Supporting information

Alicyclic Tertiary Amine Based Hyperbranched Polymers with Excitation-independent Emission: Structure, Fluorescence and Applications

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Experimental section

Materials

Ethylene glycol (≥98%, Aladdin Reagent) was dried by refluxing in toluene for removal of water. Tetrahydrofuran (≥99.0%), chloroform (≥99.0%), acetone (≥99.5%), which were purchased from Sinopharm Chemical Reagent Co. Ltd., and acryloyl chloride (≥96%, Aladdin Reagent) were purified by distillation under reduced pressure before use. Dichloromethane (≥99.5%), methanol (≥99.5%), sodium bicarbonate (≥96%), piperazidine (≥99.0%), N-methylpiperazine (≥99.5%), 1-dodecanethiol (≥98.0%), D-galactose (≥98.0%), zinc chloride (≥98.0%), thiourea (≥99.0%), thionyl chloride(≥99.0%), 2-ethyl-2-hydroxymethyl-1,3-propanediol (≥98.0%) and anhydrous magnesium sulfate with analytical grade were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received. N-aminoethyl piperazine (AEPZ, ≥99%, Aldrich), Doxorubicin (DOX, ≥99%, Aldrich), ethylene sulfide, 1, 3-propanedithiol (≥98%), glycidyl methacrylate (GMA, ≥98%) and tetrabutylammonium bromide (≥98%) were used as received. Tris(2-mercaptoethyl) amine (TMEA) was synthesized according to our previous method.[11] ^{1}H NMR ($CDCl_3$, 400 MHz, ppm from TMS): 2.83-2.50 (12H, HSCH_2CH_2-); 1.75 (3H, *H*SCH_2CH_2-); ^{13}C NMR ($CDCl_3$, ppm): 57.08 (CH_2*C*H_2-); 23.04 (HS*C*H_2-); HRMS (EI+): m/z calcd for $C_6H_{15}NS_3$: 197.388, found: 198.0442

Characterization

Nuclear Magnetic Resonance (*NMR*). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer using $CDCl_3$, DMSO-d_6 or D_2O as solvent.

Size exclusion chromatography (SEC) measurements. Molecular weight and molecular weight distribution were determined by triple detection size exclusion chromatography (TD-SEC) at 25°C. The instrumentation consists of the following: a Waters 1515 isotratic HPLC pump with 5 μm Waters styragel columns (guard, 0.5 HR, 1 HR, 3 HR, 4 HR and 5 HR, the molecular weight ranges of these columns are 0-1000, 100-5000, 500-30000, 5000-500000 and 50000-4000000 g/mol, respectively); a Waters 717 PLUS autosamples; a Waters 2414 differential refractive index (DRI) detector, the wavelength is 880 nm; a multi angle laser light scattering (MALLS) detector (Wyatt mini Dawn TRISTRA

light scattering, three detection angles are 45°, 90° and 135°, the wavelength and power are 690 nm and 220 w); a Wyatt Visco Star viscometer detector; a Waters Breeze data manager. The eluent was HPLC grade THF delivered at 1.0 mL/min. The refractive index increment (dn/dc) was determined using a Wyatt Optilab REX (λ=640 nm) interferometric differential refractometer in bath model at 25 ºC.

Measurement of fluorescence spectra. Fluorescence spectra of the resultant polymers were recorded at room temperature on on a Hitachi F-4600 fluorometer (Hitachi Co. Ltd., Japan).

Fluorescence microscopy. Fluorescence microscopy images were recorded on an Olympus IX70 microscope with three kinds of filter (WU: 330–385, WB: 460–490, and WG: 510–550 nm).

Laser confocal scanning microscope. Laser confocal fluorescence microscope images were performed on a Carl Zeiss LSM 510 with three kinds of filter (WU: 330–385, WB: 460–490, and WG: 510–550 nm).

UV-visible spectroscopy. UV-visible spectra were acquired on a Shimadzu UV-2401PC UV-Visible scanning spectrophotometer at room temperature.

The lifetime of fluorescence. The measurements of fluorescence lifetime were conducted on a FLS 920 spectrometer (Edinburgh Instruments, UK) under excitation wavelength of 375nm.

High-resolution mass spectra. High-resolution mass spectra were recorded on a Micromass GCT TOF mass spectrometer at resolutions of 7000 FWHM (EI) by direct introduction at a nominal electron energy of 70 eV for EI, a source temperature of 180◦C for EI, or recorded on LTQ Orbitrap XL (Thermo-Fisher Scientific) with Nano ESI^+, source spray voltage 3kV, APCI vaporizer temperature 250.00℃, and capillary temperature 275.00 °C.

Dynamic light scattering (DLS). The size distribution of NPs in aqueous solution measured by dynamic light scattering (DLS) were carried out on a Malvern Zetasizer Nano ZS90 with a He=Ne laser (633 nm) and 90 ° collecting optics. All samples with a concentration of 0.2 mg/mL were filtered through a 0.45 μm membrane filter (Millipore) prior to measurements.

The quantum yield. The quantum yield of the polymers was calculated according to the following equation:

$$\Phi_{SA}=\Phi_{ST}(S_{SA}/S_{ST})\,(\eta_{SA}/\eta_{ST})^2$$

Where Φ=quantum yield; S=gradient of the curve obtained from the plot of intensity versus absorbance; η=refractive index of the solvent; SA=the sample, and ST=the standard. Anthracene (quantum yield = 0.305 in $CHCl_3$) was used as a standard. The polymers and anthracene were all dispersed in $CHCl_3$. The slit width kept the same for both the standard and the samples. Absorbance was measured on a Shimadzu UV-2401PC spectrophotometer.

Dynamic light scattering. The size and size distribution of the HypTE-AlpGP NPs and DOX-encapsulated HypTE-AlpGP NPs in aqueous solution measured by dynamic light scattering (DLS) were carried out on a Malvern Zetasizer Nano ZS90 with a He-Ne laser (633 nm) and 90°collecting optics. All measurements were carried out at 25 ^{o}C, and the data were analyzed by Malvern Dispersion Technology Software 4.20.

Viability Assay

The cytotoxicity of HypET-AlpGP was evaluated in vitro by MTT assay. Human hepatocellular liver carcinoma HepG2 cells were seeded in 96-well plates at 8000 cells per well in 100 μL of complete DMEM medium and incubated at 37^{o}C in a 5% CO_2 atmosphere for 24 h. And then the cells were treated with HypTE-AlpGP and PEI in the DMEM medium at varying concentrations. After 24h, it was followed by addition of the 25 μL MTT reagent (in 20 μL of PBS, 5 mg/mL) to each well. The cells were further incubated for 3 h at 37 ^{o}C. The medium in each well was then removed and replaced by 150 μL of DMSO. The plate was gently agitated for 15 min before the absorbance (A) at 490 nm was recorded by a microplate reader (Bio-Rad). The cell viability is calculated as $A_{490,\text{treated}}/A_{490,\text{control}}\times 100\%$, where $A_{490,\text{treated}}$ and $A_{490,\text{control}}$ are the absorbance values of the cells cultured with HypTE-AlpGP and PEI, respectively. Each experiment was done in triple. The data are shown as the mean value plus a standard deviation.

Preparation of HypTE-AlpGP-DOX and cytotoxicity assay

The HypTE-AlpGP-DOX was prepared as follows. Typically, 40 mg HypTE-AlpGP was dissolved in 2 mL DMF, followed by adding a predetermined amount of Doxorubicin·HCl (DOX·HCl, two molar equivalents of triethylamine should be added) and stirred at room temperature for 2 h. Then the mixture was slowly added into 8 mL deionized water and stirred for another 2 h. Subsequently, the solution was dialyzed against deionized water for 24 h (MWCO = 3500 g mol^{-1}) and the deionized water was exchanged every 4 h. In order to determine the total loading of drug, the DOX-loaded micelle solution was lyophilized and then dissolved in DMF again. The UV absorbance of the solution at 500 nm was measured to determine the total loading of DOX. Drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following formula:

DLC (wt%) = (weight of loaded drug/weight of polymer) × 100%

DLE (%) = (weight of loaded drug/weight in feed) × 100%

DLC and DLE of HypTE-AlpGP-DOX were determined to be 4.0 % and 40% respectively.

Cytotoxicities of free doxorubicin and doxorubicin nanoparticles were determined in vitro by MTT assay, and the detailed procedure is the same as that described in the above viability assay section.

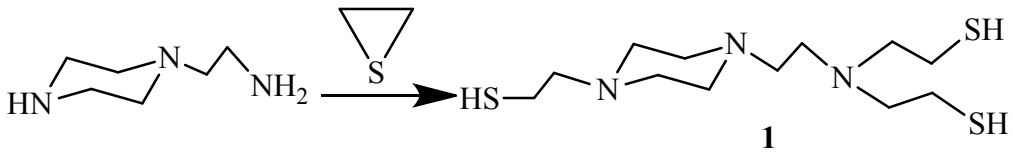
Cell Internalization

Cell Internalization. Human hepatocellular liver carcinoma HepG2 cells from the American Type Culture Collection (Manassas, VA) were maintained in Dulbecco's Modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Hyclone), 100 mg/mL streptomycin, and 100 U/mL penicillin in a humidified atmosphere (relative humidity: 95%; 5% CO_2) at 37 ^oC. Cells were seeded in a μ-Dish35 mm, high (ibidi GmbH, Germany) in culture medium for 24 h. After the cell medium was aspirated, HypTE-AlpGP in serum-free DMEM was applied at a fixed final concentration (2 or 4 mg/mL) for 9h. After being washed with phosphate buffered saline (PBS, 1 mg/mL) three times, the cells were fixed with 4% formaldehyde. The slides mounted and observed using a Zeiss LSM510 Laser Confocal Scanning Microscope imaging system.

Synthesis of ethylene glycol diacrylate (EGDA)

Ethylene glycol (6.2 g, 0.1 mol), triethylamine (TEA, 25.3 g, 0.25 mol) and anhydrous dichloromethane (80 mL) were placed into a 250 mL round-bottom flask with a magnetic stirrer. After the homogeneous solution was cooled to 0^{o}C, acryloyl chloride (22.6 g, 0.25 mol) in 20 mL of anhydrous dichloromethane was dropwise added under N_2 atmosphere, and a white precipitate of triethylammonium bromide was formed immediately. The addition was lasted for 45 min, and then the reaction mixture was warmed to room temperature in approximately 2 h, the reaction continued overnight while stirring. After the salt of TEA was removed by filtration, the filtrate was washed with aqueous solution of sodium bicarbonate and sodium chloride three times, respectively. The organic layer was dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in *vacuo,* the pure product in 50% yield was obtained as colorless oil by subsequent distillation. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.40 and 6.15 (4H, C*H*H=C*H*CO-); 5.87 (2H, CH*H*=CHCO-); 4.40 (4H, -OCH_2CH_2O-).

Synthesis of N',N',N"-tris(2-mercaptoethyl)N-aminoethyl piperazine (TMEAP).



