Electronic Supplementary Information


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1. General information and experimental procedure

1) General information:

All reactions were performed in air atmosphere unless otherwise stated. The commercially available reagents were used as supplied without further purification. Chloroform and THF were dried according to procedures described in the literatures, and other solvents were used as received without further purification unless otherwise stated. Column chromatography was performed with silica gel (200 – 300 mesh) produced by Qingdao Marine Chemical Factory, Qingdao (China). All yields were given as isolated yields. NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer (or Bruker DPX 400 MHz spectrometer) with internal standard tetramethylsilane (TMS) and solvent signals as internal references at 298 K, and the chemical shifts (δ) were expressed in ppm and J values were given in Hz. Low-resolution electrospray ionization mass spectra (LR-ESI-MS) were obtained on Finnigan Mat TSQ 7000 instruments. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on an Agilent 6540Q-TOF LCMS equipped with an electrospray ionization (ESI) probe operating in positive-ion mode with direct infusion. Dynamic light scattering (DLS) measurements were carried out on a Brookhaven BI-9000AT system (Brookhaven Instruments Corporation, USA), using a 200-mW polarized laser source (λ = 514 nm). Zeta-potential measurements were performed at 25 °C on a Zetasizer Nano Z apparatus (Malvern Instruments Ltd., UK) using the Smoluchowski model for the calculation of the Zeta-potential from the measured electrophoretic mobility. The UV-Vis absorption spectra were measured on a Perkin Elmer Lambda 35 UV-Vis Spectrometer. Transmission electron microscope (TEM) investigations were carried out on a JEM-2100 instrument.
2) Experimental procedure:

Fabrication of GalP5→G supramolecular prodrug nanoparticles. GalP5→G nanoparticles were prepared as follows: CPT-prodrug G (3.0 mg) was dissolved in water (45 mL, 1 % DMSO was added to improve the solubility of G), then an aqueous solution of GalP5 (5 mL, C = 0.8 mM) was injected. The ultimate concentrations of G and GalP5 were both 0.08 mM. After standing overnight, the prepared GalP5→G nanoparticles were purified by dialysis (molecular weight cutoff 10 000) in distilled water for several times until the water outside the dialysis tube exhibited negligible CPT absorption.

The CPT encapsulation efficiency and loading content were calculated by the following equation:

Encapsulation efficiency (%) = \( \frac{m_{CPT \cdot \text{loaded}} - m_{CPT}}{m_{CPT \cdot \text{loaded}}} \times 100 \)

Loading content (wt%) = \( \frac{m_{CPT \cdot \text{loaded}}}{m_{CPT \cdot \text{loaded}} + m_{GalP5}} \times 100 \)

where \( m_{CPT \cdot \text{loaded}} \), \( m_{CPT} \), and \( m_{GalP5} \) are mass of CPT encapsulated into the nanoparticles, mass of CPT-prodrug G and GalP5 added, respectively. The mass of CPT was measured by a UV spectrophotometer at 365 nm and calculated as relative to a standard calibration curve in the concentrations from 2 to 80 μM.

GSH-responsive behaviors of the supramolecular prodrug nanoparticles. In a typical GSH-induced release experiment, a certain amount of GSH solution was added into 10 mL of the GalP5→G nanoparticle solution, the final GSH concentration was 2 mM, 5 mM, and 10 mM, respectively. At selected time intervals, 2 mL of the release media was taken out for measuring the released CPT concentrations by UV-Vis absorbance technique, and then was returned to the original release media. A nearly 100% release of CPT from GalP5→G nanoparticles was obtained by adding Triton X-100 to the nanoparticle solution.
In vitro cell cytotoxicity assay. The relative in vitro cytotoxicities of host GalP5 and GalP5.nilG prodrug nanoparticles against MRC-5 cells (normal human fetal lung fibroblast cell line), HepG2 cells (human hepatocellular liver carcinoma cell line), and HeLa cells (human cervical carcinoma cell line) with and without pretreatment with free galactose (200 mM, 15 min) were assessed by using the MTT assay. Briefly, the cells were seeded in 96-well plates at a density of 2 × 10^4 cells per well in 200 μL of Dulbecco’s modified Eagle’s medium (DMEM) (for HepG2 and HeLa cells) or Minimum Essential Medium (MEM) (for MRC-5 cells) containing 10% fetal bovine serum (Gibco), supplemented with 50 U·mL^{-1} penicillin and 50 U·mL^{-1} streptomycin (Hyclone), and cultured in 5% CO_2 at 37 °C for 24 h. Then, the original medium was removed and 200 μL of fresh culture medium containing different concentrations of GalP5 or GalP5.nilG prodrug nanoparticles was added into each well, and the cells were further incubated for 24 h.

