JOC The Journal of Organic Chemistry

Note

pubs.acs.org/joc

Synthesis of 3,5-Bis(acrylaldehyde) Boron-dipyrromethene and Application in Detection of Cysteine and Homocysteine in Living Cells

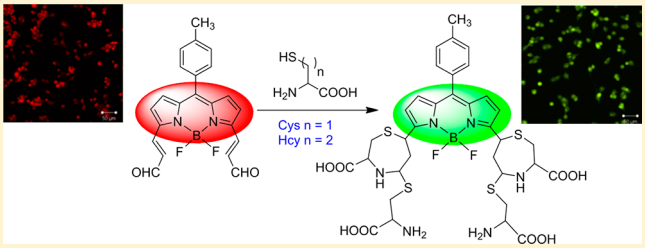
Sheri Madhu,[†] Rajesh Gonnade,[‡] and Mangalampalli Ravikanth*,[†]

[†]Department of Chemistry, Indian Institute of Technology Bombay, Mumbai 400076, India

[‡]Center for Materials Characterization, National Chemical Laboratory, Pune 411008, India

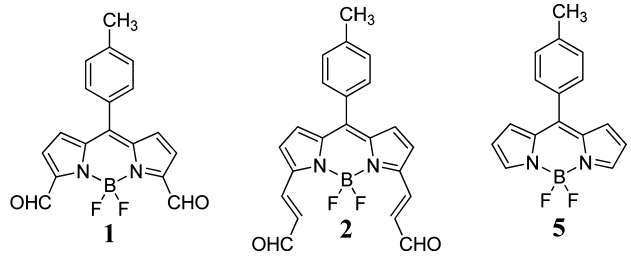
Supporting Information

ABSTRACT: Synthesis, characterization, and spectral and electrochemical properties of 3,5-bis(acrylaldehyde) BODIPY are described. The compound exhibited higher selectivity toward cysteine/homocysteine than toward other amino acids and thiol-containing compounds as shown by absorption and emission titration experiments and by experiments in living cells.



Boron-dipyrromethenes (BODIPYs) are excellent fluorescent dyes and have been used in various research fields as labeling reagents, fluorescent switches, chemosensors, light-harvesting systems, and dye-sensitized solar cells because of their advantageous photophysical properties, such as photostability, high absorption coefficients, and high fluorescence quantum yields.[1] BODIPY dyes are amenable to modifications, which allow fine-tuning of properties by introduction of suitable substituents at appropriate positions of the dipyrromethene framework.[2] Because BODIPY dyes can be easily functionalized, it is convenient to introduce the desired substituents at the BODIPY core by carrying out suitable reactions on functionalized BODIPYs.[3] For example, we and others have shown that BODIPYs can be halogenated at different positions and that the halogenated BODIPYs can be used as synthons to synthesize different types of substituted BODIPYs.[4] We recently reported the synthesis of 3,5-diformyl BODIPYs under simple reaction conditions and demonstrated their use as pH-based optical sensors and for cyanide sensing applications.[5] Because fluorophores appended with aldehyde group(s) have been shown to be useful for the detection of thiols such as cysteine[6] (Cys) and homocysteine[7] (Hcy) based on the cyclization reaction between aldehyde and thiols to form thiazolidines and thiazinanes,[8] we thought of using 3,5-diformyl BODIPY **1** (Chart 1) for sensing Cys/Hcy by this chemodosimetric approach. Interestingly, there are very few reports on fluorescent off−on molecular probes based on BODIPY for the specific detection of thiols.[9] However, we realized that 3,5-diformyl BODIPY **1** cannot be used for sensing thiols because the formyl groups are directly on the BODIPY core, and their reaction with thiols to form cyclized thiozolidine product is not stable enough to follow using any spectroscopic techniques. We anticipated that if we use acrylaldehyde in place of formyl groups, the reaction of acrylaldehyde with Cys/Hcy would form

