Electronic Supplementary Information

An Excipient-free “Sugar-coated Bullet” for the Targeted Treatment of Orthotopic Hepatocellular Carcinoma

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1. Experimental section

1.1. Materials and Instruments

All the starting materials were obtained from commercial suppliers and used as received. $^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectra were taken on a Bruker AV400 nuclear magnetic resonance spectrometer, using CDC$_3$ or DMSO-$d_6$ as solvent. Proton or carbon chemical shifts are reported in parts per million downfield from tetramethylsilane (TMS), with TMS ($\delta = 0.0$ ppm) or the solvent residue peak CDC$_3$ (7.26 ppm for $^1$H, 77.16 ppm for $^{13}$C), or DMSO-$d_6$ (2.50 ppm for $^1$H, 39.52 ppm for $^{13}$C) as the chemical shift standard. High-resolution mass spectra (HRMS) were measured on a Bruker Micro TOF II 10257 instrument with electro-spray ionization (ESI) technique and direct injection method. UV-visible absorption spectra were recorded on a Shimadzu UV-2550 spectrometer. Steady-state fluorescent spectra at room temperature was measured on an Edinburgh instrument FLS-920 spectrometer with a Xe lamp as an excitation source. Confocal laser scanning microscopy (CLSM) images were obtained with FLV 1000. In vivo images were collected using a Bruker small animal in vivo fluorescence imaging system (In Vivo Xtreme) with a Xe lamp as the excitation source.

The handling and care of the animals conformed to the guidelines of current international laws and policies (National Institutes of Health Guide for the Care and Use of Laboratory Animals, Publication No. 85–23, 1985, revised 1996). All animal experiments were performed according to procedures approved by the Fudan University Committee on Animal Care and Use. All protocols for animal studies conformed to the Guide for the Care and Use of Laboratory Animals.

1.2. Preparation of Gal-MB-DOX Nanoparticles

4.3 mg Gal-MB-DOX was dissolved in 0.5 mL of DMSO and mixed fully under ultrasound. Then the mixture was dropwise added into 4.5 mL of deionized water and stirred slightly at room temperature for 10 min. Subsequently, the solution was dialyzed at room temperature for 48 h (molecular weight cutoff = 1,000 Da).

1.3. Spectroscopic studies in in vitro

Different analysts were prepared according to our previous methods.$^{1-4}$

H$_2$O$_2$ was diluted from a 30% solution. HOCl was obtained from 14.5% NaOCl solution.

•OH was generated by Fenton reaction. To generate •OH, H$_2$O$_2$ was added in the presence of 10 equiv. of ferrous chloride. The concentration of •OH was equal to the H$_2$O$_2$ concentration.

TBHP (tert-butyl hydroperoxide) was obtained from 70% TBHP solution in ddH$_2$O.

ROO• was prepared by dissolving 2, 2′-azobis (2-amidinopropane) dihydrochloride in ddH$_2$O.

NO was prepared by dissolving SNP (sodium nitroferricyanide (III) dihydrate) in ddH$_2$O.

O$_2$•− was prepared by dissolving KO$_2$ (potassium superoxide) in DMSO.

ONOO•− was prepared using 3-morpholinosydnonimine hydrochloride.

t-BuOO• was prepared by adding TBHP in the presence of 10 equiv. of ferrous chloride. The concentration of t-BuOO• was equal to the TBHP concentration.

Prodrugs (5 µM) were treated with the required concentration of HOCl and other ROS/RNS in PB (10 mM, pH7.4) at 37 °C for 30 min. Fluorescence or absorption spectra were measured on a fluorescence or ultraviolet spectrophotometer. Unless otherwise noted, for all fluorescent measurements, the excitation wavelength was 620 nm and the emission wavelength was collected from 490 to 850 nm.

1.4. DOX release analysis of Gal-MB-DOX

DOX release analysis of Gal-MB-DOX was perform by ultraviolet spectrophotometry. Gal-MB-DOX (500 µM, 4 mL) was treated with 0 or 1000 µM of HOCl for 30 min respectively. After that, the reaction mixture was dialyzed against H$_2$O for 18 h (molecular weight cutoff = 1,000 Da), the H$_2$O was exchanged for 2 times (100 mL each time) and collected together. Finally, the absorption in 497 nm of H$_2$O was tested and the DOX release was calculated by the standard curve of free DOX.

1.5. Detection limit
The detection limit was calculated with the following equation based on the fluorescence intensity at 686 nm or absorbance at 664 nm of the probe. Detection limit = 3 \sigma / k, where \sigma is the standard deviation of blank measurement (n = 10), k is the slope between the fluorescence intensity versus HOCl concentration.

1.6. Cell culture and confocal laser scanning microscopy (CLSM) imaging

HepG2 and RAW cells were cultured in high glucose Dulbecco’s modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin. The cells were incubated at 37 °C under 5% CO₂ and split with trypsin/EDTA solution (0.25%) as recommended by the manufacturer.

Cells (5 × 10⁸ per mL) were separately plated on 14 mm glass coverslips and allowed to adhere for 12 h. The stock solution of prodrugs in DMF (1 mM) was diluted with phosphate buffered saline (PBS, 10 mM, pH 7.4) with final concentration of 10 \mu M. The cells were then incubated with different analytes for a pre-set time at 37 °C. After incubation, the cells were washed two times with PBS. CLSM imaging was performed on ZEISS LSM 880 confocal laser scanning microscope with a 60 × oil-immersion objective lens for cells. Blue channel: 470 ± 50 nm, \lambda_{ex} = 405 nm; red channel: 700 ± 50 nm, \lambda_{ex} = 633 nm.

1.7. Flow cytometry analysis

To evaluate the selectivity of prodrugs for cells, flow cytometric assay was carried out. HepG2 and RAW cells were seeded in 6-well microplates at a density of 1×10⁵ cells/mL in 2 mL medium containing 10% FBS. After 12 h of cell attachment, the plates were then washed with 100 \mu L/well PBS. The cells were then cultured in medium with prodrugs (10 \mu M) for 0.25, 0.5, 1 h. Cells in culture medium without any treatment were used as the control. After treatment, the cells were washed in PBS for twice and collected by trypsin treatment. Then the cells were diluted with 700 \mu L PBS, resuspended and determined by flow cytometry. All the experiments detected at least 20,000 cells, and these data were analyzed in the FlowJo V10.

