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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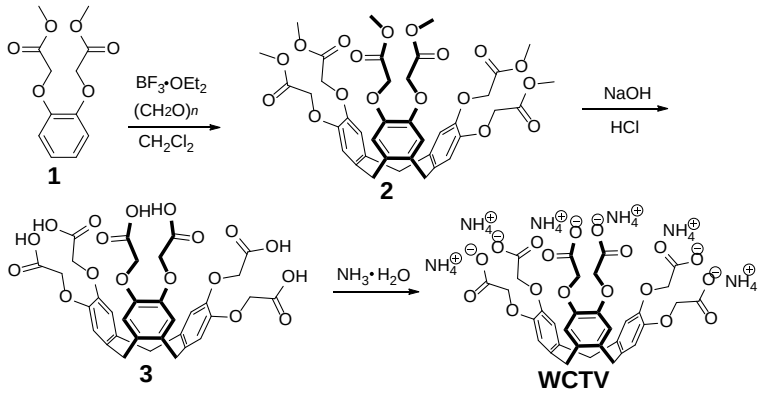
Supporting Information (13 pages)

1. Materials and methods

Compound **1** and compound **G** were commercially available. All reagents were commercially available and used as supplied without further purification. Solvents were either employed as purchased or dried according to procedures described in the literature. NMR spectra were recorded with a Bruker Avance DMX 600 spectrophotometer or a Bruker Avance DMX 400 spectrophotometer. Low-resolution electrospray ionization mass spectra were recorded with a Bruker Esquire 3000 Plus spectrometer (Bruker-Franzen Analytik GmbH Bremen, Germany) equipped with an ESI interface and an ion trap analyzer. High-resolution mass spectrometry experiments were performed with a Bruker 7-Tesla FT-ICR mass spectrometer equipped with an electrospray source (Billerica, MA, USA). The melting points were collected on a SHPSIC WRS-2 automatic melting point apparatus. Isothermal titration calorimetric (ITC) measurements were performed on a VP-ITC micro-calorimeter (Microcal, USA).The determination of the critical aggregation concentration (CAC) values was carried out on a DDS-307 instrument. Transmission electron microscopy investigations were carried out on a JEM-1200EX instrument. Atomic force microscopy (AFM) experiments were performed on a Multi-Mode Nanoscope-IIIa Scanning Probe Microscope (Veeco Company, USA) in the tapping mode. Dynamic light scattering was carried out on a Malvern Nanosizer S instrument at room temperature. The crystal structures were solved by SHELXS-97[S1] and refined by SHELXL-97.[S2] UV-vis spectra were taken on a Perkin-Elmer Lambda 35 UV-vis spectrophotometer. The confocal scanning laser microscopy investigations were carried out on a CLSM, Radiance 2100, Bio-Rad instrument.

*2. Synthetic routes for **2** and **WCTV***



Scheme S1 Synthetic routes for **2** and **WCTV**.

*3. Synthesis of **2** and **WCTV***

*3.1 Synthesis of **2***

To a solution of **1** (2.92 g, 11.5 mmol) in dichloromethane (200 mL), paraformaldehyde (0.698 g, 23.0 mmol) was added. Then boron trifluoride diethyl etherate ($BF_3{\cdot}O(C_2H_5)_2$, 1.63 g, 11.5 mmol) was added to the solution and the mixture was stirred at room temperature for 3 h. Then 200 mL of methanol was added to the reaction mixture, where product **2** precipitated as a white solid. The product was collected by vacuum filtration and then recrystallized from dichloromethane/methanol mixtures. The white needle-like crystals were dried under high vacuum (1.69 g, 44 %). Mp 198.5−199.8 °C. The proton NMR spectrum of **2** is shown in Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$, room temperature) δ (ppm): ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 6.86 (s, 6H), 4.71 (s, 12H), 4.64 (d, J = 14 Hz, 3H), 3.77 (d, *J* = 6.0 Hz, 18H), 3.49 (d, *J* = 14 Hz, 3H). The ^{1}3C NMR spectrum of **2** is shown in Figure S2. ^{13}C NMR (100 MHz, $CDCl_3$, room temperature) δ (ppm): 169.74, 146.71, 133.59, 117.66, 66.96, 52.19, 36.19. LRESIMS is shown in Figure S3: *m*/*z* 812.6 $[M + Na]^+$ (100%). HRESIMS: m/z calcd for $[M + Na]^+$ $C_{39}H_{42}O_{18}Na$, 821.2263, found 821.2271, error −1 ppm.

