Dyes and Pigments 82 (2009) 392–395

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

The synthesis and crystal structure of unsubstituted 4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacene (BODIPY)

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ARTICLE INFO

Article history:
Received 15 December 2008
Received in revised form
27 February 2009
Accepted 2 March 2009
Available online 10 March 2009

Keywords:
BODIPY
Fluorescence
Absorbance
Crystal structure
Dipyrromethane

ABSTRACT

The fully unsubstituted 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) was synthesized for the first time by oxidation of dipyrromethane followed by treatment with boron trifluoride diethyl etherate in the presence of a base. The compound was fully characterized and its X-ray crystal structure is reported.

1. Introduction

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY **1**, Fig. 1) is a borondifluoride dipyrrinato complex first reported in 1968 [1]. Many BODIPY derivatives display intense fluorescence. They are relatively insensitive to changes in pH and polarity and are stable under many biological conditions. Therefore BODIPY analogues have found useful applications in labeling proteins and nucleic acids [2–5]. A significant number of BODIPY derivatives have since been synthesized and characterized [6,7].

A general synthesis of BODIPY derivatives **4** (Scheme 1) involves the treatment of dipyrromethene (or dipyrrin) **3** with boron trifluoride diethyl etherate in the presence of a tertiary amine such as triethylamine or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Because dipyrromethenes **3** can be readily prepared by oxidation of dipyrromethanes **2** using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or *p*-chloranil as an oxidant [8], a wide range of BODIPY analogues **4** can be synthesized using this method. However, it was found that fully unsubstituted dipyrromethene (**3** where $R_1 = R_2 = R_3 = R_4 = H$) was unstable in solution at temperatures above −40 °C [9] due to the susceptibility of the unsubstituted dipyrromethene to nucleophilic attack. In this regard, dipyrromethene (**3** where $R_1 = R_2 = R_3 = R_4 = H$) has been reported to react with a variety of nucleophiles [10–12], therefore the fully unsubstituted BODIPY has not been synthesized to date. So far, work involving unsubstituted BODIPY system has relied upon physical data obtained by computational methods [13,14]. The first synthesis of the fully unsubstituted BODIPY **1**, its spectroscopic data (NMR, uv/vis, and fluorescence) and its crystal structure are reported herein.

2. Results and discussion

Unsubstituted dipyrromethene (**3** where $R_1 = R_2 = R_3 = R_4 = H$) is sensitive to nucleophilic addition, it is therefore necessary for

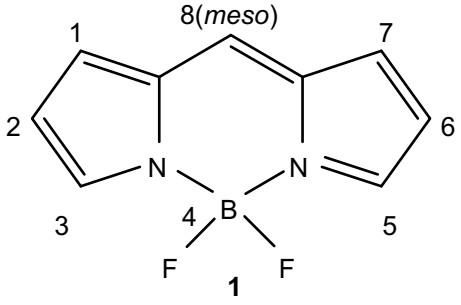

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Fig. 1. Structure of BODIPY.

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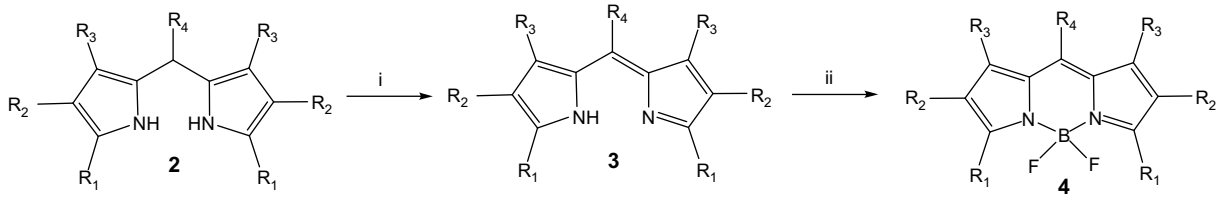
doi:10.1016/j.dyepig.2009.03.001



Scheme 1. Reagents and conditions: i, DDQ (or Chloranil); ii, $BF_3(OEt)_2$, DBU (or NEt_3).

dipyrromethene to be stabilized upon oxidation of dipyrrolemethane (**2** where $R_1 = R_2 = R_3 = R_4 = H$). Thus, the oxidation reaction was carried out at −78 °C. When the oxidation is complete, dipyrromethene was allowed to react with boron trifluoride diethyl etherate at the same temperature for 1 h prior to heating under reflux in dichloromethane. In this manner, the fully unsubstituted BODIPY **1** was isolated in 5–10% yields as a stable bright red solid. Cubic flakes were obtained after crystallization from aqueous methanol.

The fully unsubstituted BODIPY **1** was characterized by ^1H, ^{13}C, ^{11}B, and ^{19}F NMR (Fig. 2 shows its ^1H NMR spectrum) and mass spectrometry.

