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Convenient Method To Access New 4,4-Dialkoxy- and 4,4-Diaryloxy-diaza-*s*-indacene Dyes: Synthesis and Spectroscopic Evaluation

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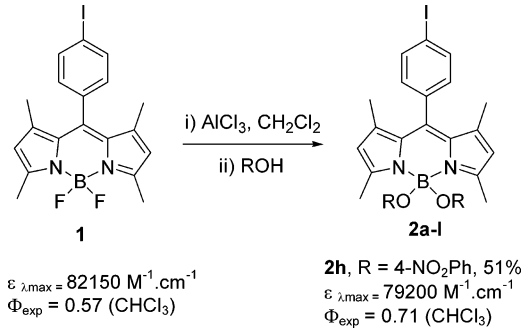
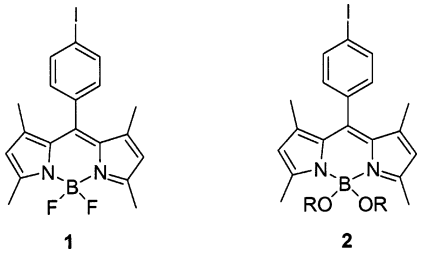


A straightforward method for the synthesis of original 4,4-dialkoxy- or 4,4-diaryloxy-diaza-*s*-indacenes (BODIPY) derivatives obtained by treatment of BODIPY **1** with various alcohols in the presence of $AlCl_3$ is described. The novel compounds are characterized by spectroscopic properties similar to those of the parent BODIPY **1**, absorption and emission spectra with similar band shapes, high molar absorption coefficients ($\epsilon_{\lambda max} \approx 80\,000\ M^{-1}\ cm^{-1}$), and for most of them high fluorescence quantum yields (Φ_{exp} from 0.52 to 0.71). Among all of the new compounds synthesized, the dye **2h** exhibits higher fluorescence quantum yield (0.71) and lifetime (4.09 ns) than compound **1** and a good chemical stability toward conditions compatible with biological cell-based assays.

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes are highly fluorescent molecules[1] that have been used for a large number of applications.[2] Recently, we synthesized various BODIPY-labeled pirenzepine derivatives to investigate their binding to GFP-labeled muscarinic M1 receptors by fluorescence resonance energy transfer (FRET).[3] BODIPY dyes are commercially available[4] in small quantities suitable for biochemical experiments, but amounts typically required for synthetic organic chemistry are prohibitively expensive. Therefore, as part of our program to develop fluorescent combinatorial libraries to identify new ligands of orphan GPCRs by FRET,[5] we were interested in the synthesis of novel and readily accessible BODIPY-like fluorescent dyes.

In this paper, we describe a rapid and flexible method for the synthesis of novel 4,4-dialkoxy- or 4,4-diaryloxy-diaza-*s*-indacenes **2** by reacting BODIPY **1** with Lewis acids. The spectroscopic properties of the resulting compounds were similar or even better than those of the parent BODIPY compound, highlighting their interest as new fluorescent dyes. Even if recent articles report on the synthesis of new BODIPY dyes,[6] to our knowledge only one structure of type **2** has been previously described in the literature.[7]



Synthesis. BODIPY dye **1** has previously been described in the literature.[2f,6e,8] Recently, Golovkova et al. have described a

