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A Polyanionic Dendritic Fluorophore for Selective Detection of Hg^{2+} in Triton X-100 Aqueous Media

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ABSTRACT

A series of water-soluble fluorescent dendritic compounds composed of phenylene-ethynylene repeating units and anionic carboxylate or cationic ammonium peripheral groups were synthesized. The first generation fluorescent dendrimer containing nine phenylene-ethynylene units and six carboxylate peripheral groups exhibited a highly selective fluorescence quenching by Hg^{2+} ions. The Stern−Volmer constant (K_{sv}) was 33,700 M^{-1} in aqueous media in the presence of Triton X-100 surfactant.

Mercury is one of the most toxic environmental pollutants generated from industrial sources. The most abundant ionic form of this element is the mercuric ion (Hg^{2+}), which can be accumulated in the organs of human or animal bodies through the food chain.[1] Inorganic mercury has been reported to produce harmful effects at a concentration of 5 ppb.[2] Development of highly sensitive and selective Hg^{2+} sensors that can provide direct determinations of the amount of Hg^{2+} in aqueous media is therefore of great interest.[3] Chemosensors for Hg^{2+} have been progressively improved using redox,[4] chromogenic,[5] or fluorogenic[6] changes as the means of detection.

Fluorescence-based methodologies have attracted much interest due to their intrinsic sensitivity and selectivity.[7] Considerable efforts have been devoted to the design of fluorescent compounds to be used as sensors for mercury. However, their poor solubility in water and low fluorescence quantum yield due to aggregation have limited the satisfactory application of these compounds in aqueous media.[8]

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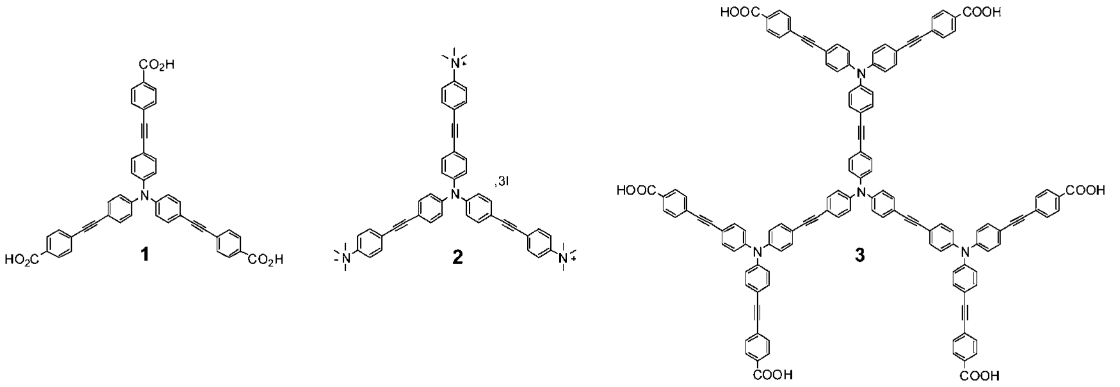

Figure 1. Structures of dendritic molecules **1**, **2**, and **3**.

Several methods have been used to prevent aggregation and increase quantum efficiencies, for example, the use of polyelectrolytes or surfactants.

Conjugated polyelectrolytes have been applied to the detection of metals due to their efficient static quenching properties with quenchers.[9] In some recent studies, surfactants were also used to enhance the fluorescence quantum yields of hydrophobic conjugated polymers containing hydrophilic side chains.[10] The dominant hydrophobic interactions between surfactants and fluorophores can significantly reduce the aggregation. An application of surfactants for amplification of the quenching effect between the fluorescent polymers and quenchers has also been reported.[11]

The fluorescent linear polymers poly(phenylene-ethynylenes) substituted by carboxylate groups have been reported to exhibit a quenching effect by Hg^{2+} in aqueous media in the absence and presence of avidin, with Stern−Volmer constant (K_{sv}) values of 10^4 and 10^5 M^{-1}, respectively.[12] Despite offering high sensivity, the unpredictable secondary and tertiary structures in solution, due to a wide number of repeating fluorophores and random molecular conformations in linear conjugated polymers, can lead to inconsistent quenching effects and inexplicable behaviors.

