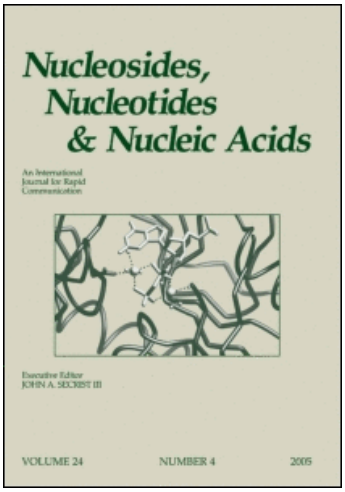

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OLIGONUCLEOTIDE LABELING USING BODIPY PHOSPHORAMIDITE

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□ *4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) derivatives were prepared and their photochemical properties were characterized. One such analogue, 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-hydroxypentyl)-3a,4a-diaza-s-indacene was transformed into the corresponding phosphoramidite and incorporated into oligodeoxyribonucleotides as a fluorescent reporter group.*

Keywords BODIPY; fluorescent labeling; oligonucleotide; solid-phase synthesis; phosphoramidite

INTRODUCTION

Fluorescent labeling of oligonucleotides is a technique commonly used in structural and functional studies. A wide range of fluorescent reporter groups have been in use, many of these are commercially available.[1] Among these fluorophores, fluorescein, rhodamine, and cyanine are the most commonly used. These fluorophores cover a wide range of excitation and emission wavelengths and are, in general, highly fluorescent; however, most of these fluorophores are charged molecules and their fluorescent properties are often pH and environment dependent. In addition, many of these fluorescent probes are not very photostable.

Numerous 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY; Figure 1) derivatives have been known to possess very high, and some near unity fluorescent quantum yields that are relatively insensitive to changes in pH and polarity and are stable under many biological conditions.[2–4] These fluorophores are often more photostable than many fluorescein, rhodamine and cyanine analogues. Therefore, the BODIPY fluorophores have found useful applications in labeling proteins, nucleic acids and lipids.[1,2]

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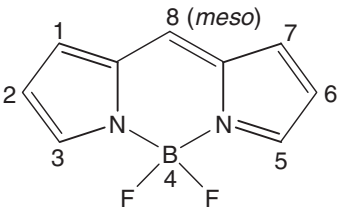
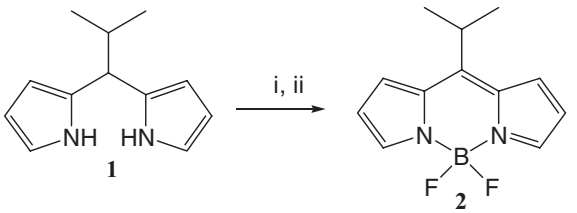


FIGURE 1 BODIPY core structure.

Contrary to these advantages, there has been little development in new labeling chemistry involving BODIPY. Most studies utilize commercially available activated BODIPY esters for labeling biological samples.[1] With few exceptions,[5] BODIPY labeling of oligonucleotides has been carried out post-synthetically between an amino modifier on oligonucleotides and an activated BODIPY carboxylate ester.[1] While this conjugation approach is efficient in most cases, a method that allows for the direct incorporation of BODIPY into oligonucleotides during solid phase synthesis would be attractive since it eliminates the need for post-synthetic modification and purification. The synthesis of phosphoramidites derived from BODIPY and their incorporation into oligodeoxyribonucleotides are reported herein.

RESULTS AND DISCUSSIONS

Unsubstituted BODIPYs, particularly at the 1,3,5,7,8-positions, are known to be unstable. Substitution at the 8-(or *meso*) position is particularly important for the stabilization of BODIPY.[3,4] It was shown previously in our lab that although the fully unsubstituted BODIPY can indeed be synthesized from the unstable dipyrromethene intermediate, efficiency in the synthesis is rather low (5–10% yields).[6,7] This effect is further confirmed by the improved yield (36%) in the preparation of 4,4-difluoro-4-bora-8-isopropyl-3a,4a-diaza-s-indacene **2** (8-isopropyl BODIPY) from 5-isopropyldipyrromethane **1** (Scheme 1).[8]



SCHEME 1 Reagents and conditions: i) DDQ, CH_2Cl_2, −30°C; ii) DBU, $BF_3{\cdot}Et_2O$, CH_2Cl_2, −30°C to reflux.