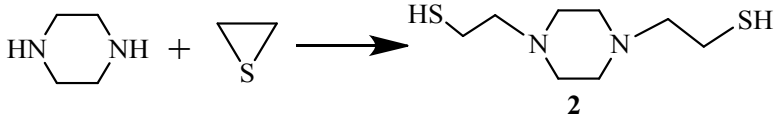
1

Scheme S1. Synthesis of N',N',N"-tris(2-mercaptoethyl)N-aminoethyl piperazine

N',N',N"-tris(2-mercaptoethyl)N-aminoethyl piperazine **1** was synthesized according to Scheme S1. Ethylene sulfide (19.8 g, 0.33 mol) was dropwise added into a solution of AEPZ (12.92g, 0.1 mol) in anhydrous THF (45 mL) under N_2 atmosphere in a 100 mL three-necked round-bottom flask, which was fitted with a dropping funnel and a reflux condenser while stirring. The addition was carried out at ambient temperature for 1h, and the reaction continued at 50°C

for 10h, then the reaction solution was cooled to room temperature. After the solvent was removed in *vacuo*, the residue was dissolved in 20 ml $CHCl_2$ and acidified by slowly adding the diluted hydrochloric acid solution. The solution was extracted with 3×25 mL of chloroform, the aqueous phase was neutralized by NaOH solution, and the neutral solution was extracted with 3×25 mL of chloroform. The extracts were dried over anhydrous magnesium sulfate, after filtration, the solvent was removed in *vacuo.* The pure product **1** was obtained in 15% yield as colorless oil. ^{1}H NMR ($CDCl_3$, ppm, TMS): 2.2-3.1(24H, -C*H*$_2$C*H*$_2$NC*H*$_2$C*H*$_2$-); 1.5-2.1(3H, *H*SCH$_2$CH$_2$-); ^{13}C NMR ($CDCl_3$, ppm): 60.85 (-N(***C***H$_2$CH$_2$SH)$_2$); 57.43 (HSCH$_2$***C***H$_2$N); 56.49 (-CH$_2$***C***H$_2$N(CH$_2$CH$_2$SH)$_2$); 54.50 (N-***C***H$_2$CH$_2$N); 53.50 (HSCH$_2$CH$_2$N(CH$_2$***C***H$_2$)$_2$N; 52.61 ((HSCH$_2$CH$_2$N(***C***H$_2$CH$_2$)$_2$N)； HRMS (APCI+): m/z calcd for $C_{12}H_{27}N_3S_3$: 309.56, found: 310.1438

Synthesis of Synthesis of N,N'-bis(2-mercaptoethyl)piperazine (BMEP) 2.

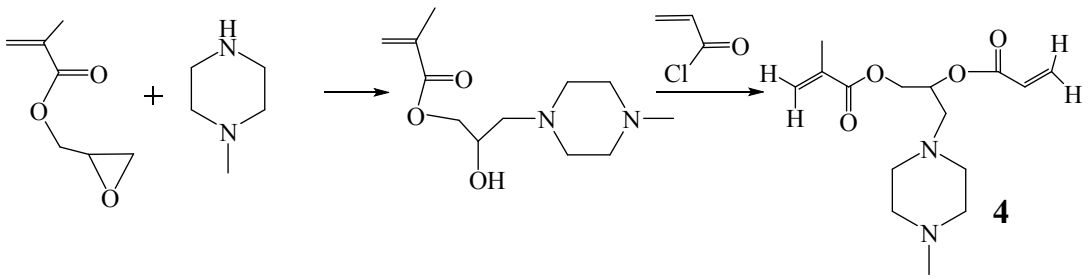


2

Scheme S2. Synthesis of N,N'-bis(2-mercaptoethyl)piperazine

The N,N'-bis(2-mercaptoethyl)piperazine **2** was synthesized according to the previous report.[2] Ethylene sulfide (10.58 g, 0.176 mol) was dropwise added into a solution of piperazidine (6.89 g, 0.08 mol) in anhydrous THF (20 mL) in a 100 mL three-necked round-bottom flask, which was fitted with a dropping funnel and a reflux condenser under N_2 atmosphere, while stirring. After addition was carried out at ambient temperature for 1h and the reaction continued at 50°C for 10h, the reaction solution was cooled to room temperature. When the solvent was removed in *vacuo*, the residue was dissolved in 40 mL CH_2Cl_2. The resultant solution was washed with 3×15 mL of H_2O and dried over anhydrous magnesium sulfate before filtration. The solution was concentrated to about 15 mL and then cooled to -20°C before filtration. The pure product **2** was obtained in 35% yield as a white solid after the solvent removed. ^{1}H NMR ($CDCl_3$, ppm, TMS): 2.2-3.1 (16H, HSC*H*$_2$C*H*$_2$ N(C*H*$_2$C*H*$_2$)$_2$NC*H*$_2$C*H*$_2$SH); 1.79 (2H, *H*SCH$_2$CH$_2$); ^{13}C NMR ($CDCl_3$, ppm): 60.85 (HSCH$_2$***C***H$_2$-); 52.62 (N(***C***H$_2$***C***H$_2$)$_2$N); 21.85 (HS***C***H$_2$CH$_2$-); HRMS (APCI+): m/z calcd for $C_8H_{18}N_2S_2$: 206.09, found: 207.0983

Synthesis of 2-(acryloyloxy)-3-(4-methylpiperazin-1-yl)propyl methacrylate 4.

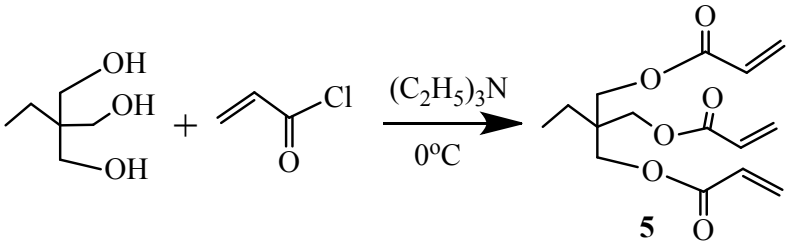


Scheme S3. Synthesis of 2-(acryloyloxy)-3-(4-methylpiperazin-1-yl)propyl methacrylate **4.**

The synthesis of monomer **4** includes two steps. The first step is synthesis of 2-hydroxy-3-(4-methylpiperazin-1-yl)propyl methacrylate **3**. GMA (12.48 g, 0.088 mol) and N-methylpiperazine (9.66 g, 0.096 mol) were added into a 50 mL three-necked flask, and then the reaction was carried out at room temperature under N_2 atmosphere for 24h while stirring. The product was extracted with 30 mL petroleum ether. After the removal of solvent under reduced pressure, the compound **3** was obtained in 80% yield by vacuum distillation.

The second step is reaction of the compound **3** with acryloyl chloride. A mixture of **3** (12.11 g, 0.05 mol), TEA (10.12 g, 0.1 mol) and anhydrous CH_2Cl_2 (70 mL) in a three-necked round-bottom flask was cooled to 0°C while stirring. Into this solution, acryloyl chloride (5.43 g, 0.06 mol) in 10 mL anhydrous CH_2Cl_2 was added slowly at 0°C under N_2 atmosphere. After the addition was completed at 0°C in 1h, the reaction continued overnight. The solid was removed by filtration, and the filtrate was washed by the solutions of saturated $NaHCO_3$, the saturated NaCl in water three times, respectively. The organic layer was then dried over anhydrous magnesium sulfate. After filtration, the solvent in the filtrate was removed in *vacuo*, the pure product **4** was obtained as colorless oil in 35% yield by silica column chromatography (petroleum ether/$CHCl_2$, 1/50, v/v). ^{1}H NMR ($CDCl_3$, ppm, TMS): 5.5-6.7 (5H, C*H*$_2$=C*H*COO and C*H*$_2$=C(CH_3)COO-); 4.46 (H, -OCH$_2$C*H*O-); 4.03 (2H, -OC*H*HCHO- and –OCH$_2$CH(C*H*H-N)O-); 3.26 (2H, -OCH*H*CHO- and –OCH$_2$CH(CH*H*-)O-); 2.86 (4H, -N(C*H*$_2$CH$_2$)$_2$N-); 2.67 (4H, -N(CH$_2$C*H*$_2$)$_2$N-); 1.95 (6H, CH$_2$=C(C*H*$_3$)COO- and -N(CH$_2$CH$_2$)$_2$NC*H*$_3$); HRMS (APCI+): m/z calcd for $C_{15}H_{24}N_2O_4$: 296.17, found: 297.1805

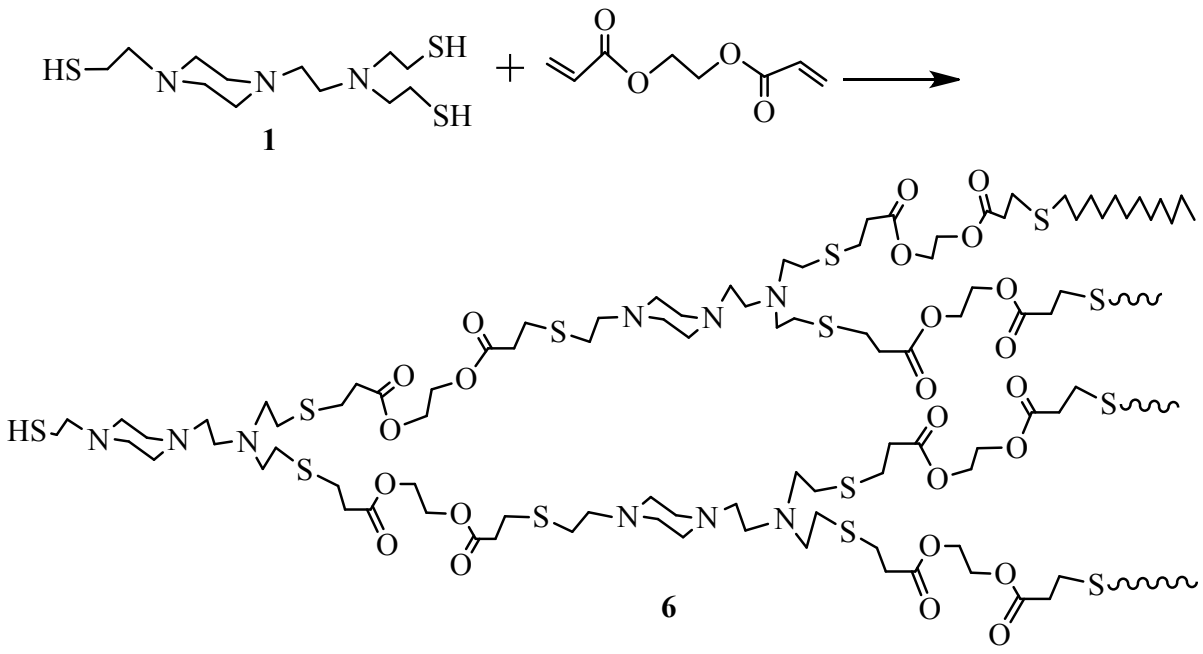
Synthesis of 1,1,1-tris(acryloyoxymethyl)propane (TAMP) 5.



Scheme S4. Synthesis of 1,1,1-tris(acryloyoxymethyl)propane 5.

The TAMP was synthesized according to previous report.[3] 1,1,1-Tris(hydroxymethyl) propane (4.47 g, 33 mmol), triethylamine (TEA, 15.29 g, 0.15 mol) and anhydrous dichloromethane (20 mL) were placed into a 100 mL round-bottom flask with a magnetic stirrer. After the homogeneous solution was cooled to 0°C, acryloyl chloride (9.97 g, 0.1 mol) in 10 mL of anhydrous dichloromethane was dropwise added under N_2 atmosphere, and a white precipitate of triethylammonium chloride was formed immediately. The addition was lasted for 50 min, and then the reaction mixture was warmed to room temperature in approximately 2 h, the reaction continued for another 24h while stirring. After the salt of TEA was filtered, the filtrate was washed with aqueous solution of sodium bicarbonate and sodium chloride three times, respectively. The organic liquid was dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in *vacuo,* the pure product TAMP **5** was obtained in 53% yield as colorless oil by silica column chromatography (2% petroleum ether/$CHCl_2$). ^{1}H NMR ($CDCl_3$, ppm, TMS): 6.38 and 6.13 (6H, 3C*H*H=C*H*CO-); 5.85 (3H, 3CH*H*=CHCO-); 4.18 (6H, 3-CH_2OOC-); 1.55 (2H, CH_3CH_2-); 0.96(3H, CH_3CH_2-)

Synthesis of hyperbranched poly(TMEAP-EGDA) 6.

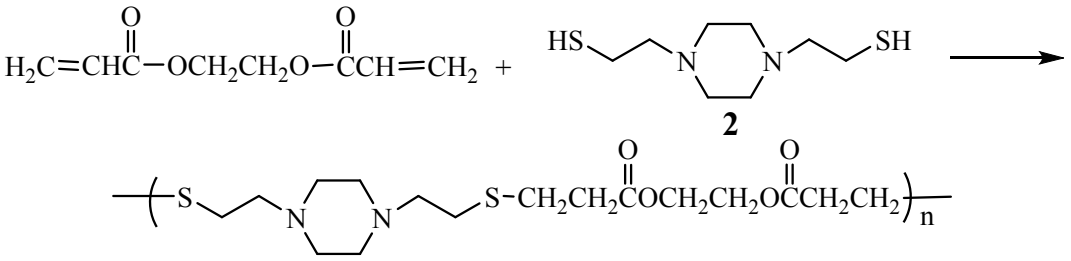


Scheme S5. Hyperbranched polymerization of TMEAP and EGDA.