Chart 1. Structure of BODIPY Compounds 1, 2, and 5

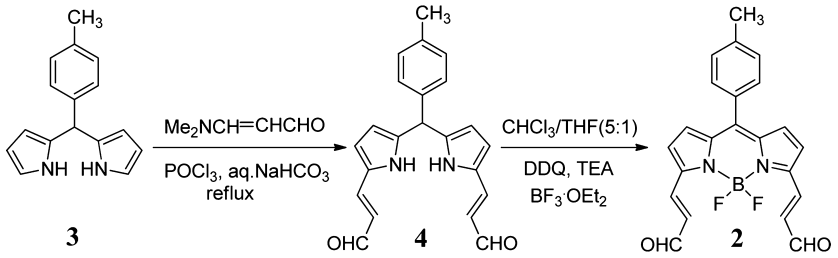


stable hexahydro-1,4-thiazepine heterocycles and thus can be used for sensing specific detection of thiols.[10] In this paper, we report synthesis, characterization, and spectral and electrochemical properties of 3,5-bis(acrylaldehyde) BODIPY **2** (Chart 1) and its use as a chemodosimetric probe for specific detection of Cys and Hcy over other amino acids including glutathione (GSH) under physiological conditions. Furthermore, confocal laser scanning fluorescence microscopy experiments demonstrated that BODIPY **2** can permeate cell membranes and could be used for ratiometric fluorescence imaging in living cells. Although several probes for the detection of Cys/Hcy are available in the literature,[6] the probe reported here absorbs and emits in a higher wavelength region compared to that of the earlier probes and shows ratiometric fluorescence response and good selectivity for Cys/Hcy over other amino acids.

The desired target BODIPY **2** was prepared in two steps starting from *meso*-(tolyl) dipyrromethane **3** as shown in Scheme 1. The dipyrromethane **3** in 1,2-dichloroethane was added dropwise to the reagent generated in situ by treating 3-

Received: March 5, 2013
Published: April 18, 2013

Scheme 1. Synthesis of BODIPY 2



dimethylaminoacrolein with $POCl_3$ at −10 °C. The reaction mixture was slowly brought to room temperature and was subsequently refluxed at 60 °C for 30 min. The reaction was quenched by aqueous $NaHCO_3$, and the resultant crude compound was subjected to silica gel column chromatography that afforded pure compound **4** as a brown solid in 87% yield. Compound **4** was confirmed by HRMS and elemental analysis and was characterized in detail by NMR spectroscopy (Figures S1−S3 in the Supporting Information). Compound **4** was treated first with DDQ in $CHCl_3$/THF (2:1) for 30 min at room temperature, neutralized with triethylamine, and reacted with $BF_3{\cdot}OEt_2$ for an additional 30 min. The crude compound was purified by silica gel column chromatography and afforded pure BODIPY **2** as a golden-green solid in 32% yield (Figures S4−S8 in the Supporting Information). In ^{1}H NMR of BODIPY **2**, the aldehyde proton appeared as a doublet at 9.85 ppm; the alkene protons appeared as a doublet and a quartet at 8.12 and 6.83 ppm, respectively (Figure S5 in the Supporting Information). In ^{19}F NMR, a quartet appeared at high field (−137 ppm) compared to normal BODIPY chromophores, such as *meso*-(tolyl) BODIPY **5** (−146 ppm), because of hydrogen bonding with adjacent alkene proton. An expected triplet was observed in BODIPY **2** at 1.1 ppm in ^{11}B NMR which was downfield shifted compared to BODIPY **5** (0.5 ppm) because of the presence of strong electron-withdrawing formyl groups. The crystal structure solved for **2** has a planar BODIPY framework comprised of two pyrrole rings and the central six-membered boron ring (Figure 1). The plane defined by F−B−F atoms was perpendicular to the BODIPY core. The dihedral angle between the *meso*-aryl ring and the BODIPY core was 51°. The acrylaldehyde functionalities at 3,5-positions and indacene plane were nearly planar, indicating significant electronic conjugation between acrylaldehyde and BODIPY core.

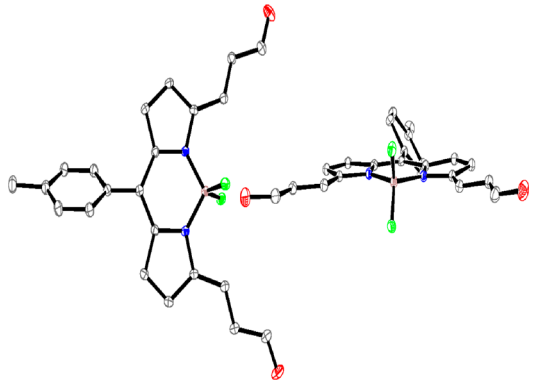


Figure 1. ORTEP diagram (left) top view, (right) side view (50% probability) of BODIPY **2**. Hydrogen atoms and solvent molecules are omitted for clarity.

