Supporting Information

The synthesis of ST-OA: ST-OA was synthesized via esterification between the hydroxyl group of a glucose monomer and the carboxyl group of octanoic acid. First, 5.0 g of starch and 0.89 g of octanoic acid were weighed and added to a 200 mL round bottom flask. Then, 3.821 g of dicyclohexyl carbodiimide (DCC) and 0.2262 g of 4-dimethylamiopryidine (DMAP) were added as a desiccant and catalyst, respectively. All the substances were dissolved in anhydrous DMSO and were allowed to react for 24 h at 60°C under nitrogen protection. After the reaction, 200 mL of water was added to the flask to separate out the insoluble byproduct, and the mixture was centrifuged for 30 min at 5000 rpm. The reaction product was collected and dialyzed with pure water for 48 h. After lyophilization, the product was purified using ethanol and then volatilized to dryness at 50°C for 24 h.

Structure certification of ST-OA: Dry solid starch, octanoic acid, and starch-octanoic acid were weighed (5 mg) and dissolved in 0.5 mL of DMSO-d each. The ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectra were obtained using a NMR spectrometer (AC-80, Bruker Biospin, Germany) at 25°C.

Critical micelle concentration determination of ST-OA: The critical micelle concentration (CMC) of ST-OA was evaluated with a fluorescence measurement using pyrene as a probe. Pyrene (12 mg) was dissolved in acetone (100 mL) and then continuously diluted to a final concentration of 0.0012 mg•mL^{-1}. Then, 0.5 mL of acetone containing pyrene was added to each of eight test tubes and evaporated naturally at 50 °C. ST-OA solutions (5 mL) of eight different concentrations ranging from 5.0×10^{-3} mg•mL^{-1} to 1.0×10^{-1} mg•mL^{-1} were added to dissolve the pyrene to a final concentration of 5.94×10^{-7} M. After the solution was treated with water-bath ultrasonication for 30 min, the fluorescence spectra of solution were recorded with a fluorometer (F-2500, Hitachi, Japan) at room temperature. The excitation wavelength was 337 nm, and the slit openings were 10 nm (excitation) and 2.5 nm (emission). The monitoring wavelength ranged from 350 to 450 nm. The intensity ratio of the first peak (I_1, 375 nm) to the third peak (I_3, 384 nm) was recorded to calculate the CMC value.

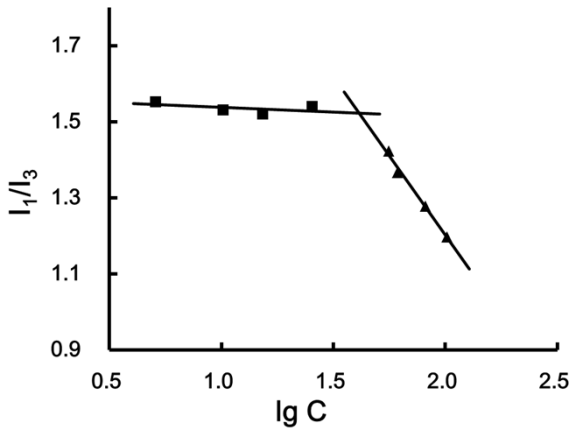

Figure. S1 Variation of the fluorescence intensity ratio for I_1/I_3 responding to the logarithm of the ST-OA micelle concentration.

The size determination and morphology observation of ST-OA micelles: The hydrodynamic diameters of the ST-OA micelles in a de-ionized water solution at the concentration of 1.0 mg•mL^{-1} were determined with dynamic light scattering using a Zetasizer (3000 HS, Malvern Instruments Ltd., UK). For morphologic observations, a drop of the sample dispersion (1 mg•mL^{-1}) was placed on a 100 mesh copper grid. After retention for 1 min, the excess dispersion was aspirated and removed with a piece of filter paper. Then, a 2% phosphotungstic acid solution (pH7.4) was added to the grid and dyed for 1 min. Finally, the solution was removed, and the copper grid was observed using transmission electron microscopy (JEOL JEM-1230, Japan).

