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SYNTHESIS AND PHOTOPHYSICAL PROPERTIES OF A NOVEL BODIPY DYE WITH A HEPTADECYL GROUP AT THE MESO POSITION

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ABSTRACT. A novel BODIPY derivative containing a 17 carbon alkyl chain at the 8th (meso) position of the BODIPY core has been synthesized by a previously reported method for making analogous compounds by way of a Knoevenagel-type condensation (Y. Tokoro, et al., Tetrahedron Lett. 2010, 26:3451, A. Nagi et al., *J. Am. Chem. Soc.* 2008, 130:15276). The compound shows a strong absorption at 520 nm and emits at 531 nm in CH_2Cl_2. Absorption and emission spectra in different solvents show little variation. The compound is highly fluorescent with a quantum yield of 0.82.

Keywords: Borondipyrromethene, fluorescence, absorption, quantum yields

INTRODUCTION

Fluorescent compounds, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacenes (BOPIPYs) (Figure 1) have received great attention and have found widespread applications in numerous fields of modern science and medicine (Haugland 2005). They have found applications in biochemical labeling of cells, proteins, DNA and other cellular components (Haugland 2005; Martin et al. 2007). BODIPY compounds have superior photochemical and photophysical characteristics such as high chemical and photostability, high absorption coefficients, high fluorescence quantum yields, and narrow absorption and emission bands (Loudet & Burgess 2007; Ulrich et al. 2008; Ziessel et al. 2007). In addition, these molecules can be fine-tuned to achieve a variety of optical properties by functionalization of the BODIPY core at the 1,2,3,5,6,7,and 8 positions (Figure 1, Loudet & Burgess 2007; Ulrich et al. 2008; Ziessel et al. 2007).

Various BODIPY derivatives with electron withdrawing and donating groups at the meso position have been synthesized (Loudet & Burgess 2007, Bonardi et al. 2008, Krumova K. Cosa G. 2010). In general, introduction of electron donating groups to the meso position (8th position) causes blue shift of the absorption while electron withdrawing groups show the opposite effect (Loudet & Burgess 2007; Nepomyashchii 2010.

BODIPY based dyes are excellent candidates for biological applications due to their high stability, high fluorescence quantum yields, narrow absorption and emission band widths. Their optimum excitation and emission wavelengths are in the visible region; hence reduces the possibility of photodamage to live cells.

Fluorescent compounds with long alkyl chains have various applications such as viscosity probes to measure live cell viscosities (Kuimova et al. 2008), unveiling mechanisms of action and therapeutic targets (Nepomyashchii 2010), and for the development of new diagnostic assays (Quesada et al. 2008) and investigating micellar properties. A BODIPY derivative with a decyl group at the meso position has been synthesized to make polymers with supramolecular self-assembled structures (Nagi et al. 2008). A Pentyl and a decyl chain containing BODIPY have been used to make nanoparticles via H-aggregation (Tokoro et al. 2010). However, according to our knowledge

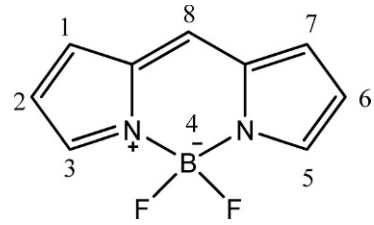

Figure 1.—The BODIPY core.

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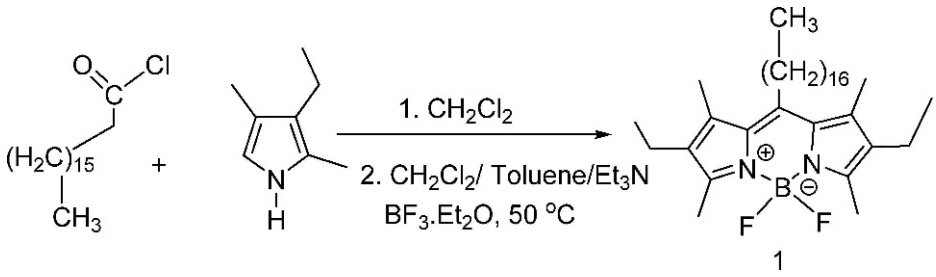

Scheme 1.—Synthetic scheme for compound **1**.

