Supporting Information

Dual-responsive dithio-polydopamine coated porous CeO_2 nanorods for targeted and synergistic drug delivery

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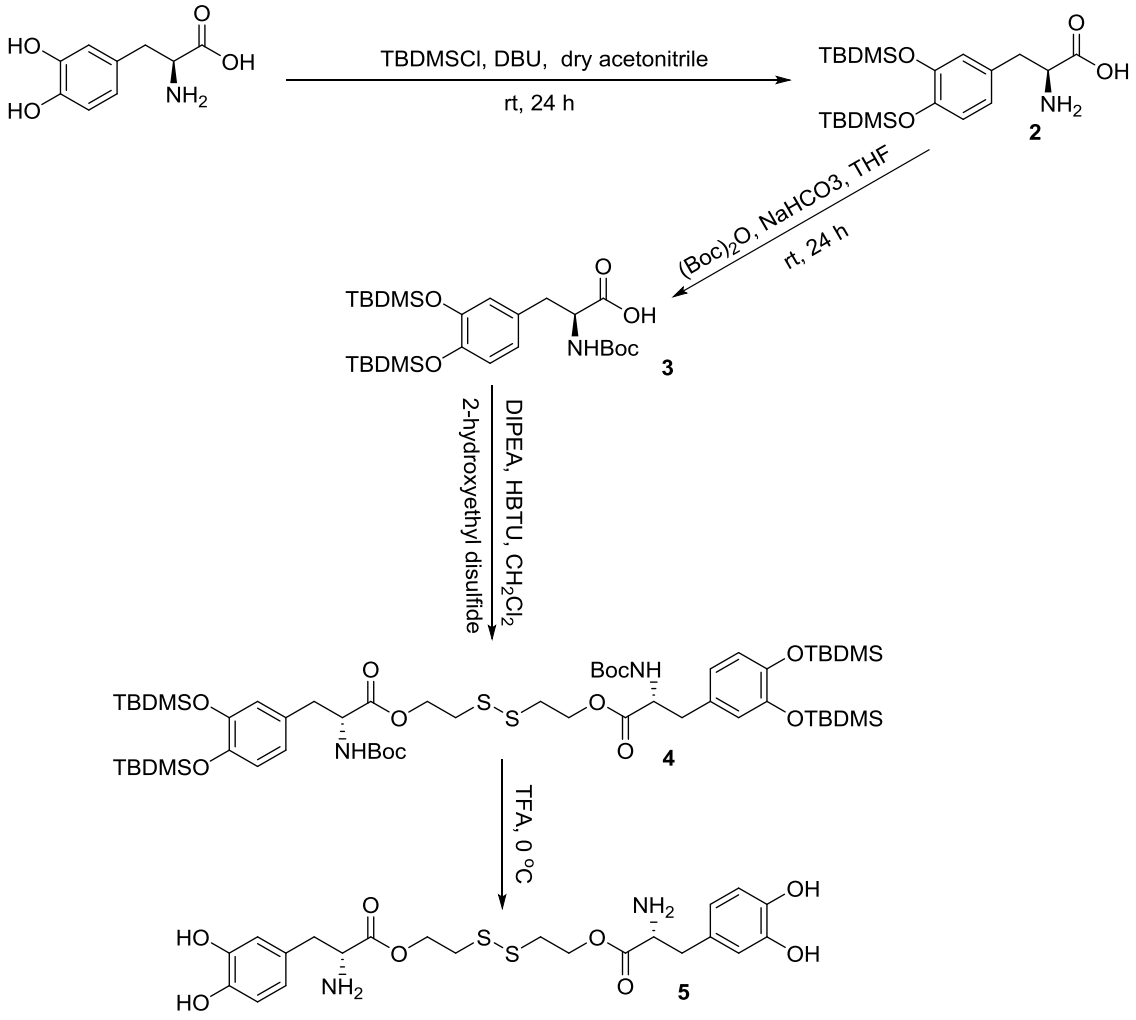
1. Materials and methods

All reagents were purchased from commercial suppliers and used without further purification unless specified. Water used in this work was triple distilled.

2. Synthesis and characterization of the compounds



Scheme S1. Synthetic route of **DOPASS** (**5**).

Synthesis of DOPASS (5)：

Compound **2**： L-DOPA (274.5 mg, 1.39 mmol) and TBDMSCl (638 mg, 4.23 mmol) were dissolved in dry acetonitrile (2.0 mL), and to the acetonitrile solution was cooled to 0 ^{o}C, and DBU (602.8 mg, 4 mmol) was added dropwise over 10 min. The mixture

solution was stirred for 24 h at room temperature. The solution was subsequently filtered. The precipitate was recrystallized from methanol/acetonitrile to give pure **2** (425.7 mg, 81 %). ^{1}H NMR (500 MHz, MeOD) δ 6.84 (dd, J = 14.0, 5.0 Hz, 2H), 6.77 (dd, J = 8.1, 1.9 Hz, 1H), 3.69 (dd, J = 9.0, 4.0 Hz, 1H), 3.21 (dd, J = 14.7, 3.9 Hz, 1H), 2.86 (dd, J = 14.6, 9.1 Hz, 1H), 1.01 (d, J = 4.6 Hz, 18H), 0.23 (d, J = 2.2 Hz, 6H), 0.20 (s, 6H) ppm.[1]

