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Synthesis of Functional Fluorescent BODIPY-based Dyes through Electrophilic Aromatic Substitution: Straightforward Approach towards Customized Fluorescent Probes

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Fluorescent materials are widely used in biological and material applications as probes for imaging or sensing; however, their customization is usually complicated without the support of an organic chemistry laboratory. Here, we present a straightforward method for the customization of BODIPY cores, which are among the most commonly used fluorescent probes. The method is based on the formation of a new C–C bond through Friedel–Crafts electrophilic aromatic substitution carried out at room temperature. The method presented can be used to obtain completely customized fluorescent materials in one or two steps from commercially available compounds. Examples of the preparation of fluorescent materials for cell staining and functionalization of silica colloids are also presented.

1. Introduction

Dyes based on 4,4-difluoro-4-bora-3 a,4 a-diaza-s-indacene (BODIPY) have been studied extensively throughout the last three decades, owing to their excellent chemical and photophysical properties. They typically possess narrow absorption peaks that can be tuned in the visible spectrum, rather high extinction coefficients and fluorescence quantum yields. In addition, they are relatively stable and easily prepared and functionalized.[1] Currently, BODIPYs are widely used as fluorescent tags in biology,[1d] as sensors,[2] laser dyes,[3] and recently they were proposed as dyes for non-linear optics,[4] as photosensitizers in solar cells[5] and for photodynamic therapy.[6]

For these reasons, a plethora of different synthetic strategies have been developed to introduce modifications on the fluorescent core to tune the spectroscopic properties, to increase the dye's solubility in polar solvents, or for conjugation to silica, proteins, or nucleosides.[1a,b] Functionalization of the dye can be performed in all of the positions indicated with numbers in Figure 1, either before or after the preparation of the fluorescent core. In the majority of synthetic approaches, these modifications are introduced in before the assembly of the aromatic core.

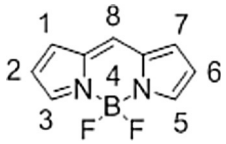

Figure 1. IUPAC numbering of the BODIPY core.

Herein, we present a new method that allows for the installation of customized molecular moieties in one step directly on the unsubstituted position 2 of a BODIPY core. To the best of our knowledge, there are only two examples of functionalization at position 2 of a fully formed BODIPY core through the direct formation of a new C–C bond; one is the introduction of a formyl group by using a Vilsmeier–Haack reaction,[7] and the second is the addition of electron-deficient alkenes through a Pd-catalyzed Heck-like reaction.[8] Alternatively, BODIPY core functionalization has been realized in a two-step procedure comprised of a halogenation reaction, followed by a Sonogashira, Heck, or Suzuki cross-coupling reaction.[9]

Our strategy for BODIPY functionalization is based on acid-catalyzed Friedel–Crafts electrophilic aromatic substitution (S_EAr) with acyl chlorides in the presence of trifluoroborate diethyletherate ($BF_3{\cdot}OEt_2$) as the Lewis acid (Scheme 1). Positions 2 and 6, owing to resonance, are the most nucleophilic and are, therefore, favored for S_EAr;[1b] on these positions, electrophilic substitutions that introduce formyl,[7] nitro,[10] and sulfonic acid groups[11] as well as halides have been reported.[9a,b] However, Lewis-acid-catalyzed acylations like Friedel–Crafts reactions have not been reported for the functionalization of BODIPYs. This is most probably because of the lability of the