The absorption spectrum of BODIPY **2** showed a sharp $S_0 \rightarrow S_1$ band at 602 nm that was bathochromically shifted by ∼55 nm (Figure S9 in the Supporting Information) compared to BODIPY **1** because of an increase in the π-electron delocalization. BODIPY **2** exhibited a bathochromically shifted emission band at 615 nm (Figure S9 in the Supporting Information) and increased quantum yield (Φ = 0.4) and singlet state lifetime (τ = 6.5 ns) compared to those of BODIPY **1** (λ_{em} = 556 nm, Φ = 0.3, τ = 5.5 ns). Thus, BODIPY **1** is green fluorescent, and BODIPY **2** is red fluorescent under a UV lamp. The absorption and emission bands of BODIPY **2** were shifted hypsochromically with a decrease in quantum yield and singlet state lifetime as the solvent polarity increases, which is in agreement with the general behavior of BODIPY chromophores (Table S1 in the Supporting Information). BODIPY **2** exhibited two reversible reductions but no oxidation like BODIPY **1** (Figure S12 in the Supporting Information). However, compared to that of BODIPY **1**, the first reduction potential was shifted negatively by 100 mV, indicating that BODIPY **2** was relatively less electron deficient (Figure S13 in the Supporting Information).

As mentioned above, aldehydes are known to react with thiols such as Cys/Hcy to form thiazolidine or thiazinane derivatives, and the aldehydes present directly on the fluorophores on reaction with thiols are expected to produce significant changes in the electronic properties of the fluorophore that are clearly reflected in their absorption and emission properties. Thus, we investigated the recognition of thiols by BODIPY **2** and monitored the changes in the absorption and emission spectra of BODIPY **2** upon formation of hexahydro-1,4-thiazepine derivatives. The systematic changes in the absorption spectra of BODIPY **2** on titration with Cys in pH 7.4 PBS/CH_3CN (9:1, v/v) media are shown in Figure 2a, and the same study on the fluorescence spectra is shown in Figure 2b. Upon addition of increasing amounts of Cys to a solution of BODIPY **2** in buffer media, the absorption band at 596 nm was decreased gradually, and at the same time a new band at 552 nm increased with a clear isosbestic point at 568 nm, supporting the formation of a hexahydro-1,4-thiazepine derivative. This is also clearly evident in the fluorescence studies. Upon addition of Cys, the fluorescence band at 612 nm decreased slowly, and a new band appeared at 567 nm with an isoemissive point at 598 nm. Similar observations were also made on systematic addition of Hcy to BODIPY **2** (Figure S14 in the Supporting Information). These changes were also associated with a visually detectable change in solution color from red to fluorescent green under a UV lamp, indicating that BODIPY **2** can be used as a possible colorimetric sensor for the detection of Cys and Hcy (Figure S19 in the Supporting Information). To investigate the selectivity, the studies were

dx.doi.org/10.1021/jo4004597 | J. Org. Chem. 2013, 78, 5056−5060

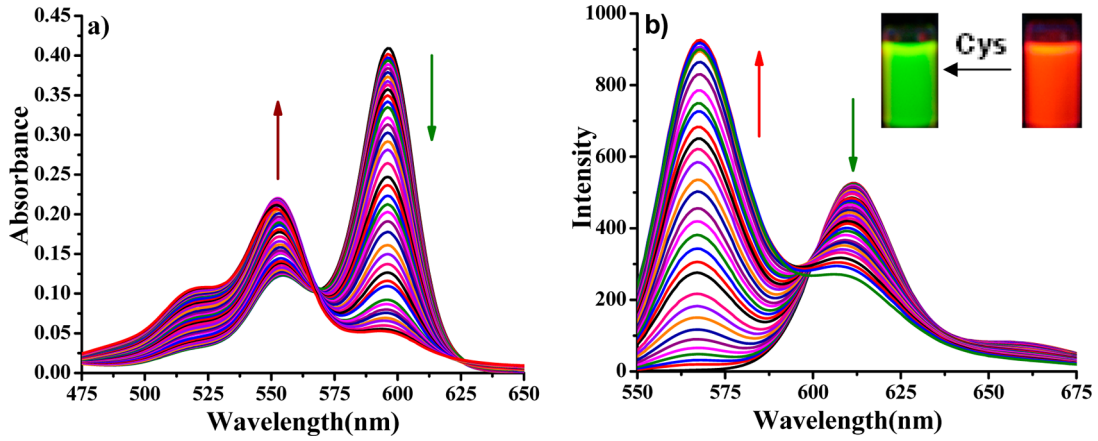


Figure 2. (a) Absorption and (b) emission spectral changes of BODIPY **2** (5 μM) upon titration with Cys (0–35 equiv) in pH 7.4 PBS/CH_3CN (9:1, v/v). The spectra were recorded after incubation of the probe with Cys for 30 min (λ_{ex} = 510 nm). (Inset: Fluorescence color change of BODIPY **2** upon titration with Cys).

also carried with other amino acids such as Arg, GSH, Lys, Phe, Thr, Trp, His, Asp, Gln, Glu, Leu, Ser, Val, Pro, and Tyr and the proteins bovine serum albumin (BSA) and HepG2 cell total protein (Figure 3), and we did not observe significant changes

