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Highly Water-Soluble Neutral BODIPY Dyes with Controllable Fluorescence Quantum Yields

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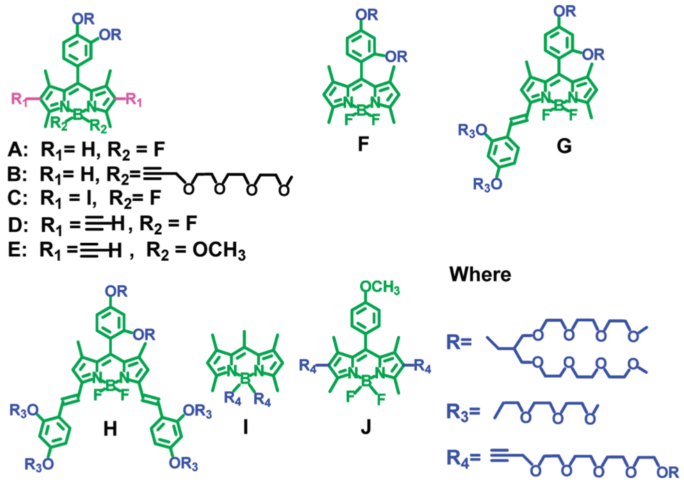
ABSTRACT



A series of novel highly water-soluble neutral BODIPY dyes have been obtained by functionalization of BODIPY dyes with branched oligo(ethylene glycol)methyl ether groups at positions 8, 2 and 6 or 4 and 4′. Use of an ortho-substituent group of branched oligo(ethylene glycol)methyl ether on the meso-phenyl ring of BODIPY dyes and replacement of the fluorine atoms of BODIPY dyes at positions 4 and 4′ with methyloxy or ethynyl subunits significantly enhance fluorescence quantum yields of BODIPY dyes.

BODIPY (4,4′-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene) dyes have gained a great deal of attention recently because of their many distinctive and desirable properties such as high extinction coefficients, narrow absorption and emission bands, high quantum efficiencies of fluorescence, relative insensitivity to environmental perturbations, and resistance to photobleaching.[1] Biological and medical applications of the BODIPY dyes require good water solubility and resistance to the formation of nonfluorescent dimer and higher aggregates. Reported strategies to make these dyes water-soluble typically involve introduction of oligo(ethylene glycol), *N*,*N*-bis(2-hydroxyethyl) amine, carbohydrates, nucleotides, or ionic hydrophilic groups such as carboxylic acid, sulfonic acid, or ammonium groups to BODIPY dyes.[2] However, neutral water-soluble BODIPY dyes have advantages over ionic ones because they avoid potential nonspecific interactions through electrostatic interactions between BODIPY dyes and proteins or other biomolecules in biologi-

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(1) (a) Loudet, A.; Burgess, K. *Chem. Rev.* **2007**, *107*, 4891. (b) Ulrich, G.; Ziessel, R.; Harriman, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 1184.

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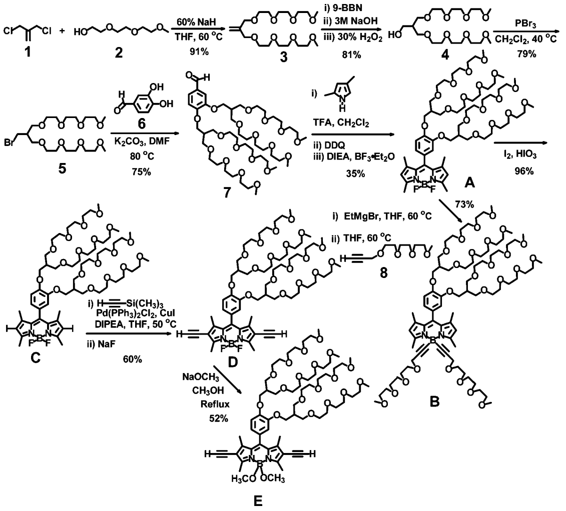
cal and medical applications. It is very important to develop general approaches to significantly enhance the water solubility of neutral BODIPY dyes with controlled fluorescence quantum yields.