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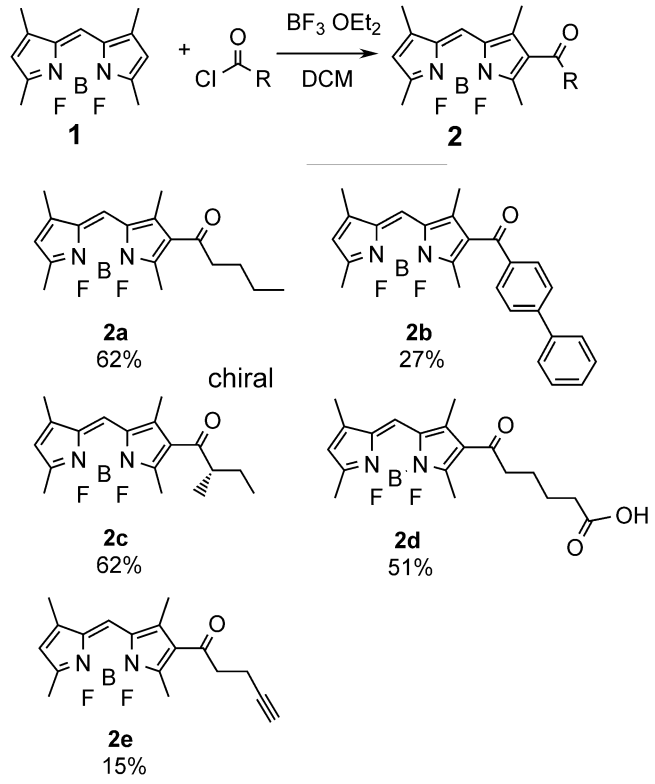


Scheme 1. Reaction scheme for the method proposed and products prepared.

BF_2 group under acidic conditions. For example, the archetypal Lewis acid used for Friedel–Crafts S_EAr ($AlCl_3$) was used to substitute the fluorine atoms for alkoxy or carboxylate groups;[3,12] whereas, Brønsted acids were used to remove the BF_2 group.[13] There are only limited examples of the Friedel–Crafts acylation of aromatic substrates from acyl chlorides using $BF_3{\cdot}OEt_2$,[14] which possess a lower relative acidity compared to $AlCl_3$.[15] It is reasonable to think that $BF_3{\cdot}OEt_2$ will not majorly affect the BODIPY core as it is commonly used to introduce the boron difluoride group during the synthesis of the dye.[16] Such an approach therefore allows for selective acylation of position 2 without the need to protect the other positions, which are disfavored by resonance.

2. Results and Discussion

2.1. Synthesis of the Materials

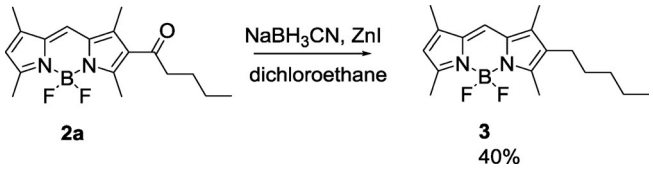
We prepared the substrate tetramethyl-BODIPY **1** from 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde following a previously reported one-step process;[17] however, this compound is commercially available, giving us the possibility to reduce the proposed method to only a single step. The Friedel–Crafts reaction was then carried out in the presence of $BF_3{\cdot}OEt_2$ as a reactant (1–3 equiv) and an acyl chloride (1.3 equiv), which can be commercially available or can be prepared following usual lab procedures.[18] After stirring for several hours (4–24 h) at room temperature, the excess $BF_3{\cdot}OEt_2$ was quenched with water. The yields of BODIPYs **2a–e** after purification (15–60%) are competitive with the traditional methodologies for functionalization of BODIPY in position 2 (overall yields of two-step methods are in the 25–60% range). With this reaction, we are able to introduce aliphatic (**2a**, **2c–2e**) and aromatic (**2b**) substituents, including functional (**2d**, **2e**) groups, which can be directly used for further conjugation through the increasingly popular copper-catalyzed azide–alkyne click reaction (**2e**),[19] or by using standard *N*-hydroxy succinimide (NHS) ester chemistry (**2d**).[20]

Aliphatic acyl chlorides gave the highest yields. Double acylation products (where also the 6-position is substituted) were not observed in the analysis; in fact, even with a four-fold excess of acyl chloride, no significant amounts of doubly substituted BODIPYs were found (see Figure S4 in the Supporting Information for analysis of the crude reaction mixture). The prevalence of mono-substitution is attributed to the relatively low acidity of BF_3 and the inductive effect of the formed ketone, which withdraws electrons from the BODIPY, deactivating position 6.