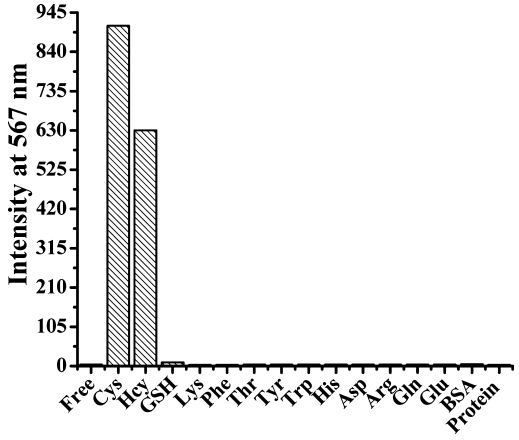


Figure 3. Histogram showing the fluorescence enhancement (567 nm band) during the titration of BODIPY **2** (5 μM) with different amino acids (excess of equivalents) in PBS/CH_3CN (9:1, v/v; pH 7.4) solution.

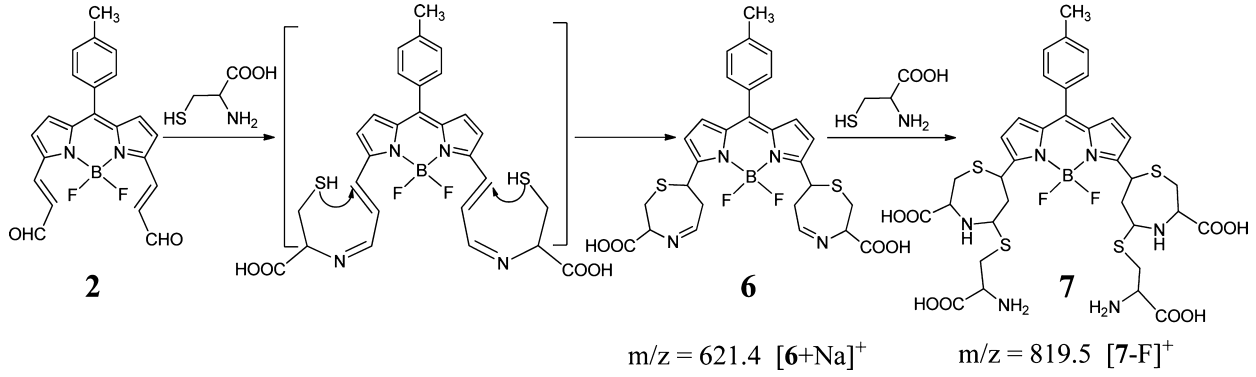
in absorption and fluorescence spectra, suggesting that BODIPY **2** is a specific sensor for detection of Cys/Hcy over other biospecies, including GSH (Figures S15–S18 in the Supporting Information).

A possible sensing mechanism of BODIPY **2** based on the reaction between α,β-unsaturated aldehydes and Cys to form a stable hexahydro-1,4-thiazepine ring is shown in Scheme 2. Initially, the amino group of Cys reacts with the aldehyde group to afford imine intermediate, which could then undergo ring closure to form perhydrothiazepine derivative **6**.

Finally, in the presence of excess equivalents of Cys, the hexahydro-1,4-thiazepine **7** is expected to form. To gain insight into the above proposed mechanism, we monitored the progress of the reaction of BODIPY **2** with Cys by mass spectrometry. After treatment of BODIPY **2** with Cys for 30 min, an intense peak at m/z 621.4 corresponding to $[\mathbf{6} + Na]^+$ was present in an ESI-MS spectrum (Figure S20 in the Supporting Information). However, after addition of excess equivalents of Cys to the solution, an intense peak was observed at m/z 819.5 (Figure S21 in the Supporting Information), corresponding to the formation of $[7 - F]^+$. Thus, the mass spectral study supports the proposed sensing mechanism.

To examine the function of the probe for its application in biological imaging, the living HepG2 cells were incubated with BODIPY **2** (10 μM) at 37 °C for 30 min. Upon excitation at 543 nm, HepG2 cells exhibited bright intracellular red fluorescence (Figure 4I). However, the cells that were pretreated with Cys followed by incubation with BODIPY **2**

