Synthesis of Aromatic Macrocyclic Amphiphiles and their Self-Assembling Behavior in Aqueous Solution

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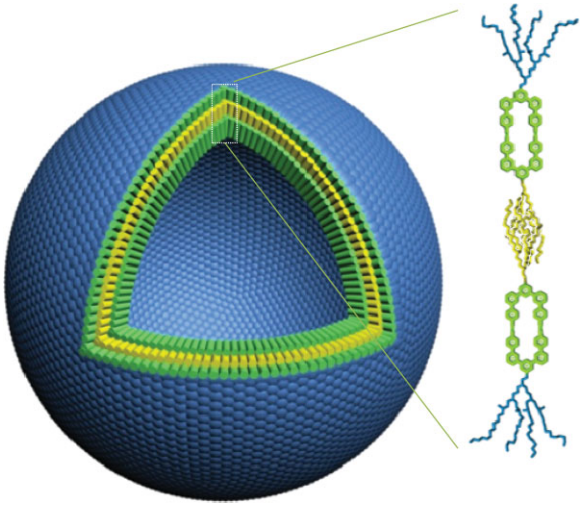
A triblock amphiphilic macrocycle consisting of a macrocyclic aromatic segment, a hydrophilic oligo(ethylene oxide) branch, and a hydrophobic alkyl dendron is successfully synthesized and characterized. The resulting cyclic amphiphile is observed to self-assemble into hollow double-layered capsules in aqueous solution, as confirmed by dynamic light scattering and cryogenic transmission electron microscopy investigations. The capsules are able to encapsulate hydrophobic guest molecules through aromatic interactions with high stability.



Introduction

The creation of supramolecular structures through the self-assembly of synthetic molecules has been one of the major research topics in the field of materials science, nanochemistry, and biomimic chemistry.[1] Among these self-assembling systems, block molecules that are able to mimic lipid amphiphilicity have been well known to form diverse aggregates in solution.[2] The incorporation of a rigid aromatic segment into an amphiphilic molecular architecture has been reported to enhance aggregation stability.[3] Furthermore, these aromatic amphiphiles can give rise to a variety of nanoscopic structures with well-defined size and shape, that include spheres, toroids, networks, and vesicles.[4]

In particular, extensive efforts have been devoted to investigate the self-assembly of macrocycles into supramolecular structures.[5] With few exeptions,[6] most of synthetic macrocycles based on an aromatic segment as a cyclic building block self-organize to form cylindrical supramolecular structures in solution by stacking on top of one another through the strengthened π–π interaction of flat aromatic segments.[7] Recently, we reported that novel amphiphilic aromatic macrocycles with an elliptical shape can self-assemble into spheres, helical coils, and into monolayered vesicles as the molecular length of the elliptical macrocycle increases.[8] This result led us to envision that the incorporation of hydrophobic branches into a macrocycle will enable a further extension of the supramolecular organization capabilities as a result of enhanced hydrophobic interactions. In this context, we have synthesized triblock aromatic macrocycles based on a hydrophilic oligoether dendron at one end and hydrophobic branched alkyl chains at the other end of the aromatic cyclic segment, and have characterized the self-assembly

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behavior of the amphiphilic triblock molecule in aqueous solution.

Experimental Part

Materials and Methods

Tetrakis(triphenylphosphine) palladium(0) (99%), and trimethylsilylacetylene from TCI were used as received. Copper(I) iodide (98%), potassium carbonate from Aldrich, and the conventional reagents were used as received. All atmosphere-sensitive reactions were performed under a nitrogen atmosphere. Dry triethylamine was prepared freshly by distillation over calcium hydride.

^{1}H NMR spectra were recorded from $CDCl_3$ solutions on a Bruker 250 NMR spectrometer. Microanalyses were performed with a Perkin Elmer 240 elemental analyzer. Matrix-assisted laser desorption–ionization time-of-flight mass spectroscopy (MALDI-TOF-MS) was performed on a Bruker Microflex LRF20 using α-cyano-4-hydroxy cinnamic acid as a matrix. Recycling preparative high performance liquid chromatography (HPLC) was performed with three 20 mm × 600 mm polystyrene gel columns (JAIGEL-H1, H1.5, and H2) on a Japan Analytical Industry LC-908 system, equipped with UV detector 310 and refractive index detector RI-5. UV-Vis absorption spectra were obtained using a Shimadzu 1601 UV spectrometer. The fluorescence spectra were obtained using a Hitachi F-4500 fluorescence spectrometer. Fluorescence microscopy (FM) images were obtained by a Nikon Eclipse TE2000-U inverted fluorescence microscope equipped with a DXM1200C digital camera.