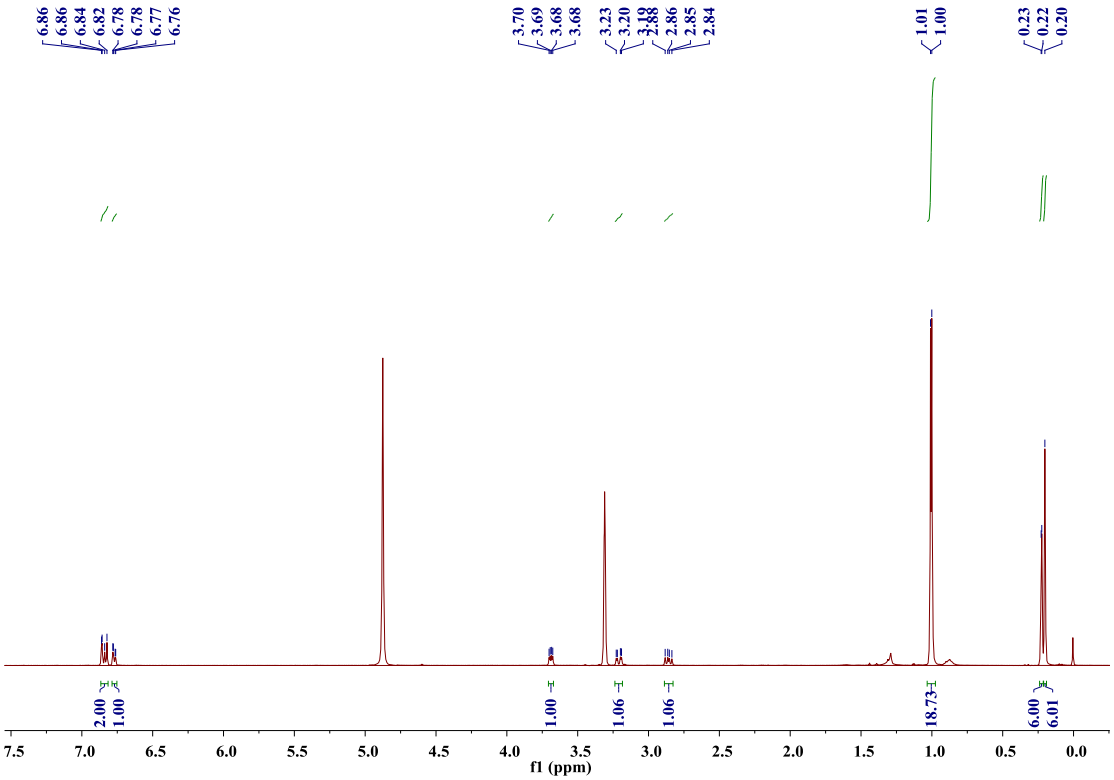


Figure S1. ^{1}H NMR spectrum (500 MHz, MeOD) of **2**.

Compound **3**: Compound **2** was added in 1 mL of deionized water containing $NaHCO_3$ (15.5 mg, 0.18 mmol). The solution of di-*tert*-butyl dicarbonate in 1 mL of tetrahydrofuran was added to the suspension. The reaction mixture was stirred for 24 h at room temperature, then tetrahydrofuran was evaporated, then water was added to the residue and the solution extracted with diethyl ether. The aqueous layer was acidified with citric acid to pH = 5-6 and extracted three times with ethyl acetate. The organic phase was dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (eluent: ethyl acetate/hexane = 1:2) to give **3** as a yellow oil (66 mg, 74 %). ^{1}H NMR (500 MHz,

MeOD) δ 6.76 (t, J = 5.3 Hz, 2H), 6.69 (d, J = 8.1 Hz, 1H), 4.29 (dd, J = 8.2, 4.9 Hz, 1H), 3.02 (dd, J = 13.9, 4.8 Hz, 1H), 2.80 (dd, J = 13.8, 8.7 Hz, 1H), 1.40 (s, 9H), 0.99 (d, J = 6.2 Hz, 18H), 0.21 (d, J = 1.8 Hz, 6H), 0.18 (s, 6H) ppm.[2]

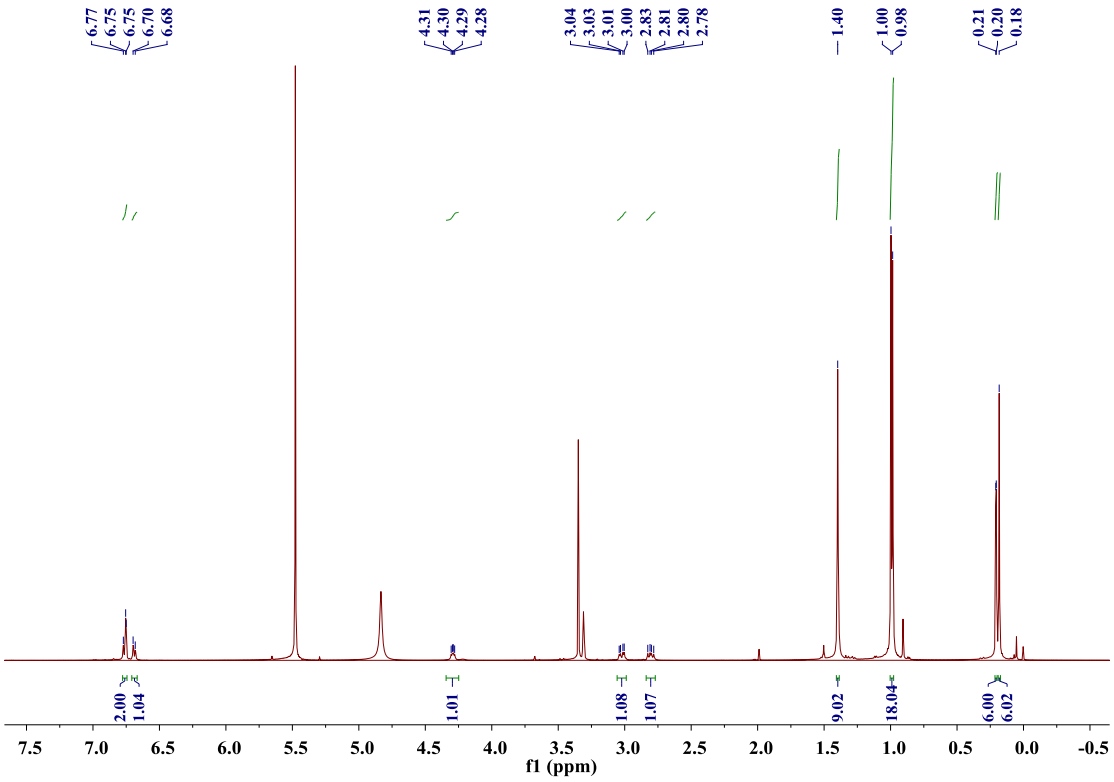


Figure S2. ^{1}H NMR spectrum (500 MHz, MeOD) of **3**.

