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Abstract

BODIPY which consists of a dipyrromethene complex with disubstituted boron has emerged as a superior fluorophore in various research fields. BODIPY typically shows high quantum yield with environment-insensitive fluorescence emission, sharp excitation and emission peaks, high water solubility and biocompatibility, and photostability. So far, various kinds of BODIPY derivatives have been developed and applied in not only academia such as chemistry, biochemistry, biomedical engineering, and medicine but also industries. BODIPY shows dramatic photophysical property changes upon substitution of functional groups or pi bond elongation on the main core structure. Among them, the blue-emitting BODIPY dyes with their synthesis and photophysical analysis were recently reported. In this chapter, the key information of the blue-emitting BODIPY dyes and their recent cutting-edge applications are summarized.

Keywords: BODIPY dyes, fluorophore, blue-emitting, bioimaging, probe

1. Introduction

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (boron-dipyrromethene, abbreviated as BODIPY) is a small molecule that emits strong fluorescence with relatively environment-insensitive photophysical property and reasonably high stability in biological conditions (see their structure and numbering in Figure 1) [1, 2]. To date, many BODIPY-based fluorescence dyes, molecular probes, and protein-labeling reagents have been developed by tuning of their fluorescence character and functionality [3]. The emission wavelength is readily tunable by modification of the BODIPY framework (BDP, Figure 1) according to purpose. In progress, various kinds of the expanded or combined structure of BODIPY derivatives which show a fluorescence emission peak in the red- or near-infrared (NIR) region have been developed for bioimaging applications [4]. Recently, in vitro fluorescence study of specific analytes and its
bioimaging applications in the blue wavelength region also came into the highlight due to the multi-color analysis purpose [5]; thus, the preparation and photophysical property analysis of blue-emitting BODIPY derivatives received attention. In this chapter, a brief explanation of blue-emitting BODIPY derivatives with photophysical properties, their synthetic method, and recently reported applications such as fluorescent labeling and molecular probes for monitoring biologically important species are described.

2. Blue-emitting BODIPY dyes

Generally, BODIPY dyes show fluorescence emission in the green region (500–530 nm) [2]. The fluorescence emission wavelength of BODIPY can be exquisitely controlled by appropriate...
substitution of chemical moieties such as aliphatic carbon, aromatic ring, pi-conjugation, halide element, and electron pushing-/donating-group. The pi-bond elongation on the alpha (α)- and beta (β)-positions of BODIPY core gives the red-shifted fluorescence emission wavelength, and the addition of nitrile (CN) moiety on the meso-position (8-position) also gives red-shifted emission [1]. In contrast, the preparation of blue-emitting BODIPY dyes is very limited to few approaches: (i) substitution of electron donating moiety (amine, alkoxy) on the meso-position of BODIPY (Figure 1b, c) and (ii) aza-/diaza-BODIPY (BTAA in Figure 1d), imidazole-/thiazole-based BODIPY (Figure 1d).

2.1 Photophysical properties of blue-emitting BODIPY dyes

2.1.1. 8-Amino-BODIPY

In 2007, Biellmann and co-workers reported the synthesis of 8-heteroatom-substituted BODIPY derivatives including 8-thiomethyl-BODIPY (8-SMe-BODIPY), 8-vinylid-thioether-BODIPY (8-VT-BODIPY), and 8-amino-BODIPY (Figure 1a) [6]. The thiomethyl group of 8-SMe-BODIPY displayed high reactivity for the nucleophilic substitution reaction. In the follow-up study, Peña-Cabrera and co-workers found that the substitution reaction of thiomethyl group and amine moiety proceed with high yield (>90%), and the resulting products showed bright blue fluorescence (Figure 1b, Table 1) [7, 8].

