

# Synthesis of the Core Compound of the BODIPY Dye Class: 4,4'-Difluoro-4-bora-(3a,4a)-diazas-indacene

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**Abstract** We report the synthesis and characterization of the missing reference core compound 4,4-Difluoro-4-bora-(3a,4a)-diazas-indacene **1** of the BODIPY fluorescent dye class. The compound exhibits a fluorescence lifetime of 7.2 ns and has a high photostability.

**Keywords** Bodipy · Fluorescence spectroscopy · Fluorescent probes · Single molecule studies

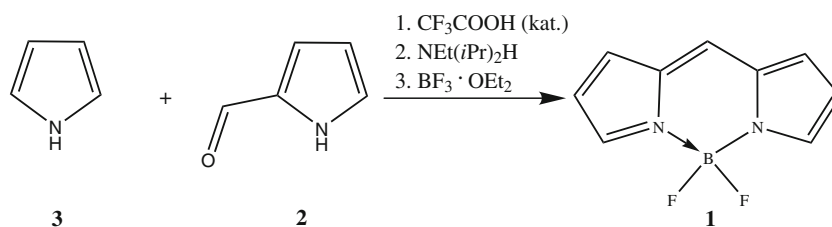
The development of emissive dyes for applications in personal diagnostics and organic fluorescent devices is steadily growing in recent years. The BODIPY (BORon-DIPYrromethene) dye class earned increasing interest in biomedical and bioanalytical applications in the last two decades. The first synthesis of a BODIPY dye was accidentally achieved in 1968 by Treibs and Kreuzer [1]. BODIPY dyes are nowadays used as active media in dye lasers [2–8] due to their high extinction coefficients, high fluorescence quantum yields, high photostability and a convenient excitation with Ar-ion Lasers (~500 nm). Owing to these fluorescence properties, various BODIPY dyes are also commercially available for protein labelling, modification of nucleotides and dextrans, as well as fluorescent substrates for enzymes and compounds with long wavelength excitation maxima [7]. Even more applications in biological systems, as fluorescent switches [9] or as system for light harvesting [10], photodynamic therapy [11] or sensor molecule [12] are reported. Several

reviews were recently published summarizing most of the actual knowledge about BODIPY dyes [13, 14]. As the name of the dye class suggests, the BODIPY chromophore is composed of two units of pyrrole which are connected by a methene bridge in the 2-position and a boron atom which is coordinated by the heteroatoms. The frame of carbon atoms of the molecule is planar and due to the heteroatoms slightly polarized, which provides nucleophilic and electrophilic reaction sites at different positions of the molecule. These reaction sites enable substitutions directly on the carbon frame of the BODIPY chromophore and were made responsible for the low stability of the core compound described here in this work [14]. Hence, the synthesis of the core compound of the BODIPY dye class, the 4,4'-Difluoro-4-bora-(3a,4a)-diazas-indacene **1**, has not been reported yet. The synthesis of BODIPY dyes can be achieved by different reaction paths [15–18], which are all based on pyrrole condensation with aldehydes. The differences in these paths originate from the desired symmetric or asymmetric substitution patterns of the obtained product. Our strategy here follows the asymmetric synthesis. By that we could avoid an extra oxidation step from the dipyrromethane to the dipyrromethene, which is required in the symmetric preparation routes [13, 14]. The one pot synthesis involves the trifluoroacetic acid-catalyzed direct condensation of 2-formylpyrrole **2** and 1H-pyrrole **3**. This is followed by deprotonation with diethyl-diisopropylamine and subsequent complexation of the intermediate dipyrromethene with borontrifluoroetherate (Scheme 1).

The appearance of many side products like polymers or porphyrins demanded repeated purification steps by fast filtration and column chromatography over silica gel and aluminum oxide. The yield of the product was low (<10%). In addition, the compound is temperature-sensitive. Elevated temperatures (>50 °C) lead to rapid loss of fluorescence

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**Scheme 1** Synthesis of 4,4-Difluoro-4-bora-(3a,4a)-diazas-indacene **1**



which we trace back to the lack of stabilizing substituents like methyl groups. However, once purified, no further condensation to porphyrins is noticed. The purified product was characterized via ESI<sup>-</sup> and ESI<sup>+</sup> (Electro Spray Ionization) mass spectroscopy. Assignment of the signals of the structure was achieved by one and two dimensional <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopy (Fig. 1a). All available data are in agreement with the proposed structure, which was verified by x-ray crystallography (Fig. 1b).