Compound **4**: 2-Hydroxyethyl disulfide (7.0 mg, 0.045 mmol) and the compound **3** (52.4 mg, 0.1 mmol) were dissolved in dry dichloromethane, and to the solution was added HBTU (60 mg, 0.16 mmol). The mixture was cooled to 0 ^{o}C, and DIPEA (319 μL, 0.18 mmol) was added dropwise over 10 min. The resulting mixture was stirred at room temperature for 15 h, followed by brine wash. The organic phase was separated, and the aqueous phase was extracted with dichloromethane. The combined organic phase was dried over $MgSO_4$. The organic layer was concentrated under reduced pressure and purified by silica-gel column chromatography (ethyl acetate/hexane = 1:20) to give 67 mg (57 %) of **4**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.73 (d, J = 8.1 Hz, 2H), 6.62 (s, 2H), 6.56 (d, J = 7.9 Hz, 2H), 4.94 (d, J = 7.4 Hz, 2H), 4.51 (d, J = 6.0 Hz, 2H), 4.35 (d, J = 2.4 Hz, 4H), 2.96 (m, J = 29.9, 13.8, 6.1 Hz, 4H), 2.87 (t, J = 6.6 Hz, 4H), 1.42 (s, 18H), 0.98 (d, J = 5.1 Hz, 36H), 0.19 (d, J = 1.7 Hz, 12H), 0.18 (s,

12H) ppm.[2]

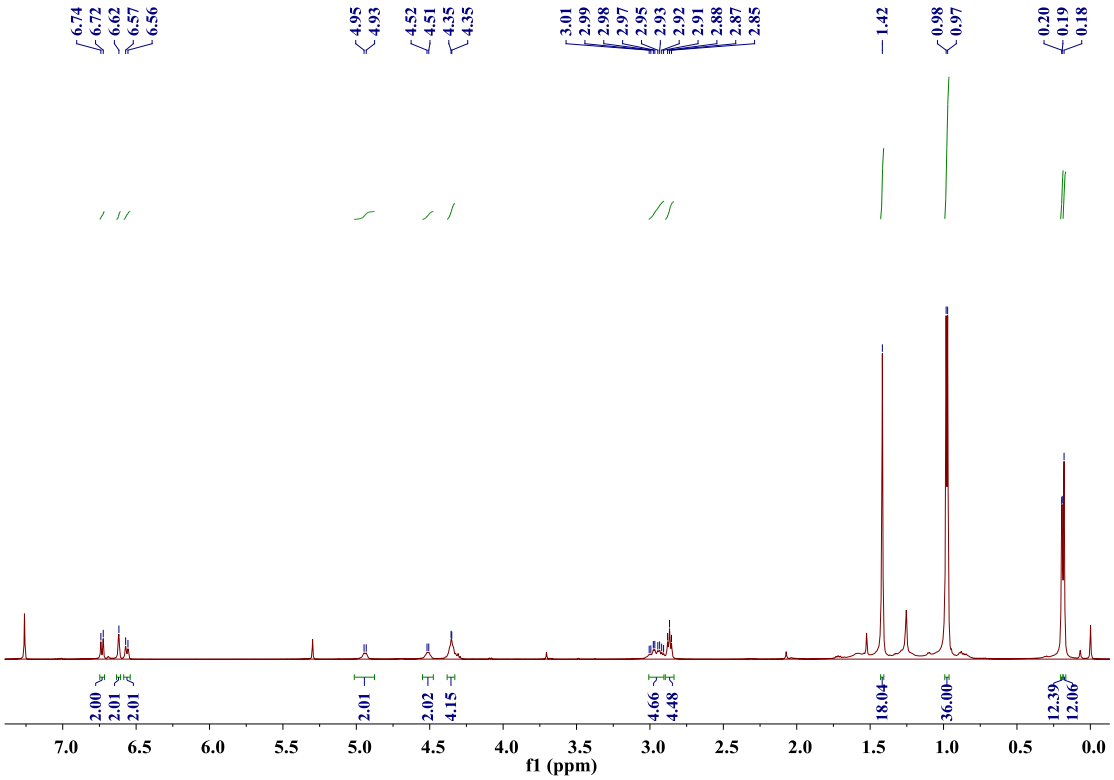


Figure S3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **4**.

Compound **5** (DOPASS): Compound **4** (65.5 mg, 0.06 mmol) was added in THF (5 mL) at 0 ^{o}C for 60 min, and then was stirred for 24 h at room temperature. The solution was concentrated under reduced pressure. The crude was recrystallized from dry diethyl ether to give **5** (20 mg, 70 %). ^{1}H NMR (500 MHz, DMSO) δ 8.40 (s, 2H), 6.67 (d, J = 8.0 Hz, 2H), 6.60 (t, J = 1.8 Hz, 2H), 6.46 (dd, J = 8.0, 1.8 Hz, 2H), 4.44 – 4.29 (m, 2H), 4.18 (s, 2H), 3.02 – 2.84 (m, 8H) ppm.[2]

