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Synthesis and Optical Properties of Water-Soluble Biperylene-based Dendrimers

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Abstract

We report the synthesis and photophysical properties of three biperylene-based dendrimers, which show red fluorescence in water. Fluorescence microscopy study demonstrated uptake of biperylene-based dendrimers in living cells. Our results indicate that these biperylene-based dendrimers are promising in fluorescence imaging applications with the potential as therapeutic carriers.

Rylene derivatives are well-known fluorophores for their exceptional photochemical stability and high fluorescence quantum yields.^{1,2} As such, they have become attractive materials for various applications such as solar cells,³ laser dyes,⁴ organic light-emitting field-effect transistors,⁵ and optical switches.⁶ Recent years, some rylene derivatives were developed as versatile fluorescent probes for biological imaging.^{7–10} Rylene derivatives also have potential as photosensitizers for photodynamic therapy.¹¹ However, the rigid aromatic structures make rylene derivatives highly hydrophobic and prone to aggregate, especially in aqueous solutions, leading to fluorescence quenching.¹² To overcome this challenge, several strategies have been developed. One method is to attach charged groups, such as sulfonic acid and quaternized amine groups, to the bay region of the rylene rings. The charges on the rylene structure inhibited the intermolecular aggregation due to the electrostatic repulsion forces.¹³ Another strategy is to attach bulky substituents to rylene structures to shield π - π stacking.^{14,15} For example, Würthner et al. introduced dendronized polyglycerol to the end imide positions and successfully developed polyglycerol-dendronized perylene bisimide

dyes which are highly fluorescent in water.¹⁴ Water-soluble dendrons are efficient building blocks to increase hydrophilicity and reduce intermolecular stacking. In addition, dendrimers with surface functional groups are attractive biomaterials that allow for targeting capability, imaging applications and drug delivery.¹⁶ Inspired by these findings, we set out to develop a dendrimeric biperylene dye with the potential for biomedical imaging and therapy applications.

In our previous study, we developed a dendrimeric quaterrylenediimide dye by covalently attaching polyamide dendrons to the bay regions of a quaterrylenediimide structure.¹⁷ We have demonstrated that such strategy was effective in increasing hydrophilicity, reducing aggregation and introducing functionality. Using similar strategy, here we developed water-soluble and functional biperylene dyes with four polyamide-based dendrons (Scheme 1). Compared to quaterrylenediimide, biperylene has the advantage of reduced aggregation due to the fact that the two perylene units connected by C-C bond are twisted instead of locating in the same plane. We report herein the synthesis and photophysical properties of three biperylene-based dendrimers, BiPI-G0, BiPI-G1 and BiPI-G2, which possess four, twelve and thirty-six carboxylic acid groups on the surface. To our best knowledge, these are the first water-soluble, functional and dendrimeric biperylene dyes. To demonstrate the potential of these dendrimers in biomedical imaging, we imaged the uptake of the dendrimers in living cells using fluorescence microscopy. Together, these new biperylene-based dendrimers hold great promise for biomedical applications.

BiPI-G0 was synthesized by following the pathway shown in Scheme S1. The key precursor **3** was prepared by selective nucleophilic substitution of the two bromine atoms of **2** using potassium carbonate as the base. Due to the undesired substitution reaction on the 9-bromine atom of perylene **2**, the reaction yield was moderate (44%). Biperylene **4** was synthesized by Yamamoto homocoupling reaction of **3** and the following deprotecting reaction of *t*-butyl groups using trifluoroacetic acid afforded BiPI-G0 in a high yield (95%). BiPI-G0 was then used as the core structure to synthesize the two generations of dendrimers BiPI-G1 and BiPI-G2. Dendron **5** and **7** was conjugated to the four carboxylic acid groups of BiPI-G0 using HBTU/HOBt as the coupling reagent to yield **6** and **8** respectively. It is noteworthy that initial attempt of chromatographic purification of **6** and **8** using silica gel failed due to the difficulty of eluting the products off the column. Therefore, thin layer chromatography with large silica gel plates was used to purify **6** and **8**. Because of the loss during purification, **6** and **8** were obtained in low yields (51% and 30% respectively). At last, the termini of **6** and **8** were deprotected using trifluoroacetic acid resulting in BiPI-G1 and BiPI-G2 with 12 and 36 carboxylic acid groups, respectively. The complete deprotection was confirmed by the disappearance of the *t*-butyl protons at around 1.4 ppm in the ¹H NMR spectra.

