Organic LETTERS

Letter

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A Water-Soluble Cyclotriveratrylene-Based Supra-amphiphile: Synthesis, pH-Responsive Self-Assembly in Water, and Its Application in Controlled Drug Release

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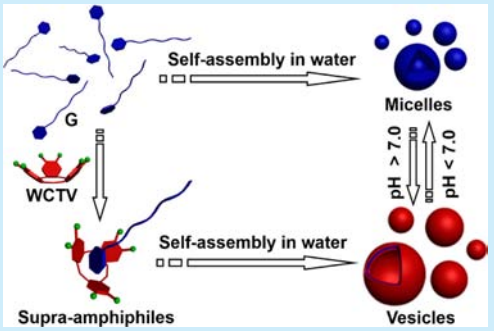
Supporting Information

ABSTRACT: A new water-soluble cyclotriveratrylene (WCTV) was designed and synthesized, and benzyldimethyldodecylammonium chloride (G) was chosen as the guest molecule to construct a supra-amphiphile by the host–guest interaction between WCTV and G in water, which is pH responsive. The supra-amphiphiles self-assembled into vesicles in water. When the pH of the solution was below 7.0, the supra-amphiphile disassociated, and the vesicles collapsed. Then, the pH-responsive self-assembly system was utilized for controlled drug release.



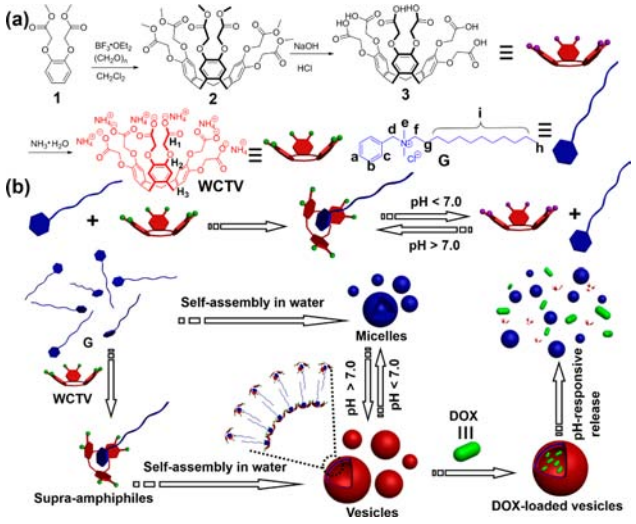
Self-assembly, the spontaneous organization of components into patterns or structures by different noncovalent interactions, is a constantly growing research area because it is not only a practical strategy for generating ensembles of complex nanostructures, which can be used for smart materials and self-healing structures, but also for better understanding living systems because living cells self-assemble.[1] Specifically, supramolecular chemistry in water has attracted increasing attention because noncovalent interactions in aqueous media are of great importance for accessing better understanding and control of major processes in biology and life.[2−4] Supra-amphiphiles, the hydrophilic and hydrophobic parts of which are connected by noncovalent interactions or dynamic covalent bonds, are a typical model to construct various supramolecular architectures in water.[5,6] Owing to the advantage that functional groups can be easily attached to amphiphiles via noncovalent interactions without tedious organic synthesis, supra-amphiphiles can be useful for extended applications in stimuli-responsive self-assemblies, control release, drug delivery systems, sensors, and so on.[7−11] Host–guest molecular recognition based on macrocyclic hosts, such as crown ethers,[12] cyclodextrins,[13,14] calixarenes,[15,16] cucurbiturils,[17,18] and pillararenes,[19−22] are one of the most important approaches to prepare supra-amphiphiles.[23] Cyclotriveratrylenes (CTV), another class of macrocycle hosts like those just mentioned, have attracted much attention due to their small cavity, stable conformation, good chemical stability, and easy modification.[24] However, the host–guest interactions and self-assemblies based on CTV in water have rarely been reported.[25,26] Moreover, the study of supra-amphiphiles constructed from CTV-based host–guest recognition in water and their self-assembly behavior have never been reported. Therefore, to expand CTV-based host–guest recognition in water and enrich the field of supra-amphiphiles, it is of great importance to prepare CTV-based supra-amphiphiles.

