Synergistic and Targeted Drug Delivery Based on Nano-CeO_2 Capped with Galactose Functionalized Pillar[5] arene via Host–Guest Interactions

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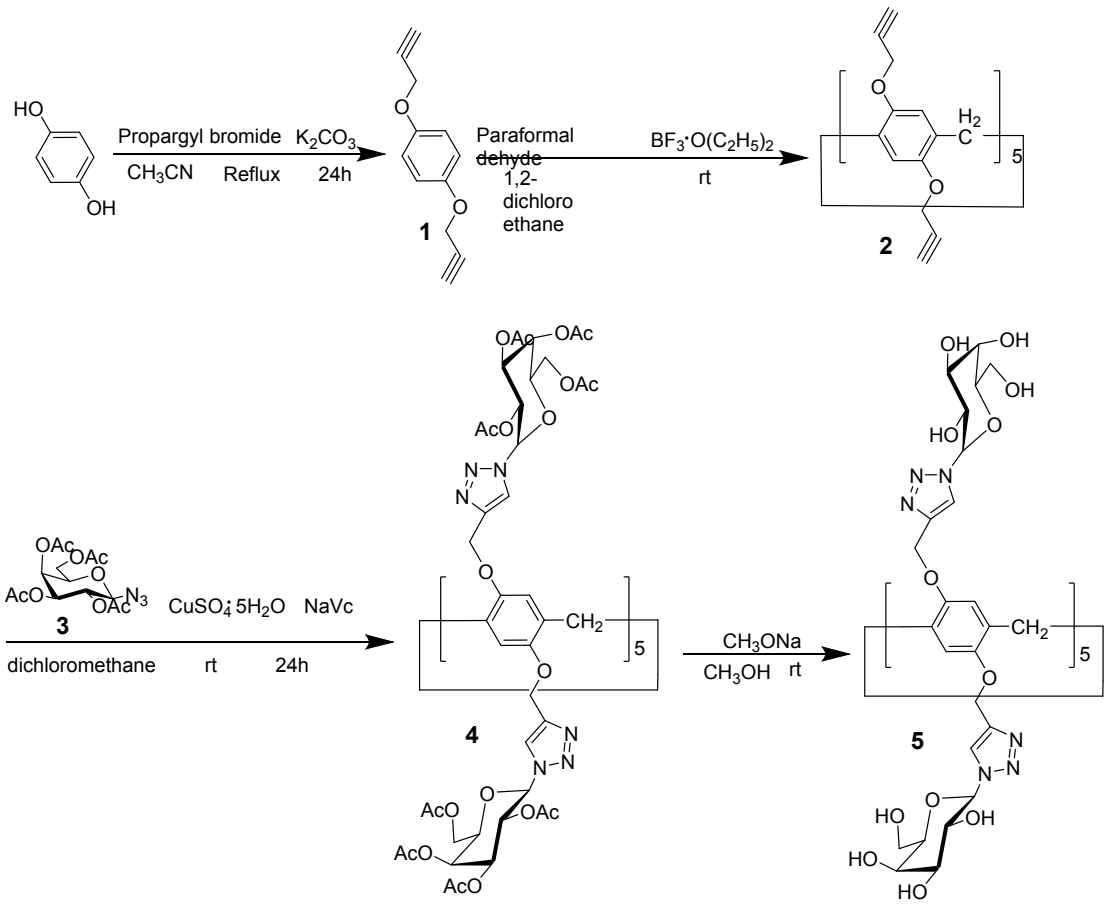
Supporting Information (pages)

I. Instrumentation and chemicals

All reagents were purchased from commercial suppliers and used without further purification unless specified. Water used in this work was triple distilled. Doxorubicin hydrochloride (DOX) was purchased from Sangon Biotech. 2,2'-dithiodipyridine and (3-mercaptopropyl) trimethoxysilane were purchased from Shang Hai D&B Chemical Technology Co. ltd. and Aladdin Industrial Corporation respectively. NMR spectra were recorded on a Bruker 500 MHz Spectrometer, with working frequencies of 500 MHz for ^{1}H. The residual signals from DMSO-d_6 (^{1}H: δ 2.50 ppm; ^{13}C: δ 39.52 ppm) or $CDCl_3$ (^{1}H: δ 7.26 ppm; ^{13}C: δ 77.16 ppm) were used as internal standards. Negative-stained TEM images were taken on a HT7700 instrument (Hitachi Ltd., 80 kV). The samples for negative-stained TEM were prepared by dropping a droplet of the sample solution onto a TEM grid (copper grid, 300 meshes, coated with carbon film). UV-Vis spectra were recorded with Shimadzu 1750 UV-Visible spectrophotometer (Japan) at 298 K. Cell culture was carried out in an incubator with a humidified atmosphere of 5 % CO_2 at 37 ^{o}C. The surface area was measured by nitrogen physisorption (Quantachrome, Autosorb-iQ) based on the Brunauer–Emmet–Teller (BET) method (ASAP 2020, Micromeritics Inc).