1.8. Cytotoxicity experiments

A Cell Counting Kit-8 (CCK-8) was employed in this experiment to quantitatively evaluate HepG2 and RAW viability. Cells were seeded in 96-well plates at a density of 1 × 10⁴ cells per well, then cultured in 5% CO₂ at 37 °C for 24 h. After the cells were incubated with prodrugs at different concentrations (0, 10, 20, 40, 60, 80, 100 and 120 \mu M in DMF/cell culture medium without FBS = 1:100) for 24 h, 100 \mu L DMEM containing 10% CCK-8 was added to each well of the 96-well assay plate for 4 h at 37 °C, six parallel replicates were prepared. The absorbance at 450 nm was determined using a microplate reader (Bio Tek Instruments, Inc). All samples were analyzed in triplicate.

1.9. In vivo fluorescence or bioluminescence imaging

All animal experiments were performed according to procedures approved by the Fudan University Committee on Animal Care and Use. All protocols for animal studies conformed to the Guide for the Care and Use of Laboratory Animals. HepG2-Luc cells were used for in vivo studies. Balb/c mice were surgically implanted tumor tissue (1-2 mm³) of HepG2-Luc cells for orthotopic hepatocellular carcinoma model, and tumor growth is tracked by bioluminescence. The mice were given an intravenous injection (i.p.) of prodrugs (0.2 mM, 100 \mu L) every two days. The body weight of each group was monitored every 2 days.
during a period of 15 days. In addition, bioluminescence imaging was performed on 1th, 4th, 8th, 12th and 15th day for monitoring therapeutic efficacy. After treatment, all mice were euthanized and tumors were collected. The tumor size and weight among each group were compared, and the tumor growth inhibitory rate was calculated.

1.12. Blood routine analysis of prodrugs

Healthy female F1 mice were randomly divided into four groups, (a) saline, (b) free DOX, (c) MB-DOX and (d) Gal-MB-DOX (n = 3) and given intravenous injection with prodrugs (100 μL, 0.2 mM) once every two days for one week. The blood was collected 24 h post the final injection and serums isolated for measurements of representative blood parameters (red blood count (RBC), white blood count (WBC), platelets (PLT), and hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were then measured by semiautomatic biochemistry analyzer.

1.13. Histopathological studies

At the end of treatment, major organs and tumor of each group mice were fixed in 4% paraformaldehyde (PFA) immediately following sacrifice, stained with hematoxylin and eosin (H&E).
2. Synthesis

Scheme S1. Synthetic route of the Gal-MB-DOX

Reagents and conditions: (a) NaOH, KMnO₄, H₂O, 95 °C, 16 h, 94%; (b) 1 N BH₃ in THF, THF, 0 °C to r.t., 48 h, 89%; (c) Pd/C, N₂H₄•H₂O, MeOH, 70 °C, 8 h, 73%; (d) imidazole, TBSCI, DMF, 0 °C to r.t., 5 h, 96%; (e) FDOCl-2, K₂CO₃, toluene, 110 °C, 12 h, 11%; (f) TsOH, MeOH, r.t., 30 min, 57%; (g) 1) Et₃N, DMAP, P-nitrophenyl chloroformate, DCM, 0 °C to r.t., 2 h, 2) Et₃N, compound 8, 0 °C to r.t., 3 h, 55%; (h) TsOH, MeOH, r.t., 1 h, 86%; (i) 1) Et₃N, DMAP, P-nitrophenyl chloroformate, DCM, 0 °C to r.t., 2) Et₃N, DOX, 0 °C to r.t., 3 h, 46%; (j) MeONa, MeOH, r.t., 2 h, 84%.
To the mixture of 2, 6-dimethyl-1-nitrobenzene (8 g, 53 mmol) in 400 mL of H₂O was added with NaOH (3.2 g, 79 mmol) at room temperature, then heated to 95 °C. KMnO₄ (34 g, 212 mmol) was added to the reaction system in batches over a period of 3h. The reaction was proceeded overnight, TLC suggested the completion of the reaction. Then the reaction was stopped and cooled to room temperature, filtered to remove solid impurities. The mixture was slowly added with HCl and a large amount of solid was precipitated out, filtration to give the white solid 2 (11.4g, 94%). ¹H NMR (400 MHz, DMSO-d₆) δ 8.19 (d, J = 7.8 Hz, 2H), 7.81 (t, J = 7.8 Hz, 1H). ¹³C NMR (100 MHz, DMSO-d₆) δ 164.61, 149.21, 134.95, 131.61, 125.43.

To the solution of 2 (11 g, 52.10 mmol) in 60 mL of THF was dropwise added 1 N BH₃ (260 mL, 260 mmol) solution dissolved in THF at 0 °C through dropping funnel under the protection of N₂. After that, the reaction was restored naturally to room temperature and stirred for 48 h. TLC suggested the completion of the reaction, then the reaction was quenched by cooling to 0 °C and slowly added 50 mL of MeOH. The solvent was removed in vacuo and purified by silica gel column chromatography to give 3 as a white solid (8.5 g, 89%). ¹H NMR (400 MHz, DMSO-d₆) δ 7.57 (m, 3H), 4.54 (s, 4H). ¹³C NMR (100 MHz, DMSO-d₆) δ 147.86, 134.79, 131.23, 128.01, 59.71.

To a mixture of 3 (8 g, 43.43 mmol) and Pd/C (0.15 g, 5wt %) in 30 mL of MeOH was slowly added N₂H₄•H₂O (6.6 mL, 130 mmol) under N₂ atmosphere. After that, the mixture was heated to 70 °C and stirred for 8 h. TLC suggested the completion of the reaction, the solution was cooled to room temperature and filtered to remove the catalyst (Try not to let the cake dry when pumping, otherwise Pd/C is easy to catch fire). The filtrate was collected and the solvent was removed in vacuo, then purified by silica gel column chromatography to give 4 as a light yellow solid (5 g, 73%). ¹H NMR (400 MHz, DMSO-d₆) δ 7.01 (d, J = 7.4 Hz, 2H), 6.53 (t, J = 7.4 Hz, 1H), 5.04 (t, J = 5.3 Hz, 2H), 4.82 (s, 2H), 4.43 (d, J = 5.3 Hz, 4H). ¹³C NMR (100 MHz, DMSO-d₆) δ 144.80, 127.52, 125.88, 115.91, 61.90.