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§ In fond memory of Dr. Philippe Klotz, our dear friend, colleague, and mentor who died untimely on June 9th, 2005, aged 40.
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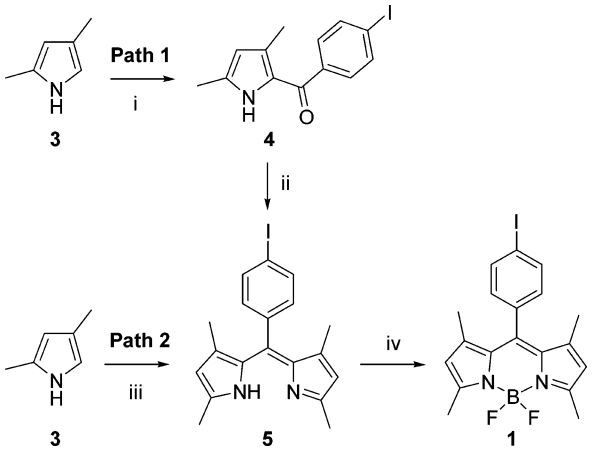
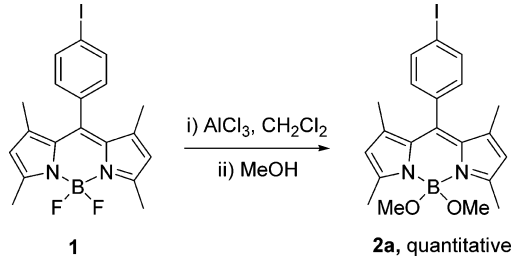
SCHEME 1[a]



[a] Key: (i) IC_6H_4COCl, CH_3MgBr, ether, 81%; (ii) **3**, $POCl_3$, CH_2Cl_2/pentane, 0 °C, 46%; (iii) IC_6H_4COOH, $POCl_3$, 24%; (iv) $BF_3{\cdot}OEt_2$, NEt_3, toluene, 80%.

SCHEME 2



two-step process to access compound **1** in a 10% overall yield.[2f] Scheme 1 depicts our strategy, which consists of the preparation of dipyrromethene intermediate **5** and its subsequent reaction with $BF_3{\cdot}OEt_2$ in the presence of Et_3N in toluene.[8b] Dipyrromethene **5** was obtained following two synthetic routes. In the first approach, a Grignard reaction of pyrrole **3** in the presence of 4-iodobenzoyl chloride afforded 2-ketopyrrole **4** in a good 81% isolated yield.[9] Ketopyrrole was subsequently reacted with pyrrole **3** and phosphorus oxychloride to provide dipyrromethene **5** in 46% yield.[10] The second route investigated involved the direct condensation of pyrrole **3** with 4-iodobenzene acid in phosphorus oxychloride used both as solvent and as reagent. Dipyrromethene **5** was thus obtained in one step but with a lower yield (24% vs 37%).[11] Noteworthy, the isolated yields were found to be highly dependent from the purity of the starting pyrroles and $POCl_3$. Prior to the reactions, these materials were thus freshly distilled.

Surprisingly, treatment of BODIPY **1** with $AlCl_3$ in dry dichloromethane and subsequent addition of dry methanol resulted in a total substitution of the two fluorine atoms to quantitatively afford molecule **2a** (Scheme 2).

Its structure was unambiguously confirmed by single-crystal X-ray analysis, which showed roughly the same angles between

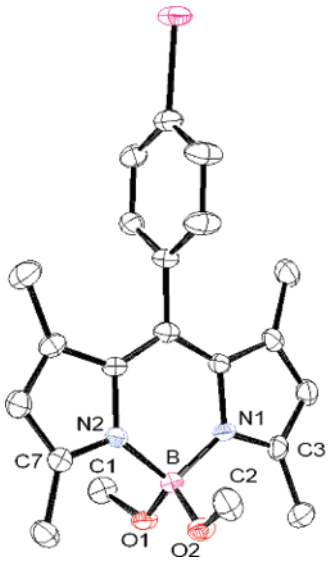


FIGURE 1. ORTEP view of compound **2a** (displacement ellipsoids at the 50% probability level).

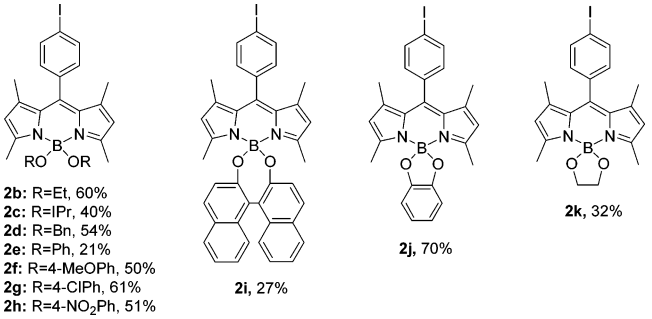


FIGURE 2. Set of BODIPY dyes **2b**−**2k** obtained by reacting compound **1** with various alcohols in the presence of $AlCl_3$ (isolated yield, %).

