Supporting information for:

Epoxy-functional sterically-stabilized diblock copolymer nanoparticles via RAFT aqueous emulsion polymerization: comparison of two synthetic strategies

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Experimental Section

Materials

Glycerol monomethacrylate (GMA; 99.8%) was donated by GEO Specialty Chemicals (Hythe, UK) and used without further purification. Benzyl methacrylate (BzMA, Sigma Aldrich, 99%) was passed through a column of basic alumina to remove inhibitor and then stored at −20 °C prior to use. 2-Cyano-2-propyldithiobenzoate (CPDB), 4,4'-azobis(4-cyanopentanoic acid) (ACVA or V-501, 99%), 2,2-azobisisiobutyronitrile (AIBN), 2-phenylethanethiol, carbon disulfide, cysteamine hydrochloride, 3-mercaptopropanoic acid, sodium thiosulfate, glycidol, and regenerated cellulose dialysis membrane (Spectra/Por 6, molecular weight cut-off = 3 500 Da) were purchased from Sigma-Aldrich (UK) and used as received. 4-Dimethylaminopyridine (DMAP; 99 %) and *N,N'*-dicyclohexylcarbodiimide (DCC; 99 %) were purchased from Alfa Aesar (UK). Sodium hydride (60 % in mineral oil) was obtained from Acros Organics (China). NMR solvents (d_4-methanol, d_2-dichloromethane and d_6-DMSO) were purchased from Goss Scientific (Nantwich, UK). Iodine, absolute ethanol (maximum water content = 0.1%) and 1,4-dioxane were supplied by VWR International S.A.S (Fontenay-sous-Bois France). Deionized water was used to prepare all the aqueous solutions described in these experiments.

Synthesis of PETTC RAFT agent

4-Cyano-4-(2-phenylethanesulfanylthiocarbonyl)sulfanylpentanoic acid (PETTC) was synthesized according to a previously reported protocol.[1] Sodium hydride (60% in mineral oil, 7.0 g, 175 mmol) and 400 mL diethyl ether was added to a 1 L round-bottomed flask equipped with a magnetic stir bar. 2-Phenylethanethiol (21.6 g, 156 mmol) was added dropwise to the stirred suspension. Hydrogen evolution was observed and after 45 min the grey suspension turned white. Carbon disulfide (13.5 g, 177 mmol) was added dropwise and a yellow precipitate of 2-phenylethanetrithiocarbonate was formed over 2 h. This crude product was collected *via* filtration and dried under vacuum overnight. Iodine (23.0 g, 90.6 mmol) was added to the suspension of the 2-phenylethanetrithiocarbonate (35.7 g, 151 mmol) in 400 mL diethyl ether. After 1.5 h stirring at 20 °C, the resulting white precipitate of sodium iodide was removed *via* filtration. The brown filtrate was washed with saturated sodium thiosulfate solution (4 x 150 mL), dried over magnesium sulfate and the solvent was

removed under reduced pressure to afford bis-(2-phenylethanesulfanylthiocarbonyl)disulfide (32.0 g, 75 mmol) as a yellow-brown oil. A 1 L two-neck round-bottom flask equipped with magnetic stirrer was charged with bis-(2-phenylethanesulfanylthiocarbonyl)disulfide (32.0 g, 75 mmol), ACVA (32.0 g, 114 mmol) and ethyl acetate (500 mL). This reaction mixture was purged with nitrogen for 45 min, then refluxed under nitrogen atmosphere overnight. The solution was washed with water (4 x 200 mL), dried using magnesium sulfate and ethyl acetate was removed under reduced pressure. The crude product was purified by silica gel column chromatography, initially using pure dichloromethane followed by 95:5 v/v dichloromethane/ethanol after the first fraction had been removed to yield PETTC as an orange oil (72% yield). ^{1}H NMR (400 MHz, CD_2Cl_2, 25 °C): δ 1.91 (s, 3H, –(CN)C***H₃***), 2.40–2.62 (m, 2H, –(CH3)(CN)-C***H₂***CH_2C(=O)OH), 2.64-2.87 m, 2H, –(CH_3)(CN)-CH_2C***H₂***C(=O)OH), 3.02–3.06 (t, 2H, –PhC***H₂***CH_2S(C=S)S), 3.60–3.66 (t, 2H, –PhCH_2C***H₂***S(C=S)S), 7.25–7.40 (m, 5H, –***Ph***CH_2CH_2S(C=S)S). ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ 24.9 (***C***H_3), 29.5 (CH_2***C***H_2C(=O)OH), 33.5 (PhCH_2***C***H_2S), 34.0 (***C***H_2CH_2C(=O)OH), 38.0 (Ph***C***H_2CH_2S), 46.3 (S***C***(CH_3)(CN)CH_2), 118.9 (SC(CH_3)(***C***N)CH_2), 126.9-128.6, 139.2 (***Ph***CH_2), 177.1 (***C***=O), 216.4 (***C***=S). MS (ES$^+$) m/z calcd: 339.0 Found: 339.0 Anal. Calcd for $C_{15}H_{17}NO_2S_3$: C, 53.07; H, 5.05; N, 4.13; S, 28.33 Found: C, 53.02; H, 5.72; N, 3.88; S, 27.21.