In addition, in order to verify that the cellular uptake of GalP5.nilG prodrug nanoparticles is mediated via galactose-binding protein ASGP-R, a competing inhibition experiment was performed. Before the addition of analyte, free galactose (200 mM) were added and incubated with HepG2 and HeLa cells for 15 min. After washing with PBS buffer to remove the free galactose, 200 μL of fresh culture medium containing different concentrations of GalP5 or GalP5.nilG prodrug nanoparticles was added into each well, and the cells were further incubated for 24 h.

Subsequently, 20 μL of MTT solution (5 mg/mL) was added into each well and incubated for another 4 h. After that, the medium containing MTT was removed, and dimethyl sulfoxide (DMSO, 150 μL) was added to each well to dissolve the MTT formazan crystals. Finally, the plates were shaken for 30 min, and the absorbance of formazan product was measured at 570 nm by a microplate reader (Thermo Scientific). Untreated cells in medium were used as the blank control. All experiments were
carried out with five replicates. The cytotoxicity was expressed as the percentage of the cell viability relative to the blank control.

**Cellular uptake and intracellular localization observed by CLSM.** The cellular uptake and intracellular localization of CPT were examined in HepG2 cell line. Briefly, HepG2 cells were plated onto glass-bottomed Petri dishes in 2.0 mL of complete DMEM culture medium at a density of $4 \times 10^4$ cells for 24 h before treatment. For competition experiments, before adding the analyte, free galactose (200 mM) was added to the Petri dishes and incubated with HepG2 cells for 15 min and then washing with PBS buffer to remove the free galactose. Next, the cells were treated with GalP5 Gos prodrug nanoparticles (10 μM) for designated time periods. Then, the culture medium was removed and cells were washed with PBS thrice. Thereafter, the LysoTracker Red DND-99 (Thermo Fisher, USA) was added to the medium with a final concentration of 50 nM and incubated for 1 h to label lysosomes. Finally, after removing the culture medium and washing with PBS thrice, the cells were fixed with 4% paraformaldehyde and investigated by fluorescence microscopy (LSM710, CarlZeiss). The fluorescence characteristics of GalP5 Gos prodrug nanoparticles were used to directly monitor the localization of CPT without utilizing additional dye.

**Statistical analysis.** Differences between treatment groups were statistically analyzed using the paired Student’s test. A statistically significant difference was reported if $p < 0.05$ or less. The data were expressed as mean ± standard deviation from at least three separate experiments.
2. Synthesis of host GalP5

The synthetic procedures for host GalP5 was shown in Scheme S1.

Scheme S1. Synthetic route for host GalP5.

Synthesis of compound $1^{S1}$

Compound 1 was prepared according to previously reported method.$^{S1}$ $^1$H NMR (400 MHz, CDCl$_3$, 298 K) $\delta$ (ppm): 6.82 (s, 10H), 4.53 (d, $J = 2.4$ Hz, 20H), 3.81 (s, 10H), 2.27 (t, $J = 2.4$ Hz, 10H).