II. Synthesis and characterization of the compounds



Scheme S1 synthesis of compound **5**

Compound **1**: Hydroquinone (11 g, 100 mmol) and K_2CO_3 (27.6 g, 200 mmol) were added to a solution of propargyl bromide (47.5 g, 200 mmol) in CH_3CN (180 mL). The mixture was heated in a three-necked flask under nitrogen atmosphere at reflux for 24 h. The solid was filtered off and the solvent was removed. The residue was dissolved in dichloromethane and washed with water five times. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to afford the crude product, which was purified by silica gel chromatography (dichloromethane/petroleum ether, v/v = 1:2 to yield the white solid (16.554 g, 89 %).^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (s, 4H), 4.64 (d, J = 2.4 Hz, 4H), 2.51 (t, J = 2.4 Hz, 2H) ppm.[1, 2]

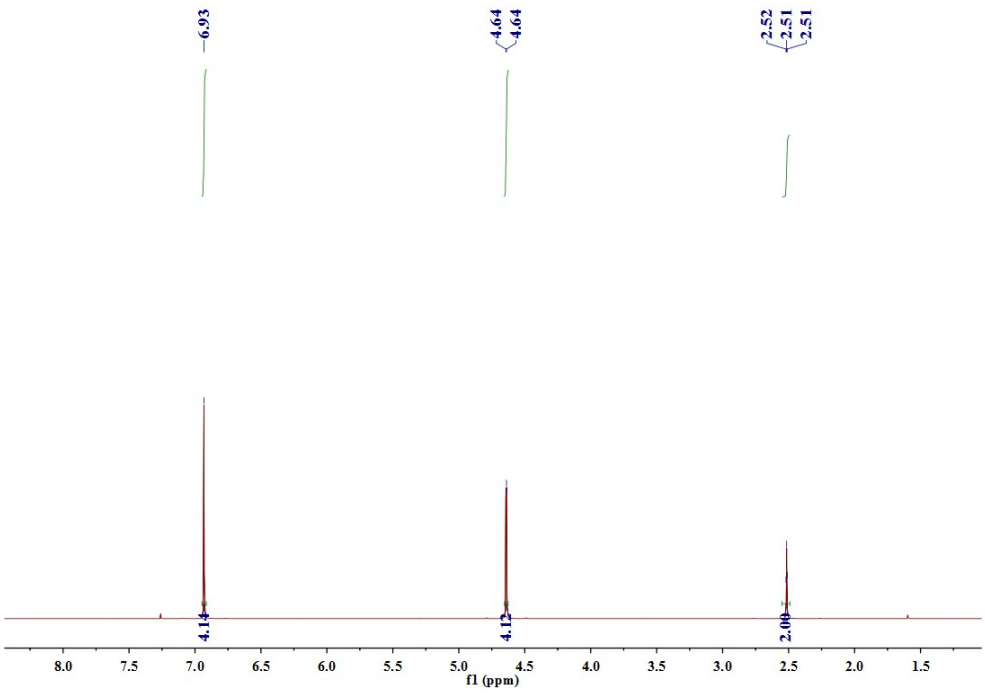


Figure S1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **2**.

Compound **2**: To a solution of **1** (3.72 g, 20 mmol) in 1,2-dichloroethane (60 mL), paraformaldehyde (1.398 g, 40 mmol) was added under nitrogen atmosphere. Then boron trifluoride diethyl etherate (4 mL) was added to the solution and the mixture was stirred at room temperature for 1 h. Water (20 mL) was added to quench the reaction. The mixture was filtered and the solvent was removed. The residue was dissolved in dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to afford the crude product, which was isolated by silica gel chromatography using dichloromethane/petroleum ether (v/v = 1:4) to give **2** as a white solid (2.97 g, 75 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (s, 10H), 4.53 (d, J = 2.1 Hz, 20H), 3.81 (s, 10H), 2.27 (s, 10H) ppm.[2]