BODIPY derivatives with alkyl chains longer than 10 carbons have not yet been synthesized. Here we report the synthesis and photophysical properties of a novel BODIPY derivative (**1**) with the heptadecyl group at the meso position. According to our knowledge, **1** has the longest alkyl chain of all the meso alkyl containing BODIPY derivatives.

The goal of this research is to synthesize a fluorescent probe, bearing a BODIPY fluorophore. Fluorescent compounds that contain long alkyl chains have been known to localize in membranes (Mukherjee & Chattopadhyay, 2005). Due to the heptadecyl chain, we expect that compound **1** will be soluble in cell membranes.

GENERAL METHODS

All inert manipulations were performed under an argon atmosphere using Schlenk techniques. Anhydrous solvents (diethyl ether, THF, hexane, dichloromethane, methanol and DMF) and other chemicals were purchased from Aldrich and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz JEOL nuclear magnetic resonance spectrophotometer. Chemical shifts are reported in parts per million (ppm), in $CDCl_3$, using TMS as the internal reference (0.00). ^{1}H data are reported as follows: multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad). UV–visible spectra were recorded using a Varian Cary Bio 300 UV-Vis spectrophotometer. Fluorescence spectra were recorded using a PTI QuantaMaster fluorimeter. Quantum yield was measured using Rhodamine B as the reference in MeOH. High-resolution mass spectral analyses were performed by the Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, IL. Melting point was recorded using a Thermo Scientific melting point apparatus.

SYNTHESIS

4,4-DIFLUORO-8-HEPTADECYL-2,6-DIETHYL-1,3,5,7-TETRAMETHYL-4-BORA-3A,4-ADIAZA-S-INDACENE (1)

Compound **1** was synthesized according to a literature procedure which has been used to make similar compounds (Tokoro et al. 2010, Nagi et al. 2008) and was purified by silica gel column chromatography eluted with CH_2Cl_2/hexane (20:80) to yield a dark orange solid in 62% yield, mp 40–43°C. ^{1}H NMR (300 MHz, $CDCl_3$,δ): 2.96 (t, $J_{H\text{-}H}$ = 9 Hz, 2H), 2.48 (s, 6H), 2.39 (q, $J_{H\text{-}H}$ = 7.6 Hz, 4H), 2.32 (s, 6H), 1.27 (broad s, 31H), 1.04 (t, $J_{H\text{-}H}$ = 7.6 Hz, 6H), 0.87 (t, $J_{H\text{-}H}$ = 6.6 Hz). ^{13}C NMR (300 MHz, $CDCl_3$,δ): 151.1, 145.1, 135.7, 132.5, 131.1, 32.0 (d), 31.9, 30.0–29.0 (m), 28.7, 22.8, 17.3, 14.9, 14.2, 13.4, 12.5. HRMS (ESI): m/z calcd. for $C_{32}H_{58}BN_2F_2$ $[M+H]^+$: 543.4664; found: 543.4661.

RESULTS

A novel BODIPY dye with a 17 carbon chain at the meso position was successfully synthesized from the corresponding acid chloride and the 3-ethyl-2,4 dimethylpyrrole according to a literature procedure (Nagi et al. 2008, scheme **1**)

The compound was characterized by ^{1}H, ^{13}C and high resolution mass spectrometry. 2,6-diethyl-1,3,5,7-tetramethyl substituted BODIPY derivatives with methyl, propyl, pentyl and decyl substituents at the meso position have been reported (Nagi et al. 2008; Nepomyashchii 2010).

^{1}H NMR (Figure 2a) of the compound **1** showed typical peaks corresponding to the BODIPY core. The peak at 2.96 ppm is due to two hydrogens of the carbon (of heptadecyl chain) adjacent to the 8^{th} position of the BODIPY core. Other hydrogens of the heptadecyl chain appeared as a broad peak at 1.27 ppm. The ^{1}H