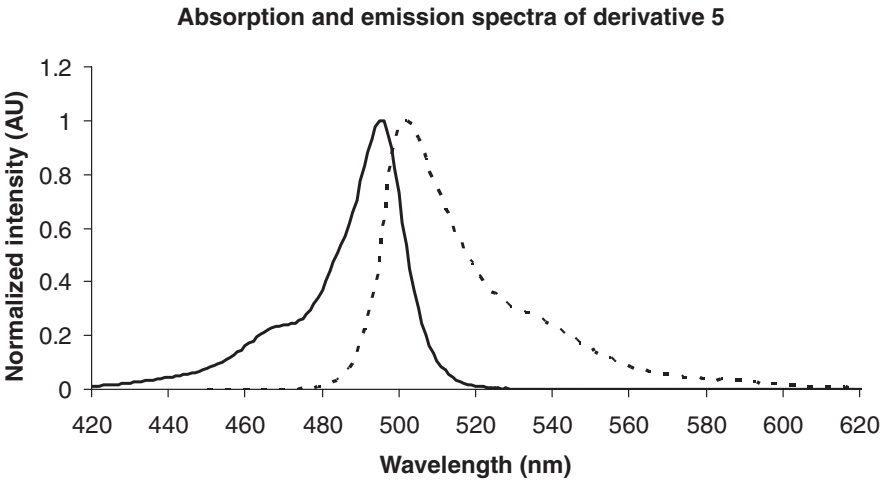


FIGURE 2 Absorption and emission spectra of 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-brom opentyl)-3a,4a-diaza-*s*-indacene **5**. Solid line: absorption; dashed line: emission.

In order to further stabilize the BODIPY core for labeling purposes, 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-bromopentyl)-3a,4a-diaza-*s*-indacene **5** was synthesized in moderate yields (62%) by condensation of 2,4-dimethylpyrrole **3** with 6-bromohexanoyl chloride **4** followed by treatment with boron trifluoride diethyl etherate and triethylamine (steps i and ii, Scheme 2).[9] Substitution of the bromine by acetate followed by hydrolysis yielded 8-(5-hydroxypentyl) BODIPY analogue **7** in moderate yields (58% for steps iii and iv, Scheme 2).[9,10] Compound **7** was subsequently transformed into its phosphoramidite **8** (step v, Scheme 2) for incorporation into oligonucleotides under conventional conditions.[11]

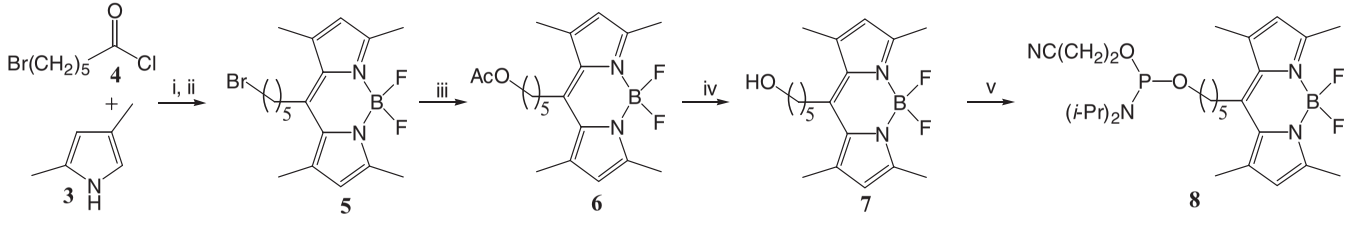
As can be seen from Figure 2 and Table 1, 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-bromopentyl)-3a,4a-diaza-*s*-indacene **5** showed a typical BODIPY UV and fluorescent profile, with a Stoke's shift of 7 nm. The relative fluorescent quantum yield of **5** was determined to be 0.98 (in methanol) using the literature procedure[12] with fluorescein as the standard ($\Phi_f = 0.95$).