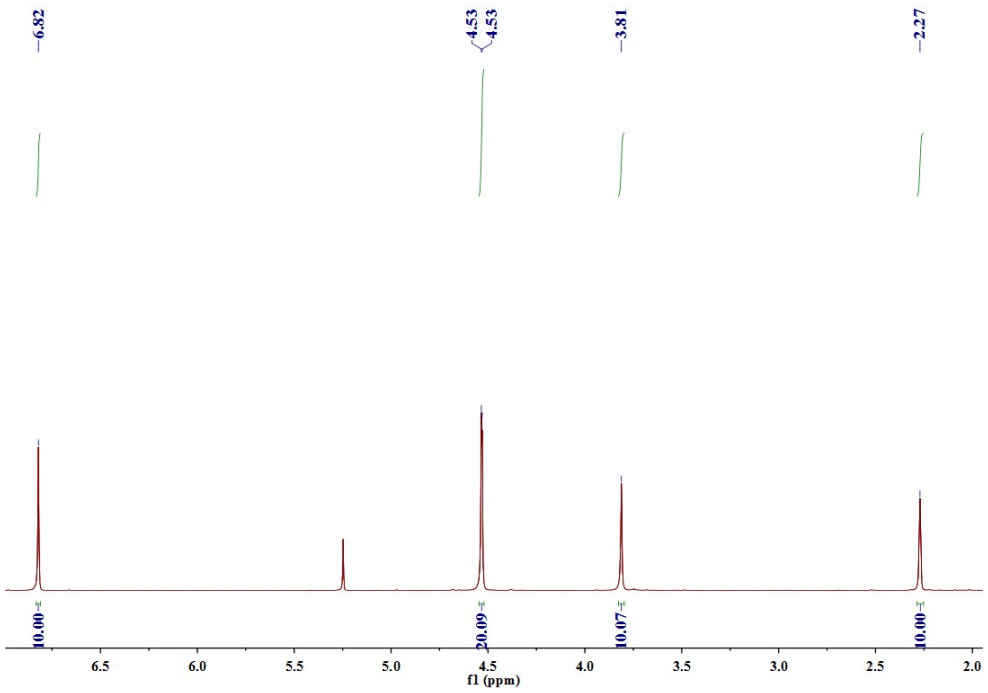


Figure S2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **3**

Compound **4**: The synthesis of **3** was performed according to the previously published procedure.[3] Compound **3** (500 mg, 1.33 mmol), copper sulfate pentahydrate (30 mg, 0.12 mmol) and sodium ascorbate (80 mg, 0.57 mmol) were added to a solution of **2** (66 mg, 0.067 mmol) in dichloromethane (10 mL), then added 10 mL H_2O. The mixture was stirred under nitrogen atmosphere in room temperature for 24 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed with water (3 × 10 mL). The organic phase was dried over magnesium sulfate and filtered. The solvent was removed under reduced pressure. Purification via silica gel chromatography (dichloromethane/methanol = 40:1) afforded **4** as a light yellow solid (177 mg, 56 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.14 (s, 10H), 7.00 (s, 10H), 6.06 (d, J = 9.3 Hz, 10H), 5.78 (t, J = 9.8 Hz, 10H), 5.60 (d, J = 3.0 Hz, 10H), 5.35 (dd, J = 10.2, 3.3 Hz, 10H), 5.11 (d, J = 11.9 Hz, 10H), 4.95 (d, J = 11.9 Hz, 10H), 4.35 (t, J = 6.6 Hz, 10H), 4.28 – 4.15 (m, 4H), 2.28 (s, 30H), 2.12 – 2.04 (m, 120 H) ppm.[2]

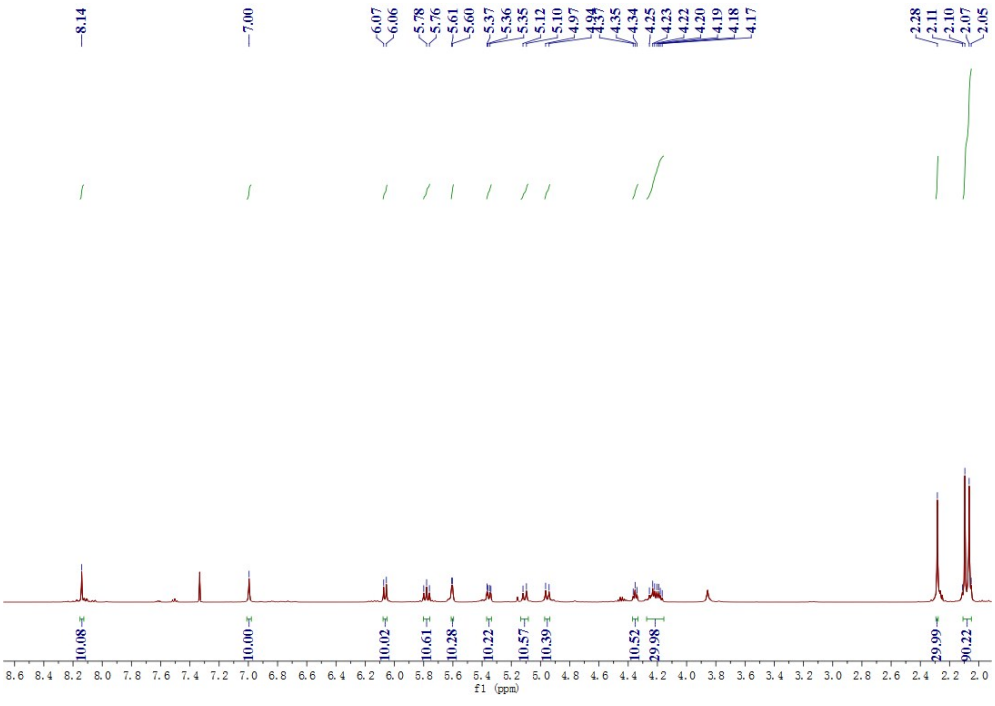


Figure S3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **4**

Compound **5**: Compound **4** (2.36 g, 0.5 mmol) was dissolved in a sodium methoxide solution (35 mL, 0.15 M) in a clean dry round bottom flask. The solution was stirred for 12 h. The precipitate was filtered under suction and washed with CH_3OH (5 × 20 mL) to give **5** as a white solid (1.10 g, 73 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 (s, 10H), 7.08 (s, 10H), 5.53 – 5.43 (m, 10H), 5.41 – 5.20 (m, 20H), 5.01 (s, 10H), 4.92 – 4.80 (m, 10H), 4.73 (d, J = 4.6 Hz, 10H), 3.76 (s, 10H), 3.68 (d, J = 6.5 Hz, 10H), 3.68 – 3.62 (m, 20H) ppm.[2]

