



Nanoparticles via H-aggregation of amphiphilic BODIPY dyes

Yuichiro Tokoro, Atsushi Nagai, Yoshiki Chujo *

Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

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ABSTRACT

We have synthesized wedge-shaped BODIPY dyes with hydrophilic chains and studied their aggregational behavior in THF-containing water by UV–vis and photoluminescence spectroscopies, transmission electron microscopy, and dynamic light scattering. One of these dyes showed an aggregate derived from H-dimers and forms a high density nanoparticle in THF-containing water solution.

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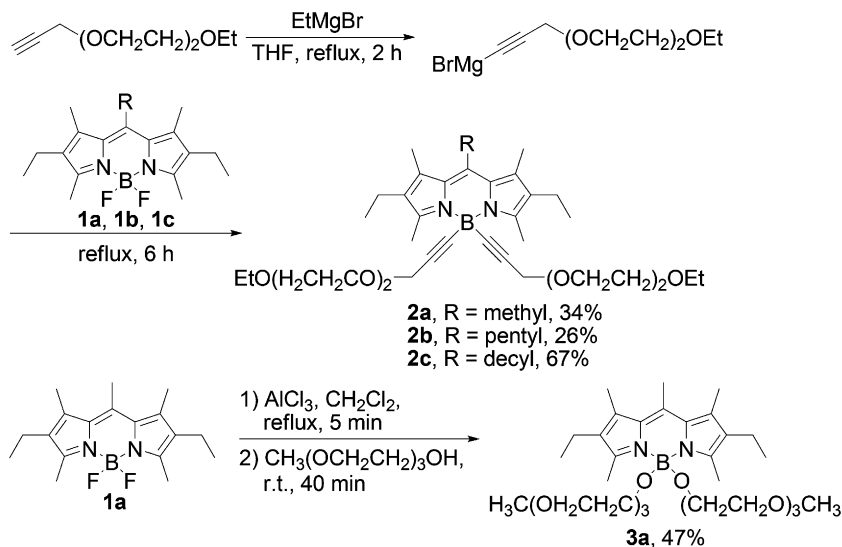
4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dyes are an important class of highly luminescent materials that have been found in widespread applications for biochemical labeling, photonic molecular systems, laser dyes, organogelators, and light-emitting devices because they tend to be strongly UV-absorbing molecules that emit relatively sharp fluorescence peaks with high quantum yields.¹ The absorption spectrum of BODIPY exhibits an intense $S_0 \rightarrow S_1$ transition with its maximum at 505 nm due to a large transition dipole on the line which links 2-position with 6-position of the BODIPY core.² They are relatively insensitive to the polarity and pH of their environment and are reasonably stable under physiological conditions.³

The BODIPY dyes show the tendency to form aggregates and a very low solubility in polar solvents. Monomeric BODIPY dyes can stack in two geometrically distinct forms: H- and J-dimers.⁴ The former is most likely a result of intermolecular dimerization. In the H-dimer, two BODIPY planes stack with both almost parallel $S_0 \rightarrow S_1$ transition dipoles and antiparallel electric dipole moments. This dimer is practically non-fluorescent and exhibits blue-shifted absorption relative to that of the monomer. The J-dimer, in which the $S_0 \rightarrow S_1$ transition dipoles are oriented in planes at 55° , is fluorescent and shows a red-shifted absorption relative to that of the monomer. Dimers of BODIPY form only upon orbital contact, that is, at distances within a few Ångström, so that these dimers are expected to be applied for understanding folding mechanisms of proteins, which are derived from hydrophobic interaction, by detection of two close sub-regions labeled by BODIPY.⁵

Hydrophilic and hydrophobic interactions are important not only for protein foldings but also for self-assembly of amphiphilic molecules, consisting of hydrophilic and hydrophobic moieties.⁶ The self-assembly results in highly organized aggregates with various morphologies such as spheres, rods, planar bilayers, vesicles, and others. The formation of these morphologies depends on solvent environments, molecular structure, and shapes, as well as the relative fraction of hydrophilic and hydrophobic parts. Some examples of BODIPY dyes with hydrophilic part have been reported.^{3,7} Although these dyes seem amphiphilic molecules, their solubility in polar solvents was so high that the aggregation or the self-assembly of BODIPY dyes was prevented. Here, we report the synthesis of BODIPY dyes moderately soluble in the polar solvents and the first case of nanoparticles formed by the hydrophilic interaction and the BODIPY's strong aggregation.