The primary-amine product, 8-AB, is characterized by maximum absorption and emission wavelengths at 399 and 437 nm with high-fluorescence quantum yield (0.92). Interestingly, the secondary-amine products, 8-PAB (propargyl amine substituted) and 8-MAB (monomethylamine substituted), show different photophysical properties each other, particularly quantum yield. Both emit fluorescence in the blue wavelength region, at 405 and 394 nm, upon excitation at 464 and 440 nm, respectively. However, 8-MAB gives lower quantum yield (0.1) than 8-PAB (0.52), because the higher electron-donating capacity of the amine group toward BODIPY core leads to a higher instability of the LUMO orbitals as well as enhancement of the non-radiative decay processes [7, 8]. For 8-PAB, the electron-deficient alkyne moiety decreases the electron-donating ability of amine; thus, 8-PAB keeps higher fluorescence quantum. The tertiary amine product, 8-DMAB, shows a similar photophysical property of 8-MAB.

The aniline derivative, 8-PhB, shows less blue-shift of the absorption and emission wavelengths than aliphatic amine-substituted derivatives due to the less electron-donating ability of substituents, like 8-PAB.

2.1.2. 8-Alkoxy-BODIPY

Hydroxyl group-substituted 8-alkoxy-BODIPY, 8-OH-B, was identified by Ahn and co-workers in 2012 while developing a molecular probe for mercury ions using 8-SMe-BODIPY [9]. 8-OH-B showed the most blue-shifted absorption and emission wavelengths from the BODIPY core at 370 and 490 nm, respectively, but the quantum yield and other photophysical properties were not characterized due to its low stability. The other 8-alkoxy-BODIPY derivatives were reported by Peña-Cabrera, Boens, and co-workers in 2013 [10–12]. The significant blue shift of absorption and emission was not observed for these derivatives, probably due to the less electron-donating ability of their substituent.
2.1.3. BTAA

Difluoro-boron-triaza-anthracene complex (BTAA) was reported by Arbeloa and co-workers in 2011 [13]. Newly synthesized diaza-BODIPY-type derivative, BTAA, showed an absorption and fluorescence emission peak at 384 and 398 nm, respectively, with high quantum yield (0.43). Their systematic analysis data represented the environment-insensitive photophysical property of BTAA. The properties of other derivatives (2,6-IM, TM, SM) were predicted by quantum mechanical calculation in their report, not by the experiments.

2.2. Synthesis of blue-emitting BODIPY dyes

2.2.1. 8-Amino-BODIPY/8-alkoxy-BODIPY

For the 8-amino-BODIPY derivatives or 8-alkoxy-BODIPY derivatives, 8-SMe-BODIPY has been used as a starting material (Figure 2a). 8-SMe-BODIPY can be prepared by three-step synthesis: (i) reaction of pyrrole with thiophosgene, (ii) methylation of the intermediate using methyl iodide, and (iii) boronation of methylated intermediate using boron trifluoride in the presence of organic base (triethylamine) [7, 9]. The thiomethyl (-SMe) moiety at the meso-position of 8-SMe-BODIPY acts as a good leaving group to prepare N– and O–based nucleophilic substitution reaction with excellent reactivity. Thus, 8-SMe-BODIPY can be converted in a $S_N$Ar-like process by amines or alkoxy moieties to produce meso-amine-substituted