Synthesis of epoxy-functional RAFT agent (E-PETTC) via Steglich esterification

Glycidol was distilled under vacuum at 60-62 °C prior to use. A 100 mL flame-dried round-bottom flask equipped with a magnetic stirrer was charged with PETTC (5.00 g, 14.73 mmol), DMAP (0.180 g, 1.47 mmol) and anhydrous dichloromethane (50 mL). The solution was stirred and purged with N_2 for 20 min before adding glycidol (1.20 g, 16.20 mmol) under an inert atmosphere. A 25 mL flame-dried round-bottom flask was charged with DCC (3.34 g, 16.20 mmol) and anhydrous dichloromethane (8 mL) and stirred for 5 min. This solution was then transferred via cannula into the PETTC solution under a nitrogen atmosphere and stirred for 20 h at 20 °C. The orange solution was filtered to remove the insoluble dicyclohexylurea by-product as a white solid. The orange filtrate was then washed with water (4 x 20 mL), dried with magnesium sulfate and the solvent was removed under reduced pressure. The crude product was eluted through a silica gel column using dichloromethane as the mobile phase to yield E-PETTC (3.62 g, 62%). ^{1}H NMR (400 MHz, CD_2Cl_2, 25 °C): δ 1.91 (s, 3H, –(CN)C***H₃***), 2.40–2.62 (m, 2H, –(CH_3)(CN)-C***H₂***CH_2C(=O)OCH_2CHCH_2(O)), 2.65-2.87 (m, 4H, –(CH_3)(CN)-CH_2C***H₂***C(=O)OCH_2-O-CH_2-(CH-C***H₂***(O)), 3.00–3.10 (t, 2H, –PhC***H₂***CH_2S(C=S)S), 3.20–3.25 (t, 1H, –(C***H***CH_2(O)), 3.60–3.66 (t, 2H, –PhCH_2C***H₂***S(C=S)S), 3.80-4.50 (m, 2H, CH_2CH_2C(=O)OC***H₂***$CHCH_2$(O)), 7.25–7.40 (m, 5H, –***Ph***CH_2CH_2S(C=S)S). ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ 24.9 (***C***H_3), 29.6 (CH_2***C***H_2C(=O)OCH_2CHCH_2(O)), 33.8 (PhCH_2***C***H_2S), 34.1 (***C***H_2CH_2C(=O)OCH_2CHCH_2(O)), 38.0 (Ph***C***H_2CH_2S), 44.7 (OCH_2CH***C***H_2(O)), 46.4 (S***C***(CH_2)(CN)CH_2), 49.1 (OCH_2***C***HCH_2(O)), 65.6 (O***C***H_2CHCH_2(O)), 118.9 SC(CH_3)(***C***N)-CH_2), 126.9-128.6, 139.2 (***Ph***CH_2), 171.2 (***C***=O), 216.5 (***C***=S). HRMS (ES$^+$) m/z calcd: 395.1 found: 395.1. Anal. Calcd. for $C_{18}H_{21}NO_3S_3$: C, 54.66; H, 5.35; N, 3.54; S, 24.32%. Found: C, 54.80; H, 5.39; N, 3.63; S, 23.93%.

Synthesis of epoxy-functional poly(glycerol monomethacrylate) macro-CTA (E-PGMA$_{44}$) via RAFT solution polymerization of GMA in ethanol using E-PETTC

E-PETTC RAFT agent (1.05 g, 2.65 mmol), GMA monomer (17.00 g, 106.18 mmol), AIBA (144.0 mg, 0.53 mmol; [E-PETTC] / [AIBA] molar ratio = 5.0) and anhydrous ethanol (18.20 g) were added to a 100 mL round-bottomed flask equipped with a magnetic stirrer. The resulting orange 50 % w/w alcoholic solution was cooled to 0 °C using an ice bath and purged with N_2 for 45 min. The sealed flask was immersed in an oil bath set at 56 °C for 130 min and the GMA polymerization was quenched by immersing the reaction flask in an ice bath followed by exposure of its contents to air. A final GMA conversion of 80 % was determined by ^{1}H NMR analysis. The reaction solution was diluted with methanol and purified by five successive precipitations into dichloromethane (ten-fold excess). The macro-CTA was dissolved in water and residual dichloromethane was removed under vacuum. The resulting aqueous solution was freeze-dried for 48 h to yield a yellow powder. ^{1}H NMR analysis indicated a mean degree of polymerization of 44 (E-PGMA$_{44}$). DMF GPC analysis using a refractive index detector and a series of near-monodisperse poly(methyl methacrylate) calibration standards indicated an M_n of 11 900 g mol^{-1} and an M_w/M_n of 1.15.