The uv/vis absorption and fluorescent emission spectra of a methanol solution of fully unsubstitute BODIPY **1** were measured and displayed a maximal absorbance at 497 nm with a molar extinction coefficient (ε) of 64,000 $M^{-1}\,cm^{-1}$. The maximal emission wavelength was found to be 504 nm (Fig. 3), with a Stokes shift of 7 nm.

Using a time-correlated photon counting method [15], the fluorescent lifetimes of BODIPY **1** and fluorescein (standard) were found to be 6.89 ns and 3.95 ns, respectively (in the literature fluorescent lifetime of fluorescein was found to be 4.16 ns [16]). The literature and measured fluorescent lifetimes of fluorescein were used to adjust the fluorescent quantum yield of BODIPY.

The relative fluorescent quantum yield (Φ_f) of BODIPY was determined in methanol using the literature procedure with fluorescein as the standard ($\Phi_f = 0.95 \pm 0.03$) [17]. The raw Φ_f of BODIPY **1** was found to be 1.03 (based on a $\Phi_f = 0.95$ for fluorescein). After correction using the measured and literature lifetimes for fluorescein, the Φ_f of BODIPY was determined to be 0.97 ± 0.03.

The X-ray crystal structure of BODIPY reveals that the B–N bond lengths are very similar, at 1.551(8) and 1.547(8) Å for B1–N1 and B1–N2 distances, respectively, indicating the delocalization nature of the BODIPY core. The molecule is virtually planar, however, B1 is displaced from the mean plane formed by C4, C5 and C6 by $8 \pm 1°$. A view of the molecule is shown in Fig. 4. Some selected bond lengths [Å] and angles [°] are shown in Table 1.

3. Experimental

3.1. Synthesis of fully-unsubtituted BODIPY

Dipyrromethane [18] (50 mg, 0.342 mmol) was co-evaporated with dry toluene (3 ml) and then dissolved in dry dichloromethane (10 ml). In a separate round bottom flask, DDQ (90 mg, 0.396 mmol) was co-evaporated with dry toluene (3 ml) and then dissolved in dry dichloromethane (5 ml). After the dipyrromethane solution was cooled to −78 °C (dry ice–acetone bath), the solution of DDQ in dichloromethane was added by cannulation under nitrogen. The reaction mixture was allowed to proceed for 1 h at the same temperature, and then DBU (0.31 ml, 2.07 mmol) followed by boron trifluoride diethyl etherate (0.43 ml, 3.42 mmol)

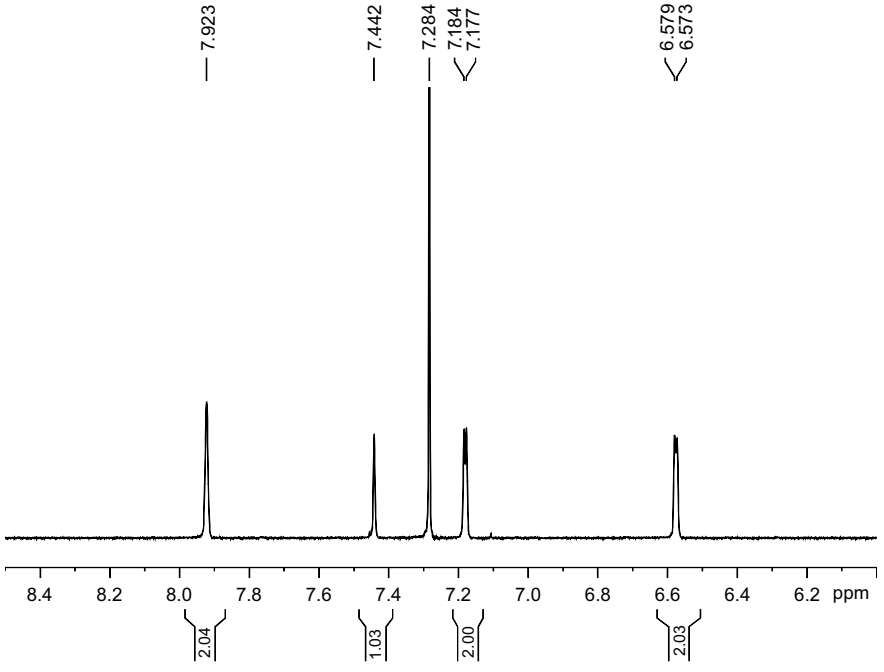


Fig. 2. ^1H NMR spectrum of BODIPY **1** in $CDCl_3$.