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\text{emi}}$ (nm)</th>
<th>Q.Y.</th>
<th>Solvent</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDP</td>
<td>497.0</td>
<td>507.0</td>
<td>0.87</td>
<td>MeOH</td>
<td>[8, 11]</td>
</tr>
<tr>
<td>8-PAB</td>
<td>405.0</td>
<td>464.5</td>
<td>0.52</td>
<td>MeOH</td>
<td>[7, 8, 14]</td>
</tr>
<tr>
<td>8-AB</td>
<td>399.0</td>
<td>437.5</td>
<td>0.92</td>
<td>MeOH</td>
<td>[8]</td>
</tr>
<tr>
<td>8-MAB</td>
<td>394.5</td>
<td>440.0</td>
<td>0.10</td>
<td>MeOH</td>
<td>[8, 11]</td>
</tr>
<tr>
<td>8-DMAB</td>
<td>395.8</td>
<td>438.0</td>
<td>0.99</td>
<td>MeOH</td>
<td>[8]</td>
</tr>
<tr>
<td>8-PhB</td>
<td>403.5</td>
<td>461.0</td>
<td>0.16</td>
<td>MeOH</td>
<td>[10, 12]</td>
</tr>
<tr>
<td>8-OH-B</td>
<td>370.0</td>
<td>409.0</td>
<td>n.r.</td>
<td>Buffer/ACN</td>
<td>[9, 15]</td>
</tr>
<tr>
<td>8-OMe-B</td>
<td>441.0</td>
<td>484.0</td>
<td>0.85</td>
<td>MeOH</td>
<td>[11, 12, 16]</td>
</tr>
<tr>
<td>8-OPh-B</td>
<td>459.0</td>
<td>495.0</td>
<td>0.97</td>
<td>c-Hex</td>
<td>[16]</td>
</tr>
<tr>
<td>BTAA</td>
<td>384.0</td>
<td>398.4</td>
<td>0.43</td>
<td>MeOH</td>
<td>[13]</td>
</tr>
<tr>
<td>2,6-IM/TM/SM$^a$</td>
<td>n.r.</td>
<td>400–430</td>
<td>n.r.</td>
<td>n.r.</td>
<td>[13]</td>
</tr>
</tbody>
</table>

The numbers indicate the highest-intensity wavelengths for the absorption and fluorescence emission spectra in the described solvent. Abbreviations: Q.Y.: quantum yield, n.r.: not reported, MeOH: methanol, c-Hex: cyclohexane, buffer: HEPES buffer (10 mM, pH 7.4), ACN: acetonitrile.

$^a$Predicted blue-emitting BODIPY derivatives from quantum mechanical calculation.

Table 1. Photophysical properties of blue-emitting BODIPY dyes.

2.1.3. BTAA

Difluoro-boron-triaza-anthracene complex (BTAA) was reported by Arbeloa and co-workers in 2011 [13]. Newly synthesized diaza-BODIPY-type derivative, BTAA, showed an absorption and fluorescence emission peak at 384 and 398 nm, respectively, with high quantum yield (0.43). Their systematic analysis data represented the environment-insensitive photophysical property of BTAA. The properties of other derivatives (2,6-IM, TM, SM) were predicted by quantum mechanical calculation in their report, not by the experiments.

2.2. Synthesis of blue-emitting BODIPY dyes

Synthesis of blue-emitting BODIPY dyes follows established synthetic routes (Figure 2).

2.2.1. 8-Amino-BODIPY/8-alkoxy-BODIPY

For the 8-amino-BODIPY derivatives or 8-alkoxy-BODIPY derivatives, 8-SMe-BODIPY has been used as a starting material (Figure 2a). 8-SMe-BODIPY can be prepared by three-step synthesis: (i) reaction of pyrrole with thiophosgene, (ii) methylation of the intermediate using methyl iodide, and (iii) boronation of methylated intermediate using boron trifluoride in the presence of organic base (triethylamine) [7, 9]. The thiomethyl (-SMe) moiety at the meso-position of 8-SMe-BODIPY acts as a good leaving group to prepare N– and O–based nucleophilic substitution reaction with excellent reactivity. Thus, 8-SMe-BODIPY can be converted in a $S_N$Ar-like process by amines or alkoxy moieties to produce meso-amine-substituted
BODIPYs (8-amino-BODIPY) or meso-alkoxy-substituted BODIPYs (8-alkoxy-BODIPY) in mild reaction conditions with high yield [16, 17].

2.2.2. BTAA

In 2011, Arbeloa and co-workers reported the synthesis of BTAA dye. They successfully synthesized BTAA via boron complex formation of N-(2-pyridinyl)-2-pyridinamine with boron trifluoride in toluene solvent with organic base (triethylamine) with high yield (80%) (Figure 2b) [13].