Scheme 2. Proposed Mechanism for the Reaction of BODIPY 2 with Cys



dx.doi.org/10.1021/jo4004597 | J. Org. Chem. 2013, 78, 5056–5060

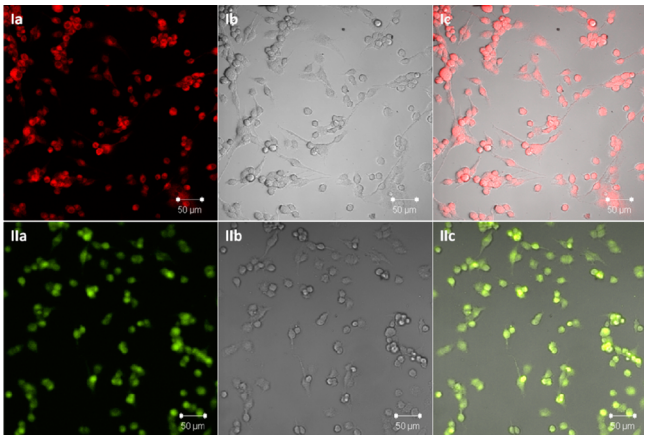


Figure 4. Confocal laser scanning fluorescence images of living HepG2 cells before (I) and after (II) incubation with BODIPY **2** (10 μM) with Cys. The left panel represents the fluorescence, the center panel the bright-field transmission images, and the right panel respective bright-field and overlay images; incubation was performed at 37 °C for 30 min. Scale 50 μm.

for 30 min exhibited bright yellow-green fluorescence (Figure 4II). Further bright-field measurements confirmed that the cells treated with BODIPY **2** were viable throughout the imaging experiments (Figure 4Ia,IIb). The overlay of fluorescence and bright-field images revealed that the fluorescence signals are localized in the full area of the cell, indicating a cellular distribution of Cys and good permeation of the cell membrane by BODIPY **2** (Figure 4Ic,IIc). These results demonstrate the practical applicability of BODIPY **2** for imaging Cys in living cells.

In conclusion, we have synthesized 3,5-bis(acrylaldehyde) BODIPY **2** in 32% yield from *meso*-aryl dipyrromethane under simple reaction conditions. The compound absorbs and emits at higher wavelengths compared to those of our earlier reported 3,5-diformyl BODIPY **1**. However, 3,5-diformyl BODIPY **1** cannot be used for sensing thiols such as Cys/Hcy because of the instability of the resulting thiazolidinine derivatives. 3,5-Bis(acrylaldehyde) BODIPY can be used for selective sensing of Cys/Hcy over other amino acids and thiols at physiological pH. The probe displays a 45 nm blue-shift in emission with associated visually detectable fluorescent color change from red to fluorescent green upon addition of Cys/Hcy. Furthermore, confocal laser fluorescence scanning microscopic experiments demonstrated that the probe can permeate cell membranes and can readily be used to detect the intracellular Cys/Hcy in living HepG2 cells.

EXPERIMENTAL SECTION

General. The NMR experiments were performed with a 400 MHz spectrometer, and chemical shifts are expressed in parts per million with TMS as an internal reference. Cyclic voltammetric (CV) studies were carried out with an electrochemical system utilizing a three-electrode configuration consisting of a glassy carbon (working) electrode, a platinum wire (auxiliary) electrode, and a saturated calomel (reference) electrode. The experiments were performed in dry CH_2Cl_2 with 0.1 M TBAP as the supporting electrolyte. All potentials were calibrated versus the saturated calomel electrode by the addition of ferrocene as an internal standard, taking $E_{1/2}$ (F_c/F_c^+) = 0.42 V versus SCE.[15] The quantum yields were calculated using Rhodamine 6G (Φ_f = 0.88 in ethanol).[16] All Φ values are corrected for changes in refractive index. HRMS (ESI/TOF) data were recorded with a mass spectrometer.

Preparation of the Test Solution. A stock solution of BODIPY **2** (5×10^{-4} M) was prepared in CH_3CN. The test solution of BODIPY **2** (5 μM) in pH 7.4 PBS (containing PBS/CH_3CN, 9:1, v/v) was prepared by placing 0.03 mL of the probe stock solution in CH_3CN (0.27 mL of CH_3CN, 2.70 mL of pH 7.4 PBS). The solutions of various testing species were prepared from Cys, Arg, Hcy, Phe, Pro, Tyr, Val, Ala, Gly, Lys, Leu, Glu, Ser, and GSH. The resulting solution was shaken well and incubated for 30 min at room temperature before recording the spectra.

Materials. Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), trypsin-EDTA, L-glutamine, penicillin (50 units/mL), and streptomycin (50 μg/mL) were obtained from a commercial source.

