Synthesis of well-defined conjugated copolymers by RAFT polymerization using cysteine and glutathione-based chain transfer agents

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Experimental Section

Materials. All solvents, monomers, and other chemicals were purchased from Aldrich at the highest purity available unless otherwise stated. N-acetyl glutathione was synthesized according to a literature method (see Levy, E. J.; Anderson, M. E.; Meister, A. *Anal. Biochem.* **1993**, *214*, 135). Methyl acrylate (MA), butyl acrylate (BA), methyl methacrylate (MMA), N,N-dimethylacrylamide (DMA) and styrene (St) were passed through a basic alumina (Brockmann I) column to remove the inhibitor before use. N-isopropylacrylamide (NIPAM) was recrystallized twice from hexane/toluene mixture prior to use. Dioxane was distilled over CaH_2. 2,2'-Azobisisobutyronitrile (AIBN) was recrystallized twice from ethanol. Other chemicals were of analytical grade and used as received.

Synthesis of Chain Transfer Agents. To a stirred solution of N-acetyl glutathione (1.40 g, 4.0 mmol) in 50 mL of methanol was added dropwise a solution of sodium methoxide in methanol (25 wt%, 2.59 g, 12 mmol) under nitrogen. After stirring for 30 min, CS_2 (0.45 g, 5.9 mmol) was added dropwise to the solution, and the mixture was then stirred at ambient temperature for 5 h. To the yellow solution was added benzyl bromide (98%, 0.75 g, 4.3 mmol), and the mixture was stirred overnight under nitrogen. The mixture was concentrated, partitioned between water (150 mL) and dichloromethane (DCM), and acidified by concentrated hydrochloric acid. After standing for 10 min, three layers were formed, and 2-acetamido-*S*-(benzylsulfanylthiocarbonyl)glutathione (ABSG) was precipitated as an intermediate layer due to its poor solubility in both water and DCM. The yellow solid was collected by filtration, washed thoroughly with water and DCM, and dried in vacuum oven under reduced pressure at 40 ^{o}C until constant weight. ABSG (2.06 g, 4.0 mmol) was obtained as a yellow solid in quantitative yield. ^{1}H NMR (DMSO-d_6), δ: 8.40 (d, *J*8.4, 1H, NH), 8.34 (t, *J*5.8, 1H, NH), 8.16 (d, *J*7.8, 1H, NH), 7.2-7.4 (m, 5H, PhH), 4.67 (s, 2H, CH_2S), 4.62 (m, 1H, CH, $H_{\alpha\text{-cys}}$), 4.16 (m, 1H, CH, $H_{\alpha\text{-glu}}$), 3.87 and 3.49 (m, each 1H, CH_2, $H_{\beta\text{-cys}}$), 3.75 (m, 2H, CH_2, H_{gly}), 2.19 (t, *J*8.0, 2H, CH_2, $H_{\gamma\text{-glu}}$), 1.95 and 1.76 (m, each 1H, CH_2, $H_{\beta\text{-glu}}$), 1.84 (s, 3H, CH_3CO). ^{13}C NMR (DMSO-d_6), δ: 223.2 (C=S), 173.6, 171.8, 171.0, 169.9, 169.6 (C=O), 135.2, 129.4, 128.8, 127.9 (PhC), 51.6, 50.6, 41.0, 40.6, 38.7, 31.8, 27.0, 22.5. FT-IR (cm^{-1}): 3279, 3060, 3030, 2979, 2918, 1711, 1640, 1526, 1495, 1452, 1415, 1370, 1346, 1215, 1192, 1137, 1064, 985, 927, 826, 798, 773, 694. Anal. Calcd for $C_{20}H_{25}N_3O_7S_3$: C, 46.59%; H, 4.89%; N, 8.15%; S, 18.66%. Found: C, 46.56%; H, 4.92%;

N, 8.12%; S, 18.61%. ESI-MS: *m/z* = 514.0786 [M − H]$^-$, theoretical value = 514.0782 (Calcd for $C_{20}H_{24}N_3O_7S_3$).