Figure S1. $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of compound 1.
Synthesis of compound 2

Compound 2 was prepared according to previously reported method.\textsuperscript{S2} \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, 298 K) \(\delta\) (ppm): 5.39 (dd, \(J = 3.4, 0.9\) Hz, 1H), 5.23 (dd, \(J = 10.5, 8.0\) Hz, 1H), 5.01 (dd, \(J = 10.5, 3.4\) Hz, 1H), 4.55 (d, \(J = 8.0\) Hz, 1H), 4.15 (tt, \(J = 11.3, 5.6\) Hz, 2H), 4.04 (ddd, \(J = 10.7, 4.7, 3.5\) Hz, 1H), 3.92 (td, \(J = 6.6, 1.0\) Hz, 1H), 3.68 (ddd, \(J = 10.7, 8.5, 3.3\) Hz, 1H), 3.50 (ddd, \(J = 13.2, 8.5, 3.5\) Hz, 1H), 3.29 (ddd, \(J = 13.4, 4.6, 3.4\) Hz, 1H), 2.14 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H).

\textbf{Figure S2.} \textsuperscript{1}H NMR spectrum (400 MHz, CDCl\textsubscript{3}, 298 K) of compound 2.

Synthesis of compound 3

A mixture of 1 (0.1 g, 0.1 mmol), 2 (0.63 g, 1.5 mmol), CuSO\textsubscript{4}·5H\textsubscript{2}O (0.01 g, 0.05 mmol) and sodium ascorbate (0.02 g, 0.1 mmol) in 5 mL of DMF was stirred under argon atmosphere for 24 h at 65 °C. Then, 40 mL of H\textsubscript{2}O was added. The aqueous layer was extracted with dichloromethane for 3 times and organic layer were dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and concentrated. The crude product was purified by silica gel chromatography using dichloromethane/MeOH (20:1, v/v) as the eluent to afford compound 3 as a white solid (0.44 g, 0.086 mmol, 86 %). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, 298 K) \(\delta\) (ppm): 7.97 (s, 10H), 7.00 – 6.93 (m, 10H), 5.39 (s, 10H), 5.16 (s, 10H), 5.01 (d, \(J = 6.5\) Hz, 30H), 4.65 (s, 10H), 4.54 (d, \(J = 17.5\) Hz, 20H), 4.28 (s, 10H), 4.14 (s, 20H), 3.97 (s, 20H), 3.81 (s, 10H), 2.14 (d, \(J = 3.2\) Hz, 30H), 2.03 (s, 30H), 1.97 – 1.94 (m, 60H). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}, 298 K) \(\delta\) (ppm): 170.5, 169.8,
169.6, 149.7, 144.3, 128.6, 124.0, 115.6, 101.0, 77.3, 77.1, 76.7, 70.7, 68.5, 67.7, 67.0, 62.4, 61.1, 50.0, 20.9, 20.5.

Figure S3. $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of compound 3.

Figure S4. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 298 K) of compound 3.
Synthesis of host GalP5

A solution of MeONa in MeOH was added to 10 mL MeOH of compound 3 (0.1 g, 0.02 mmol) until the pH value reached to 11. The mixture was stirred at room temperature for 12 h. After removal of the solvent under reduced pressure, 10 mL of H₂O was added. And the solution was neutralized by Amberlite IR 120 (H⁺ resin form), the resulted solution was filtered, and residues were washed by H₂O several times. The obtained filtrate was concentrated to afford the target host molecule GalP5 as white powder (0.065 g, 0.018 mmol, 94 %). ¹H NMR (400 MHz, DMSO-鱀, 298 K) δ (ppm): 8.32 (s, 10H), 6.98 (s, 10H), 5.05 (s, 10H), 4.92 (s, 10H), 4.75 (d, J = 11.5 Hz, 20H), 4.53 (s, 30H), 4.17 (d, J = 6.8 Hz, 10H), 4.10 – 4.02 (m, 10H), 3.87 (s, 10H), 3.63 (s, 20H), 3.51 (d, J = 5.8 Hz, 20H). ¹³C NMR (100 MHz, D₂O, 298 K) δ (ppm): 150.1, 143.3, 128.9, 125.4, 103.1, 75.1, 72.6, 70.6, 69.0, 68.6, 68.2, 65.7, 60.9, 50.3, 20.4, 14.0.

