Support Information

pH and Singlet Oxygen Dual-Responsive GEM Prodrug micelles for Efficient Combination Therapy of Chemotherapy and Photodynamic

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Materials and Methods

2-Mercaptoethanol was purchased from Chengdu Best Reagent Co., Ltd. (Chengdu, China), 1, 2-Dichloroethylene, triethylamine, 4-cyano-4 ((phenylcarbonthioy)thio) pentanoic acid(CAT), gemcitabine hydrochloride (GEM · HCl), Chlorin e6 (Ce6), acryloyl chloride, 4-nitrophenyl carbonochloridate, Azodiisobutyronitrile (AIBN), methoxypolyethylene glycol monomethacrylate (mPEG-MA, Mw = 300), N-(2-hydroxyethyl) hexamethyleneimine and DCFH-DA were purchased from Adamas Reagent, Ltd. (Shanghai, China). CCK8 Kit, Annexin V-FITC/PI Apoptosis Detection Kit (Dojindo) and SOSG reagent were obtained from Sigma-Aldrich.

Synthesis of compound 1

Compound 1 was synthesized according to previous work.[1] Typically, NaOH (3.07 g; 78.78 mmol) was added to 30 mL of ethanol containing 2-mercaptoethanol (6.00 g; 76.78 mmol) at 0 °C and the mixed solution was stirred at 0 °C for 30 min. Then 2 mL ethanol containing cis-1, 2-dichloroethylene (1.86 g; 19.19 mmol) was added into above mixed solution dropwise, the resulting solution was heated at 80 °C for 18 h. Then, the resulting solution was diluted with water and washed with diethyl ether. We get the concentrated crude product from the combined organic layer which

were washed with water again and dried over anhydrous $MgSO_4$. At last, compound-1 was purified by column chromatography on silica gel as colourless liquid, eluting with a mixture of ethyl acetate and petroleum ether (6 : 4) (yield: 70%).

Synthesis of compound 2

A mixture of triethylamine (2.94 mL; 21.15 mmol) and Compound 1 (2.25 g) in dichloromethane was placed in an ice bath. After the dropwise addition of acryloyl chloride (1.24 mL; 13.19 mmol) in ice bath, the resulting solution was stirred at room temperature for 24 h in the dark. Compound 2 was purified by silica gel column chromatography as colourless liquid, eluting with a mixture of ethyl acetate and petroleum ether (1 : 4) (yield: 46%). HRMS (ESI+): calcd for $C_9H_{14}O_3S_2$ $[M+Na]^+$ 257.0282, found 257.0280.

Synthesis of compound 3

The solution of dichloromethane containing the compound 2 (0.7 g; 8.24 mmol) and triethylamine (0.76 mL; 5.46 mmol) was placed in an ice bath, and a solution of dichloromethane containing p-nitrophenyl chloroformate (0.91 g; 4.51 mmol) was added dropwise, then the mixture was stirred in the dark at room temperature for 24 h. After the reaction was completed, the resulting solution was further extracted with saturated sodium hydrogen carbonate and saturated brine solution for three times, respectively. The obtained organic layer was dried with anhydrous $MgSO_4$. At last, compound 3 was purified by gel column chromatography as faint yellow liquid, eluting with ethyl acetate: petroleum ether (1:4) (yield: 43%). HRMS (ESI+): calcd for $C_{16}H_{17}O_7S_2$ $[M+Na]^+$ 422.0344, found 422.0340.

Synthesis of mPEG

A solution of CAT (0.217 g; 0.78 mmol), mPEGMA (M_w = 300) (7 g; 23.33 mmol) and AIBN (0.051 g; 0.31 mmol) were dissolved in 12 mL tetrahydrofuran (THF) in a schlenk tube, after the solution was degassed by three times of freeze-evacuate-thaw cycles，the mixed solution was stirred under Ar atmosphere at 70 °C for 24 h, followed by being dialyzed against deionized water and

lyophilized to obtain the final product mPEG (faint red, yield: 71%).