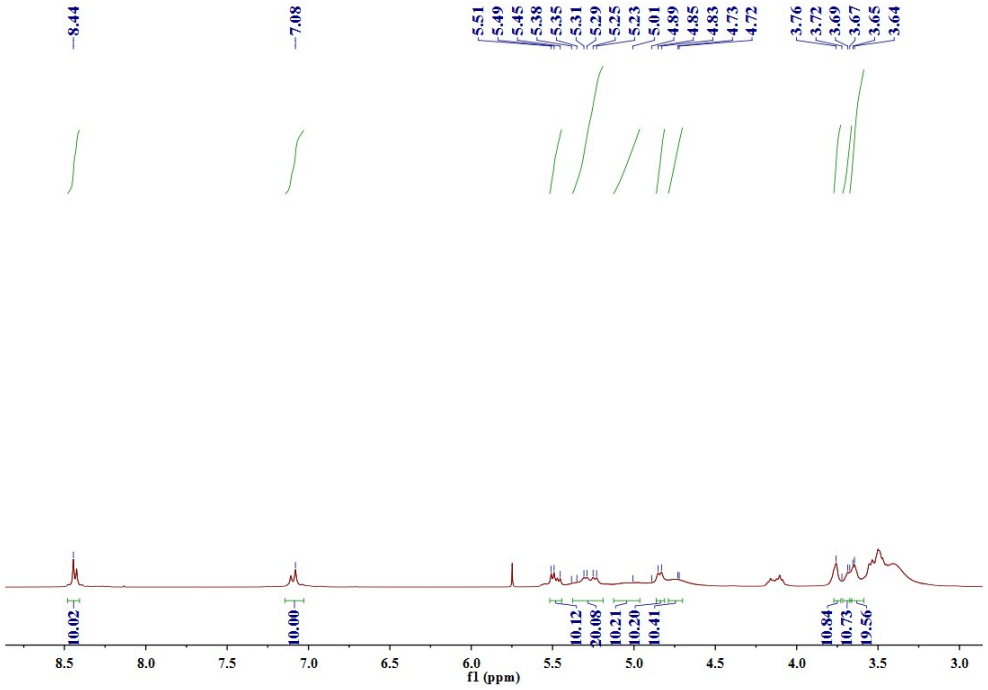


Figure S4. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **5**

III. Synthesis and characterization CeONRs

CeO_2 (10 mg) disperse in ethanol (15 mL) and water (133 μL) with sonication, (3-mercaptopropyl) trimethoxysilane (3.34 mg) added into the turbid liquid, stirring for 24 h. After centrufugaition, the solid was redispersed in ethanol, the 2,2'-dithiodipyridine (3.73 mg) was dissolved in 5 mL ethanol, and was added dropwise into the turbid liquid, then stirred for 12 h. In the end, the solid wash with ethanol for 5 times after centrifugation.[4]

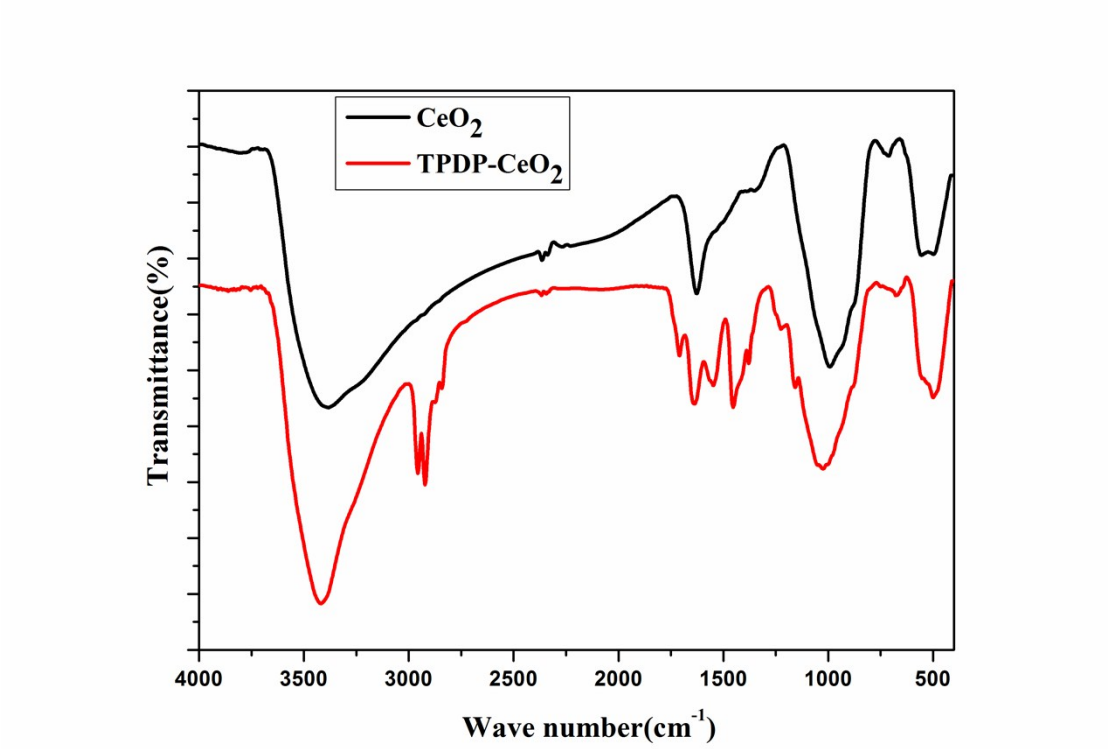


Figure S5. FT-IR spectroscopy of CeO_2 and TPDT-CeO_2.

Study of TPDP's characteristic absorption peak: 22 mg 2,2'-dithiodipyridine was dissolve in 1mL methanol, and the same concentration of (3-mercaptopropyl) trimethoxysilane, and their mixture was stirred overnight. These three solutions were detected by the UV-Vis spectroscopy to confirm the characteristic absorption and the success of modification. By test concentration of 2,2'-dithiodipyridine in liquid supernatant of the mixture to confirm the amount of TPDP on the CeO_2. The concentration of 2,2'-dithiodipyridine was determined by measurement of absorbance at 300 nm using a standard absorbance vs. concentration curve constructed for 2,2'-dithiodipyridine.

The content of TPDP on CeONRs = $(C_1-C_2)/C_1\times100$ %

(C_1: concentration of 2,2'-dithiodipyridine before reaction; C_2 :concentration of 2,2'-dithiodipyridine after reaction).