Figure S5. ¹H NMR spectrum (400 MHz, DMSO-鱀, 298 K) of compound GalP5.
Figure S6. $^{13}$C NMR spectrum (100 MHz, D$_2$O, 298 K) of compound GalP5.

3. Synthesis of CPT prodrug guest G

The synthetic procedures for CPT prodrug guest G was shown in Scheme S2.

Scheme S2. Synthetic route for CPT prodrug guest G.
**Synthesis of compound 4**

Compound 4 was prepared according to previously reported method with some modifications.

Bis(2-hydroxyethyl) disulfide (7.7 g, 50.0 mmol) and propargyl bromide (3.0 g, 25.0 mmol) were dissolved in THF (200 mL). Then, NaH powder (80 wt %, 1.5 g, 37.5 mmol) was added into the reaction mixture in three successive batches under nitrogen atmosphere within 2 h at 0 °C. The mixture was further stirred for another 8 h at room temperature, and then a few drops of water were added to quench the reaction. The mixture was filtered, and the solvent was removed by evaporation. The crude product was purified by column chromatography, using dichloromethane/ethyl acetate (4:1, v/v) as eluent to afford a pale-yellow clear oil (2.1 g, 11.2 mmol, 45 %).

$^1$H NMR (400 MHz, CDCl$_3$, 298 K) δ (ppm): 4.20 (d, $J = 2.4$ Hz, 2H), 3.90 (t, $J = 5.8$ Hz, 2H), 3.81 (t, $J = 6.4$ Hz, 2H), 2.91 (dt, $J = 11.6, 6.1$ Hz, 4H), 2.47 (t, $J = 2.4$ Hz, 1H), 2.11 (s, 1H).

![Figure S7](image)

*Figure S7.* $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of compound 4.

**Synthesis of compound 5**

A mixture of triphosgene (0.12 g, 0.4 mmol) and DMAP (0.49 g, 4.0 mmol) in 10 mL of anhydrous chloroform was stirred under argon atomosphere for 0.5 h at room temperature. Then a solution of camptothecin (0.38 g, 1.1 mmol) in anhydrous
chloroform was added slowly. The resulting mixture was stirred at room temperature for additional 2 h, and then the solution of compound 4 (0.21 g, 1.1 mmol) in CHCl₃ was added. The resulting mixture was stirred at room temperature for 12 h. After removal of the solvent under reduced pressure, the crude product was purified by silica gel chromatography using ethyl acetate/petroleum ether (v/v, 3:1) as the eluent to afford compound 5 as a pale yellow solid (0.28 g, 0.5 mmol, 45 %). ¹H NMR (400 MHz, CDCl₃, 298 K) δ (ppm): 8.49 (s, 1H), 8.34 (d, J = 8.5 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.90 (t, J = 7.7 Hz, 1H), 7.72 (t, J = 7.5 Hz, 1H), 7.51 (s, 1H), 5.71 (d, J = 17.3 Hz, 1H), 5.45 – 5.30 (m, 3H), 4.38 (dt, J = 10.7, 5.4 Hz, 2H), 4.13 (d, J = 2.4 Hz, 2H), 3.73 (t, J = 6.3 Hz, 2H), 2.99 – 2.86 (m, 4H), 2.45 (t, J = 2.4 Hz, 1H), 2.29 – 2.15 (m, 2H), 1.01 (t, J = 7.5 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃, 298 K) δ (ppm): 167.3, 157.3, 153.5, 152.3, 148.9, 146.5, 145.6, 132.1, 131.3, 130.8, 129.7, 128.5, 128.2, 128.1, 120.3, 96.1, 78.0, 74.9, 67.9, 67.1, 66.7, 58.2, 50.0, 38.6, 36.5, 31.9, 29.7, 7.7. ESI-MS: m/z calcld [M + Na]+ 589.11, found 589.00. HR-ESI-MS: m/z calcld [M + Na]+ 589.1079, found 589.1077.