When the aromatic biphenyl acyl chloride was used as the electrophile (to form **2b**), the yield was slightly lower. This example shows, however, that although the acyl chloride used carries competing acylation sites (the biphenyl), the reaction is selective for the BODIPY core.

2.2. Spectroscopic Properties

The carbonyl group inserted with the proposed method is conjugated to the BODIPY core, as evidenced by the IR vibrations of the C=O bond at relatively short wavenumbers (around $\nu \approx 1650\ cm^{-1}$). The conjugation of the carbonyl can allow us to tune the fluorescent properties of the materials prepared. For example, the ketone resulting from the acylation reaction can be removed under mild conditions with $NaBH_3CN$ and ZnI.[21] As an illustration, we reduced **2a** to give the alkyl-substituted BODIPY analogue **3** (Scheme 2). We notice that, although the



Scheme 2. Reduction of compound **2a**.

absorption maxima of acylated products **2a–e** remain the same as starting material **1**, the Stokes shifts and the extinction coefficient increased, as described in Table 1. Moreover, in **3**, where the ketone is reduced, the Stokes shift is again reduced and the absorption maximum increases to $\lambda_{max} = 521$ nm (Figure 2).

2.3. Direct Applications of the Materials

To demonstrate direct application of the materials prepared with our method, we used compound **2d**, prepared in one step from tetramethyl-BODIPY **1** and adipoyl chloride (both

Table 1. Spectroscopic properties of the products. Measurements were carried out in dichloromethane. The absorption maximum and emission maximum excitation wavelengths were 400 nm and a slit of 5 nm was used. The Stokes shift ($\Delta\lambda$), extinction molar coefficient (ε), and quantum yield (ϕ) were calculated by using Rhodamine B in absolute ethanol as the reference ($\phi = 0.5$ at 22 °C).

Product	Absorption [nm]	Emission [nm]	$\Delta\lambda$ [nm]	ε [$M^{-1}cm^{-1}$]	ϕ
1	506	512	6	98 500	0.95
2a	505	515	10	163 500	0.80
2b	509	519	10	151 000	0.82
2c	506	516	10	121 000	0.79
2d	499[a]	508[a]	9[a]	23 500[a]	0.62[a]
2e	503	513	10	147 500	0.85
3	521	528[b]	7	82 500	0.75[b]

[a] Measurement carried out in acetonitrile. [b] Excitation wavelength 450 nm.

the substrates are commercially available), to stain Madin Darby Canine Kidney (MDCK) cells. The endosomes of compound **2d** present in the cytosol, as shown in the fluorescent confocal microscopy image (3D reconstruction of optical sections) of the dye in MDCK confluent cells (Figure 3a), demonstrate that amphiphilic **2d** was adsorbed on the cellular membrane and then internalized by endocytosis. The cell nuclei of the cells were stained with DAPI (blue in Figure 3a).

In addition, compound **2d** can be easily converted in a single step to the corresponding NHS ester **4**; this is a versatile intermediate for the connection of molecular moieties, because it quickly and quantitatively reacts with amines to give amides. Such functional dyes are of particular interest to biomedical science for protein tagging.[22,23] We coupled **4** to (3-aminopropyl)-trimethoxysilane to obtain **5** (Figure 3); this trimethylsilyl-containing dye can be used for the formation of fluorescent monolayers on glass, silica, and indium tin oxide (ITO).[24] As a proof-of-principle, we also functionalized silica colloids (7.3 μm). Such labelled colloids can be used to create colloidal assemblies in liquid-crystalline media.[25] The superimposed fluorescence and bright-field microscope images of one silica colloid are shown in Figure 3b.