Herein, we designed and synthesized a new water-soluble cyclotriveratrylene (WCTV) with a crown conformation that is more stable than the saddle conformation[27,28] and chose benzyldimethyldodecylammonium chloride (G) as the guest molecule to form a supra-amphiphile by the host–guest interaction between WCTV and G in water (Scheme 1). In addition, the reversible pH-responsive self-assembly of the supra-amphiphile in water was also investigated. The amphiphilic guest itself self-assembled into micelles. Interestingly, upon the addition of WCTV to the solution of G, the supra-amphiphile formed, and the aggregation morphology changed to vesicles. When the pH of the solution decreased below 7.0, the CTV-based host–guest recognition disassociated, and the supra-amphiphile disassembled; correspondingly, the vesicles collapsed and micelles formed again. Moreover, when the pH of the solution increased over 7.0, the supra-amphiphiles reassembled, leading to the reformation of the vesicles. The vesicles formed from the supra-amphiphiles were used as delivery vehicles because the vesicles contain hydrophilic cavities that can encapsulate hydrophilic guest molecules and release them in response to a decrease in pH.[29,30] The hydrophilic anticancer drug doxorubicin (DOX)[31] was then used as a model payload (Scheme 1). The resulting DOX-loaded vesicles presented rapid DOX release in cancer cells. Furthermore, the materials for the preparation of the vesicles are facile to obtain, have low cytotoxicity, loading of DOX by the vesicles does not affect the therapeutic effects of

Received: April 29, 2016
Published: June 7, 2016

DOI: 10.1021/acs.orglett.6b01264
Org. Lett. 2016, 18, 2910–2913

Scheme 1. (a) Synthetic Route to WCTV and Cartoon Representation of WCTV, G, and the Supra-amphiphile and (b) Cartoon Representation of the pH-Responsive Self-Assembly of the Amphiphile and its Application in Controlled Drug Release



DOX for cancer cells, and the vesicles are pH responsive. These properties endow the vesicles with great potential for application in the field of highly efficient drug delivery systems

For the supra-amphiphile to be synthesized, a new water-soluble CTV (WCTV) was synthesized. First, compound CTV **2** was obtained through a new and easy method with a high yield by cyclooligomerization of monomer **1** (Scheme 1a), and its single crystals were successfully obtained by diffusing ethanol into its saturated DMF solution (Figure 1). Next, water-soluble cyclotriveratrylene (WCTV) was synthesized through the hydrolysis of **2** and then refluxed in ammonium hydroxide.

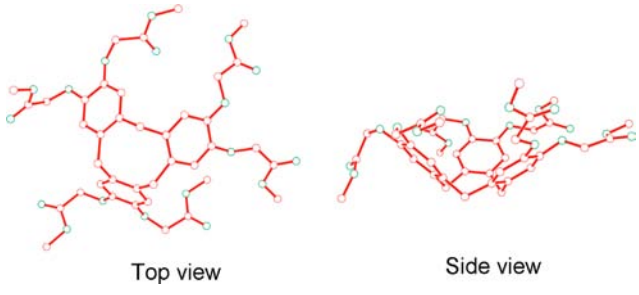


Figure 1. Ball–stick views of the crystal structure of **2**. Hydrogen atoms are omitted for clarity. Color code: C, red; O, green.

^{1}H NMR spectroscopy was used to investigate the host–guest interactions between WCTV and G. Compared with free WCTV and G (Figure 2a and c, respectively), significant chemical shift changes of the signals for the protons on G occurred in the presence of equimolar WCTV (Figure 2b). The peaks related to H_a, H_b, H_c, H_d, H_e, H_f, and H_g shifted upfield ($\Delta\delta$ = −0.20, −0.20, −0.37, −0.19, −0.70, −0.54, and −0.43 ppm, respectively), indicating that these protons are located within the cavity of WCTV and shielded by the electron-rich cyclic structure because of the complexation between WCTV and G. Moreover, these peaks became broad due to complexation dynamics. Additionally, protons H_3 on WCTV also slightly shifted downfield and split. In addition, a 2D