3. Application of blue-emitting BODIPY dyes

Blue-emitting BODIPY derivatives have been used in various research areas. In particular, their unique photophysical property serves feasible application in biological study. Recently, a few examples of notable applications, such as fluorescent labeling and molecular sensing probes, were reported using blue-emitting BODIPY derivatives. As described above, fluorescence imaging in shorter wavelengths (blue-channel) is undoubtedly an essential and useful tool in biological study, because it can be used to avoid the interference of general fluorescent materials which have emission in green and red channels. Moreover, the high quantum yield with negligible solvent/media-dependence of BODIPY allows bioimaging of targeting substrate efficiently.

In this chapter, recently reported fluorescent labeling probes and molecular sensing probes based on blue-emitting BODIPY are summarized.
3.1. Labeling

Labeling of biomolecules with a signaling unit such as a fluorophore or radioisotope is an essential tool for studying molecular interactions in biological systems [18, 19]. In particular, a labeling technique based on fluorophore has received great attention because it enables researchers to monitor specific components in a complex biological environment [18].

A few blue-emitting dyes have been reported for biomolecule labeling, mainly based on coumarin backbone, which has drawbacks such as environment-sensitive fluorescence change. To overcome this issue, new labeling probes based on blue-emitting BODIPY have been recently reported.

In 2013, Peña-Cabrera and co-workers presented the fluorescent tagging of alcoholic and phenolic biomolecules using 8-SMe-BODIPY via a $S_N$Ar-type reaction (Figure 3) [16]. They demonstrated the labeling of cholesterol (alcoholic) and estrone (phenolic) at the meso-position of BODIPY core in the presence of CuTC (Copper(I) thiophene-2-carboxylate) and sodium bicarbonate. Interestingly, the cholesterol-labeled product gives high blue fluorescence regardless of the media at 485 nm, but the estrone-labeled product gives poor fluorescence at 488 nm in polar solvents due to intramolecular charge transfer (ICT) [20] quenching.

For protein labeling, Kim and co-workers reported new approaches in 2017 (Figure 3). They found that 8-SMe-BODIPY could be useful for protein labeling in mild conditions via $S_N$Ar-type reaction toward the lysine residues that have a secondary amine moiety [21]. As a model protein, a lysozyme (six lysine in the total 129 amino acid) undergo the substitution reaction, and the resulting product shows bright blue fluorescence, maximum absorption and emission at 375 and 409 nm, respectively. In the course of their ongoing research using 8-SMe-BODIPY, they developed a bio-conjugalatable group containing blue-emitting 8-amino-BODIPY derivatives (BP-1–BP-4, Figure 3) [22]. They demonstrated the labeling of bovine serum albumin (BSA) using BP-2 by amide-bond formation and BP-3 by thiol-ene addition reaction. The labeling was successfully proceeded in a mild condition, and the resulting products showed bright blue fluorescence and absorption and emission maximums at 390–402 nm and 462–465 nm in deionized water, respectively.

In 2018, Chang and co-workers reported that 8-amino-BODIPY derivatives containing the azide and cyclooctyne moiety (AzA-1, COA-1, Figure 3) are applicable for copper-free click chemistry [23, 24]. The probes, namely “tame probes,” show high biocompatibility with no background noise after labeling in live cells.

3.2. Fluorescent probes

3.2.1. Metal ions (mercury ions; Hg$^{2+}$, zinc ions; Zn$^{2+}$)

Monitoring of metal ions in the biological system is very important to understand molecular interactions and processes [25]. So far, various kinds of monitoring techniques for metal ions have been developed which mostly depend on expensive instruments. Recently, new approaches based on fluorescence are highlighted for analyte sensing because of their ease of use, low cost, high efficiency, and biocompatibility [26–28].
For toxic metal ion analysis, a few fluorescent probes based on blue-emitting BODIPY have been introduced, particularly for mercury ion. Mercury ion is one of the poisonous elements to environmental and biological systems. It can readily penetrate biological membranes and cause serious damage to the central nervous system (CNS) [29].