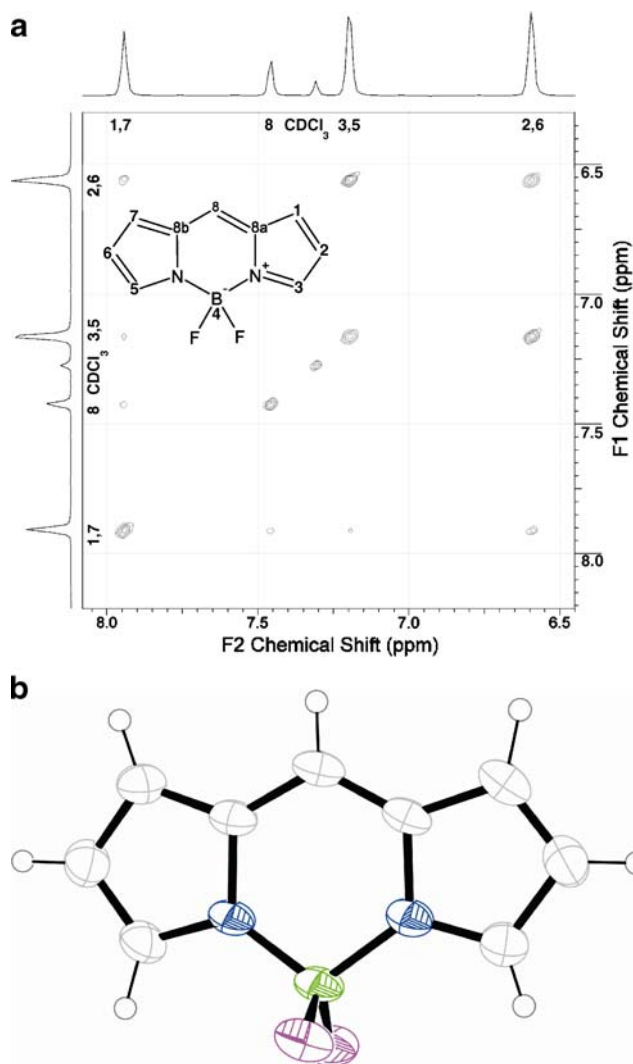
We further characterized **1** by absorption ( $\lambda_{\max}$ =503 nm) and emission ( $\lambda_{\max}$ =512 nm) spectroscopy which revealed the typical pattern for green fluorescent BODIPY dyes (Fig. 2), with a strong vibronic shoulder. The energy spacing between the 0–0 transition and the shoulder is about 740 cm<sup>-1</sup>, which can be tentatively assigned to a C-H out-of-plane vibration.

The fluorescence lifetime  $\tau_{\text{F1}}$  via TCSPC (Time-Correlated-Single-Photon-Counting) [19] and fluorescence quantum yield  $\Phi_{\text{F1}}$  [20] were determined (Table 1). It turned out that the fluorescence quantum yield of the fluorescent dye is even superior to other BODIPY dyes [13, 14] and comparable to dyes like rhodamine or fluoresceine.

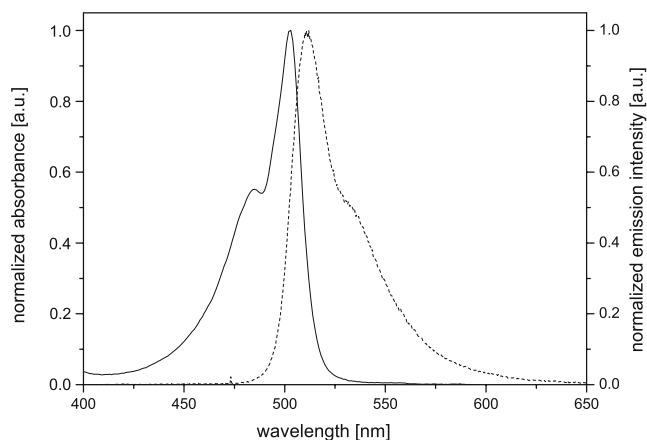
The comparison of the photophysical properties of **1** with the commercially available Borondipyrromethene PM 546 (Lambdachrome Laser Dyes) [21], allows by a rough assessment an explanation of the high fluorescence lifetime of **1**. The molar absorptivity of the latter is reported to be about 80,000 M<sup>-1</sup>/cm<sup>-1</sup> [22] and its fluorescence quantum yield is about 1 [22]. The oscillator strength *f* which we calculated for PM546 and compound **1** are 0.42 and 0.32, respectively. This reduction of *f* coincides with a prolongation of the radiative lifetime from 5.8 ns to 7.2 ns, for the here described compound **1** [23].