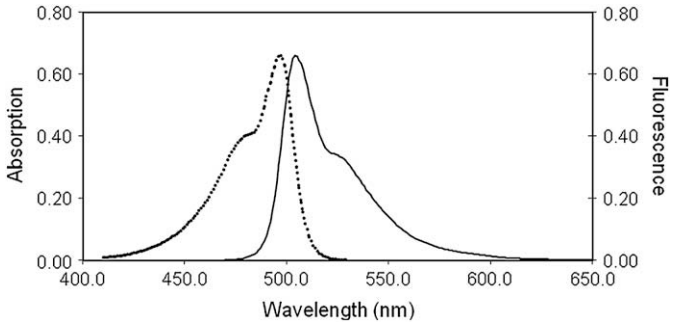


Fig. 3. The absorption and emission spectra of BODIPY (▪▪▪▪▪: excitation at 10.0 μM in methanol; —: emission at 1.84 μM in methanol).

were added. The reaction mixture was maintained at the same temperature for an additional 1 h, and then heated under reflux. After 1 h, the products were cooled to room temperature and then filtered through a bed of Celite and washed with dichloromethane (10 ml). The filtrate and washing were combined and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane, were combined and evaporated under reduced pressure to give the fully-unsubtituted BODIPY **1** as a red solid (yields range between 5 and 10%). Recrystallization of the solid from aqueous methanol gave bright red cubic flakes. M.p. 166–167 °C. (Found, in material recrystallized from aqueous methanol: C, 56.52; H, 3.66; N, 14.34. Calc. for $C_9H_7BF_2N_2$: C, 56.31; H, 3.68; N, 14.59%.) δ_H[$CDCl_3$, 600.2 MHz]: 6.57 (2 H, d, $J = 3.6$, H-2 and H-6), 7.18 (2 H, d, $J = 3.6$, H-3 and H-5), 7.44 (1 H, s, H-8), 7.92 (2 H, s, H-1 and H-7). δ_C[$CDCl_3$, 150.9 MHz]: 118.8 (C-2 and C-6), 131.3 (C-8), 131.4 (C-3 and C-5), 135.2, 145.1 (C-1 and C-7). δ_B[$CDCl_3$, 96.3 MHz]: (0.31, t, $J = 29$). δ_F[$CDCl_3$, 282.4 MHz]: 145.2 (q, $J = 29$). R_f (hexane–diethyl ether 1:1 v/v): 0.58.

3.2. Relative fluorescent quantum yield determination

Five solutions with increasing concentrations (BODIPY in methanol and fluorescein in 0.1 M NaOH) were prepared and their absorbance were measure at 497 and 492 nm, respectively. The concentrations of these solutions were kept low so that the absorbance does not exceed 0.10. The integrated fluorescence intensity of the solutions were then measured on a fluorimeter.

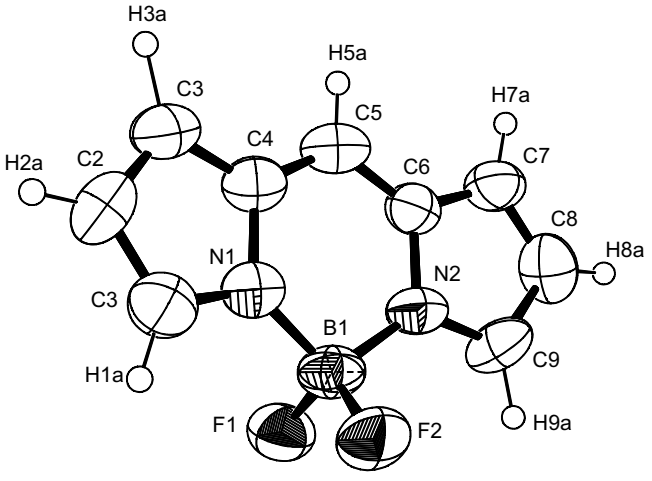


Fig. 4. The molecular structure of unsubstituted BODIPY with atom labels and 50% probability displacement ellipsoids for non-H atoms (prepared with ORTEP).

Table 1
Bond lengths [Å] and angles [°].