In 2012, Ahn and co-workers reported a new fluorescent probe for mercury ions based on 8-SMe-BODIPY which shows a ratiometric fluorescence behavior (Figure 4) [9]. The mercury ion promoted hydrolysis of thiomethyl (-SMe) at the meso-position of BODIPY core and generated 8-hydroxy-BODIPY (8-OH-B). In the sensing media of this work (HEPES buffer, 10% acetonitrile), 8-SMe-BODIPY shows an absorption maximum at 485 nm and an emission maximum at 525 nm, whereas the hydrolyzed product 8-OH-B shows blue-shifted absorption and emission peaks at 370 and 409 nm, respectively. Strong blue emission was only observed upon adding mercury ions among the other metal ions. However, the low chemical stability of 8-OH-B was observed in the NMR study.

Turn-on-type fluorescent probe based on blue-emitting BODIPY for mercury ion was also reported in 2016. Zhao and co-workers prepared an 8-amino-BODIPY linked to a thiourea unit which can be hydrolyzed via mercury ion-promoted cyclization (Hg Probe 1, Figure 4) [15]. Hg Probe 1 showed absorption and emission maximums at 400 and 465 nm in PBS buffer solution (0.5% DMSO) with very weak fluorescence. Upon adding mercury ions, a new
absorption peak appeared at 370 nm, and fluorescence was dramatically increased with a new emission peak at 420 nm. The peak difference of 8-OH-B in Ahn’s study and this work seems to be coming from the sensing media.

A coordination-based fluorescent probe using 8-amino-BODIPY for the zinc ion was reported by Peña-Cabrera and co-workers in 2017 [30]. In this study, they have synthesized several aza-crown 8-amino-BODIPY derivatives and analyzed their photophysical properties. Among them, the compound 5 gives the turn-on property toward zinc ions with emission maximum at 429 nm when excited at 330 nm (Figure 4).

3.2.2. Amino acid (cysteine; Cys, homocysteine; Hcy)

Biothiols such as cysteine (Cys) and homocysteine (Hcy) play crucial roles in the balance of biological system. The concentration of Cys and Hcy is associated with many diseases such as cancer, Alzheimer’s disease (AD), Parkinson’s disease (PD), osteoporosis, diabetes, and hematopoiesis decrease [31].

Recently, Ahn and co-workers reported a Cys/Hcy-selective probe (Figure 5, upper) [32] and a Cys-selective probe (Figure 5, bottom) [33], based on 8-amino-BODIPY.

For Cys/Hcy sensing, they used 8-SMe-BODIPY by mimicking the native chemical ligation strategy [32]. The methylthio group might be readily exchanged with thiol moiety in Cys/Hcy,
and the amine moiety in their amino acid backbone could further undergo intramolecular displacement to give the corresponding 8-amine-BODIPY (Figure 5). The original emission peak of 8-SMe-BODIPY at 524 nm disappeared upon adding Cys/Hcy, while a new peak in the shorter wavelength at 467 nm appeared which corresponded with 8-amine-BODIPY. After confirming selectivity and sensitivity, they applied the probe for the bioimaging of biothiols in living species, zebrafish. A brighter blue emission was observed from all the organs than the green emission, demonstrating the ratiometric fluorescence bioimaging application of blue-emitting BODIPY.

A fluorescence resonance energy transfer (FRET)-based ratiometric-type probe for Cys was also reported [33]. In the course of their ongoing research using 8-amine-BODIPY, they prepared a FRET couple between fluorescein (FITC, green emission) and 8-amine-BODIPY (P1, Figure 5). The FITC linked with diacrylate showed no fluorescence, thus P1 gives only blue fluorescence at 452 nm when excited at 400 nm. Upon treatment with Cys, the diacrylate moiety could be hydrolyzed in aqueous media and generate fluorescent FITC. A new emission band appeared at 520 nm when excited at 400 nm, indicating FRET. P1 has been applied for the analysis of Cys level in cell lines (B16F10, Rat1, N2A, HeLa, C6, and HT22) as well as human plasma.