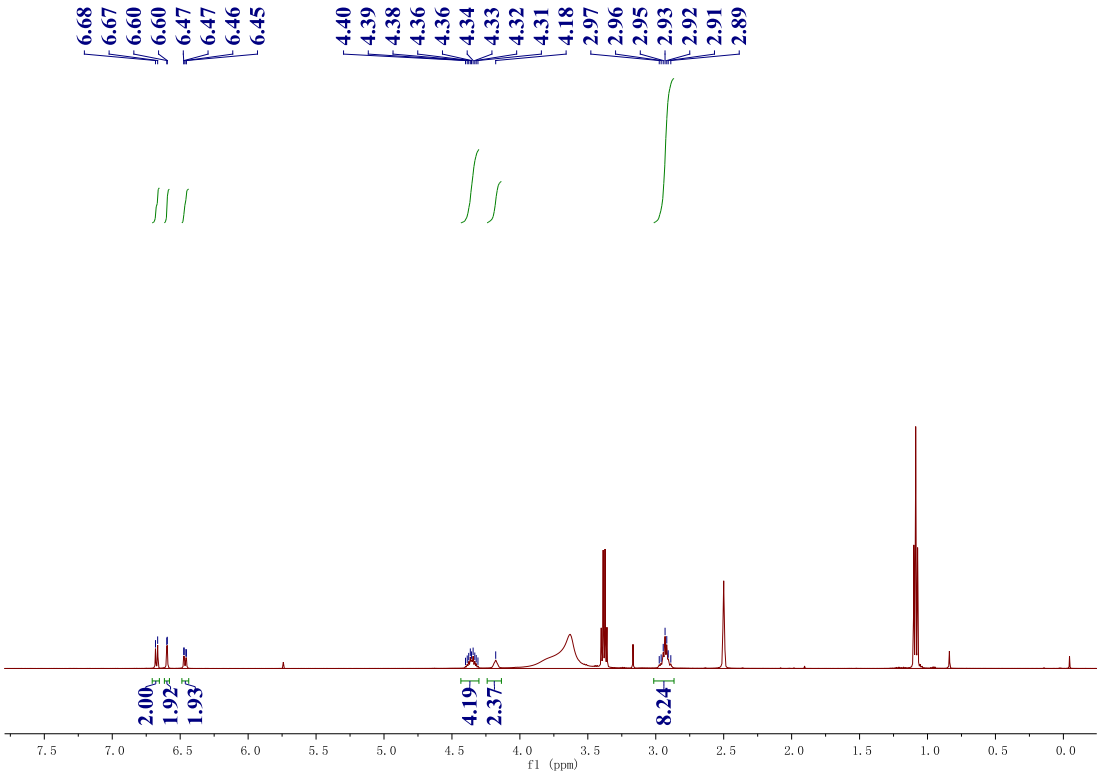
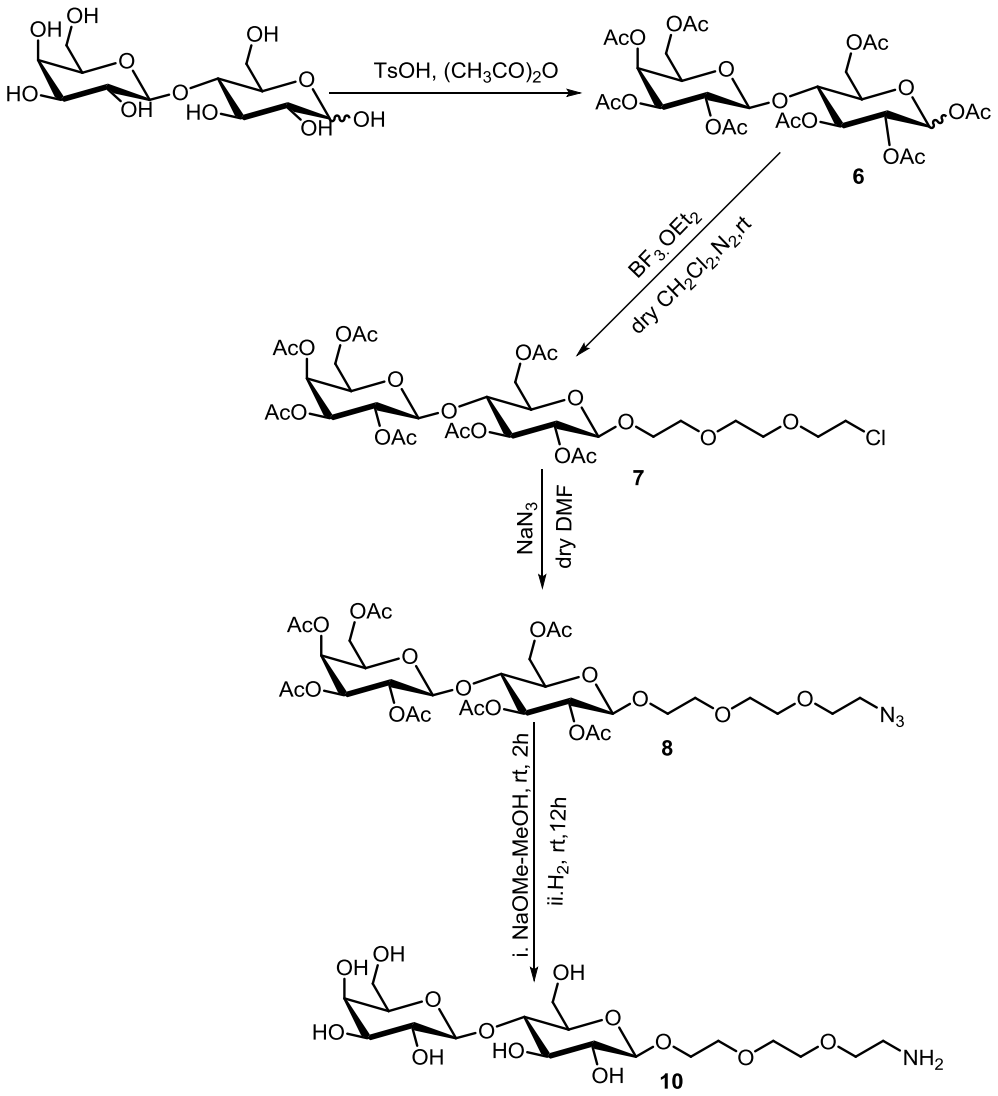


Figure S4. ^{1}H NMR spectrum (500 MHz, DMSO) of **5**.



Scheme S2. Synthetic route of **10** (Lac-NH_2).

Compound **7**: To a solution of compound **6**(2.7 g, 4.0 mmol) in dry CH_2Cl_2 (20 mL) was added freshly activated 3 Å molecular sieves (1.0 g) and 2-[2-(2-chloroethoxy)ethoxy]ethanol (1 g, 0.86 mL, 5.9 mmol) at room temperature for 1 h under N_2 atmosphere. The reaction mixture was cooled at 0 ^oC, and $BF_3 \cdot OEt_2$ (2.0 mL) was added. The reaction mixture was stirred at ^oC for 24 h, and filtered over a Celite bed. The filtrate was diluted with EtOAc, and the organic layer was washed with saturation $NaHCO_3$ and saturation NaCl, dried over $MgSO_4$, and concentrated. The residue was purified by silica-gel column chromatography (ethyl acetate/hexane = 3:2) to give **7** (1.7 g, 56%).[3]

Compound **8**: Compound **7** (1.5 g, 1.9 mmol) was added to a suspension of NaN_3 (0.6

g, 9.2 mmol) in dry DMF (15 mL) at 80 °C for 16 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with $CHCl_3$. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica-gel column chromatography (ethyl acetate/hexane = 3:1) to give **8** (1.0 g, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (d, J = 2.5 Hz, 1H), 5.18 (t, J = 9.3 Hz, 1H), 5.09 (dd, J = 10.3, 8.0 Hz, 1H), 4.91 (ddd, J = 17.5, 9.9, 5.8 Hz, 2H), 4.56 (d, J = 7.9 Hz, 1H), 4.51–4.43 (m, 2H), 4.14–4.04 (m, 3H), 3.88 (ddd, J = 18.6, 11.4, 5.5 Hz, 2H), 3.78 (t, J = 9.4 Hz, 1H), 3.68–3.64 (m, 6H), 3.64–3.60 (m, 6H), 2.14 (s, 3H), 2.11 (s, 3H), 2.07–2.01 (m, 12H), 1.95 (s, 3H) ppm.[3]