Synthesis of P (AEMA-*co*-$^1O_2$)-PEG

A solution of compound 3 (0.37 g; 0.92 mmol), AEMA (0.281 g; 1.33 mmol)，mPEG (1 g; 2.45 mmol) and AIBN (7.3 mg; 44.51 mmol) were dissolved in 5 mL THF in a schlenk tube, after the solution was degassed by three times of freeze-evacuate-thaw cycles，the mixed solution was stirred under Ar atmosphere at 70 °C for 24 h. Afterwards, the resulted solution was dialyzed against deionized water and lyophilized to obtain P (AEMA-*co*-$^1O_2$)-PEG (faint red, yield: 68%).

Synthesis of P (AEMA-*co*-$^1O_2$ -GEM)-PEG

The P (AEMA-*co*-$^1O_2$)-PEG (0.79 g; 0.06 mmol) and gemcitabine hydrochloride (GEM·HCl) (0.11 g; 0.37 mmol) were dissolved in 20 mL DMSO under argon and then triethylamine (0.76 mL; 0.54 mmol; 0.0546 g) was added. The resulted solution was stirred at room temperature for 48 h in the dark and then dialyzed against deionized water for 24 h. The product was obtained by lyophilization (faint red, yield: 72%).

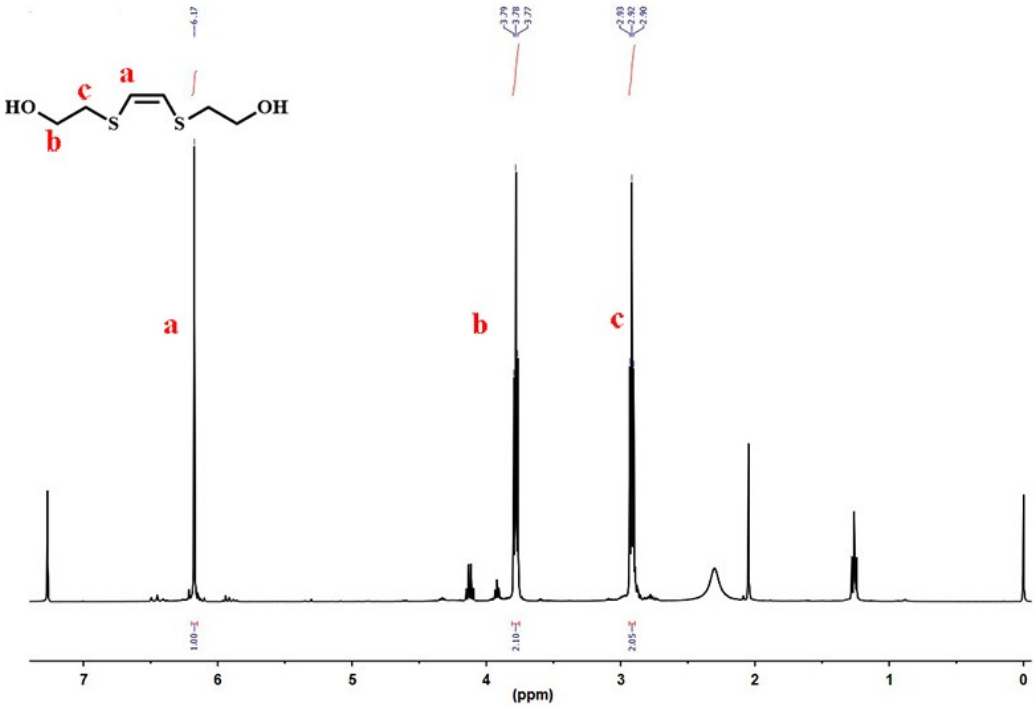

Figure S1. ^1H NMR spectrum of **compound 1** in $CDCl_3$

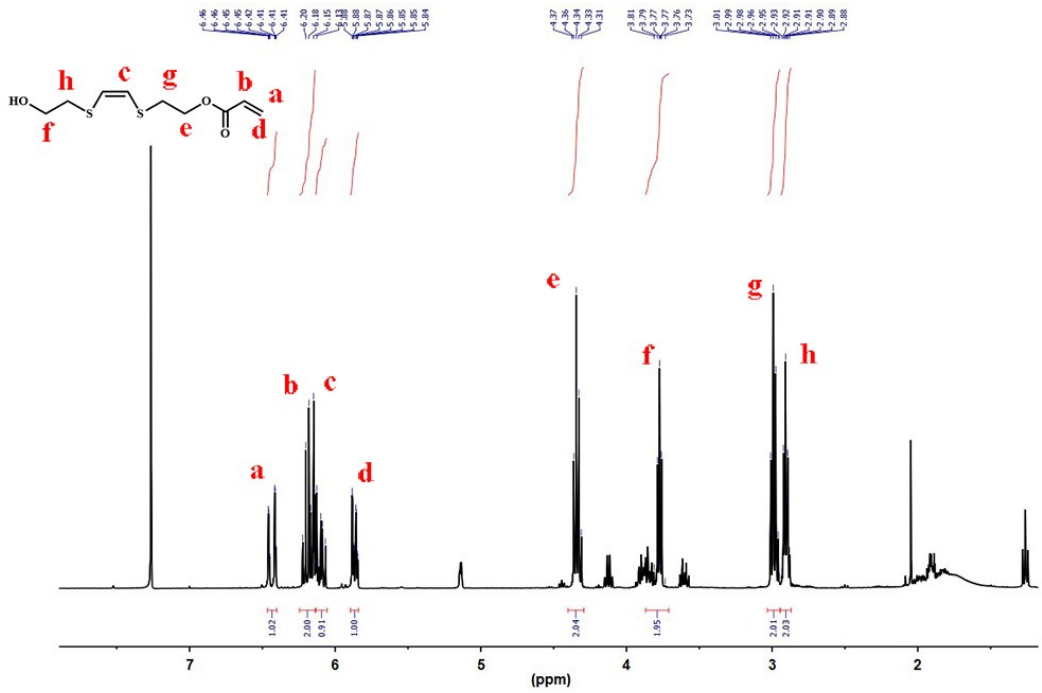

Figure S2. ^{1}H NMR spectrum of **compound 2** in $CDCl_3$

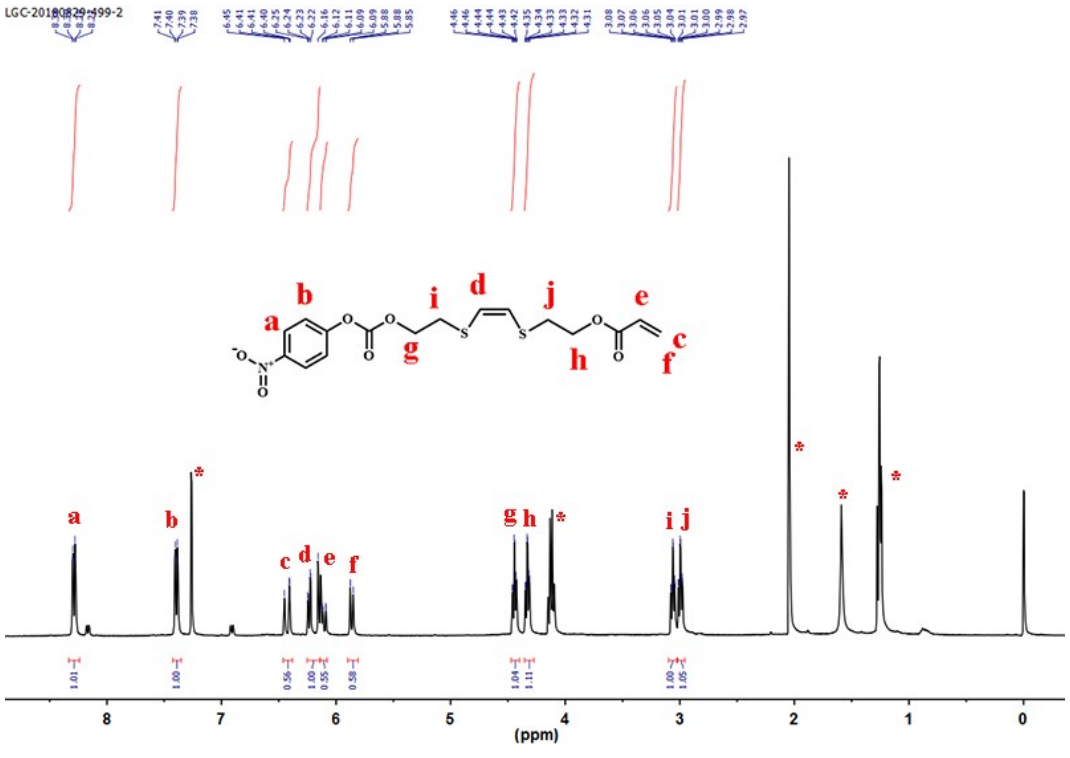

Figure S3. ^{1}H NMR spectrum of **compound 3 -$^{1}O_2$(reactive oxygen species)** in $CDCl_3$