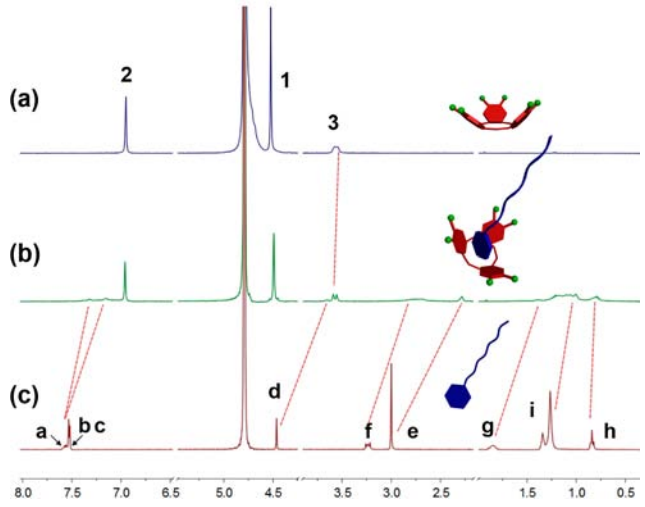


Figure 2. Partial ^{1}H NMR spectra (400 MHz, D_2O, room temperature): (a) WCTV (2.50 mM), (b) G (2.50 mM) and WCTV (2.50 mM), and (c) G (2.50 mM).

NOESY NMR experiment was also performed to confirm the ^{1}H NMR results (Figure S7). Moreover, the isothermal titration calorimetry (ITC) experiments were carried out to determine the association constant (K_a) and stoichiometry of this host–guest complex. The K_a value was estimated to be $(1.34 \pm 0.30) \times 10^4$ M^{-1} for WCTV⊃G in the 1:1 complexation mode (Figure S8). These phenomena provided convincing evidence for the formation of an inclusion complex between WCTV and G (Figure 2).

The molecular recognition of WCTV to G in water not only has high binding strength but is also pH responsive. As shown in the ^{1}H NMR spectra (Figure S9), when the solution pH was 5.0, the carboxylic groups of WCTV were protonated to insoluble carboxylic acid groups to become compound **3**. The signals related to the protons on the host disappeared in the ^{1}H NMR spectrum as the host precipitated from the solution (Figure S9b). The complexation between the host and G was destroyed. Thus, there were only the signals for the protons on water-soluble guest G in the ^{1}H NMR spectrum. When the pH of the solution recovered to 7.0, WCTV formed again. The peaks corresponding to the protons on G turned back to the original state (Figure S9c), indicating the formation of WCTV⊃G. These results demonstrated the reversible pH-responsive complexation between WCTV and G.

Moreover, the inclusion complex WCTV⊃G also acted as a supra-amphiphile with the alkyl chains as the hydrophobic part and WCTV head as the hydrophilic portion. Hence, we further explored its self-assembly behavior in water. First, by using the concentration-dependent conductivity measurements, the critical aggregation concentrations of the amphiphilic G and the supra-amphiphile were measured to be 8.95×10^{-5} and 3.63×10^{-5} M, respectively (Figure S10). The self-assembly behaviors of G and the supra-amphiphile in water were then investigated via transmission electron microscopy (TEM), dynamic light scattering (DLS), and atomic force spectroscopy (AFM) experiments. As shown in Figure 3a, G formed micelles with an average diameter of ∼3 nm because of its amphiphilicity. Upon the addition of WCTV, vesicles were obtained as the supra-amphiphiles WCTV⊃G formed because of the steric hindrance and the electrostatic repulsion between the supra-amphiphiles.[15,32] As shown in Figure 3b, the vesicles

DOI: 10.1021/acs.orglett.6b01264
Org. Lett. 2016, 18, 2910–2913

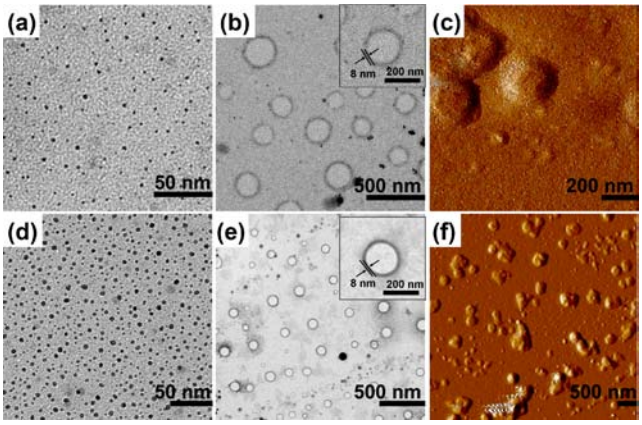


Figure 3. (a) TEM image of G (1.25×10^{-4} M) aggregates in water. (b) TEM image of WCTV with G aggregates (1.25×10^{-4} M for both) in water. (c) AFM image of b. (d) After adding enough HCl solution of b. (e) After adding enough NaOH solution of d. (f) AFM image of e.