In order for the BODIPY building blocks to be incorporated by the phosphoramidite chemistry-based solid phase synthesis, they should be stable during chain assembly and subsequent deprotection conditions once the

TABLE 1 Photospectroscopic characterization of BODIPY derivative **5**

Maximal absorption wavelength (nm)	Maximal emission wavelength (nm)	Molar extinction coefficient ε ($M^{-1}\bullet cm^{-1}$)	Quantum yield Φ_f (in CH_3OH)[a]
495	502	89,600	0.98

[a]Using fluorescein as the standard ($\Phi_f = 0.95$).



SCHEME 2 Reagents and conditions: i) CH_2Cl_2, 40°C, 3 hours; ii) NEt_3, $BF_3{\cdot}Et_2O$; iii) KOAc, DMF, 50°C, 3 days; iv) aq. LiOH; (v) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $Et_2N(i\text{-}Pr)$, THF.

desired sequence is assembled on the solid support. One such key deprotection step involves a treatment with a base to free the oligonucleotide products from the solid support. This treatment also removes the acyl protecting groups on the base residues and *O*-cyanoethyl from the internucleotide phosphate linkages. A common deprotection condition is to incubate the immobilized oligonucleotide product in concentrated aqueous ammonia solution at 55°C for 12–16 hours. It was found that the BODIPY derivative **5** is not stable under the above unblocking conditions, presumably due to displacement of fluorine by hydroxide.[13] In order to ensure that the BODIPY moiety remains unchanged during the deprotection step, *i.e.* incubation with concentrated aqueous ammonia solution, the Q-linker[14] and 5′-*O*-dimethoxytrityl ultramild nucleoside phosphoramidites were used for solid phase oligonucleotide synthesis (Figure 3, thymidine phosphoramidite is not protected on thymine). Use of the Q-linker and the ultramild phosphoramidites allows for fast cleavage of oligonucleotide products from the solid support and deprotection of base acyl protecting groups under mild basic conditions (i.e., incubation with concentrated aqueous ammonium hydroxide at room temperature for 2 hours). It was indeed found that incubation of compound **7** in a mixture of methanol and concentrated ammonium hydroxide (1:7 v/v, methanol was added to solubilize compound **7**) for 2 hours at room temperature did not lead to the formation of highly polar by-products.[15]

Solid phase syntheses of oligodeoxyribonucleotides labeled with BODIPY at the 5′-end were performed on an ABI 3400 DNA synthesizer. Built-in ABI 1.0 μmol DNA synthesis cycle was used. All phosphoramidites were prepared as a 0.10 *M* solution in dry acetonitrile. *S*-Ethyltetrazole (0.25 *M* in acetonitrile) was used as the activator and coupling time of 20 seconds was used. Trichloroacetic acid solution (3% in dichloromethane) was used for detritylation (110 seconds continuous flow through the column). After assembly of the sequence is completed, the products were deprotected (cleavage from solid support and removal of base protecting groups) by incubation in concentrated ammonium hydroxide solution at room

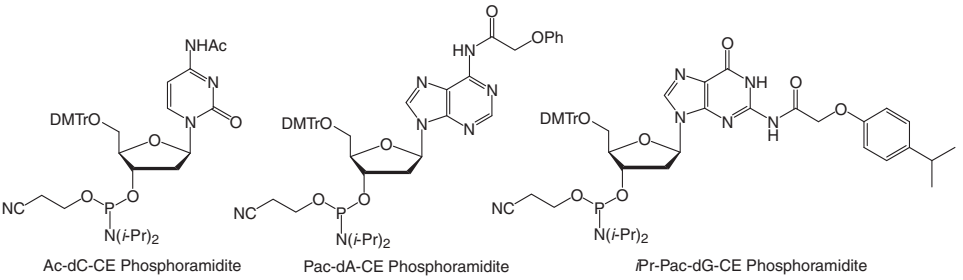


FIGURE 3 Ultramild phosphoramidites used in solid phase oligonucleotide synthesis. DMTr: dimethoxytrityl.