Next, we studied the spectroscopic properties of BiPI dendrimers. As expected, the twisted structure of biperylene can efficiently inhibit intermolecular aggregation. Figure 1 shows the normalized absorption spectra change of BiPI dendrimers in different concentrations from 0.5 to 10 μ M in water. All three dendrimers showed strong broad absorption band in a region of 450–650 nm in water, with the maximum absorption at 535, 540 and 545 nm for BiPI-G0, BiPI-G1 and BiPI-G2 respectively. With the concentration increased, all three

dyes showed increased absorption intensity (Figure S1), whereas the band profiles of the dendrimers, including shape, position and molar extinction coefficients, showed negligible change as demonstrated in Figure 1. It has been reported that perylene dyes showed distinct absorption curves at different concentrations due to aggregation.¹⁴ These results indicate that BiPI-G0, BiPI-G1 and BiPI-G2 exist mainly as monomeric dye molecules in water at the concentration range from 0.5 to 10 μM .

The concentration-dependent fluorescence behavior of these biperylene dendrimers was also investigated. As illustrated in Figure 1, BiPI-G0, BiPI-G1 and BiPI-G2 showed broad fluorescence from 600 to 800 nm, with peaks at 663, 661 and 646 nm respectively. Similar to the concentration-dependent absorption results, the fluorescence intensity increased gradually with increasing concentration from 0.5 to 10 μM (Figure S1) and the emission band profiles remained almost the same at the concentrations studied.

To further verify the inhibited dye aggregation due to the twisted configuration of BiPI dyes, we studied temperature-dependent absorption and emission profiles. Similar to the concentration-dependent results, the absorption and emission spectra profiles of all three dyes showed insignificant change when temperature was increased from 20 $^{\circ}\text{C}$ to 70 $^{\circ}\text{C}$ (Figure 2), indicating that no significant aggregation existed. We also found that the fluorescent peaks of BiPI-G0 and BiPI-G1 showed slight blue-shift as well as intensity decrease (Figure 2 and S2); while the fluorescent intensity of BiPI-G2 decreased dramatically when temperature increased (Figure S2), which is likely due to the significantly increased nonradiative decays of the highly flexible system of BiPI-G2 at high temperatures.^{18,19}

When dissolved in water at a concentration of 1 μM , BiPI-G0, BiPI-G1 and BiPI-G2 exhibited broad and structureless absorption band from 450 to 650 nm (Figure 3) and detectable fluorescence with quantum yields of 0.57%, 0.85% and 1.24%, respectively (Table 1). The fluorescence intensity of the three biperylene dyes in water increased as the size of the dendron enlarged, although the dendritic effect is not as large as what was observed in perylene bisimide dyes.¹⁴ There are two possible reasons: (1) Nonradiative decays (such as intramolecular rotation and vibration) other than intermolecular aggregation are critical in the fluorescence quenching; (2) Fluorescence of the hydrophobic biperylene core is quenched by water. It has been previously reported that polar solvents could cause fluorescence quenching of hydrophobic core.^{20,21} Nevertheless, the fluorescence quantum yield increases over two fold from G0 to G2 (0.57% for BiPI-G0 and 1.24% for BiPI-G2). Importantly, higher generation of BiPI dendrimers has improved hydrophilicity and drug loading capability, due to the increased number of surface carboxylic acid groups and larger volume available for drug encapsulation. To suppress intramolecular movements and water quenching, we used two surfactants, Pluronic P123 and cetyltrimethylammonium bromide (CTAB), to coat the BiPI dyes. Researchers have used Pluronic P123 and CTAB to enhance fluorescence of perylene and terrylene dyes.^{10,22,23} In our study, we found that Pluronic P123 and CTAB were able to enhance the fluorescence of BiPI dendrimers in aqueous solutions. As shown in Figure 3, all three BiPI dendrimers exhibited enhanced, narrowed and structural absorption bands in Pluronic P123 and CTAB solutions. The absorption change is due to restricted movements of BiPI molecules by the copolymer Pluronic P123