We hypothesized that incorporation of branched oligo(ethylene glycol)methyl ether into BODIPY dyes could effectively enhance enthalpic interactions of BODIPY dyes with water and significantly increase the water solubility of BODIPY dyes, and that introduction of steric hindrance at the meso, 4,4'-positions of BODIPY dyes could significantly reduce their aggregation through $\pi-\pi$ stacking interactions between BOIDIPY cores in aqueous solution and considerably enhance their fluorescence quantum yields. In this letter, we have introduced branched oligo(ethylene glycol)methyl ether to the meso, 2,6-, 4,4'-positions BODIPY dyes and demonstrated significantly enhanced fluorescence quantum yields of the new dyes (the abstract scheme). These neutral BODIPY dyes are highly water-soluble because of the strong hydrophilic nature of oligo(ethylene glycol)methyl ether residues. These approaches offer very efficient ways to prepare different BODIPY dyes with emission ranging from green to deep red regions.

In order to demonstrate the feasibility of using branched oligo(ethylene glycol)methyl ether to enhance the water solubility of BODIPY dyes, we first introduced branched oligo(ethylene glycol)methyl ether to BODIPY dyes at the meso position (Scheme 1). Compound **4** was prepared according to a reported procedure[3] and further brominated with PBr_3 in methylene chloride at 40 °C, affording brominated branched oligo(ethylene glycol)methyl ether (**5**). The benzaldehyde derivative bearing branched oligo(ethylene glycol)methyl ether residues (**7**) was prepared by reacting 3,4-dihydroxybenzaldehyde with compound **5** under basic conditions. BODIPY dye substituted with branched oligo(ethylene glycol)methyl ether at the meso position (**A**) was prepared through the condensation of the benzaldehyde derivative (**7**) with 2,4-dimethylpyrrole in the presence of a catalytic amount of trifluoroacetic acid (TFA) and followed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and chelation with BF_3-etherate in the presence of *N*,*N*-diisopropylethylamine (DIPEA).[4] BODIPY dye (**A**) is highly water-soluble. Highly fluorescent E-BODIPY dye (**B**) was also prepared by replacing the usual fluorine atoms of BODIPY dye (**A**) with ethynyl tri(ethylene glycol)methyl ether (**8**) via a Grignard reaction. In order to evaluate the effect of functionalization of BODIPY dye (**A**) on water solubility, we iodized BODIPY dye **A** at positions 2 and 6, affording 2,6-diiodo BODIPY dye (**C**), and palladium-catalyzed Sonogashira coupled 2,6-diiodo BODIPY dye (**C**) with trimethylsilylacetylene, followed by deprotection of trimethylsilyl groups in the presence of NaF, affording 2,6-diethynyl BODIPY dye (**D**).[4b,c] Replacement of the usual fluorine atoms of BODIPY dye **A** with methyloxy subunits introduced some steric hindrance at the BODIPY core and resulted in BODIPY dye with methyloxy subunits at positions 4,4′ (**E**). BODIPY dyes (**B**, **C**, **D**, and **E**) are highly water-soluble, and further functionalization of BODIPY dye (**A**) does not affect water solubility of new BODIPY dyes, indicating that the strong hydrophilic character of branched oligo(ethylene glycol)methyl ether residues considerably enhances interactions of BODIPY dyes with water, thus significantly increasing their water solubility.