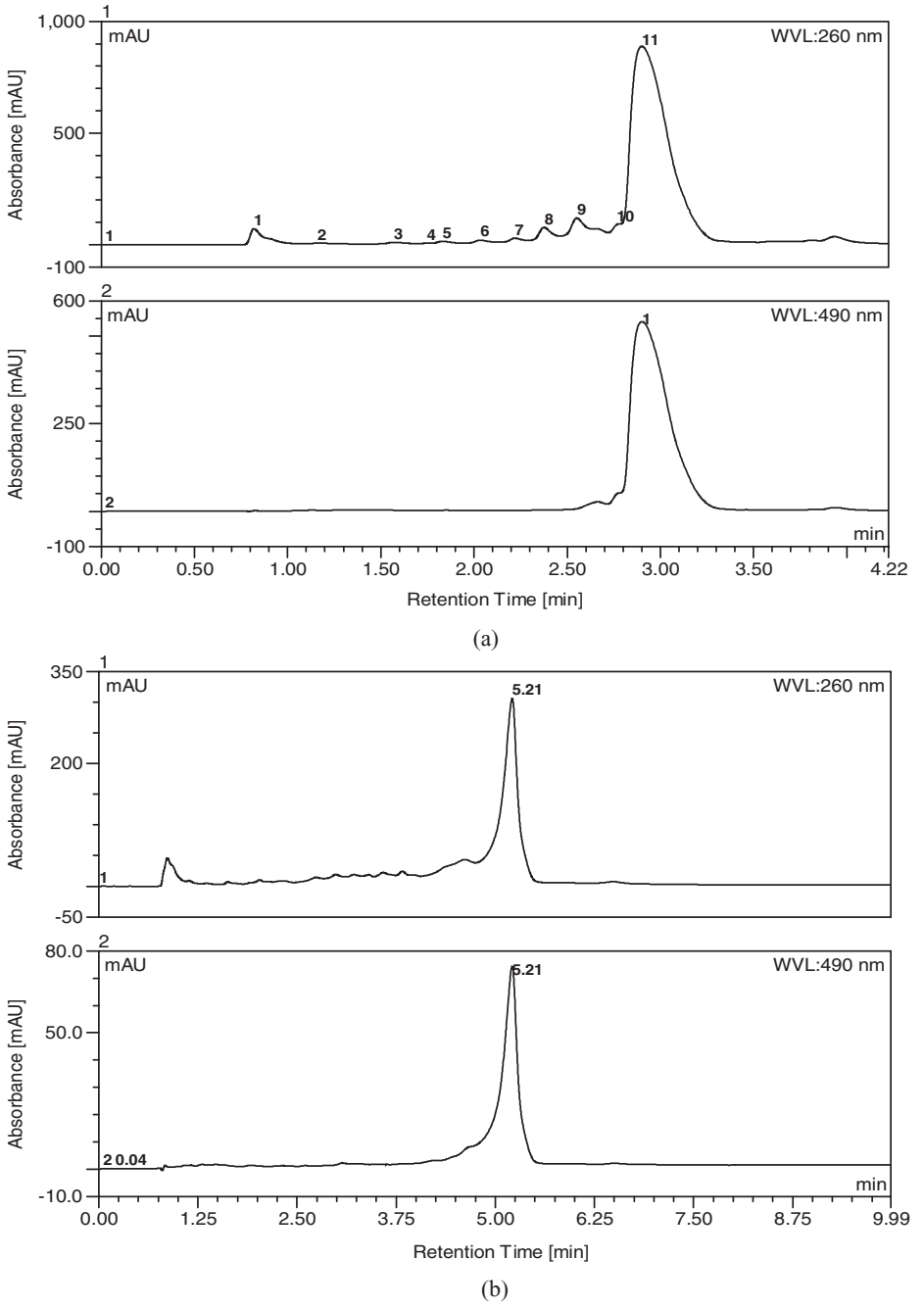


FIGURE 4 Anion exchange HPLC chromatograms of oligonucleotides labeled with BODIPY at the 5′-end. a) Sequence A: top and bottom profiles monitored at 260 and 490 nm, respectively; b) Sequence B: top and bottom profiles monitored at 260 and 490 nm, respectively.