Cell Culture and Fluorescence Imaging. HepG2 cells were cultured in complete medium at 37 °C under 5% CO_2 in an incubator. The complete medium comprised of DMEM supplemented with 10% FCS, 2 mM L-glutamine, penicillin (50 units/mL), and streptomycin (50 μg/mL). Before imaging, the cells were washed with PBS (pH 7.4) three times and then incubated with BODIPY **2** (10 μM) in PBS (containing 1% CH_3CN as a cosolvent) for 30 min in an atmosphere of 5% CO_2 and 95% air at 37 °C. After the sample had been washed with PBS three times to remove the remaining probe, the fluorescence images were visualized under a laser scanning confocal microscope. Red and green emissions were observed after excitation at 543 and 514 nm, respectively.

1,9-Bis(3-oxo-1-propenyl)-*meso*-(tolyl)-dipyrromethane (4). 3-(Dimethylamino) acrolein (1.26 g, 4.20 mmol) was added to a 100 mL three-neck round-bottom flask that was then cooled to 5−10 °C and flushed with nitrogen for 5 min. $POCl_3$ (1.94g, 4.23 mmol) was added dropwise over 15 min with stirring. Dry dichloroethane (10 mL) was then added, and the mixture was stirred at room temperature for 15 min. The mixture was cooled to 0 °C, and *meso*-(tolyl)-dipyrromethane **3** (1g, 1.40 mmol) in dry dichloroethane (20 mL) was added dropwise over 1 h; the solution turned a deep purple color. The solution was warmed to 60 °C for 30 min and then allowed to cool to room temperature. Saturated sodium carbonate solution (50 mL) was added, and the reaction mixture was stirred vigorously at room temperature for 2 h. The mixture was extracted with ethylacetate (3 × 100 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The crude compound was subjected to silica gel column chromatography eluting with petroleum ether/ethylacetate (30:70) and afforded pure 1,9-bis(3-oxo-1-propenyl)-*meso*-(tolyl) dipyrromethane **4** as a brown solid in 87% yield (1.26 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, $-CH_3$), 5.46 (s, 1H, *meso*-CH), 5.84−5.85 (d, ^3J (H, H) = 3.68 Hz, 2H, Py), 6.39−6.45 (dd, ^3J (H, H) = 15.57 Hz, 2H, −CH), 6.58−6.59 (d, ^3J (H, H) = 3.68 Hz, 2H, Py), 7.07−7.09 (d, ^3J (H, H) = 8.16 Hz, 2H, Ar), 7.13−7.15 (d, ^3J (H, H) = 7.92 Hz, 2H, Ar), 7.37−7.41 (d, ^3J (H, H) = 15.57 Hz, 2H, −CH), 9.41−9.43 (d, ^3J (H, H) = 8.04 Hz, 2H, −CHO), 11.61 (br s, 2H, −NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.8, 43.2, 107.2, 110.4, 117.3, 121.4, 128.1, 128.3, 129.1, 136.1, 138.3, 140.4, 142.8, 193.1. HRMS calcd $[M + 1]^+$ for $C_{22}H_{20}N_2O_2$ 345.1715, found 345.1691. Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 76.68; H, 5.80; N, 8.20.

3,5-Bis(3-oxo-1-propenyl)-*meso*-(tolyl)-4-bora-3a,4a-diaza-*s*-indacene (2). 1,9-bis(3-oxo-1-propenyl)-*meso*-(tolyl) dipyrromethane **4** (0.5 g, 1.46 mmol) was dissolved in $CHCl_3$/THF (2:1; 100 mL) and oxidized with DDQ (0.4 g, 1.75 mmol) at room temperature. The reaction mixture was allowed to stir at room temperature for 30 min. Triethylamine (5.88 g, 58.4 mmol) and then $BF_3{\cdot}OEt_2$ (10.3g, 73 mmol) were added to the reaction mixture successively without any time delay, and the reaction mixture was stirred at room temperature for an additional 30 min. The reaction mixture was evaporated, and the crude product was purified using silica gel column chromatography with petroleum ether/ethylacetate (75:25) and afforded pure 3,5-bis(3-oxo-1-propenyl)-*meso*-(tolyl) BODIPY **2** as a golden-green solid in 32% yield (0.182 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.50 (s, 3H, $-CH_3$), 6.78−6.83 (dd, ^3J (H, H) = 16.05 Hz, 2H, −CH), 6.99−7.02 (dd, ^3J (H, H) = 4.48 Hz, 4H, Py), 7.37−7.39 (d, ^3J (H, H) = 8.0 Hz, 2H, Ar), 7.45−7.47 (d, ^3J (H, H) = 8.0 Hz, 2H, Ar), 7.98−8.03 (d, ^3J