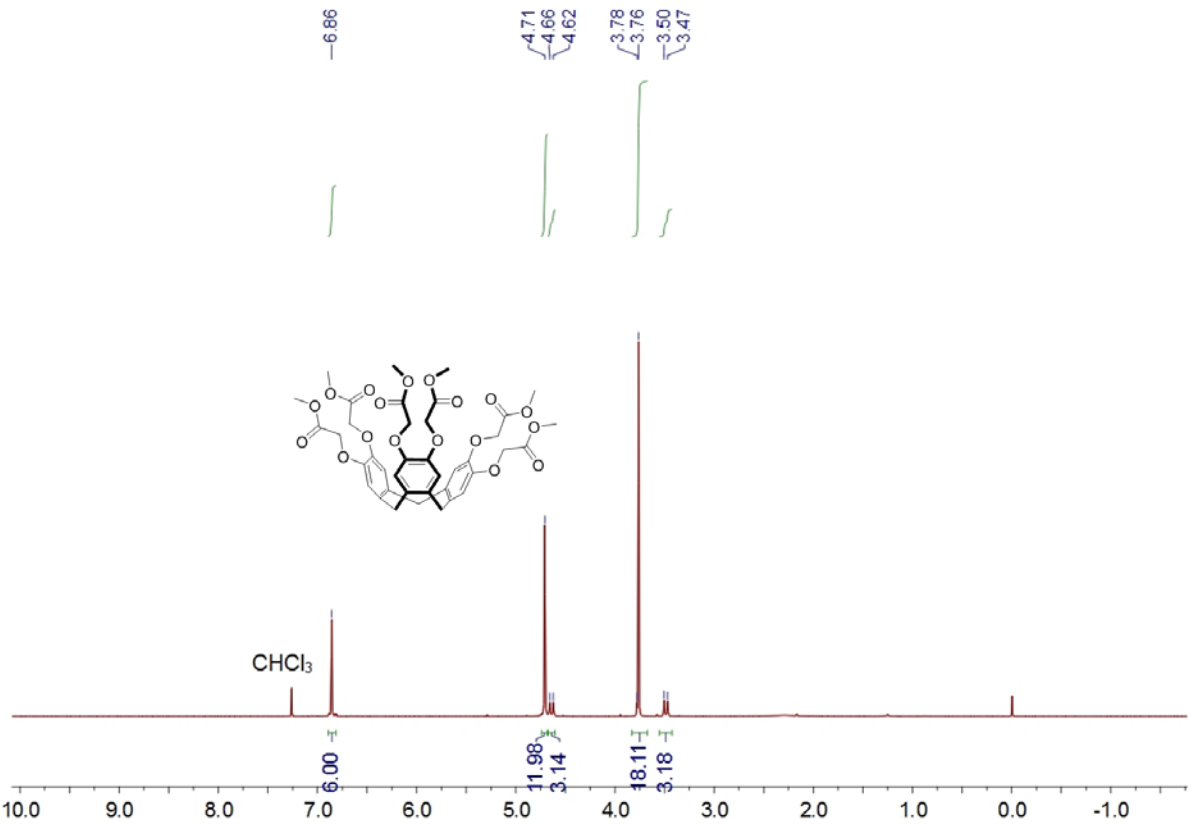


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **2**.

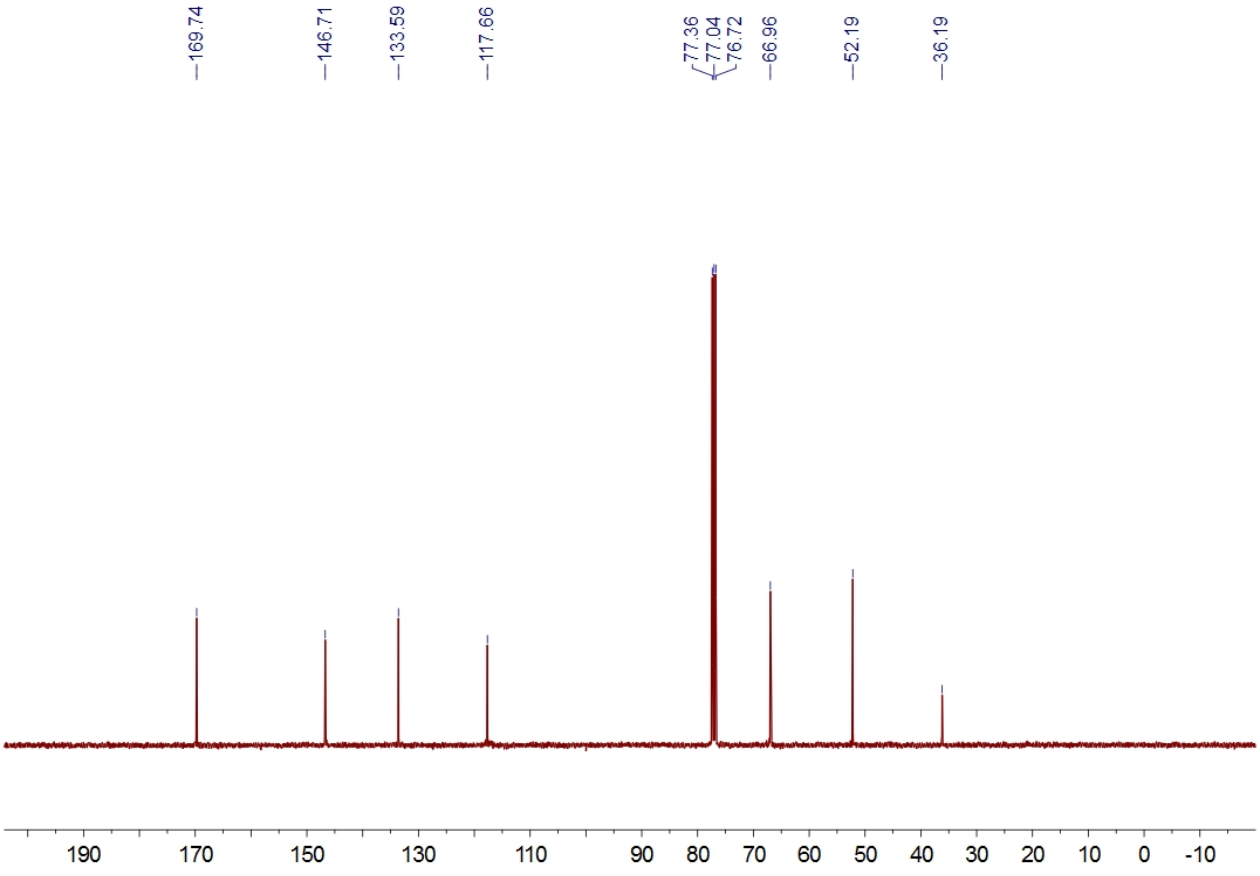


Figure S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 293K) of **2**.