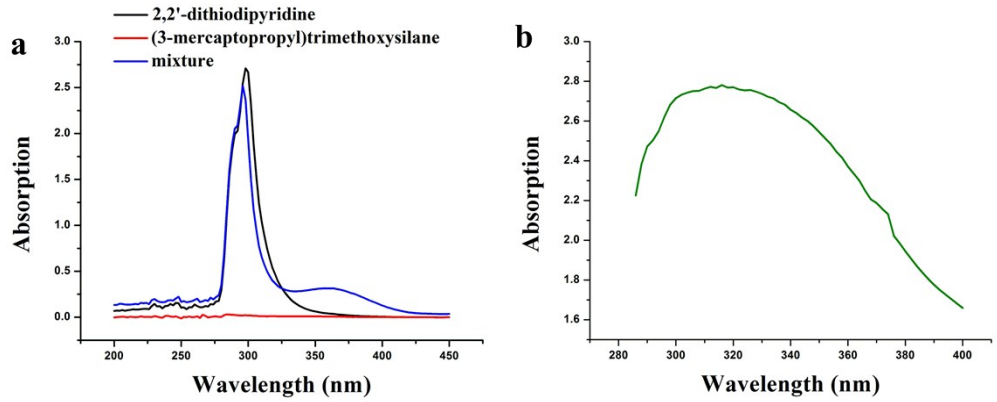


Figure S6. UV-Vis spectroscopy of 2,2'-dithiodipyridine, (3-mercaptopropyl)trimethoxysilane and their mixture (a); UV/Vis spectroscopy of TPDP-CeO_2 (b).

BET surface (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
206.3525	0.667707	9.182

Table S1

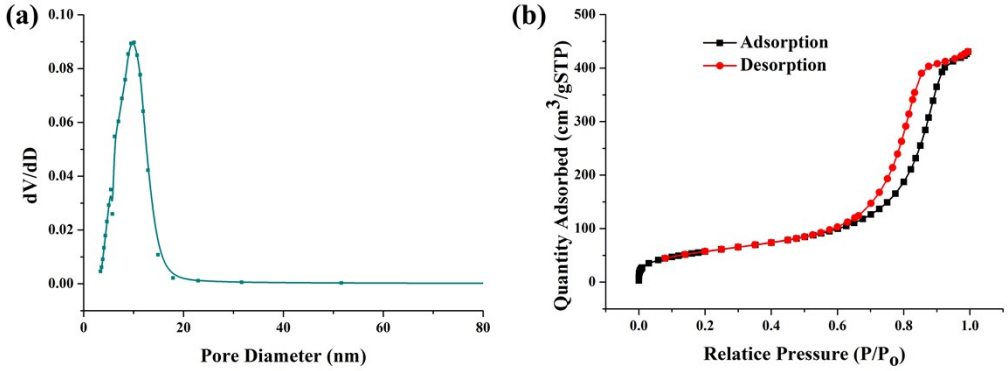


Figure S7. (a) Pore size distribution of TPDP-CeONRs; (b) N_2 adsorption-desorption isotherms of the TPDP-CeONRs.

IV. Study of host-guest interaction

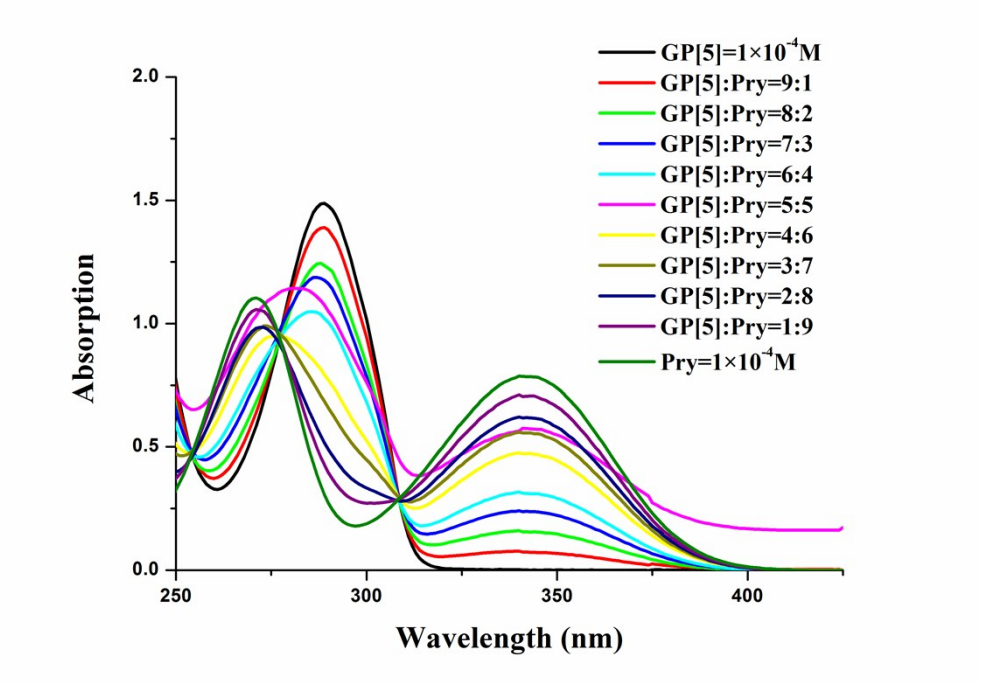


Figure S8 UV-Vis absorption of the mixture of GP[5] and 2-Mercaptopyridine in water at different molar ratios while GP[5] + Pry = 1.0×10^{-4} M.