Initially, F-BODIPYs (**1**) as precursors of PEG-yne-BODIPYs (**2**) were prepared through the condensation of acyl chlorides with 2,4-dimethyl-3-ethylpyrrole in dry CH_2Cl_2 followed by chelation with an excess boron trifluoride diethyl etherate in the presence of triethylamine.⁸ As shown in Scheme 1, PEG-yne-BODIPYs (**2a–c**) were obtained by displacement of fluoride from F-BODIPYs (1 equiv) in tetrahydrofuran (THF) under reflux conditions using 3-(2-(2-ethoxyethoxy)ethoxy)-1-propynylmagnesium bromide, which was prepared from the Grignard change reaction of ethylmagnesium bromide in THF (1.0 M, 2.2 equiv) and 3-(2-(2-ethoxyethoxy)ethoxy)-1-propyne (2.4 equiv).⁹ These compounds (**2a–c**) were purified by silica gel column chromatography eluted with hexane/ethyl acetate. The compound **2a** was a red solid. In contrast, the compounds **2b** and **2c** having long alkyl chains were obtained as red viscous liquids. The tetra-coordination state of the boron atoms in **2** was confirmed by the ^{11}B NMR spectroscopy in

* Corresponding author. Tel.: +81 75 383 2604; fax: +81 75 383 2605.
E-mail address: chujochujo@synchem.kyoto-u.ac.jp (Y. Chujo).



Scheme 1. Synthesis of amphiphilic BODIPYs.

CDCl_3 [**2a**: $\delta_{\text{B}} = -13.98$ ppm, **2b**: $\delta_{\text{B}} = -14.07$ ppm, **2c**: $\delta_{\text{B}} = -14.07$ ppm]. The chemical shifts of **2a–c** are upfield-shifted compared to those of F-BODIPYs (**1a–c**; around 0 ppm), indicating that fluorides on the boron were replaced by sp-carbons whose electronegativity is less than that of fluoride. The compounds **2a–c** were fusible and soluble in common organic solvents including tetrahydrofuran (THF), acetone, ethyl acetate, and chloroform, whereas, low solubility in water. To examine the role of sp-carbons connecting the BODIPY core with the hydrophilic chain, PEG-BODIPY (**3a**) was also synthesized by the treatment of F-BODIPY (**1a**) with 2-[2-(2-methoxyethoxy)ethoxy]ethanol in the presence of AlCl_3 .¹⁰ The obtained compounds were red liquids and soluble in THF, acetone, chloroform, and water.

The UV–vis absorption and photoluminescence experiments of PEG-yne-BODIPYs and PEG-BODIPY were carried out in THF and THF-containing water (1.0×10^{-5} M). In THF, all compounds showed strong and sharp absorption maxima at ~ 517 nm assignable to the $\text{S}_0 \rightarrow \text{S}_1$ transition of the monomeric BODIPY core (Fig. 1) and showed almost the same emission maxima at ~ 532 nm, which was excited at absorption maxima. Meanwhile, in THF-containing water, an increase of the water ratio is related to a decrease of molar absorption coefficient at the absorption maxima. The absorption spectra of **2a** display an appearance of a new peak at 465 nm when the ratio of THF is below 5% (v/v)

(Fig. 1A). Moreover, an isosbestic point was observed at 480 nm, indicating that H-dimers of **2a** grew as the solvent's polarity increased (Fig. 2). The H-dimers probably concentrate the hydrophobic cores at the center and extend the hydrophilic chains to the surrounding polar solvent. The absorption spectra of **2b** and **2c** show no appearance of new peak derived from H-dimers, and the wavelengths of the absorption maxima in THF-containing water (below 5%) were slightly longer than those in THF (Figs. 2 and 3). This would mean that the BODIPY cores of **2b** or **2c** cannot stack with parallel $\text{S}_0 \rightarrow \text{S}_1$ transition dipoles due to the long alkyl chain at the *meso* position. The compounds **2b** and **2c**, however, aggregate in the way their cores interact weakly.¹¹ The peak shift of **2b** appeared when the ratio of THF in the solvent was below 10%, whereas that of **2c** appeared when the ratio of THF was below 20%. It seems to be that the hydrophobic chains on the *meso* positions of BODIPYs mainly cause their peak shifts. Figure 4 illustrates the concentration dependence of BODIPYs (**2a** and **2b**) exhibiting H-dimer formation. The UV–vis spectra of **2a** and **2b** in diluted THF-containing water solution [2% (v/v), 1.0×10^{-6} M] are resemblant to that of the monomeric BODIPY dye, so that the aggregation requires the moderately high concentration (above 1.0×10^{-5} M). In contrast to PEG-yne-BODIPYs, PEG-BODIPY (**3**) exhibited small change in absorption spectra by the ratio of water and THF (Fig. 5). It can be deduced that the relatively large hydrophilic part

