Fluorescent water-Soluble Probes Based on Ammonium Cation Peg Substituted Perylenebisimides: Synthesis, Photophysical Properties, and Live Cell Images

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Abstract. To synthesize perylenebisimides (PBI) fluorescent probes that will improve the water-soluble ability and the cytocompatibility, the synthesis and properties of fluorescent water-soluble probes based on dendritic ammonium cation polyethylene glycol (PEG) substituted perylenebisimides(GPDIs) are presented. As we expected, with increased ammonium cation PEG, the aggregation of the PBI in an aqueous solution is completely suppressed by the hydrophilic ammonium cation PEG groups. And the fluorescence quantum yield increases from 25% for GPDI-1 to 62% for GPDI-2. When incubated with Hela cells for 48 h, the viabilities are 71% (for GPDI-1) and 76% (for GPDI-2). Live cell imaging shows that these probes are efficiently internalized by HeLa cells. The study of the photophysical properties indicated increasing the ammonium cation PEG generation can increase the fluorescence quantum yield. Live cell imaging shows that with the ammonium cation PEG chains of perylenebisimides has high biocompatibility. The exceptionally low cytotoxicity is ascribed to the ammonium cation PEG chains, which protect the dyes from nonspecifically interacting with the extracellular proteins. Live cell imaging shows that ammonium cations PEG chains can promote the internalization of these probes.

1. Introduction
Due to fluorescence molecular imaging is noninvasive, rapid, highly sensitive and inexpensive; it plays a major role in living tissues and cells research. [1-3] Many biological species in living cells can be targeted and visualized by appropriate fluorescent probes.[4-5] Characteristics of a successful fluorescent probe in live-cell imaging include water solubility, brightness, photo stability, low cytotoxicity and live-cell permeability.[6-7]

As we all know, perylenebisimides (PBI) fluorescent dyes display exceptional chemical, thermal, and photochemical stabilities, as well as high fluorescence quantum yields close to unity in organic solvents.[8-9] However, PBIs exhibit poor water solubility and very weak fluorescence in water because of the aggregation of perylenechromophores.[10-11] Therefore, high fluorescence quantum yields has become the new attention of water-soluble PBI over the last few years. Our group is interested in the development of photochemically stable and biocompatible dyes with high fluorescence in aqueous solutions for bioimaging.[12] In our study, the synthesis and properties of fluorescent water-soluble probes based on dendritic ammonium cation PEG-substituted perylenebisimides (GPDIs) are presented. Scheme 1 shows the chemical structures of the GPDIs. The GPDIs are PBI moieties as fluorescence cores, ammonium cation PEG chains as hydrophilic shells for inducing water solubility and reducing cytotoxicity. Based on the number of the hydrophilic dendritic ammonium cation PEG chain, these compounds can be divided into two classes: GPDI-1 and GPDI-2.
2. Experimental

**Synthesis.** Scheme 2 is the Synthesis approaches of GPDIs

3. Characterization of DPPBIs

(A)UV-vis absorption spectral changes of probe GPDI-1 (10μM) in aqueous solution
Figure 1. Spectroscopic analyses of specific GPDI-1 and GPDI-2 probes.
3.1. Aggregation Behavior and Optical Properties.

In aqueous solution, GPDI-1 probe showed aggregation behavior. Which was investigated via concentration-dependent UV-Vis absorption spectroscopy. In a low-concentration aqueous solution (4.0 × 10^{-6} mol/L), the absorption bands of GPDI-1 showed maximum absorption at 533 nm (Figure 1A). When the aqueous solution concentration increased from 4.0 × 10^{-6} mol/L to 1.0 × 10^{-4} mol/L, a blue shift of the absorption maximum was observed (from 533 nm to 497 nm), implying the formation of H-type cofacial π-π stacking in a high-concentration aqueous solution. However, when the aqueous solution concentration increased from 4.0 × 10^{-6} mol/L to 1.0 × 10^{-4} mol/L, compound GPDI-2 has a nonaggregation UV-vis absorption with the absorption maximum at 537 nm (Figure 1C). This result suggests that the hydrophilic bulky ammonium cation PEG groups suppressed the aggregation of the PBI in an aqueous solution.

Table 1. Fluorescence data for the GPDIs

|         | GPDI-1 | GPDI-2 |
|---------|--------|--------|
| $\lambda_{max}$ (nm) | 533    | 537    |
| $\Phi_c$ (%) | 25     | 62     |

10^5 mol L^{-1}, excitation wavelength is 492 nm. N,N-Dicyclohexyl-perylene-3,4:9,10-tetracarboxylic acid bisimide in chloroform was used as reference ($\phi = 100\%$).

We determined the fluorescence quantum yields of the GPDIs in the aqueous solution (Table 1). As expected, GPDI-1 exhibited a very low fluorescence quantum yield (25%), which could be attributed to fluorescence quenching by strong aggregation in water. With increasing ammonium cation PEG generation, there was a continuous increase in fluorescence quantum yields from 25% for GPDI-1 to 62% for GPDI-2. It suggests that PBI fluorophores are efficient emitters in water if their aggregation is completely suppressed.

3.2. Cytocompatibility Properties and Live Cell Images

![Figure 2](image)

*Figure 2.* *In vitro* viability of HeLa cells treated with GPDI-1 (left) and GPDI-2 (right) for 24 h (Black) and 48 h (Red).
To demonstrate the potential utility of GPDIs probes for cellular imaging, their cytotoxicity was assessed using MTT cell-viability assay. We have reported that the perylenebisimide probes with PEG groups show very low cytotoxicity. [16] Incorporating ammonium cation PEG groups on the PBI, GPDIs probes have slight increase in cytotoxicity but still remain low cytotoxicity. The cell viability was over 85% even though 10μM GPDIs were added for 24h. And the cell viability was over 70% when 10μM GPDIs was added for 36h (Figure 2), indicating that GPDIs probes have a good cytocompatibility. We ascribed the exceptionally low cytotoxicity to PEG chains which protect the dyes from interacting nonspecifically with the extracellular proteins and triggering immunogenicity and antigenicity inside the cells. [17]

![Figure 3](image_url) Bright-field image of HeLa cells stained by GPDI-1 (A), and confocal fluorescence image of HeLa cells stained by GPDI-1(B), image of Overlay of two channels (C).

![Figure 4](image_url) Bright-field image of HeLa cells stained by GPDI-2 (A), and confocal fluorescence image of HeLa cells stained by GPDI-2 (B), image of Overlay of two channels (C).

To examine the intracellular fluorescence imaging of compounds, live cell imaging based on GPDIs was investigated with confocal laser scanning microscopy (CLSM). Hela cells were tested to demonstrate the utility of GPDIs in cell imaging. The fluorescence images of GPDI-1stained HeLa cells are shown in Figure 3. Strong fluorescence was observed for HeLa cells, implying that GPDI-1 was efficiently internalized by HeLa cells (Figure 3B). And HeLa cells were incubated with GPDI-2, weakened fluorescence was observed in living cells (Figure 4B). These results indicate that ammonium cations PEG chains can promote the internalization of these probes but inhibit the fluorescence intensity.

4. Conclusion
In summary, the synthesis and properties of fluorescent water-soluble probes based on ammonium cation PEG substituted perylenebisimides are reported. With increased ammonium cation PEG generation, the aggregation of the PBIs in aqueous solution is completely suppressed, and the
fluorescence quantum yield is increased. High fluorescence quantum yields, good water solubility, photostability, and low cytotoxicity make these GPDIs probes excellent candidates as a new class of probes for live cell imaging.

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6. References
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