Scheme 1. Synthetic Route to Highly Water-Soluble BODIPY Dyes at the Meso Position



BODIPY dye **A** displays weak fluorescence in 0.5 M phosphate buffer solution (PBS) (pH 7.4) with a fluorescence quantum yield of only 4.2% while it becomes highly fluorescent with fluorescence quantum yields of 68% and 61% in methylene chloride and ethanol, respectively. The low fluorescence quantum yield of BODIPY dye **A** may be attributed to self-quenching because of the likely aggregation of the dye in aqueous solution through $\pi-\pi$ stacking or hydrophobic interactions between BODIPY cores. In order to prove this hypothesis, we replaced the usual fluorine atoms of BODIPY dye **A** with ethynyl subunits to introduce steric hindrance to the BODIPY core, resulting in BODIPY dye (**B**) with a significantly enhanced fluorescence quantum yield of 35.7% in PBS solution. The side chains of ethynyl tri(ethylene glycol)methyl ether in BODIPY dye (**B**) provide steric hindrance and reduce the aggregation of BODIPY dye in aqueous solution. Iodization of BODIPY dye (**A**) at positions 2 and 6 results in 2,6-diiodo BODIPY dye (**C**),

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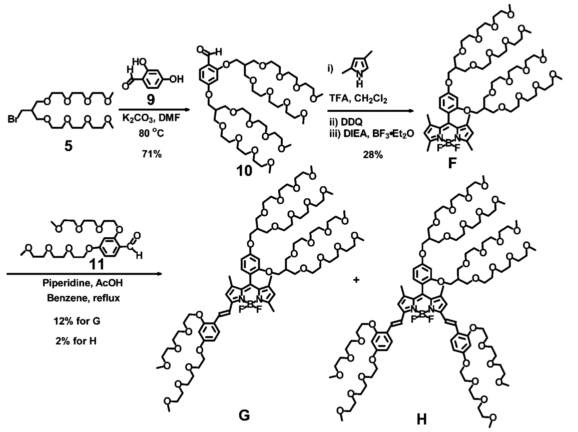
which has an extremely low fluorescence quantum yield of 1.2% in PBS solution because of efficient intersystem crossing induced by the heavy atom effect of iodine.[4a,b] Replacement of iodo groups of BODIPY dye (**C**) at positions 2 and 6 with ethynyl groups yielded 2,6-diethynyl BODIPY dye (**D**), which shows a low fluorescence quantum yield of 1.9%, attributed to potential dye aggregation with enhanced π-conjugation in aqueous solution. Replacement of the fluorine atoms of BODIPY dye (**D**) with methyloxy subunits gave BODIPY dye (**E**), which displays an enhanced fluorescence quantum yield of 8.9% due to the slightly increased steric hindrance of the methyloxy subunits on the BODIPY core.

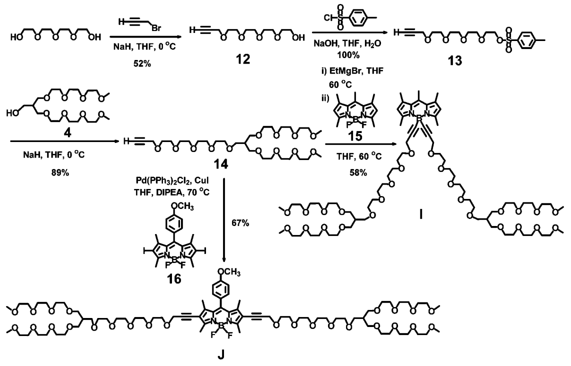
We hypothesized that further introduction of a bulky group to an ortho position on the meso-phenyl ring of BODIPY dyes would additionally enhance fluorescence quantum yields of BODIPY dyes through a greater disruption of $\pi-\pi$ stacking interactions between the BODIPY cores. In order to prove our hypothesis, we prepared BODIPY dye **F** with one ortho-substituent group of branched oligo(ethylene glycol)methyl ether from the aldehyde **10** via a similar approach to that used to prepare BODIPY dye **A** (Scheme 2). As we expected, BODIPY dye **F** is highly fluorescent with a fluorescence quantum yield of 31.2% in PBS solution which we attribute to enhanced steric hindrance of the bulky ortho-substituent group on the meso-phenyl ring of BODIPY dye **F**. BODIPY dyes with extended π-conjugation systems have strong hydrophobic features and are typically insoluble in water. In order to further demonstrate the feasibility of using branched oligo(ethylene glycol)methyl ether to promote the water solubility of BODIPY dyes with an extended π-conjugation system, we prepared BODIPY dyes bearing monostyryl and distyryl groups at positions 3, 3 and 5 (**G** and **H**) by condensation of methyl substituents of BODIPY dye **F** at positions 3, 3 and 5 with aldehyde derivative **11**.[4d] Both BODIPY dyes **G** and **H** are readily soluble in aqueous solution and have fluorescence quantum yields of 21.2% and 1.3%, respectively. The unexpectedly low fluorescence quantum yield of BODIPY dye **H** may arise from rotation of vinyl bonds caused by interactions between flexible oligo(ethylene glycol)methyl ether at the meso position with those at positions 3 and 5, suggested by a strong absorption peak at 383 nm, attributed to phenyl units at 3,5-positions, the relatively weak absorption of the BODIPY core at 507 nm, and a shoulder peak at 605 nm (please see Figure 40 in the Supporting Information).