Synthesis

Aromatic derivatives **2**, **6**, and $\mathbf{R_2}$ were synthesized according to procedures described previously.[8,9]

Synthesis of Compound **3**

Compound **2** (0.25 g, 0.38 mmol), tosylated second-generation dendritic decyl chain ($\mathbf{R_2}$) (0.35 g, 0.35 mmol) and excess K_2CO_3 were dissolved in 70 mL of anhydrous acetonitrile. The mixture was heated at reflux for 48 h. The resulting solution was extracted with dichloromethane/ethyl acetate and water, and dried over anhydrous magnesium sulfate. The crude product was purified by column chromatography using dichloromethane/hexane as eluent to yield 0.38 g (71%) of a colorless waxy solid. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.78–7.75 (m, 6Ar-*H*), 7.65–7.36 (m, 11Ar-*H*), 7.18 (d, 2Ar-*H*, *o* to Ar-O, J = 1.17 Hz), 4.17 (d, 2H, –CH_2OAr, J = 5.43 Hz), 3.60–3.31 (m, 24H, –OCH_2CH_2), 2.35–2.12 (m, 3H,–C*H*(OCH_2)$_2$), 1.50–1.48 (m, 8H, –OCH$_2$CH_2), 1.24 (s, 56H, –CH_2CH_2), 0.89–0.84 (m, 12H, –CH$_2$CH_3).

$C_{82}H_{124}I_2O_7$: Calcd. C 66.74, H 8.47; Found C 66.81, H 8.42.
MALDI-TOF-MS m/z = 1 498.23 $[M + Na]^+$, Calcd. 1496.74.

Synthesis of Compound **4**

Compound **3** (0.38 g, 0.27 mmol), trimethylsilylacetylene (53 mg, 0.54 mmol), and copper(I) iodide (0.6 mg, 0.003 mmol) were added to a suspension of tetrakis(triphenylphosphine) palladium(0) (5.9 mg, 0.005 mmol) in 50 mL of dry triethylamine. The mixture was degassed and heated at 70 °C for 24 h. The resulting solution was extracted with dichloromethane and water, and dried over anhydrous magnesium sulfate. The crude product was then purified by column chromatography using dichloromethane/hexane as eluent to yield 0.34 g (87%) of a colorless waxy solid. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.83 (s, 2Ar-*H*, *o* to Ar–≡–TMS), 7.64–7.45 (m, 15Ar–*H*), 7.19 (d, 2Ar-*H*, o to ArO, J = 1.18 Hz), 4.15 (d, 2H, –CH_2OAr, J = 4.71 Hz), 3.60–3.31 (m, 24H, –OCH_2CH_2), 2.45–2.13 (m, 3H, –C*H*(OCH$_2$)$_2$), 1.51–1.49 (m, 8H, –OCH$_2$CH_2), 1.24 (s, 56H, –CH_2CH$_2$), 0.89–0.85 (m, 12H, –CH$_2$CH_3), 0.27 (s, 18H, –SiCH_3).

$C_{92}H_{142}O_7Si_2$: Calcd. C 78.02, H 10.11; Found C 77.87, H 10.05.
MALDI-TOF-MS: m/z = 1 437.89 $[M + Na]^+$, Calcd. 1 438.28.

Synthesis of Compound **5**

Compound **4** (0.34 g, 0.24 mmol) and K_2CO_3 (0.34 g, 2.40 mmol) in tetrahydrofuran (THF)/methanol (1 : 1, 50 mL) were stirred for 2 h at room temperature. The resulting mixture was then extracted with ether and water. The combined organic layer was dried over anhydrous magnesium sulfate. The crude products were purified by column chromatography using dichloromethane/hexane as eluent to yield 0.28 g (93%) of a colorless waxy solid. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.83 (d, 2Ar-*H*, *o* to Ar–≡, J = 1.45 Hz), 7.65–7.53 (m, 15Ar-*H*), 7.18 (d, 2Ar-*H*, *o* to ArO, J = 1.23 Hz), 4.17 (d, 2H, –CH_2OAr, J = 4.88 Hz), 3.59–3.31 (m, 24H, –OCH_2CH_2), 3.14 (s, 2H, ≡C*H*), 2.43–2.05 (m, 3H, –C*H*(OCH$_2$)$_2$), 1.50–1.47 (m, 8H, –OCH$_2$CH_2), 1.25 (s, 56H, –CH_2CH$_2$), 0.90–0.84 (m, 12H, –CH$_2$CH_3).