The preparation of blank $CaCO_3$ nanoparticles: The boiled 0.25% starch solution and ST-OA solution at three different concentrations (0.25%, 0.125%, 0.0625%) were set as templates to prepare $CaCO_3$ nanoparticles. All the

ST-OA solutions received sufficient water-bath ultrasonication and were kept at 60°C. $CaCl_2$ and Na_2CO_3 were weighed and dissolved in de-ionized water at a concentration of 0.5 M. Then, 200 μL of a $CaCl_2$ solution was added to 50 mL of a template solution and stirred for 30 min in a 30°C water bath. Finally, 200 μL of a $NaHCO_3$ solution was quickly injected into the bottom of the solution and vigorously stirred for an additional 10 min.

The preparation of DOX-loaded $CaCO_3$ nanoparticles: The templates were set for the preparation of $CaCO_3$ nanoparticles. First, 200 μL of a $CaCl_2$ solution (0.5 M) and 1.0 mL of DOX·HCl (10 mg•mL^{-1}) were added to 50 mL of a template solution and stirred for 30 min in a 30°C water bath. Then, 200 μL of a $NaHCO_3$ solution (0.5 M) was quickly injected into the bottom of the solution and vigorously stirred for an additional 10 min.

Near infrared (NIR) spectrometer analysis of $CaCO_3$ nanoparticles: ST-OA and the $CaCO_3$ nanoparticles (prepared in water and in four templates) were weighed and ground. After 5 h drying at 120°C, the powders were collected and analyzed with an infrared spectrometer (IR-4100, JASCO, Japan).

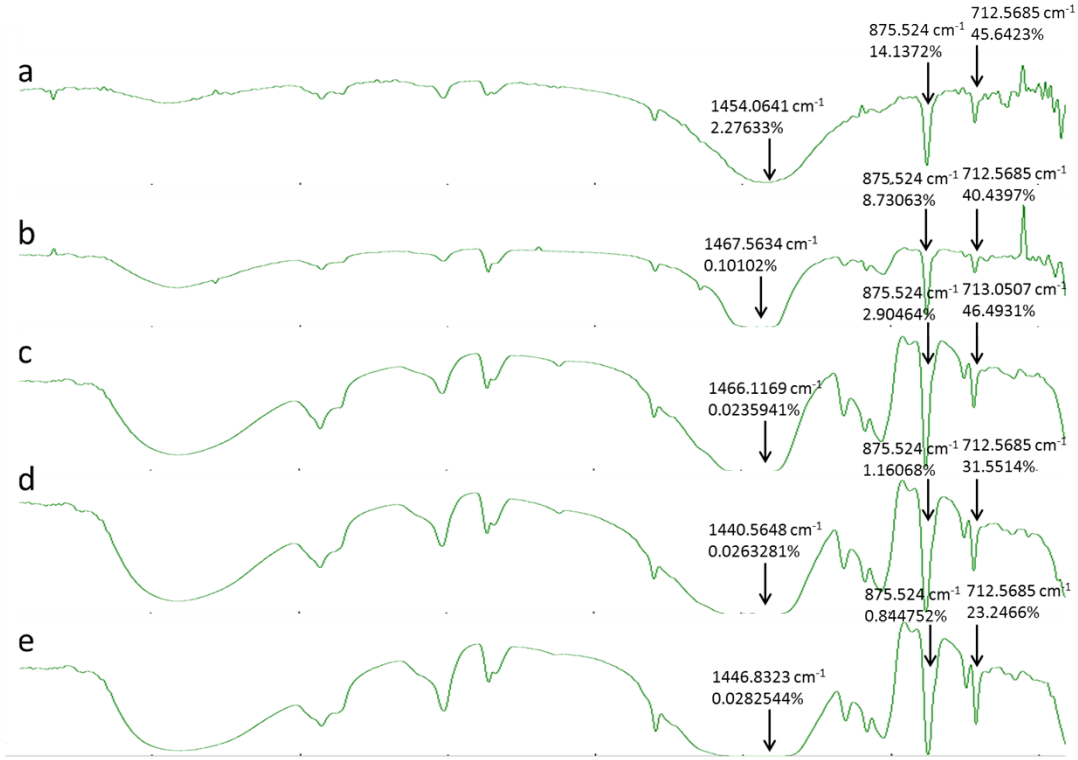

Figure. S2 The IR spectrometer analysis of $CaCO_3$ nanoparticles.