Synthesis of E-PGMA$_{44}$-PBzMA$_{250}$ diblock copolymer spheres by RAFT aqueous emulsion polymerization of BzMA

E-PGMA$_{44}$-PBzMA$_{250}$ diblock copolymer spheres were prepared *via* polymerization-induced self-assembly (PISA) using the following RAFT aqueous emulsion polymerization formulation: E-PGMA$_{44}$ macro-CTA (0.119 g, 0.016 mmol), BzMA monomer (0.700 g, 4.0 mmol) and ACVA (1.5 mg, 0.019 mmol; macro-CTA/ACVA molar ratio = 3.0) were added to a 15 mL sample vial, followed by addition of water (3.82 mL) to produce a 20% w/w aqueous solution. This reaction solution was purged with nitrogen gas for 30 min at 20 °C before being immersed in an oil bath set at 70 °C. The reaction mixture was stirred for 3 h to ensure essentially complete conversion of the BzMA monomer (> 99% as judged by the disappearance of the vinyl signals at 6.1 ppm using ^{1}H NMR spectroscopy) and the polymerization was then quenched by exposure to air, followed by cooling to 20 °C. DMF GPC analysis indicated that the resulting diblock copolymer had an M_n of 36 900 g mol^{-1} and an M_w/M_n of 1.53 (relative to a series of near-monodisperse poly(methyl methacrylate) standards).

Synthesis of a P(GMA$_{47}$-*co*-GlyMA$_1$) macro-CTA *via* RAFT solution copolymerization in ethanol

GMA (1.53 g, 9.50 mmol), GlyMA (0.34 g, 2.30 mmol), CPDB (0.53 g, 2.40 mmol; target DP = 5), and AIBN (0.079 g, 0.48 mmol; CPDB/AIBN molar ratio = 5.0) were accurately weighed into a 250 mL round-bottomed flask. Anhydrous ethanol was then added to produce a 50% w/w solution, which was placed in an ice bath and purged under nitrogen for 45 min at 0 °C. The sealed flask was then immersed in an oil bath set at 70 °C to initiate the RAFT solution

copolymerization. After 2 h, an aliquot of the reaction mixture was taken and analysis by ^{1}H NMR indicated a 71 % conversion. At this point, a 50% w/w solution of GMA (23.47 g, 147 mmol) in anhydrous ethanol (previously degassed under nitrogen in an ice bath for 45 min) was added to the reaction solution. This was allowed to react for a further 2 h at 70 °C before being quenched by exposure to air, followed by cooling to 20 °C. ^{1}H NMR analysis indicated a monomer conversion of 69%. Methanol (20 mL) was added to this solution, followed by precipitation into a ten-fold excess of dichloromethane to remove unreacted GMA and GlyMA monomers. The precipitate was isolated *via* filtration and washed with excess dichloromethane before being dissolved in methanol (60 mL). The crude polymer was precipitated into excess dichloromethane again and isolated *via* filtration. It was then dissolved in water and freeze-dried overnight to afford a pink solid. ^{1}H NMR studies indicated the incorporation of approximately one unit of GlyMA per copolymer chain and an overall mean degree of polymerization of 48 *via* end-group analysis. Thus the mean chemical composition of this epoxy-functional macro-CTA was P(GMA_{47}-*co*-$GlyMA_1$). DMF GPC studies (refractive index detector; calibration relative to a series of near-monodisperse poly(methyl methacrylate) standards) indicated an M_n of 12 800 g mol^{-1} and an M_w/M_n of 1.19.

Synthesis of P(GMA_{47}-*co*-$GlyMA_1$)-$PBzMA_{250}$ diblock copolymer spheres by RAFT aqueous emulsion polymerization of BzMA

Spherical P(GMA_{47}-*co*-$GlyMA_1$)-$PBzMA_{250}$ diblock copolymer nanoparticles were prepared *via* polymerization-induced self-assembly (PISA) using the following RAFT aqueous emulsion polymerization formulation: P(GMA_{47}-*co*-$GlyMA_1$) macro-CTA (0.528 g, 68 µmol), BzMA monomer (3.00 g, 17.0 mmol) and ACVA (6.40 mg, 22 µmol; macro-CTA/ACVA molar ratio = 3.0) were added to a 50 ml round-bottomed flask, followed by addition of water (14.98 mL) to produce a 20% w/w aqueous solution. This reaction solution was purged with nitrogen gas for 30 min at 20 °C before being immersed in an oil bath set at 70 °C. The reaction mixture was stirred for 3 h to ensure essentially complete conversion of the BzMA monomer (> 99% as judged by disappearance of the vinyl signals at 6.1 ppm using ^{1}H NMR analysis) and was quenched by exposure to air, followed by cooling to 20 °C. DMF GPC analysis indicated that the diblock copolymer possessed a relatively narrow molecular weight distribution (M_w/M_n = 1.27) and an M_n of 35 800 g mol^{-1} (relative to a series of near-monodisperse poly(methyl methacrylate) standards).