TMEAP **1** (0.1545 g, 0.5 mmol), EGDA (0.17 g, 1 mmol) and 1 mL chloroform were successively added into a 5 mL glass tube with a magnetic bar, and then the system was degassed by three freeze-pump-thaw cycles. The tube was sealed under vacuum, and then the sealed tube was placed in an oil bath at 50°C. After the polymerization was carried out for 10h, 22h, 29h or 46h, the tube was cooled to room temperature and opened. Into the reaction mixture, 1-dodecanethiol (0.52 g) was added, and the tube was sealed again after three freeze-pump-thaw cycles. The reaction was

carried out for additional 24h at 50°C. After cooling to room temperature, the tube was opened, and the solution was poured into methanol while stirring vigorously, the polymer was precipitated. After filtration and drying in *vacuo* at room temperature for 24h, the target hyperbranched polymers, which were respectively marked as HypTE10, HypTE22, HypTE29 and HypTE46 obtained from the polymerization 10h, 22h, 29h and 46h, respectively, were obtained.

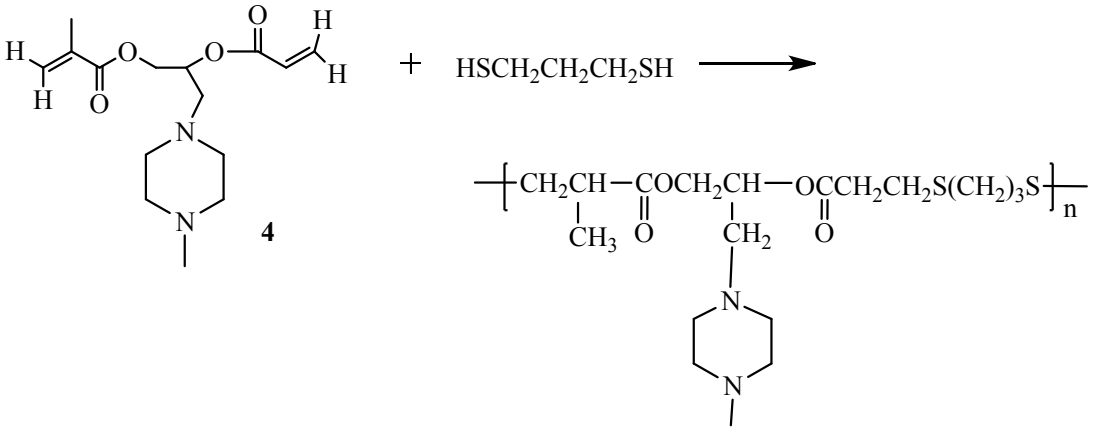
Synthesis of linear polymer from EGDA and N, N'-bis(2-mercaptoethyl)piperazine (BMEP).



Scheme S6. Linear Michael addition polymerization of BMEP and EGDA.

N,N'-bis(2-mercaptoethyl)piperazine **2** (0.307 g, 1.05 mmol), EGDA (0.17 g, 1 mmol) and 1 mL chloroform were successively added into a 5 mL glass tube with a magnetic bar. After three freeze-pump-thaw cycles, the tube was sealed under vacuum, and then the sealed tube was placed in an oil bath at 50°C for 26h. The product was obtained in 56.7% yield after precipitation in diethyl ether, filtration and drying in *vacuo*.

Synthesis of linear polymer with side piperazine group.

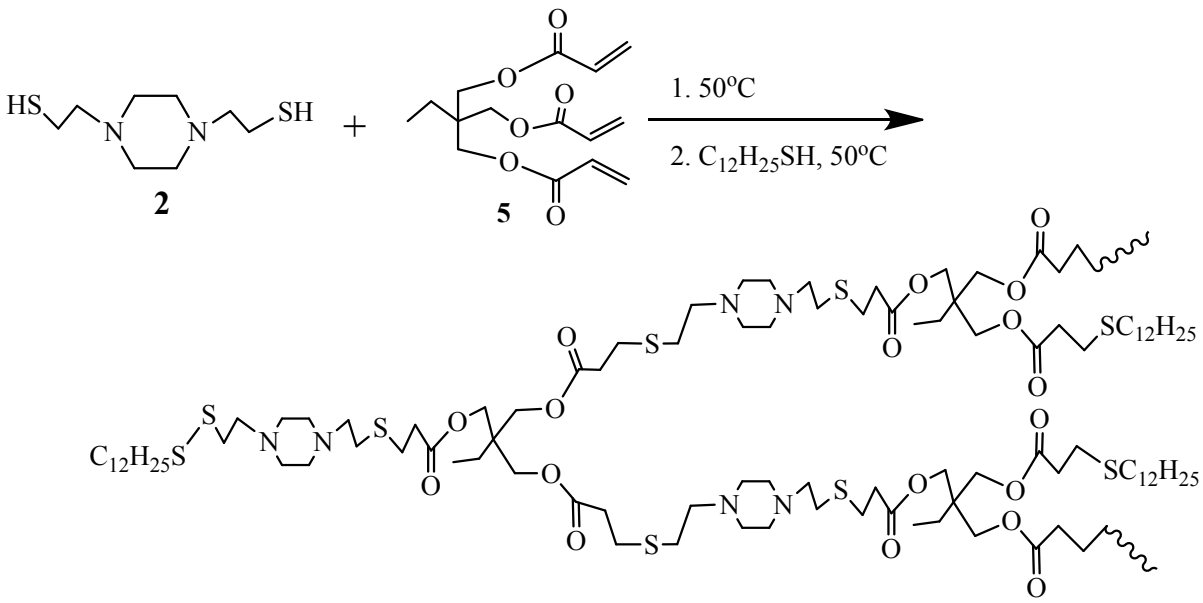


Scheme S7. Michael addition polymerization of 2-(acryloyloxy)-3-(4-methylpiperazin-1-yl) propyl methacrylate **4** and 1,3-propanedithiol.

The compound **4** (0.297 g, 1 mmol), 1,3-propanedithiol (0.108 g, 1 mmol) and tetrabutyl ammonium bromide (2.10g, 6.5 mmol) were successively added into a 5 mL glass tube with a magnetic bar, and then the system was degassed by three freeze-pump-thaw cycles. The tube was sealed under vacuum, and then the sealed tube was placed in an oil bath at

100°C. After the polymerization was carried out for 24h, the tube was cooled to room temperature and opened. The product in methanol was precipitated from distilled water while stirring vigorously. Then the solid was dissolved in THF and precipitation was performed in petroleum ether. The linear polymer was obtained in 38. 3% yield after drying at room temperature under vacuum for 1 day.

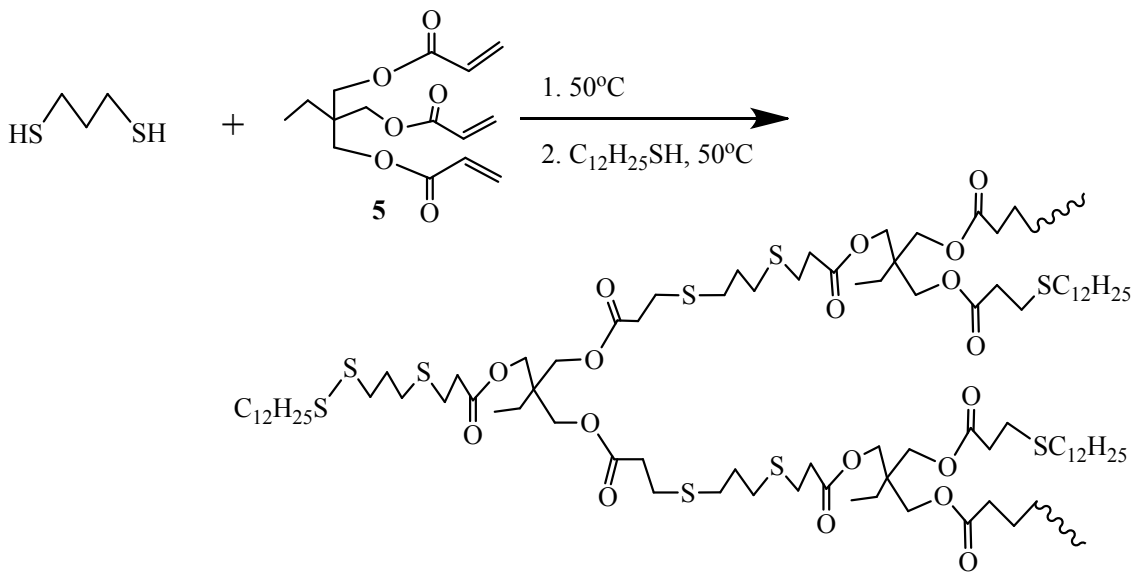
Synthesis of hyperbranched poly(BMEP-TAMP).



Scheme S8. Michael addition polymerization of 1,1,1-tris(acryloyoxymethyl)propane **5** and BMEP **2**.

BMEP **2** (0.163 g, 0.5 mmol), TAMP **5** (0.148 g, 0.5 mmol) and 1 mL chloroform were successively added into a 5 mL glass tube with a magnetic bar. After three freeze-pump-thaw cycles, the tube was sealed under vacuum, and then the sealed tube was placed in an oil bath at 50°C. After the polymerization was carried out for 23h, the tube was cooled to room temperature and opened. Into the reaction mixture, the 1-dodecanethiol (0.304 g) was added, and the tube was sealed again after three freeze-pump-thaw cycles. The reaction was carried out for additional 24h at 50°C. After cooling to room temperature, the tube was opened, and the solution was poured into methanol while stirring vigorously, the polymer was precipitated. After filtration and drying in vacuo at room temperature for 24h, the target hyperbranched polymers, which is marked as HypME23, was obtained in 64.0% yield.

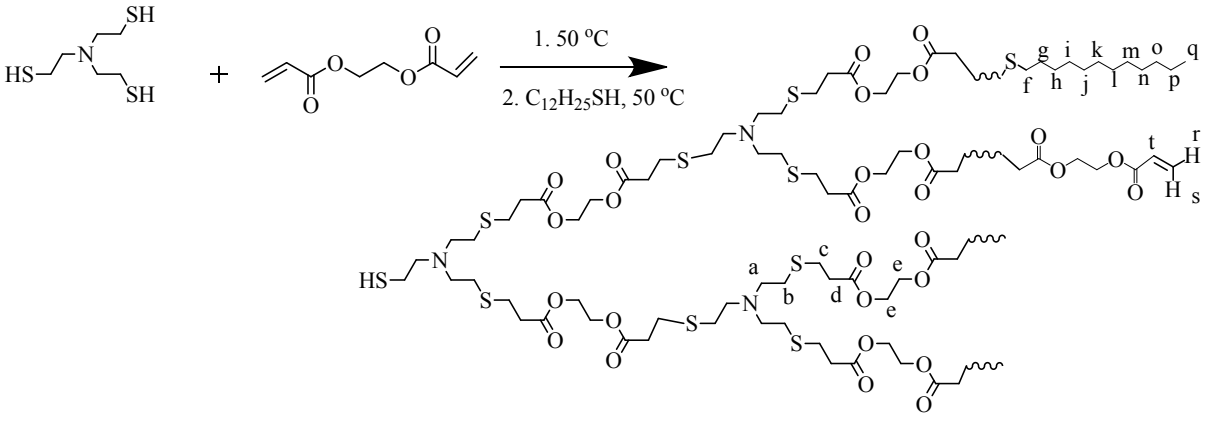
Synthesis of hyperbranched HypAD.



Scheme S9. Michael addition polymerization of 1,1,1-tris(acryloyoxymethyl)propane **5** and 1, 3-propanedithiol.

Hyperbranched HypAD was prepared according to the following procedure. 1, 3-Propanedithiol (54 mg, 0.5 mmol), TAMP **5** (0.148 g, 0.5 mmol) and 1 mL chloroform were successively added into a 5 mL glass tube with a magnetic bar. After three freeze-pump-thaw cycles, the tube was sealed under vacuum, and then the sealed tube was placed in an oil bath at 50°C. After the polymerization was carried out for 23h, the tube was cooled to room temperature and opened. Into the reaction mixture, the 1-dodecanethiol (0.304 g) was added, and the tube was sealed again after three freeze-pump-thaw cycles. The reaction was carried out for additional 24h at 50°C. After cooling to room temperature, the tube was opened, and the solution was poured into methanol while stirring vigorously, the polymer was precipitated. After filtration and drying in vacuo at room temperature for 24h, the target hyperbranched polymers, which is marked as HypAD, was obtained in 83.0% yield.