$C_{86}H_{126}O_7$: Calcd. C 81.21, H 9.98; Found C 81.25, H 9.89.
MALDI-TOF-MS: m/z = 1 293.11 $[M + Na]^+$, Calcd. 1 293.92.

Synthesis of Compound **1**

Compound **5** (0.28 g, 0.22 mmol) and **6** (0.34 g, 0.22 mmol) in anhydrous triethylamine (10 mL) was added to a suspension of (triphenylphosphine) palladium(0) (5.9 mg, 0.005 mmol) and copper(I) iodide (0.6 mg, 0.003 mmol) in anhydrous triethylamine (150 mL) over 100 h at 50 °C, using a dual syringe pump. After the addition, the mixture was stirred for an additional 24 h at room temperature to complete the reaction. The mixture was then extracted with dichloromethane and water. The organic layer was dried over anhydrous magnesium sulfate. The crude products were purified by column chromatography using ethyl acetate/methanol as eluent, and the product was further purified by recycling preparative HPLC to yield 68 mg (12%) of a white waxy solid. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.92 (s, 4Ar-*H*, *o* to Ar–≡), 7.68–7.50 (m, 30Ar-*H*), 7.23 (d, 4Ar-*H*, *o* to ArO, J = 1.35 Hz), 4.19 (d, 4H, –CH_2OAr, J = 5.25 Hz), 3.62–3.43 (m, 84H, –OCH_2CH_2), 3.37 (m, 12H, OCH_3), 2.46–2.13 (m, 6H, –C*H*(OCH$_2$)$_2$), 1.52–1.49 (m, 8H, –OCH$_2$CH_2), 1.24 (s, 56H, –CH_2CH$_2$), 1.11 (s, 12H, –OCHCH_3), 0.89–0.84 (m, 12H, –CH$_2$CH_3).

$C_{160}H_{232}O_{26}$: Calcd. C 74.73, H 9.09; Found C 74.80, H 9.05.
MALDI-TOF-MS: m/z 2 595.02 $[M + Na]^+$, Calcd. 2 593.54.

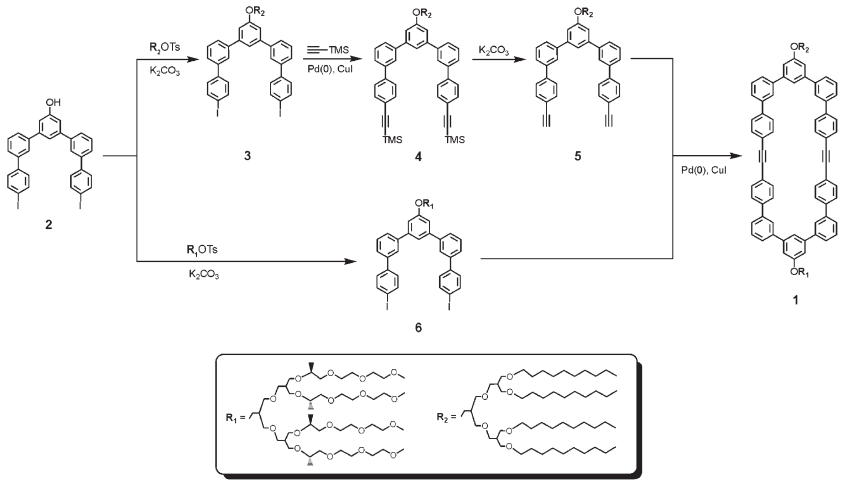
Solution Preparation

Aqueous solutions of the macrocycle were prepared by mixing compound **1** and deionized water in clean glass vials (25 mL). The

samples were sealed with para film at room temperature. Before using in each experiment they were sonicated for two hours and stabilized for more than one day.