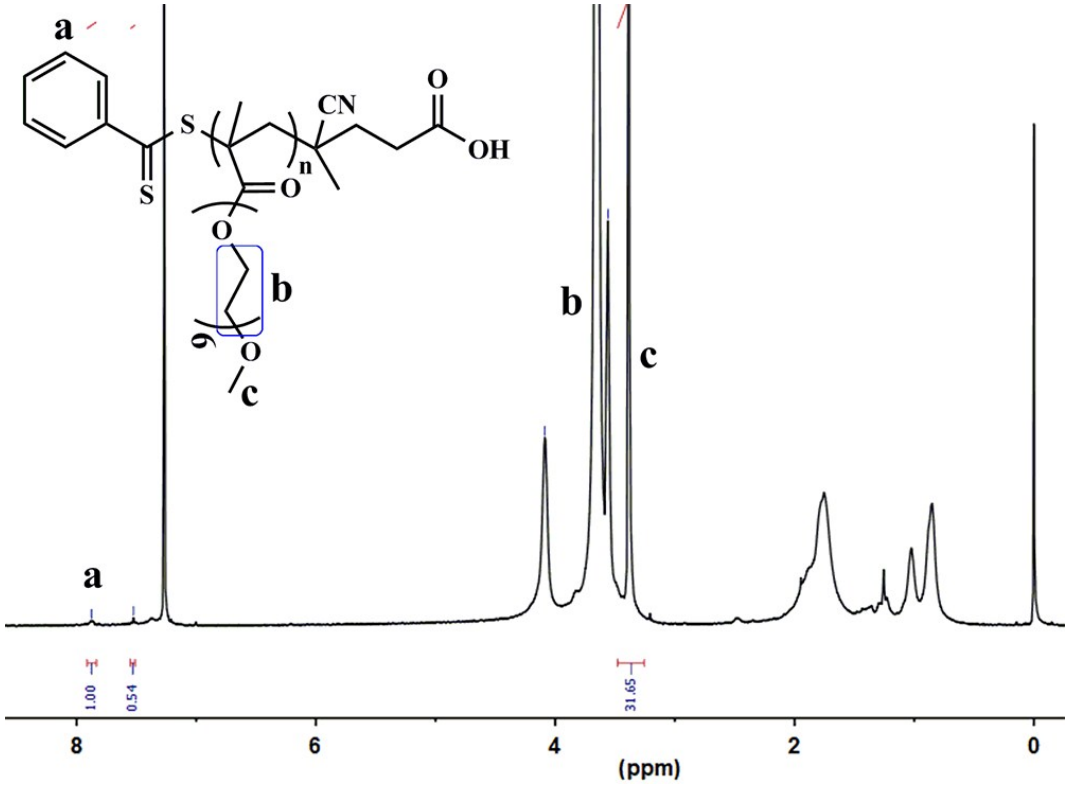

Figure S4. ^{1}H NMR spectrum of **PEG** in $CDCl_3$