Post-polymerization modification of P(GMA_{47}-*co*-$GlyMA_1$) macro-CTA and P(GMA_{47}-*co*-$GlyMA_1$)-$PBzMA_{250}$ diblock copolymer spheres using epoxy-thiol chemistry

The P(GMA_{47}-*co*-$GlyMA_1$) macro-CTA was derivatized with cysteamine using the following protocol: a 10% w/w aqueous solution of P(GMA_{47}-*co*-$GlyMA_1$) macro-CTA (0.100 g polymer, 0.013 mmol epoxy groups) and cysteamine hydrochloride (29.0 mg, 0.253 mmol, cysteamine/epoxy molar ratio = 20) were weighed into a 6 mL vial. This reaction solution was adjusted to approximately pH 8.5 using KOH and then stirred for 16 h at 20 °C. The resulting derivatized macro-CTA was purified by dialysis against water to remove excess

cysteamine hydrochloride. The same protocol was used for the reaction of 3-mercaptopropanoic acid instead of cysteamine.

P(GMA_{47}-*co*-$GlyMA_1$)-$PBzMA_{250}$ diblock copolymer spheres were derivatized with cysteamine using the following protocol: a 20% w/w aqueous dispersion of P(GMA_{47}-*co*-$GlyMA_1$)-$PBzMA_{250}$ nanoparticles (4.00 g, 0.015 mmol epoxy groups) and cysteamine hydrochloride (35.0 mg, 0.307 mmol, cysteamine/epoxy molar ratio = 20) were weighed into a 15 mL vial. This reaction solution was adjusted to approximately pH 8.5 using KOH and stirred for 16 h at 20 °C. The resulting derivatized nanoparticles were purified by dialysis against water to remove excess cysteamine hydrochloride. Essentially the same protocol was used for the reaction of 3-mercaptopropanoic acid instead of cysteamine. The same reaction conditions were also used for the reaction of either cysteamine or 3-mercaptopropanoic acid with the E-$PGMA_{44}$-$PBzMA_{250}$ nanoparticles.

Instrumentation

NMR spectroscopy. ^{1}H NMR spectra were recorded in d_4-methanol, d_2-dichloromethane or d_6-DMSO using a 400 MHz Bruker Avance-500 spectrometer (64 scans were averaged per spectrum).

Gel Permeation Chromatography (GPC). Molecular weights and dispersities were determined using an Agilent 1260 infinity set-up comprising two Polymer Laboratories PL gel 5 µm Mixed C columns and a refractive index detector operating at 60 °C. The mobile phase was HPLC-grade DMF containing 10 mmol LiBr at a flow rate of 1.0 mL min^{-1}. Calibration was conducted using a series of ten near-monodisperse poly(methyl methacrylate) standards ranging from 2 380 to 988 000 g mol^{-1}.

DLS studies were conducted using a Malvern Zetasizer NanoZS instrument on 0.1% w/w aqueous dispersions at 25 °C in disposable cuvettes at a fixed scattering angle of 173°. The solution pH of the initially basic copolymer dispersion was adjusted using HCl. Intensity-average hydrodynamic diameters were calculated via the Stokes-Einstein equation using a non-negative least-squares (NNLS) algorithm. All data were averaged over three consecutive runs.

Aqueous Electrophoresis. Measurements were performed on 0.1% w/w aqueous copolymer dispersions containing 1 mM KCl as background electrolyte using a Malvern Zetasizer NanoZS instrument operating at 25 °C. The solution pH of the initially basic copolymer dispersions was adjusted using HCl. Zeta potentials were calculated from the Henry equation using the Smoluchowski approximation. All data were averaged over three consecutive runs.

Scanning Electron Microscopy (SEM). Copolymer dispersions were diluted with deionized water at 20 °C to produce 0.1% w/w dispersions. One droplet of each dilute dispersion was then placed onto a bare silicon wafer and allowed to dry overnight at 20 °C. The silicon wafers were mounted onto SEM stubs using electrically conductive adhesive pads. The stubs were gold-coated for 2 min prior to analysis to prevent sample-charging. SEM studies were performed using an Inspect F microscope operating at 5 kV.