had an average diameter of ~180 nm, and the wall thickness of the vesicles was ~8 nm. In addition, the AFM experiments confirmed the vesicular morphology and the wall thickness of the vesicles (Figure 3c and Figure S11). The thickness was approximately two extended lengths of the supra-amphiphile, which had antiparallel packing in water, suggesting that the vesicles had a bilayer wall structure.[33] Moreover, the DLS results of the vesicles were consistent with the TEM results (Figure S12a). Interestingly, when the solution was added with HCl solution, the supra-amphiphile disassembled, and the vesicles turned back to micelles (Figure 3d). After adding NaOH solution, the vesicles formed again (Figure 3e and f and Figure S12b).

The pH-responsive vesicles formed by the supra-amphiphile were then used for controlled drug release. DOX was used as a model drug to measure the encapsulation efficiency and release behavior of the vesicles. First, the DOX encapsulation experiment was carried out, and encapsulation efficiency was studied. As shown in Figure S13, compared with the unloaded vesicle solution, the absorption of the DOX-loaded one shifted from 440 to 550 nm, which is characteristic absorption of DOX in water, and its absorption became stronger. Additionally, the DOX-loaded vesicle solution was light red whereas the unloaded one was colorless. Furthermore, from the TEM image (Figure S14a), the average diameter of DOX-loaded vesicles was 220 nm, which was larger than that of the unloaded vesicles, and the interior of the vesicles was darker. These results demonstrated that DOX was successfully encapsulated into the vesicles. DLS results confirmed that the average diameter of the DOX-loaded vesicles is 220 nm (Figure S14b), which is in agreement with the size of the vesicles increasing after encapsulating guests molecules as previously reported.[30,34] Meanwhile, the DOX encapsulation efficiency of the vesicles was calculated to be 12.8% according to UV−vis absorption spectra.[35]

Then, the controllable DOX release *in vitro* experiments were carried out. The total release of DOX was only approximately 10.3% within 24 h under the physiological conditions, indicating that the vesicles are highly stable toward leakage when the solution pH is 7.4. In acidic conditions at pH 6.5 and 4.0, DOX was released in a larger amount from the DOX-loaded vesicles, resulting in approximately 62.9 and 92.2% release efficiencies, respectively. Additionally, in acidic conditions, rapid release could be observed in the first 5 min (Figure 4). As the microenvironment of tumor cells is acidic,[31]

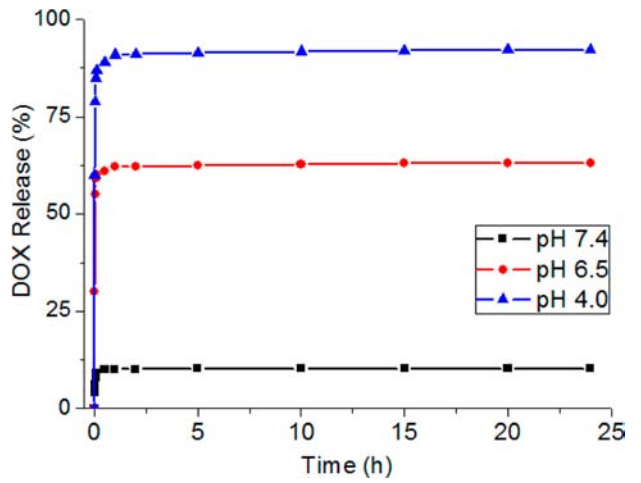


Figure 4. Release percentage of the DOX-loaded vesicles in the release media of different pH values.

the rapid release of DOX drugs from the DOX-loaded vesicles can be induced by the acidic microenvironment of tumor cells, which is of great importance for specific targeted therapy. As a result, this rapid release phenomenon, which presents a good response to physiologically relevant pH (pH 4.0−7.4), makes these DOX-loaded vesicles ideal candidates for a drug delivery system.