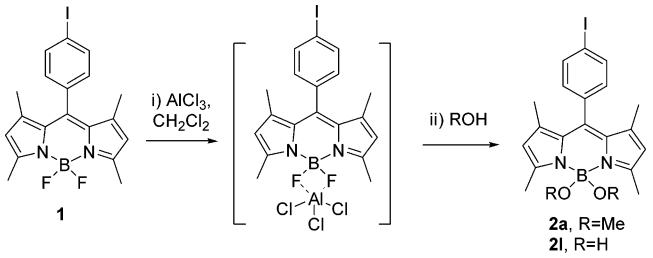


FIGURE 3. Proposed mechanisms for the formation of BODIPY dyes **2**.

F−B−N in **1** (110°2′) and O−B−N in **2a** (111°55′) (Figure 1).

This compound was found fully stable to air and moisture. In addition, its spectroscopic properties showed good quantum yield and molar absorption coefficient ($\phi_{exp} = 0.52$; $\epsilon_{\lambda max} = 79\,650\ M^{-1}\ cm^{-1}$) as compared to those of parent BODIPY **1**.

This unexpected result prompted us to investigate further the scope and limitations of the reaction by changing the nature of both the Lewis acid and the alcohols. Noteworthy, the substitution of fluorine atoms did not occur in the absence of $AlCl_3$. Replacing $AlCl_3$ by $GaCl_3$ still allowed one to access compound **2a**, whereas reactions with $Sc(OTf)_3$ or $TiCl_4$ led to the decomposition of BODIPY **1**. To establish the scope of our

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TABLE 1. Molar Absorption Coefficients ($\epsilon_{\lambda max}$) at the Absorption Maximum Wavelengths (λ_{max}), fwhm of Absorption and Emission Spectra, and Stokes Shift Determined between the Maxima of These Spectra for Compounds 1 and 2a−l in Chloroform Solution at 20 °C

compound	substituent	$\epsilon_{\lambda max}$ M^{-1} cm^{-1}	λ_{max} nm	fwhm (cm^{-1}) absorption	fwhm (cm^{-1}) emission	Stokes shift cm^{-1}
1	F	82 150	504	766	855	526
2a	OMe	79 650	505	766	904	482
2b	OEt	79 950	505	749	923	523
2c	OiPr	83 800	504	769	900	526
2d	OBn	75 550	506	803	1004	603
2e	OPh	80 100	506	745	903	439
2f	OPh−4-OMe	83 300	507	759	948	437
2g	OPh−4-Cl	85 200	506	736	887	521
2h	OPh−4-NO_2	79 200	505	718	863	441
2i	bisnaphthol	71 050	506	779	923	398
2j	catechol	78 000	510	764		
2k	ethyleneglycol	52 600	507	782	959	519
2l	OH	66 950	504	783	950	693

TABLE 2. Experimental Fluorescence Quantum Yields (ϕ_{exp}) and Lifetimes (τ) for Compounds 1 and 2a−2l[a]

compound	ϕ_{exp}	τ ns	τ_0 ns	k_f $\times 10^8$	k_{nr} $\times 10^8$	R_0 Å
1	0.57 ± 0.02	3.11 ± 0.02	5.25	1.83	1.38	46.0
2a	0.52 ± 0.02	2.85 ± 0.05	5.68	1.82	1.69	44.3
2b	0.545 ± 0.02	3.13 ± 0.04	5.45	1.74	1.45	44.9
2c	0.67 ± 0.02	3.79 ± 0.04	5.13	1.77	0.87	47.6
2d	0.65 ± 0.02	3.94 ± 0.03	5.56	1.65	0.89	45.8
2e	0.06 ± 0.015	0.38 ± 0.02	5.73	1.58	24.74	31.2
2f	0.004	nd	5.26	nd	nd	nd
2g	0.065 ± 0.015	0.37 ± 0.02	5.24	1.76	25.27	31.7
2h	0.71 ± 0.02	4.09 ± 0.02	5.67	1.74	0.71	47.2
2i	0.002	nd	5.86	nd	nd	nd
2j	0	nd	nd	nd	nd	nd
2k	0.14 ± 0.015	0.93 ± 0.02	7.90	1.51	9.24	33.0
2l	0.54 ± 0.02	3.21 ± 0.05	6.14	1.68	1.44	41.9