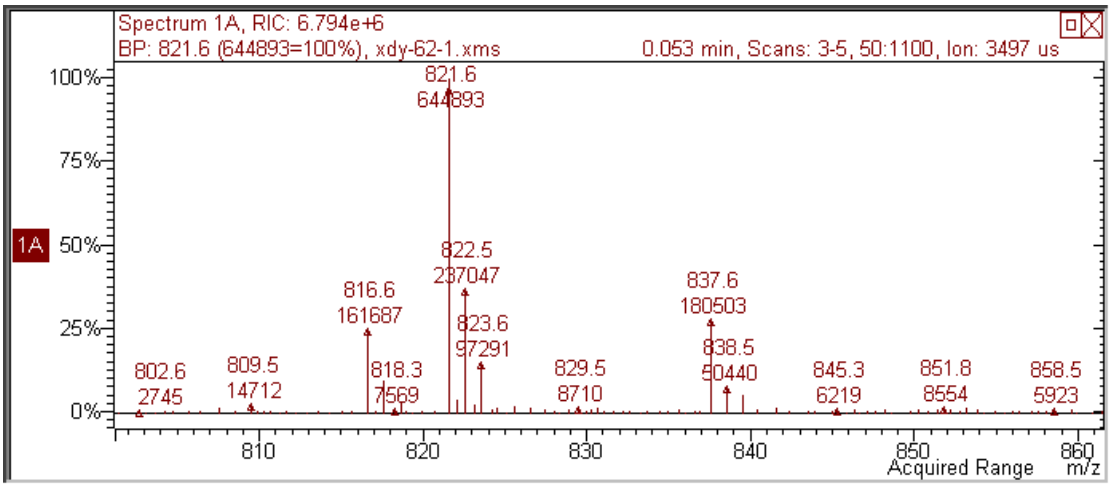


Figure S3. Electrospray ionization mass spectrum of **2**. Main peak: *m/z* 821.6 $[M + Na]^+$ (100%).

*3.2 Synthesis of **WCTV***

A solution of **2** (1.20 g, 1.50 mmol) in enthanol (120 mL) was treated with 40% aqueous sodium hydroxide (120 mL) at reflux for 10 h. The mixture was concentrated under reduced pressure, diluted with water (30 mL), and acidified with HCl solution. The precipitated product **3** was collected by filtration, washed with water, and dried under vacuum (0.998 g, 93%). A solution of **3** (0.900 g, 1.25 mmol) and 40% ammonium hydroxide (100.0 mL) were stirred at reflux for 5 h. The mixture was concentrated under reduced pressure to get the precipitated product **WCTV**. Then it was collected by filtration, washed with ethanol and dried under vacuum to obtain **WCTV** as a white solid (1.03 g, 99%). Mp: over 250 °C. The proton NMR spectrum of **WCTV** is shown in Figure. S4. ^{1}H NMR (400 MHz, D_2O, room temperature) δ (ppm): 6.95 (s, 6H), 4.79 (s, 3H), 4.52 (s, 12H), 3.57 (s, 3H). The ^{13}C NMR spectrum of **WCTV** is shown in Fig. S2. ^{13}C NMR (100 MHz, D_2O, room temperature) δ (ppm): 179.37, 148.49, 135.68, 117.11, 70.26, 38.10. LRESIMS is shown in Fig. S3: *m/z* 713.5 $[M - 6NH_4 + 5H]^-$ (100%). HRESIMS: m/z calcd for $[M - 2NH_4 - 10NH_3]^{2-}$ $C_{66}H_{58}O_{36}$, 145.0319, found 145.0325, error 4 ppm.