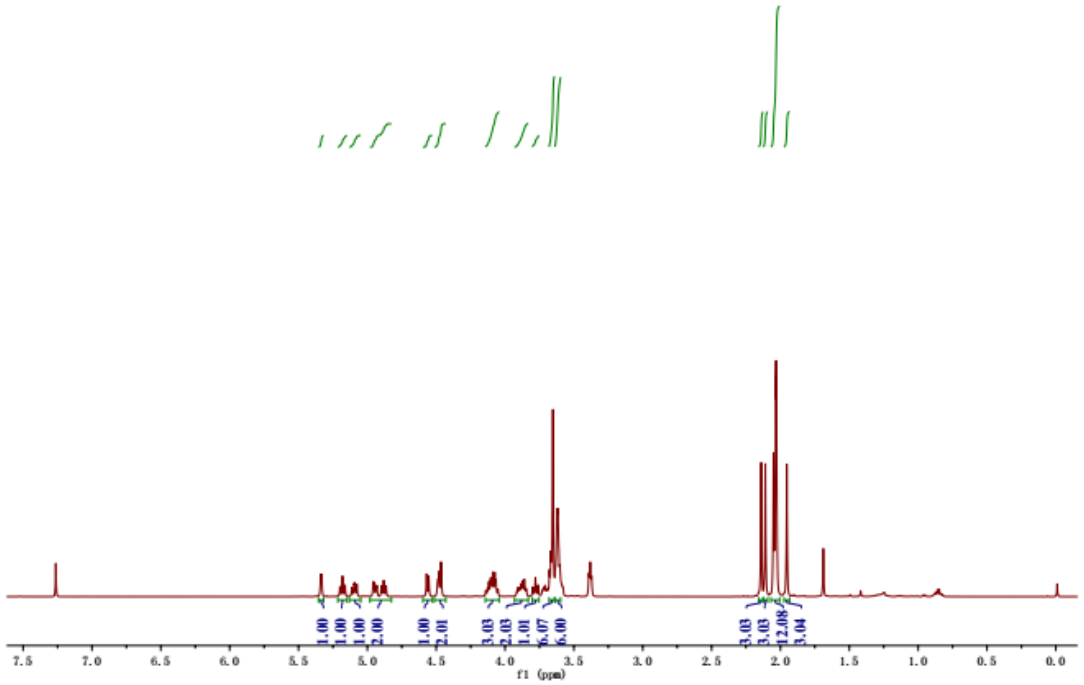


Figure S5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **8**.

Compound **9**: Compound **8** (1.0 g, 1.3 mmol) was deacetylated with a catalytic amount of NaOMe in MeOH (15 mL) to give **9** as a colorless solid.[3]

Compound **10** (Lac-NH_2): A mixture of **9** (200 mg, 0.40 mmol) and Pd/C (10 %, catalytic amount) in 20 mL CH_3OH was stirred at room temperature under hydrogen atmosphere (100 psi) for 8h. The resulting mixture was filtered, and the filtrate was concentrated under reduced pressure to give **10** as a white solid (120 mg, 92 %). ^{1}H

NMR (500 MHz, D_2O) δ 4.52 (dd, J = 8.0, 1.6 Hz, 1H), 4.45 (d, J = 7.8 Hz, 1H), 4.10–4.05 (m, 1H), 3.99 (dd, J = 12.2, 1.8 Hz, 1H), 3.93 (d, J = 3.2 Hz, 1H), 3.86 (dd, J = 10.6, 5.9 Hz, 1H), 3.82 (d, J = 5.3 Hz, 1H), 3.80 (d, J = 4.2 Hz, 1H), 3.78–3.75 (m, 3H), 3.75–3.69 (m, 6H), 3.69–3.63 (m, 5H), 3.62 (d, J = 5.3 Hz, 1H), 3.55 (dd, J = 9.8, 8.0 Hz, 1H), 2.97–2.84 (m, 1H), 2.82 (t, J = 5.4 Hz, 1H) ppm.[3]