To the mixture of 4 (5 g, 32.64 mmol) and imidazole (8.9 g, 130.56 mmol) in 50 mL of DMF was slowly added TBSCl (15g, 98 mmol) dissolved in 50 mL of DMF over a period of 1 h at 0 °C. After that, the mixture was restored naturally to room temperature and stirred for 5 h. TLC suggested the completion of the reaction, H₂O (400 mL) was added to the reaction solution, extracted with EA (50 mL × 3). The combined organic layers were washed with brine (400 mL × 2), dried with Na₂SO₄ and
concentrated in vacuo. The crude product was purified by silica gel column chromatography to give 5 as a light-yellow oil (12 g, 96%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.05 (d, $J = 7.5$ Hz, 2H), 6.57 (t, $J = 7.5$ Hz, 1H), 4.72 (s, 2H), 4.63 (s, 4H), 0.89 (s, 18H), 0.06 (s, 12H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 146.00, 128.81, 126.85, 118.18, 65.39, 28.29, 20.43, -2.81.

To the mixture of 5 (5 g, 13.1 mmol) and FDOCl-2 synthesized according our previous work $^2, 3$ (4.6 g, 13.1 mmol) in 50 mL of toluene was added K$_2$CO$_3$ (5.3 g, 33.7 mmol) and the mixture was heated to 110 $^\circ$C and stirred for 12 h under N$_2$ atmosphere. TLC suggested the consumption of 5, the solution was cooled to room temperature and filtered to remove the solid impurities. The filtrate was collected and concentrated in vacuo and then purified by silica gel column chromatography to give 5 as a brown solid (860 mg, 11%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.40 (s, 1H), 7.32 (dd, $J = 15.8, 8.1$ Hz, 4H), 7.25 – 7.17 (m, 1H), 6.76 (s, 2H), 6.66 (d, $J = 8.8$ Hz, 2H), 4.62 (s, 4H), 2.89 (s, 13H), 2.89 (s, 12H), 0.82 (s, 18H), -0.00 (s, 12H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 154.19, 148.98, 136.96, 134.43, 133.81, 128.74, 127.33, 126.91, 125.61, 111.42, 111.21, 62.86, 40.67, 26.05, 18.43 (s), -5.10. HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{37}$H$_{56}$N$_4$O$_3$SSi$_2$: 693.3684, found 693.3680.

To the solution of 6 (800 mg, 1.15 mmol) in 5 mL of MeOH was added p-toluene sulfonic acid (160 mg, 0.92 mmol). The mixture was stirred at room temperature for 30 min. When most of MeOH was removed in vacuo, 30 mL of saturated NaHCO$_3$ solution was added to the residue, white solid was precipitated out and was purified by silica gel column chromatography to give 7 as a light blue solid (380 mg, 56.9%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.65 – 7.49 (m, 3H), 7.45 (s, 1H), 7.29 (d, $J = 4.1$ Hz, 2H), 6.89 – 6.64 (m, 4H), 4.77 (s, 2H), 4.67 (s, 2H), 4.31 (s, 1H), 3.06 (s, 12H), 0.88 (s, 9H), 0.03 (s, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 156.27, 149.03, 137.54, 135.71, 135.33, 134.44, 133.81, 128.74, 127.33, 126.91, 125.61, 111.42, 111.21, 62.86, 40.67, 26.05, 18.43 (s), -5.10. HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{31}$H$_{42}$N$_4$O$_3$: 579.2820, found 579.2828.

To a 50 mL round bottom flask was added 7 (300 mg, 0.52 mmol), Et$_3$N (0.22 mL, 1.55 mmol), DMAP (63 mg, 0.52 mmol) and DCM (5 mL). P-nitrophenyl chloroformate (260 mg, 0.62 mmol) dissolved in DCM (5 mL) was added dropwise at 0 $^\circ$C, and then the reaction mixture was stirred at room temperature for 2 h. TLC showed the completely consumption of 7. The reaction mixture was then washed with 1 N HCl solution (10 mL), dried over sodium sulfate, filtered and concentrated to give the intermediate which was used directly in next step. To the intermediate with DCM (5 mL) and Et$_3$N (0.22 mL, 1.55 mmol), 8 (650 mg, 0.52 mmol) dissolved in DCM (5 mL) was added dropwise at 0 $^\circ$C, the mixture was then restored naturally.
to room temperature and stirred for 3 h. H$_2$O (10 mL) was added to the reaction solution. The organic layer was concentrated in vacuo, the crude product was purified by silica gel column chromatography to give 9 as a light blue solid (500 mg, 55%).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.88 (s, 2H), 7.53 (s, 2H), 7.33 (d, $J$ = 7.5 Hz, 3H), 7.20 (t, $J$ = 7.6 Hz, 1H), 7.08 (d, $J$ = 8.8 Hz, 1H), 6.71 (s, 3H), 5.91 (d, $J$ = 9.1 Hz, 2H), 5.63 – 5.59 (m, 4H), 5.41 – 5.14 (m, 5H), 4.77 – 4.58 (m, 6H), 4.36 (s, 1H), 4.30 – 4.13 (m, 6H), 4.02 (s, 2H), 3.76 – 3.51 (m, 18H), 3.37 (s, 2H), 2.98 (s, 12H), 2.63 (d, $J$ = 1.0 Hz, 1H), 2.24 (s, 6H), 2.05 (d, $J$ = 12.4 Hz, 12H), 1.86 (d, $J$ = 12.2 Hz, 6H), 0.86 (s, 9H), 0.02 (s, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.32, 170.03, 169.85, 168.97, 156.36, 154.18, 145.38, 145.31, 137.49, 134.07, 127.83, 127.63, 126.12, 121.56, 86.13, 86.09, 73.95, 70.93, 70.84, 70.56, 70.35, 70.07, 68.76, 67.95, 66.90, 64.37, 63.75, 62.76, 61.13, 47.89, 40.92, 26.02, 20.65, 20.51, 20.19, -5.19. HRMS (ESI): m/z [M + Na]$^+$ calcd for C$_{79}$H$_{110}$N$_{12}$O$_{29}$Si: 1773.6884, found 1773.6882.