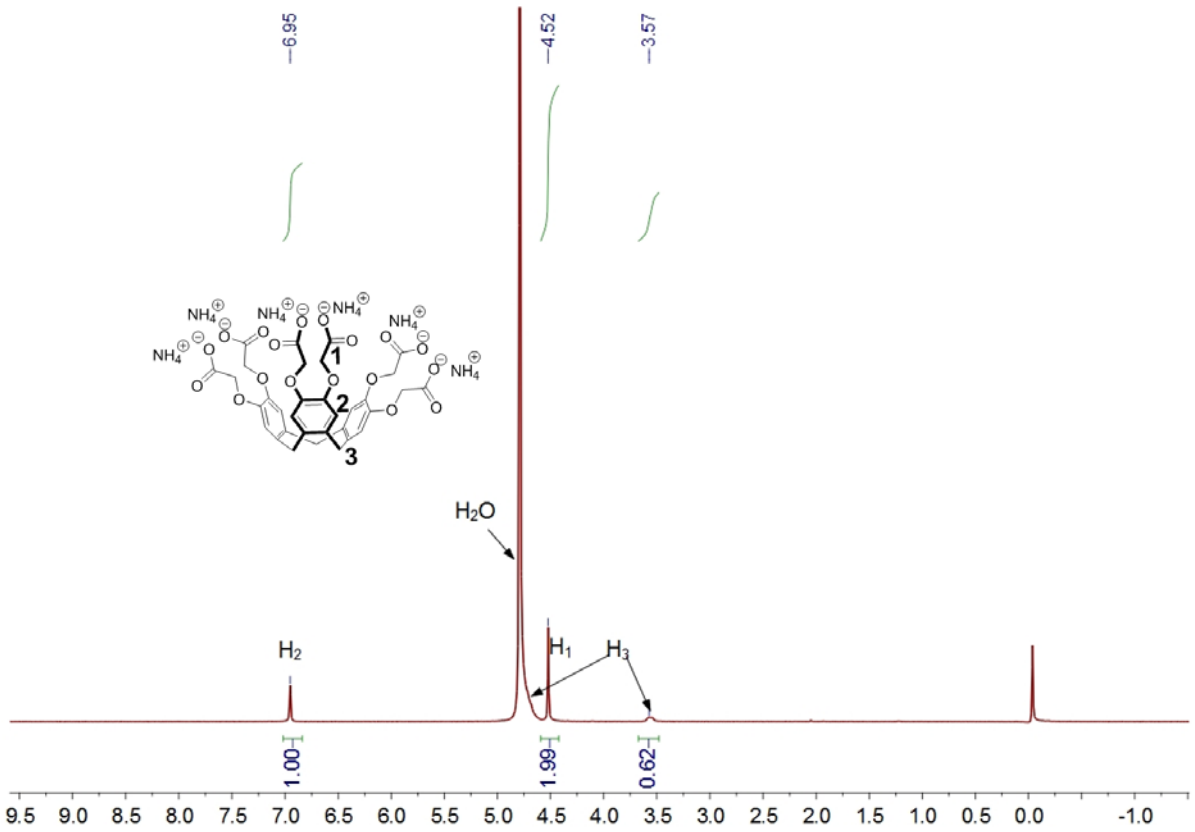


Figure S4. ^{1}H NMR spectrum (400 MHz, D_2O, room temperature) of **WCTV**.

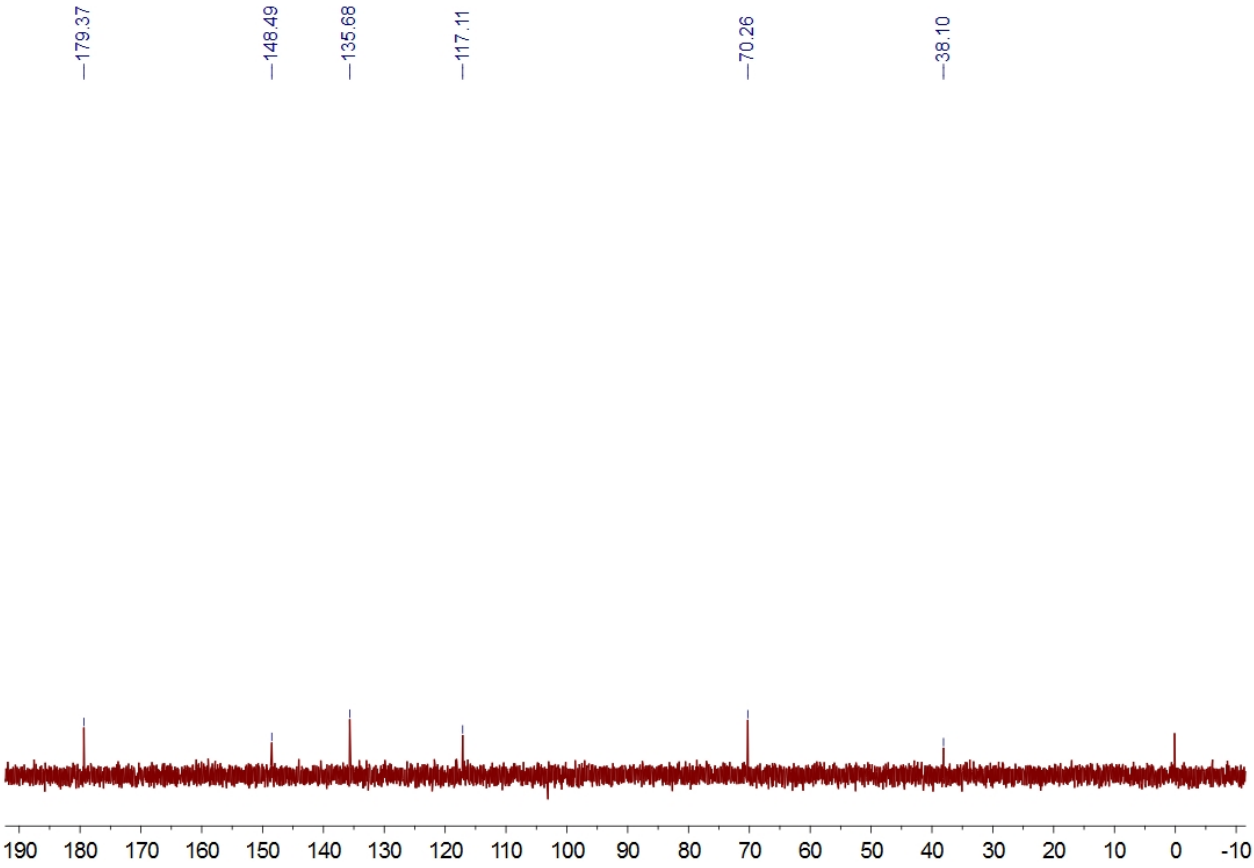


Figure S5. ^{13}C NMR spectrum (100 MHz, D_2O, room temperature) of **WCTV**.

