Supporting information

Synthesis of polymeric micelles with a dual-functional sheddable PEG stealth for enhanced tumor-targeted drug delivery

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1 Materials and Experimental section

Oligo (ethylene glycol) monomethyl ether methacrylate (OEGMA, M_n=300 g/mol and pendent EO units DP ~4.5) and 2-hydroxyethyl methacrylate (HEMA, 99%) were purchased from Aldrich and purified by passing through a column to remove the inhibitor. Poly (ethylene glycol) methyl ether (mPEG, M_n =2000 g/mol) was freeze-dried one day before use. Folic acid (Aldrich, 97%), 2,2-dimethoxypropane (J&K, 98%), 2,2-bis (hydroxymethyl) propinoic acid (bis-MPA, J&K, 99%), Ethyl bromoacetate (J&K, 97%), Hydrazine hydrate (J&K, 80 wt % solution in H_2O), 4-carboxybenzaldehyde (J&K, 97%), *p*-toluenesulfonic acid monohydrate (J&K, 99%), 2-Hydroxyethyl-2′-(bromoisobutyryl)ethyl disulfide double-head initiator (OH-SS-iBuBr) was prepared according to literature, [1] Dess-Martin periodinane (J&K, 98%), 4-pentynoic acid (J&K, 95%), L-lactide (J&K, 99%), Acetyl chloride (Rionlon(Tianjin), 99.0%), 2-bromoisobutyric acid (Adrich, 98%), 2-hydroxyethyl disulfide (Adrich, 90%), copper(Ⅰ)bromide (CuBr, Adrich, 99.999%), N,N,N,N',N'-pentamethyldiethylenetriamine (PMDETA, Adrich,99%), N,N'-dicyclohexylcarbodiimide (DCC, Aladdin, 99.0%), 4-(dimethylamino) pyridine (DMAP, J&K, 99%), anhydrous ethyl ether (Rionlon(Tianjin), 99.0%) , tetrahydrofuran (THF, Rionlon(Tianjin), 99.0%), anhydrous methanol (Rionlon(Tianjin), 99.5%), dichloromethane (DCM, Rionlon(Tianjin), 99.5%), tetrahydrofuran anhydrous (THF, stailized with BHT, J&K, 99.5%), triethylamine (Rionlon(Tianjin), 99.0%), sodium azide (J&K, 98.0%) and other chemicals were used as received. The balance (Sartorius group) used in this study has a sensitivity of

1/10000.

Synthesis of isopropylidene-2,2-bis (methoxy)propionic acid

This product was prepared according to literature.[2] Briefly, bis-MPA (10.10040 g, 74.55 mmol), 2,2-dimethoxypropane (11.88479 g, 111.83 mmol), and catalyst content of *p*-toluenesulfonic (1.07439 g, 5.59 mmol) were dissolved in 50 mL of acetone. After stirring for 2 h, the solution was neutralized by adding NH_3/EtOH (50:50 v/v) solution. The evaporated residue was dissolved into 250 mL DCM and washed by distill water (20 mL×2). Subsequently, the organic phase was dried by anhydrous $MgSO_4$ and filtrated, evaporated to precipitate the final white product bis-MPA. Yield=78.35%. ^{1}H NMR ($CDCl_3$,ppm) : δ 1.14 (s, 3H, C***H₃***CC(O)O-) , 1.36 (s, 3H, C***H₃***C(CH_3)OCH_2-), 1.39 (s, 3H, CH_3C(C***H₃***)OCH_2-), 3.67 (d, J=28 Hz, 2H, -OC***H₂***C(CH_3)C(O)O-), 4.17 (d, J=24 Hz, 4H, -OC***H₂***C(CH_3)-) . ^{13}C NMR ($CDCl_3$): δ 18.49, 22.06, 25.14, 41.77, 65.81, 98.35, 180.44.

Synthesis of bis-MPA-SS-iBuBr

Bis-MPA (0.31597 g, 1.815 mmol), was dissolved in 80 mL DCM, and the solution was cooled to 0 ^{o}C under the condition of N_2. Subsequently, DCC (0.37827 g, 1.815 mmol) and DMAP (0.02036 g, 0.165 mmol) were added in solid state. OH-SS-iBuBr (0.50035 g, 1.65 mmol) in 6 mL DCM was added dropwise to the reaction mixture. The solution was future stirred overnight after stirring for 30 min in an ice bath. The precipitated dicyclohexyl urea (DCU) formed during the reaction was removed by

vacuum filtration. Subsequently, the solvent was removed by rotary evaporation, and the product was purified by column chromatography with mixtures of ethyl acetate/hexane (1/6 v/v). The first spot is the product. The final light yellow oily product was isolated by evaporation and dried in a vacuum overnight. Yield=79.36%. ^{1}H NMR ($CDCl_3$,ppm) : δ 1.13 (s, 3H, C***H₃***CC(O)O-) , 1.32 (s, 3H, C***H₃***C(CH_3)OCH_2-), 1.37 (s, 3H, CH_3C(C***H₃***)OCH_2-), 1.88 (s, 6H, -O(O)CC(C***H₃***)$_2$Br), 2.85-2.95 (m, 4H, -CH_2C***H₂***-SS-C***H₂***CH_2-), 3.58 (d, J=28 Hz, 2H, -OC***H₂***C(CH_3)C(O)O-), 4.13 (d, J=24 Hz, 4H, -OC***H₂***C(CH_3)-), 4.3-4.4 (m, 4H,-C***H₂***CH_2-SS-CH_2C***H₂***-). Mass expected (found) (M+ H+): 461.04(461.10).

Synthesis of bis-MPA-SS-iBuBr

Bis-MPA-SS-iBuBr (0.20040 g, 0.436 mmol), *p*-toluensulfonic (0.50288 g, 2.617 mmol), and 1 mL distilled water were dissolved in 15 mL THF. The reaction mixture was stirred for 2 h at room temperature (rt) and subsequently evaporated to obtain the crude product. The crude product was added 7mL distilled water and the mixture was dissolved into 32 mL CH_2Cl_2 and washed by distilled water (4 mL×3). The organic phase was dried by anhydrous $MgSO_4$, filtrated, evaporated and dried in a vacuum overnight to form a light yellow oily residue. Yield=98.55%. ^{1}H NMR ($CDCl_3$,ppm) : δ 1.01 (s, 3H, C***H₃***CC(O)O-), 1.88 (s, 6H, -O(O)CC(C***H₃***)$_2$Br), 2.87-2.95 (m, 4H, -CH_2C***H₂***-SS-C***H₂***CH_2-), 3.67 (d, J=28 Hz, 2H, HOC***H₂***C(CH_3)C(O)O-), 3.85 (d, J=28 Hz, 4H, HOC***H₂***C(CH_3)-), 4.34-4.42 (m, 4H,-C***H₂***CH_2-SS-CH_2C***H₂***-). Mass expected (found) (M+ H^+): 421.01(421.00).

Synthesis of Alkynyl-bis-MPA-SS-iBuBr

bis-MPA-SS-iBuBr (0.10000 g, 0.23846 mmol), DCC (0.04744 g, 0.228 mmol), and DMAP (0.00268 g, 0.022 mmol) were dissolved in 20 mL DCM. The mixture was cooled to 0 ^{o}C under the condition of N_2. 4-pentynoic acid (0.02239 g, 0.217 mmol) in 2 mL DCM was added dropwise to the reaction mixture and stirred overnight after stirring for 30 min in an ice bath. The precipitated dicyclohexyl urea (DCU) precipitate formed during the reaction was removed by vacuum filtration. Subsequently, the solvent was removed by rotary evaporation, and the product was purified by column chromatography with mixtures of ethyl acetate/hexane (1.5/3.5 v/v). The oily product was isolated by evaporation and dried in a vacuum overnight. Yield=73.29%. ^{1}H NMR ($CDCl_3$,ppm) : δ 1.22 (s, 6H, -O(O)CC(C***H*****$_3$**$)_2$Br), 1.93 (s, 3H, C***H*****$_3$**CC(O)O-) , 1.98 (t, 1H, C***H***≡CCH_2CH_2-), 2.44-2.61 (m, 4H, CH≡CC***H*****$_2$**C***H*****$_2$**-, O***H***-CH_2-), 2.9-3.0 (m, 4H, -CH_2C***H*****$_2$**-SS-C***H*****$_2$**CH_2-), 3.64-3.74 (m, 2H, HOC***H*****$_2$**C(CH_3)C(O)O-), 4.22-4.46 (m, 6H, CH≡CCH_2CH_2COOC***H*****$_2$**-and -C***H*****$_2$**CH_2-SS-CH_2C***H*****$_2$**-). Mass expected (found) (M- H+): 499.04(499.00).

Synthesis of ethyl azidoacetate

To a solution of ethyl bromoacetate (6.88659 g, 40 mmol) in 160 mL acetone, NaN_3 (6.63367 g, 100 mmol) was added and the reaction mixture was stirred for 6 h at 60 ^{o}C. After cooling to room temperature, 50 mL ethyl acetate was added. In the next step, the mixture was washed by saturated NaCl (100 mL×3). The result solution was dried by anhydrous Na_2SO_4. The light yellow oily product was precipitated after the

solvent was filtrated and evaporated. Yield=85.07%. ^{1}H NMR ($CDCl_3$, ppm): δ 1.31 (t, J=24 Hz, 3H, -$CH_2C\boldsymbol{H_3}$), 3.83 (s, 1H, -$C\boldsymbol{H_2}N_3$), 4.19-4.28 (m, 2H, -$C\boldsymbol{H_2}CH_3$). ^{13}C NMR ($CDCl_3$): δ 14.26, 62.01, 77.16, 168.44.

Synthesis of 2-azidoacetohydrazide

Hydrazine monohydrate (1.20130 g, 24 mol, 1.2 equiv) was added to a solution of ethyl azidoacetate (3.02200 g, 20 mol, 1 eq) in 12 mL of ethanol. The mixture was stirred with reflux at 80 °C for 4 h. The progress of the reaction was monitored by thin layer chro-matography (TLC), and a small amount of hydrazine was added, if required, until the reaction was complete. Subsequently, the solvent was removed by rotary evaporation, and the product was purified by column chromatography with mixtures of ethyl acetate/DCM (1/9 v/v). The oily product was isolated by evaporation and dried in a vacuum overnight. Yield=86%. ^{1}H NMR ($CDCl_3$, ppm): δ 3.91(s, 2H, -NHN$\boldsymbol{H_2}$), 4.03(s, 1H, -$C\boldsymbol{H_2}N_3$), 7.61(s, 1H, -$N\boldsymbol{H_1}NH_2$).

Synthesis of monomethoxypolyethylene glycol-aldehyde (mPEG-CHO)

Dess-Martin periodinane (0.18394 g, 0.425 mmol) was added to a solution of mPEG (0.50000 g, 0.425mmol) in 200 mL DCM. The mixture was stirred at 40 °C for 5 d. Subsequently, the solvent was removed by rotary evaporation, and the crude product was precipitated in an excess of ice extane. The product was further purified by dialysis (MWCO 0.5 kDa) against distilled water and freeze-dried to precipitate the product as white power. Yield=76.60%. ^{1}H NMR ($CDCl_3$, ppm): δ 3.37 (s, 3H, -$OC\boldsymbol{H_3}$), 3.44-3.84 (m, -$OC\boldsymbol{H_2}C\boldsymbol{H_2}O$-), 4.16 (s, 2H, -$OC\boldsymbol{H_2}$CHO), 9.73 (s, 1H, -C***H***O).