![Diagram of molecule 9]

To the solution of 9 (500 mg, 0.29 mmol) in 5 mL of MeOH was added p-toluene sulfonic acid (50 mg, 0.29 mmol), the mixture was stirred at room temperature for 1 h. After the completion of the reaction, MeOH was removed in vacuo. The residual was purified by silica gel column chromatography to give 10 as a light blue solid (400 mg, 86%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.87 (s, 2H), 7.74 – 7.30 (m, 5H), 7.23 (s, 1H), 7.07 (d, $J$ = 8.2 Hz, 1H), 6.78 (d, $J$ = 33.3 Hz, 3H), 5.90 (d, $J$ = 9.0 Hz, 2H), 5.67 – 5.46 (m, 4H), 5.28 (s, 3H), 5.00 (s, 2H), 4.72 – 4.55 (m, 6H), 4.37 – 4.10 (m, 7H), 3.97 (s, 2H), 3.68 – 3.45 (m, 18H), 3.25 (s, 2H), 2.98 (s, 12H), 2.24 (s, 6H), 2.04 (d, $J$ = 11.7 Hz, 12H), 1.86 (d, $J$ = 11.0 Hz, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.33, 170.03, 169.82, 168.95, 156.09, 145.28, 135.23, 132.36, 131.18, 129.90, 127.70, 126.75, 121.55, 86.14, 73.95, 70.83, 70.54, 70.49, 70.34, 70.25, 69.92, 68.71, 67.96, 66.89, 64.35, 64.25, 62.54, 61.13, 47.89, 40.89, 20.66, 20.51, 20.22. HRMS (ESI): m/z [M + Na]$^+$ calcd for C$_{73}$H$_{90}$N$_{12}$O$_{29}$S: 1659.6019, found 1659.6019.

![Diagram of molecule 10]

The synthetic method of 12 is similar to that of 9. 12 was produced as a red solid (280 mg, 46%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 13.97 (s, 1H), 13.26 (s, 1H), 8.04 (d, $J$ = 7.6 Hz, 1H), 7.91 – 7.71 (m, 3H), 7.52 – 7.29 (m, 4H), 7.24 – 7.00 (m, 4H), 6.70 (s, 3H), 5.89 (d, $J$ = 7.7 Hz, 2H), 5.64 – 5.38 (m, 6H), 5.28 (s, 3H), 5.17 – 4.86 (m, 5H), 4.74 (s, 1H), 4.66 – 4.57 (m, 4H), 4.35 – 3.93 (m, 13H), 3.78 – 3.44 (m, 21H), 3.27 (d, $J$ = 19.0 Hz, 4H), 3.03 (d, $J$ = 18.7 Hz, 2H), 2.93 (s, 12H), 2.39 – 2.14 (m, 8H), 2.02 (d, $J$ = 11.1 Hz, 13H), 1.84 (d, $J$ = 7.1 Hz, 7H), 1.25 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 213.97, 187.13, 186.75, 170.35, 170.06, 169.90, 169.01, 161.09, 156.27, 155.74, 155.17, 145.27, 140.79, 135.75, 135.57, 134.58, 133.81, 133.68, 128.98, 127.71, 126.61, 121.60, 120.98, 119.85, 118.48, 114.42, 111.59, 111.41, 100.88, 86.10, 73.93, 70.83, 70.53, 70.47, 70.27, 69.97, 69.47, 68.77, 67.96, 67.48, 66.91, 65.55, 64.32, 63.46, 61.13, 56.71, 47.93, 47.05, 40.89, 35.67, 33.99, 29.86, 20.65, 20.51, 20.20, 16.94. HRMS (ESI): m/z [M + Na]$^+$ calcd for C$_{106}$H$_{122}$N$_{13}$O$_{41}$S: 2206.7733, found 2206.7733.
12 (280 mg, 0.13 mmol) was dissolved in 5 mL of MeOH. When pH value of the mixture was adjusted to 8-9 by MeONa, the mixture was stirred at room temperature for 2 h. After the completion of reaction, cation exchange resin was added to adjusted pH = 7, the crude product was purified by recrystallization in MeOH to give Gal-MB-DOX as a light red solid (200 mg, 84%).

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 13.30 (s, 2H), 8.22 (s, 2H), 7.92 (s, 2H), 7.78 (s, 1H), 7.66 (s, 1H), 7.53 (s, 1H), 7.29 (d, $J = 25.7$ Hz, 5H), 6.89 (s, 1H), 6.69 (s, 1H), 6.69 (s, 1H), 5.77 (s, 2H), 5.48 (s, 3H), 5.25 (s, 4H), 4.99 (d, $J = 48.9$ Hz, 7H), 4.79 – 4.46 (m, 9H), 4.23 – 3.96 (m, 8H), 3.88 (s, 2H), 3.74 (d, $J = 22.1$ Hz, 4H), 3.51 (s, 20H), 3.14 (d, $J = 33.3$ Hz, 4H), 2.87 (s, 1H), 2.17 (d, $J = 20.4$ Hz, 1H), 1.99 (s, 1H), 1.86 (s, 1H), 1.51 (s, 1H), 1.14 (s, 3H).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 214.21, 186.85, 170.76, 169.60, 161.25, 156.59, 155.67, 154.35, 149.23, 144.25, 136.63, 135.19, 135.00, 134.40, 128.57, 127.87, 126.68, 123.32, 120.48, 120.15, 119.47, 111.68, 111.27, 111.10, 110.87, 100.73, 88.56, 88.21, 78.87, 77.30, 75.46, 74.17, 73.73, 73.59, 70.64, 70.22, 69.99, 69.81, 69.51, 68.92, 68.40, 67.12, 64.09, 62.50, 60.90, 57.05, 48.37, 47.65, 37.17, 32.60, 30.30, 21.12, 17.49. HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{85}$H$_{108}$N$_{13}$O$_{33}$S: 1870.6888, found 1870.6888.

**Scheme S2.** Synthetic route of the intermediate 8

Reagents and conditions: (a) (Boc)$_2$O, MeOH, 0 °C to r.t., 12 h, 94%; (b) propargyl bromide, KOH, THF, 0 °C to r.t., 12 h, 75.1%; (c) 1) trifluoroacetic acid, DCM, r.t., 12 h, 2) Et$_3$N, chloroacetyl chloride, 0 °C to r.t., 2 h, 62%; (d) compound 8-4, KOH, DMF, 0 °C to r.t., 12 h, 58.4%; (e) β-D-galactopyranosyl azide, sodium ascorbate, CuSO$_4$·5H$_2$O, THF/H$_2$O, r.t., 2 h, 85.3%; (f) trifluoroacetic acid, DCM, r.t., 6 h, 99%.
To the mixture of aminopropanediol (5 g, 54.9 mmol) in 80 mL of MeOH was dropwise added (Boc)₂O (14.4 g, 65.9 mmol) at 0 °C. The reaction was restored naturally to room temperature and stirred for 12 h. TLC suggest the completion of the reaction, the solvent was removed in vacuo and the residue was purified by recrystallization in ethyl acetate to give 8-1 as a white solid (9.85 g, 94%).