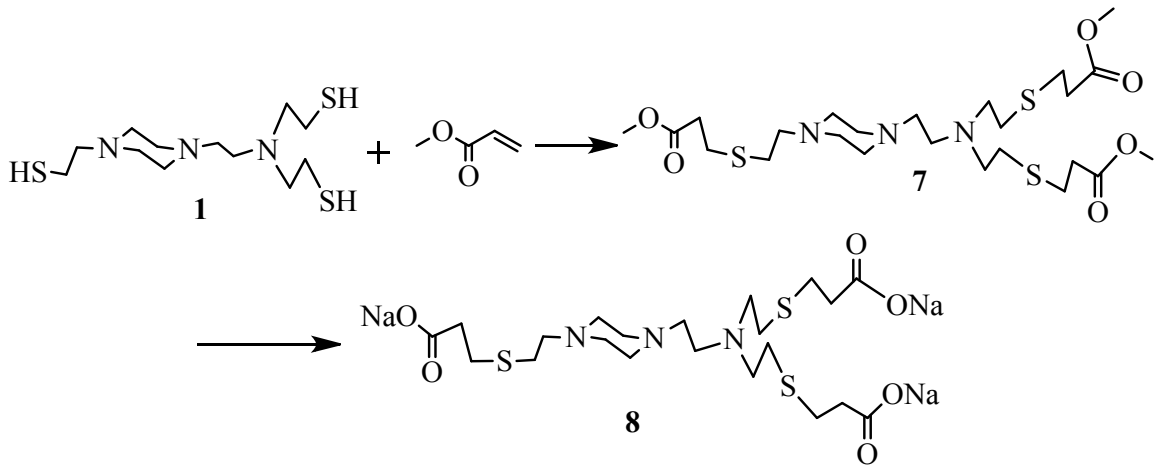
Synthesis of hyperbranched poly(EGDA-TMEA)s (HypETs).



Scheme S10. The HypETs were prepared by Michael addition polymerization of EGDA and TMEA

Tris(2-mercaptoethyl) amine (98.5 mg, 0.5 mmol), EGDA (0.17 g, 1 mmol) and 1 mL chloroform were successively added into a 5 mL glass tube with a magnetic bar, and then the system was degassed by three freeze-pump-thaw cycles. The tube was sealed under vacuum, and then the sealed tube was placed in an oil bath at 50°C. After the polymerization was carried out for 9h, or 13h, or 23h, or 29h, or 33h respectively, the tube was cooled to room temperature and opened. Into the reaction mixture, the 1-dodecanethiol (0.506g) was added, and the tube was sealed again after three freeze-pump-thaw cycles. The reaction was carried out for additional 24h at 50°C. After cooling to room temperature, the tube was opened, and the solution was poured into diethyl ether while stirring vigorously, the polymer was precipitated. After filtration and drying in vacuo at room temperature for 24h, the target hyperbranched polymers, which were respectively marked as HypET9, HypET13 , HypET23, HypET29 and HypET33 for the polymers obtained from 9h, 13h, 23h, 29h and 33h polymerization, were obtained.

Syntheses of N',N',N"-tris(carboxylateethylthioethyl) N-aminoethyl piperazine (TCEAP) 8.

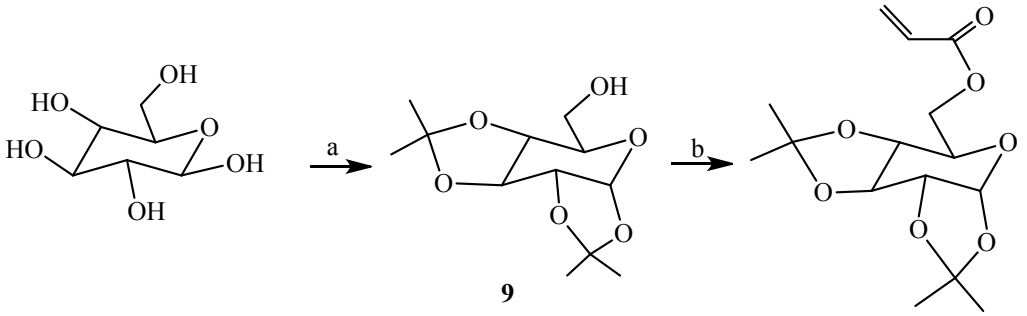


The compound **1** (0.309 g, 1 mmol) and methyl acrylate (0.284 g, 3.3 mmol) were added into a 5 mL glass tube with a magnetic bar. After three freeze-pump-thaw cycles, the tube was sealed under vacuum, the reaction was carried out at room temperature for 24h. The compound **7** was obtained in a yield of 97.3% after removing unreacted methyl acrylate under reduced pressure. ^{1}H NMR ($CDCl_3$, ppm, TMS): 3.69 (9H, -COOCH_3); 2.2-3.0 (36H, the rest H).

The compound **8** was prepared by hydrolysis of the compound **7**. The synthetic procedure is as follows: **7** (323.5 mg, 0.6 mmol) was added into a solution of THF/H_2O (9/1, v/v) containing NaOH (72 mg, 1.8 mmol), and then the hydrolysis was carried out at room temperature under N_2 atmosphere for 24h. After removal of the solvents under reduced pressure, the hydrolyzed product **8** was obtained after dried in *vacuo*. ^{1}H NMR (D_2O, ppm, TMS): 1.9-3.28 (36H, all the H).

Hydrolysis of HypTE. Hydrolysis of the HypTE29 (323.5 mg) was carried out in THF/water (9/1,v/v) solution containing 72 mg of NaOH at 30°C under N_2 for 24h, and then the resultant product was dried in *vacuo*. ^{1}H NMR (D_2O, ppm, TMS): 1.9-3.28 (36H, NC*H*$_2$C*H*$_2$- and C*H*$_2$C*H*$_2$COO-); 1.22 and 0.81 (H from the terminal group).

Synthesis of 1,2:3,4-di-O-isopropylidene-6-O-acryloyl-*α-D*-galactopyranose (DIAlGP) 10.



a. Anhydrous $ZnCl_2$, acetone
b. Acryloyl chloride, Basic Al_2O_3, Anhydrous CH_3CN

Anhydrous $ZnCl_2$ (15.6 g, 114.5 mmol) was dissolved in acetone (163 mL) under N_2 atmosphere while stirring, and a very small amount of $Zn(OH)_2$ presented was dissolved by dropwise addition of H_2SO_4 through septum. Finely ground anhydrous galactose (13 g, 0.072 mol) was added into the solution, and then the solution was stirred for 4 h at room temperature. A suspension of 26 g Na_2CO_3 in 46 mL H_2O was added to the solution and the mixture was stirred until the liquid layer contained zinc ions and filtrated. The solid on the filter was washed with acetone (3×15 mL) and the combined filtrates were evaporated using rotary evaporator under reduced pressure (30 °C, 200 mmHg). The residue was extracted with diethyl ether (3×35mL) and the ethereal extract was dried over $MgSO_4$. After the solvent was removed, the residue was dried in vacuum overnight to afford a sirup-like raw product (16.3g). The raw product was distilled and the pure product was collected at 131–135 °C/0.3 mmHg to afforded 14.5 g of product **9** as a colorless viscous liquid (77.1% of yield). ^{1}H NMR ($CDCl_3$, TMS, ppm): 5.58 (1H, anomeric C*H*), 4.62 (1H, sugar moiety, C*H*), 4.35-4.28 (2H, sugar moiety C*H*), 3.90-3.70 (4H, *H*OC*H*$_2$ and sugar moiety C*H*), 1.54-1.35 (12H, C*H*$_3$).

To a stirred suspension of 1,2:3,4-di-O-isopropylidene-*α-D*-galactopyranose **9** (8.00 g , 3.07×10^{-2} mol) and basic alumina (6.24 g , 14.11×10^{-2} mol) in distilled acetonitrile (80 mL), acryloyl chloride (12.80 mL, 15.82×10^{-2} mol) was added at room temperature. The reaction mixture was stirred for 3 days. The solids were removed by filtration though a micromembrane. Reaction completion was monitored using thin layer chromatography with hexane/ethyl acetate (2:1) as the solvent. The solvent was removed under reduced pressure to yield light yellow oil, which was purified via column chromatography (hexane/ethyl acetate, 2:1). Viscous light yellow oil was obtained in 56% of yield. 1H NMR ($CDCl_3$, ppm, TMS): 6.40 (1H, C*H*H=CH-), 6.17 (1H, CH$_2$=C*H*), 5.84 (1H, CH*H*=CH), 5.53 (1H, anomeric C*H*), 4.61 (1H, sugar moiety C*H*H), 4.13 (4H, sugar moiety C*H*H and 2C*H*), 4.10 (1H, sugar moiety C*H*), 1.33-1.53 (12H, C*H*$_3$).

Synthesis of hyperbranced polymer from EGDA, TMEAP and AlpGP (HypTE-DIAlpGP).

The compound **1** (0.4326 g, 1.4 mmol), EGDA (0.119 g, 0.7 mmol), DIAlpGP (0.2198 g, 0.7 mmol) and chloroform (2 mL) were successively added into a 5 mL glass tube with a magnetic bar. After three freeze-pump-thaw cycles, the tube was sealed under vacuum, and then the sealed tube was placed in an oil bath at 50°C for 33h. The product was obtained in 34.4% yield after precipitation in diethyl ether, filtration and drying in *vacuo*.

The product 0.2 g was dissolved in 10 mL THF, into this solution, aqueous solution of HCl (2 mol/L) was slowly added until the pH reached to about 3, and then the reaction solution was stirred at room temperature for 20h. The HypTE-AlpGP was obtained after dialysis (molecular weight (M_w) cutoff: 3500 Da) against deionized water for 24h.

Table S1. Characterization and properties of the hyperbranched polymers, HypTE10, HypTE22, HypTE29 and HypET46

No[a]	M_n[b] (g/mol)	M_w/M_n[b]	DB[c]	Φ[d]	τ (ns)
HypTE10	5710	3.44	0.67	0.12	—
HypTE22	28460	4.37	0.71	0.15	2.20
HypTE29	51670	3.44	0.82	0.35	2.25
HypTE46	70290	3.18	0.88	0.49	2.26

[a)]HypTE10, HypTE22, HypTE29 and HypTE46 were prepared by polymerization with molar ratio of EGDA/TMEAP=2/1 at 50°C for 10, 22, 29 and 46h, respectively. [b)] Obtained from TD-SEC measurements. [c)] Calculated based on the ^{1}H NMR data. [d)] Quantum yield was measured according to the Williams' method.