Synthesis of mPEG-hydrazone

A mixture of 2-azidoacetohydrazide (57.55 mg, 0.5 mmol) and mPEG-CHO (0.40000 g, 0.2 mmol) in 4 mL of methanol was stirred at 80 ^{o}C for 8 h in Schlenk flask in the presence of a drop of glacial acetic acid. After cooled to room temperature, the mixture was precipitated in an excess of ice diethyl ether, and the product was future dried in a vacuum overnight. Yield=92.10%. ^{1}H NMR ($CDCl_3$, ppm): δ 3.38 (s, 3H, -OC***H₃***), 3.44-3.84 (m, -OC***H₂***C***H₂***O-), 4.00-4.27 (m, 5H, -CH_2OC***H₂***C***H***NNHCOC***H₂***N_3), 9.39 (s, 1H, -NN***H***-).

Synthesis of HEMA-LA

After three freeze-pump-thaw cycles, a mixture of L-lactide (8.73515 g, 60 mmol) and HEMA (3.94364 g, 30 mmol) was stirred at 110 ^{o}C until the L-lactide was molten. Subsequently, a small amount of $SnOct_2$ (131.39 mg, 0.3 mmol) dissolved in 80 μL toluene was added in a nitrogen atmosphene. Finally, the reaction mixture was degassed with another three freeze-pump-thaw cycles and then sealed followed by immersing the flask into an oil bath at 110 ^{o}C for 40 min. The reaction mixture was diluted with THF (20 mL) and then precipitated in 180 mL ice-cold water. The formed precipitate was collected by centrifugation, dissolved in 40 mL ethyl acetate, and centrifugation to remove remaining solids. The solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. An oily product was precipitated and dried in a vacuum overnight. Yield=63.37%. ^{1}H NMR ($CDCl_3$, ppm): δ 1.40-1.62 (m, 12H, -CH (OH) C***H₃***, - (CO) CH (O) C***H₃***), 1.94 (s, -(CH_2)CHC***H₃***), 2.72 (s, 1H, -

OH), 4.23-4.50 (m, 5H, -OC***H*****$_2$**C***H*****$_2$**O-, HOC***H***-), 5.09-5.31 (m, 3H, -(CO)C***H***(O)CH_3), 5.60 (s, 1H, - (CH3)CC***H***), 6.12 (s, 1H, - (CH3)CC***H***).

Synthesis of HEMA-*g*-LA

To the solution of HEMA-LA (28.03459 g, 16.92mmol) in 25mL DCM which was in an ice bath under the condition of N_2, acetyl chloride (7.74 ml, 101.53 mmol) in 20 mL DCM was added dropwise. After 0.5 h, the mixture was stirred for another 24 h at rt. Subsequently, the solvent was removed by rotary evaporation, and the crude product dissolved in 20 mL THF was precipitated in an excess of ice-cold water (200 mL). The formed precipitate was collected by centrifugation, dissolved in 40 mL ethyl acetate, and the solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. An oily product was precipitated and dried in a vacuum overnight. Yield=82.01%. ^{1}H NMR ($CDCl_3$, ppm): δ 1.47-1.62 (m, 12H, -CH (OH) C***H*****$_3$**, - (CO) CH (O) C***H*****$_3$**), 1.94 (s, -(CH_2)CHC***H*****$_3$**), 2.13 (s, 3H, -OCOC***H*****$_3$**), 4.30-4.48 (m, 4H, -OC***H*****$_2$**C***H*****$_2$**O-), 5.06-5.27 (m, 4H, -(CO)C***H***(O)CH_3, -COC***H***(CH_3)$COCH_3$), 5.60 (s, 1H, - (CH3)CC***H***), 6.12 (s, 1H, - (CH3)CC***H***).

ATRP of OEGMA and HEMA-*g*-LA using alkynyl-bis-MPA-SS-iBuBr as an initiator

P(OEGMA300)-*st*-P(HEMA-*g*-LA) was synthesized by ATRP using alkynyl-bis-MPA-SS-iBuBr as the initiator and CuBr/bpy as the catalyst. The typical procedure was as follows: a solution of alkynyl-bis-MPA-SS-iBuBr (0.35960 g. 0.72 mmol), OEGMA300 (1.23120 g, 4.104 mmol), HEMA-*g*-LA (1.80000 g, 3.6 mmol), bpy

(229.49 mg, 1.44 mmol) in 9 mL anisole was added into a 25 mL Schlenk flask. After three freeze-pump-thaw cycles, CuBr (103.29 mg, 0.72 mmol) was introduced into the flask under N_2. Finally, the reaction mixture was degassed with another three freeze-pump-thaw cycles and then sealed followed by immersing the flask into an oil bath at 60 ^oC for 18 min. The polymerization was quenched by immersing the flask in the liquid nitrogen and diluted with THF. The resulting solution was passed through a short neutral Al_2O_3 column to remove the copper catalyst using THF as an eluent. The resulting solution was concentrated by rotary evaporation and poured into hexane/anhydrous ethyl ether (7/1 v/v) to precipitate the product for three times. An oily product was precipitated and dried in a vacuum overnight.

Preparation of folate-conjugated P(OEGMA300)-*st*-P(HEMA-*g*-LA)

To a solution of FA (0.32448 g, 0.713mmol) in 100 mL DMF which was in an ice bath under the condition of N_2, was added DCC (0.89167 g, 4.28 mmol) and DMAP (0.52797 g, 4.28 mmol). The solution was stirred for 1 h at 0 ^oC and another 12 h under dark at rt, then P(OEGMA300)-*st*-P(HEMA-*g*-LA) (0.50000 g, 0.14 mmol) in 3 mL DMF was added under N_2. The reaction mixture was srirred for 3 d. The precipitated dicyclohexyl urea (DCU) precipitate formed during the reaction was removed by vacuum filtration, and then the solution was dialysis (MWCO, 0.5 kDa) against DMF for 24 h, followed by further dialysis against distilled water for another 24 h. The polymer solution was freeze-dried to precipitate the product.

Preparation of mPEG(FA)-P(OEGMA300)-*st*-P(HEMA-*g*-LA)

A solution of FA-P(OEGMA300)-*st*-P(HEMA-*g*-LA) (0.13000g, 0.03 mmol), a solution of mPEG-NNH-N_3 (256.21 mg, 0.09 mmol), PMDETA (14.90 μL, 0.07 mmol) in 5 mL DMF was added into a 25 ml Schlenk flask. After three freeze-pump-thaw cycles, CuBr (10.03 mg, 0.07 mmol) was introduced into the flask under N_2. Finally, the reaction mixture was degassed with another three freeze-pump-thaw cycles and then sealed followed by immersing the flask into an oil bath at 45 ^oC for 3 d. The polymerization was quenched by immersing the flask in the liquid nitrogen and dialysis (MWCO, 3.5 kDa) against phosphate buffer (PB, 10 mM, pH 7.4) before it was purified by Ultrafiltration centrifuge tube (100 kDa).

Stability and degradation of micelles

Aqueous self-assembly of polymeric micelles was prepared using the classical dialysis method. Briefly, mPEG(FA)-P(OEGMA300)-*st*-P(HEMA-*g*-LA) (5 mg) was dissolved into 6 mL of DMSO, the solution was next added dropwise into 4 mL of phosphate buffer (PB, 10 mM, pH 7.4) under vigorous stirring for 1 h. The resultant solution was dialysis (MWCO, 3.5 kDa) against certain buffer solution. Then the solution was introduced into 5 mL microcentrifuge tubes that were kept in a horizontal laboratory shaker thermostated at a constant temperature of 37°C and a stirring speed of 120 rpm. At predetermined time intervals, the mean hydrodynamic size and size distribution (polydispersity index, PDI) of the micelles were examined by DLS.

***In vitro* drug loading and drug release study**

Briefly, DOX•HCl (0.50 mg) and TEA (10 μL) were dissolved in 2 mL of DMSO and stirred overnight under dark at rt. Next, mPEG(FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ (P1) (5.00 mg) dissolved in 4 mL of DMSO was added to the above DOX solution and stirred at room temperature for 1 h. The resultant solution was added into 4 mL of phosphate buffer (PB, 10 mM, pH 7.4) under vigorous stirring. After stirring for another 1 h, the solution was dialyzed (MWCO, 3.5 kDa) against 5 L of phosphate buffer (PB, 10 mM, pH 7.4) for 24 h, during which the phosphate buffer (PB, 10 mM, pH 7.4) was renewed every 3 h at the initial 12 h.

For *in vitro* drug release study, the solution of drug-loaded micelles in phosphate buffer (PB, 10 mM, pH 7.4) was split in equal volumes into four dialysis tubes with a MWCO of 3.5 kDa, which were then immersed in a Falcon tube containing 25 mL of three different release media of phosphate buffer (PB, 10 mM, pH 7.4), acetate buffer (10 mM, pH 5.0), and phosphate buffer (PB, 10 mM, pH 7.4) with 10 mM DTT at 37 ^{o}C with constant shaking (120 rpm). At predetermined time intervals, 3mL of release medium was sampled and replenished with equal volume of fresh medium. The samples were determined by measuring the absorbance at 499 nm with UV-vis spectrometer using a calibration curve obtained from free DOX·HCl in the corresponding release buffers. The experiment was performed in quadruplicate for each sample, and the data were presented as mean values.

The encapsulation efficiency (EE) and the drug loading content (DLC) of the drug-loaded micelles were determined using the UV-vis spectrometer at 499 nm. The EE and DLC were calculated using the following equations.[3-4]

$$EE\ (\%) = W_{DOX\ loaded\ in\ micelles} / W_{DOX\ fed\ for\ encapsulation} \times 100\% \quad (1)$$

$$DLC\ (\%) = W_{DOX\ loaded\ in\ micelles} / W_{DOX\ loaded\ micelles} \times 100\% \quad (2)$$

Cell Viability Assay

The *in vitro* cytotoxicity of various formulations was evaluated by the MTT assay. HeLa cells were seeded in 96-well plates at a plating density of 2500 cells per well in 100 μL of complete growth medium and incubated in an incubator maintained at 37 °C and 5% CO_2 environment for 24 h. A concentrated P1 stock solution at pH 7.4 was prepared by ultrafiltration centrifugation using a molecular-weight cut-off (MWCO 30.0 kDa) and the buffer solution of the DOX-loaded P1 micelles at pH 5.0 was prepared by a large dilution of the concentrated P1 stock solution prepared at pH 7.4 using the buffer of pH 5.0. Finally, all of the polymer solutions were diluted in 5-fold in Opti-MEM medium (Invitrogen). The cells were rinsed once with PBS and incubated with 100 μL of the sample solutions with different polymer or DOX concentrations at 37 ^{o}C for 24 h. Cells were next rinsed with PBS and the medium was replaced with 100 μL of culture medium. After addition of 20 μL of MTS reagent to each well, cells were then incubated at 37 °C and 5% CO_2 for 3 h. The absorbance of each well was measured at a wavelength of 490 nm on a Tecan Safire2 plate reader (Männerdorf, Switzerland). Cell viability for each treatment condition was determined by normalizing to the cells only signal.