The mixture of 8-1 (5 g, 26.2 mmol) and propargyl bromide (6.2 mL, 78.4 mmol) in 30 mL of THF was stirred 30 min at 0 °C, to this solution was partially added KOH (7.33 g, 130.7 mmol) over a period of 1 h. After that, the reaction was restored naturally to room temperature and stirred for 12 h. TLC suggested the completion of the reaction, H₂O (100 mL) was added to the reaction solution, extracted with EA (50 mL x 3). The combined organic layers were dried with Na₂SO₄ and concentrated in vacuo, the crude product was purified by silica gel column chromatography to give 8-2 as a brown oil (5.3 g, 75.1%). ¹H NMR (400 MHz, CDCl₃) δ 4.92 (s, 1H), 4.18 (d, J = 2.4 Hz, 4H), 3.94 (s, 1H), 3.69 – 3.51 (m, 4H), 2.45 (t, J = 2.4 Hz, 2H), 1.46 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 155.50, 79.49, 74.62, 68.57, 58.46, 49.45, 28.36.

To the solution of 8-2 (5.3 g, 19.83 mmol) in 24 mL of DCM was dropwise added 6 mL of trifluoroacetic acid. The mixture was stirred at room temperature for 12 h. TLC suggested the completion of the reaction, then most of the solvent was removed in vacuo, 30 mL of saturated NaHCO₃ solution was added to the residue, extracted with EA (50 mL x 3). The combined organic layers were dried with Na₂SO₄ and concentrated in vacuo to give the intermediate. To the intermediate was added 15 mL of DCM, then Et₃N (8.3 mL, 59.48 mmol) and chloroacetyl chloride (1.89 mL, 23.79 mmol) dissolved in 5 mL of DCM was added dropwise. After that, the mixture was stirred at r.t. for 2 h, TLC suggested the completion of the reaction. The mixture was washed with 1 N HCl (10 mL), saturated NaHCO₃ solution (10 mL) and saturated NaCl solution (10 mL) respectively. The organic layer was collected and concentrated to give the crude product, which was further purified by silica gel column chromatography to give 8-3 as a brown oil (3 g, 62%). ¹H NMR (400 MHz, CDCl₃) δ 6.93 (s, 1H), 4.32 – 4.26 (m, 1H), 4.19 (s, 4H), 4.06 (s, 2H), 3.73 – 3.59 (m, 4H), 2.47 (s, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 165.87, 79.27, 74.91, 67.92, 58.50, 48.76, 42.60.

The mixture of 8-3 (1 g, 4.1 mmol) and 8-4 (1.2 g, 4.1 mmol) in 5 mL of DMF was stirred 30 min at 0 °C. To this solution was partially added KOH (345 mg, 6.2 mmol) over a period of 1 h. After that, the reaction was restored naturally to room temperature and stirred for 12 h. TLC suggested the completion of the reaction. H₂O (40 mL) was added to the reaction solution, extracted with EA (10 mL x 3). The combined organic layers were washed with brine (10 mL x 3), dried with Na₂SO₄ and
concentrated in vacuo, the crude product was purified by silica gel column chromatography to give 8-5 as a light-yellow oil (1.2 g, 58.4%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.25 (s, 1H), 4.24 (s, 2H), 4.18 (d, $J = 2.1$ Hz, 4H), 3.99 (s, 1H), 3.74 – 3.54 (m, 16H), 3.33 (t, $J = 4.8$ Hz, 2H), 2.47 (s, 2H), 1.46 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 169.77, 156.01, 79.47, 74.77, 70.97, 70.63, 70.54, 70.36, 70.27, 68.38, 58.42, 47.74, 40.37, 28.43. HRMS (ESI): m/z [M + Na]$^+$ calcd for C$_{24}$H$_{40}$N$_2$O$_9$: 523.2626, found 523.2636.

To the solution of 8-5 (400 mg, 0.8 mmol) and $\beta$-D-galactopyranosyl azide (360 mg, 1.76 mmol) in a mixture of THF (8 mL) and H$_2$O (2 mL) was added sodium ascorbate (80 mg, 0.32 mmol) and CuSO$_4$•5H$_2$O (32 mg, 0.16 mmol). The mixture was stirred at room temperature under N$_2$ atmosphere for 2 h. TLC suggested the completion of the reaction, the solvent was then removed in vacuo and purified by silica gel column chromatography to give 8-6 as an off-white spumy solid (850 mg, 85.3%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.89 (s, 2H), 7.09 (d, $J = 8.9$ Hz, 1H), 5.92 (d, $J = 9.3$ Hz, 2H), 5.59 (dd, $J = 16.6$, 6.5 Hz, 4H), 5.32 – 5.26 (m, 2H), 4.76 – 4.61 (m, 4H), 4.41 – 4.33 (m, 1H), 4.32 – 4.14 (m, 6H), 4.03 (s, 2H), 3.73 – 3.53 (m, 18H), 3.31 (t, $J = 5.1$ Hz, 2H), 2.24 (s, 6H), 2.09 – 2.00 (m, 12H), 1.86 (d, $J = 12.5$ Hz, 6H), 1.45 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.32, 170.04, 169.83, 169.78, 168.97, 168.93, 155.98, 145.39, 145.31, 121.54, 121.49, 86.17, 86.13, 73.97, 70.96, 70.83, 70.61, 70.57, 70.52, 70.35, 70.23, 68.75, 68.70, 67.94, 66.87, 64.37, 61.12, 47.87, 40.51, 28.44, 20.68, 20.52, 20.23, 20.19. HRMS (ESI): m/z [M + Na]$^+$ calcd for C$_{52}$H$_{78}$N$_8$O$_{27}$: 1269.4869, found 1269.4900.