3. Conclusions

We have presented a novel approach for the selective functionalization of a BODIPY core at position 2 through an electrophilic aromatic substitution carried out by using trifluoroborate diethyletherate ($BF_3{\cdot}OEt_2$) as the Lewis acid and acyl chlorides as electrophiles. The method presented is specific for position 2 and allows functionalization without the need for further protection of other free positions on the BODIPY core. The products were obtained in reasonably good yields and the reaction also allows the connection of functional groups to the fluorescent cores. Besides, we have demonstrated some direct biological applications of one dye that could be obtained in one step at room temperature from commercially available starting materials, in addition to further manipulation of the same dye to obtain a fluorescent trimethoxysilyl material for silica functionalization. This approach presents a good alternative to multistep and metal-catalyzed functionalization strategies for BODIPY dyes and can be used to create customized fluorescent probes as alternatives to commercially available fluorescent compounds. The fact that the process consists of only one step, in which the reagents are all commercially available, allows for a fast preparation of customized fluorescent molecules with tailor-made characteristics. However, there is still room for improvement, and the approach presented could, in principle, be applied to different electrophiles[26] and substrates.

Experimental Section

General Procedure

A) Electrophilic Aromatic Substitution on BODIPY Cores

Tetramethyl BODIPY **1** (1 equiv) was placed in a round-bottom flask and dissolved in dichloromethane in an argon atmosphere

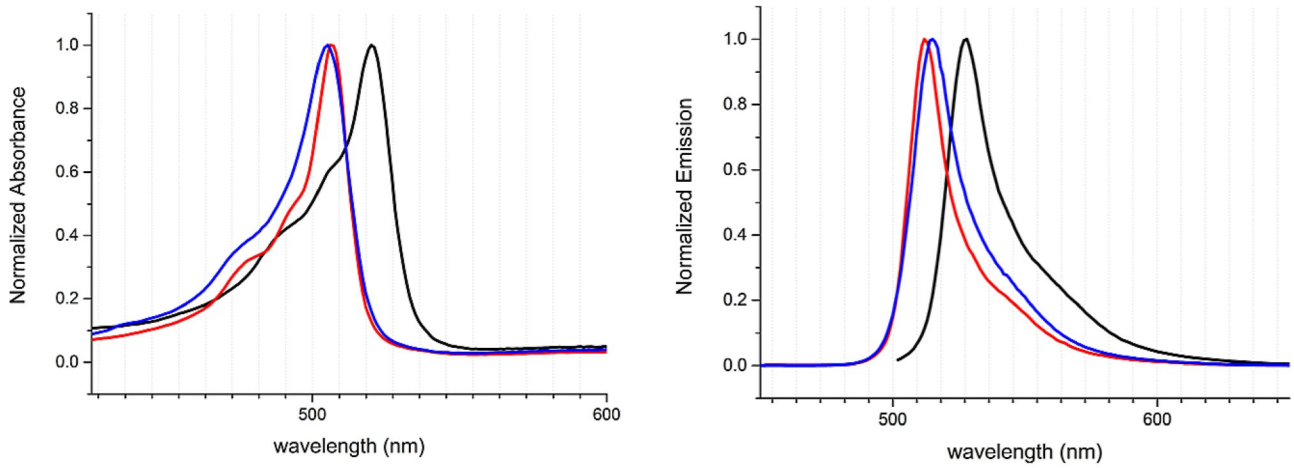


Figure 2. Normalized UV/Vis absorption (left) and fluorescence emission (right) of **1** (red), **2a** (blue), and **3** (black).