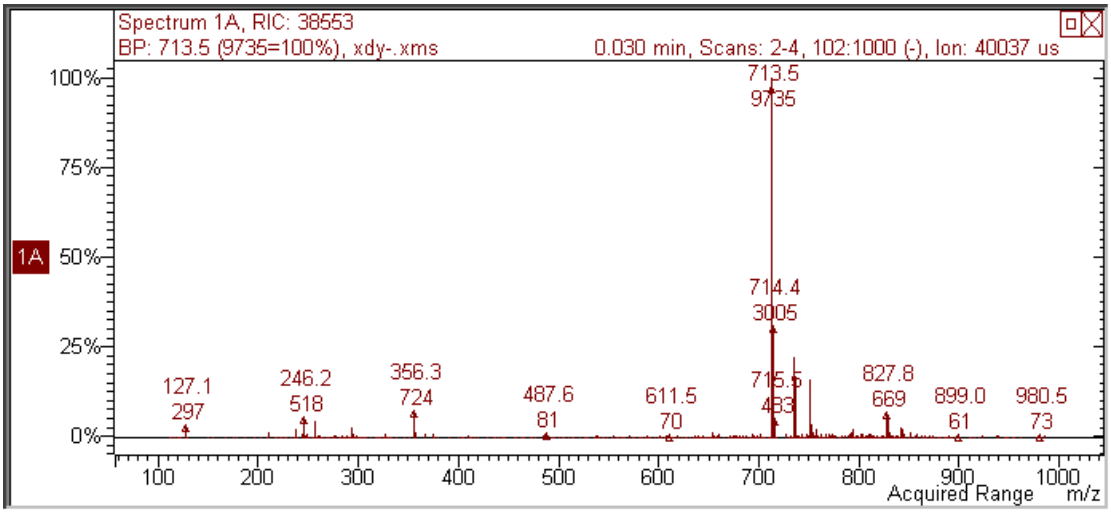


Figure S6. Electrospray ionization mass spectrum of **WCTV**. Main peak: *m/z* 821.6 $[M - 6NH_4+5H]^-$ (100%).

*4. X-ray crystal data of **2***

Crystal data of **2**: colourless, $C_{39}H_{42}O_{18}$, *FW* 798.73, monoclinic, space group $C1_2/c1$, a = 39.372(2), b = 8.0823(2), c = 27.4716(14) Å, α = 90.00°, β = 121.374(7)°, γ = 90.00°, V = 7463.8(6) Å^3, Z = 8, D_c = 1.422 g cm^{-3}, T = 172 K, μ = 0.965 mm^{-1}, 27172 measured reflections, 6583 independent reflections, 540 parameters, 0 restraints, $F(000)$ = 3360.0, R_1 = 0.0617, wR_2 = 0.1431 (all data), R_1 = 0.0508, wR_2 = 0.1304 [$I > 2\sigma(I)$], max. residual density 0.711 e•Å^{-3}, and goodness-of-fit (F^2) = 1.032. CCDC-1030157.

5. *Partial 2 D NOESY spectra of an equimolar solution of **WCTV⊃G***

2 D NOESY NMR experiment was employed to study the relative positions of the components in complex **WCTV⊃G.** NOE correlation signals were observed between protons H_f on the benzene ring of **G** and proton H_1 and H_3 of **WCTV** (Fig. S7, A and B), between protons H_a on the benzene ring of **G** and proton H_2 of **WCTV** (Fig. S7, C) and between protons H_c on the benzene ring of **G** and proton H_1 of **WCTV** (Fig. S7, D)

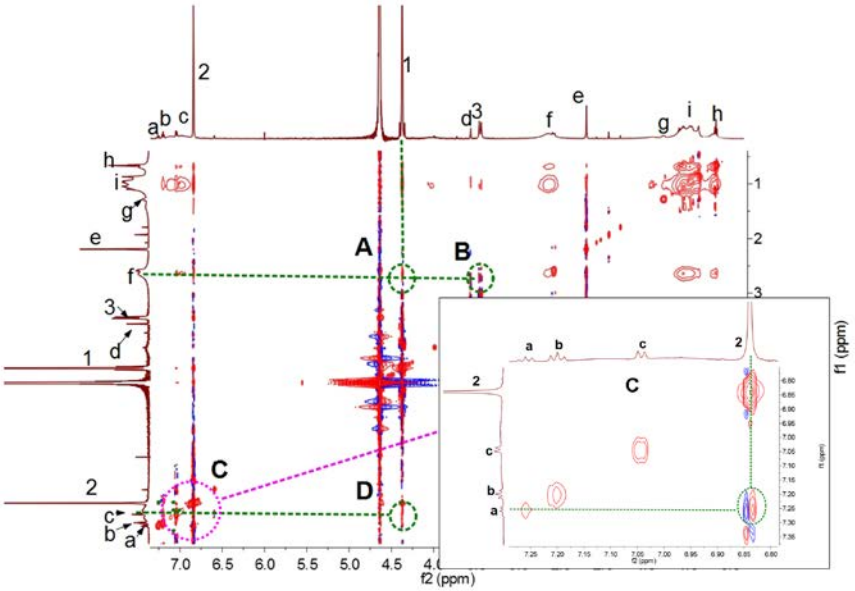


Figure S7. Partial 2D NOESY spectra of an equimolar mixture **G** and **WCTV** (2.50 mM) (600 MHz, D_2O, room temperature).