TABLE 2 Masses of BODIPY-labelled oligonucleotides as determined by negative ESI-MS. Mass values correspond to singularly (negatively) charged species, that is, $[M\text{-}H]^-$

Sequence	Observed (Da)	Calculated (Da)
A	3374.7	3375.1
B	6438.9	6439.1

temperature for 2 hours. The supernatants were lyophilized and analyzed by anion exchange chromatography.

Two sequences with BODIPY labeling at the 5′-termini were synthesized (sequence A: 5′-BODIPY-T_{10}-3′; sequence B: 5′-BODIPY-d[TCCC GCCTGTGACATGCATT]-3′). As can be seen in the anion exchange HPLC chromatogragms of the fully deprotected oligonucleotides (Figure 4), both sequences were successfully labeled with BODIPY at the 5′-end. Since the BODIPY derivative **5** shows maximal absorption at 495 nm where nucleos(t)ides do not absorb, the HPLC profiles clearly indicated that the major component that appears in the profile monitored at 260 nm is labeled with BODIPY.

Both fully-deprotected products (sequences A and B) were analyzed by electrospray (ESI) mass spectroscopy in the negative mode, and the observed molecular masses of both sequences were in good agreement with calculated values (Table 2).

CONCLUSION

In summary, a phosphoramidite derived from a BODIPY analogue was synthesized and incorporated into oligodeoxyribonucleotides at the 5′-termini as a fluorescent labeling agent. Due to the advantages of BODIPY fluorophores, this approach may provide a useful alternative to other commercially available fluorophores for DNA/RNA labeling. Work is underway to improve the acid and base stability of the BODIPY core structure so that this approach could be more readily adapted to conventional phosphoramidite building blocks other than the ultramild phosphoramidites. This approach is also being expanded to cover other excitation and emission ranges, particularly the red and purple regions for oligonucleotide labeling.

EXPERIMENTAL

Instrumentation

Melting points were measured with a Stuart Scientific melting point apparatus (Bibby Scientific Ltd., UK) and are uncorrected. ^{1}H NMR spectra were measured at 300 and 600 MHz with Bruker AV300 and AV600 spectrometers (Germany); spectra were calibrated to residual undeuterated

NMR solvents; J values are given in Hz. ^{13}C NMR spectra were measured at 75.5 or 150.9 MHz with the same spectrometers. ^{31}P NMR spectra were measured at 121.5 or 242.9 MHz with the same spectrometers, and were referenced to external 85% H_3PO_4. Chemical shifts are given in ppm. Low and high resolution mass spectra were obtained with Kratos Concept 1S high resolution mass spectrometer (Mass Spectrometry Instruments Ltd., UK) using electron impact or fast atom bombardment sources interfaced with DART 32 bit acquisition system through a Sun Sparcstation 10 and Mach 3 software. Bruker HCT Proteineer with electrospray (Bruker, Germany) interfaced with an Agilent 1100 series LC system with built-in auto-sampler and diode array detector were used for LC-MS. UV/vis spectrums were obtained using a Thermospectronic/Unicam UV/vis spectrometer (USA) configured to the Vision32 software. Fluorimetric data were gathered using QuantaMaster model QM-2001-4 cuvette-based L-format scanning spectrofluorometer from Photon Technology International (PTI, USA) interfaced with FeliX32 software.

Chromatography

Merck silica gel 60 Art 7734-3 was used for flash column chromatography.