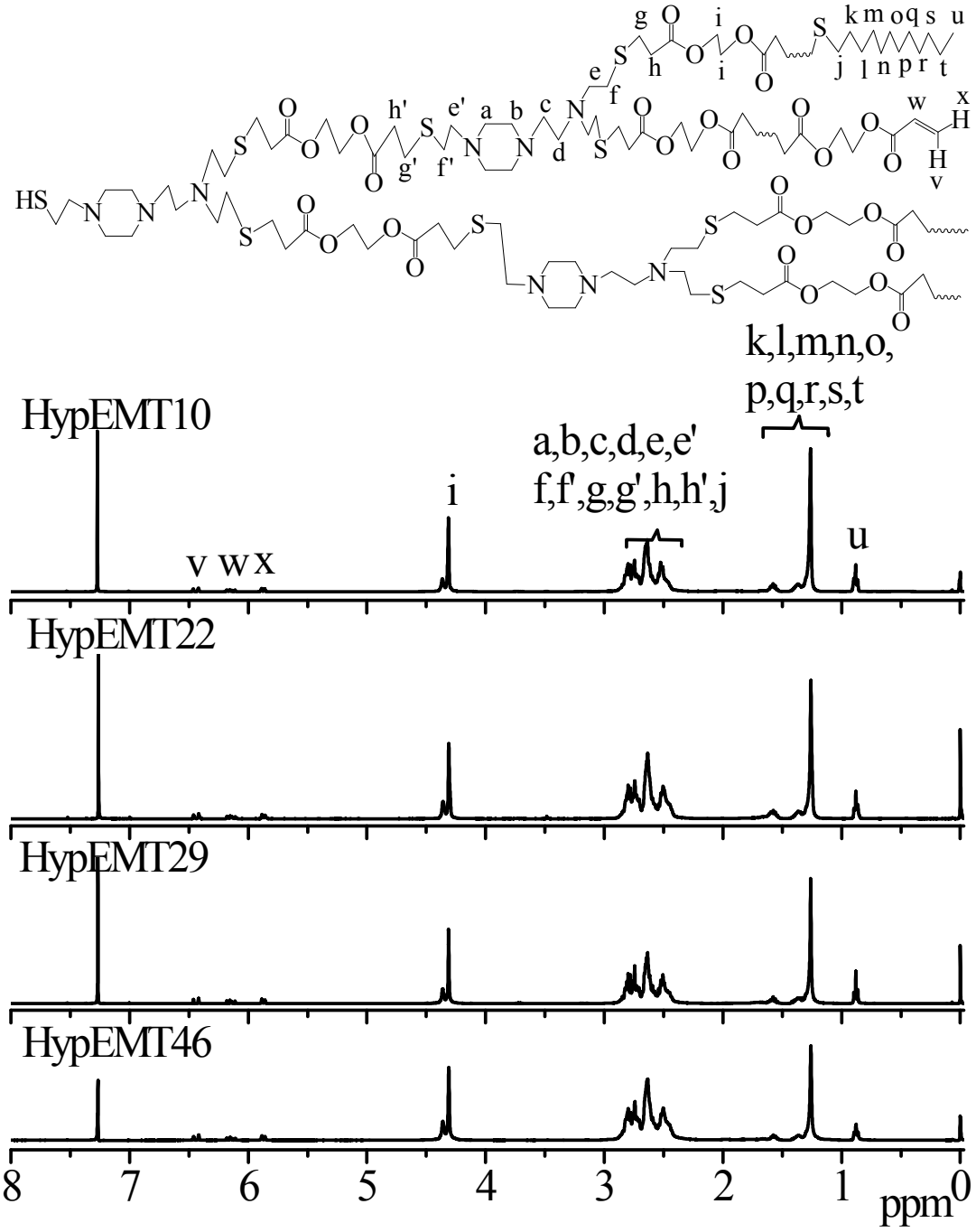


Figure S1.[1]H NMR spectra of HypTE10, HypET22, HypET29 and HypET46 prepared by Michael addition polymerization of EGDA and TMEAP with molar ratio of 2/1 at 50°C for 10h, 22h, 29h and 46h.

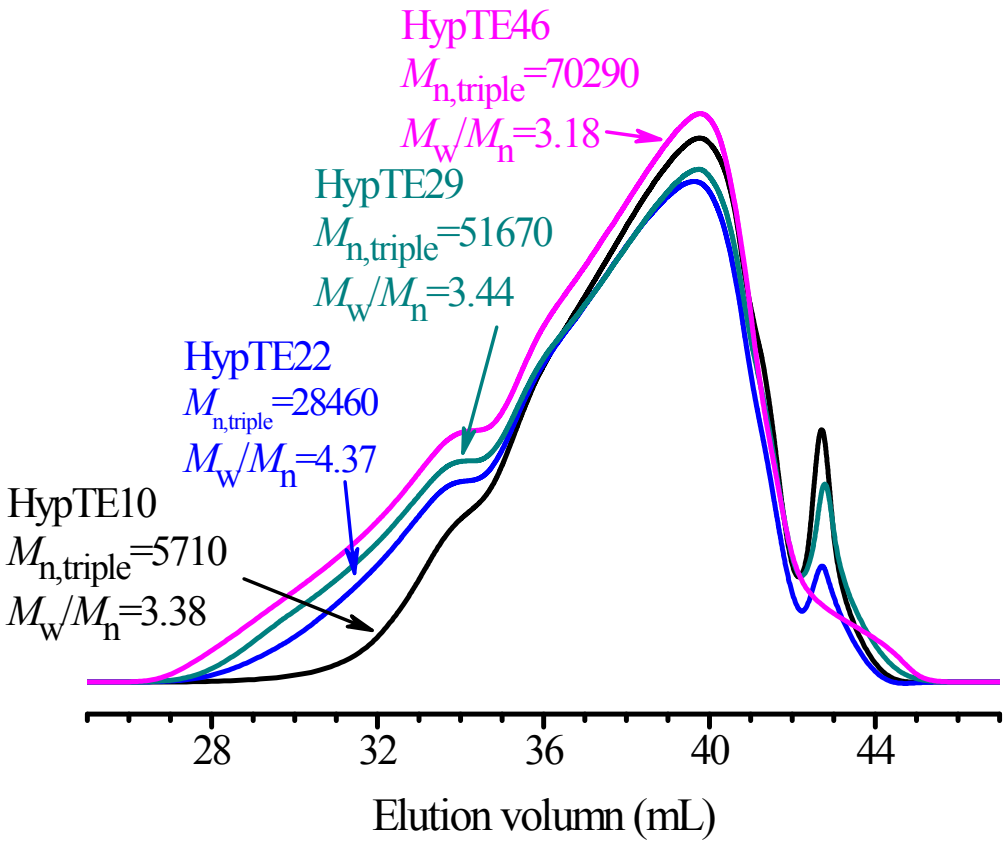


Figure S2. TD-SEC traces of the HypTE10 , HypTE22 , HypTE29 and HypTE46 prepared by Michael addition polymerization of EGDA and TMEAP with molar ratio of 2/1 at 50°C for 10h , 22h , 29h and 46h.

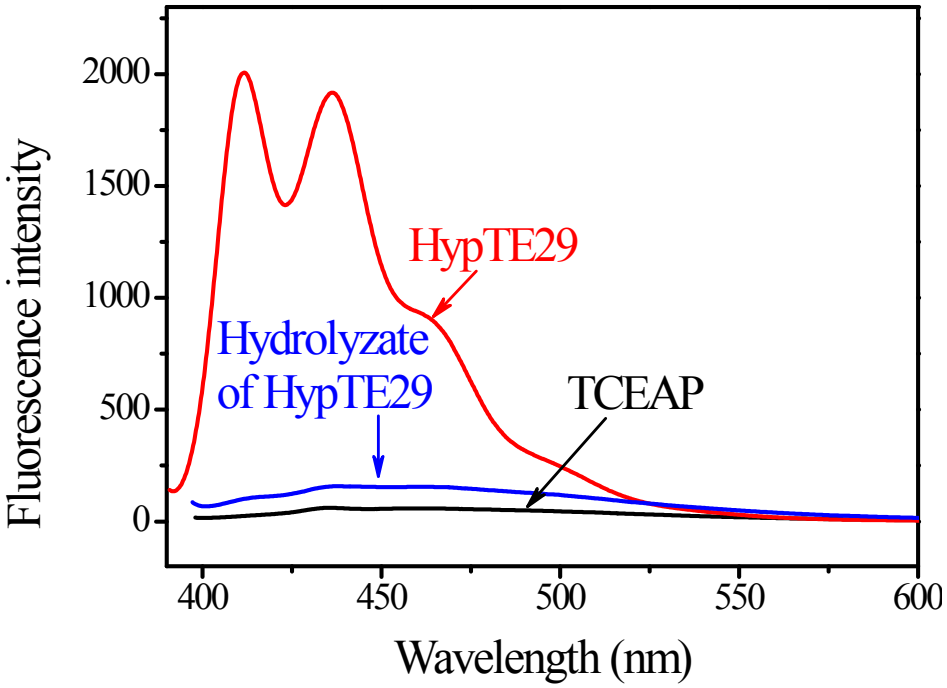


Figure S3. Fluorescence spectra of HypTE29 prepared by Michael addition polymerization with feed molar ratio of EGDA/TMEAP=2/1 at 50°C for 29h, and its hydrolyzed products as well as TCEAP synthesized by reaction of TMEAP and methyl acrylate, and then hydrolysis in a NaOH solution in THF/H_2O (9/1, v/v).

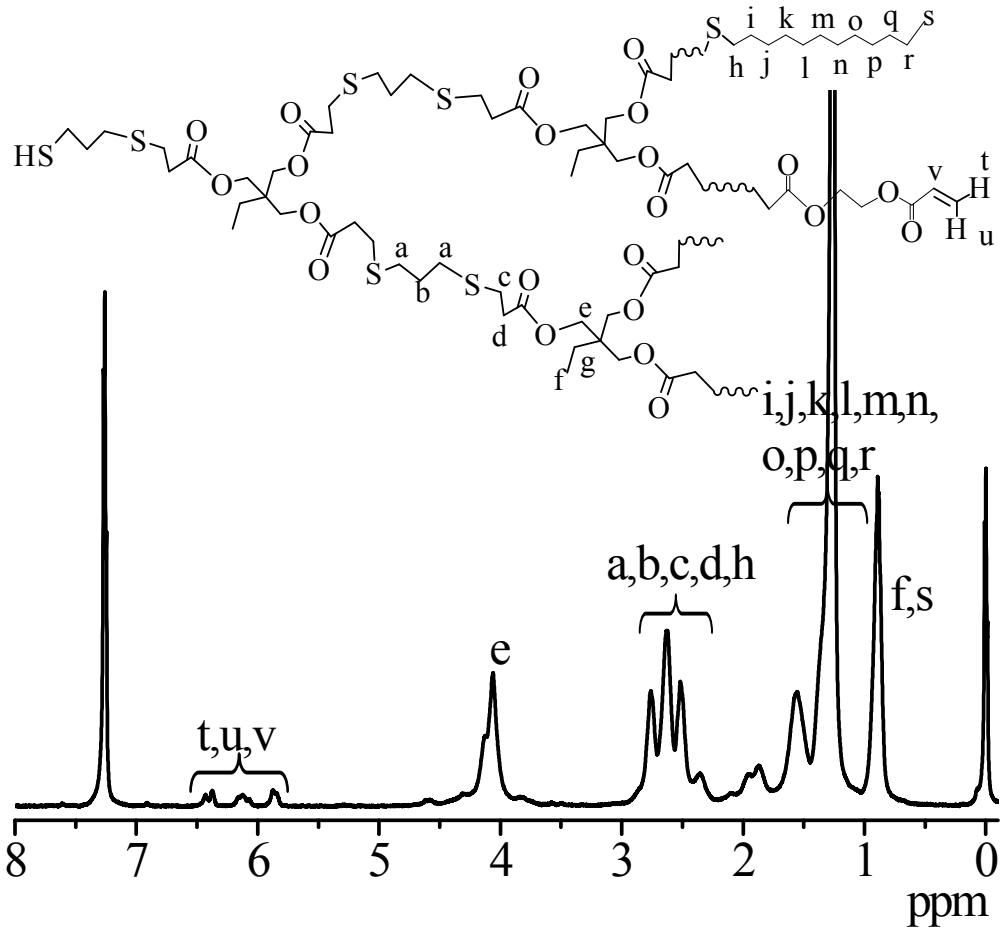


Figure S4. ^{1}H NMR spectrum ($CDCl_3$) of hyperbranched poly(PDT-TAMP) prepared by Michael addition polymerization of 1, 3-Propanedithiol and TAMP with molar ratio of 1/1 at 50°C for 23h.