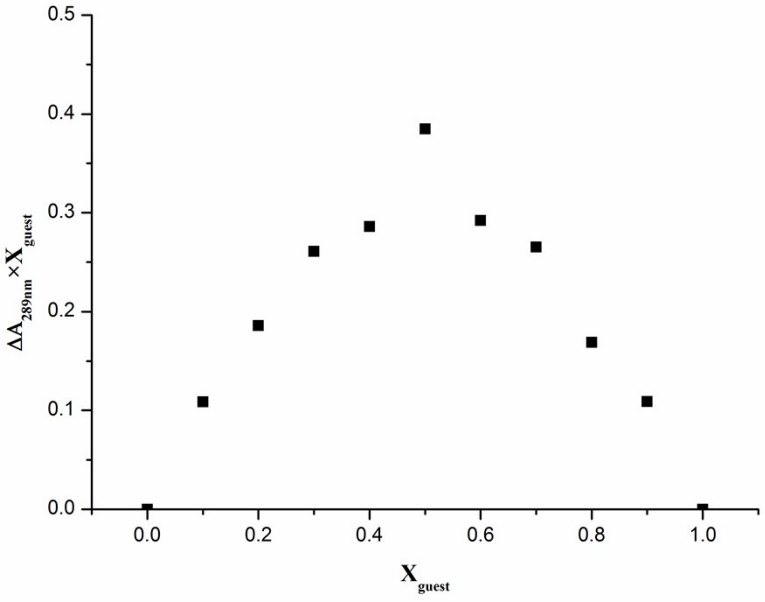


Figure S9 Job plot of the difference in absorption at 289 nm (a characteristic absorption peak of GP[5]) against the mole fraction of 2-Mercaptopyridine at an invariant total concentration of 1.0×10^{-4} M in aqueous solution. It indicated that the complex between GP[5] and 2-Mercaptopyridine has 1:1 stoichiometry.

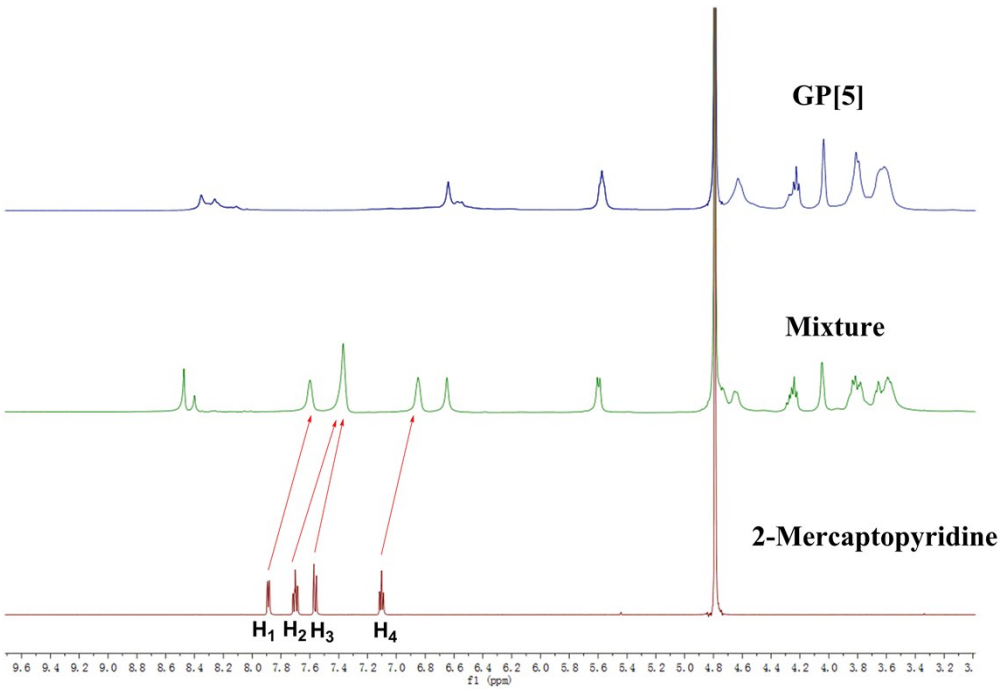


Figure S10 ^{1}H NMR spectra (500 MHz, D_2O, 298 K) of GP[5], 2-Mercaptopyridine and their mixture.

V. The study of release profile

The release profile of DOX loaded CeONRs@GP[5] was studied as the following. 5 mg (0.029 mmol) of CeONRs was dispersed in 1 ml PBS containing 1M DOX. The mixture was subjected to ultrasonication for 20 minutes, and then left to a shaker for 24 h to reach the max absorption. After that 14.01 mg GP[5] was added, and continuous stirring for 5 h. Then it was purified by water washing after centrifugation. The amount of unloaded DOX was quantitatively measured by microplate reader at 490 nm.

Drug loading capacity = ($W_{\text{initial DOX}} - W_{\text{DOX in supernatant}}$) / W_{CeONRs} ($mg \cdot g^{-1}$)

The DOX release from DOX-loaded CeONRs and CeONRs@GP[5] were studied at different circumstance—— with or without GSH (2 mM) in PBS. 3 mg of DOX-loaded CeONRs was added into 10 mL of corresponding release medium at room temperature. At specified time intervals, 0.1 mL of the release medium was taken out for measuring the DOX released DOX concentration with an Epoch microplate spectrophotometer (Biotek). The concentration of DOX was determined by measurement of absorbance at 490 nm using a standard absorbance vs. concentration curve constructed for DOX in the corresponding release medium.

VI. Cell culture and cell viability

HepG-2 cells and 293T cells were cultured in 1640 medium and DMEM which containing 10% FBS, 1% penicillin/streptomycin in 5% CO_2 at 37 °C in a humidified atmosphere with 100% humidity and 5 % CO_2 at 37 °C.

The relative cytotoxicities of CeONRs, GP[5], CeONRs@GP[5], DOX and CeONRs@GP[5] were evaluated in vitro by MTT assay, respectively. The cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 μL complete DMEM or 1640 medium and grew for 24 h at 37 °C. Subsequently, cells were incubated with CeONRs, GP[5], CeONRs@GP[5], DOX and CeONRs@GP[5] at different concentrations for 24 h, 48 h, and72 h. The cells were washed and the fresh medium containing MTT was added into each plate. The cells were incubated for another 4 h. After that, the medium containing MTT was removed, dimethyl sulfoxide (100 μL) was added to each well, and the plates were gently shaken for 10 min to dissolve the formazan crystals. Finally, the absorbance at 490 nm was recorded with a microplate reader.