To the solution of 8-6 (800 mg, 0.64 mmol) in 12 mL of DCM was dropwise added 3 mL of trifluoroacetic acid. The mixture was stirred at room temperature for 6 h. TLC suggested the completion of the reaction, most of solvent was then removed in vacuo, 30 mL of saturated NaHCO$_3$ solution was added to the residue, extracted with DCM (50 mL x 3). The combined organic layers were dried with Na$_2$SO$_4$ and concentrated in vacuo to give 8 as an off-white spumy solid which was used in next step directly without further purification (800 mg, 99%).
To the solution of 8-5 (400 mg, 0.8 mmol) in 12 mL of DCM was dropwise added 3 mL of trifluoroacetic acid. The mixture was stirred at room temperature for 3 h. TLC suggested the completion of the reaction, most of solvent was then removed in vacuo, 30 mL of saturated NaHCO$_3$ solution was added to the residue, extracted with EA (50 mL x 3). The combined organic layers were dried with Na$_2$SO$_4$ and concentrated in vacuo to give amino intermediate 13 as a brown oil which was used directly in next step without further purification. To a 50 mL round bottom flask was added 7 (290 mg, 0.5 mmol), Et$_3$N (0.21 mL, 1.5 mmol), DMAP (61 mg, 0.5 mmol) and DCM (5 mL). P-nitrophenyl chloroformate (121 mg, 0.6 mmol) dissolved in DCM (5 mL) was added dropwise at 0 $^\circ$C, and then the reaction mixture was stirred at room temperature for 2 h. TLC showed the completely consumption of 7. The reaction mixture was washed with 1 N HCl solution (10 mL), dried over sodium sulfate, filtered and concentrated to give the intermediate 14 which was used directly in next step. To the intermediate 14 was added DCM (5 mL) and Et$_3$N (0.21 mL, 1.5 mmol). Amino intermediate 13 (200 mg, 0.5 mmol) dissolved in DCM (5 mL) was added dropwise at 0 $^\circ$C, the mixture was then restored naturally to room temperature and stirred for 3 h, TLC suggested the completion of reaction. H$_2$O (10 mL) was added to the reaction solution. The organic layer was concentrated in vacuo, the crude product was purified by silica gel column chromatography to give 15 as a light blue solid (260 mg, 52%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.50 (d, J = 8.7 Hz, 2H), 7.34 (t, J = 6.4 Hz, 2H), 7.20 (t, J = 7.2 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 6.75 (s, 2H), 6.66 (d, J = 8.2 Hz, 2H), 5.30 (s, 1H), 5.21 (s, 2H), 4.66 (s, 2H), 4.40 – 4.28 (m, 1H), 4.18 (s, 4H), 4.02 (s, 2H), 3.82 – 3.47 (m, 18H), 3.37 (s, 2H), 2.96 (s, 12H), 2.48 (s, 2H), 0.87 (s, 9H), 0.03 (s, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 169.74, 156.32, 154.35, 154.35, 149.04, 137.55, 134.48, 134.16, 133.30, 128.58, 127.84, 127.62, 127.48, 126.03, 111.35, 111.00, 79.50, 74.80, 70.97, 70.63, 70.54, 70.36, 70.32, 70.09, 68.38, 63.88, 62.70, 58.42, 47.73, 40.93, 40.69, 26.04, 18.40, -5.18. HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{45}$H$_{58}$N$_6$O$_{11}$S: 1005.4822, found 1005.4815.
To the solution of 15 (260 mg, 0.26 mmol) in 5 mL of MeOH was added p-toluene sulfonic acid (45 mg, 0.26 mmol), the mixture was stirred at room temperature for 1 h. TLC suggested the completion of reaction, MeOH was removed in vacuo, the residual was purified by silica gel column chromatography to give 16 as a light blue solid (200 mg, 87%).

\[ ^1H \text{NMR (} 400 \text{ MHz, CDCl}_3 \text{)} \delta 7.60 (s, 1H), 7.50 – 7.40 (m, 3H), 7.28 (d, J = 7.4 Hz, 1H), 7.20 (t, J = 7.4 Hz, 1H), 7.01 (d, J = 8.3 Hz, 1H), 6.76 (s, 2H), 6.66 (d, J = 7.6 Hz, 2H), 5.09 (s, 1H), 4.97 (s, 2H), 4.60 (s, 2H), 4.37 – 4.23 (m, 1H), 4.15 (d, J = 1.6 Hz, 4H), 3.95 (s, 2H), 3.79 – 3.33 (m, 18H), 3.23 (d, J = 4.5 Hz, 2H), 2.94 (s, 12H), 2.45 (s, 2H). \]

\[ ^{13}C \text{NMR (} 100 \text{ MHz, CDCl}_3 \text{)} \delta 169.75, 156.40, 156.02, 149.13, 138.09, 135.75, 134.75, 132.21, 131.32, 129.91, 128.34, 127.58, 126.69, 111.34, 111.10, 79.49, 74.80, 70.91, 70.60, 70.51, 70.36, 70.27, 69.95, 68.36, 64.36, 62.54, 58.42, 47.72, 40.88, 40.72. \]

HRMS (ESI): m/z [M + H]^+ calcd for C_{45}H_{58}N_6O_{11}S: 891.3957, found 891.3965.

The synthetic method of MB-DOX is similar to that of 9. MB-DOX was produced as a red solid (140 mg, 43%).

\[ ^1H \text{NMR (} 400 \text{ MHz, DMSO-d}_6 \text{)} \delta 13.29 (s, 1H), 7.92 (d, J = 3.5 Hz, 2H), 7.78 (s, 1H), 7.66 (s, 1H), 7.47 (d, J = 9.3 Hz, 1H), 7.34 – 7.21 (m, 5H), 6.87 (s, 1H), 6.73 (s, 2H), 6.64 (d, J = 8.3 Hz, 2H), 5.48 (s, 1H), 5.23 (s, 1H), 4.92 (s, 5H), 4.73 (s, 1H), 4.57 (s, 2H), 4.13 (s, 6H), 3.98 (s, 3H), 3.88 (s, 2H), 3.69 (s, 1H), 3.50 (dd, J = 27.2, 13.8 Hz, 19H), 3.08 (s, 2H), 2.98 (s, 2H), 2.86 (s, 12H), 2.16 (d, J = 16.2 Hz, 2H), 1.85 (s, 1H), 1.50 (s, 1H), 1.12 (s, 3H). \]

\[ ^{13}C \text{NMR (} 100 \text{ MHz, CDCl}_3 \text{)} \delta 213.97, 186.99, 186.64, 169.93, 161.03, 156.34, 156.23, 155.64, 155.30, 154.97, 149.13, 135.73, 135.47, 134.58, 133.83, 133.66, 129.45, 128.97, 128.43, 127.61, 126.68, 120.87, 119.79, 118.47, 111.62, 111.52, 111.33, 111.21, 100.86, 79.49, 74.86, 70.89, 70.57, 70.47, 70.29, 70.03, 69.39, 68.87, 68.39, 67.54, 65.52, 64.25, 63.51, 58.41, 56.67, 47.80, 47.15, 40.81, 35.64, 33.92, 29.78, 16.91. \]