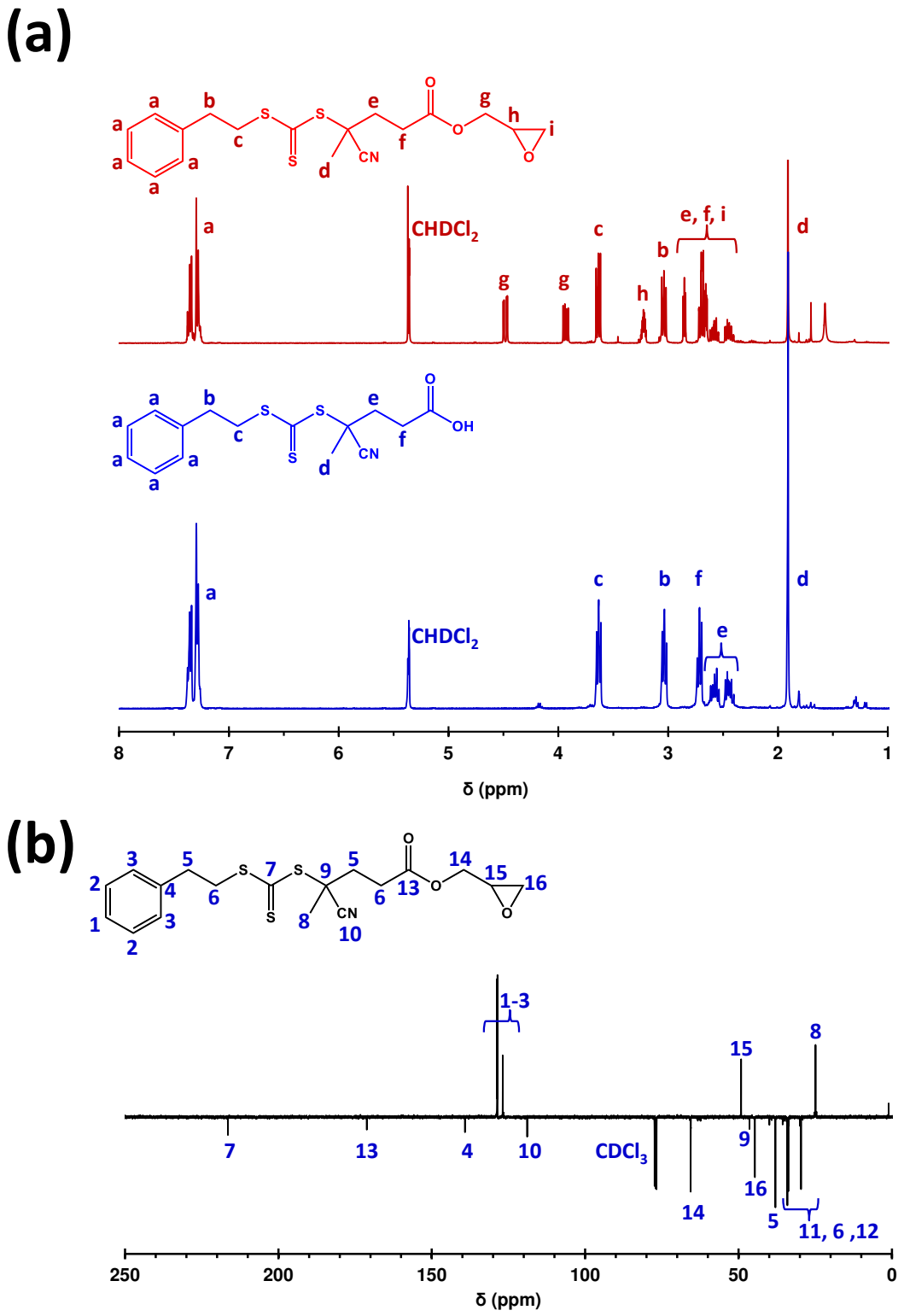

Figure S1. (a) Fully assigned ^{1}H NMR spectra (CD_2Cl_2) recorded for the epoxy-functional chain transfer agent E-PETTC (red, upper spectrum) and the PETTC precursor (blue, lower spectrum). (b) Fully assigned ^{13}C NMR spectra ($CDCl_3$) recorded for the epoxy-functional chain transfer agent E-PETTC.