[a] The natural radiative fluorescence lifetimes (τ_0) were calculated from the Strickler−Berg equation. The radiative k_f and nonradiative k_{nr} rate constants were calculated from the experimental quantum yield and lifetime by $k_f = \phi_{exp}/\tau$ and $k_f + k_{nr} = \tau^{-1}$, respectively. The Förster critical distance for homotransfer (R_0) was calculated from the absorption and emission spectra. Experimental conditions are as in Table 1.

reaction, we were next interested in determining the extent to which the fluorine atoms of BODIPY could be replaced by various alkyl and aryl alcohols substituted with electron-withdrawing or -donating groups. Compounds **2a**−**k** were thus obtained in low to excellent yields (Figure 2). Nevertheless, the substitution with 1,3-propanediol did not permit one to access the corresponding cyclic compound.

To shed light on the mechanism of formation of compound **2**, some additional experiments were conducted. We hypothesized that $AlCl_3$ activates B−F bonds, thus allowing subsequent nucleophilic substitution with alcohols (Figure 3).

To reinforce this hypothesis, compound **1** was reacted with $AlCl_3$ without any alcohol. By TLC monitoring, we observed the disappearance of the starting BODIPY **1** and the formation of a more polar product, which was converted rapidly into the expected compound **2a** upon addition of methanol. Attempts to isolate the polar intermediate by column chromatography on a deactivated basic alumina failed. The only compound obtained was the dihydroxy derivative **2l** probably formed during the purification step, because of the presence of water used to deactivate basic alumina.

NMR studies were also investigated to support this mechanism. ^{11}B NMR analysis (64 MHz) of BODIPY **1** displayed a triplet pattern due to B−F coupling (δ −2.45 ppm, J = 32.8 Hz), which evolved upon $AlCl_3$ addition to a broad signal at 5.77 ppm. ^{19}F NMR analysis (188 MHz) confirmed this observation with a conversion of the quartet observed for BODIPY **1** (δ −146.75 ppm) to a broad signal at −142.42 ppm. These results combined with the above studies tend to indicate that $AlCl_3$ interacts with fluorine atoms to facilitate the substitution by alcohols.

Spectroscopic Properties. The spectroscopic properties of all new dyes are collected in Table 1. The introduction of various alcohols produced only minor changes in the absorption maxima (λ_{abs} = 504−510 nm). High molar absorption coefficients (ϵ) were measured, with values standing around 80 000 M^{-1} cm^{-1}, with only a few exceptions: a higher value was found for B(OPh−Cl) **2g** and lower values for B(bisnaphthol) **2i** and mainly for B(ethyleneglycol) **2k**. Absorption and fluorescence spectra of all compounds showed good mirror symmetry with similar band shapes for absorption and emission spectra as can be checked by measurement of their full width at half-maximum (fwhm). The Stokes shifts were ranging between 400 and 700 cm^{-1} (11−17 nm). Fluorescence parameters are summarized in Table 2.