The size determination and morphology observation of the $CaCO_3$ nanoparticles and DOX-loaded $CaCO_3$ nanoparticles: The hydrodynamic diameters of the nanoparticles in de-ionized water solution at the concentration of 1.0 mg•mL^{-1} were determined with dynamic light scattering using a Zetasizer (3000 HS, Malvern Instruments Ltd., UK). For morphologic observations, scanning electron microscopy and transmission electron microscopy (JEOL JEM-1230, Japan) were both conducted. After centrifugation (18000 rpm, 30 min) and lyophilization, the products were re-dissolved in de-ionized water and observed.

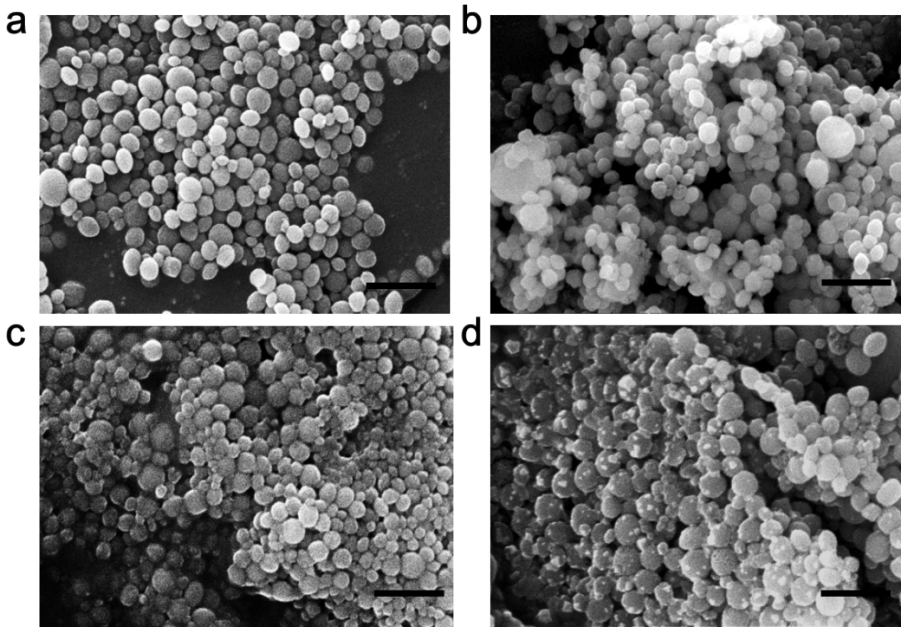

Figure. S3 Scanning electron microscopy of blank $CaCO_3$ nanoparticles synthesized in 0.25% starch solution (a), 0.25% starch-octanoic acid solution (b), 0.125% starch-octanoic acid solution (c) and 0.0625% starch-octanoic acid solution (d). The bar represents 200 nm.

Determination of the drug encapsulation efficiency: To determine the DOX content, a fluorescence spectrophotometer was used. The excitation wavelength was set at 505 nm, the emission wavelength at 565 nm, and the slit openings at 5 nm. First, 9 mL of a DOX-loaded $CaCO_3$ nanoparticle solution was centrifuged at 18000 rpm for 30 min. The supernatant was collected, and the DOX concentration was measured. The drug encapsulation efficiency (EE %) of the DOX-loaded nanoparticles was calculated using the following equation:

$$EE\ \% = (C_0\text{-}C_1) / C_0 \times 100\%; \quad (1)$$

Where C_0 is the total DOX content and C_1 is the DOX content in the supernatant; both units are in micrograms.