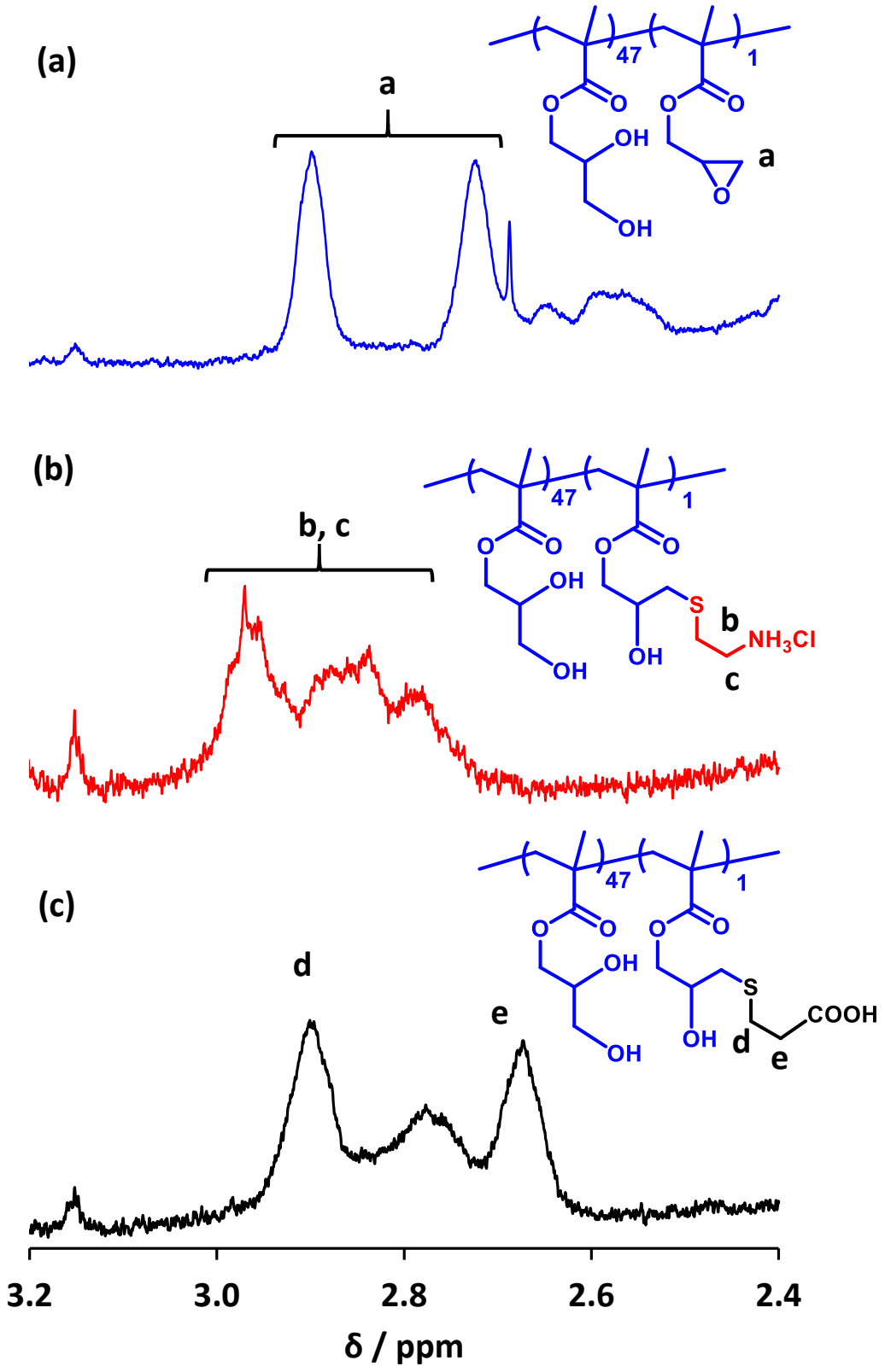

Figure S2. Partial ^{1}H NMR spectra (CD_3OD) recorded for (a) the pristine P(GMA_{47}-*co*-$GlyMA_1$) macro-CTA and after its reaction with either (b) cysteamine or (c) 3-mercaptopropanoic acid in aqueous solution at pH 8.5.