dx.doi.org/10.1021/jo4004597 | J. Org. Chem. 2013, 78, 5056−5060

(H, H) = 16.1 Hz, 2H, −CH), 9.84−9.86 (d, ^3J (H, H) = 7.76 Hz, 2H, −CHO). ^{11}B NMR (128.3 MHz, $CDCl_3$): δ 1.07 (t, ^1J (B−F), 1B). ^{19}F NMR (376.4 MHz, $CDCl_3$): δ −137.2 (q, ^1J (F−B), 2F). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.7, 29.9, 119.3, 129.7, 130.6, 130.8, 131.9, 133.9, 138.4, 139.8, 142.3, 146.2, 152.2, 193.3. HRMS calcd $[M - F]^+$ for $C_{22}H_{17}BF_2N_2O_2$ 371.1367, found 371.1378. Anal. Calcd for $C_{22}H_{17}BF_2N_2O_2$: C, 67.72; H, 4.39; N, 7.18. Found: C, 67.79; H, 4.30; N, 7.25.

X-ray Crystallography. X-ray intensity data measurements of BODIPY **2** were carried out on a SMART APEX II CCD diffractometer with graphite-monochromatized (Mo Kα = 0.71073 Å) radiation at 297(2) K. Data were collected with an ω scan width of 0.5° at different settings of φ and 2θ with a frame time of 10 s, keeping the sample-to-detector distance fixed at 50 mm. The X-ray data collection was monitored by the APEX2 program.[11] The data were corrected for Lorentzian, polarization, and absorption effects using the SAINT[12] and SADABS[13] programs. SHELX-97 was used for structure solution and full matrix least-squares refinement on F^2.[14] All the H atoms were placed in geometrically idealized position and constrained to ride on their parent atoms. The asymmetric unit contained two molecules of acetonitrile along with one molecule of BODIPY **2**; thus the host to guest ratio was 1:2.

Crystal Data of BODIPY 2. (CCDC 913899) $C_{22}H_{17}BF_2N_2O_2{\cdot}2(CH_3CN)$, M = 472.29, colorless plate, 0.31 × 0.24 × 0.12 mm^3, triclinic, space group $P\bar{1}$, a = 6.7710(4) Å, b = 12.0890(7) Å, c = 14.8586(9) Å, α = 99.663(3)°, β = 93.799(3)°, γ = 91.628(3)°, V = 1195.39(12) Å^3, Z = 2, T = 90(2) K, $2\theta_{max}$ = 60.00°, D_{calc} (g cm^{-3}) = 1.312, F(000) = 492, μ (mm^{-1}) = 0.095, 30409 reflections collected, 6940 unique reflections (R_{int} = 0.0276), 6254 observed ($I > 2\sigma(I)$) reflections, multiscan absorption correction, T_{min} = 0.971, T_{max} = 0.989, 319 refined parameters, S = 1.024, R1 = 0.0374, wR2 = 0.1008 (all data R = 0.0414, wR2 = 0.1054), maximum and minimum residual electron densities; $\Delta\rho_{max}$ = 0.23, $\Delta\rho_{min}$ = −0.26 (e Å^{-3}).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

All NMR spectra of compounds **2** and **4**, fluorescence and absorption spectral traces, and photophysical data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*ravikanth@chem.iitb.ac.in

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.R. is thankful for the financial support from BRNS and DST, Government of India. S.M. thanks IIT Bombay for a fellowship. We also acknowledge SAIF, IIT Bombay, Mumbai, India, for the confocal laser scanning microscopy facility.

■ REFERENCES

(1) (a) Loudet, A.; Bugess, K. *Chem. Rev.* **2007**, *107*, 4891−4932. (b) Ulrich, G.; Ziessel, R.; Harriman, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 1184−1201. (c) Boens, N.; Leen, V.; Dehaean, W. *Chem. Soc. Rev.* **2012**, *41*, 1130−1172.

(2) (a) Buyukcakir, O.; Bozdemir, O. A.; Kolemen, S.; Erbas, S.; Akkaya, E. U. *Org. Lett.* **2009**, *11*, 4644−4647. (b) Komkaew, A.; Lim, S. H.; Lee, H. B.; Kiew, L. V.; Chung, L. Y.; Burgess, K. *Chem. Soc. Rev.* **2013**, *42*, 77−88.

(3) (a) Palma, A.; Alvarez, L. A.; Scholz, D.; Frimannsson, D. O.; Grossi, M.; Quinn, S. J.; O'Shea, D. F. *J. Am. Chem. Soc.* **2011**, *133*, 19618−19621. (b) Verbelen, B.; Leen, V.; Wang, L.; Boens, N.; Dehaen, W. *Chem. Commun.* **2012**, *48*, 9129−9131. (c) Harriman, A.; Izzet, G.; Ziessel, R. *J. Am. Chem. Soc.* **2006**, *128*, 10868−10875. (d) Killoram, J.; O'Shea, D. F. *Chem. Commun.* **2006**, 1503−1505.