In vitro DOX release from the DOX-loaded $CaCO_3$ nanoparticles: pH 4.5 and pH 7.4 phosphate-buffered saline (PBS) were used for the dissolution medium. The DOX-loaded $CaCO_3$ nanoparticles solutions (9 mL) were centrifuged at 18000 rpm for 30 min. The supernatant was collected and measured at 481 nm, and the sediments were dissolved in 1.5 mL of PBS (pH7.5 or pH4.5) and mixed with vibration for 30 s (HZ-8812S, Scientific and Educational Equipment plant, Tai Cang, China). The release test was conducted in a 37 °C water-bath and with horizontal shaking at 60 rpm. At 0.5, 2, 4, 6, 8, 24, 48, and 72 h, the samples were centrifuged at 13400 rpm for 3 min, and 1 mL of the supernatant was measured. Then, 1 mL of fresh dissolution medium was added and mixed with vibrations for 30 s. The concentration of DOX was determined with a fluorescence spectrophotometer. All drug release tests were performed thrice.

Cell culture: A549 cells were cultivated in RPMI-1640 medium with 10% (v/v) newborn bovine serum and 1% antibiotics (penicillin and streptomycin, 10^4 U/L, respectively). All cells were cultured in a humidified incubator with 5% CO_2 at 37°C (MODEL3111, Thermo Forma, USA).

Cellular uptake assay of the DOX-loaded $CaCO_3$ nanoparticles: A549 cells were seeded into 24-well micro plates (Nalge Nunc International，Naperville，IL，USA) at a density of 3×10^4 cells per well. After the attachment of the cells, 0.5 μg of DOX-loaded $CaCO_3$ nanoparticles was added into each well with a final concentration of 0.5 μg per well. DOX·HCl was also added at a concentration of 0.5 μg/well and set as the control. After incubating for 0.5, 3, 6 or 12 h, 20 μL of Hochest33342 (10 μg/mL) was added into each well and allowed to continue incubating. After 10 min, the medium was aspirated, and the cells were lightly washed twice with PBS. The cells were observed using a fluorescence inverted microscope (CMS GmbH, Leica, Germany).

In vitro cytotoxicity of blank $CaCO_3$ nanoparticles: The *in vitro* cytotoxicity of the $CaCO_3$ nanoparticles was evaluated using a methyl thiazolyl tertazolium (MTT) assay in A549 cells. The cells were seeded in a 96-well microplate (Nalge Nunc International, Naperville, IL, USA) at a concentration of 1.0×10^4 cells per well. After growing at 37 °C for 24 h, a blank $CaCO_3$ nanoparticle solution (1 mg•mL^{-1}) was added at various volumes to final concentrations of 2, 5, 10, 20, 50, 100, and 200 μg/mL. After 48 h, 20 ìL of a MTT solution (5 mg•mL^{-1}) was added, and the cells were incubated for an additional 4 h. Then, the medium was replaced with DMSO (100 μL/well). After gently shaking the microplates for 10 min, formazan was dissolved in DMSO. Finally, each sample with three replicates (n=3) was analyzed on a microplate reader (BioRad, Model 680, USA) at a wavelength of 570 nm at room temperature. The survival percentage was calculated compared to non-adjunction groups (100% survival).

In vitro anti-tumor activity of DOX-loaded $CaCO_3$ nanoparticles: A549 cells were seeded in 96-well microplates (Nalge Nunc International, Naperville, IL, USA) at a concentration of 1.0×10^4 cells per well. After growing at 37 °C for 24 h, various volumes of the DOX-loaded $CaCO_3$ nanoparticle solution were added to a final concentration of 0.05, 0.25, 0.5, 1, 2, 3, or 5 μg/mL and incubated for an additional 48 h. The experiment was then finished using the same protocol as the *in vitro* cytotoxicity assay. The cells treated with DOX·HCl were set as the control group