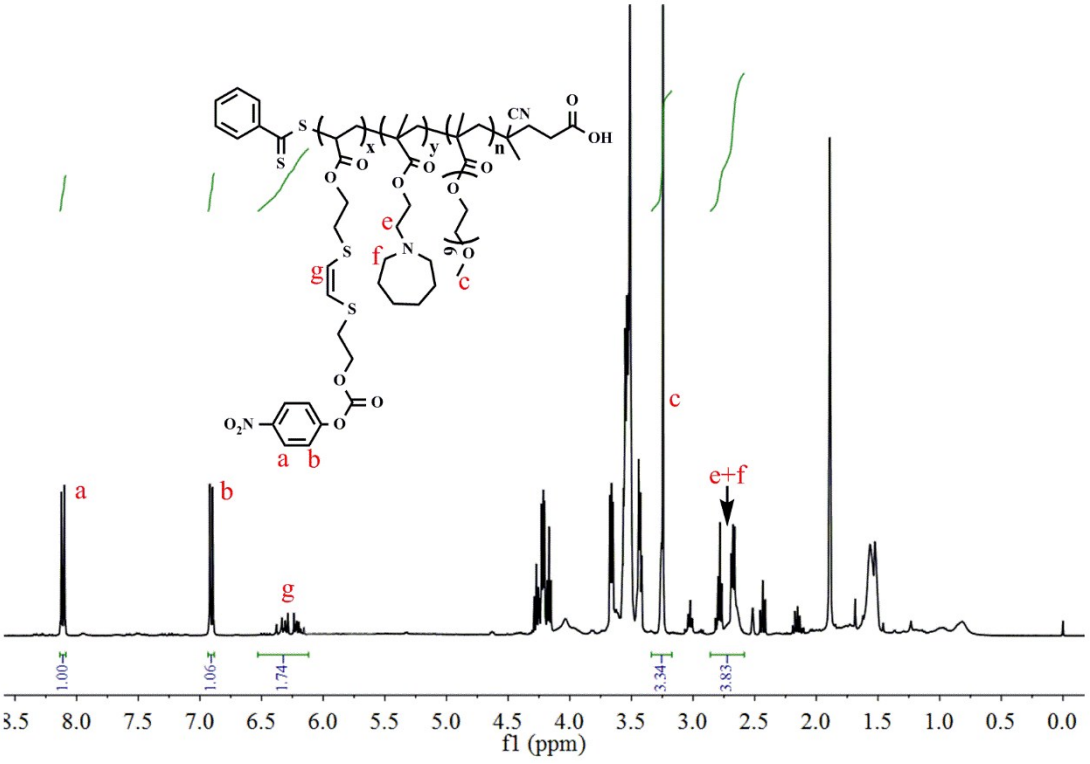

Figure S5. ^{1}H NMR spectrum of **P (AEMA-*co*-$^{1}O_2$)-PEG** in DMSO-d_6.