Evaluation of Cellular Uptake by Flow Cytometry (FCM) Analysis

HeLa cells were seeded in 24-well plates at a density of 140000 cells per well in 1.0

mL of complete growth medium and incubated for 24 h at 37 °C in 5% CO_2 environment. After the cells were rinsed once with PBS, fresh MEM containing DOX, was added to replace the original medium, and the cells with phosphate buffer (PB, 10 mM, pH 7.4) were set as a control. Note that the buffer solution of the DOX-loaded P1 micelles at pH 6.5 or 5.0 was prepared by a large dilution of a concentrated polymer stock solution prepared at pH 7.4 using the buffer of pH 6.5 or 5.0 to guarantee an identical DOX concentration of 6.5 μg mL^{-1} for the three different pH conditions. The DOX concentration for free DOX·HCl, P1 micelles at pH 7.4, pH 6.5 and pH 5.0 in MEM was set at 6.5 μg/mL. After incubation for certain time, the polymer solutions were aspirated, and the cells were rinsed twice with PBS. Cells were then harvested by incubation with 200 μL of Trypsin-EDTA, followed by resuspension with 1 mL of complete growth medium. Cells were transferred to 1.5 mL microcentrifuge tubes and pelleted at 300 g for 5 min at 4 °C. The supernatant was aspirated, and the cell pellets were resuspended in 200 μL of PBS. Cells were analyzed for uptake of fluorescent polymer using a BD Accuri C6 Plus flow cytometer (BD Biosciences) with an excitation wavelength and emission wavelength of 488 nm and 595 nm. A minimum of 10 000 cells was analyzed each sample with the fluorescence intensity.

Fluorescence imaging

HeLa cells were seeded in 12-well plates at a plating density of 150000 cells per well in 1 mL of complete growth medium and incubated in 5% CO_2, 37 ^oC environment

for 24 h. Solutions of free DOX and P1 micelles at pH7.4 and pH5.0 were prepared in MEM setted at the concentration of 6.5 μg/mL and were later added to the wells and incubated at 37 °C for 6 h. After incubation, cells were rinsed with PBS and counterstained with DAPI. Coverslips were mounted onto glass slides and imaged using Leica DM4000 biological microscope.

2. **Characterization**

^{1}H NMR spectra were recorded on a JNM-ECS spectrometer at 400 MHZ using $CDCl_3$ and d_6-DMSO as the solvents, respectively. The average hydrodynamic diameter (D_h) at various temperatures was measured by dynamic light scattering (DLS) on a Zetasizer (Nano ZS, Malvern, Worcestershire, UK) at a fixed detection angle of 173°. The size exclusion chromatography and multi-angle laser light scattering (SEC-MALLS) was carried out to determine the molecular weight and molecular weight distribution of the polymers. SEC using HPLC-grade DMF containing 0.1 wt% LiBr at 60^{o}C as the eluent at a flow rate of 1 mL/min Tosoh TSK-GEL R-3000 and R-4000 columns (Tosoh Bioscience) were connected in series to a Agilent 1260 series (Agilent Technologies), an interferometric refractometer (Optilab-rEX, Wyatt Technology) and a MALLS device (DAWN EOS, Wyatt Technology). The MALLS detector was operated at a laser wavelength of 690.0 nm. The FT-IR spectroscopic measurements were conducted on a NEXUS 670 FT-IR spectrometer (Nicolet, WI, USA) and solid or liquid samples were pressed into potassium bromide (KBr) pellet prior to the measurements. Gel permeation chromatography (GPC) plots were recorded on a Shimadzu LC-20AD instrument with a calibration standard of PEG and an eluent solvent of tetrahydrofuran (THF). The flow rate was 1.0 mL min^{-1} and the column temperature was fixed at 40 °C. Number-average molecular weights (M_n), weight-average molecular weights (M_w), and $Đ$ (M_w/M_n) were achieved for the PEGs end functionalized with L. Ultraviolet-visible (UV-vis) absorption of the sample solution was measured on an UV-1780 spectrometer (SHIMADZU, Kyoto, Japan).

TEM measurements were carried out on a JNM-2010 instrument operating at an acceleration voltage of 200 keV. The TEM samples were made by dropping 10 μL of the solution onto a carbon-coated copper grid for 15 min, and the excess solution was slightly blotted up by a filter paper. Thereafter, one drop of phosphotungstic acid solution (PTA, 1%, pH 6.5) was added onto the copper grid and kept for 5 min for staining. The final grid was dried overnight under ambient environment.

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3. Confirmation of significant detachment of mPEG shielding corona by the SEC and NMR analyses of the degraded products of mPEG (FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ after incubation at pH 5.0 for 24 h

We further investigated the cleavage of mPEG shielding by SEC and NMR measurements. Because the SEC-MALLS system (using DMF as an eluent) used in this study was currently out of order and has been sent back to the factory for repair, we had to turn to another SEC system based on a Shimadzu LC-20AD instrument with a calibration standard of PEG and an eluent of tetrahydrofuran (THF) for the SEC analysis of various samples, including mPEG, FA-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$, mPEG (FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ and mPEG (FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ after incubation at pH 5.0 for 24 h (Fig. S18a). Since the solubility of samples in THF was not as good as that in DMF, an unknown elution peak was recorded at approximately 10.5 min. The elution peak at 11.5 min is attributed to the solvent. Nevertheless, the notable appearance of an elution peak assigned to the cleaved mPEG moiety at 10.9 min after incubation of mPEG(FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ at pH 5.0 for 24 h supports significant removal of mPEG shielding by acidic pH-triggered cleavage of a hydrazone link. Further attempts to quantify the detachment efficiency of mPEG segment by ^{1}H NMR spectroscopy were unsuccessful due to the quite low signal-to-noise ratio of the aldehyde signal (Fig. S18b).

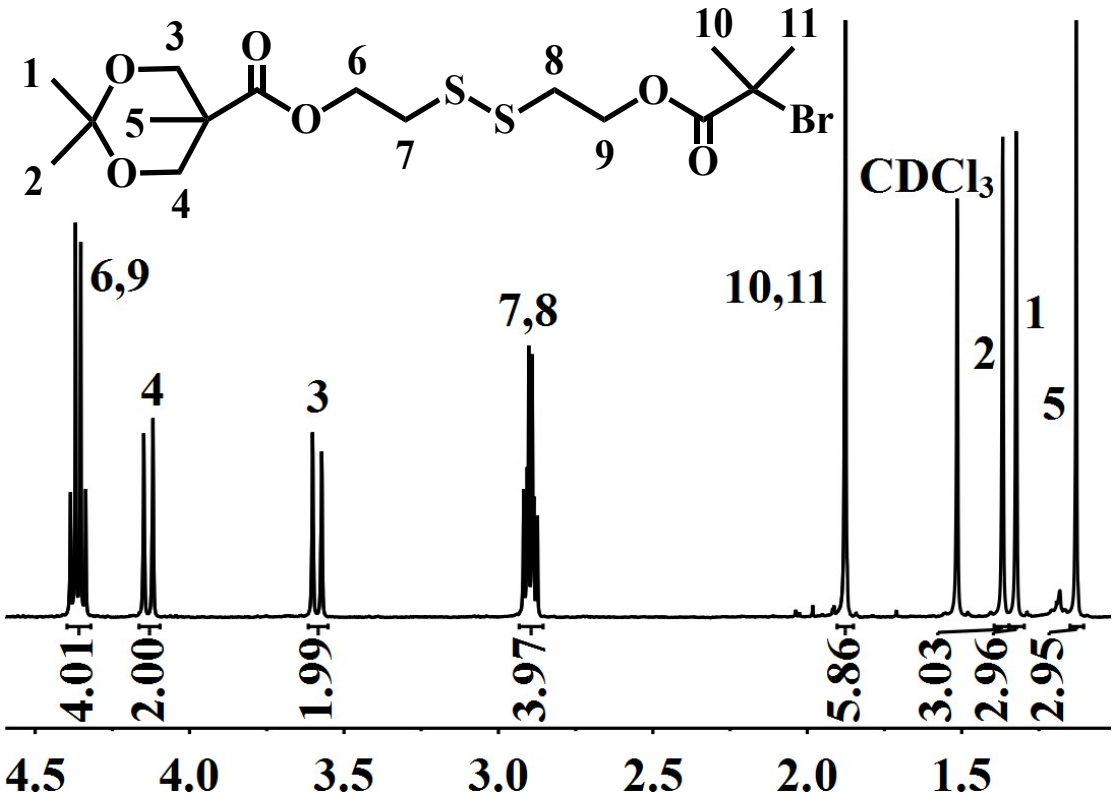

Fig. S1 ^{1}H NMR spectrum of Bis-MPA-SS-iBuBr ($CDCl_3$).

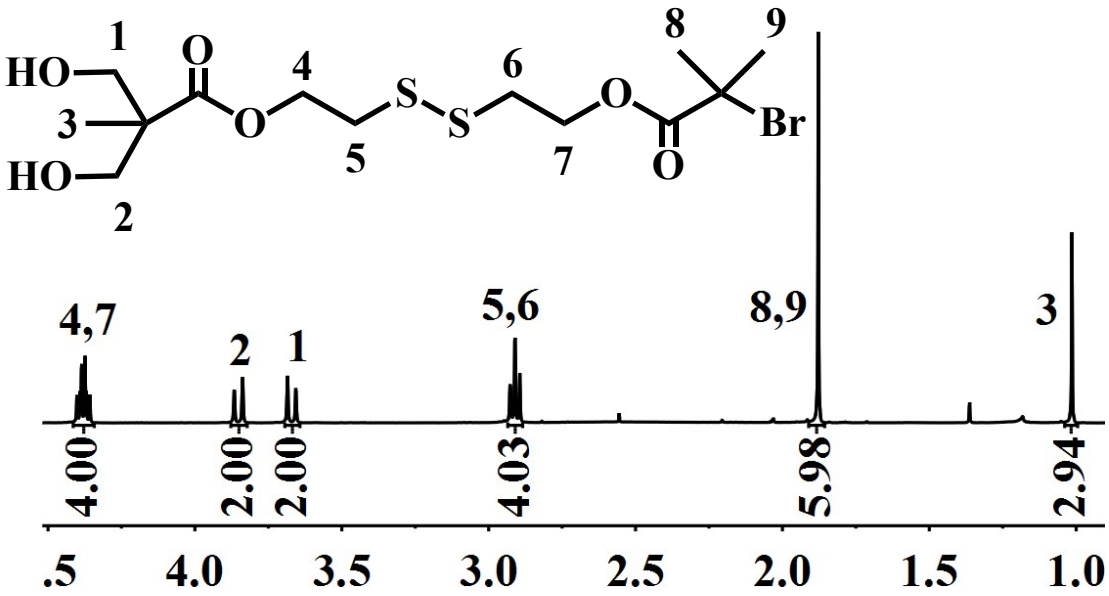

Fig. S2 ^{1}H NMR spectrum of Bis-MPA-SS-iBuBr ($CDCl_3$).