Dynamic Light Scattering (DLS) Experiments

DLS experiments were performed with solutions at a scattering angle of 90° at 25 °C, using a UNIPHASE HeNe laser operating at 632.8 nm. The maximum operating power of the laser was 30 mW. The detector optics employed optical fibers coupled to an ALV/SOSIPD/DUAL detection unit, which employed an EMI PM-28B power supply and a ALV/PM-PD preamplifier/discriminator.



Scheme 1. Synthetic route to molecule **1**.

Transmission Electron Microscopy (TEM) Experiments

TEM experiments were carried out with a JEOL JEM-2010 operated at 120 kV. The cryogenic TEM (cryo-TEM) experiments were performed with a thin film of an aqueous solution of the macrocycles (5 μL) transferred to a perforated supported grid. The excess liquid was blotted with filter paper for 2–3 s, and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. The grid was transferred, on a Gatan 626 cryoholder, to a JEM-2010 TEM. Direct imaging was carried out at a temperature of approximately −175 °C, and the images were acquired with a Dual Vision 300 W and SC 1000 CCD camera (Gatan, Inc.; Warrendale, PA).

Results and Discussion

Synthesis of the Amphiphilic Macrocycle

The synthesis of aromatic macrocyclic amphiphile (**1**) is outlined in Scheme 1 and started with the preparation of a dendritic oligoether dendron and a branched alkyl chain. The aromatic core (**2**) was etherificated with hydrophobic and hydrophilic dendrons, respectively. The hydrophobic segment was further modified through a Sonogashira reaction and deprotection of the trimethylsilyl group for the final reaction. The final macrocycle **1** was prepared from the ring closure reaction by a Sonogashira reaction of **5** and **6** in the presence of Pd and copper iodide as catalysts.[10] To alleviate side reactions such as homo-coupling and polymerization of precursors, and to improve the yield of the product, the reaction was performed under high-dilution conditions by dropping the two reactants into the catalyst solution, using a dual syringe pump over four days. The resulting aromatic macrocycle (**1**) was characterized by ^{1}H NMR spectroscopy, elemental analysis, and MALDI-TOF-MS and was shown to be in good agreement with the expected chemical structure. As shown in Figure 1, the MALDI-TOF-MS spectrum of the molecule exhibits two signals that can be assigned as the Na and K adducts of the molecular ions.

Self-Assembly Behavior of the Amphiphilic Macrocycle

The macrocyclic molecule can lead to supramolecular assemblies in a solvent selective for the oligoether dendron because of its amphiphilic characteristics. Subsequently, the self-aggregation behavior of **1** (0.01 wt.-%) was investigated by using UV-vis and fluorescence spectroscopies (Figure 2a). The absorption maximum of the macrocycle in aqueous solution is blue-shifted with respect to that recorded in THF, and the fluorescence is quenched, which indicates H-type aggregation of the aromatic segments. DLS experiments were performed with a solution of **1** to further investigate aggregation behavior (Figure 2b). The CONTIN

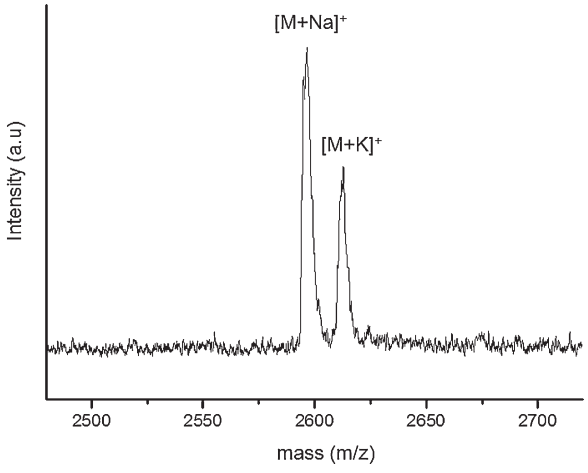


Figure 1. MALDI-TOF-MS spectrum of amphiphilic macrocycle **1**.