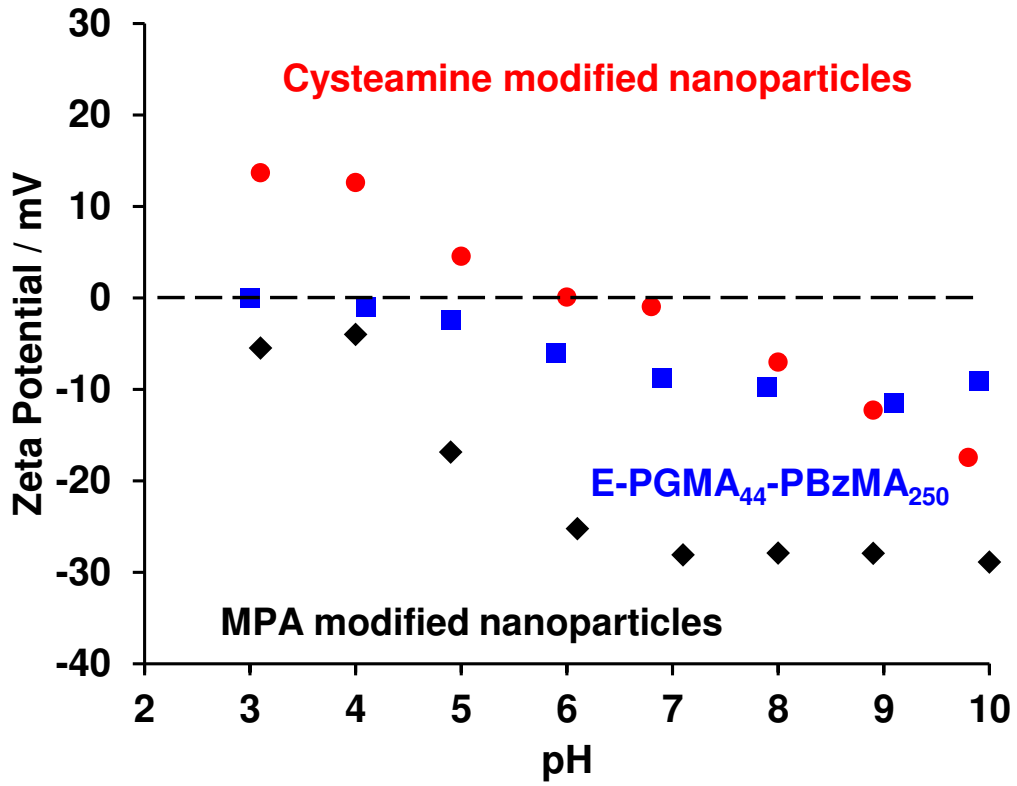

Figure S3. Aqueous electrophoresis data obtained for the E-PGMA$_{44}$-PBzMA$_{250}$ nanoparticles before and after reaction with a twenty-fold excess of either cysteamine or 3-mercaptopropanoic acid. Comparison with the electrophoretic data shown in Figure 4 suggests that there is premature loss of terminal epoxy groups prior to their reaction with cysteamine, leading to more weakly cationic nanoparticles being obtained at low pH. In the case of the 3-mercaptopropanoic acid, premature loss of the terminal epoxy group via ester hydrolysis leads to the formation of a carboxylic acid end-group so appreciable anionic character is still observed at high pH. This side-reaction also leads to weakly anionic precursor nanoparticles (zeta potential ~ −10 mV at pH 9-10).