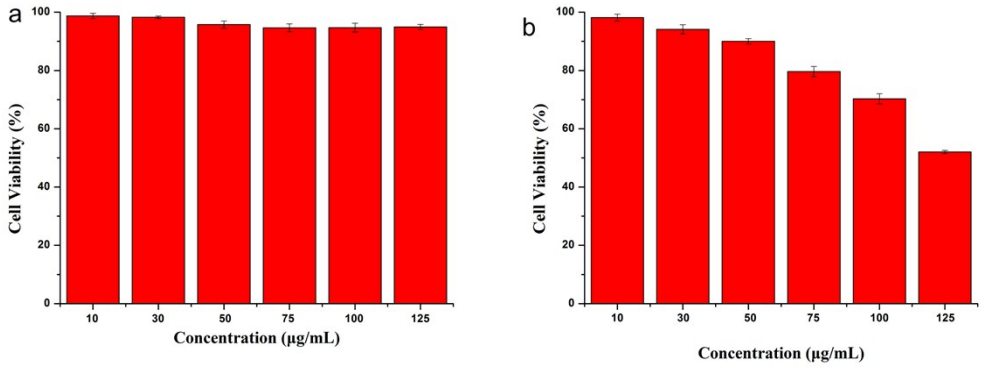


Figure S11. Cell viability of 293T cells incubated with (a) GP[5], (b) TPDP-CeONRs, in different concentration for 24 h.

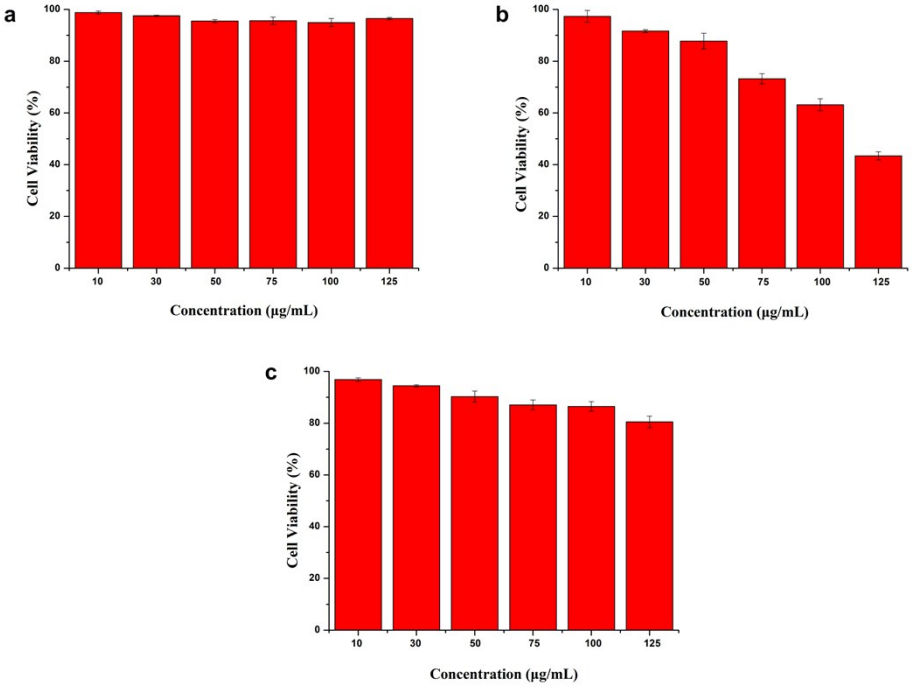


Figure S12. Cell viability of 293T cells incubated with (a) GP[5], (b) TPDP-CeONRs, (c) CeONRs@GP[5] in different concentration for 48 h.

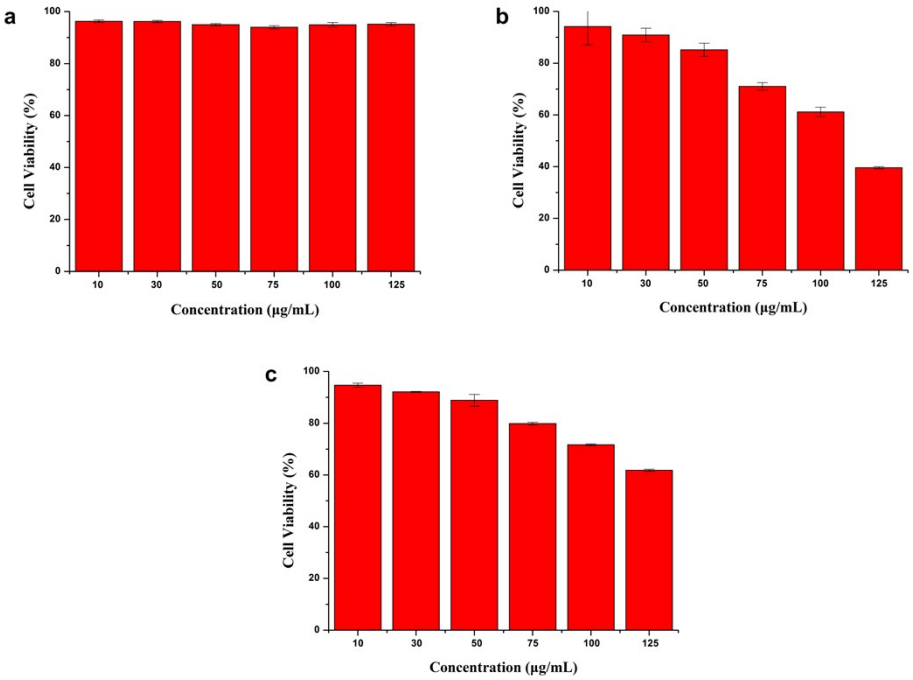


Figure S13. Cell viability of 293T cells incubated with (a) GP[5], (b) TPDP-CeONRs, (c) CeONRs@GP[5] in different concentration for 72 h.

