fluorescence. The rotational freedom of two pyrrolic units is likely to allow geometric reorganizations in the excited state giving rise to non-radiative decay pathways. The insertion of a single BF$_2$ unit (**20a**), leads to a red-shift of 26 nm due to an intensified electronic stabilization of the *meso* position, the fluorescence quantum yield, however, remains below 1%. Only the introduction of the second BF$_2$ unit ensures complete structural rigidity increasing the fluorescence quantum yield up to 79%, accompanied by a further red-shift of about 30 nm (**21a**).[20]

Since the unsubstituted BOIMPY π-system is characterized by a strong electronic depletion, localized predominantly at the benzimidazolium or tetrazolium moiety, counterbalancing this property through additional electron donors appeared appealing. A few years ago, Reissig and co-workers described the synthesis of extremely electron-rich pyrroles engaging allenyl enol ethers of type **27** (Scheme 6).[24] These precursors are easily obtained from alcohols such as **25** and a propargylic tosylate (**26**). The vicinal hydrogen atom at the allene adjacent to the oxygen is acidic enough to be deprotonated by *n*-butyllithium. The organolithium species was then reacted with tosylated imines and the crucial ring-closure to β-alkoxy substituted pyrroles (**28a-28c**) enforced under the influence of potassium *tert*-butoxide. Despite their sensitivity, these pyrrole species proved to be suitable substrates for our established synthetic procedures and furnished compounds of type **23a-23c** and **24a-24c**, respectively, as (Aza-)BOIMPY variants with an even more intensified push-pull effect.[25] Scheme 6 depicts the product scope realized. The alkoxy substituents and the phenyl rings in **23a/24a** cause a bathochromic shift of the main absorption by about 100 nm compared to the native system. Replacing the phenyl groups by more electron-rich thienyl (**23b/24b**) or furyl rings (**23c/24c**), facilitates another red-shift of 100 nm. However, this red-shift comes at the expense of the emission capability, which might be the result of a greater displacement of potential energy surfaces (S$_0$ and S$_1$) under the influence of introduced rotational freedom (alkoxy and aryl groups) and the maximized push-pull quality. Species **23a/24a** exhibit higher Stokes shifts (ca. 600 cm^{-1}) compared to parent structures (**7a**, **21a**). Inspired by the design of so-called *keiofluors* and their convincing fluorescence,[26,17a] we also employed a furopyrrole as building block (**24d**). We assume that the increased rigidity of this fused structure is responsible for the normalized fluorescence of 39% at ca. 700 nm compared to the equally red-shifted species **23a/24a**. This value is within the expectations of the *energy gap law*.

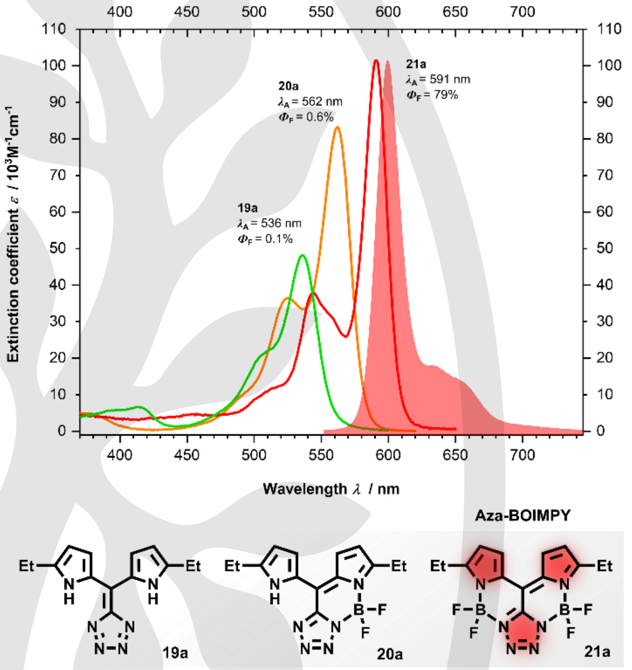


Figure 9. Effects of electron-withdrawing BF$_2$ units on the absorption and fluorescence quantum yields. The shaded spectrum represents the emission spectrum of 21a.