*6. The determination of the association constant of complex **WCTV⊃G** by ITC experiment*

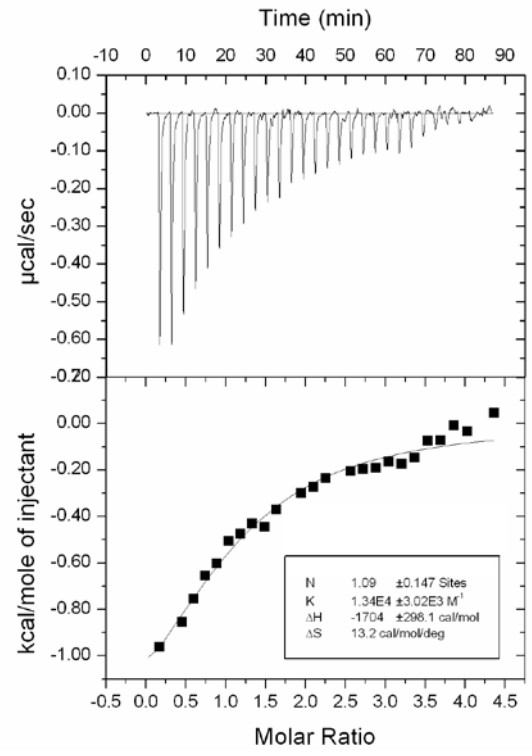


Figure S8. Microcalorimetric titration of **WCTV** with **G** in water at 298.15 K. (Top) Raw ITC data for 29 sequential injections (10 µL per injection) of a **G** solution (2.00 mM) into a **WCTV** solution (0.100 mM). (Bottom) Net reaction heat obtained from the integration of the calorimetric traces.

*7. pH-Responsive complexation of **WCTV⊃G***

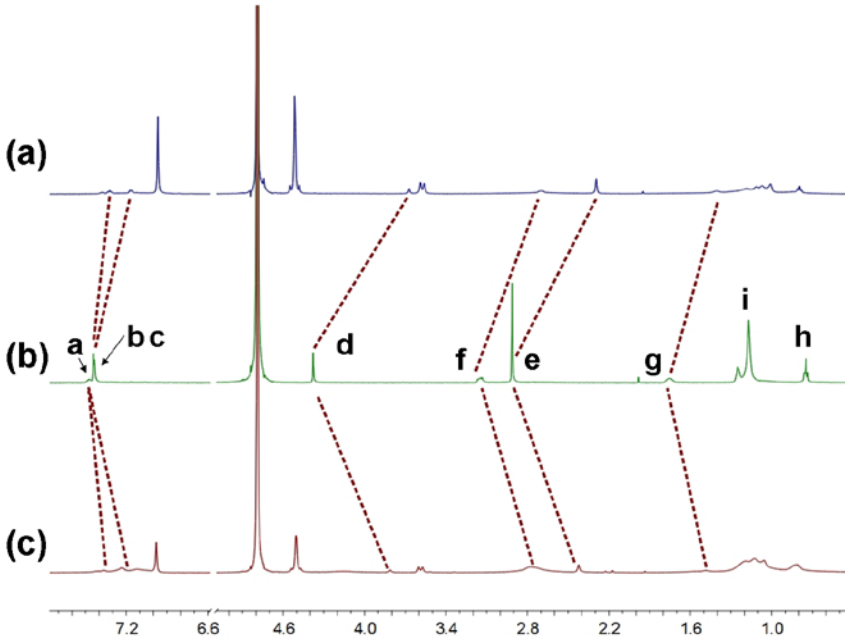


Figure S9. Partial ^{1}H NMR spectra (400 MHz, D_2O, room temperature): (a) **WCTV⊃G** (2.50 mM) when the solution pH was 7.0; (b) **WCTV⊃G** (2.50 mM) when the solution pH decreased to below 7; (c) **WCTV⊃G** (2.50 mM) when the solution pH recovered to 7.0.

*8. Critical aggregation concentration (CAC) determination of **G** and **WCTV⊃G***

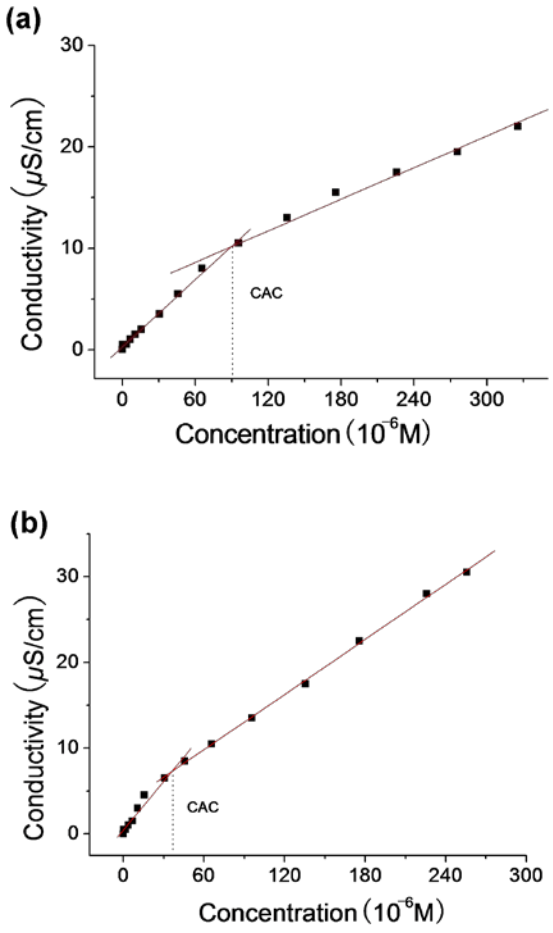
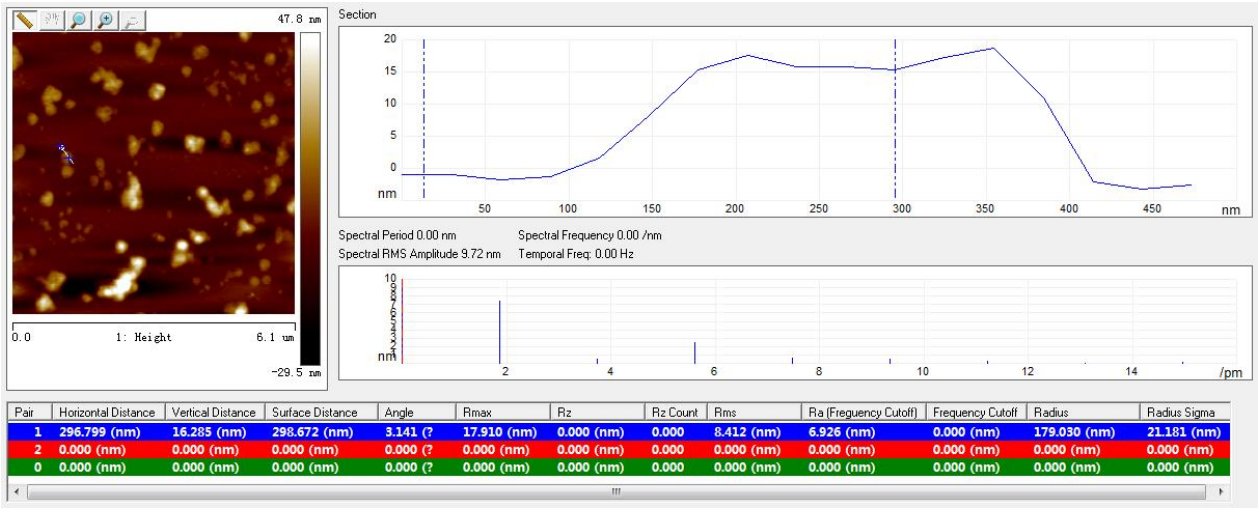