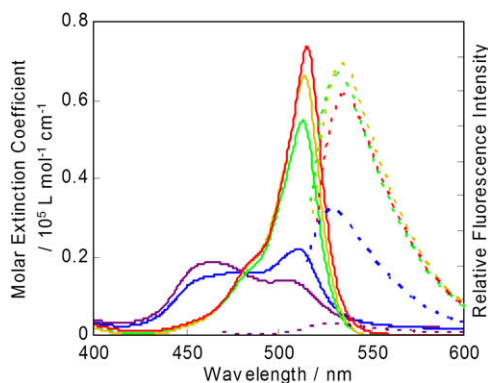


Figure 1. UV–vis spectra (solid lines) and emission spectra (dotted lines) of **2a** in THF-containing water (1.0×10^{-5} M). The ratios of THF (v/v) in the solvents are 2% (purple), 5% (blue), 10% (green), 20% (yellow), and 100% (red).

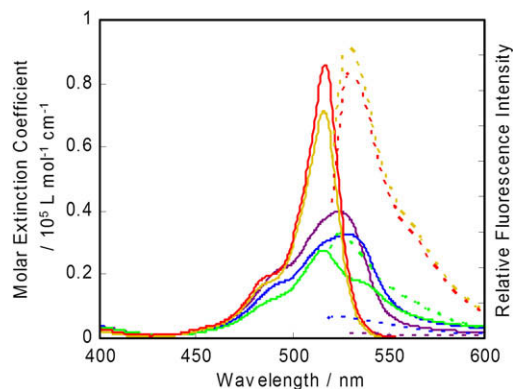


Figure 2. UV–vis spectra (solid lines) and emission spectra (dotted lines) of **2b** in THF-containing water (1.0×10^{-5} M). The ratios of THF (v/v) in the solvents are 2% (purple), 5% (blue), 10% (green), 20% (yellow), and 100% (red).

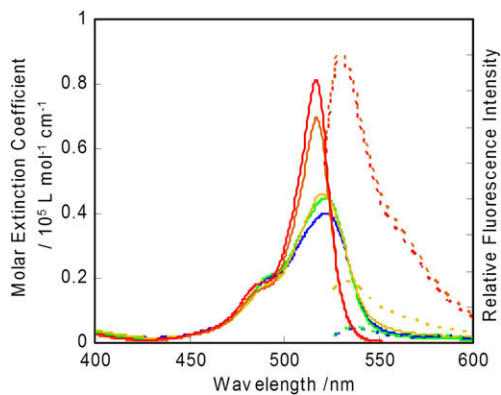


Figure 3. UV-vis spectra (solid lines) and emission spectra (dotted lines) of **2c** in THF-containing water (1.0×10^{-5} M). The ratios of THF (v/v) in the solvents are 5% (blue), 10% (green), 20% (yellow), 30% (orange), and 100% (red).

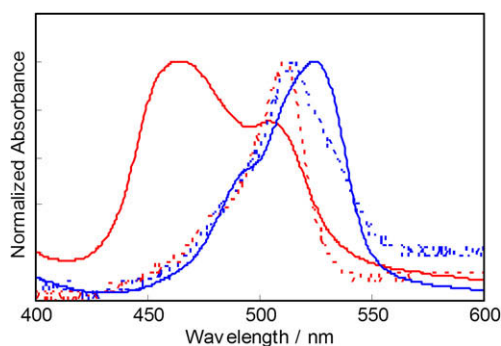


Figure 4. UV-vis spectra of **2a** (red) and **2b** (blue) in THF-containing water. The concentrations are 1.0×10^{-5} M (solid lines) and 1.0×10^{-6} M (dotted lines). The ratio of THF (v/v) in the solvents is 2%.