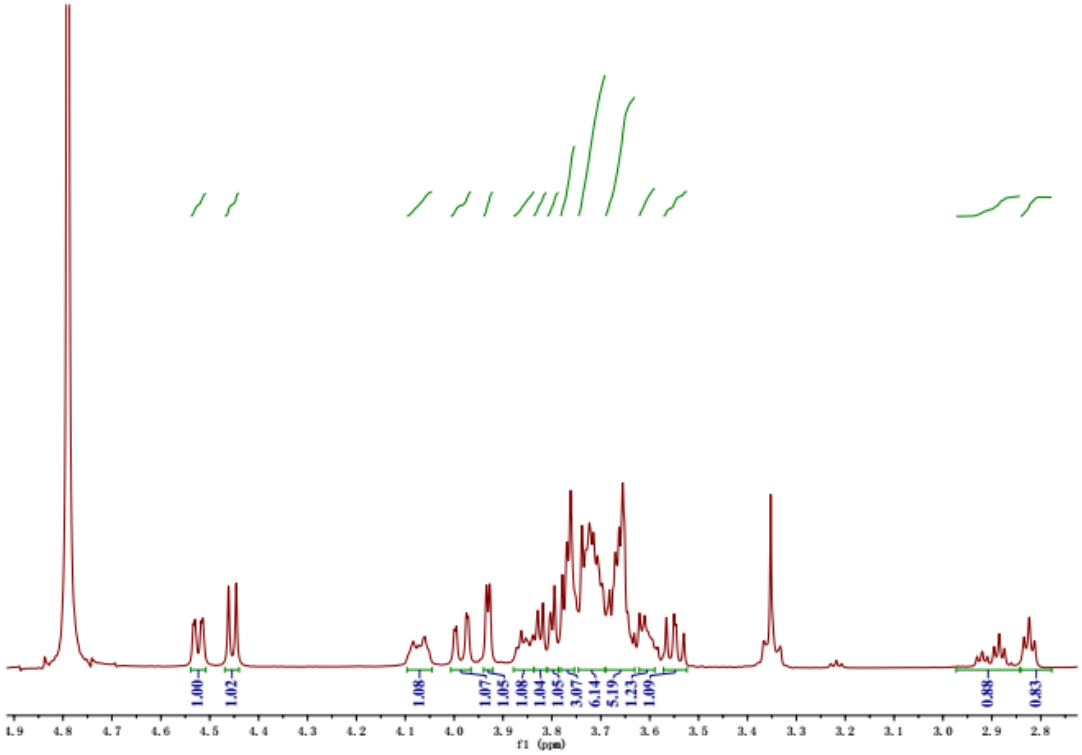


Figure S6. ^{1}H NMR spectrum (500 MHz, D_2O) of **10**

2. FT-IR of the DDS

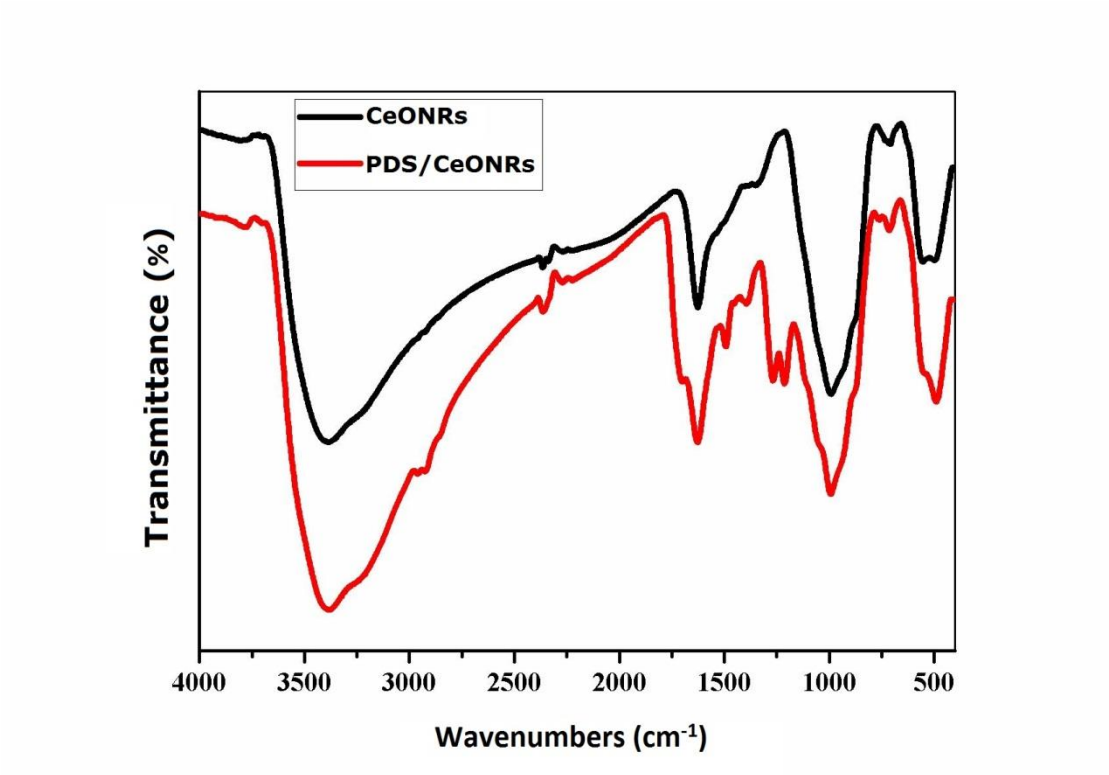


Figure S7. FT-IR spectroscopy of CeONRs and PDS/CeONRs.

3. Analysis of Lac-PDS/CeONRs by DLS and SEM.

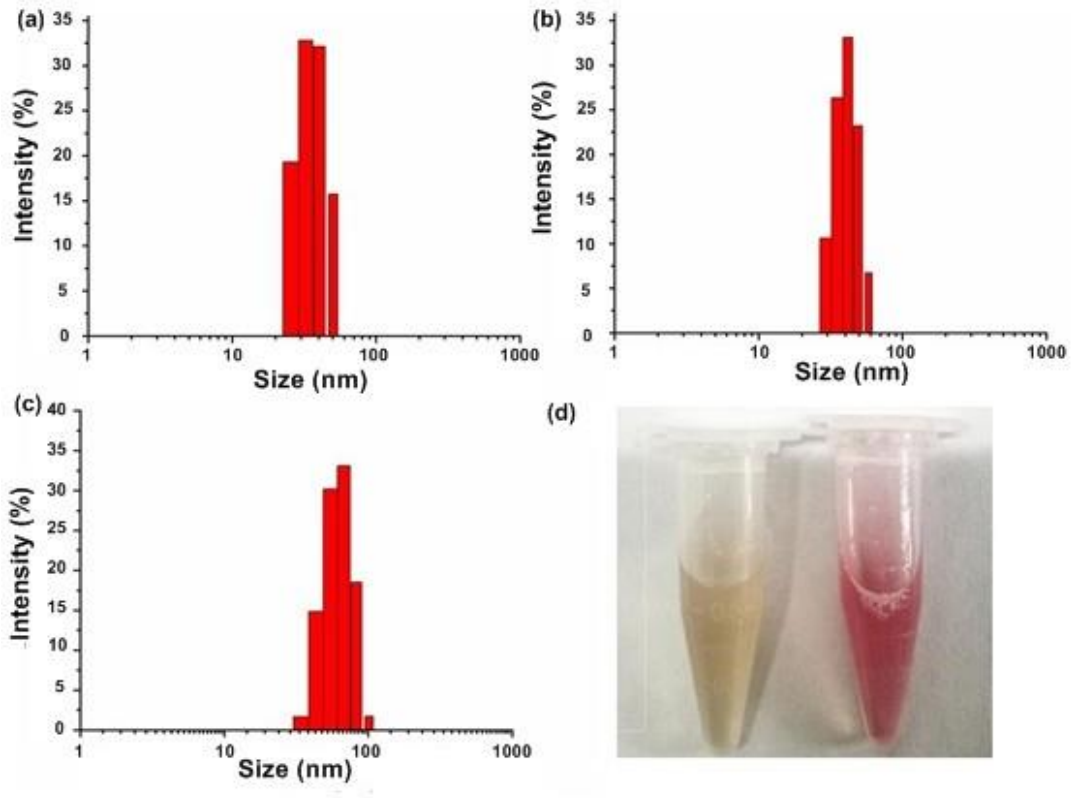


Figure S8. DLS analysis, (a) CeONRs; (b) PDS/CeONRs; (c) Lac-PDS/CeONRs; Digital photos of Lac-PDS/ CeONRs dispersions in PBS buffer and RPMI 1640 culture medium containing 10% FBS for at least 1 day (d).

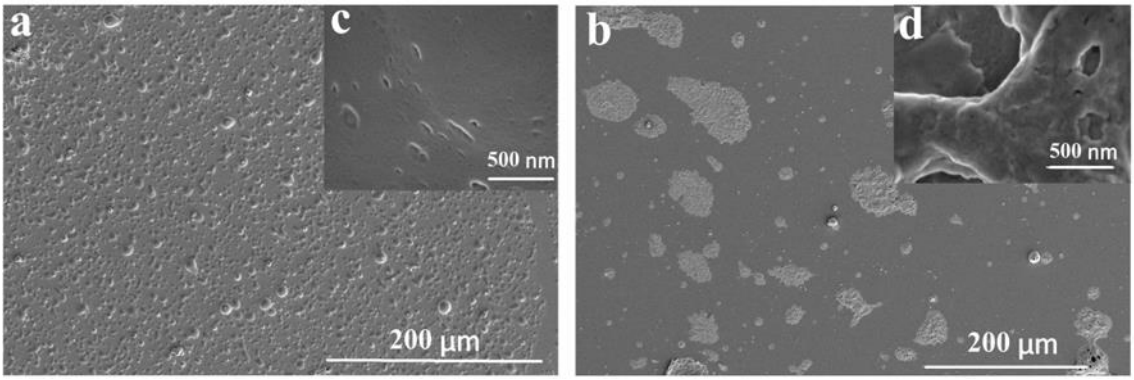


Figure S9. (a) SEM images of the PDS coated silicon slice surface; the inset shows an enlargement of the image; (b) the PDS coated silicon slice surface after being treated with 10 mM GSH for 4 h. The enlarged images of box (c, d).