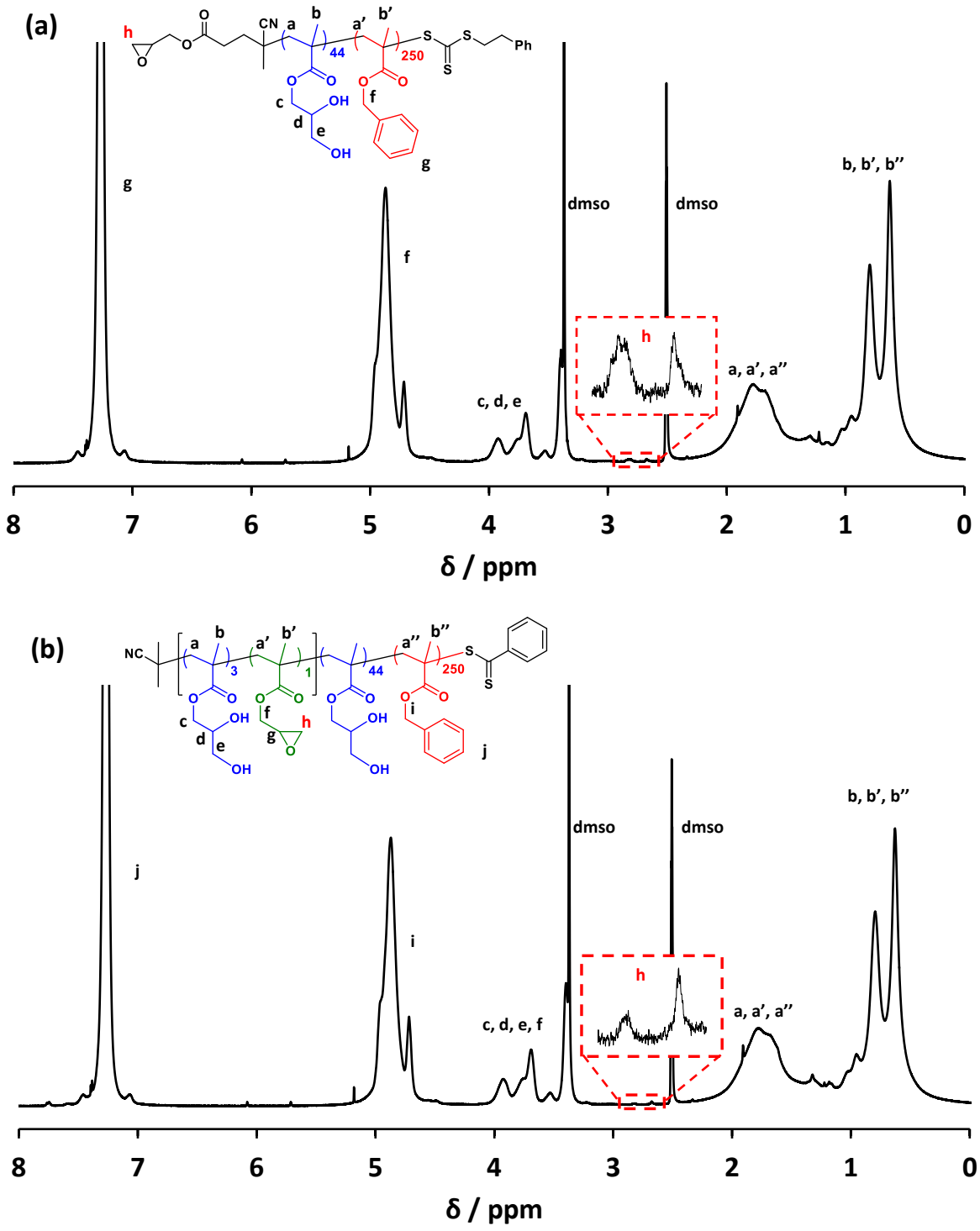

Figure S4. Assigned ^{1}H NMR spectra (d_6-DMSO) obtained for (a) E-PGMA$_{44}$-PBzMA$_{250}$ and (b) P(GMA$_{47}$-*co*-GlyMA$_{1}$)-PBzMA$_{250}$. Note that in both cases the desired epoxy-functionality is retained after the BzMA polymerization (see inset in dashed red box).

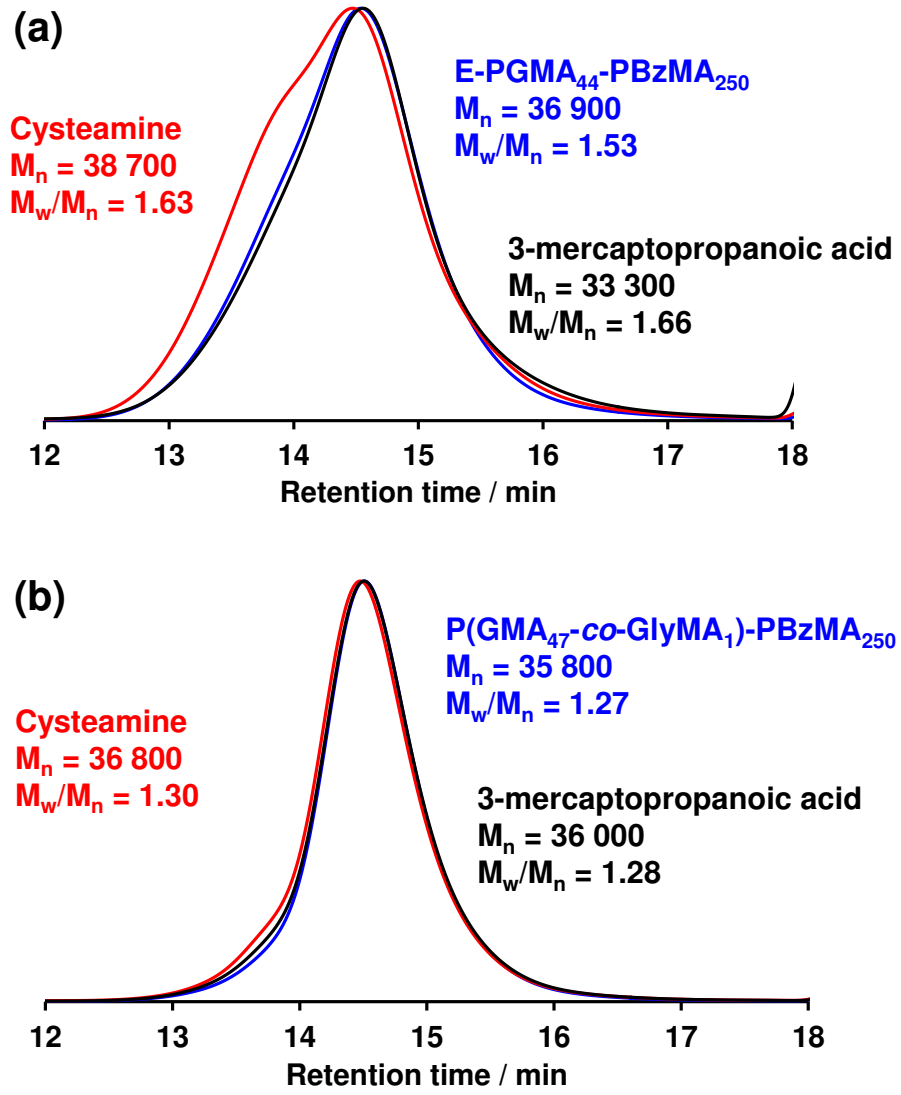

Figure S5. DMF GPC curves recorded for: (a) the E-PGMA$_{44}$-PBzMA$_{250}$ diblock copolymer before and after reaction with either cysteamine or 3-mercaptopropanoic acid at pH 8.5; (b) the P(GMA$_{47}$-*co*-GlyMA$_1$)-PBzMA$_{250}$ diblock copolymer before and after its reaction with either cysteamine or 3-mercaptopropanoic acid at pH 8.5. In the latter case, the molecular weight distribution of the precursor diblock copolymer remains essentially unchanged after epoxy-thiol derivatization.

References

1. E. R. Jones, M. Semsarilar, A. Blanazs and S. P. Armes, *Macromolecules*, 2012, **45**, 5091-5098.