and quaternized amine CTAB micelles. When the BiPI dyes were incorporated into the hydrophobic micelles center, the intramolecular movements such as rotation and vibration were significantly inhibited and quenching effect from water was avoided. Therefore, their fluorescence quantum yields increased dramatically. When treated with 10 wt% P123, the fluorescent quantum yield of BiPI-G0 increased 43 fold from 0.57% to 24.38% and that of BiPI-G1 increased 13 fold from 0.85% to 11.63%. No significant increase of quantum yield was observed when BiPI-G2 was treated with P123. In contrast, CTAB appeared to be more effective on large dye molecules. Treatment of CTAB did not show significant effect on the quantum yields of BiPI-G0 and BiPI-G1, but greatly increased the quantum yield of BiPI-G2 by 18 fold from 1.24% to 29.52%. The improved fluorescence performance indicated that the hydrophobic environment and confined dye configuration in micelle efficiently reduced the nonradioactive decay and water quenching of the BiPI dyes. Besides the fluorescence intensity change, the fluorescence peaks of these dyes showed significant blue shift at the presence of Pluronic P123 or CTAB (from 663 to 597 nm for BiPI-G0 with P123; from 661 to 602 nm for BiPI-G1 with P123; and from 646 to 607 nm for BiPI-G2 with CTAB). Generally, two kinds of excited states contribute to the fluorescence of symmetrical biaryl dyes, including locally excited (LE) state and charge transfer (CT) state. The contribution from CT state typically increases in polar solvents;^{24,25} therefore, the increase of fluorescence from biperylene dyes is mainly due to the increased contribution from LE state that relates to the non-polar environment of inner micelle. Importantly, increased contribution from LE state causes blue shift,²² which matches our observations.

To evaluate the cytotoxicity of BiPI dyes, we used the hemocytometer-based trypan blue dye exclusion method as we previously described.¹⁷ Wild type astrocytoma delayed brain tumor (WT-DBT) cells were treated with indicated concentration (0, 1, 5 and 10 μ M) of BiPI-G0, BiPI-G1 and BiPI-G2 for 24 h before evaluating the cytotoxicity. As shown in Figure S3, BiPI-G0, BiPI-G1 and BiPI-G2 exhibited comparably low cytotoxicity in WT-DBT cells as indocyanine green (ICG), the clinically approved near infrared fluorescent dye. As the positive control, doxorubicin caused cell death at high concentrations. These results indicate that BiPI-G0, BiPI-G1 and BiPI-G2 may be considered as a safe fluorescent agent for cellular imaging studies.

To evaluate the potential of BiPI-G0, BiPI-G1 and BiPI-G2 as fluorescence imaging probes in living cells, WT-DBT cells were incubated with 5 μ M of dendrimers at 37 °C for 3 h. Con-focal like fluorescence images was captured using a Zeiss Axio Observer equipped with ApoTome 2 System. The ApoTome is a low-cost illumination microscopy technique that greatly reduces the photobleaching effect of fluorescent dyes caused by laser on regular con-focal microscope.²⁷ In order to capture a con-focal like image, three raw images are acquired with the grid projected onto the in-focus specimen section. Because out-of-focus parts of the projected grid is invisible, ApoTome technology can delineate the areas of a fluorescence image containing object structures located within the focus of the objective and from that of out-of-focus region. Strong fluorescence signal was observed from cells treated with BiPI-G0, BiPI-G1 or BiPI-G2, which was mainly located in the cytoplasm (Figure 4). These images indicate that biperylene dendrimers may be potentially used as new fluorescent imaging probes in living cells. To quantify the cellular uptake, we incubated WT-DBT cells with 5 μ M of BiPI dendrimers for 3 h and measured the fluorescence