Fluorescence quantum yields (ϕ_{exp}) as well as fluorescence lifetimes (τ) were first determined experimentally. The fluorescence decays were monoexponential for all compounds, except for the three of low quantum yield that present a small additional component (<5%; not presented in Table 2). Radiative lifetimes (τ_0) calculated from the Strickler−Berg equation are in excellent agreement with the inverse of the experimentally determined radiative rate constant, indicating that neither interaction of these molecules with the solvent nor change in

their excited-state geometries are occurring. Moreover, while k_f remains relatively constant for all compounds, large differences appear in their k_{nr} values, which thus govern the values of the fluorescence lifetime and quantum yield. Finally, Förster radii for homotransfer were all ranging from 42 and 48 Å, except for the low quantum yield probes (**2e**, **2g**, and **2k**), for which they are more than 10 Å lower.

In summary, the spectroscopic measurements show that, in addition to being original constructs, the novel BODIPYs herein described display interesting fluorescence properties. In particular, compound **2h** exhibits a higher fluorescence quantum yield ($\phi_{exp} = 0.71$) and lifetime ($\tau = 4.09$ ns) than those for BODIPY **1** (0.57 and 3.11 ns). Thus, it represents an interesting tool with potential application for biological labeling provided that it displays a good chemical stability toward conditions compatible with biological cell-based assays. Therefore, BODIPY **2h** was dissolved in a Tris-Hcl 50 mM, pH 7.4/$MgCl_2$ 5 mM/BSA 1 mg/mL/DMSO 4% mixture at 37 °C. One UV−visible spectrum per hour was then recorded over a 12 h period, confirming the excellent stability of compound **2h** in this experimental condition.

In conclusion, we have developed an efficient, rapid, and low-cost method to access new 4,4-dialkoxy- and 4,4-diaryloxy-diaza-*s*-indacene (BODIPY) dyes **2a**−**l** following treatment of known BODIPY **1** with various alcohols in the presence of $AlCl_3$. Spectroscopic analysis showed only minor changes in the absorption maxima and the molar absorption coefficients as compared to BODIPY **1** and very similar band shapes for absorption and emission spectra. Among the new structures synthesized, BODIPY **2h** exhibits higher fluorescence quantum yield and lifetime than BODIPY **1** combined with an excellent stability in water. Our method represents an efficient, straightforward, and versatile entry to a new class of fluorescent BODIPY dyes with potential applications in biochemistry and molecular biology.

Experimental Section

General Procedure for the Synthesis of Compounds 2a−l. BODIPY **1** (200 mg, 0.445 mmol) was dissolved in dry CH_2Cl_2 (10 mL) in the presence of aluminum chloride (89 mg, 0.668 mmol) under argon. The resulting mixture was refluxed for 5 min prior to addition of alcohol (5 mL). After 5 min at room temperature, the crude mixture was concentrated under reduced pressure, and compounds **2a**−**l** were isolated by column chromatography on deactivated basic alumina (CH_2Cl_2/MeOH 95/5) to afford a red solid. Data for compound **2a** (210 mg, quantitative): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.83 (d, $J = 8.3$ Hz, 2H), 7.07 (d, $J = 8.3$ Hz, 2H), 5.97 (s, 2H), 2.93 (s, 6H), 2.52 (s, 6H), 1.42 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 156.1, 141.0, 138.2, 135.3, 132.6, 130.3, 121.2, 94.4, 49.1, 14.8, 14.6; ^{11}B NMR ($CDCl_3$, 64 MHz) δ 2.11 (s); ESI-TOF (135 eV) calcd for $C_{21}H_{24}BINaN_2O_2$ 497.0872, found 497.1193. Anal. Calcd for $C_{21}H_{24}BIN_2O_2$: C, 53.20; H, 5.10; N, 5.91. Found: C, 52.80; H, 5.05; N, 5.71.

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Supporting Information Available: Crystallographic data for compound **2a**; experimental details and characterizations for compounds **1**, **2a**−**l**, **4**, and **5**; ^{1}H and ^{13}C spectra for compounds **2a**−**l**; ^{11}B NMR and ^{19}F NMR spectra for compound **1** in the absence or in the presence of $AlCl_3$; absorption and fluorescence emission spectra for compounds **1** and **2h**; and UV−visible spectra for the stability study of compound **2h**. This material is available free of charge via the Internet at http://pubs.acs.org.

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