HRMS (ESI): m/z [M + H]^+ calcd for C_{73}H_{83}N_7O_{23}SN: 1460.5490, found 1460.5437.
3. Supplementary Table

Table S1. Some recently published prodrugs for orthotopic HCC based on different response substances.a.

| Agents | Formulation | Targeted strategy | Activator | Concentration of activator | Response time | Biological application | References |
|--------|-------------|-------------------|-----------|---------------------------|---------------|------------------------|------------|
| DOX@Gal-micelles/siRNA | Physically encapsulated by polymer | Galactose | Acidic pH | pH = 5 | 11 h (About 83% drug release) | Chemo- and gene therapy for subcutaneous and orthotopic HCC mice | 5 |
| PRS@SF | Porous RNA nanospheres | Aptamer of EpCAM, Cytoplasmic Dicer enzymes | -- | -- | Chemo- and gene therapy for subcutaneous and orthotopic HCC mice | 6 |
| Trojan Horse-like NBs | Nanobowls | EPR effect | Acidic pH | pH = 5.3 | > 60 h | Chemotherapy for orthotopic HCC mice | 7 |
| Tf-Ps-Dox | Physically encapsulated by polymer | Transferrin | GSH | 2–10 mM | -- | Chemotherapy for orthotopic HCC mice | 8 |
| GE11-CPs | Physically encapsulated by polymer | GE11 peptides | GSH | 10 mM | -- | Chemotherapy for orthotopic HCC mice | 9 |
| ZnPc/SFB@BSA | Bovine serum albumin (BSA) nanocapsules | -- | Trypsin | 1 mg mL⁻¹ | 90.1% of drug release after 6 d | Chemotherapy for orthotopic HCC mice | 10 |
| PLGA-PEG-AEAA-DOX/ICT | Physically encapsulated by polymer | EPR effect | Acidic pH | pH = 5.5 | > 80% drug release at 24 h | Chemotherapy and immunotherapy for orthotopic HCC mice | 11 |
| SP94-PB-SF-Cy5.5 | Prussian blue (PB) nanoparticle | HCC-targeting peptide SP94 | Temperature | 42 °C | ~74% of drug release at 24 h | Multimodal therapy for subcutaneous and orthotopic HCC mice | 12 |
| LBP/pCas9 | Lactose-derived branched cationic biopolymer | Lactose | Dithiothreitol | 10 mM | 24 h | Genome editing to treat orthotopic HCC mice | 13 |
| DOX@Gal-PCN-224 | Glyco-MOF nanostructures | Galactose | Acidic pH | pH = 5.6 | 65% of drug release at 2 h | Interventional photodynamic and chemotherapy for orthotopic HCC mice. | 14 |
| Nano-FdUMP and Nano-Folox | Lipid nanoparticle | Aminoethyl Anisamide (AEAA) | Acidic pH | pH = 5.5 | > 95% of drug release at 24 h | Chemo-immunotherapy for orthotopic HCC mice. | 15 |
| NGR@DDP | Bottlebrush-Like Nano-Riceball | CD13-targeted | Ultrasound and H₂O₂ | 0.1 mM H₂O₂ and ultrasound (5 min) | ≈83.5% of drug release at 60 h | Sono-chemotherapy for orthotopic HCC mice. | 16 |
| Gal-MB-DOX | Monocomponent nanoformulation | Galactose, EPR effect | ROS | 10 μM | < 30 s | Chemotherapy for orthotopic HCC mice | 17 |

a If no actual data were provided in the references, data were estimated according to the original published figures.
4. Supplementary Figures

**Fig. S1.** Absorption spectra of (A) **Gal-MB-DOX** (5 μM) and (B) **MB-DOX** (5 μM) before/after addition of HOCl (5 μM). The data was recorded in PB (10 mM, 0.5% DMF).

**Fig. S2.** (A) Fluorescent spectra of **MB-DOX** (5μM) before/after addition of different concentration of HOCl (0 to 10 μM), \( \lambda_{ex} = 620 \) nm.
Fig. S3. (A) Pseudo-first-order kinetic plot of the reaction of 5 μM prodrugs to 10 μM HOCl. (B) Linear relationship of the fluorescence intensity at 686 nm of Gal-MB-DOX with the concentration of HOCl, the data was recorded after 20 min in PB (10 mM, pH 7.4, 0.5% DMF). (C) The observed rate constant and detection limit of the prodrugs.

| Prodrugs   | K (S⁻¹) | Detection limit/nM |
|------------|---------|--------------------|
| MB-DOX     | 0.0016  | -                  |
| Gal-MB-DOX | 0.06402 | 94.74              |

Scheme S4. The proposed elimination reaction between Gal-MB-DOX and ROS.
Fig. S4. Fluorescent spectra of DOX released from MB-DOX (5μM) (A) and Gal-MB-DOX (5μM) (B) after addition of different concentration of HOCl (0 to 10 μM), λ<sub>ex</sub> = 470 nm. The data was recorded in PB (10 mM, 0.5% DMF).

Fig. S5. DOX release analysis of Gal-MB-DOX after treatment of different concentration of HOCl. (a) The linear fit of the absorbance at 497 nm versus the concentration of free DOX from 5 to 80 µM; (b) absorption of the deionized water after dialysis of the prodrug or the mixture of prodrug and HOCl; (c) DOX release efficiency from Gal-MB-DOX at different concentrations of HOCl.
**Fig. S6.** Mass spectroscopic analysis of Gal-MB-DOX after addition of HOCl in PB solution for 30 min.