The modified frontier orbital energy levels (increased HOMO and decreased LUMO) are well reflected by cyclic voltammetric investigations (Figure 10). Two reversible waves are found in the reductive region corresponding to the formation of mono- and dianionic species, respectively. In contrast to the parent BOIMPY **7a** the electron-rich (Aza-)BOIMPYs **23a** and **24a** tolerate a reversible oxidation process visible by the oxidative wave at ca. 0.8 V.[25] The increased electron deficiency of the Aza-BOIMPY π-system compared to its BOIMPY counterpart is also evident in the CV spectra, as indicated by lower reduction potentials for **24a** than for **23a.**

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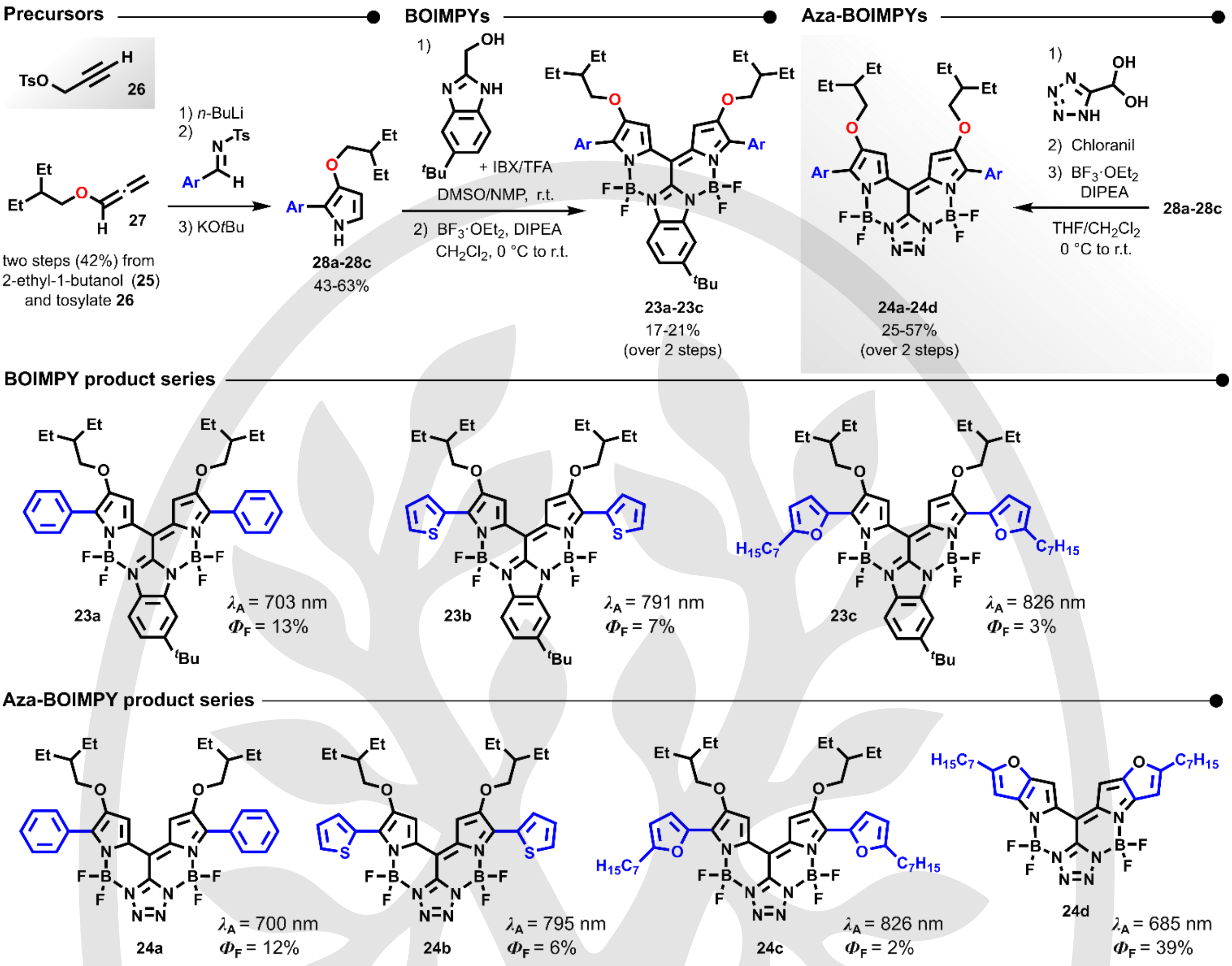


Scheme 6. (Aza-)BOIMPY synthesis with electron-rich pyrroles and corresponding product scopes. Spectroscopic data in CH_2Cl_2 at r.t.