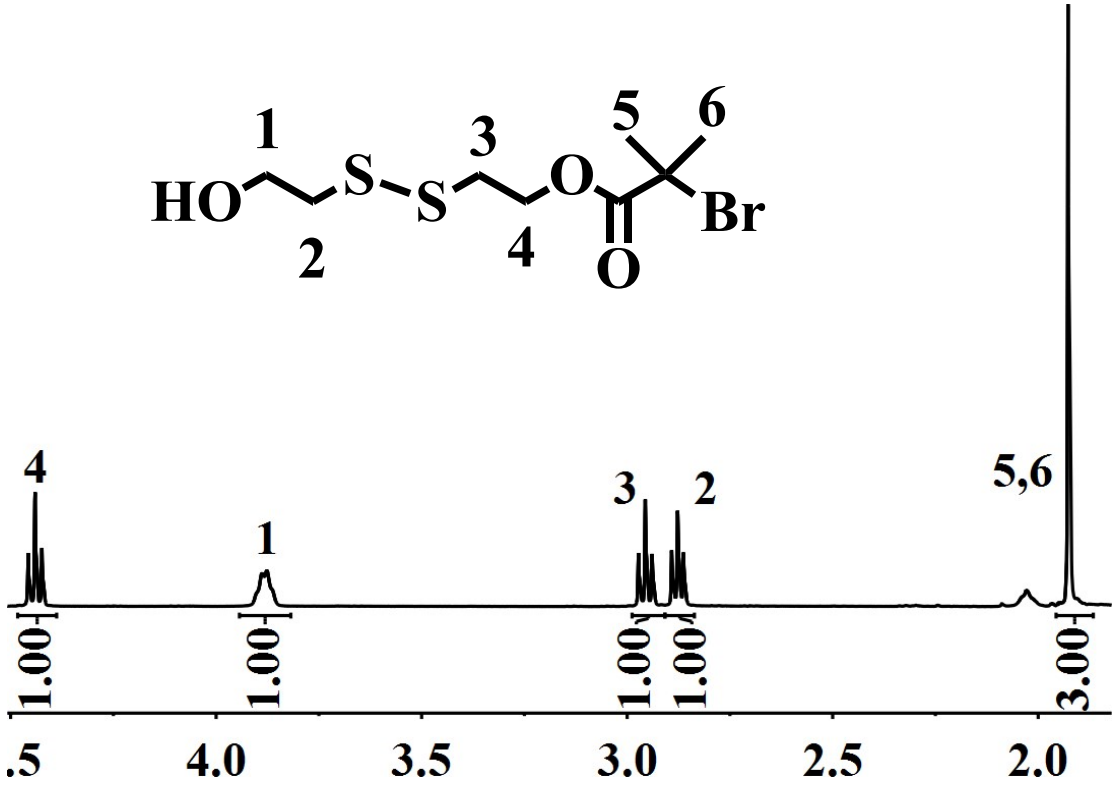

Fig. S3 ^{1}H NMR spectrum of OH-SS-iBuBr ($CDCl_3$).

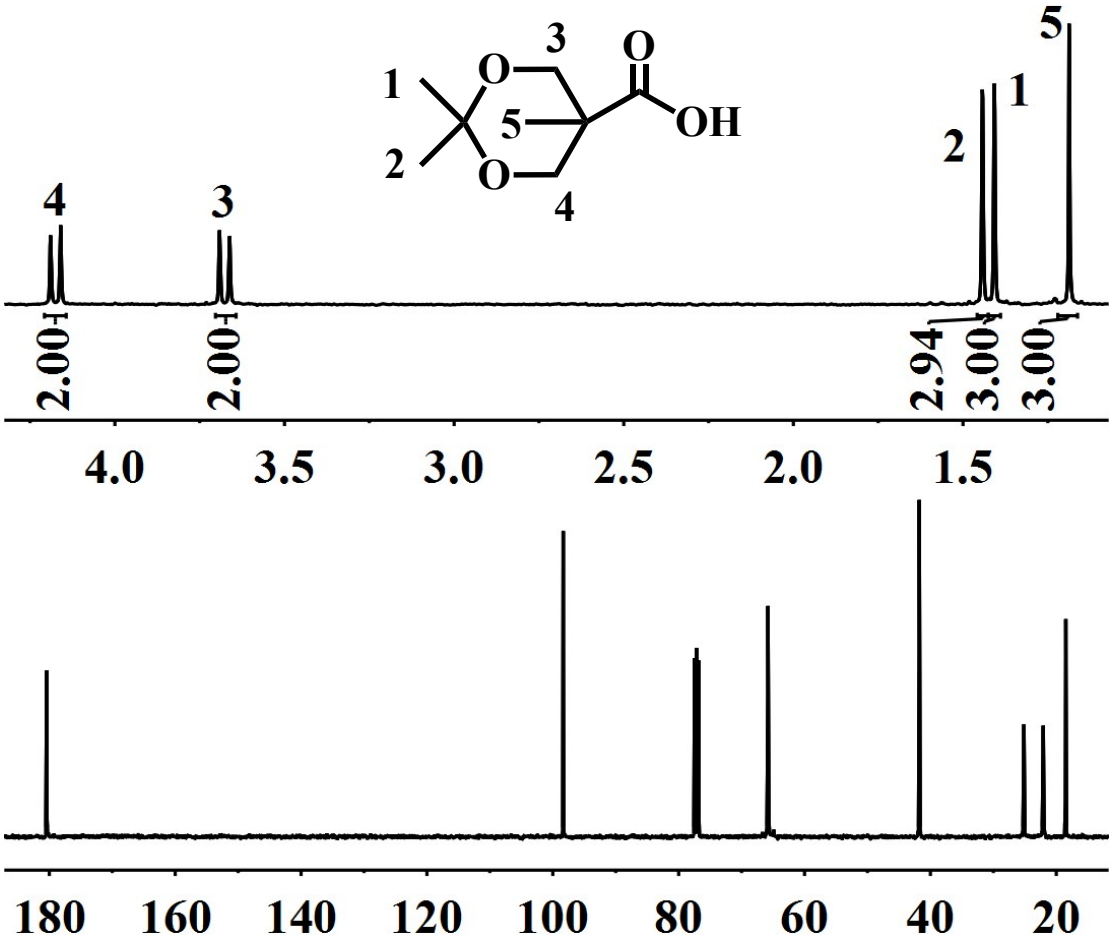

Fig. S4 ^{1}H and ^{13}C NMR spectra of isopropylidene-2,2-bis (methoxy)propionic acid ($CDCl_3$).

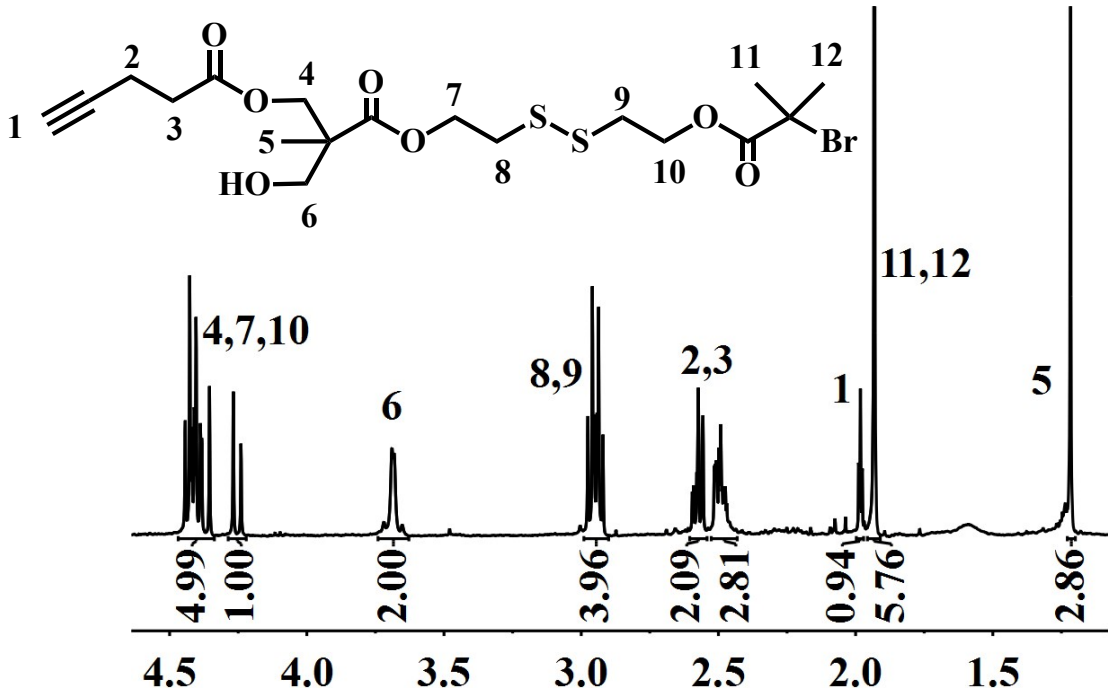

Fig. S5 ^{1}H NMR spectrum of alkynyl-bis-MPA-SS-iBuBr ($CDCl_3$).

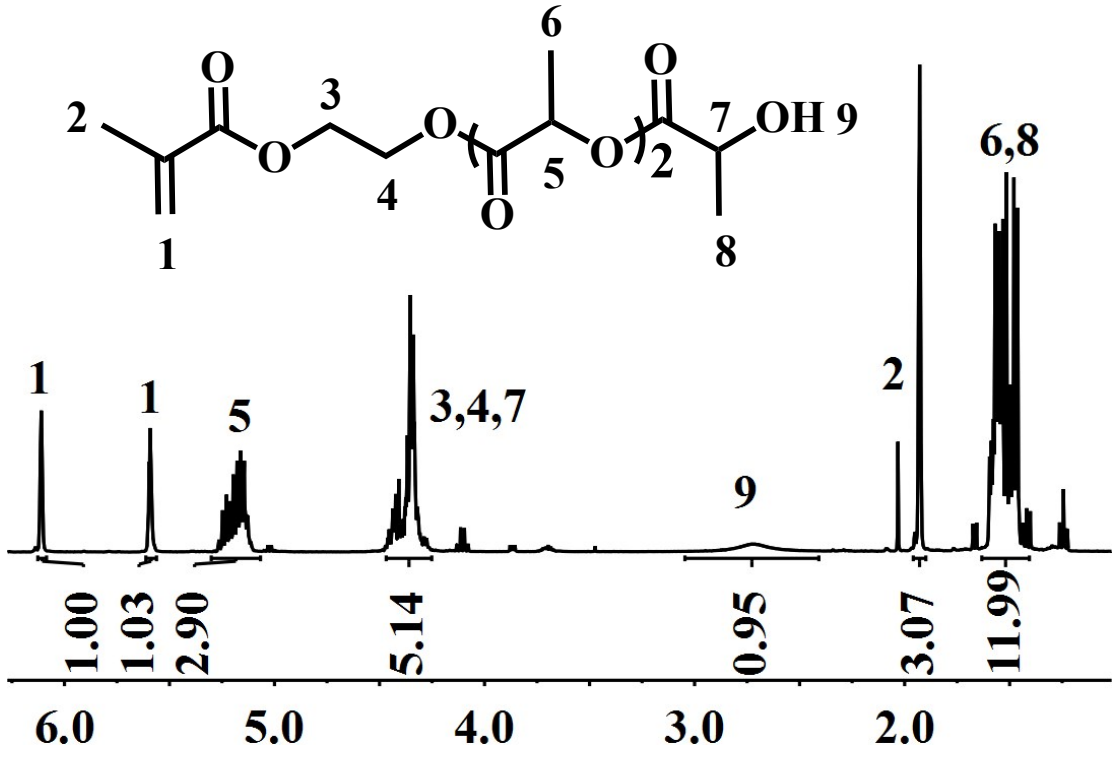

Fig. S6 ^{1}H NMR spectrum of HEMA-LA ($CDCl_3$).