In comparison with linear polymers, the numbers of fluorophore units in dendrimers can be controlled by a stepwise synthesis. This should reflect in the more predictable fluorescence property and other structure-related behaviors of dendritic molecules. We thus decided to synthesize dendritic compounds **1**−**3** (Figure 1) and study their fluorescent sensing applications.

Diphenylacetylene is selected as the repeating fluorescent unit for its known high fluoresence quantum yield and structural rigidity. To make the dendritic compounds water-soluble, carboxyl or quaternary ammonium groups have been installed as the peripheral groups. The compounds were synthesized by a convergent approach as outlined in Scheme 1. The reactive core, 4,4′,4″-triiodotriphenylamine **4**, was prepared from the iodination of triphenylamine using benzyltrimethylammonium iododichloride (BnMe$_3$-ICl$_2$).[13] The peripheral building blocks, methyl 4-ethynyl benzoate **5** and *N*,*N*-dimethyl-4-ethynylaniline **6**, were obtained through the Sonogashira coupling[14] of trimethylsilylacetylene with the corresponding aryl iodide and a subsequent base-catalyzed desilylation. With the required building blocks in hands, we proceeded with the Sonogashira coupling between **4** and **5** followed by the hydrolysis of triester **7** to afford ionizable fluorophore **1**. Similarly, the reaction between **4** and **6** gave rise to the triamine **8**, which was treated with an excess of MeI to provide the polycationic fluorescent compound **2**. In order to obtain the first generation fluorescent dendrimer **3**, we carried out a reaction of the core **4** with 1 molar equiv of trimethylsilylacetylene to obtain the branch building blocks **9**. The Sonogashira coupling of **9** with **5** followed by desilylation gave the dendron **10**, which was coupled with **4** to afford hexaester **11**. The hydrolysis of **11** eventually afforded first generation fluorescent dendrimer **3** in moderate yield.[15]

The effects of surfactants on the photophysical properties of **1**−**3** were studied. Without the surfactant, compounds **1**−**3** displayed absorption peaks around 370−375 nm. In the presence of the surfactant Triton X-100, these bands were slightly red-shifted to 379−383 nm. The spectral shifts

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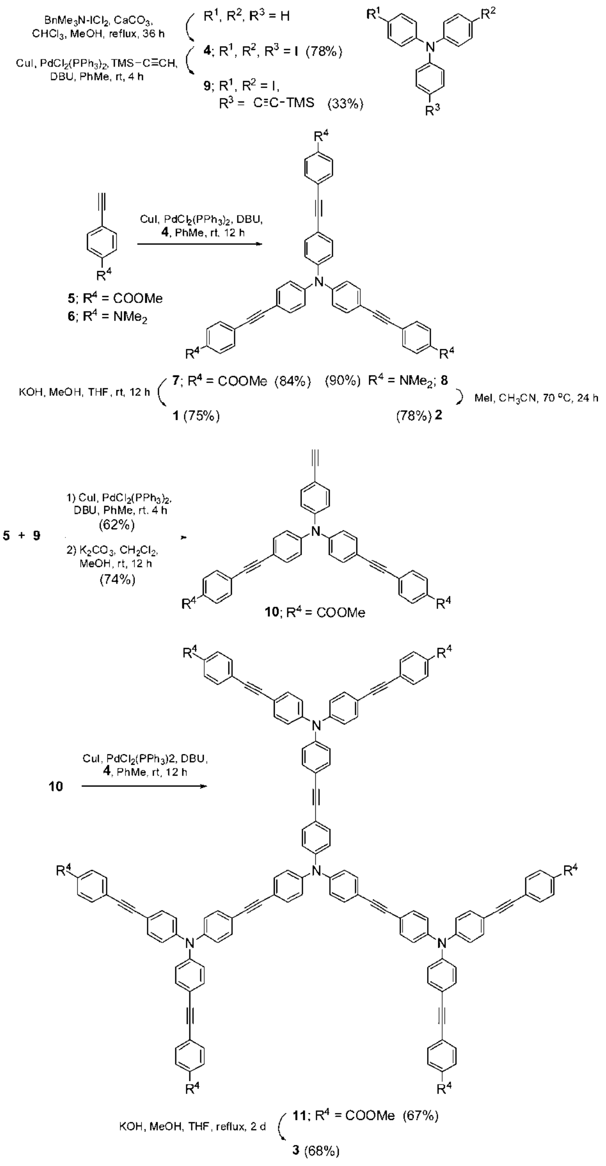
Scheme 1. Synthesis of **1**, **2**, and **3**