In addition, fluorescence correlation spectroscopy was used to determine the rate constant for intersystem crossing (ISC)  $k_{23}$ .  $k_{23}$  (Fig. 3) and  $\tau_{\text{F1}}$  are used to calculate the intersystem crossing quantum yield  $\Phi_{\text{ISC}}$  ( $\Phi_{\text{ISC}}=k_{23} \tau_{\text{F1}}$ ). The calculated  $\Phi_{\text{ISC}}=1.1\%$  for **1** is high for a dye of this structure as it is lacking heavy atoms or ketogroups. We synthesized a partially deuterated compound **1** by using perdeuterated pyrrole for comparison. No significant change of  $k_{23}$  or  $\tau_{\text{F1}}$  could be detected. This excludes overtones of C-H vibrations as accepting modes according to the energy gap law [24]. The value of the quenching rate constant  $k_{31}$ ,

is in agreement with a diffusion controlled quenching of the triplet state by molecular oxygen [25]. Moreover, from the FCS-experiments, the photostability as a key parameter for single-molecule experiments can be estimated by measuring

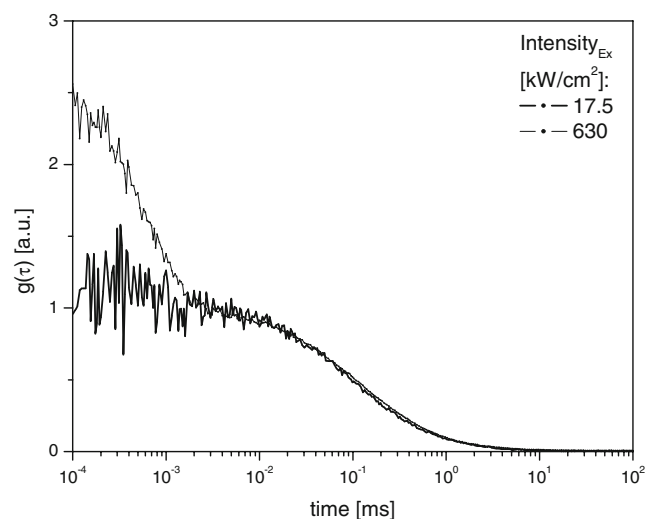


**Fig. 1** a H,H-Cosy-NMR spectrum of **1** in Deuteriochloroform. Integrations of the signals are (from low to high ppm) 2, 2, 1, 2. Strong coupling is visible at signals of vicinal atoms (e.g. 1–2, 7–6), weaker constants for the <sup>3</sup>J-couplings (e.g. 1–3, 5–7). The H-atom in *meso*- position (8) exhibits weak coupling to H(1, 7). b X-Ray crystallographic structure of **1**



**Fig. 2** Normalized absorption (solid line) and fluorescence emission (dashed line) of **1** in dichloromethane

the diffusional time  $\tau_D$  [26].  $\tau_D$  is the dwell time which a molecule needs in average to pass the observation volume. Photobleaching is detected as a shortening of this time. Most remarkably, despite the distinct population of the triplet state no change of  $\tau_D$  upon increasing excitation intensity is detected. Reversely, the fact that  $\tau_D$  is not affected by higher excitation intensities, indicates that no photobleaching occurs during the residence time of the dye in the excitation area. From comparison to fluoresceine, we conclude that the photostability of **1** is superior to fluoresceine, in agreement with our previous findings [26].



**Fig. 3** Normalized fluorescence correlation function  $g(\tau)$  of the molecule at two different excitation intensities. The solubility of **1** in water is high enough to record FCS. The increase of the correlation function at short times ( $<10^{-2}$ ms) shows that the molecule is strongly transferred into the triplet state with increasing excitation intensity. The good overlapping of both correlation functions at longer timescales ( $>10^{-2}$ ms) indicates good photostability of the molecule [26]

**Table 1** Spectroscopic data of **1**

$\lambda_{\max}(\text{abs.})$	$\lambda_{\max}(\text{em.})$	$\Phi_{\text{F1}}$	$\Phi_{\text{ISC}}$	$\epsilon$	$\tau_{\text{F1}}$	$k_{23}$	$k_{31}$
[nm]	[nm]	[%]±5%	[%]	$\text{M}^{-1}\text{cm}^{-1}$	[ns]	$[\mu\text{s}^{-1}]$	$[\mu\text{s}^{-1}]$
503 <sup>a</sup>	512 <sup>a</sup>	90 <sup>b</sup>	1.1	$5 \times 10^4$	7.2 <sup>a</sup>	1.52 <sup>c</sup>	0.58 <sup>c</sup>