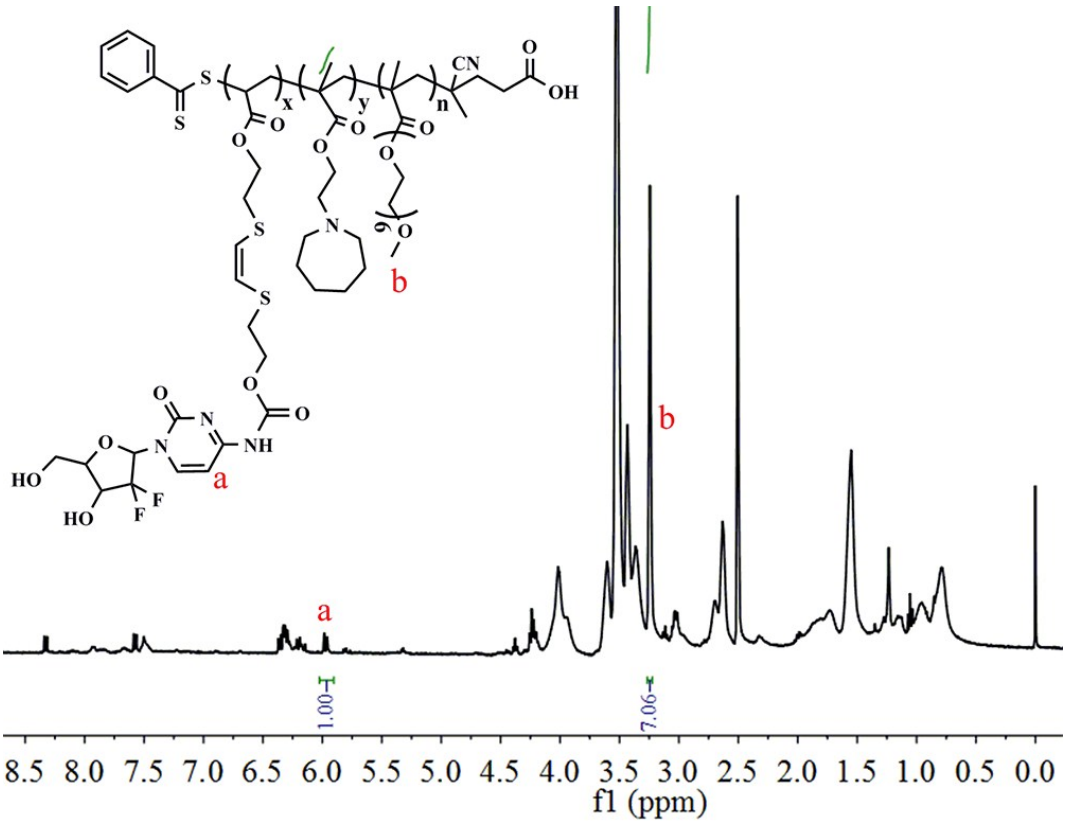

Figure S6. ^{1}H NMR spectrum of **P (AEMA-*co*-$^{1}O_2$ -GEM)-PEG** in DMSO-d_6.

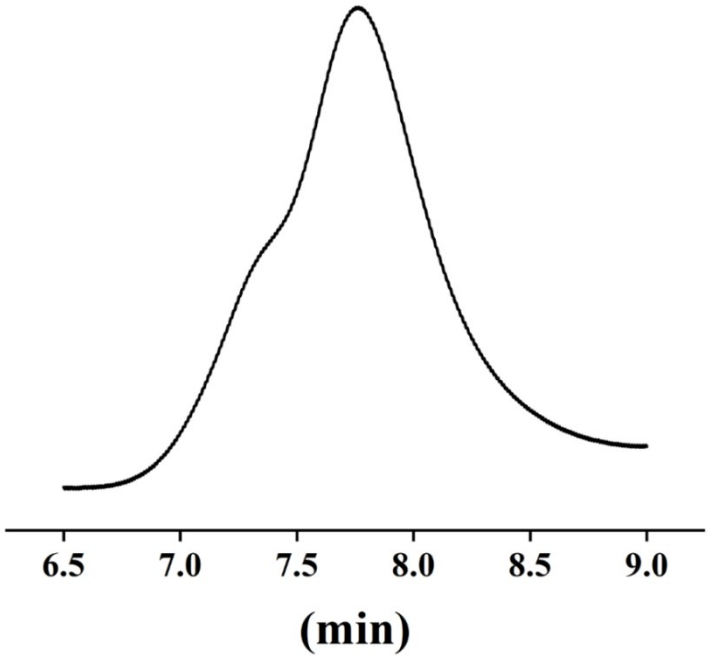

Figure S7. GPC trace of **P (AEMA-*co*-$^{1}O_2$ -GEM)-PEG** in THF

Reference:

1. G. Saravanakumar, J. Lee, J. Kim and W. J. Kim, *Chemical Communications*, 2015, **51**, 9995-9998.