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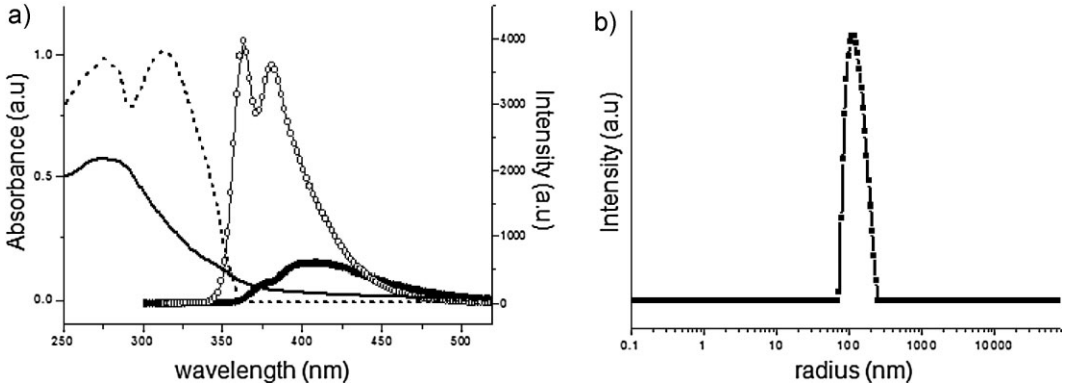

Figure 2. a) Absorption of **1** (0.01 wt.-%) in THF (dot line) and water (solid line), emission spectra of **1** in THF (circles) and water (dots). b) Size distributions of 0.01 wt.-% aqueous solution of **1** obtained from CONTIN analysis of the autocorrelation functions.

analysis of the autocorrelation function showed a monomodal size distribution, which suggests the presence of equilibrated aggregates in a water environment. The average hydrodynamic radius (R_H) of **1** was observed to be approximately 110 nm. Evidence for the formation of spherical aggregates was provided by FM and TEM. FM investigations revealed that **1** was self-assembled into spherical objects as large as a few hundred nanometer in diameter (Figure 3a). The formation of spherical aggregates was further confirmed by TEM experiments. The TEM micrograph negatively stained with a 2 wt.-% aqueous solution of uranyl acetate shows spherical objects with an average diameter of about 200 nm (Figure 3b). This dimension exceeds the extended molecular length (≈7 nm by Corey–Pauling–Koltun (CPK) modeling) by a factor of about 30, which strongly suggests that the aggregates are capsule-like entities rather than simple micelles. To further corroborate the formation of hollow capsules, we performed cryo-TEM investigations. As shown Figure 3c, the micrograph shows hollow spherical objects with diameters that range from a few hundred nanometers to a few micrometers with a uniform wall thickness of approx. 10 nm against the vitrified solution background. It should be noted that the hydrated oligoether dendrons may not provide sufficient contrast for direct visualization. Thus, the dimension of 10 nm for the wall thickness can be considered to correspond to approximately twice the extended hydrophobic segment including the aromatic segment and alkyl chains (≈5 nm by CPK modeling), which implies a bilayer packing of the macrocyclic molecules (Figure 3d). These results imply that the aromatic segment adopts a planar conformation within the aromatic domain. Considering that a similar macrocyclic amphiphile based on only hydrophilic chains self-assembles into helical coils based on a boat conformation,[8] the planar conformation seems to force the macrocyclic segment to pack into layers.

Remarkably, these vesicular aggregates encapsulate hydrophobic dyes in the shell of the capsules without