(4) (a) Leen, V.; Braeken, E.; Luckermans, K.; Jackers, C.; Auweraer, M. V.; Boens, N.; Dehaen, W. *Chem. Commun.* **2009**, 4515−4517. (b) Lakshmi, V.; Ravikanth, M. *J. Org. Chem.* **2011**, *76*, 8466−8471. (c) Lakshmi, V.; Ravikanth, M. *Dalton Trans.* **2012**, *41*, 5903−5911.

(5) (a) Madhu, S.; Rao, M. R.; Shaikh, M. S.; Ravikanth, M. *Inorg. Chem.* **2011**, *50*, 4392−4400. (b) Madhu, S.; Ravikanth, M. *Inorg. Chem.* **2012**, *51*, 4285−4292. (c) Madhu, S.; Basu, S. K.; Jadhav, S.; Ravikanth, M. *Analyst* **2013**, *138*, 299−306.

(6) (a) Rusin, O.; Luce, N. N.; Agbaria, R. A.; Escobedo, J. O.; Jiang, S.; Warner, I. M.; Dawan, F. B.; Lian, K.; Strongin, R. M. *J. Am. Chem. Soc.* **2004**, *126*, 438−439. (b) Lim, S.; Escobedo, J. O.; Lowry, M.; Xu, X.; Strongin, R. M. *Chem. Commun.* **2010**, *46*, 5707−5709. (c) Yang, X.; Guo, Y.; Strongin, R. M. *Angew. Chem., Int. Ed.* **2011**, *50*, 10690−10693. (d) Chen, X.; Zhou, Y.; Peng, X.; Yoon, J. *Chem. Soc. Rev.* **2010**, *39*, 2120−2135.

(7) (a) Long, L.; Lin, W.; Chen, B.; Gao, W.; Yuan, L. *Chem. Commun.* **2011**, *47*, 893−895. (b) Zhang, X.; Ren, X.; Xu, Q.-H.; Loh, K. P.; Chen, Z.-K. *Org. Lett.* **2009**, *11*, 1257−1260. (c) Lin, W.; Long, L.; Yuan, L.; Cao, Z.; Chen, B.; Tan, W. *Org. Lett.* **2008**, *10*, 5577−5580. (d) Yuan, L.; Lin, W.; Yang, Y. *Chem. Commun.* **2011**, *47*, 6275−6277.

(8) (a) Li, H.; Fan, J.; Wang, J.; Tian, M.; Du, J.; Sun, S.; Sun, P.; Peng, X. *Chem. Commun.* **2009**, 5904−5906. (b) Jun, M. E.; Roy, B.; Ahn, H. *Chem. Commun.* **2011**, *47*, 7583−7601. (c) Yang, Z.; Zhao, N.; Sun, Y.; Miao, F.; Liu, Y.; Liu, X.; Zhang, Y.; Ai, W.; Song, G.; Shen, X.; Yu, X.; Sun, J.; Wong, W.-Y. *Chem. Commun.* **2012**, *48*, 3442−3444.

(9) (a) Shao, J.; Guo, H.; Ji, S.; Zhao, J. *Biosens. Bioelectron.* **2011**, *26*, 3012−3017. (b) Niu, L.-Y.; Guan, Y.-S.; Chen, Y.-Z.; Wu, L.-Z.; Tung, C.-H.; Yang, Q.-Z. *J. Am. Chem. Soc.* **2012**, *134*, 18928−18931. (c) Niu, L.-Y.; Guan, Y.-S.; Chen, Y.-Z.; Wu, L.-Z.; Tung, C.-H.; Yang, Q.-Z. *Chem. Commun.* **2013**, *49*, 1294−1296.

(10) (a) Starkenmann, C.; Brauchli, R.; Maurer, B. *J. Agric. Food Chem.* **2005**, *53*, 9244−9248. (b) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.

(11) *APEX2*; Bruker AXS Inc.: Madison, WI, 2006.

(12) *SAINT*; Bruker AXS Inc.: Madison, WI, 2006.

(13) *SADABS*;. Bruker AXS Inc.: Madison, WI, 2006.

(14) Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112.

(15) Masui, M.; Sayo, H.; Tsuda, Y. *J. Chem. Soc. B* **1968**, 973−976.

(16) Qin, W.; Leen, V.; Rohand, T.; Dehaen, W.; Dedecker, P.; Van der Auweraer, M.; Robeyns, K.; Van Meervelt, L.; Beljonne, D.; Van Averbeke, B.; Clifford, J. N.; Driesen, K.; Binnemans, K.; Boens, N. *J. Phys. Chem. A* **2009**, *113*, 439−447.