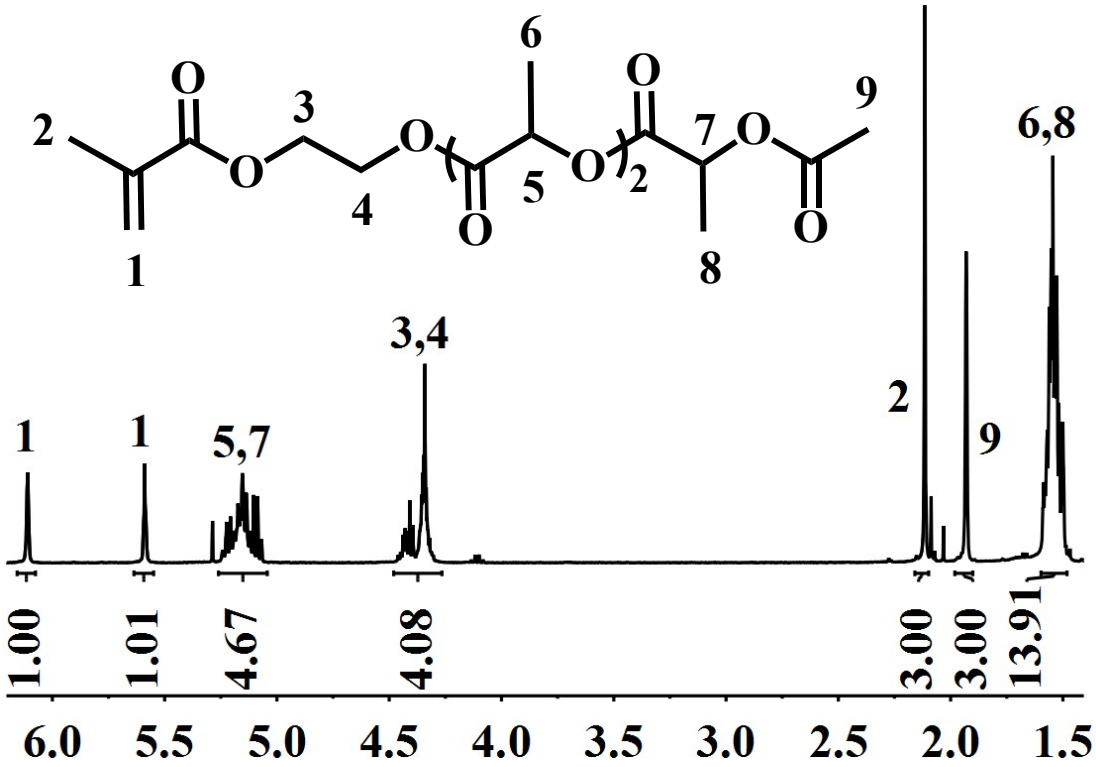

Fig. S7 ^{1}H NMR spectrum of HEMA-*g*-LA ($CDCl_3$).

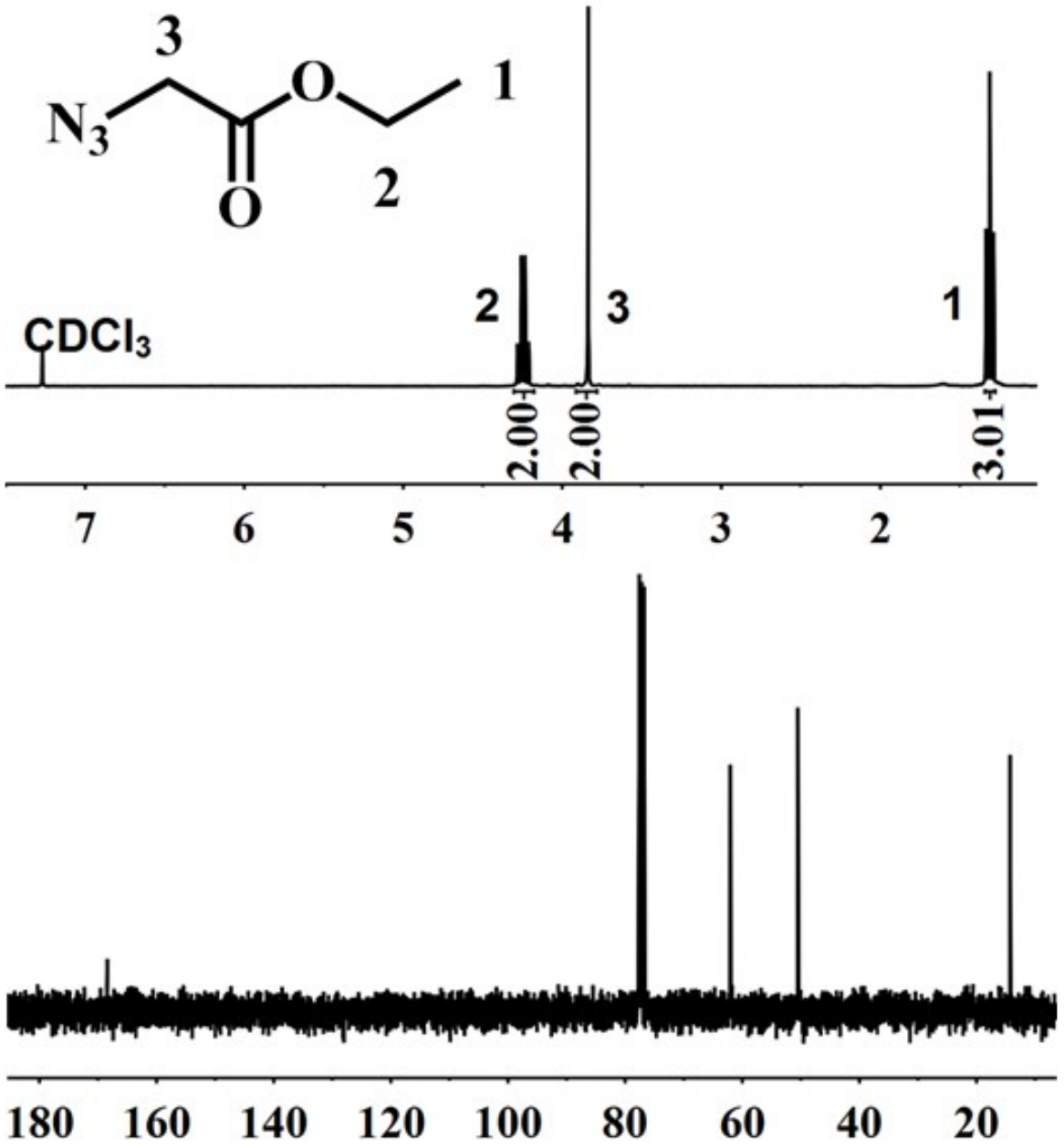

Fig. S8 ^{1}H and ^{13}C NMR spectra of ethyl azidoacetate ($CDCl_3$).

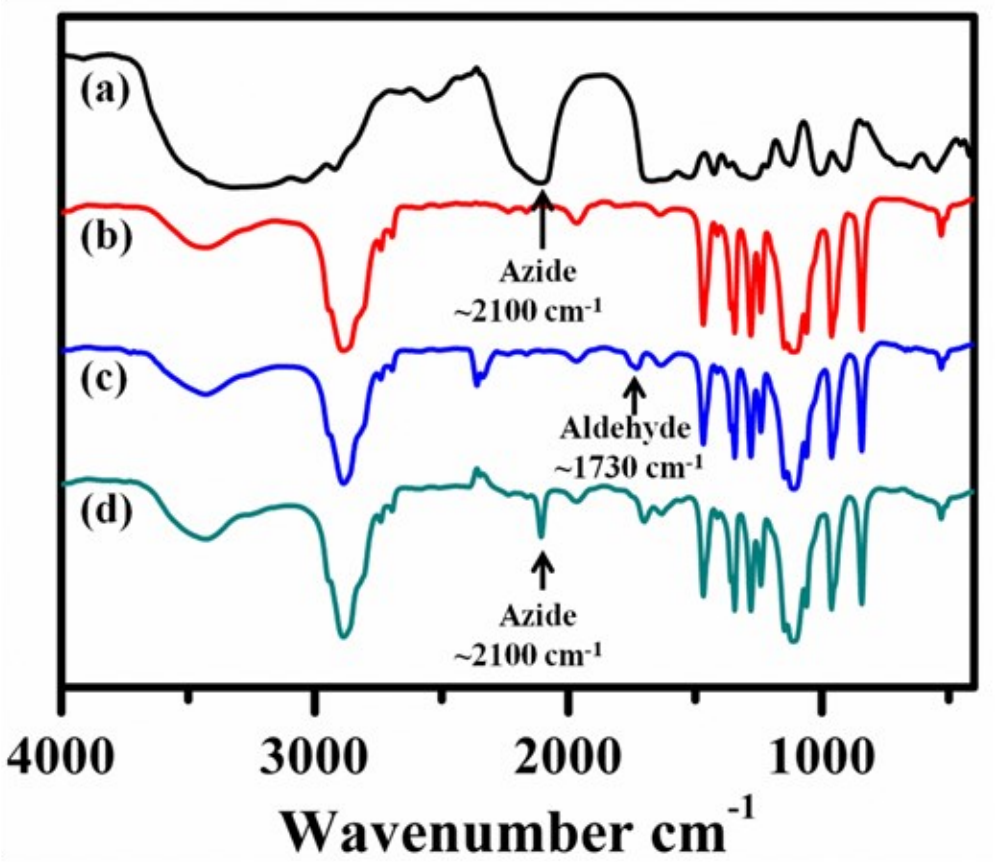

Fig. S9 FT-IR spectra of (a) $NH_2NH\text{-}N_3$, (b) mPEG, (c) mPEG-CHO, (d) mPEG-$NNH\text{-}N_3$.