intensity of the uptaken (in cells) and unbound (in cell medium) molecules using a microplate reader. We found that $7.64 \pm 0.30\%$ of BiPI-G0, $2.89 \pm 0.12\%$ of BiPI-G1, and $2.81 \pm 0.42\%$ of BiPI-G2 was uptake by WT-DBT cells (Figure S4). In addition, we found that BiPI-G0 dendrimer was uptaken by cells in a both concentration-dependent and incubation time-dependent manner, with stronger fluorescence signal observed at higher concentrations and longer incubation time. BiPI-G1 and BiPI-G2 also showed concentration-dependent cell uptake, whereas incubation time did not show large effect on the dendrimer uptake after 5 minutes (Figure S5).

In conclusion, we have developed three polyamide-based biperylene dendrimers with four, twelve and thirty-six carboxylic acid termini. We found that the twisted configuration of the biperylene dyes efficiently suppressed the dye aggregation. The dendrimeric biperylene dyes showed red fluorescence in water and the fluorescence quantum yields increased with increase of the dendron size. Moreover, these biperylene dendrimers showed low cytotoxicity and strong fluorescence signal in living cells. Additionally, the prospect that targeting, signaling and therapeutic molecules can be attached to or incorporated into these dendrimers offers BiPI dyes great promise in imaging and therapeutic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The normalized concentration-dependent absorption and emission ($\lambda_{\text{ex}} = 530 \text{ nm}$) spectra of (a) BiPI-G0, (b) BiPI-G1 and (c) BiPI-G2 in water. The insets show the extinction coefficients at different concentrations.

Figure 2.

The normalized temperature-dependent absorption and emission ($\lambda_{\text{ex}} = 530 \text{ nm}$) spectra of (a) BiPI-G0, (b) BiPI-G1 and (c) BiPI-G2 in water at a concentration of $10 \mu\text{M}$.

Figure 3.

The UV-vis absorption (solid) and emission spectra (dash) of (a) BiPI-G0, (b) BiPI-G1 and (c) BiPI-G2 in water (black), in water in presence of 0.5 wt %/wt CTAB (red), and in water in present of 10 wt %/wt Pluronic P123 (green) at a concentration of 1 μ M. (λ_{ex} = 500 nm)

Figure 4.

Fluorescence microscopy images of BiPI-G0, BiPI-G1 and BiPI-G2. WT-DBT cells were incubated with 5 μM of BiPI-G0, BiPI-G1 and BiPI-G2 at 37 $^{\circ}\text{C}$ for 3 h before imaging. Left: fluorescence images. Right: differential interference contrast (DIC) images. Scale bars: 20 μm .

scheme 1.
The structures of BiPI dendrimers.

Table 1
Photophysical properties of BiPI dendrimers in different solvents at a concentration of 1 μ M.

Solvent	Sample	λ_{abs} /nm	$\epsilon/10^4 \text{cm}^{-1}$ M^{-1}	λ_{em} /nm	$\phi/\%$ ^a
CH ₃ CN	BiPI-G0	524	4.62	633	3.99
	BiPI-G1	528	5.25	634	5.42
	BiPI-G2	536	6.20	656	3.18
DMSO	BiPI-G0	532	7.14	607	0.85
	BiPI-G1	531	7.65	628	0.98
	BiPI-G2	534	7.31	611	1.19
water	BiPI-G0	535	4.05	663	0.57
	BiPI-G1	540	4.49	661	0.85
	BiPI-G2	545	4.45	646	1.24
Water, 10wt% P123	BiPI-G0	530	7.66	597	24.38
	BiPI-G1	536	7.53	602	11.63
	BiPI-G2	545	7.59	607	1.58
Water, 0.5wt% CTAB	BiPI-G0	537	5.89	633	2.78
	BiPI-G1	538	7.55	624	3.08
	BiPI-G2	543	7.74	607	29.52

^a Rhodamine B was used as the standard ($\phi = 65\%$ in ethanol, ref 26).