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An amphiphilic [2]biphenyl-extended pillar[6]arene: Synthesis, controllable self-assembly in water and application in cell-imaging

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ABSTRACT

Synthesis and functionalization of novel macrocyclic host molecules are important topics in supramolecular chemistry. In this work, the first amphiphilic [2]biphenyl-extended pillar[6]arene (AM-[2]BP-ExP6) was designed and synthesized with poly(ethylene glycol) chains as the hydrophilic tails and a rigid cavity as the hydrophobic core. Due to its amphiphilic nature, AM-[2]BP-ExP6 could self-assemble to stable fibers in water. What's more, AM-[2]BP-ExP6 could associate with quaternary ammonium modified tetraphenylethylene guest (QTPE) to form a 2:1 host-guest complex, accompanied by significant enhanced fluorescence. Interestingly, different like AM-[2]BP-ExP6, AM-[2]BP-ExP6⊃QTPE host-guest complex self-assembled into fluorescent particles with diameter about 310 nm, the obtained fluorescent particles can be further employed in living cell imaging.

Macrocyclic hosts (*e.g.,* crown ethers [1,2], cyclodextrins [3,4], calixarenes [5,6], and cucurbiturils [7,8]) have been widely concerned by scientists not only due to their unique molecular structures, but also due to their diverse host-guest properties and potential applications [9–12]. So, they are the "star" molecules in the field of supramolecular chemistry [13–18]. For example, Prof. Yan and co-workers constructed mechanically interlocked vitrimers through crown ether-based host-guest molecular recognition and dynamic covalent bonds to realize in reversibly breaking the mechanical bonds [1]. Design and synthesis of macrocyclic compounds with novel structures has always been the goal of supramolecular organic chemists due to it can not only bring new host-guest molecular recognitions, but also open the application fields of supramolecular chemistry [19–24]. Pillar[*n*]arenes, mainly including pillar[5]arenes and pillar[6]arenes, are the latest classical macrocyclic compounds, which were oligomeric cyclics formed from 1,4-alkoxybenzenes bridged by methylene at their 2,5-position and first reported by the Ogoshi's group in 2008 [25–37]. During the past more than ten years, scientists have paid much attention to the syntheses [38,39], host-guest properties [40,41], and various applications of pillar[*n*]arenes because they contain a symmetrical pillar-like cavity and 2n functionalizable sites, which makes them modification easily and superior to other macrocyclic hosts [42–46]. For example, based on the rigid and symmetrical cavity of pillar[*n*]arene, Prof. Li built functional materials through the marriage of macrocyclic chemistry and co-crystal engineering. And the resulting macrocycle co-crystals (MCCs), P5-PDIα exhibit selective vapochromic responses to volatile organic compounds of haloalkanes [46].

In 2016, Prof. Yang *et al.* prepared a new macrocyclic compound called [2]biphenyl-extended-pillar[6]arenes ([2]BP-ExP6). Compared with traditional pillar[6]arene, [2]BP-ExP6 contains two biphenyls units, the biphenyl unit and two 1,4-dimethoxy-benzene groups connect one by one by -CH_2- alternately, endowing the symmetric structures [47]. Then in 2018, their group synthesized the first water-soluble [2]BP-ExP6 containing eight pyridinium groups (CWBP-ExP6), which exhibited high host-guest interaction towards sulfonate-bearing guests. Significantly, 2,6-naphthalene-disulfonate could be removed from water by CWBP-ExP6 due to the host–guest complexation induced precipitation [48]. Although some development has been made in the investigation of BP-ExP6 [49–53], to the best of our knowledge, amphiphilic [2]BP-ExP6, which process both self-assembly characteristics like conventional linear amphiphiles and host-guest properties like macrocyclic compounds, has not been reported before.

Herein, in order to enrich the molecular structure types of [2]BP-ExP6s and develop the its applications in biomaterials, especially in cell imaging, we designed and prepared the first amphiphilic [2]BP-ExP6 (AM-[2]BP-ExP6) with two poly(ethylene glycol) chains as the hydrophilic tail and rigid cavity as the hydrophobic part. When dispersed in water, AM-[2]BP-ExP6 could