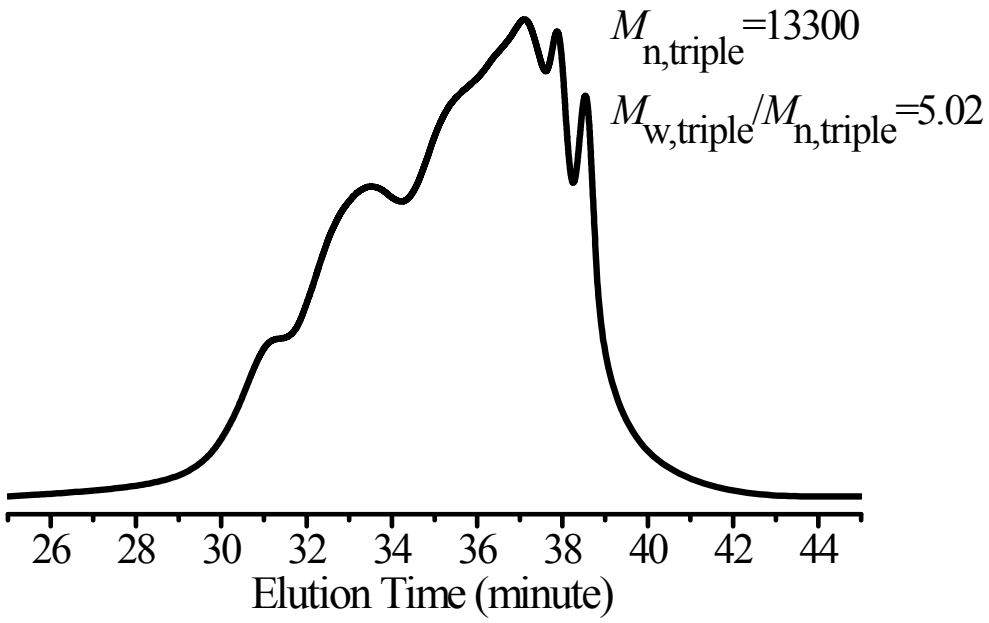


Figure S5. TD-SEC trace of the HypAD23 which was prepared by Michael addition polymerization with molar ratio of 1, 3-propanedithiol/1,1,1-tris(acryloyoxymethyl)propane =1/1 at 50^{o}C for 23h.

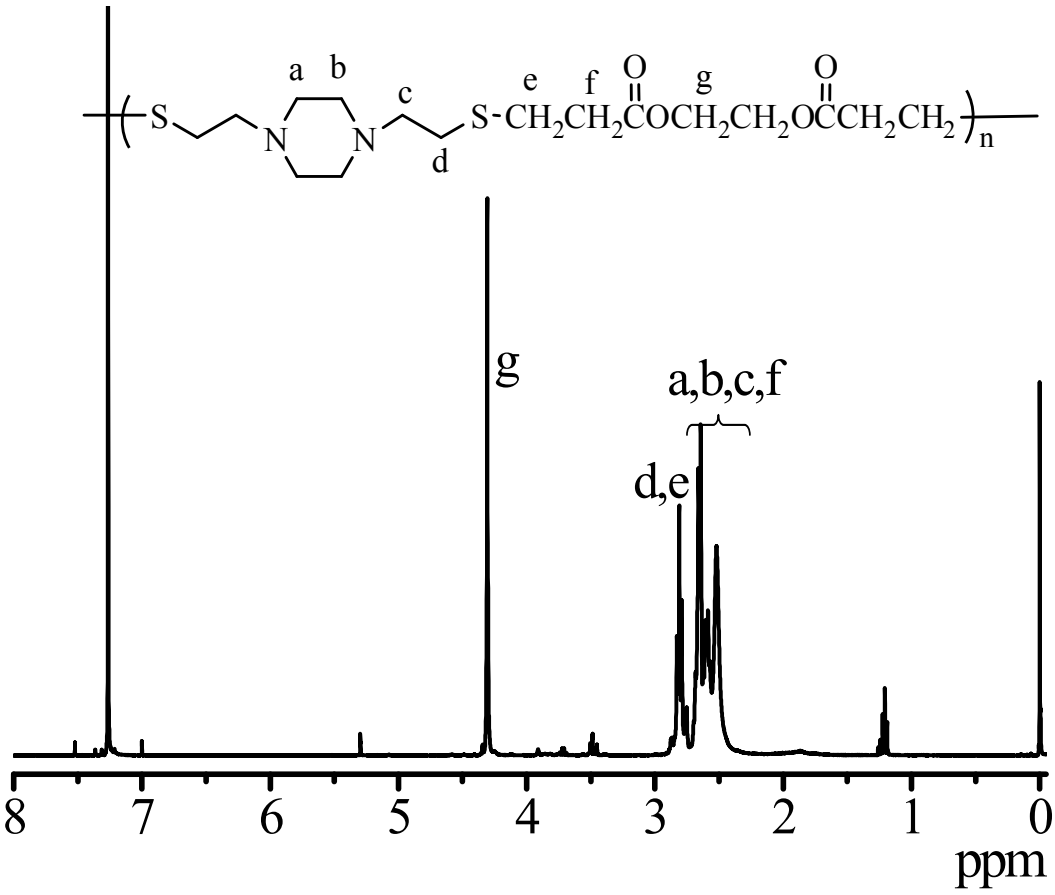


Figure S6. ^{1}H NMR spectrum of the linear polymer with tertiary amine in the backbone, l-P(BMEP-EGDA) obtained from Michael addition polymerization of EGDA and N,N'-bis(2-mercaptoethyl) piperazine with molar ratio of 1/1 at 50^{o}C for 26h.

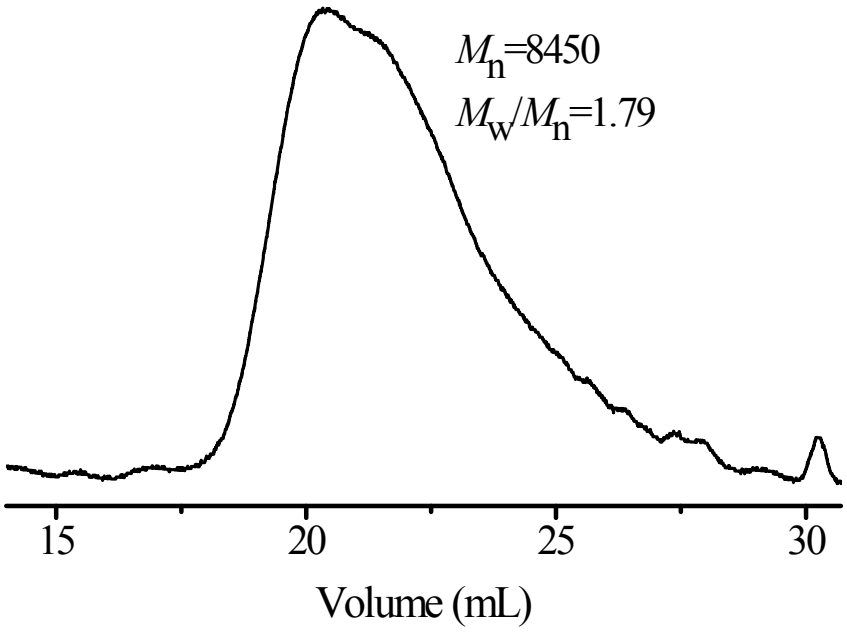


Figure S7. SEC curve of the linear polymer with tertiary amine in the backbone, l-P(BMEP-EGDA) obtained from Michael addition polymerization of EGDA and N,N'-bis(2-mercaptoethyl)piperazine with molar ratio of 1/1 at 50°C for 26h.

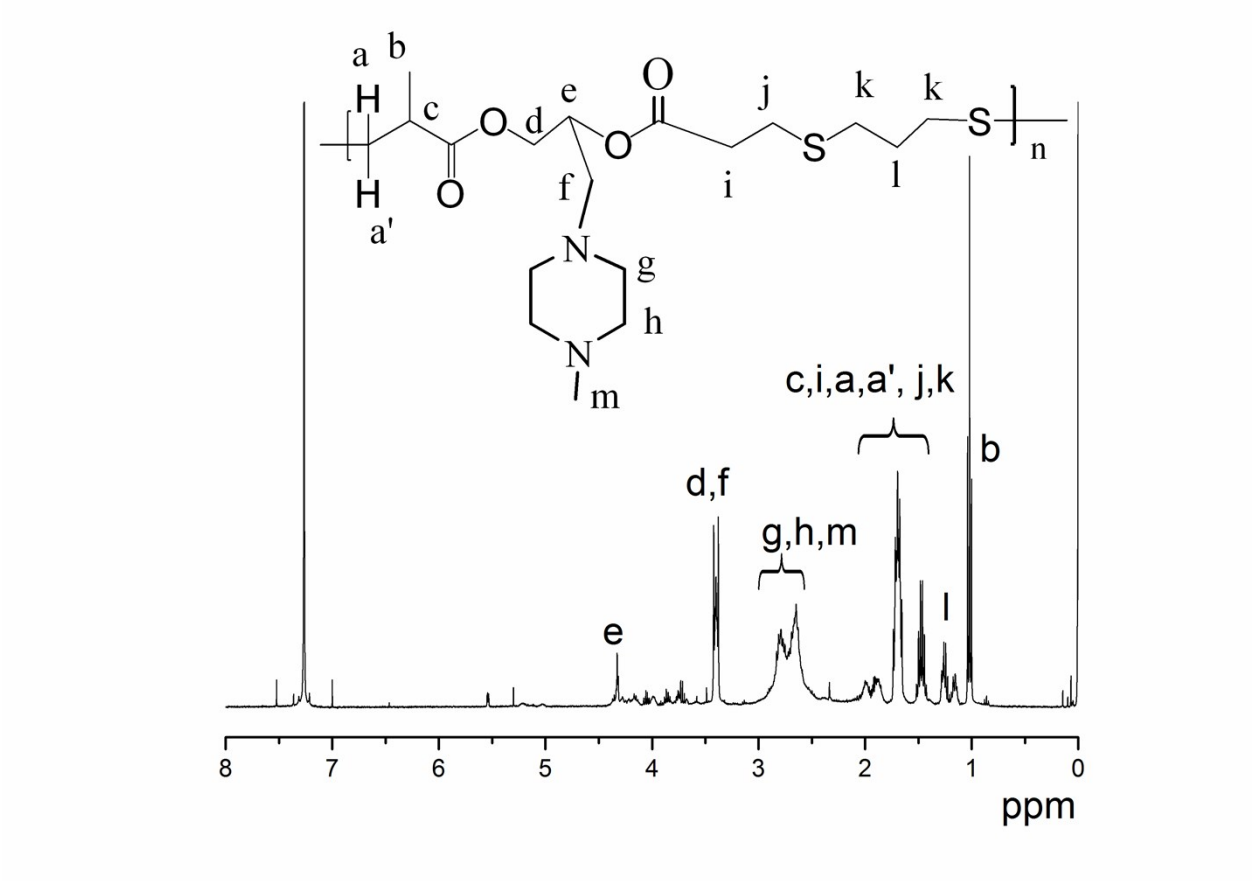


Figure S8. ^{1}H NMR spectrum ($CDCl_3$) of the linear polymer with tertiary amine as side group, l-P(AMPMA-PDT) obtained from Michael addition polymerization in tetrabutyl ammonium bromide with molar ratio of PDT/AMPMA=1/1 at 100^{o}C for 24h.

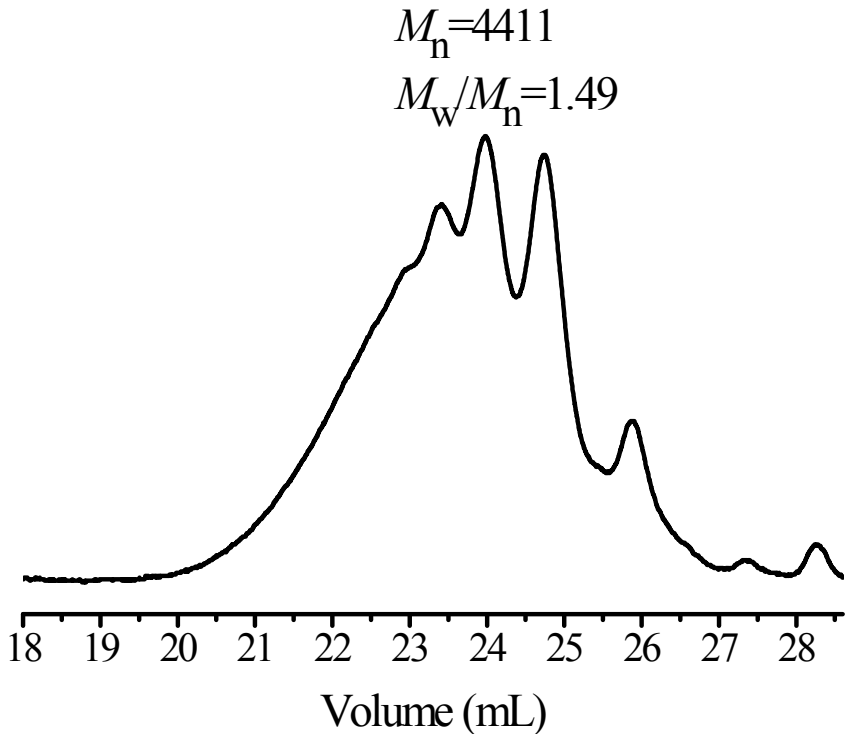


Figure S9. SEC curve of the linear polymer with tertiary amine as side group, l-P(AMPMA-PDT) obtained from Michael addition polymerization of 2-(acryloyloxy)-3- (4-methylpiperazin-1-yl)propyl methacrylate (AMPMA) and 1,3-propanedithiol (PDT) in tetrabutyl ammonium bromide with molar ratio of 1/1 at 100^{o}C for 24h.