Anion exchange chromatography (HPLC) was carried out using a Dionex ICS 3000 System on a 4 × 250 mm Dionex DNA Pac100 column (Dionex Corp., USA). The column was eluted with the following program: Eluent A: 0.25 *M* Tris (constant at 10%), pH 8.0; eluent B: water; eluent C: NaCl (1.0 *M*). Gradient: convex gradient (curve 5) of 10% C to 55% in 20 minutes. Flow rate: 1.5 ml/min.

Chemicals and Solvents

Suitably protected nucleoside phosphoramidites and solid supports were purchased from Glen Research. The other chemicals were purchased from Aldrich or Alfa Aesar and used without further purification unless stated otherwise. DMF, THF, and dichloromethane were purified by Pure-Solv Solvent Purification Systems (Innovative Technology), and stored over activated 4 Å molecular sieves. Triethylamine and N,N-diisopropylethylamine were dried by heating, under reflux, over calcium hydride and then distilled in a nitrogen atmosphere.

4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-bromopentyl)-3a,4a-diaza-s-indacene 5

A solution of 6-bromohexanoyl chloride **4** (0.56 ml, 3.66 mmol) in dry dichloromethane (25 ml) was added dropwise over a period of 5 minutes to a solution of 2,4-dimethylpyrrole **3** (1.0 ml, 9.71 mmol) in dry dichloromethane (10 ml). The mixture was heated under reflux for 3 hours

and then concentrated to dryness under reduced pressure. The residue was dissolved in dry dichloromethane (10 ml) followed by addition of dry hexane (100 ml). After the mixture was left at −4°C overnight, the supernatant was decanted and the residue was washed with cold hexane (2 × 20 ml) and then dissolved in dry toluene (30 ml). Dry triethylamine (1.0 ml, 7.17 mmol) was added and the reaction mixture was stirred for 15 minutes. Boron trifluoride diethyl etherate (1.2 ml, 9.50 mmol) was then added and the mixture was heated at 40°C for 3 hours. Upon cooling to room temperature, the products were successively washed with water (30 ml) and brine (3 × 30 ml). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure to give a dark red oil. The mixture was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (1:1 v/v), were pooled and concentrated under reduced pressure to yield the title compound as a bright orange solid (0.78 g, 62%).

HR-FAB found $M^+ = 396.11746$, $C_{18}H_{24}BBrF_2N_2^+$ requires 396.11840; δ_H[$CDCl_3$, 600.2 MHz]: 1.68 (4 H, m, br), 1.95 (2 H, m, br), 2.44 (6 H, s), 2.54 (6 H, s), 2.99 (2 H, br), 3.46 (2 H, t, $J = 6.5$ Hz), 6.08 (2 H, s); δ_C[$CDCl_3$, 150.9 MHz]: 14.5, 16.4, 28.2, 28.6, 30.9, 32.2, 33.4, 121.7, 131.4, 140.2, 145.9, 154.0; R_f: 0.57 (hexane–diethyl ether 1:1, v/v).

4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-hydroxypentyl)-3a,4a-diaza-s-indacene 7

4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-bromopentyl)-3a, 4a-diaza -*s* -indacene **5** (0.20 g, 0.504 mmol) and potassium acetate (0.17 g, 1.73 mmol) were purged with nitrogen in a septum-capped flask. The solids were then dissolved in dry DMF (10 ml) and stirred at 50°C for 3 days. After the products were cooled to room temperature, ethyl acetate (50 ml) was added and the resulting mixture was washed successively with distilled water (50 ml) and brine (3 × 50 ml). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure and the products (4,4-difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-acetoxypentyl)-3a,4a-diaza-*s*-indacene **6**) were used for the next step without further purification.

The residue above was dissolved in a mixture of THF–methanol (10 ml, 2:8 v/v) and sparged with nitrogen for 15 minutes. In a separate flask, lithium hydroxide monohydrate (41 mg, 0.98 mmol) was dissolved in distilled water (5 ml) and sparged with nitrogen for 15 minutes. The latter solution was then added dropwise to the former over 5 minutes. After 3 hours, the products were concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (98:2 v/v) were combined and concentrated under reduced pressure to give the title compound as a bright orange solid (98 mg, 58%).