<sup>a</sup> Measurement taken in Dichloromethane

<sup>b</sup> Measurement taken in Acetonitrile

<sup>c</sup> Measurement taken in Water

In conclusion, we reported the synthesis of the core compound of the BODIPY dye class, 4,4-Difluoro-4-bora-(3a,4a)-diazas-indacene. This compound is suitable for single-molecule experiments, and exhibits a high photochemical but low thermal stability. Although the synthesis itself is straightforward, the purification of the product is quite extensive. Currently, work is in progress to elucidate the subtle effects of methylation on the photophysical properties in the BODIPY dye family. As unsubstituted reference molecule of the dye class, compound **1** may prove to be helpful in fundamental research of photostability and photochemistry of fluorescent dyes, which was the motivation of this work.

## Experimental section

**General instrumentation** Mass spectra (MS) were measured with a Waters LCMS-system consisting of a HPLC-system coupled with a single quadrupole mass spectrometer for ESI. NMR spectra were recorded on an Bruker BioSpin GmbH Avance 500 instrument.

**Optical spectroscopy (UV/Vis)** Absorption spectra were measured on a Perkin-Elmer Lambda 5 two-beam spectrophotometer. Fluorescence and emission spectra were recorded on different (Fluorolog 3, Jobin-Yvon, FP-6500, Jasco and SD2000 Fibre Optic Spectrometer, Ocean Optics). The latter was fibre-coupled which allowed us to take fluorescence spectra directly from the TLC-foils.

**Fluorescence correlation spectroscopy** Experiments were performed with a home-built confocal setup based on an inverted microscope [26]. A frequency-doubled diode laser ( $\lambda_{\text{ex}}=488$  nm) was directed to the microscope body and deflected by a dichroic mirror (495DCLP). The laser was focused by a water immersion objective lens into a drop of dye solution on top of a cover slide. Fluorescence was collected by the same objective and was focussed by the tube lens to a pinhole with a diameter of 50  $\mu\text{m}$ . After additional filtering with a bandpass filter (HQ525/50), fluorescence was split by semitransparent mirror and detected by two avalanche photodiode modules. TTL-

signals, each corresponding to a detected photon, were cross-correlated by a hardware correlator finally yielding the autocorrelation function  $g^2(\tau)$ . Analysis of all curves was done by fitting with

$$g^2(\tau) = \frac{1}{N} \left( \frac{1}{1 + \left(\frac{\tau}{\tau_d}\right)} \right) \left( 1 + \frac{k_{23}^{eff}}{k_{31}} \cdot \exp\left(-\left(k_{23}^{eff} + k_{31}\right)\tau\right) \right) + Offset$$

$\tau_d$  and  $1/k_{31}$  are the diffusional time and the triplet lifetime, respectively. From the intensity dependent  $k_{23}^{eff}$ , the intersystem crossing rate constant  $k_{23}$  can be computed as described previously [25, 26]. The depicted curves were normalized to  $N=1$ .

**Fluorescence lifetime measurements**  $\tau_f$  were determined by time-correlated single photon counting under magic-angle conditions. A picosecond excitation laser (LDH-P-C-470B, Picoquant;  $\lambda_{ex}=470$  nm, pulse width  $\sim 70$  ps, repetition rate 20 MHz) was focused into a cuvette. Fluorescence was collected in  $90^\circ$  geometry and detected after spectral filtering with the same filter as in FCS by an avalanche-photodiode (DM 100ct, MPD). The instrumental response function was  $\sim 200$  ps and electronic signals were recorded on a stand-alone TCSPC-module (PicoHarp 300, Picoquant). The fluorescence lifetime was determined by monoexponential reconvolution-fit using commercial software (SymPhoTime, Picoquant).

**Synthesis of 4,4'-Difluoro-4-bora-(3a,4a)-diazas-indacene (1)** A solution of pyrrole (2 mmol) and a drop of trifluoroacetic acid in 50 mL dichloromethane is stirred at room temperature. Then 2-formylpyrrole (2.3 mmol), dissolved in 25 ml dichloromethane, is added dropwise. The reaction mixture is stirred until reaction control by thin layer chromatography shows complete consumption of the aldehyde. Seven milliliter of N-ethyl-diisopropylamine in 20 ml dichloromethane and 10 ml borontrifluoride-etherate in 20 ml dichloromethane are consecutively and slowly added. Ice cooling was applied during these additions. Purification is achieved by column chromatography with silica gel and alumoxan (solvent:  $CH_2Cl_2$ ). Several cycles of column chromatography were needed to completely purify the product from the starting materials. A stable fluorescent solution of **1** could only be achieved by completely purifying the product. The overall yield was about 8%.