Figure S8. ¹H NMR spectrum (400 MHz, CDCl₃, 298 K) of compound 5.
Figure S9. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 298 K) of compound 5.

Figure S10. LR-ESI-MS spectrum of compound 5.

Figure S11. HR-ESI-MS spectrum of compound 5.
Synthesis of compound $6^{S4}$

Compound 4 was prepared according to previously reported method.$^{S4}$ $^1$H NMR (400 MHz, CDCl$_3$, 298 K) δ (ppm): 3.41 (t, $J = 6.7$ Hz, 2H), 3.28 (t, $J = 6.9$ Hz, 2H), 1.91 – 1.83 (m, 2H), 1.63 – 1.60 (m, 2H), 1.51 – 1.37 (m, 4H).

$^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of compound 6.

Synthesis of compound 7

Compound 6 (0.41 g, 0.2 mmol) and trimethylamine (33% in ethanol, 0.7 g, 2.0 mmol) were added to ethanol (15 mL). The resulting solution was refluxed for 24 h in a sealed flask. Then, the mixture was concentrated under reduced pressure and washed by diethyl ether for several times to give compound 7 as a white solid (0.43 g, 0.16 mmol, 81 %). $^1$H NMR (400 MHz, CDCl$_3$, 298 K) δ (ppm): 3.70 – 3.66 (m, 2H), 3.45 (s, 9H), 3.30 (t, $J = 6.7$ Hz, 2H), 1.80 (dd, $J = 8.5$, 6.1 Hz, 2H), 1.61 (dd, $J = 13.7$, 6.8 Hz, 2H), 1.51 – 1.40 (m, 4H). ESI-MS: m/z calcd [M – Br]$^+$ 185.18, found 185.15. HR-ESI-MS: m/z calcd [M – Br]$^+$ 185.1761, found 185.1759.
Figure S13. $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of compound 7.

Figure S14. LR-ESI-MS spectrum of compound 7.

Figure S15. HR-ESI-MS spectrum of compound 7.
Synthesis of compound G

A mixture of 5 (0.25 g, 0.44 mmol), 7 (0.15 g, 0.58 mmol), Cu(CNCH$_3$)$_4$PF$_6$ (0.016 g, 0.044 mmol) and TBTA (0.023 g, 0.044 mmol) in dichloromethane/MeOH (1:1, v/v, 20 mL) was stirred under argon atmosphere for 6 h at room temperature. After removal of the solvent under reduced pressure, the crude product was purified by silica gel chromatography using dichloromethane/ethyl acetate (4:1, v/v) as the eluent to afford compound G as a pale yellow solid (0.31 g, 0.37 mmol, 84 %). $^1$H NMR (400 MHz, DMSO-$d_6$, 398 K) $\delta$ (ppm): 8.72 (s, 1H), 8.19 – 8.14 (m, 2H), 8.03 (s, 1H), 7.88 (s, 1H), 7.74 (d, $J$ = 7.6 Hz, 1H), 7.09 (s, 1H), 5.53 (s, 2H), 5.33 (s, 2H), 4.44 (s, 2H), 4.32 (t, $J$ = 6.7 Hz, 4H), 3.59 (s, 2H), 3.51 (s, 2H), 3.02 (s, 9H), 2.88 (d, $J$ = 6.0 Hz, 2H), 2.18 (s, 2H), 1.81 (s, 2H), 1.60 (d, $J$ = 30.5 Hz, 4H), 1.24 (s, 4H), 0.92 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 298 K) $\delta$ 144.1, 132.1, 129.0, 128.3, 124.2, 119.7, 94.9, 78.4, 68.1, 66.9, 66.7, 65.6, 63.7, 55.4, 52.6, 51.0, 49.6, 38.3, 36.7, 29.8, 28.4, 26.1, 25.8, 22.4, 8.0. ESI-MS: m/z calcld [M – Br]$^+$ 751.29, found 751.15. HR-ESI-MS: m/z calcld [M – Br]$^+$ 751.2942, found 751.2937.