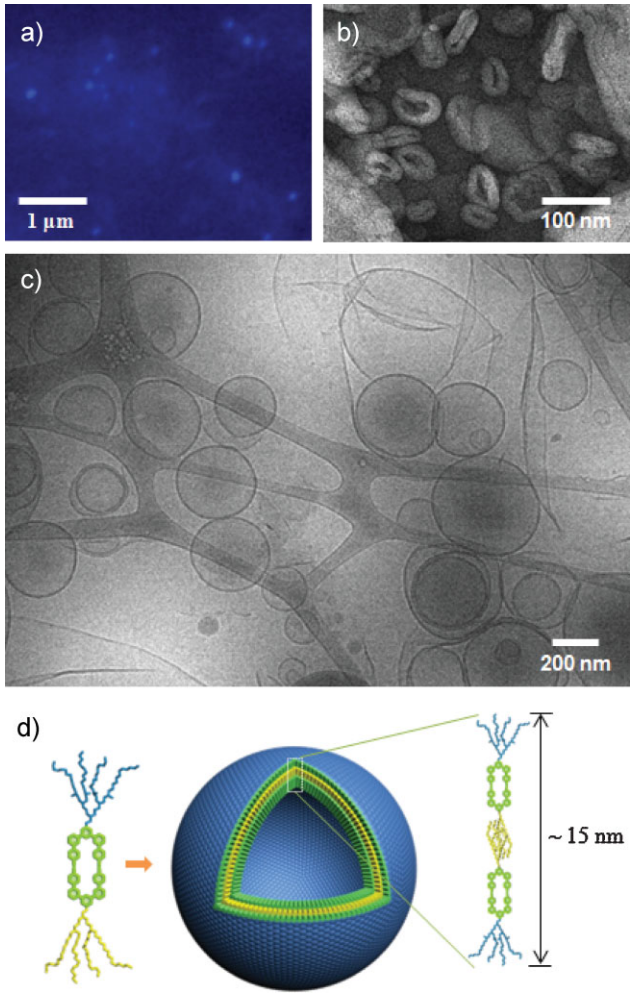

Figure 3. a) FM, b) TEM, and c) cryo-TEM images of an aqueous solution of **1** (0.01 wt.-%) showing the vesicular objects. d) Schematic representation of bilayered vesicular objects formed from **1** (yellow: hydrophobic dendron, green: macrocyclic aromatic segment, blue: hydrophilic dendron).

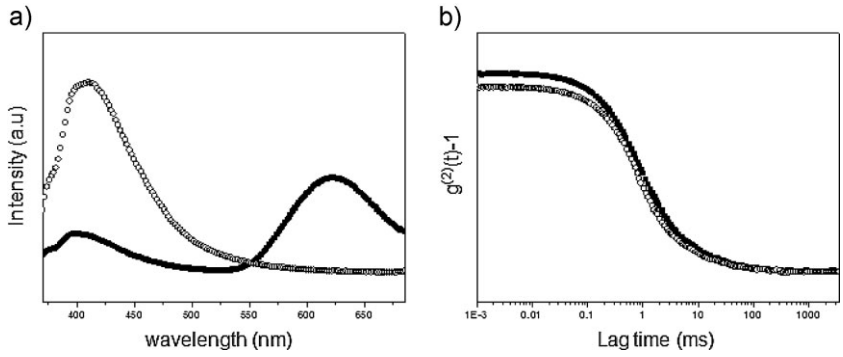

Figure 4. a) Emission spectra and b) autocorrelation functions (obtained by DLS at a scattering angle of 90°) of **1** in water (circles) and a Nile Red-encapsulated aqueous solution of **1** (dots) (0.01 wt.-%).

any disruption of their shape. The encapsulation experiments were performed in an aqueous solution of **1** with a hydrophobic guest, Nile Red.[11] Approximately 0.01 mg of Nile Red was added to 2 mL of an aqueous solution (0.01 wt.-%) of **1**. After sonication for two hours, the mixed solution was stabilized for one day at room temperature to obtain equilibrated aggregates, and then filtered to remove trace amounts of insoluble Nile Red. The intercalation of Nile Red within the aromatic segments was confirmed by fluorescence spectroscopy (Figure 4a). When a solution of **1** containing Nile Red was excited at 280 nm, where most of the radiation is absorbed, the fluorescence intensity is significantly suppressed while a strong emission emerges at 620 nm. This result indicates energy transfer from **1** to Nile Red, which demonstrates that Nile Red is efficiently encapsulated in the shell of the vesicles.[12] In great contrast to reported rod amphiphiles, the vesicular objects formed by the macrocyclic triblock molecules maintain their assemblies without any change in shape and size even after encapsulating guest molecules (Figure 4b). This result indicates that the vesicles have a high aggregation stability mainly as a result of the self-assembly of rigid aromatic macrocycles.

Conclusion

In this work, a triblock amphiphilic macrocycle composed of a hydrophilic oligoether dendron at one end and a hydrophobic branched alkyl chain at the other end of an aromatic macrocyclic segment has been synthesized. The macrocyclic molecules self-assemble into vesicular aggregates with an average diameter of ≈200 nm and a uniform wall thickness in a water environment, as confirmed by DLS and TEM experiments. The vesicles persist in their shape and size even after aromatic guest intercalation, which indicates a high aggregation stability. Consequently, introduction of such a macrocycle and a hydrophobic branch into amphiphilic molecules should enable the construction of highly stable hollow capsules that can be used as efficient delivery vectors for both hydrophobic and hydrophilic cargo molecules.

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