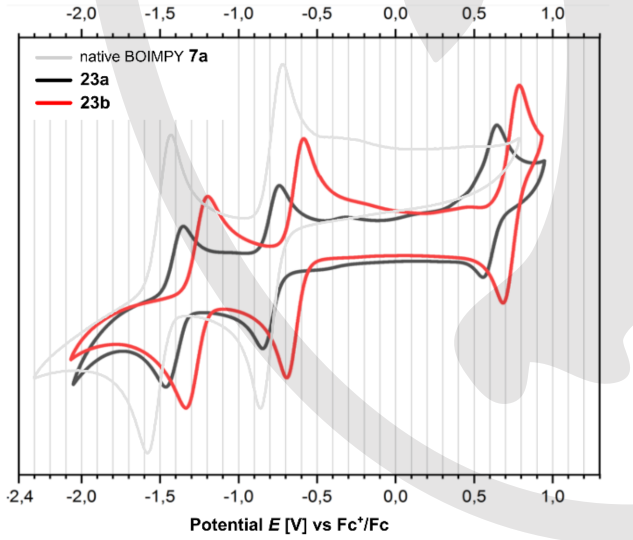
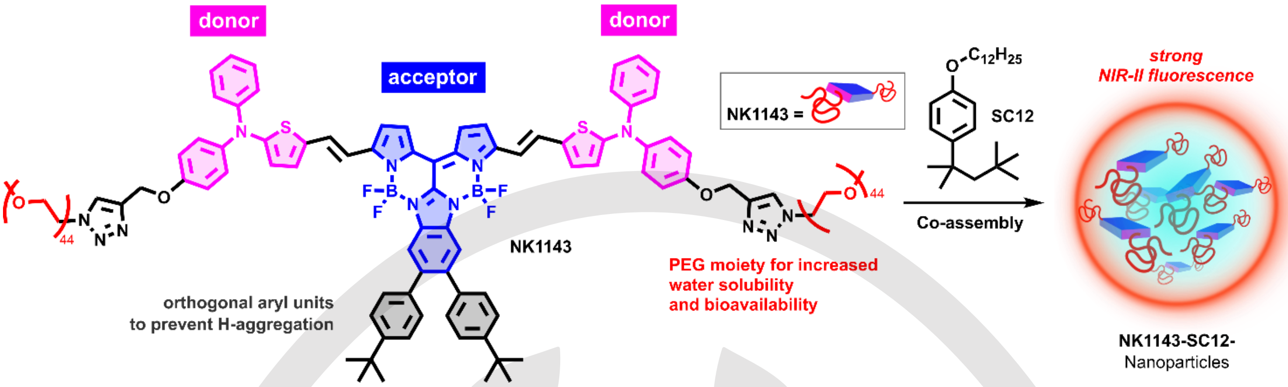


Figure 10. Cyclovoltammetric measurements of BOIMPY **23a** and Aza-BOIMPY **24a** contrasted with unsubstituted species **7a**.

Understanding the photophysical background of spectroscopic data is especially interesting and valuable in case of chromophores as it can assist and facilitate further optimization strategies. Computational studies proved to be a powerful tool to gain insights into the complexity of excitation processes and decay pathways. After our initial experimental report on BOIMPY fluorophores[4] Jacquemin and co-workers performed in-depth *ab initio* investigations of the novel scaffold.[22] Their methods combined TD-DFT and second-order Coupled Cluster approaches to elucidate the excited state behaviour. Their studies confirmed the intrinsic push-pull quality of the BOIMPY motif and could verify the absence of a charge transfer process, an exclusion which is usually mandatory for a dominant radiative decay and a strong fluorescence. The vibronic characteristics were unraveled as well and could successfully simulate the unique spectral signature of the chromophore. Specifically, the low influence of the *meso* benzimidazole auxochrome and its substitution pattern on the excitation energies was verified as a result of the pyrrolic localization of the excitation event. Beyond the *meso* imidazolium unit ($N\text{-}C^{m}{=}N$) derivatizations only show a minor effect on excitation energies as evident when comparing BOIMPYs with their aza analogs. However, if the benzimidazole



Scheme 7. BOIMPY-based D-A-D type NIR-II fluorophore **NK1143** for fluorescence imaging by Xiong et al.[27]

subunit reaches certain energy levels a charge transfer process can indeed take place and completely quench emission, e.g. by using naphthimidazole.[4] Finally, the studies suggested further structural modifications, mainly with acetylene units at the pyrrole moieties, for which excitation energies far in the NIR region were computationally predicted.