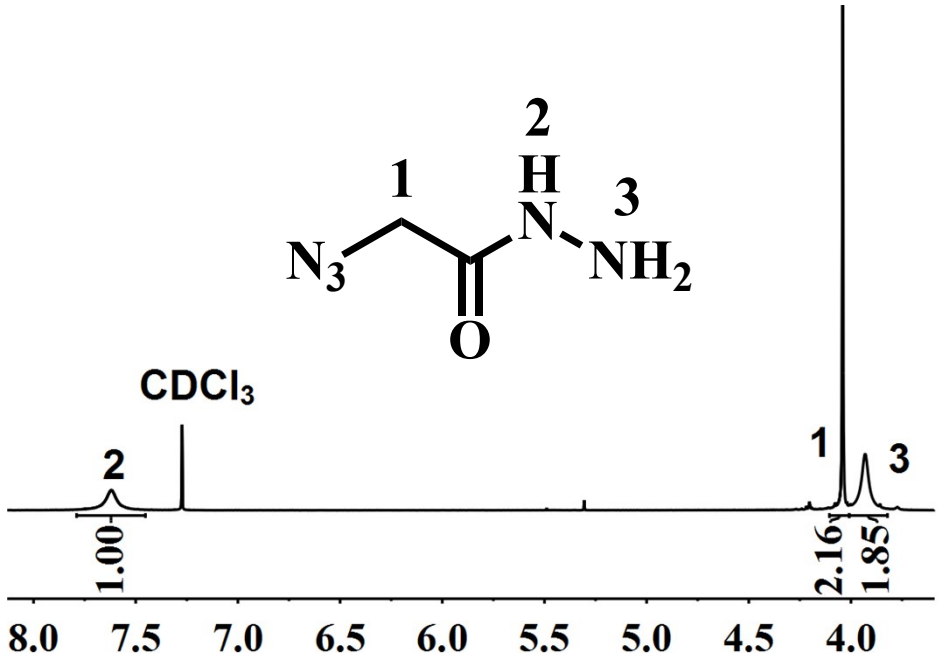

Fig. S10 ^1H NMR spectrum of $NH_2NH\text{-}N_3$ ($CDCl_3$).

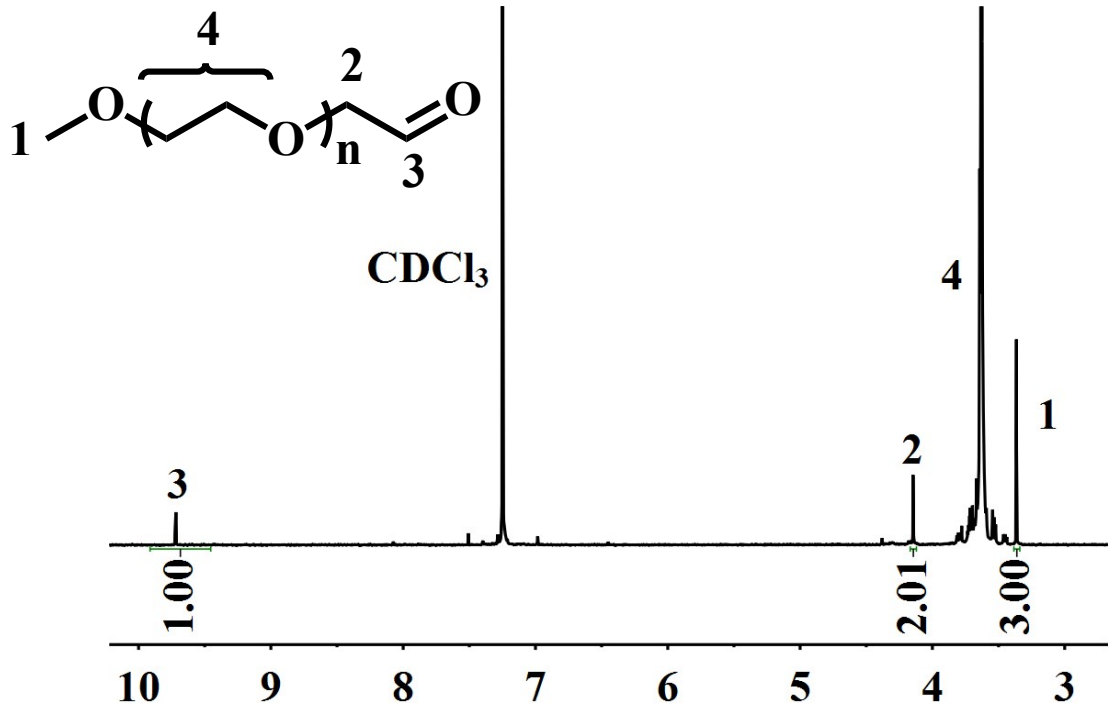

Fig. S11 ^{1}H NMR spectrum of mPEG-CHO ($CDCl_3$).

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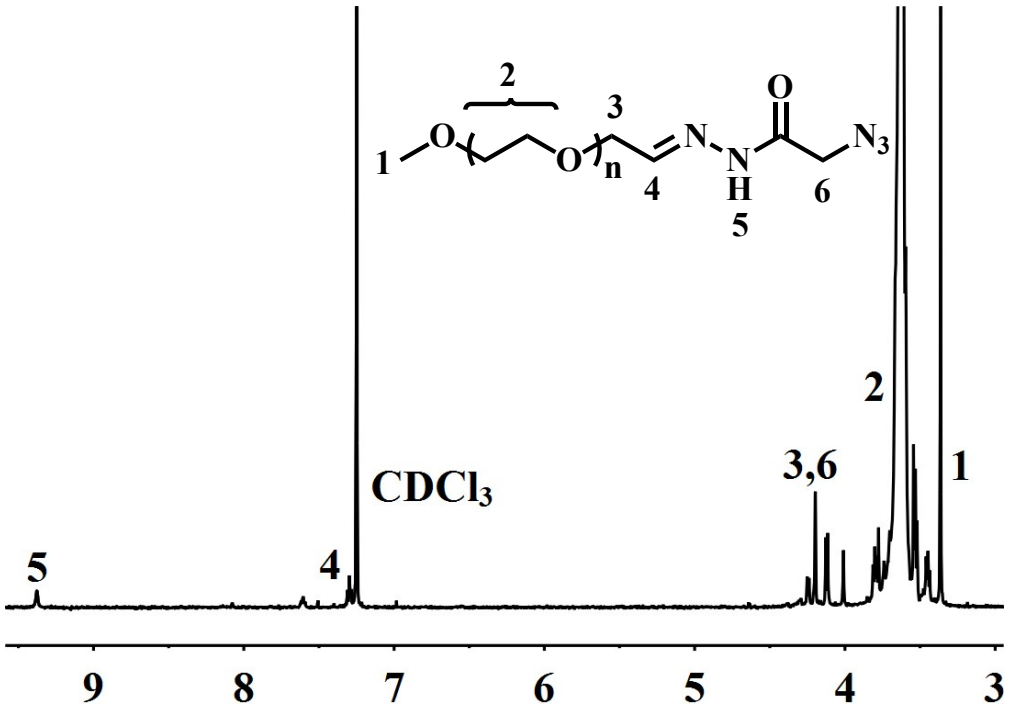

Fig. S12 ^{1}H NMR spectrum of mPEG-NNH-N_3 ($CDCl_3$).

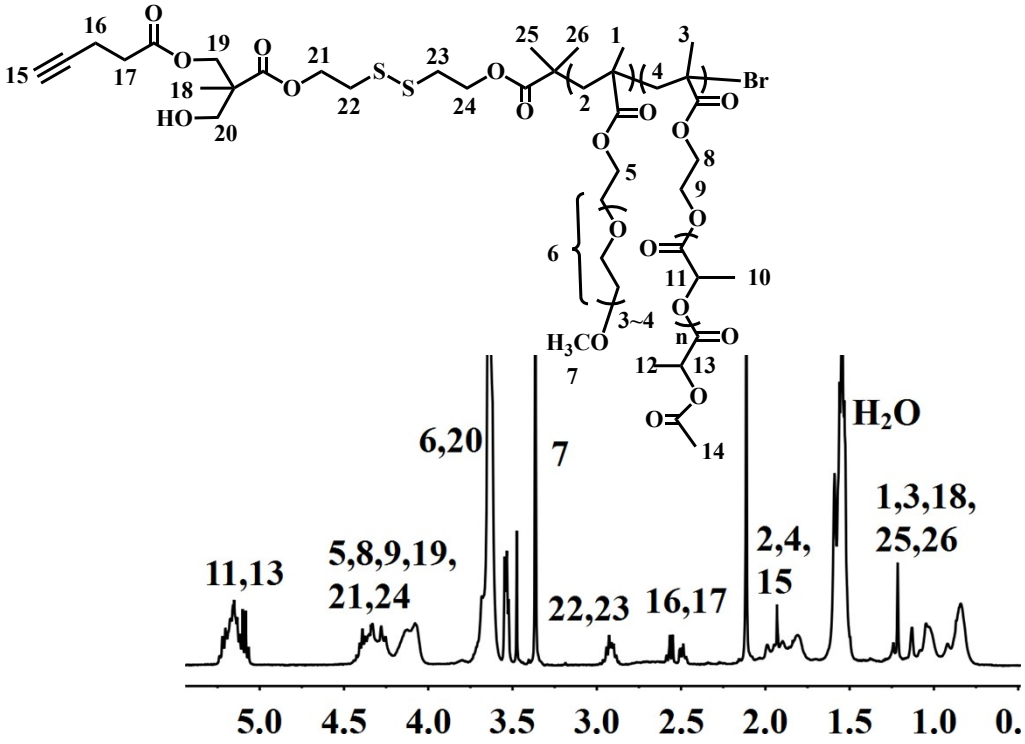

Fig. S13 ^{1}H NMR spectrum of P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ ($CDCl_3$).

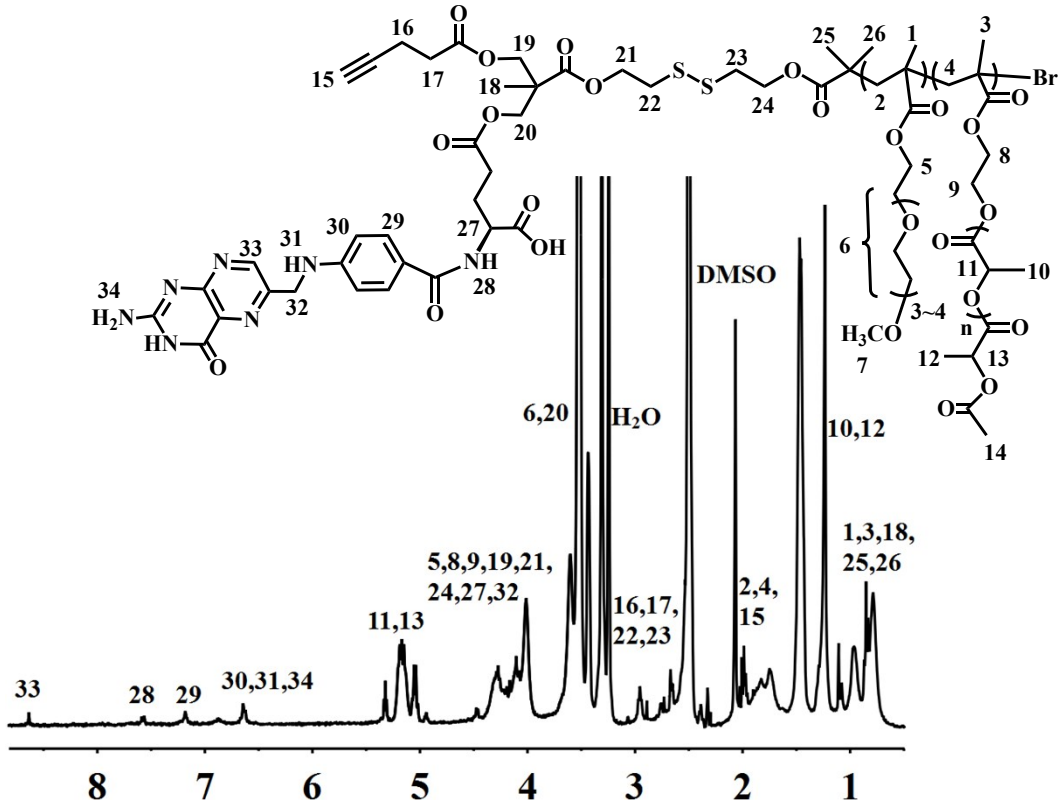

Fig. S14 ^{1}H NMR spectrum of FA-P(OEGMA300)$_8$-*st*-P(HEMA-*g*-LA)$_4$ (d_6-DMSO).