4. DOX loading and release

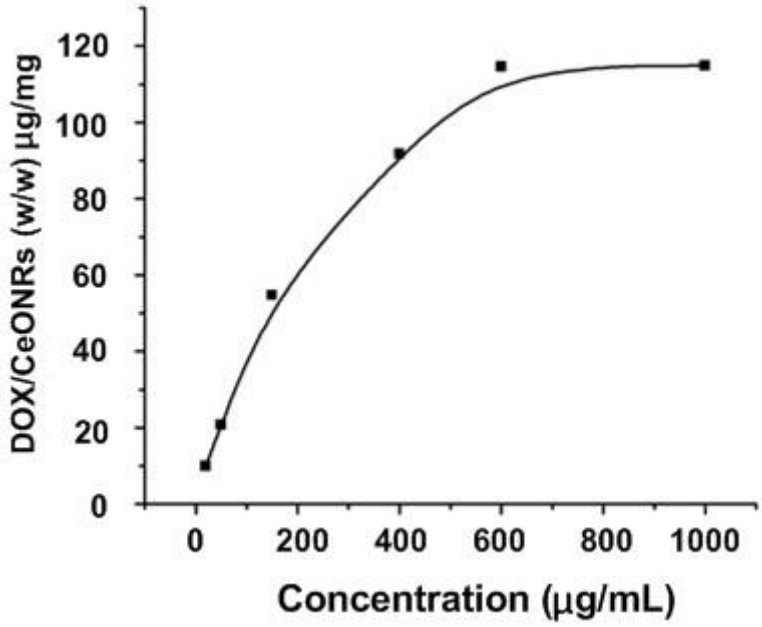


Figure S10. Profile of DOX loading CeONRs

In vitro drug release from DOX-loaded CeONRs was detected as described previously. 10 mg freeze-dried DOX@CeONRs was dispersed in 1 mL of PBS. Then the dispersion was transferred into a dialysis bag (MWCO = 3500), and the bag was submerged in 10 mL PBS of different pH values (7.4, 5.0) and GSH (2.5 mM, 10.0 mM), respectively, and stirred at 37 ^{o}C. At specified time intervals, the concentration of DOX was determined by UV-Vis spectrophotometry. The cumulative release of DOX from Lac-PDS/DOX@CeONRs was also prepared by the same procedure.

5. Analysis of CeONRs by Zeta potential and BET.

Table S1. The Zeta potential of NPs

Sample	ζ potential (mV)
CeONRs	-1.84 ±0.35
PDS/CeONRs	-8.29 ±0.43
Lac-PDS/CeONRs	-14.64 ±0.17

Table S2. BET data for the surface, pore volume and pore size

BET surface (m^2/g)	**Pore volume (cm^3/g)**	**Pore size (nm)**
104.41	0.36	11.98

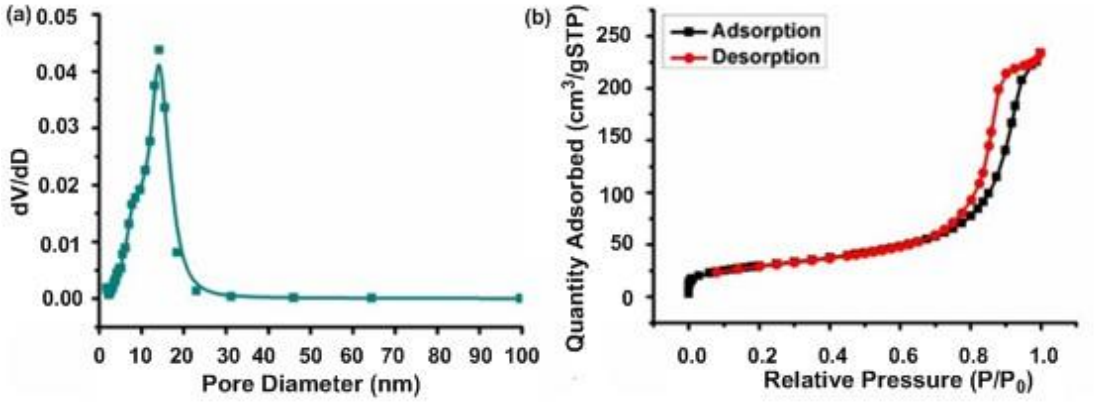


Figure S11. (a) Pore size distribution of CeONRs; (b) N_2 adsorption-desorption isotherms of the CeONRs.