observed in the emission spectra were in the opposite direction and of more significance. The emission peaks of **1**, **2**, and **3** were at 454, 485, and 489 nm in the absence of Triton X-100. The surfactant caused the emission bands to blue-shift by 20, 47, and 68 nm, respectively (Table 1).

The blue shift and increase in fluorescence quantum yields (Φ_F) suggested that the deaggregation of **1**−**3** was caused by the addition of the surfactants. The enhancement of Φ_F of **3** was greater than that of **1** implied that compound **1**, a smaller molecule, was only partially deaggregated.

We also investigated the fluorogenic behaviors of **1**−**3** in the presence of metal ions in the +2 oxidation state, such as Cr^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}. Without the surfactant, the fluorescence signal of **3** can be quenched by Hg^{2+} ions (Figure 2a), whereas there are no significant effects on the signals of **1** and **2**. With Triton X-100, the fluorescence signal of **1** was quenched by all metal ions listed above, but the signal of **2** was not affected. On the other hand, the fluorophore **3** still exhibited a selective quenching by Hg^{2+} ions.

Table 1. Photophysical Properties of **1**−**3** in 50 mM Phosphate Buffer (pH 8.0) without and with Triton X-100

	absorption		fluorescence	
compd	λ_{max} (nm)	ε (M^{-1} cm^{-1})	λ_{max} (nm)	$\Phi_F{}^a$
		Without Triton X-100		
1	374	5900	454	0.097
2	370	26823	485	0.14
3	375	63500	489	0.037
		With Triton X-100		
1	383	9592	434	0.47
2	382	21310	438	0.46
3	379	49820	421	0.65

[a] Quinine sulfate in 0.1 M H_2SO_4 ($\Phi_F = 0.45$) was the reference.

A Stern−Volmer plot was made in order to access a quantitative measurement of fluorescence quenching. K_{sv} for the system with Triton X-100 was 33,700 M^{-1}, whereas it was only 5,800 M^{-1} for the system without Triton X-100 (Figure 3). It is clear that Triton X-100 could amplify this selective quenching effect.

The nonselective quenching of **1** suggested that positively charged metal ions reduce the electrostatic repulsion between