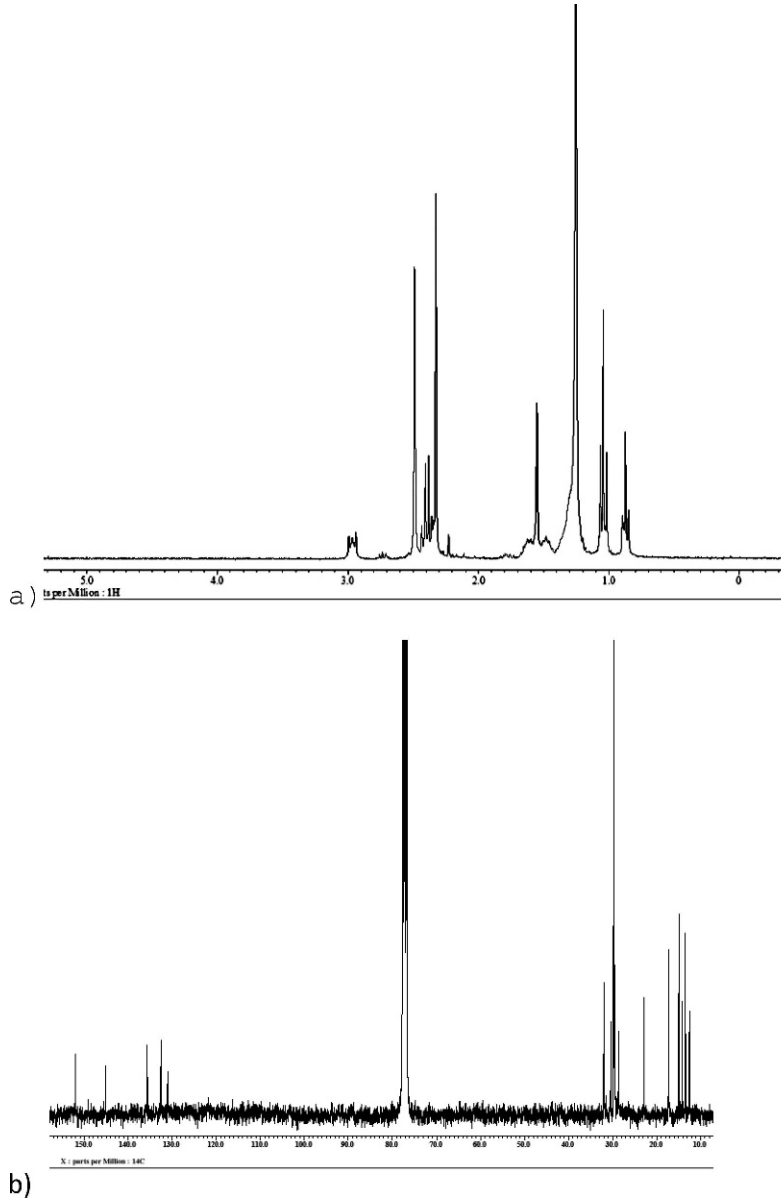

Figure 2.—a) ^{1}H NMR and b) ^{13}C NMR of compound **1** in $CDCl_3$.

resonances at 2.48, 1.70, 2.32 ppm are assigned to the methyl substituents at 1, 7 and 2, 5 positions. ^{13}C NMR (Figure 2b).showed five separate peaks for five different carbons of the BODIPY core (152–130 ppm). The carbons of the alkyl chain adjacent to the ring appeared at 32.0 ppm. All the other secondary carbons of the heptadecyl chain appeared together between 30–29 ppm. Carbons of the methyl and ethyl groups appeared between 17–14.2 ppm. The terminal methyl group of the alkyl chain was located at 12.4 ppm.

STEADY STATE ABSORPTION AND EMISSION SPECTRA

UV/Vis absorption and emission spectra were recorded at room temperature in pentane,

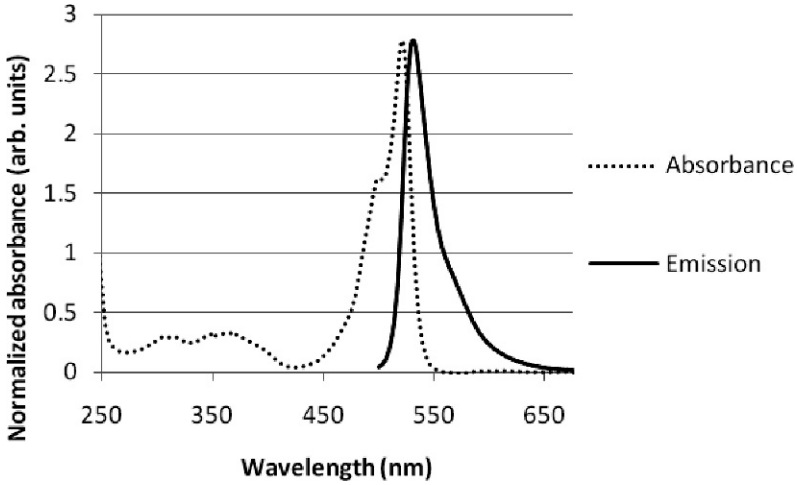

Figure 3.—Normalized absorption and emission spectra of 1 in CH_2Cl_2.