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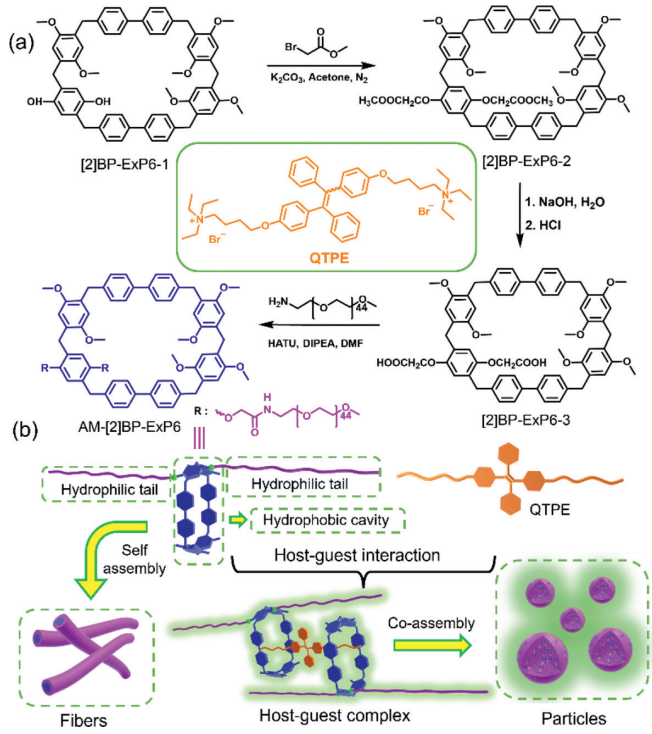

Scheme 1. (a) Synthetic route to amphiphilic [2]biphenyl-extended-pillar[6]arenes (AM-[2]BP-ExP6). (b) Cartoon representation of AM-[2]BP-ExP6 and AM-[2]BP-ExP6⊃QTPE self-assembly in water.

self-assembly into fibers with width about 200 nm. However, due to AM-[2]BP-ExP6 contain a rigid cavity, it could associate with quaternary ammonium modified tetraphenylethylene guest (QTPE) to form host-guest complex and further self-assembly into fluorescent particle (Scheme 1). Importantly, the obtained fluorescent particles can be further applied in living cell imaging.

As described in Scheme 1a, AM-[2]BP-ExP6 was synthesized from a hydroquinone functionalized [2]BP-ExP6 ([2]BP-ExP6-1) through three steps. With AM-[2]BP-ExP6 in hand, we then investigated whether it could self-assemble into highly ordered aggregates in aqueous environment. Firstly, ^{1}H NMR of AM-[2]BP-ExP6 spectra in different solvents were carried out. As shown in Fig. S6 (Supporting information), compared with in $CDCl_3$, only proton resonance of the PEG fragment was observed in D_2O, suggesting that AM-[2]BP-ExP6 molecule self-assembled into nanostructures with stacked pillar[n]arene frameworks, where the inner protons of pillararene-framework were efficiently shielded by the PEG chains [54]. In order to investigate the self-assembly behavior of AM-[2]BP-ExP6 on water, we first prepared the solution and left it for 1 day to reach a stable state. The critical aggregate concentration (CAC) value of AM-[2]BP-ExP6 was calculated to be 2.6×10^{-3} mmol/L in aqueous solution using pyrene as a fluorescent probe (Fig. 1a). Tyndall effect was observed when the concentration of AM-[2]BP-ExP6 is 3.0×10^{-3} mmol/L, which indicated that aggregates were formed in the solution (Fig. 1a, inset). DLS studies showed that with the increase of concentration, the particle size of the assemblies increased gradually (Fig. S16 in Supporting information), which in accordance with previous report [55]. However, when the concentration is up to 3.0×10^{-3} mmol/L, larger than CAC value the size of AM-[2]BP-ExP6-based assemblies was arranging from 100 nm to 2000 nm, indicating the assemblies was not a standard spherical structure (Fig. 1b). The morphology of the assemblies was examined by TEM and SEM technologies. When the concentration was 3.0×10^{-3} mmol/L, the overview of the assemblies demonstrated that it is fibers with the width ~200 nm