For the cellular uptake of the WCTV⊃G supra-amphiphile-based pH-responsive drug release system to be further investigated, a simple evaluation of cytotoxicity for WCTV, G, the unloaded vesicles, and the DOX-loaded vesicles at different concentrations against HepG2 (human liver hepatocellular carcinoma) cell lines was carried out using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.[29] HepG2 cells were incubated with WCTV, G, WCTV⊃G, DOX, and DOX-loaded vesicles, and the relative viabilities of HepG2 cells in the different groups were recorded. As shown in Figure S15a, we found that the relative viability of HepG2 cells incubated with WCTV, G, and unloaded vesicle groups was over 75% after 24 h. Additionally, the half-maximal inhibitory concentrations (IC_{50}) of WCTV, G, and WCTV + G are approximately 77.88, 26.37, 35.70 μg/mL, respectively. These results indicate low cytotoxicity of the materials to prepare the vesicles, suggesting that the vesicles constructed from the supra-amphiphiles are promising drug carriers. Moreover, as shown in Figure S15b, the cytotoxicity assay demonstrated that, compared with free DOX, DOX-loaded vesicles have a similar relative cell viability to that of the cancer cells under the same conditions, and the IC_{50} value of DOX and DOX-loaded vesicles was 9.553 and 9.185 μg/mL, respectively. These results indicated that, for the cancer cells employed in this work, encapsulation of DOX by the vesicles has almost no influence on the therapeutic effects of DOX. The cellular internalization of the DOX-loaded vesicles was examined on HepG2 cells by confocal laser scanning microscopy (CLSM) following the dye-specific fluorescent signature (Figure 5). After exposure to the blank, vesicles, DOX, and DOX-loaded vesicles groups for 4 h, respectively, the DOX was internalized into the cells with a fraction of the DOX being delivered into

DOI: 10.1021/acs.orglett.6b01264
Org. Lett. 2016, 18, 2910−2913

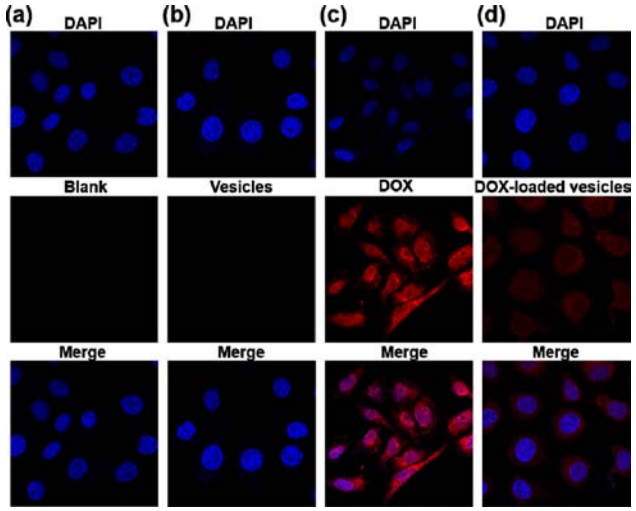


Figure 5. Confocal laser scanning microscopic images of HepG2 cells in (a) blank, (b) vesicles, (c) DOX, and (d) DOX-loaded vesicles.

nuclei, and the DOX-loaded vesicles were enriched around the nuclei of the HepG2 cells, indicating that the present system may be used to deliver a chemotherapeutic agent (DOX) into cells.

In conclusion, a pH-responsive supra-amphiphile based on the host−guest interactions between a new water-soluble cyclotriveratrylene (WCTV) and an amphiphilic guest (G) was constructed. The supra-amphiphile WCTV⊃G self-assembled into vesicles in water, which were used for controlled drug release. The anticancer drug DOX could be successfully encapsulated into these vesicles and controllably released. The host WCTV, guest G, and supra-amphiphile WCTV⊃G showed low cytotoxicity and that encapsulation of DOX by the self-assembled vesicles does not affect the therapeutic effects of DOX for cancer cells. These results make the vesicles applicable in the field of controlled drug release. This work expands cyclotriveratrylene-based host−guest recognition in water and enriches the field of supra-amphiphiles.

■ ASSOCIATED CONTENT

Supporting Information

These materials are available free of charge via the Internet at The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01264.

Synthesis and characterization of **2** and WCTV, ^{1}H NMR data, 2D NOESY NMR data, ITC data, UV−vis data, DLS results, and other materials (PDF)

X-ray crystallographic files for **2** (CCDC 1030157) (CIF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Fundamental Research Funds for the Central Universities.

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DOI: 10.1021/acs.orglett.6b01264
Org. Lett. 2016, 18, 2910−2913