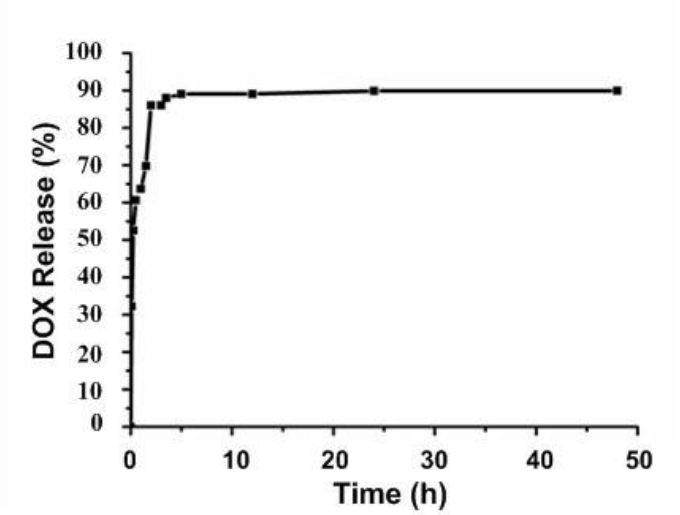


Figure S12. DOX release profile of CeONRs in PBS

6. Cytotoxicity evaluation

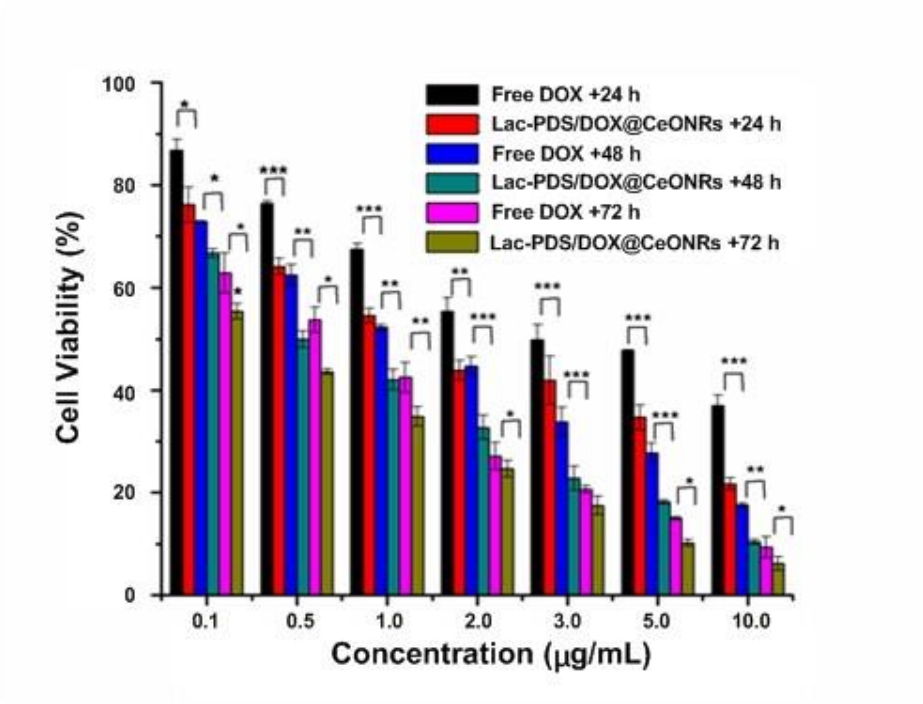


Figure S13. Cell viability of HepG2 cells incubated with Lac-PDS/DOX@CeONRs and Free DOX at different concentrations for 24 h, 48 h, 72 h. (***: p<0.001;**: *p*<0.01; *: *p*<0.5).

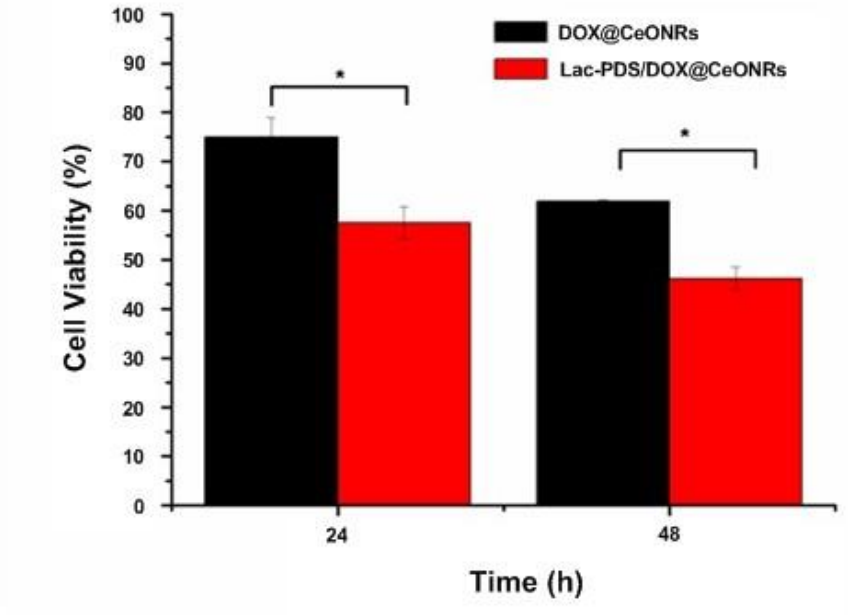


Figure S14. Cell viability of HepG2 cells incubated with Lac-PDS/DOX@CeONRs and DOX@CeONRs at different concentrations for 24 h, 48 h and the DOX concentration is 1 μg/mL.(*: *p*<0.5)

7. Confocal laser scanning microscopy (CLSM)

HepG2 cells were seeded in 35 mm plastic bottomed μ-dishes for 24 h, and then the medium was replaced with a fresh one. The cells were then incubated with Lac-PDS/DOX@CeONRs for 4 h at the concentration of 5 μM. The dishes were then washed with PBS for three times. Thereafter, the cells were stained with DAPI for 15 min. Finally, the cells were washed with PBS and then observed under a confocal fluorescence microscope (OLYMPUS FV1000).

8. TEM image

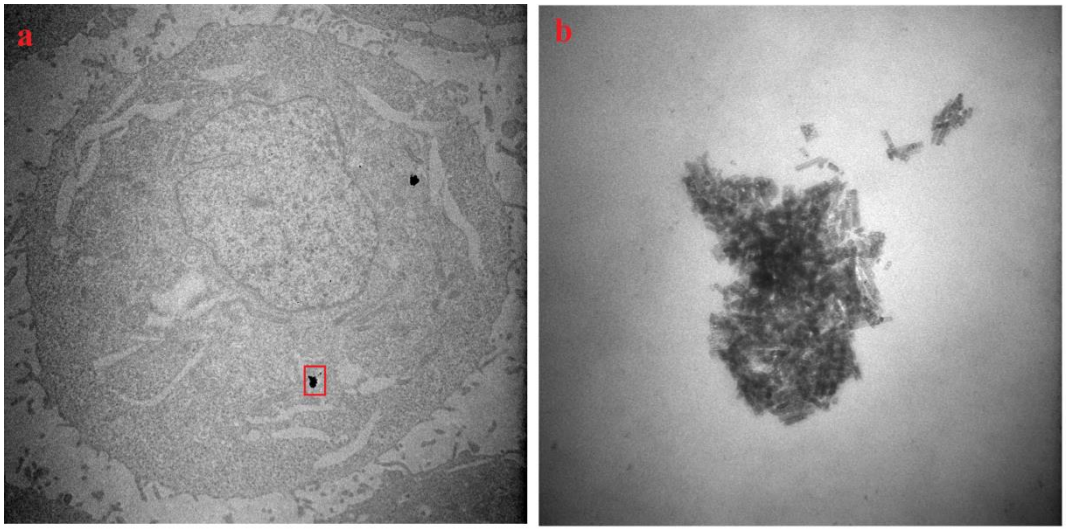


Figure S15. (a) TEM image of HepG2 incubated with CeONRs for 24 h; (b) Enlarged view of the marked area of panel a.

9. Reference

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3. Kato H, Uzawa H, Nagatsuka T, et al. Preparation and evaluation of lactose-modified monoliths for the adsorption and decontamination of plant toxins and lectins. *Carbohydrate research.* 2011;346(13):1820-1826.