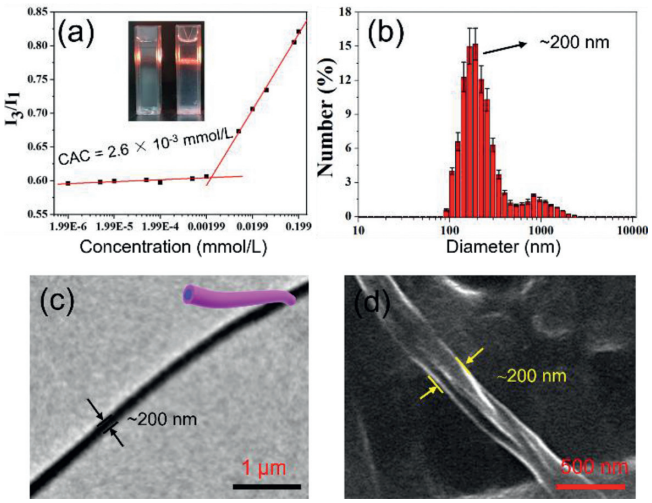

Fig. 1. (a) CAC measurement of AM-[2]BP-ExP6 in water using pyrene as a fluorescent probe (inset: Tyndall effect). (b) DLS study of AM-[2]BP-ExP6 in water. (c) TEM image of AM-[2]BP-ExP6 self-assembly in water. (d) SEM image of AM-[2]BP-ExP6 self-assembly in water. [C] = 3.0×10^{-3} mmol/L.

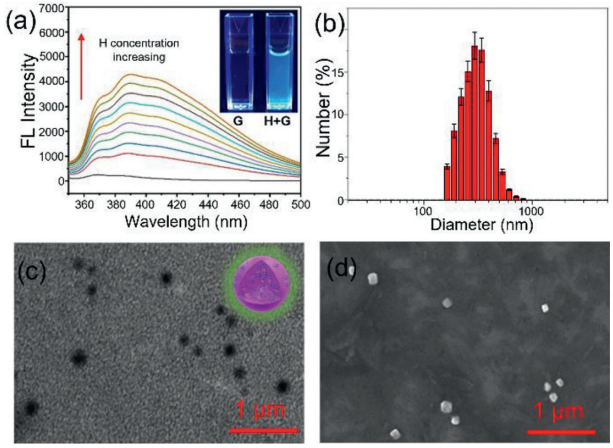

Fig. 2. (a) Fluorescence spectra of G (QTPE) in the presence of H (AM-[2]BP-ExP6, λ_{ex} = 312 nm). [QTPE] = 1.0 μmol/L. [AM-[2]BP-ExP6] concentrations were 0, 0.2, 0.4, 0.6, 0.8 1.0, 1.2, 1.4, 1.6, 1.8 μmol/L, respectively. (b) DLS study of AM-[2]BP-ExP6⊃QTPE in water. (c) TEM image of AM-[2]BP-ExP6⊃QTPE self-assembly in water. (d) SEM image of AM-[2]BP-ExP6⊃QTPE self-assembly in water. 2 [QTPE] = [AM-[2]BP-ExP6] = 3.0×10^{-3} mmol/L.

and the length longer than 1 μm (Figs. 1c and d). It is important to note that at a concentration of 3.0×10^{-3} mmol/L, freshly prepared solutions first form irregular aggregates of larger size, which rearrange over time. Finally, the stable fiber-like structure is formed (Fig. S17 in Supporting information) [56,57].

From previous reports, we know that [2]BP-ExP6 contains a pillar[6]arene-like rigid cavity, which can complex with cationic guest effectively [47]. In this work, because the guest QTPE contained two quaternary ammonium units, we wondered whether QTPE could complex with AM-[2]BP-ExP6 to induce the morphologies of the assemblies transformation. Firstly, from UV-vis spectra we found that QTPE has a characteristic absorption peak at 312 nm (Fig. S18 in Supporting information). Then, we used NMR spectroscopy (Figs. S21 and S22 in Supporting information) and fluorescence titration experiments (Fig. 2a and Fig. S15 in Supporting information) to determine the association constant (K) between AM-[2]BP-ExP6 and QTPE and applied UV-vis titration and Job's plot curve to calculate the host-guest stoichiometry (Fig. S20 in Supporting information). After the gradual addition of AM-[2]BP-

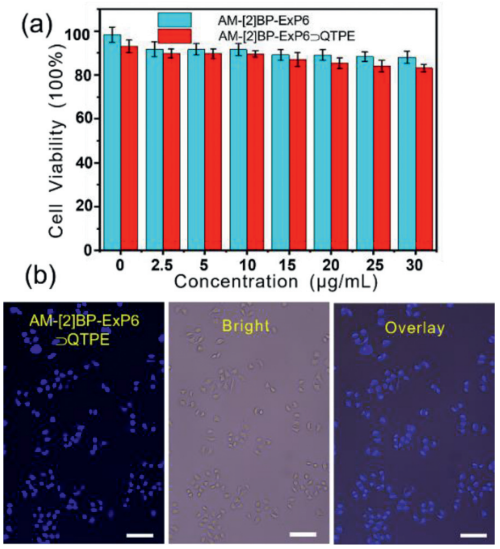

Fig. 3. (a) The viabilities of HeLa cells cultivated with different concentration of AM-[2]BP-ExP6 and AM-[2]BP-ExP6⊃QTPE for 4 h. (b) Confocal laser scanning microscopy images of HeLa cells cultivated with AM-[2]BP-ExP6⊃QTPE for 4 h. [C] = 30 μg/mL. Scale bar = 100 μm.