Scheme 2. Synthetic Route to BODIPY Dyes Bearing Monostyryl and Distyryl Groups at Positions 3 and 5



In order to further show the versatility of these approaches, we introduced branched oligo(ethylene glycol)methyl ether to BODIPY dyes at 4,4'-, 2,6-positions. We introduced a tethered spacer of tetra(ethylene glycol) to the branched oligo(ethylene glycol)methyl ether in order to reduce spatial hindrance around the BODIPY dye by reacting compound **4** with ethynyl-functionalized tetra(ethylene glycol) tosylate (**13**) in dry THF in the presence of sodium hydride. BODIPY dye (**I**) bearing branched oligo(ethylene glycol)methyl ether residues at positions 4 and 4′ was prepared by replacing the usual fluorine atoms of F-BODIPY dye (**15**) with ethynyl-functionalized oligo(ethylene glycol)methyl ether (**14**) via a Grignard reaction. BODIPY dye **J** bearing branched oligo(ethylene glycol)methyl ether residues at the positions 2 and 6 was prepared by palladium-catalyzed Sonogashira coupling of 2,6-diiodo BODIPY dye (**16**) with ethynyl-functionalized branched oligo(ethylene glycol)methyl ether (**14**) (Scheme 3). Both BODIPY dyes **I** and **J** are extremely soluble in water, and both are highly fluorescent in aqueous solution. Thus, introduction of side chain serves the dual purposes of aqueous solubility and inscreasing fluorescence quantum yields by reducing aggregation. BODIPY dye **I** with the side chains at the 4,4′-positions is considerably more fluorescent in PBS solution (fluorescence quantum yield 68%) than BODIPY dye **J** substituted at the 2,6-positions (fluorescence quantum yield 34%). So, substitution at the 4,4′-positions is more effective at preventing aggregation than 2,6-substitution (Table 1).

Scheme 3. Synthetic Route to BODIPY Dyes Bearing Branched Oligo(ethylene glycol)methyl Ether at 4,4'-, and 2,6-Positions



All BODIPY dyes (**A**−**J**) are easily soluble not only in water but also in common solvents. The absorption properties of BODIPY dye **A** in PBS solution are characterized by a strong $S_0 \rightarrow S_1$ ($\pi-\pi^*$) transition at 499 nm and a weaker broad band around 350 nm attributed to the $S_0 \rightarrow S_2$ ($\pi-\pi^*$) transition (Table

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Table 1. Absorption and Emission Maxima, and Fluorescence Quantum Yields of BODIPY Dyes in Organic Solvents and 0.5 M Phosphate Buffer Solution (PBS) (pH 7.4)[a]