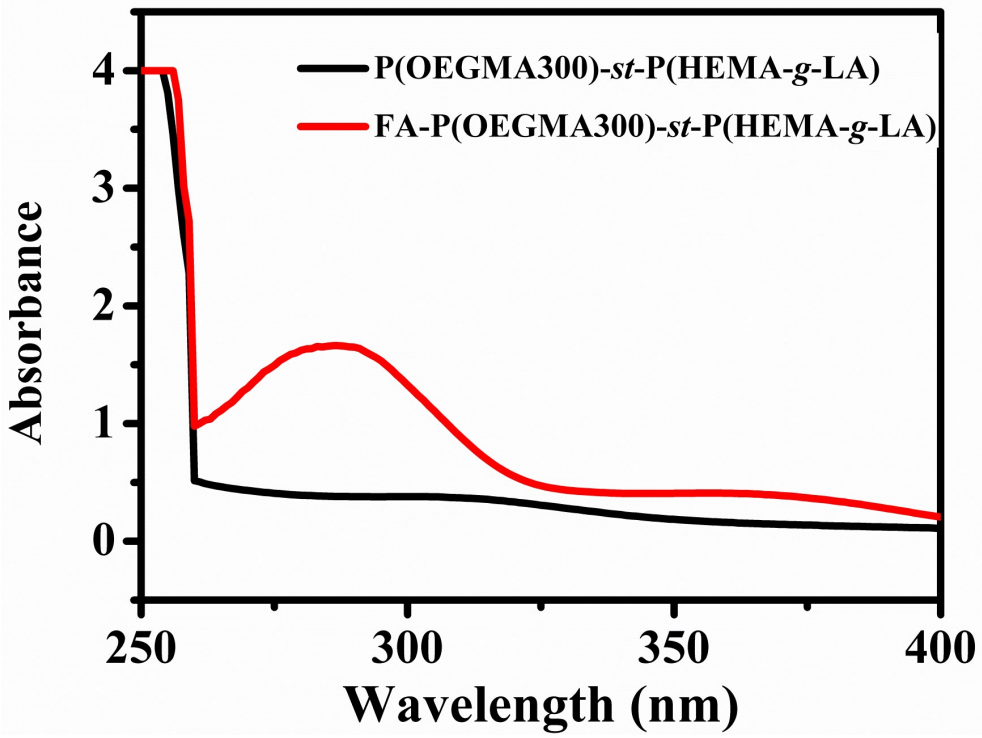

Fig. S15 UV-Vis absorbance of P (OEGMA300)$_4$-*st*-P (HEMA-*g*-LA)$_4$ and FA- P (OEGMA300)$_4$-*st*-P (HEMA-*g*-LA)$_4$ in DMSO.

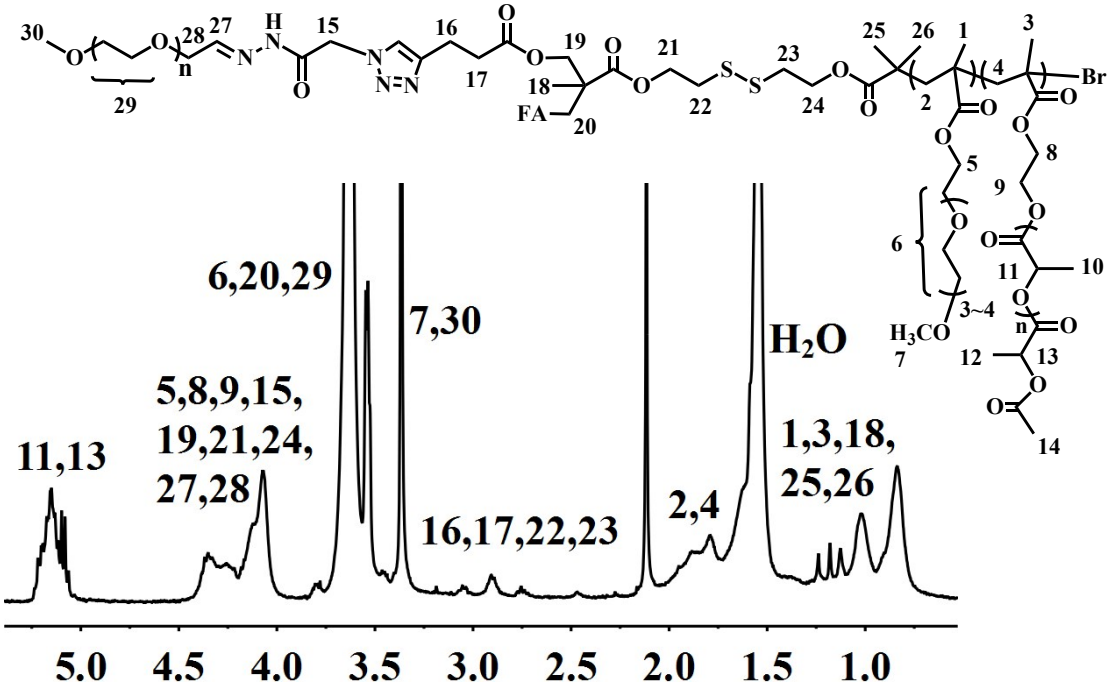

Fig. S16 ^{1}H NMR spectrum of mPEG(FA)-P(OEGMA300)$_8$-*st*-P(HEMA-*g*-LA)$_4$ ($CDCl_3$).

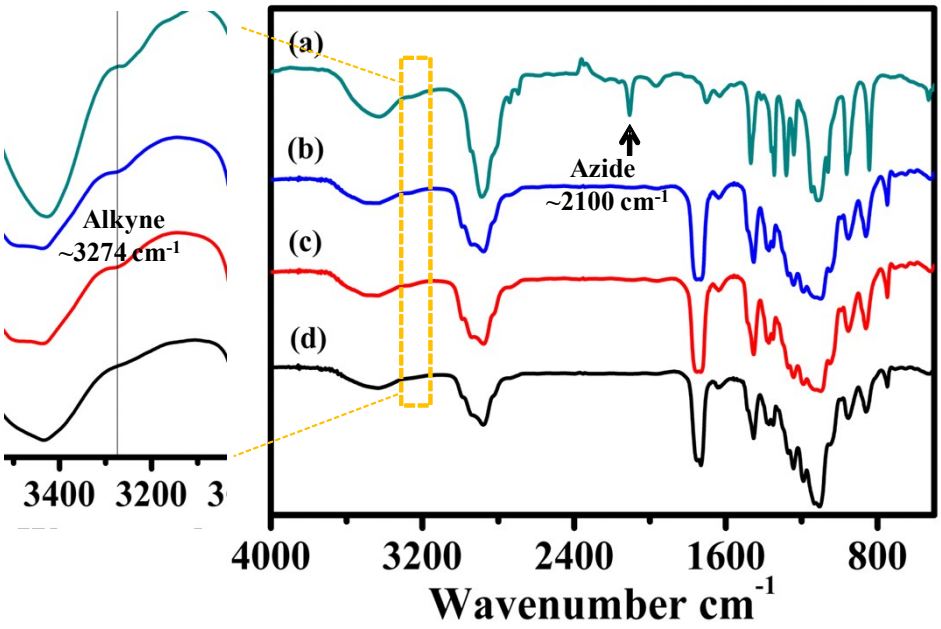

Fig. S17 FT-IR spectra of (a) mPEG-NNH-N_3, (b) P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$, (c)FA-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$, (d) mPEG(FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$.

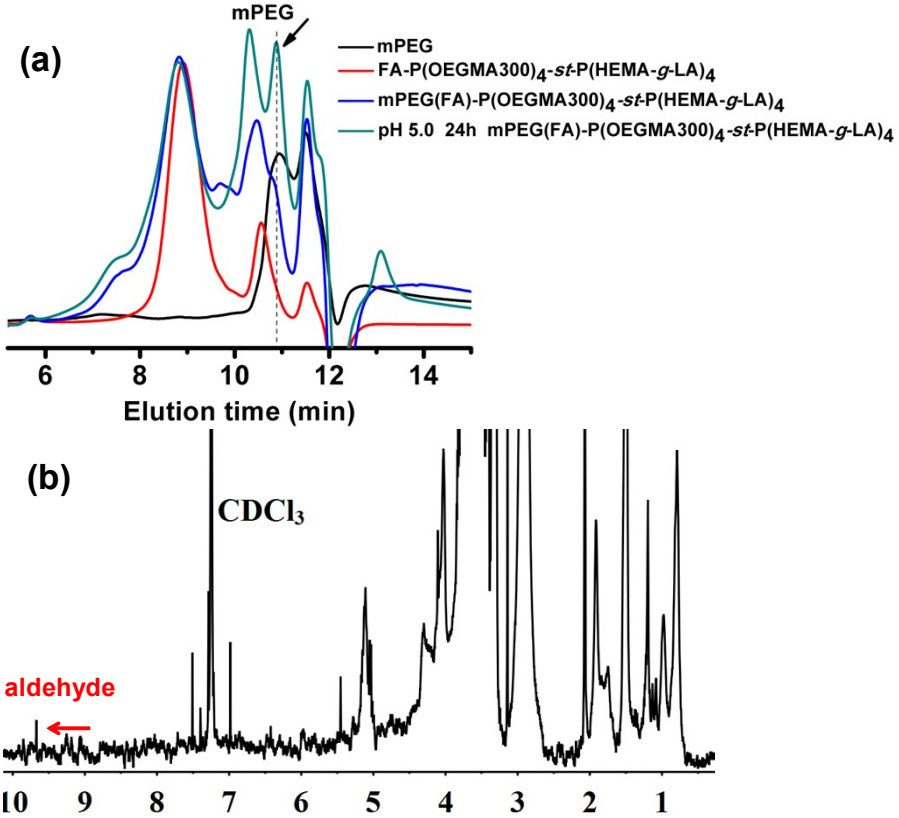

Fig. S18 (a) SEC elution traces of mPEG, FA-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$, mPEG(FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ and mPEG(FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ at pH 5.0 for 24 h using THF as the eluent, (b) ^{1}H NMR spectrum of mPEG(FA)-P(OEGMA300)$_4$-*st*-P(HEMA-*g*-LA)$_4$ at pH 5.0 for 24 h ($CDCl_3$).