CH_2Cl_2, and DMF. The absorption spectrum of **1** shows a strong absorption at 520 nm in pentane and 521 nm in CH_2Cl_2 (Figure 3). It was slightly blue shifted (517 nm) in DMF. The absorption band near 520 nm is characteristic to BODIPY derivatives and has been assigned to the S_0-S_1 (π-π^*) transition (Kollmannsberger et al. 1998). The broad absorption at 371 nm is due to the S_0-S_2 (π-π^*) transition (Karolin et al. 1994). The molar absorption coefficient of the compound at 521 nm was found to be 82,800 cm^{-1} mol^{-1}. It is known that BODIPY derivatives have high molar absorption coefficiencies (Ziessel et al. 2007). That of compound **1** falls between the meso-decyl substituted (115,000 cm^{-1} mol^{-1}, Nagai et al. 2008), meso-methyl substituted (75,000 cm^{-1} mol^{-1}, Nepomyashchii et. al. 2010) derivatives.

Emission spectra were recorded at room temperature providing emission maxima in CH_2Cl_2 (Figure 2) pentane and DMF of 531, 529 and 535 nm respectively. Quantum yield of **1** in CH_2Cl_2 was measured using Rhodamine B as the reference (Φ= 0.65 in ethanol). The reference and **1** were irradiated at 500 nm. The quantum yield of **1** was found to be 0.82 while literature reported values for the compounds with a methyl or decyl substituent at the meso position are 0.99 and 0.92 respectively (Nepomyashchii 2010, Nagai, 2008).

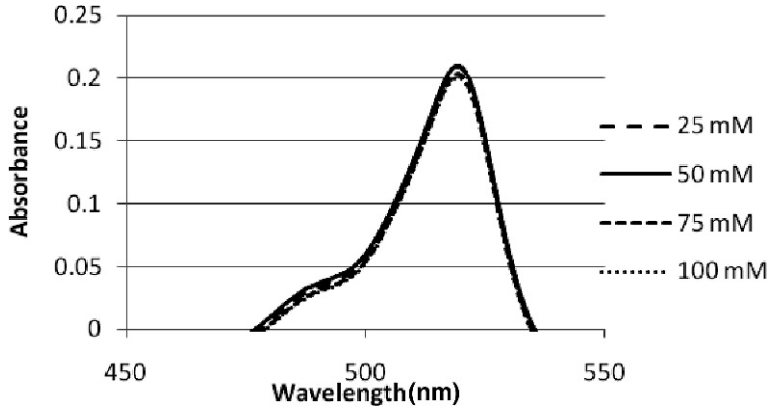

Figure 4.—Absorbance of **1** in DMF with increasing TBAF concentrations.

SENSITIVITY TO FLUORIDE ION

It is known that some BODIPY derivatives are sensitive to anions such as fluorides (Huh et al. 1998; Meng et al. 2009; Bozdemir 2010) The presence of fluorine ions causes a decrease of the absorption by BODIPY due to decomposition (Meng et al. 2009). It has been suggested that the decomposition is due to nucleophilic displacement which breaks a B-N bond and forms a new B-F bond (Meng et al. 2009).

In order to test the stability of **1** in the presence of fluoride ions, absorption of **1** at 521 nm in DMF was monitored in the presence of varying millimolar concentrations of tetrabutylammonium fluoride (TBAF). No significant change in absorption was observed (Figure 4). This suggests that the B-N bond of compound **1** is stable in the presence of anions such as fluoride.

In summary, we successfully synthesized and characterized a novel BODIPY dye **1** which has the longest alkyl chain at the meso position reported to date. According to our preliminary studies, **1** is readily taken up and accumulated in live human osteosarcoma (Sacs-2) cells and mouse fibroblasts (NIH3T3) cells. Details of ongoing cellular uptake studies with compound **1** and several other BODIPY derivatives will be published elsewhere.

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