Data of mass spectroscopy: ( $m/z_{theo}=192$  g/mol;  $ES^+$ :  $m/z_{exp}=210$  [ $M+NH_4^+$ ], 211 [ $M+H_3O^+$ ];  $ES^-$ :  $m/z_{exp}=192$  [ $M$ ], 191 [ $M-H$ ]), Thin Layer Chromatography:  $R_f=0.6$ .

$^1H-NMR$  (400 MHz,  $CDCl_3$ )  $\delta/ppm$  6.53 (d,  $J=4.0$  Hz, 2H, 3/5) 7.13 (d,  $J=4.0$  Hz, 2H, 2/6) 7.13 (d,  $J=4.0$  Hz, 2H, 3/5).

$^{13}C-NMR$  (125 MHz,  $CDCl_3$ )  $\delta/ppm$  118.53 (sec. C, 3/5) 131.00 (sec. C 8) 131.06 (sec. C, 2/6) 134.93 (quart. C, 8a/8b) 144.81 (sec. C, 1/7)

Crystallographic data: “CCDC 702859 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif)”.

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## References

- Treibs A, Kreuzer F-H (1968) *Justus Liebigs Ann Chem* 718:208–223
- Falk H, Hofer O, Lehner H (1974) *Monatsh Chem* 105:169–178
- VosdeWael E, Pardoen JA, Vankoevinge JA, Lugtenburg J (1977) *Recl Trav Chim Pays-Bas* 96:306–309
- Wories HJ, Koek JH, Lodder G, Lugtenburg J, Fokkens R, Driessen O, Mohn GR (1985) *Recl Trav Chim Pays-Bas* 104:288–291
- Haughland RP, Kang HC (1988) US Patent US4774339
- Monsma FJ, Barton AC, Kang HC, Brassard DL, Haughland RP, Sibley DR (1989) *J Neurochem* 52:1641–1644
- Haughland RP (2005) *The Handbook, A Guide to Fluorescent Probes and Labeling Technologies*, 10th edn, Invitrogen Corp, Eugene. <http://probes.invitrogen.com>
- Boyer JH, Haag AM, Sathyamoorthi G, Soong M-L, Thangaraj K, Pavlopoulos TG (1993) *Heteroat Chem* 4:39–49
- Coskun A, Deniz E, Akkaya EU (2005) *Org Lett* 7(23):5187–5189
- Burghart A, Thoresen LH, Chen J, Burgess K, Bergstrom F, Johansson LBA (2000) *Chem Comm* 2203–2204
- Yogo T, Urano Y, Ishitsuka Y, Maniwa F, Nagano T (2005) *J Am Chem Soc* 127:12162–12163
- Kollmannsberger M, Resch-Genger U, Daub J (2000) *J Am Chem Soc* 122:968–969
- Ulrich G, Ziessel R, Harriman A (2008) *Angew Chem Int Ed* 47:1184–1201
- Loudet A, Burgess K (2007) *Chem Rev* 107:4891–4932
- Burghart A, Kim H, Welch MB, Thorensen LH, Reibenspies J, Burgess K (1999) *J Org Chem* 64:7813–7819
- Kang HC, Haughland RP (1993) US Patent 5187288
- Ulrich G, Ziessel R (2004) *J Org Chem* 69:2070–2083
- Morgan LR, Boyer JH (1993) US Patent 5446157
- Lakowicz JR (1999) *Principles of Fluorescence Spectroscopy*, 2nd edn. Kluwer Academic/Plenum Publishers, New York
- Horiba Jobin Yvon, A Guide to Recording Fluorescence Quantum Yields, <http://www.jobinyvon.com/SiteResources/Data/MediaArchive/files/Fluorescence/applications/quantumyieldstrad.pdf>
- Lambda Physik, Lambdachrome Laser dyes, [http://bcp.phys.strath.ac.uk/ultrafast/Blue\\_book/Lamdachrome-laser-dyes.pdf](http://bcp.phys.strath.ac.uk/ultrafast/Blue_book/Lamdachrome-laser-dyes.pdf)
- Shah M, Thangaraj K, Soong M-L, Wolford LT, Boyer JH (1990) *Heteroatom Chem* 1(5):389
- Strickler SJ, Berg RA (1962) *J Chem Phys* 37:814–822
- Siebrand W, Williams DF (1967) *J Chem Phys* 49:403–404
- Widengren J, Mets Ü, Rigler R (1995) *J Phys Chem* 99:13368–13379
- Hinkeldey B, Schmitt A, Jung G (2008) *ChemPhysChem* 9:2019–2027