HR-FAB found $M^+ = 334.20448$, $C_{18}H_{25}BF_2N_2O^+$ requires 334.20280; δ_H[$CDCl_3$, 600.2 MHz] include the following signals: 1.64–1.72 (4 H, m), 2.44 (6 H, s), 2.54 (6 H, s), 2.98 (2 H, m), 3.71 (2 H, t, $J = 5.8$ Hz), 6.07 (2 H, s); δ_C[$CDCl_3$, 150.9 MHz]: 14.5, 16.4, 26.6, 28.4, 29.7, 31.7, 32.4, 62.7, 121.6, 131.4, 140.3, 146.3, 153.8; R_f: 0.46 (dichloromethane–methanol 95:5, v/v).

Phosphoramidite 8

4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-8-(5-hydroxypentyl)-3a,4a-diaza-*s*-indacene **7** (0.40 g, 1.20 mmol) was co-evaporated with dry toluene (7 ml) and redissolved in dry THF (10 ml). *N*,*N*-Diisopropylethylamine (0.46 ml, 2.64 mmol) followed by *N*,*N*-diisopropyl phosphochloridite (0.45 g, 1.90 mmol) were added to the reaction mixture and the reaction was allowed to proceed for 30 minutes at room temperature. The products were then concentrated under reduced pressure and purified by column chromatography on silica gel. The appropriate fractions, which were eluted with acetone–hexane–triethylamine (20:78:2 v/v) were combined and evaporated under reduced pressure to yield the phosphoramidite as a bright orange oil (0.33 g, 51%).

δ_P[$CDCl_3$, 121.5 MHz]:149.9; R_f: 0.38 (dichloromethane).

Solid Phase Synthesis of BODIPY Labeled Oligonucleotides

Thymidine immobilized on the Q-linker (Glen Research, Sterling, VA, USA) was used as the solid support. Solid phase synthesis was performed on an ABI 3400 DNA synthesizer. Standard ABI 1.0 μmol DNA synthesis cycle was used for the solid phase synthesis of BODIPY labeled oligonucleotides. All phosphoramidites were prepared as 0.1 *M* solutions in dry acetonitrile. *S*-Ethyltetrazole (0.25 *M* in acetonitrile) was used as the activator and coupling time of 20 seconds was used. Trichloroacetic acid solution (3% in dichloromethane) was used for detritylation (110 seconds continuous detritylation).

Deprotection of BODIPY-Labeled Oligonucleotides

The product from solid phase synthesis was dried and emptied into an eppendorf tube, and incubated with concentrated aqueous ammonia at room temperature for 2 hours. After centrifugation at 10,000 rpm for 5 minutes, the supernatant was collected and lyophilized to give fully-deprotected BODIPY-labeled oligonucleotides.

Relative Fluorescent Quantum Yield Determination

Five solutions with increasing concentrations (BODIPY in methanol and fluorescein in 0.1 *M* NaOH) were prepared and corresponding absorbance

measured 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 intensities of the solutions were measured on a fluorimeter. Graphs of integrated fluorescence intensity versus absorbance were plotted for both BODIPY and fluorescein, and the gradients of the linear plots were used for the calculation of fluorescence quantum yields.