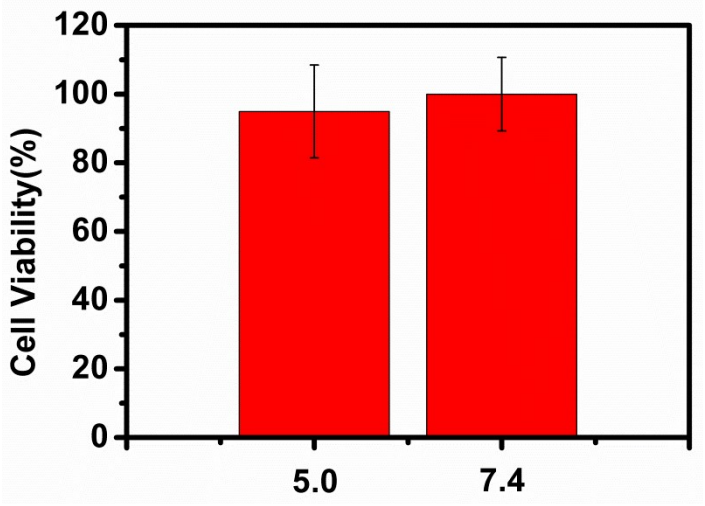

Fig. S19 *In vitro* cytotoxicity of HeLa cells in phosphate buffer (PB, pH 7.4, 10 mM) and acetate buffer (10 mM, pH 5.0) for an incubation period of 24 h. Cell viability was determined by MTS assay and expressed as % viability compared to the untreated cells control. The data were expressed as mean ±SD, *n*=3.

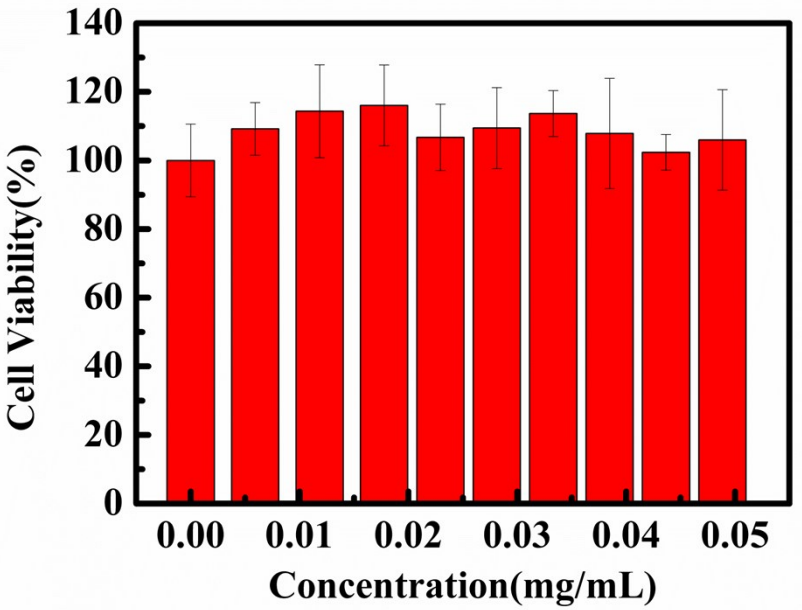

Fig. S20 *In vitro* cytotoxicity of P1 blank micelles (PB, pH 7.4, 10 mM) in HeLa cells for 24 h incubation. Cell viability was determined by MTS assay and expressed as % viability compared to the untreated cells control. The data were expressed as mean ±SD, *n*=3.

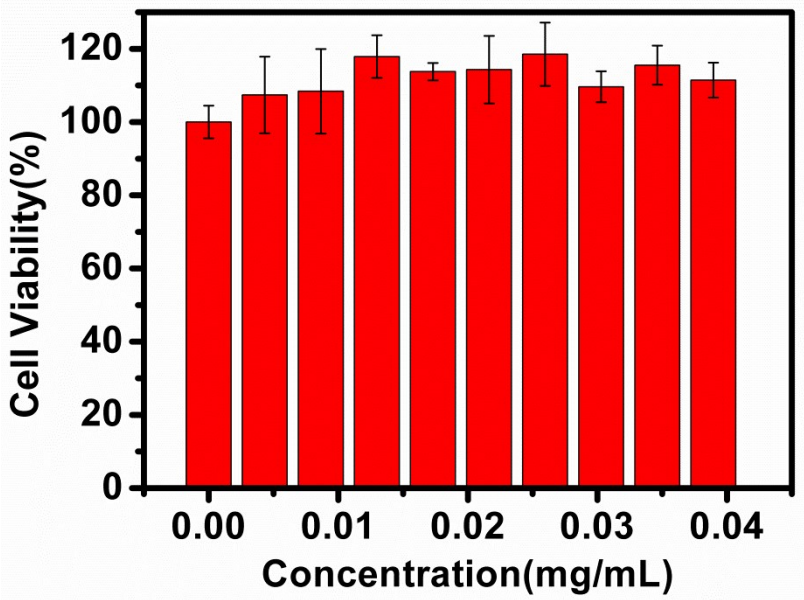

Fig. **S21** *In vitro* cytotoxicity of P1 blank micelles in HeLa cells for an incubation period of 24 h at an acidic pH of 5.0. Cell viability was determined by MTS assay and expressed as % viability compared to the untreated cells control. The data were expressed as mean ±SD, n=3.

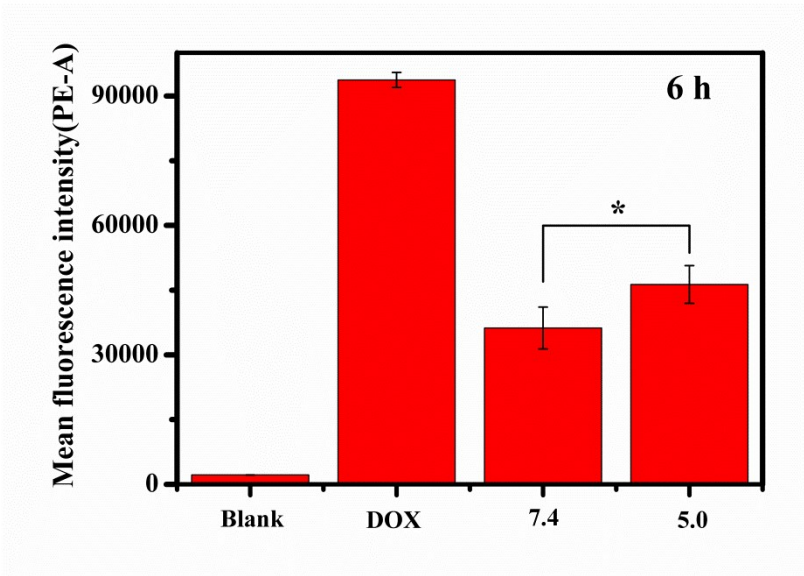

Fig. S22 Quantitative measurements of the mean fluorescence intensityafter incubation with free DOX•HCl, drug-loaded P1 micelles in different release media of phosphate buffer (PB, pH 7.4, 10 mM) and phosphate buffer (PB, pH 5.0, 10 mM) in HeLa cells using FCM (6 h incubation, DOX concentration ≈6.5 μg mL^{-1}, and 10 000 cells were counted). The data were expressed as mean ± SD (n = 3; Student's t-test, *p < 0.05).

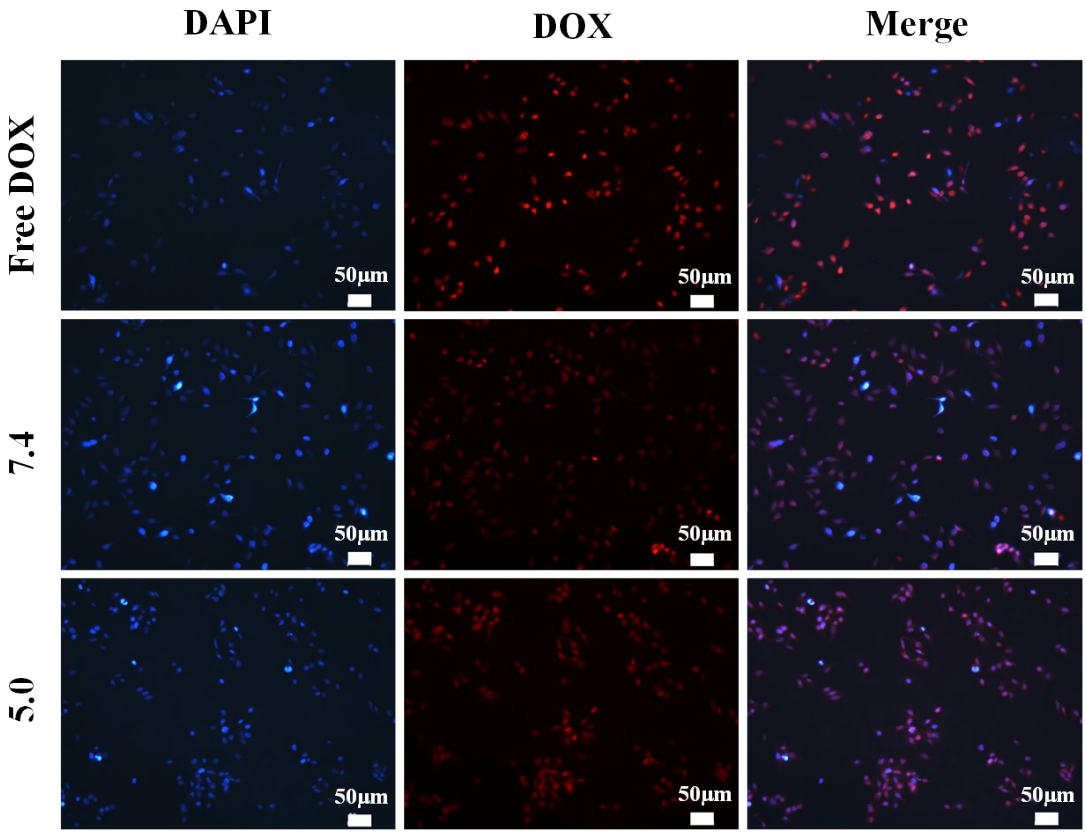

Fig. S23 Confocal images of free DOX (red), drug-loaded P1 micelles in different release media of phosphate buffer (PB, pH 7.4, 10 mM) and acetate buffer (10 mM, pH 5.0) uptake in HeLa cells (6 h, nuclei stained blue with 2-(4-amidinophenyl)- 6-indolecarbamidine (DAPI)). Cells were treated with free DOX or polymer constructs at an equivalent DOX concentration of 6.5 μg mL^{-1}.