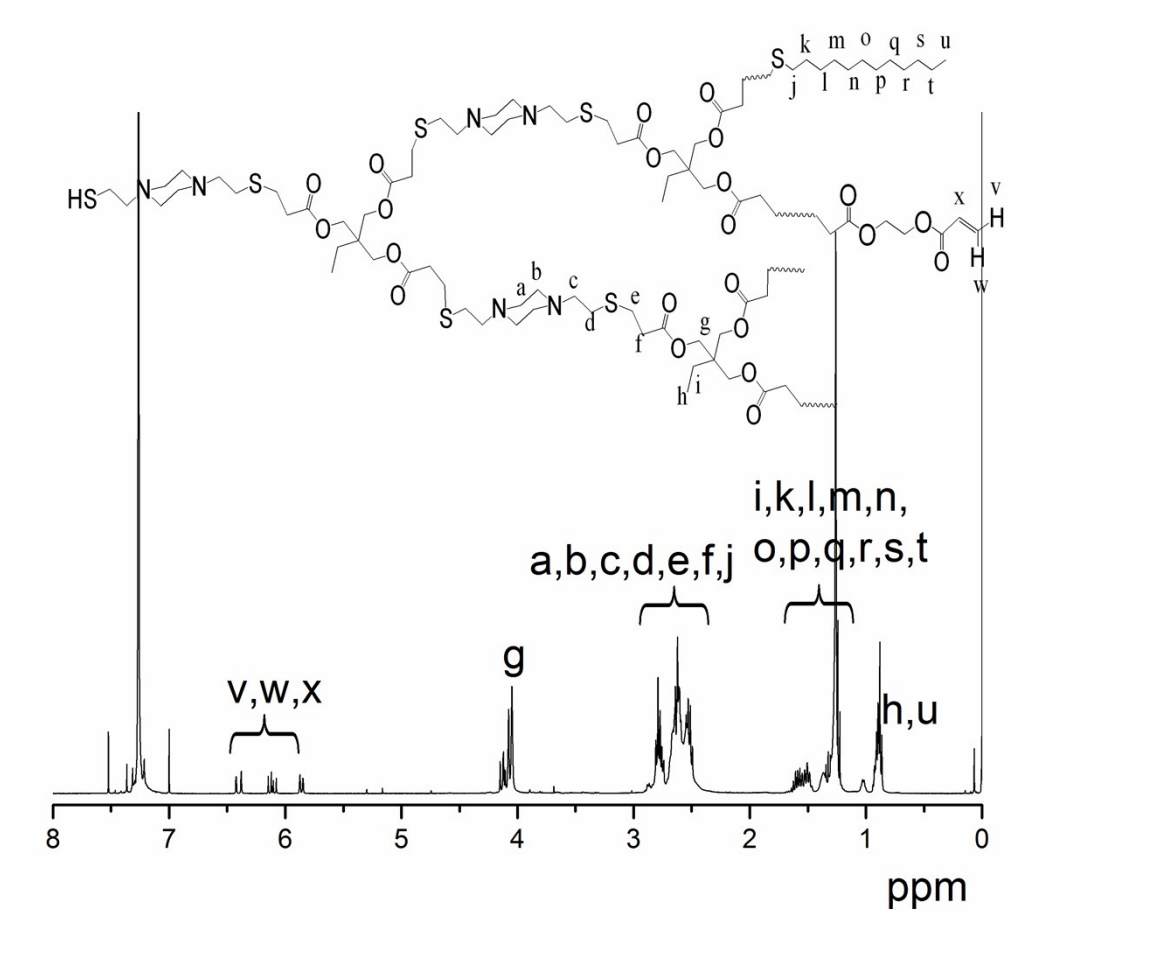


Figure S10. ^{1}H NMR spectrum, of HypME23 in $CDCl_3$. HypME23 was prepared by Michael addition polymerization with molar ratio of TAMP/N,N'-bis(2-mercaptoethyl)piperazine =1/1 at 50^{o}C for 23h.

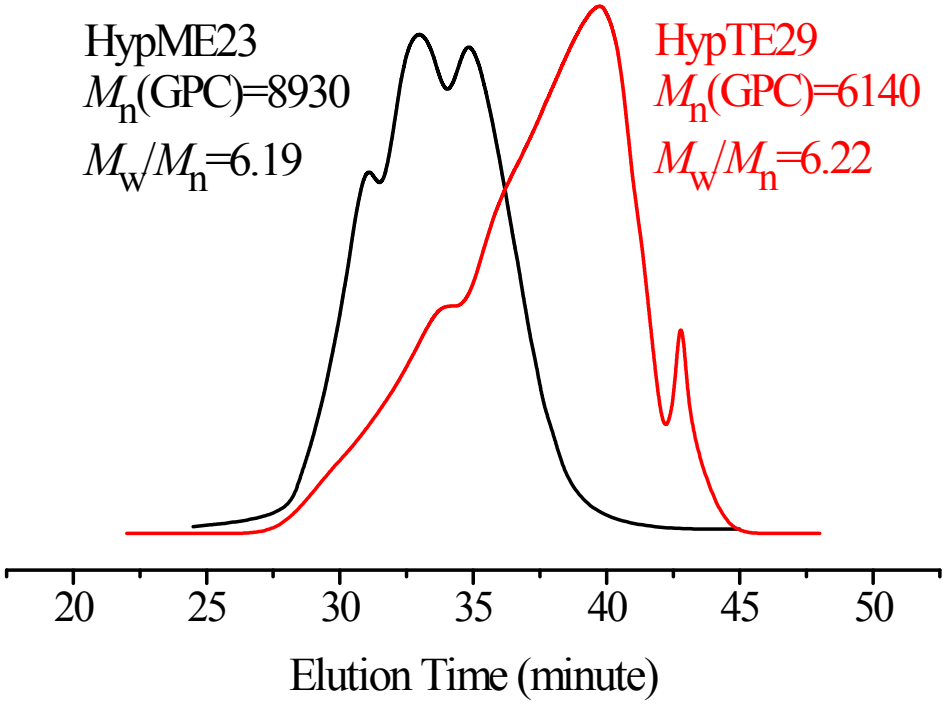


Figure S11. SEC curves of the HypME23 and HypTE29, the former was prepared by Michael addition polymerization with molar ratio of TAMP/N,N'-bis(2-mercaptoethyl)piperazine=1/1 at 50°C for 23h, and the latter was obtained from polymerization with molar ratio of EGDA/TMEAP=2/1 at 50°C for 29h.

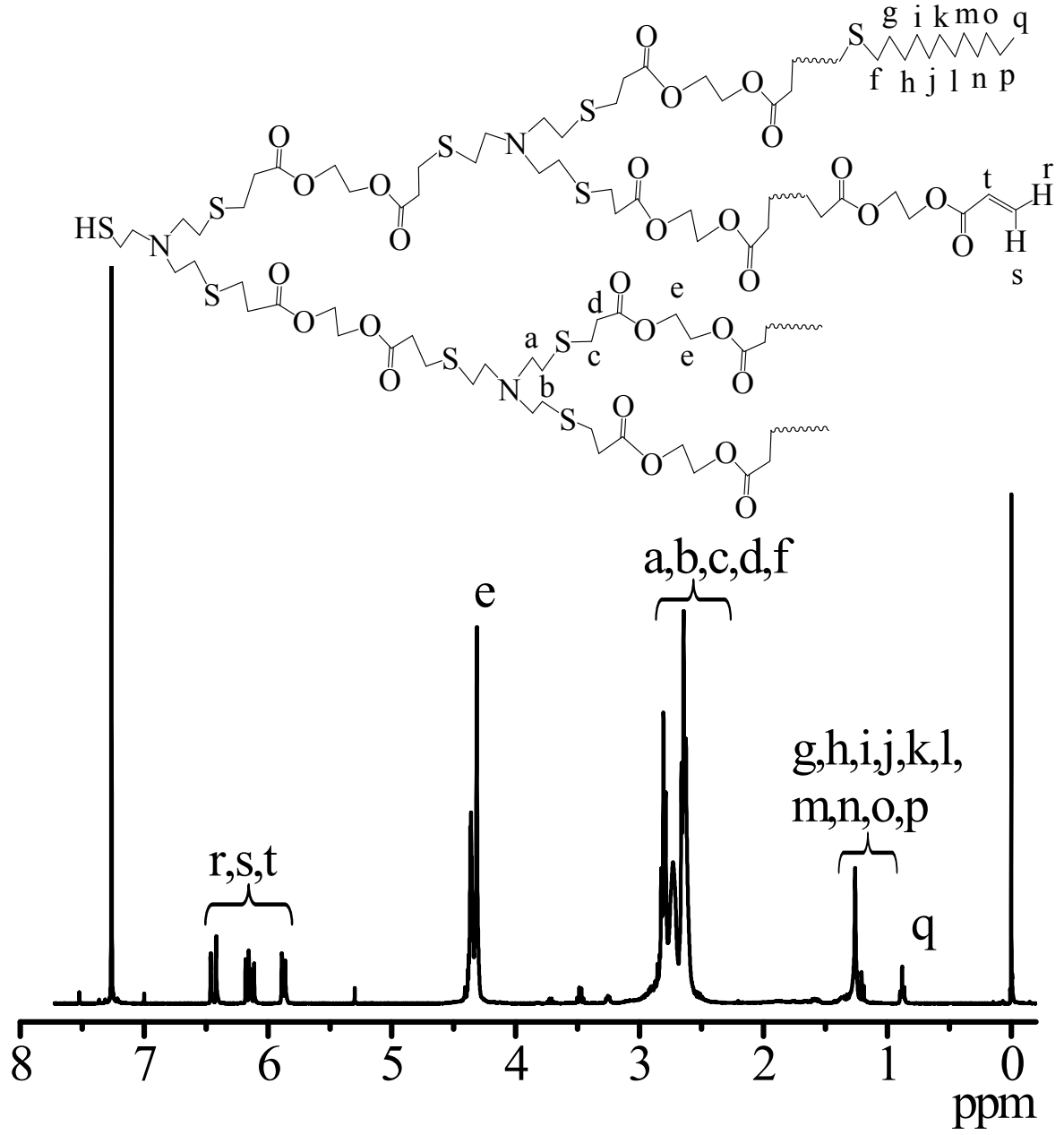


Figure S12. ^{1}H NMR spectrum of HypET9, which was prepared by polymerization with molar ratio of EGDA/TMEA=2/1 at 50°C for 9h, in $CDCl_3$.