REFERENCES AND NOTES

1. Haugland, R. P. Ed. *The handbook, a guide to fluorescent probes and labeling technologies* (10th ed.); Invitrogen Corp., Carlsbad, CA, 2005, pp. 315–412.
2. Karolin, J.; Johansson, L. B.-A.; Strandberg, L.; Ny, T. Fluorescence and absorption spectroscopic properties of dipyrrometheneboron difluoride (BODIPY) derivatives in liquids, lipid membranes, and proteins. *J. Am. Chem. Soc.* **1994**, 116, 7801–7806.
3. Loudet, A.; Burgess, K. BODIPY dyes and their derivatives: syntheses and spectroscopic properties. *Chem. Rev.* **2007**, 107, 4891–4932.
4. Ulrich, G.; Ziessel, R.; Harriman, A. The chemistry of fluorescent BODIPY dyes: versatility unsurpassed. *Angew. Chem., Int. Ed.* **2008**, 47, 1184–1201.
5. Wanninger-Weiß, C.; Di Pasquale, F.; Ehrenschwender, T.; Marx, A.; Wagenknecht, H.-A. Nucleotide insertion and bypass synthesis of pyrene- and BODIPY-modified oligonucleotides by DNA polymerases. *Chem. Commun.* **2008**, 1443–1445.
6. Tram, K.; Yan, H.; Jenkins, H.; Vassiliev, S.; Bruce, D. The synthesis and crystal structure of unsubstituted 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY). *Dyes Pigments* **2009**, 82, 392–395.
7. The fully unsubstituted BODIPY was also synthesized independently by two other groups; a) Schmitt, A.; Hinkeldey, B.; Wild, M.; Jung, G. Synthesis of the core compound of the BODIPY dye class: 4,4′-difluoro-4-bora-(3a,4a)-diaza-*s*-indacene. *J. Fluoresc.* **2009**, 19, 755–758; b) Arroyo, I. J.; Hu, R.; Merino, G.; Tang, B. Z.; Peña-Cabrera, E. The smallest and one of the brightest. Efficient preparation and optical description of the parent borondipyrromethene system. *J. Org. Chem.* **2009**, 74, 5719–5722.
8. For the crystal structure of compound **2**, refer to: Tram, K.; Jenkins, H. A.; Yan, H. Private communication (2010) to the Cambridge Structural Database, deposition number CCDC 793318.
9. Canham, S. M.; Bass, J. Y.; Navarro, O.; Lim, S.-G.; Das, N.; Blum, S. A. Toward the single-molecule investigation of organometallic reaction mechanisms: single-molecule imaging of gluorophore-tagged palladium(II) complexes. *Organometallics*, **2008**, 27, 2172–2175.
10. Shepherd, J. L.; Kell, A.; Chung, E.; Sinclar, C. W.; Workentin, M. S.; Bizzotto, D. Selective reductive desorption of a SAM-coated gold electrode revealed using fluorescence microscopy. *J. Am. Chem. Soc.* **2004**, 126, 8329–8335.
11. Beaucage, S. L.; Caruthers, M. L. Synthetic strategies and parameters involved in the synthesis of oligodeoxyribonucleotides according to the phosphoramidite method. In *Current protocols in nucleic acid chemistry*, eds. S.L. Beaucage, D.E. Bergstronm, G.D. Glick, R.A. Jones, Wiley, New York, 2001; v1, 3.3.1–3.3.20.
12. Lacowicz, J. R. Ed. *Principles of fluorescence spectroscopy* (3rd ed.), Springer, New York, 2006, pp. 54–55.
13. Wang, P.; Giese, R. W. Phosphate-specific fluorescence labeling with BO-IMI: reaction details. *J. Chromatogr. A* **1998**, 809, 211–218.
14. Pon, R. T.; Yu, S. Hydroquinone-*O*,*O*′-diacetic acid ('Q-linker') as a replacement for succinyl and oxalyl linker arms in solid phase oligonucleotide synthesis. *Nucleic Acids Res.* **1997**, 25, 3629–3635.
15. This observation was made by TLC comparisons. TLC (dichloromethane–methanol, 98:2 v/v, ran on Alumina oxide coated TLC plates) of three solutions of compound **7** in dichloromethane, methanol–water (1:7 v/v), and methanol–concentrated ammonium hydroxide (1:7 v/v) were monitored over a period of 2 hours. In all three TLC lanes, majority of compound **7** ran with an R_f of *ca.* 0.4, with weakly fluorescent spots at the baseline. Over 2 hours, intensity of the baseline material remained consistently low in all cases.