Five years after their introduction, BOIMPYs also attracted the interest of the biophysical community. The group of Xiong incorporated a BOIMPY motif into a donor-acceptor-donor (D-A-D) type triad system for fluorescence imaging (Scheme 7).[27] The BOIMPY core, employed as a strong electron-withdrawing acceptor, was decorated with 4-*tert*-butylphenyl moieties to prevent H-aggregation and further functionalized with *N,N*-diphenylthienyl-2-amine units serving as electron donors. Additionally, polyethylene glycol groups were added to the D-A-D core to enhance water-solubility and bioavailability. When the resulting chromophore cassette NK1143 was co-assembled with a suitable amphiphile and aggregation inhibitor (SC 12), an encapsulation into nanoparticles enabled a strong NIR-II fluorescence (0.16%) at 1143 nm in DMSO. In a bioimaging study an optical penetration up to 8 mm was demonstrated allowing the visualization of tumor xenografts and deep-seated abdominal metastatic tumors with excellent photostability.

Conclusions and Outlook

Like many other important dyes and fluorophores (e.g. malachite green, Cy3 and Cy5 as well as rhodamines), pyrrole-derived BODIPYs can also be traced back to the structural motif of canonical cyanines, which Walter König first investigated over 100 years ago. In contrast to polyenes, whose excitation energies approach a limit as the chain length increases, each additional double bond within the cyanine chain contributes to an approximate red-shift of 100 nm within certain boundaries. The characteristic symmetry of the cyanine π-system, along with its resonance structures, lies at the heart of its unique photophysical properties. Nevertheless, the main excitation of cyanines ($S_1 \leftarrow S_0$) can be quite correctly explained by a HOMO-LUMO transition, assigning the positions at which displaced electron density upon excitation can either be stabilized or destabilized by substituent effects. A more precise identification of these positions, however, is achieved by mapping the charge density difference (CDD). In order to close the energy gap for a red-shifted absorption, positions that lose electron density require destabilization by electron donors while positions with up-concentrated electron density demand stabilization by acceptors. The latter case is especially valid for the *meso* position of the BODIPY scaffold, allowing for the efficient lowering of the LUMO energy level by electron-withdrawing groups at this position. The rationale behind the BOIMPY design is based on this principle, according to which benzimidazole and tetrazole subunits as *meso* auxochromes evolve a synergistic effect. They not only provide two pocket regions to chelate two symmetrical BF_2 units but additionally increase the electron-withdrawing effect at the *meso* position by being simultaneously transformed into strong imidazolium or tetrazolium acceptors. While this is sufficient for a red-shift from 500 to ca. 600 nm, complexation finally ensures full planarity and rigidity in the ground and excited states, equipping BOIMPYs with their strong fluorescence.

The synthetic access to the BOIMPY motif is analogous to its BODIPY ancestor allowing engagement of aldehyde as well as carboxylic acid functionalities as electrophiles for condensation with a range of different pyrroles. Similarly, most post-functionalizations known from BODIPYs can be easily transferred to BOIMPYs, rendering them red-shifted platforms for further tailor-made derivatizations. Based on their exceptionally high electron deficiency, current research also explores a potential catalytic redox activity. We believe that a certain degree of optimism is justified in anticipating the emergence of BOIMPYs as highly effective photoredox catalysts in due course.

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Conflict of Interest

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The authors declare no conflict of interest.

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Biosketches

	**Daniel B. Werz** received his diploma (2000) and Ph.D. (2003, R. Gleiter) from the University of Heidelberg supported by a scholarship of the Studienstiftung des deutschen Volkes. After a postdoctoral stay at ETH Zürich (P. H. Seeberger) he began his independent research at the University of Göttingen in 2006 (mentor of the habilitation: L. F. Tietze). In 2013 he was appointed as Associate Professor of organic chemistry at TU Braunschweig, where he was promoted to Full Professor in 2018 before moving as a chair of organic chemistry in 2022 to the University of Freiburg. He has been JSPS Visiting Professor at the University of Kyoto/Japan, at IIT Bombay/India, at IIT Guwahati and at Tel Aviv University/Israel. His research interests include donor-acceptor cyclopropanes, catalysis, carbohydrates and organic dyes.
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