F(1)–B(1)	1.405(8)
F(2)–B(1)	1.378(7)
N(1)–C(1)	1.340(7)
N(1)–C(4)	1.377(7)
N(1)–B(1)	1.551(8)
N(2)–C(9)	1.335(6)
N(2)–C(6)	1.384(6)
N(2)–B(1)	1.547(8)
C(1)–C(2)	1.382(7)
C(1)–H(1A)	0.9300
C(2)–C(3)	1.367(7)
C(2)–H(2A)	0.9300
C(3)–C(4)	1.393(7)
C(3)–H(3A)	0.9300
C(4)–C(5)	1.373(7)
C(5)–C(6)	1.380(7)
C(5)–H(5A)	0.9300
C(6)–C(7)	1.381(7)
C(7)–C(8)	1.375(7)
C(7)–H(7A)	0.9300
C(8)–C(9)	1.401(7)
C(8)–H(8A)	0.9300
C(9)–H(9A)	0.9300
C(1)–N(1)–C(4)	105.8(5)
C(1)–N(1)–B(1)	128.2(6)
C(4)–N(1)–B(1)	125.8(6)
C(9)–N(2)–C(6)	107.1(5)
C(9)–N(2)–B(1)	128.7(6)
C(6)–N(2)–B(1)	124.1(5)
F(2)–B(1)–F(1)	108.6(6)
F(2)–B(1)–N(2)	110.7(6)
F(1)–B(1)–N(2)	110.7(6)
F(2)–B(1)–N(1)	110.8(6)
F(1)–B(1)–N(1)	109.5(6)
N(2)–B(1)–N(1)	106.6(6)
N(1)–C(1)–C(2)	111.8(6)
N(1)–C(1)–H(1A)	124.1
C(2)–C(1)–H(1A)	124.1
C(3)–C(2)–C(1)	105.7(6)
C(3)–C(2)–H(2A)	127.1
C(1)–C(2)–H(2A)	127.1
C(2)–C(3)–C(4)	107.8(6)
C(2)–C(3)–H(3A)	126.1
C(4)–C(3)–H(3A)	126.1
C(5)–C(4)–N(1)	118.9(6)
C(5)–C(4)–C(3)	132.1(7)
N(1)–C(4)–C(3)	108.8(6)
C(4)–C(5)–C(6)	123.3(6)
C(4)–C(5)–H(5A)	118.3
C(6)–C(5)–H(5A)	118.3
C(5)–C(6)–N(2)	120.3(6)
C(5)–C(6)–C(7)	131.3(7)
N(2)–C(6)–C(7)	108.3(6)
C(8)–C(7)–C(6)	108.5(6)
C(8)–C(7)–H(7A)	125.8
C(6)–C(7)–H(7A)	125.8
C(7)–C(8)–C(9)	105.5(6)
C(7)–C(8)–H(8A)	127.3
C(9)–C(8)–H(8A)	127.3
N(2)–C(9)–C(8)	110.7(6)
N(2)–C(9)–H(9A)	124.7
C(8)–C(9)–H(9A)	124.7

Graphs of integrated fluorescence intensity *vs* absorbance were plotted for both BODIPY and fluorescein, and the gradients of the linear plots were used for the calculation of fluorescence quantum yields.

3.3. Fluorescent lifetime determination

A single photon-timing apparatus based on a 407 nm pico-second pulsed diode laser (PicoQuant, PDL 800-B) was used to

measure the kinetics of fluorescence decay [15]. The instrument response function of the system had a width at one-half height of 68 ps. Samples were measured in 1 cm glass fluorescence cuvettes (BODIPY in methanol and fluorescein in 0.1 M NaOH) at low concentration, absorbance less than 0.10 at 492 nm. Fluorescence decay curves were fit with the sum of exponential decays using software written by Vasil'ev [15].

3.4. Crystal structure determination of BODIPY

A crystal of suitable size ($0.20 \times 0.20 \times 0.04$ mm^3) was mounted on a glass fibre. Data were collected at room temperature (23 °C) on a SMART APEX II diffractometer with Mo Kα radiation ($\lambda = 0.71073$ Å) located at the McMaster Analytical X-ray Diffraction Facility (MAX). Data were processed using APEX v2.2.0 and cell_-now; a multi-scan absorption correction was applied. The structure was solved by direct methods (SHELXS-97) and refined using least-squares techniques. All non-hydrogen atoms were refined anisotropically; hydrogen atoms were either located or treated as riding on their constituent atoms and updated after each cycle of refinement. In the final cycles of refinement, $R(F) = 10.29\%$ and $wR(F)^2 = 28.03\%$.

Crystal data. $C_9H_7BF_2N_2$, $M = 191.98$, monoclinic, $a = 10.126(4)$, $b = 10.196(4)$, $c = 17.143(6)$ Å, $\alpha = 90°$, $\beta = 102.285(6)°$, $\gamma = 90°$, volume $= 1729.5(11)$ Å^3, $T = 296(2)$ K, space group $C2/c$, $Z = 8$, 3845 reflections measured. Final R indices $R1 = 0.1029$.

Supplementary materials. CCDC 713483 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.

4. Conclusions

The fully unsubstituted BODIPY was synthesized for the first time. It possesses a fluorescent quantum yield of $97 \pm 3\%$, with a lifetime of 6.89 ns. Its crystal structure was solved.

Acknowledgments

The authors wish to thank the Natural Sciences and Engineering Research Council of Canada and the Research Corporation for funding this work.

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