Figure S10. (a) The concentration-dependent conductivity of **G**. The critical aggregation concentration (CAC) was determined to be 8.98×10^{-5} M and (b) The concentration-dependent conductivity of the mixture of **G** and **WCTV**. The critical aggregation concentration (CAC) was determined to be 3.63×10^{-5} M.

9. AFM result of the self-assemled vesicles



Pair	Horizontal Distance	Vertical Distance	Surface Distance	Angle	Rmax	Rz	Rz Count	Rms	Ra (Freguency Cutoff)	Frequency Cutoff	Radius	Radius Sigma
1	296.799 (nm)	16.285 (nm)	298.672 (nm)	3.141 (?	17.910 (nm)	0.000 (nm)	0.000	8.412 (nm)	6.926 (nm)	0.000 (nm)	179.030 (nm)	21.181 (nm)
2	0.000 (nm)	0.000 (nm)	0.000 (nm)	0.000 (?	0.000 (nm)	0.000 (nm)	0.000	0.000 (nm)	0.000 (nm)	0.000 (nm)	0.000 (nm)	0.000 (nm)
0	0.000 (nm)	0.000 (nm)	0.000 (nm)	0.000 (?	0.000 (nm)	0.000 (nm)	0.000	0.000 (nm)	0.000 (nm)	0.000 (nm)	0.000 (nm)	0.000 (nm)

Figure S11. (a) AFM result of the self-assembled vesicles. The height measured from the AFM experiment is the height of two walls of the vesicles. It means that the wall thickness of the vesicles is 8.145 nm.

10. Dynamic light scattering (DLS)experiments

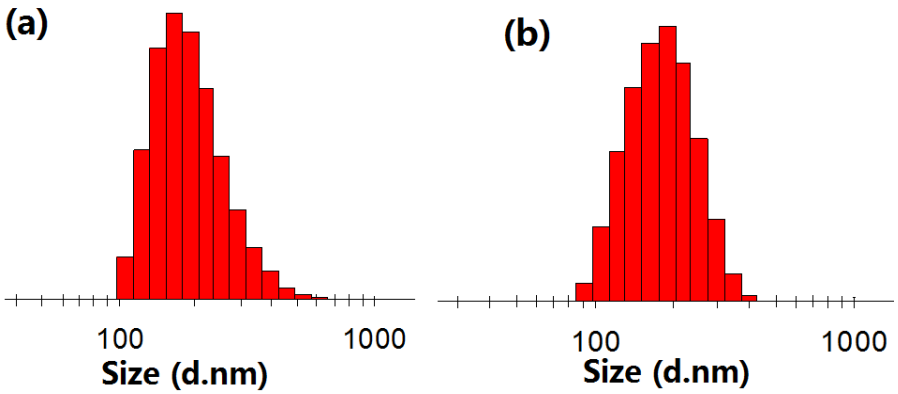


Figure S12. (a) DLS result of **WCTV⊃G** aggregates (2.50×10^{-4} M for both) in water; (b) DLS result of the reformed **WCTV⊃G** aggregates (2.50×10^{-4} M for both) in water.

11. DOX encapsulation experiment

DOX loading experiment: DOX-loaded vesicles were prepared by adding a certain amount of DOX into a freshly prepared aqueous solution of **WCTV** and **G** (2.5×10^{-4} M for both). The ultimate concentrations of DOX, WCTV, and G were 0.05, 0.25, and 0.25 mM, respectively. And then the prepared DOX-loaded vesicles were purified by dialysis (molecular weight cutoff = 3500) in distilled water for several times until the water outside the dialysis tube exhibited negligible DOX fluorescence. As a result, DOX was successfully loaded into the vesicles constructed from **WCTV⊃G**-based supra-amphiphiles. The DOX encapsulation and loading efficiency were calculated by the following equations:[S3]

Encapsulation Efficiency (%) = ($m_{DOX\text{-}loaded}$ / m_{DOX})100

$m_{DOX\text{-}loaded}$ and m_{DOX} are mass of DOX encapsulated in vesicles and mass of DOX added, respectively. The mass of DOX was measured by a UV spectrophotometer at 490 nm and calculated as relative to a standard calibration curve in the concentrations from from 5.00×10^{-3} to 2.50×10^{-2} mM in water.