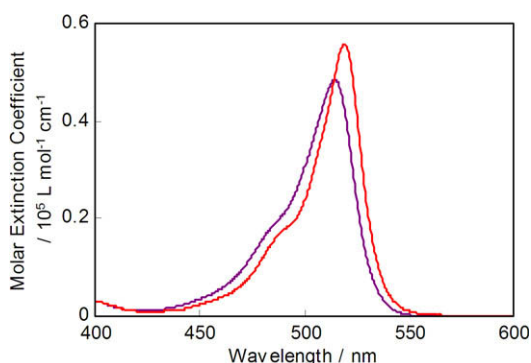


Figure 5. UV-vis spectra of **3a** in THF-containing water (1.0×10^{-5} M). The ratios of THF (v/v) in the solvents are 2% (purple) and 100% (red).

amplifies the solubility to the polar solvents, which disturbs the stacking to form dimer. In addition, the flexible hydrophilic chains, which have no rigid spacers like sp-carbons, probably refuse the wedge-shaped conformation of BODIPY molecules leading to the production of ordered aggregations. In fluorescence experiments of **2** (Fig. 1), while a decrease of THF in the solvent leads to a decrease of maximum intensity, the peak positions did not change, thereby implying that the aggregates exhibit no emission. The absolute fluorescence quantum yields of **2a** and **2b** in THF at room temperature were determined as $\Phi_F = 0.90$ and 0.84 , respectively, whereas those in THF-containing water [2% (v/v)] were determined as $\Phi_F = 0.30$ and 0.02 , respectively. This means that the monomeric **2a** slightly remains in THF-containing water [2% (v/v)], owing to

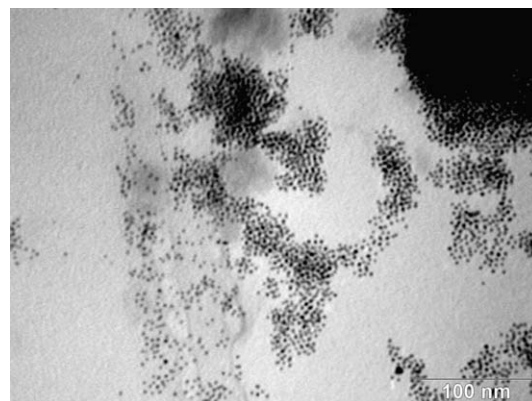


Figure 6. TEM micrograph of self-assembled **2a** dried at rt from THF-containing water (1.0×10^{-4} M). The ratio of THF (v/v) in the solvents is 2%.

the presence of a shoulder peak at around 515 nm as shown in Figure 1, and the non-fluorescent H-dimer reduces the fluorescence quantum yield. Moreover, the weak aggregates of **2b** are probably non-fluorescent.

Transmission electron microscopy (TEM) was used to visualize the assembly of **2a** after drying the solution in THF-containing water [2% (v/v), 1.0×10^{-4} M] on a grid. The TEM image of **2a** revealed the presence of spherical nanoparticles with the diameter of 3.90 ± 0.65 nm (Fig. 6). These nanoparticles are liable to aggregate to larger particles with the diameter of ~ 100 nm. Conversely, TEM images of **2b** and **2c** obtained by drying the solution in THF-containing water [2% (v/v), 1.0×10^{-4} M] on a grid showed no orderly high-density aggregates, thereby meaning that interactions of their molecules are too weak. From dynamic light scattering (DLS), the size distribution of nanoparticles of **2a** was 133.4 ± 34.4 nm (Fig. S12), which was consistent with that of the aggregated nanoparticles in the TEM image. Based on these results, it can be said that attractive interactions between the neutral hydrophilic chains make a trouble to disperse each nanoparticle.

In conclusion, the BODIPY's strong aggregation and the hydrophilic interaction drive the self-assembly of an amphiphilic BODIPY into size-controlled spherical nanoparticles. To form these nanoparticles, it is important that there are rigid spacers like sp-carbons between the BODIPY core and the hydrophilic chains and no long hydrophobic chain on the core. More detailed experiments are underway to inhibit the aggregation of nanoparticles and to make other shaped nanoaggregates.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.120.

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