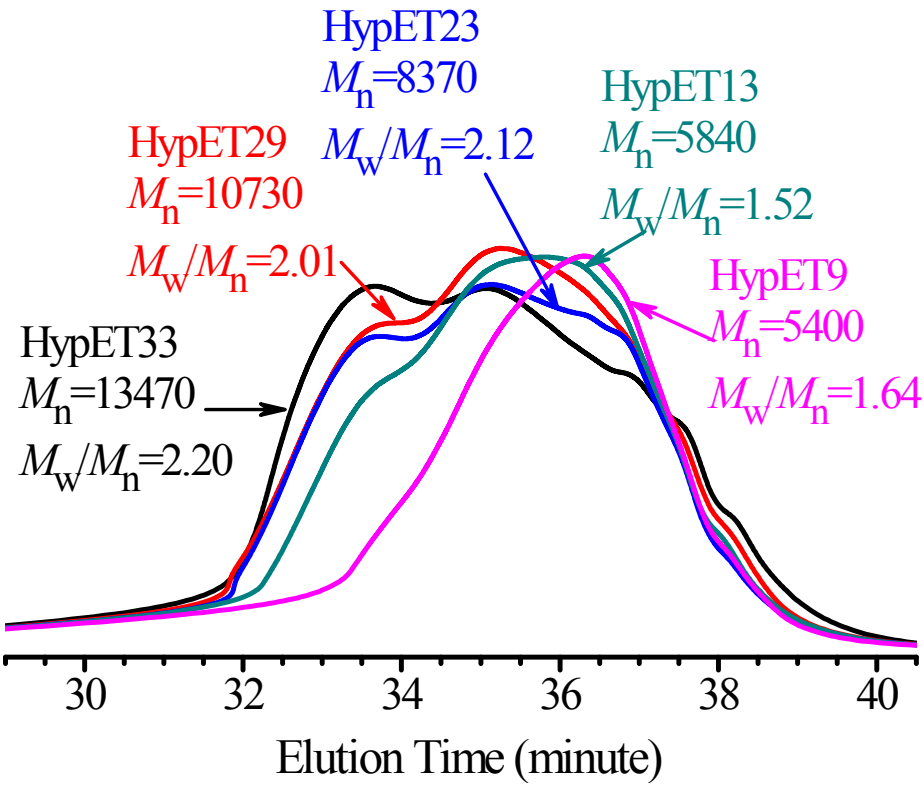


Figure S13. TD-SEC traces of the HypET9, HypET13, HypET23, HypET29 and HypET33 prepared by Michael addition polymerization with feed molar ratio of EGDA/TMEA=2/1 at 50°C for 9, 13, 23, 29 and 33h, respectively.

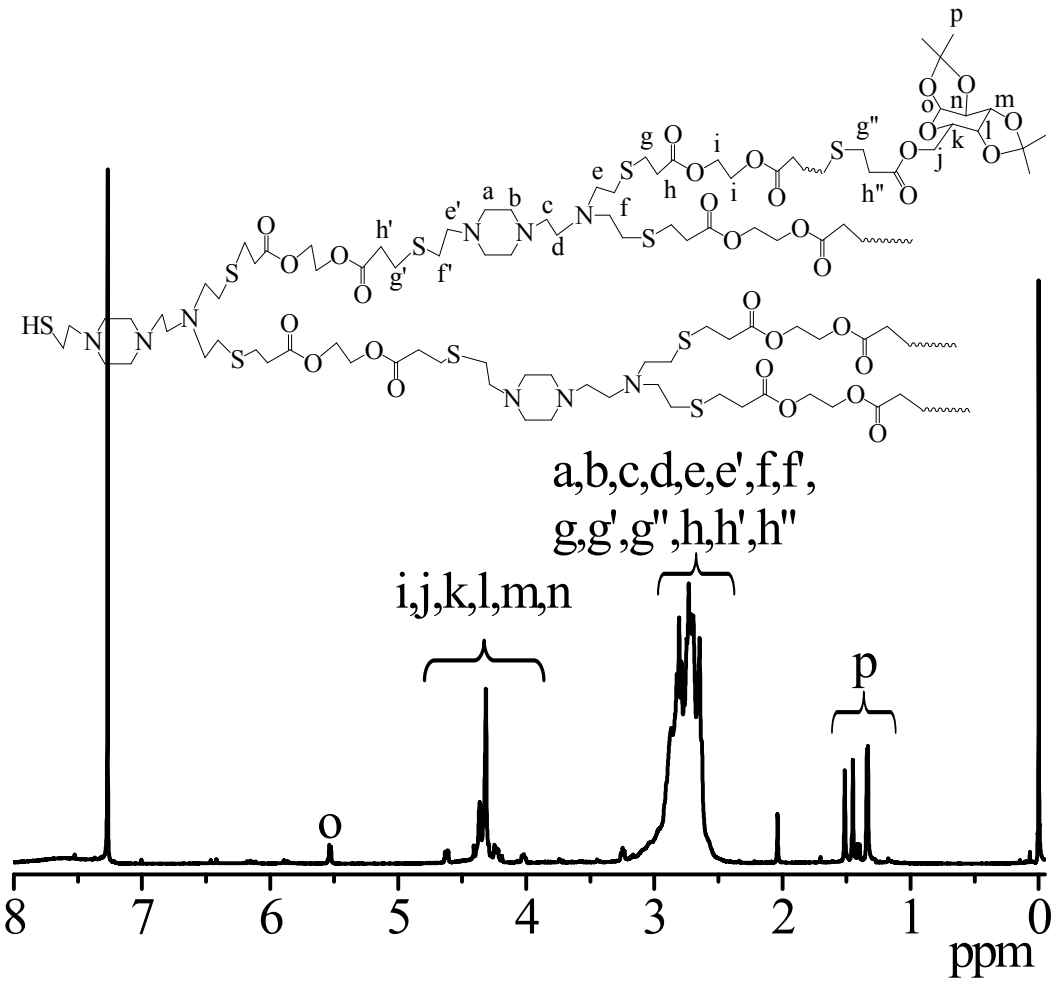


Figure S14. ^{1}H NMR spectrum ($CDCl_3$) of HypTE-DIAlpGP, which was prepared by polymerization with molar ratio of EGDA/MPEDT/DIAlpGP=1/2/1 at 50°C for 33h.

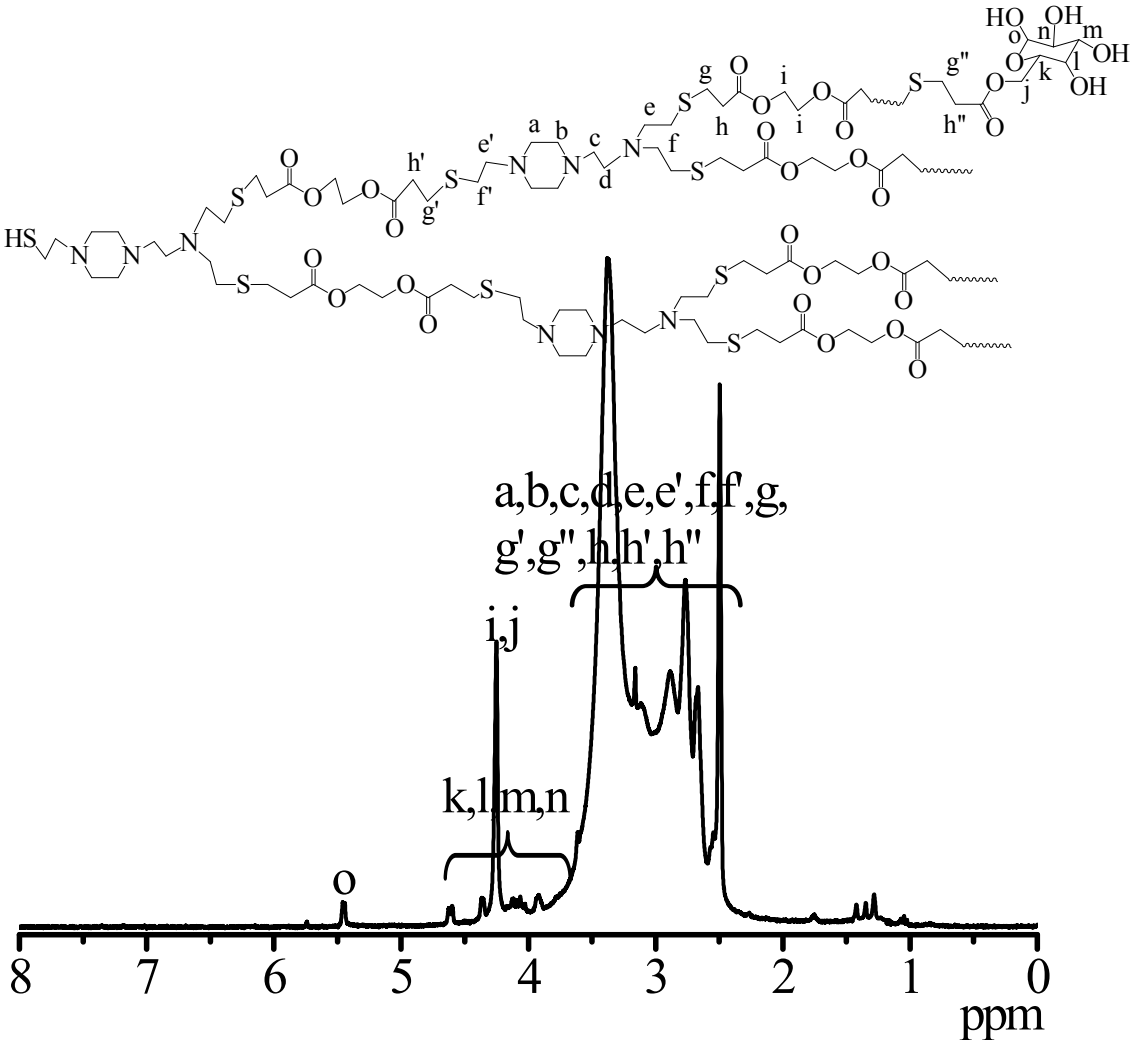


Figure S15. ^{1}H NMR spectrum (DMSO-d_6) of HypEMT-AlpGP, which was prepared by deprotection reaction of the HypTE-DIAlpGP in diluted aqueous solution of hydrochloric acid (pH=3) at room temperature for 20h.

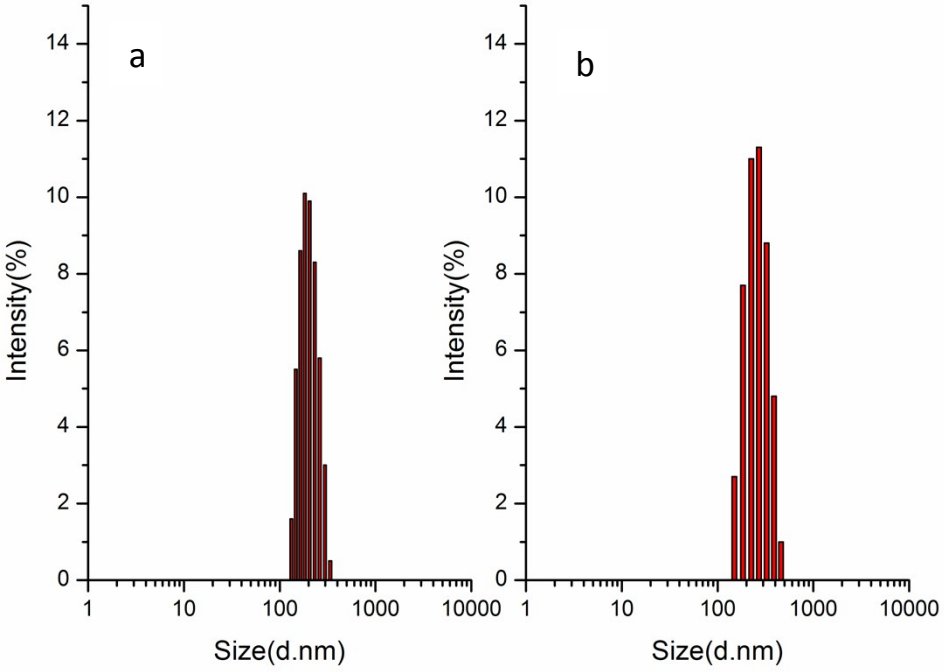


Figure S16. Size distribution of (a) HypTE-AlpGP NPs and (b) DOX-encapsulated HypTE-AlpGP NPs in H_2O measured by DLS.

References

[1] Sun, M.; Hong, C. Y.; Pan, C. Y. *J. Am. Chem. Soc*. **2012**,*134*, 20581-20584.

[2] Amoroso,A. J.; Chung, S. S. M.; Spencer, D. J. E.; Danks, J. P.; Glenny, M. W.; Blake, A. J.; Cooke, P. A. C.; Wilson, M. S. *Chem. Commun*. **2003**, 2020–2021.

[3] Huang, Z. B.; Chang, S. H. *Tetrahedron Lett*. **2005**, *46*, 5351–5355.