ExP6 to the aqueous solution of QTPE, the fluorescence intensity at 390 nm was significantly enhanced. Strong blue emission can be observed when AM-[2]BP-ExP6⊃QTPE irradiated at a 312 nm UV lamp while QTPE exhibited very weak fluorescence (Fig. 2a, inset). Furthermore, the quantum yield of AM-[2]BP-ExP6⊃QTPE in water was calculated to be 0.14, which is larger than QTPE (Fig. S19 in Supporting information). The mechanism of this enhancement may be that the complexation can effectively suppress the quenching effect and other nonradiative pathways caused by the aggregation of QTPE in water [58,59]. By using the Stern-Volmer equation, the association constant K of the complex was determined to be $(1.00 \pm 0.15) \times 10^6$ L/mol (Fig. S15). This high binding affinity favors the stability of AM-[2]BP-ExP6⊃QTPE in water.

Notably, AM-[2]BP-ExP6⊃QTPE exhibited different self-assembly behavior compared with AM-[2]BP-ExP6. DLS results (Fig. 2b) showed that the fluorescent aggregates of AM-[2]BP-ExP6⊃QTPE have an average diameter of ∼300 nm with a narrow size distribution. Furthermore, spherical structures with the size about 220 nm were observed from SEM image (Fig. 2d). At last, solid spherical structures were observed from TEM image, confirming that AM-[2]BP-ExP6⊃QTPE self-assembled into stable particles in water (Fig. 2c). The diameter of the particles calculated from TEM and SEM images is smaller than that from DLS study is due to the poly(ethylene glycol) chains being extended in aqueous solution but shrunken under dry conditions [60].

With the AM-[2]BP-ExP6⊃QTPE based particles in hand, we then investigated it as an fluorescent agent for living cell imaging. Before attempting this, we first evaluated the cytotoxicity of AM-[2]BP-ExP6, and AM-[2]BP-ExP6⊃QTPE by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at different concentrations against human cervical carcinoma cells (HeLa). After being incubated with different concentration (from 2.5 μg/mL to 30 μg/mL) of AM-[2]BP-ExP6 and AM-[2]BP-ExP6⊃QTPE for 4 h, the viability of HeLa cells all remained above 95%, indicating that both AM-[2]BP-ExP6 and AM-[2]BP-ExP6⊃QTPE have good biocompatibility and low toxicity (Fig. 3a). Then, HeLa cells were treated with AM-[2]BP-ExP6⊃QTPE for 4 h, and we used confocal laser scanning microscopy (CLSM) to monitor the intracellular distribution of the AM-[2]BP-ExP6⊃QTPE particles. It is clearly showed that when treated with AM-[2]BP-ExP6⊃QTPE, HeLa cells exhibited bright blue fluorescence emission (Fig. 3b). These observations indicated that the fluorescent AM-[2]BP-ExP6⊃QTPE-based particles can be successfully applied for imaging living cells.

In conclusion, the first amphiphilic [2]biphenyl-extended-pillar[6]arene (AM-[2]BP-ExP6) with two polyethylene glycol chains as the hydrophilic tail and a rigid cavity as the hydrophobic part was designed and synthesized successfully. AM-[2]BP-ExP6 could self-assembly into stable fibers with the width of about 200 nm and the length longer than 1 μm in aqueous solution. Furthermore, due to AM-[2]BP-ExP6 could associate well with quaternary ammonium modified tetraphenylethylene guest (QTPE) to form a 2:1 host-guest complex, fluorescent particles can be easily fabricated by employing the host-guest complex AM-[2]BP-ExP6⊃QTPE as the building block. These host-guest complex-based particles could be further applied in living cell imaging due to their strong blue emission. It is anticipated that amphiphilic macrocyclics-based host-guest complex can provide an efficient platform for constructing functional biomaterials.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.108088.

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