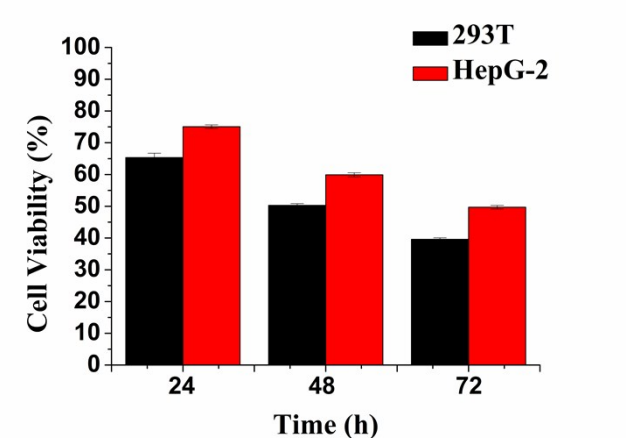


Figure S14. SH-CeONRs (TPDP-CeONRs treated with 2M GSH for 24 h) on viabilities of 293T cells and HepG-2 cells for 24, 48, and 72 h.

VII. The study of targeting ability by flow cytometry

HepG-2 cells were cultured in 1640 medium containing 10 % FBS, 1 % penicillin/streptomycin (complete 1640) in 5 % CO_2 at 37 °C. HepG-2 cells were seeded in 6-well plates (1×10^5 cells/well) and cultured in complete medium for 24 h. The fresh medium containing 5 μM free DOX, DOX-loaded CeONRs@GP[5], and DOX loaded CeONRs@GP[5] were added respectively. In contrast, one group was pretreated with lactobionic acid (2 mg/mL) for 4 h before the incubation with DOX-loaded CeONRs@GP[5]. After 4 h, the cells were harvested and washed for two times with cold PBS and resuspended in 500 μL PBS. Finally, cells were analyzed by a flow cytometer (Beckman Coulter Cytomics Altra).

VIII. Confocal laser scanning microscopy (CLSM)

HepG-2 cells were seeded in 20 mm plastic bottomed μ-dishes for 24 h. The medium was replaced with a fresh one and then incubated with DOX-loaded CeONRs@GP[5] for 6 h. The dishes were washed with PBS three times and fixed with 4.0 % paraformaldehyde at room temperature for 15 min. After washing with PBS, the cells were all stained with DAPI for 5 min. Finally, the cells were washed with PBS and then observed under a confocal fluorescence microscope.

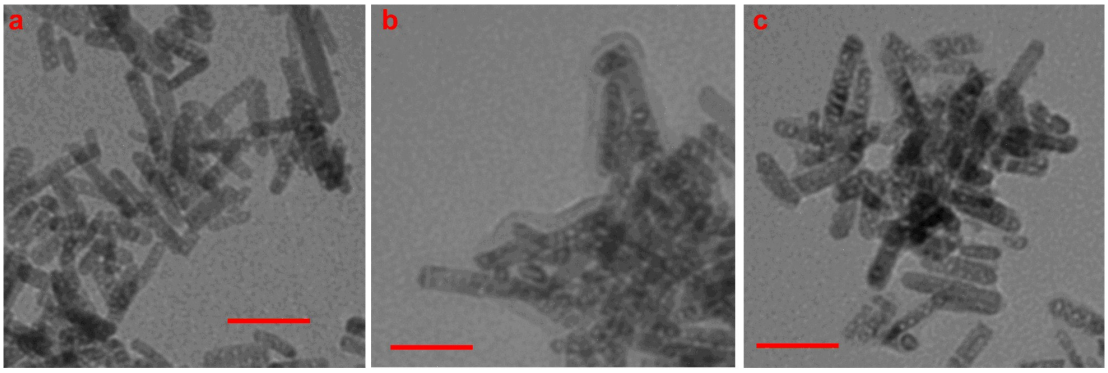


Figure S15. TEM of unmodified CeONRs (a); GP[5] capped CeONRs (b); CeONRs@GP[5] after treated with 2 μM GSH (c).

IX. Reference

1. Bi, J.; Zeng, X.; Tian, D.; Li, H., Temperature-Responsive Switch Constructed from an Anthracene-Functionalized Pillar[5]arene-Based Host–Guest System. *Organic Letters* **2016,** *18*, 1092-1095.

2. Yu, G.; Ma, Y.; Han, C.; Yao, Y.; Tang, G.; Mao, Z.; Gao, C.; Huang, F., A sugar-functionalized amphiphilic pillar[5]arene: synthesis, self-assembly in water, and application in bacterial cell agglutination. *J Am Chem Soc* **2013,** *135*, 10310-10313.

3. Su, L.; Zhao, Y.; Chen, G.; Jiang, M., Polymeric vesicles mimicking glycocalyx (PV-Gx) for studying carbohydrate-protein interactions in solution. *Polymer Chemistry* **2012,** *3*, 1560-1566.

4. Nakamura, T.; Matsushita, H.; Sugihara, F.; Yoshioka, Y.; Mizukami, S.; Kikuchi, K., Activatable 19F MRI nanoparticle probes for the detection of reducing environments. *Angew Chem Int Ed Engl* **2015,** *54*, 1007-1010.