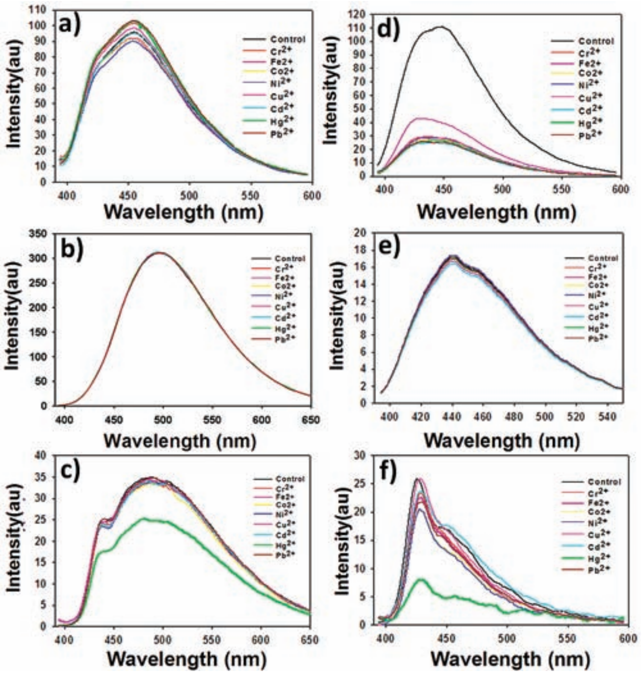


Figure 2. Emission spectra of the solutions of **1**−**3** (10 μM) upon the addition of metal ions (40 μM): without Triton X-100 for (a) **1**, (b) **2**, and (c) **3**; with 0.1 mM Triton X-100 for (d) **1** (1 μM), (e) **2** (0.1 μM), and (f) **3** (0.1 μM).

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(15) The spectroscopic data (^1H and ^{13}C NMR, MALDI-TOF MS, and ESI-MS spectra) of every compound are available in Supporting Information.

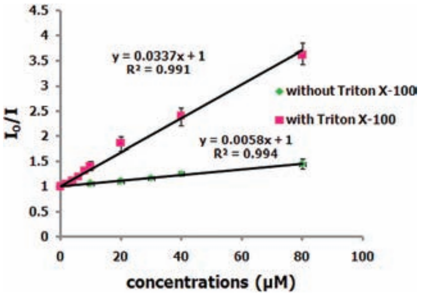

Figure 3. Stern−Volmer plots for fluorescence quenching of **3** (0.1 μM) with and without of Triton X-100 (0.1 mM).

the partially deaggregated negatively charged fluorophores **1**, resulting in the enhancement of self-quenching. Conversely, the charge repulsion among positively charged fluorophore **2**, having a comparable molecular size to **1**, cannot be reduced by the metal ions and therefore exhibits no enhancement of the self-quenching effect.

The selective fluorescence quenching of **3** by Hg^{2+} is more complicated and difficult to rationalize. With the high selectivity for Hg^{2+}, the common metal ion enhanced self-quenching mechanism is unlikely to play the key role. The quenching effect may involve selective formation of **3**·Hg^{2+} complex and efficient energy transfer between the fluorescent units in **3** to this complex at the periphery.[16] To test if the proposed complex could be reversed, a strong chelator, EDTA, was added. The addition of 2.5 molar equiv of EDTA could restore the fluorescent signal of **3** to its original level (Figure 4). The result supports the above hypothesis to some extent.

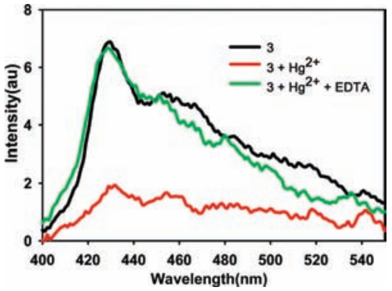

Figure 4. Emission spectra of **3** (0.1 μM) upon the addition of Hg^{2+} (40 μM) and EDTA (100 μM).

In summary, a series of diphenylacetylene dendritic compounds containing negatively and positively charged peripheral groups were successfully synthesized through a convergent approach. We have demonstrated that these diphenylacetylene based dendritic molecules with a charge decorated periphery constitute a new intriguing class of fluorophores useful for sensing applications in aqueous media. The quantum efficiencies of the fluorophores in water were considerably enhanced by the nonionic sufactant Triton X-100. In the presence of Triton X-100, the fluorescent signal of the first generation dendrimer containing a carboxylate periphery could be selectively quenched by Hg^{2+}. A wide linear fluorescence quenching response to Hg^{2+} concentration was observed in the range of 2−80 μM (0.4−16 ppm). Other applications along this line are currently under investigation, and the results will be disclosed in due course.

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Supporting Information Available: Spectroscopic data and detailed experimental procedures. This information is available free of charge via the Internet at http://pubs.acs.org.

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