BODIPY dye	solvent	absorption maximum (nm)	emission maximum (nm)	fluorescence quantum yield (%)
A	CH_2Cl_2	501	511	68.0
	ethanol	500	509	61.0
	PBS	499	510	4.2
B	CH_2Cl_2	499	508	81.0
	PBS	496	507	35.7
C	CH_2Cl_2	534	548	1.9
	PBS	533	550	1.2
D	CH_2Cl_2	539	552	47.9
	ethanol	539	554	43.0
	PBS	536	555	1.9
E	CH_2Cl_2	538	552	51.4
	PBS	534	550	8.9
F	CH_2Cl_2	504	514	91.5
	PBS	501	511	31.2
G	CH_2Cl_2	580	596	30.7
	PBS	577	598	21.2
H	CH_2Cl_2	659	676	6.1
	PBS	660	676	1.3
I	CH_2Cl_2	495	507	79.0
	PBS	491	501	68.0
J	CH_2Cl_2	547	563	39
	PBS	543	563	33.6

[a] Quantum yields of BODIPY dyes were determined by use of fluorescein (fluorescence quantum yield of 0.85 in 0.1 N NaOH) as a standard.[5]

1).[1a] Replacement of the usual fluorine atoms of BODIPY dye **A** with ethynyl subunits at 4,4′-positions causes slight blue shifts of BODIPY dye **B** in both absorption and emission compared with its precursor BODIPY dye **A**.[1b] These shifts may arise from decreased aggregation of BODIPY dye **B** in PBS solution due to the bulky ethynyl substituents, which also results in the observed increase in fluorescence quantum yield. Introduction of 2,6-diiodo substituents to the BODIPY dye **A** to give BODIPY **C** leads to large red shifts (33 and 38 nm) in both the absorption and fluorescence maxima, respectively, as 2,6-diiodo substituents function as auxochromes (Table 1). 2,6-Diethynylation of 2,6-diiodo-tetramethyl BODIPY dye (**C**) results in a slight red shift for BODIPY dye **D** due to the enhanced conjugation compared with BODIPY dye **A**. Replacement of the usual fluorine atoms of BODIPY dye **D** with methyloxy subunits at positions 4,4′ causes minor blue shifts of BODIPY dye **E** in both absorption and emission compared with its precursor BODIPY dye **D** (Table 1), which may arise from less aggregation of BODIPY dye **E** due to slightly enhanced steric hindrance from the methyloxy subunits. BODIPY dye **F** in PBS solution displays absorption and emission peaks at 501 and 511 nm, respectively. BODIPY dyes with monostyryl and distyryl substituents at 3, 3 and 5 positions (**G** and **H**) in PBS solution show significant red shifts in both absorption and emission due to their extended π-conjugation as compared with their precursor BODIPY dye (**F**). BODIPY dyes **G** and **H** in aqueous solution show absorption maxima at 577 and 660 nm and fluorescence maxima at 598 and 676 nm, respectively (Table 1 and Supporting Information). BODIPY dye **I** in aqueous solution shows absorption and fluorescence maxima at 491 and 501 nm, respectively. Absorption and fluorescence spectra of BODIPY dye **J** in PBS solution are red-shifted with absorption and fluorescence maxima at 543 and 563 nm, respectively, because of its extended π-conjugation compared with those of its precursor BODIPY dye (**15**) in methylene chloride.

In conclusion, we have developed effective ways to prepare highly water-soluble neutral BODIPY dyes with emissions from green to deep red regions through functionalization of BODIPY dyes at positions 8, 2 and 6 or 4 and 4′ with branched oligo(ethylene glycol)methyl ether residues. Fluorescence quantum yields of BODIPY dyes can be manipulated by introducing branched oligo(ethylene glycol)methyl ether residues to an ortho position on the meso-phenyl ring of BODIPY dyes, replacing the fluorine atoms of BODIPY dyes at 4,4′-positions with methyloxy or ethynyl subunits or attaching branched oligo(ethylene glycol)methyl ether residues to positions 2 and 6 of BODIPY dyes.

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Supporting Information Available: Detailed synthesis, characterization, and optical properties of BODIPY dyes. This material is available free of charge via the Internet at http://pubs.acs.org.

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