3.2.3. Chemical warfare (phosgene gas)

Chemical warfare (CW) is the use of toxic chemical substances as weapons [34]. Among them, phosgene is a kind of colorless gas and a highly lethal chemical warfare. It reacts with the amine species and generates cross-linking by urea formation. This reaction can occur with proteins in the pulmonary alveoli, the site of gas exchange, and destroy the barrier of blood-air, causing suffocation [35]. Thus, a rapid and facile method for detecting phosgene is required. Fluorescence method possesses advantages in terms of high sensitivity with fast responsibility and real-time analysis for phosgene.

![Figure 5. Reported 8-amino-BODIPY-based fluorescent probes for amino acid: cysteine (Cys), homocysteine (Hcy).](image-url)
To date, various kinds of fluorescent probes for phosgene have been introduced [36]. In 2017, Tian and co-workers reported a phosgene probe based on 8-amino-BODIPY (8-EDAB, Figure 6) [37]. Phosgene reacts with primary amine moiety in 8-EDAB, which undergoes a fast intramolecular cyclization reaction (phosgene-mediated acidylation) to afford a urea-containing 8-amino-BODIPY. The 8-EDAB emitted blue fluorescence at 445 nm with low quantum yield (0.15) upon 390 nm excitation, probably due to the intramolecular charge transfer (ICT) quenching of secondary amine toward BODIPY core. Upon addition of phosgene, a new emission peak at 512 nm appeared upon 465 nm excitation with high quantum yield (0.65) and fast response (<1.5 sec) in sensing media (acetonitrile, 80 nM triethylamine). In addition, 8-EDAB showed sub-nanomolar detection limit (0.12 nM) for phosgene with high sensitivity.

In the same year, Song and co-workers reported a similar approach using ortho-phenylenediamine (OPD)-introduced BODIPY for phosgene sensing (o-Pab, Figure 6) [38]. Unlike the ethylenediamine-substituted fluorescent probe as described above (8-EDAB), o-Pab shows no fluorescence in sensing media (chloroform, 1% triethylamine) due to photoinduced electron transfer (PET) quenching [39] from OPD to the BODIPY core and rotational deactivation along the aryl-amine-aryl single bonds. Fluorescence spectra of o-Pab exhibit a turn-on response, with an emission peak at 530 nm upon 450 nm excitation. Although the OPD-substituted 8-amino-BODIPY does not show blue emission, their experimental data give information to understand the basic photophysical property of meso-substituted BODIPY derivatives.

3.2.4. Lipid membrane

Lipid membranes (mono-/bi-layers, vesicles, biological membranes) form a barrier around all cells and play important roles in almost all living organisms as well as viruses [40, 41]. To date,
the staining of lipid membrane using fluorescence agents has been widely used for biological studies. Membrane targeting protein- or peptide-conjugated fluorophore and long aliphatic carbon chain-conjugated fluorophore are representative approaches. In the same vein, Ameloot and co-workers developed a new blue-emitting lipid membrane probe based on 8-amino-BODIPY linked with a long carbon chain (C_{18}-alkyl) at the 3,5-position and taurine substituent at the meso-position (Probe 1, Figure 7) \cite{42}. Probe 1 shows blue fluorescence in the range between 480 and 494 nm with high quantum yield (0.38–0.93) upon excitation with 415–429 nm, in the various solvents. The lipid membrane-staining and fluorescence-imaging applications were successfully carried out for small unilamellar vesicles, giant unilamellar vesicles, and biological cells (OLN-93 cells) using one-/two-photon microscopy.

4. Summary and outlook

In this chapter, the basic photophysical property, synthetic method, and application of blue-emitting BODIPY derivatives are introduced. The amino- or alkoxy-moiety substitution at the meso-position of BODIPY core is expected to provide a shorter excitation and emission wavelength at the blue region with high quantum yield, and the systematic analysis results have given evidences. In addition, their superior photostability and chemical stability with facile functioning give many possibilities for developing fluorescent tagging reagents and molecular probes to monitor biologically important species. Most of the applications using blue-emitting BODIPYs were carried out very recently; therefore, we believe that this summary will be helpful for the beginner who wishes to study the fluorophore/fluorescent probe and will inspire scientists to develop many useful systems with practical applications.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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