Figure S16. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$, 298 K) of compound G.
Figure S17. $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$, 298 K) of compound G.

Figure S18. LR-ESI-MS spectrum of compound G.

Figure S19. HR-ESI-MS spectrum of compound G.
4. Host-guest complexation of GalP5 and G

Figure S20. $^1$H NMR (400 MHz, D$_2$O, 298 K) spectra: (a) GalP5 (2.0 mM), (b) GalP5 (2.0 mM) and G (2.0 mM).

5. 2D NOESY spectra of GalP5 and GM in D$_2$O

Figure S21. 2D NOESY spectrum of GalP5 and GM (400 MHz, D$_2$O, 298 K) ([GalP5] = 4.0 mM, GM = 4.0 mM).
6. Job’s plot for GalP5⊃G_M

Figure S22. (a) UV-Vis absorption spectra of complex GalP5⊃G_M with different molar ratios in water while [GalP5] + [G_M] = 10 μM. (b) Job plot of complex GalP5⊃G_M showing a 1:1 stoichiometry between GalP5 and G_M by plotting the absorbance difference at 290 nm (a characteristic absorption peak of GalP5) against the mole fraction of GalP5.

7. Determination of the association constant (K_a) for GalP5⊃G_M

To determine the association constant between GalP5 and G_M, ¹H NMR titrations were carried out in solutions, which had a constant concentration of G_M (4.0 mM) and varying concentrations of GalP5. By a non-linear curve-fitting method, the association constant between the G_M and GalP5 was calculated.

The non-linear curve-fitting was based on the following equation: \[ \Delta \delta = (\Delta \delta_c/[G]_0) \left( 0.5[H]_0+0.5([G]_0+1/K_a)-(0.5([H]_0^2+(2[H]_0(1/K_a-[G]_0))+(1/K_a+[G]_0)^2)0.5) \right). \]
Where \( \Delta \delta \) is the chemical shift change of H_b on G_M at [H]_0, \( \Delta \delta_c \) is the chemical shift change of H_b when the guest is completely complexed, [G]_0 is the fixed initial concentration of the guest (G_M), and [H]_0 is the varying concentrations of GalP5.
Figure S23. The chemical shift changes of H₆ on G₅M upon addition of GalP₅. The red solid line was obtained from the non-linear curve-fitting using. The association constant (Kₐ) of GalP₅ and G₅M was estimated to be about (1.22 ± 0.40) × 10³ M⁻¹.

8. Zeta-potentials of the aggregates formed by GalP₅⊃G

Figure S24. Zeta-potential of the aggregates formed by GalP₅⊃G.
9. TEM images of GalP5→G aggregates under GSH-stimulus

![TEM image of GalP5→G aggregates under GSH-stimulus](image)

*Figure S25.* TEM images of GalP5→G aggregates in the presence of GSH (10.0 mM).

10. DLS data of GalP5→G aggregates in PBS and MEM cell culture medium

![DLS data graphs](image)

*Figure S26.* DLS data of GalP5→G prodrug nanoparticles in (a) PBS and (b) MEM cell culture medium.
11. Stability of GalP5→G prodrug nanoparticles in PBS, plasm, and MEM cell culture medium

**Figure S27.** Time-dependent size changes of GalP5→G nanoparticles under different conditions.

12. In vitro cell assay

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**Table S1.** Cytotoxicity of GalP5→G prodrug nanoparticles against HepG2 and HeLa cells at different concentrations after 24 h incubation.
13. References