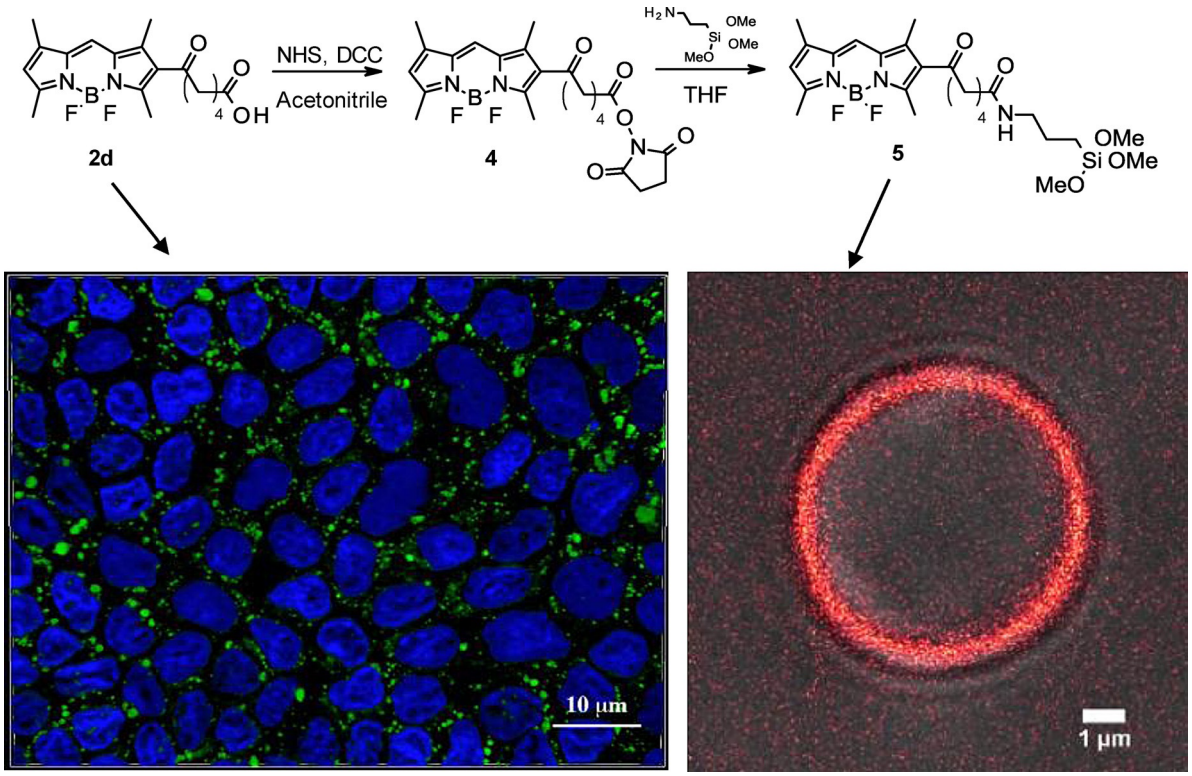


Figure 3. Reaction scheme for the preparation of a trimethoxysilane bearing BODIPY **5. a)** MDCK confluent cell culture treated with **2d** (green). The nuclei were stained with DAPI (blue) and live cells were labelled with 0.5 mM **2d** (stock solution 5 mM in DMSO and then diluted 1:10 in ADMEM medium); the cells were then fixed in 4% formaldehyde. **b)** Superimposition of fluorescence and bright-field images of a 7.3 μm silica colloid treated with **5** and dispersed in a low birefringent liquid crystal mixture CCN47-55. Fluorescence was imaged with excitation at 470 nm and emission was detected in the range of 495–574 nm (in this case the red color merely indicates high emission and does not reflect the real color of the light emitted).

(concentration of **1** ca. 0.07 M). The mixture was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (3 equiv) was added immediately. The mixture was stirred for 10 min, and then the acyl chloride (1.3 equiv) was added. The mixture was stirred for another 10 min at 0 °C and then for 4–24 h at room temperature. The reaction process was assessed by using thin-layer chromatography (TLC). The reaction was then quenched by adding water and the biphasic mixture was stirred for between 10 min and 2 h. The mixture was then separated and the aqueous layer extracted with fresh dichloromethane. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. Products **2a–e** were obtained after chromatographic purification as orange solids.

B) Acyl Chlorides from Carboxylic Acids

A commercial carboxylic acid was placed in a round-bottom flask and dissolved in dichloromethane. The mixture was cooled to 0 °C and oxalyl chloride (1–3 equiv) was added immediately.[18] The mixture was stirred for 10 min at 0 °C, and then allowed to warm to room temperature. The mixture was stirred for another 1 h and finally refluxed for 2 h. The solvent was then gently evaporated under vacuum at room temperature.

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Keywords: BODIPY · cell staining · dyes/pigments · electrophilic aromatic substitution · fluorescent colloids

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