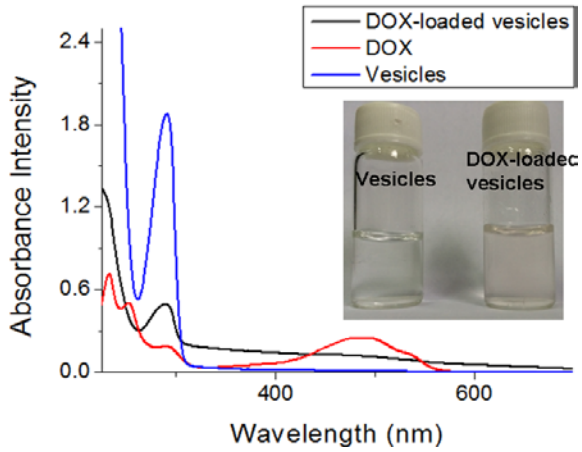


Figure S13. UV-vis absorption spectra of DOX-loaded vesicles, DOX, unloaded vesicles in water. Inset: color change of DOX-loaded vesicle (right) compared with unloaded one (left)

12. TEM and DLS results of the DOX-loaded vesicles

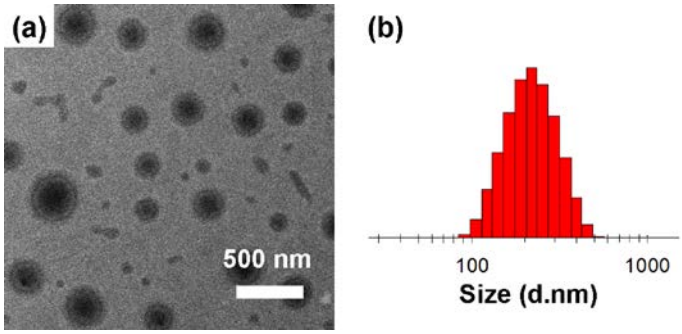


Figure S14. (a)TEM image of DOX-loaded vesicles; (b) DLS results of DOX-loaded vesicles in water

13. Controllable DOX release in vitro experiment

Controllable DOX release: 0.05 M tris-HCl (pH = 7.4), 0.2 M sodium acetate (pH = 4.0), and 0.1 M citrate (pH = 6.5) buffer solutions were used as drug release media to simulate normal physiological conditions and the intracellular conditions of tumor. In a typical release experiment, 1.60 mL of DOX-loaded vesicles were added into 8.40 mL of appropriate release medium at 37 °C. At selected time intervals, 3 mL of the release media was taken out for measuring the released DOX concentrations by the UV−vis absorption technique and then was returned to the original release media. The concentration of DOX was determined by measurement of absorbance at 490 nm using a standard absorbance verses concentration curve constructed for DOX in the corresponding release buffer. By presenting the vesicles to very low pH (the solution of HCl, pH = 2.0), a nearly 100% release of DOX from DOX-loaded vesicles could be obtained.

14. Relative cell viabilities

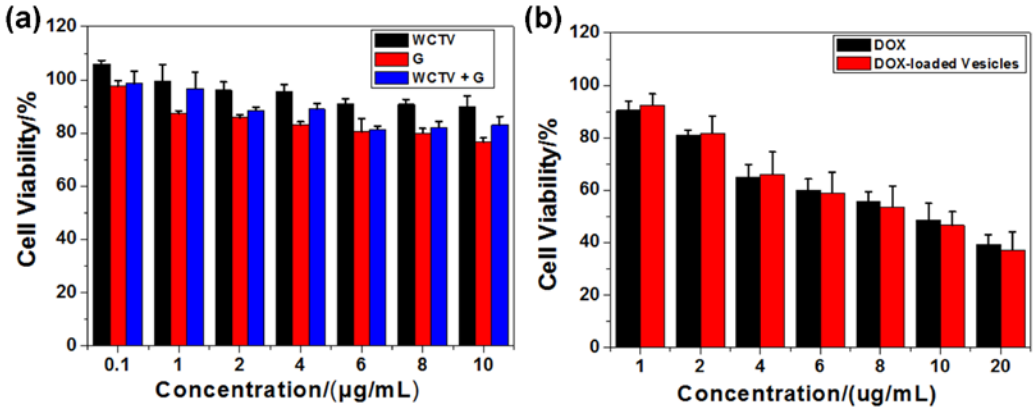


Figure S15. Relative cell viabilities of (a) HepG2 cells (24 h) incubated with **WCTV**, **G**, and **WCTV** + **G**, respectively, at different concentrations; (b) HepG2 cells (24 h) incubated with DOX and DOX-loaded vesicles at different concentrations. The half-maximal inhibitory concentration (IC_{50}) of **G**, **WCTV** + G, DOX and DOX-loaded vesicles are about 77.88 μg/mL, 26.37 μg/mL, 35.70 μg/mL, 9.553μg/mL and 9.185 μg/mL, respectively.

References:

S1. Sheldrick, G. M.; SHELXS-97, Program for solution of crystal structures, University of Göttingen, Germany, 1990.

S2. Sheldrick, G. M.; SHELXS-97, Program for refinement of crystal structures, University of Göttingen, Germany, 1997.

S3. Wang, K.; Guo, D.-S.; Wang, X.; Liu, Y. *ACS Nano* **2011**, *5*, 2880–2894.