**Fig. S7.** Confirmation of the aggregates in the solution of Gal-MB-DOX (60 μM) after addition of HOCl (60 μM). The aggregate was separated by centrifugation, the precipitation was re-dispersed in DMF and the supernatant was directly tested by UV-vis spectra.
Fig. S8. Fluorescent spectra of Gal-MB-DOX (5 μM) before/after addition of different concentration of, HOCl (0 to 10 μM) (A), ONOO⁻ (0 to 10 μM) (B), •OH (0 to 10 μM) (C) and t-BuOO• (0 to 10 μM) (D). λ_{ex} = 620 nm. The data was recorded in PB (10 mM, 0.5% DMF).
Fig S9. Fluorescence intensity of Gal-MB-DOX (5 μM in PB, pH 7.4) at 686 nm after adding different ions (A) (from 1 to 17: Gal-MB-DOX, CH₃COO⁻, CO₃²⁻, SO₄²⁻, F⁻, Cl⁻, NO₂⁻, S₂O₃²⁻, ClO₄⁻, Cu²⁺, NH₄⁺, Na⁺, Mg²⁺, Al³⁺, K⁺, Ca²⁺, Fe³⁺ (100 μM); 18: HOCl (20 μM) and amino acids (B) (from 1 to 21: Gal-MB-DOX, Leu, Pro, Gly, Gln, Glu, Met, Lys, Trp, Ser, Thr, Asp, Ile, Val, His, Ala, Cys, Phe, Asn, Tyr, Arg (100 μM); 22: HOCl (20 μM), (λex = 620 nm).
Fig. S10. Stability test of the nanoparticles prepared by Gal-MB-DOX in PB (A, B, C), 10% FBS in aqueous solution (D, E, F) and DMEM (G, H, I). (A, D, G) Absorption changes of the nano dispersion within 48 h; (B, E, H) the normalized absorption changes at 664 nm from 0 to 48 h, and after addition of HOCl; (C, F, I) DLS of the original nano dispersion and after staying for 48 h.
Fig. S11. Flow cytometry analysis of cellular uptake of \textbf{Gal- MB-DOX} (10 μM) in RAW and HepG2 cells at different time intervals from 0 to 60 min. MB channel in (a) RAW cells and (b) HepG2 cells, (c) DOX channel in HepG2 cells.
**Fig. S12.** The cell viability of **MB-DOX** and **Gal-MB-DOX** for 24 h at different concentrations (0, 10, 20, 40, 60, 80, 100 and 120 µM) in HepG2 and RAW cells for 24 h. The toxicity of the compounds was measured by CCK-8 assay. Values are the mean ± s.d. for n = 6.

**Fig. S13.** The changes of fluorescence intensity of commercial caspase-3 probe (GreenNuc™ Caspase-3 Assay Kit for Live Cells) in HepG2 cells after treated with different concentrations of **Gal-MB-DOX** (1: 0 µM; 2: 0 µM + caspase-3 inhibitor; 3: 100 µM; 4: 150 µM; 5: 200 µM; 6: 200 µM + caspase-3 inhibitor) for 24 h. n = 3, data are presented as mean values ± SD. NS: no significance, *p < 0.05, **p < 0.01, ***p < 0.001 (λexc = 485 nm, λem = 515 nm).
Fig. S14. *Ex vivo* fluorescence imaging of the major organs. Balb/c mice with orthotopic hepatocellular carcinoma were intraperitoneal injected with saline, MB-DOX, (1 mM, 100 μL) and Gal-MB-DOX (1 mM, 100 μL) for 3 h. The organs of mice were resected and imaged after euthanized. The fluorescence intensity of different organs was compared using the *In Vivo* Xtreme imaging system. 1: Heart, 2: Liver, 3: Lung, 4: Spleen, 5: Kidney and 6: Muscle. Red circle in brightfield image indicated the tumor located on liver.
Fig. S15. Biosecurity evaluation of different treatments: (a) saline, (b) DOX, (c) MB-DOX and (d) Gal-MB-DOX NPs. H&E staining of the heart, spleen, lung, and kidney of orthotopic HCC mice after 15 days of different treatments. Scale bar = 2 mm.
Fig. S16. $^1$H NMR of 2 in DMSO-$d_6$

Fig. S17. $^{13}$C NMR of 2 in DMSO-$d_6$
**Fig. S18.** $^1$H NMR of 3 in DMSO-$d_6$

**Fig. S19.** $^{13}$C NMR of 3 in DMSO-$d_6$
Fig. S20. $^1$H NMR of 4 in DMSO-$d_6$

Fig. S21 $^{13}$C NMR of 4 in DMSO-$d_6$
**Fig. S22.** $^1$H NMR of 5 in DMSO-$d_6$

**Fig. S23.** $^{13}$C NMR of 5 in DMSO-$d_6$
Fig. S24. $^1$H NMR of 6 in CDCl$_3$

Fig. S25. $^{13}$C NMR of 6 in CDCl$_3$
Fig. S26. HR MS of 6

Fig. S27. $^1$H NMR of 7 in CDCl$_3$
Fig. S28. $^{13}$C NMR of 7 in CDCl$_3$

Fig. S29. HR MS of 7
Fig. S30. $^1$H NMR of 9 in CDCl$_3$

Fig. S31. $^{13}$C NMR of 9 in CDCl$_3$
Fig. S32. HR MS of 9

Fig. S33. $^1$H NMR of 10 in CDCl$_3$
Fig. S34. $^{13}$C NMR of 10 in CDCl$_3$

Fig. S35. HR MS of 10
**Fig. S36.** $^1$H NMR of 12 in CDCl$_3$

**Fig. S37.** $^{13}$C NMR of 12 in CDCl$_3$
Fig. S38. HR MS of 12

Fig. S39. $^1$H NMR of Gal-MB-DOX in DMSO-$d_6$
Fig. S40. $^{13}$C NMR of Gal-MB-DOX in DMSO-$d_6$

Fig. S41. HR MS of Gal-MB-DOX
Fig. S42. $^1$H NMR of 8-2 in CDCl$_3$.

Fig. S43. $^{13}$C NMR of 8-2 in CDCl$_3$.
Fig. S44. $^1$H NMR of 8-3 in CDCl$_3$

Fig. S45. $^{13}$C NMR of 8-3 in CDCl$_3$
Fig. S46. $^1$H NMR of 8-5 in CDCl$_3$

Fig. S47. $^{13}$C NMR of 8-5 in CDCl$_3$
Fig. S48. HR MS of 8-5

Fig. S49. $^1$H NMR of 8-6 in CDCl$_3$
Fig. S50. $^{13}$C NMR of 8-6 in CDCl$_3$

Fig. S51. HR MS of 8-6
Fig. S52. $^1$H NMR of 15 in CDCl$_3$

Fig. S53. $^{13}$C NMR of 15 in CDCl$_3$
Fig. S54. HR MS of 15

Fig. S55. $^1$H NMR of 16 in CDCl$_3$
Fig. S56. $^{13}$C NMR of 16 in CDCl$_3$

Fig. S57. HR MS of 16
Fig. S58. $^1$H NMR of MB-DOX in CDCl$_3$. 

Fig. S59. $^{13}$C NMR of MB-DOX in CDCl$_3$. 
Fig. S60. HR MS of MB-DOX

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