Other CTAs were synthesized according to a similar method and obtained in almost quantitative yield.

2-Acetamido-3-(benzylsulfanylthiocarbonylsulfanyl)propionic acid (ABSPA): purified by silica flash column chloromatography using a gradient eluent of 1:1 hexane/ethyl acetate to pure ethyl acetate to give a yellow solid product in 99.2% of isolated yield. ^{1}H NMR (DMSO-d_6), δ: 8.43 (d, *J*8.1, 1H, NH), 7.32 (m, 5H, PhH), 4.68 (s, 2H, CH_2S), 4.51 (m, 1H, CH), 3.92 and 3.54 (m, each 1H, CH_2), 1.83 (s, 3H, CH_3CO). ^{13}C NMR (DMSO-d_6), δ: 222.9 (C=S), 171.5, 169.5 (C=O), 135.1, 129.3, 128.7, 127.8 (PhC), 50.5, 40.5, 37.7, 22.4. FT-IR (cm^{-1}): 3355, 3313, 3066, 3029, 2954, 2912, 1706, 1615, 1549, 1494, 1455, 1415, 1376, 1340, 1310, 1225, 1144, 1112, 1068, 1042, 1012, 976, 940, 916, 896, 860, 826, 800, 768, 700, 660. Anal. Calcd for $C_{13}H_{15}NO_3S_3$: C, 47.39%; H, 4.59%; N, 4.25%; S, 29.20%. Found: C, 47.42%; H, 4.60%; N, 4.23%; S, 29.15%. ESI-MS: *m/z* = 328.0147 [M − H]$^-$, theoretical value = 328.0141 (Calcd for $C_{13}H_{14}NO_3S_3$).

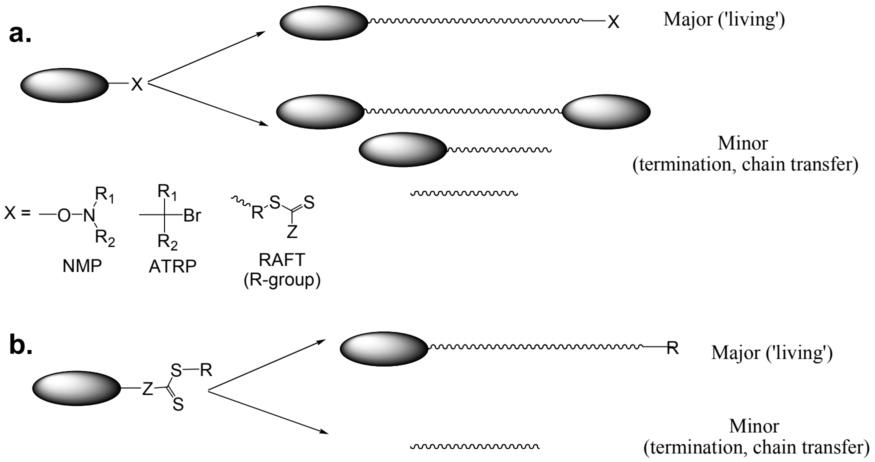
2-Acetamido-3-(methoxycarbonylphenylmethylsulfanylthiocarbonylsulfanyl)propionic acid (AMSPA): purified by silica flash column chloromatography using ethyl acetate as an eluent to give a yellow solid product in 98.5% of isolated yield. ^{1}H NMR (DMSO-d_6), δ: 8.45 (t, *J*7, 1H, NH), 7.41 (m, 5H, PhH), 5.87 (d, *J*2.4, 1H, CHS), 4.49 (m, 1H, CH), 3.88 and 3.56 (m, each 1H, CH_2), 3.68 (s, 3H, CH_3O), 1.83 (s, 3H, CH_3CO). ^{13}C NMR (DMSO-d_6), δ: 221.3 (C=S), 171.6, 169.9, 168.8 (C=O), 132.6, 129.5, 129.4, 128.9 (PhC), 57.6, 53.4, 50.7, 38.2, 22.5. FT-IR (cm^{-1}): 3337, 3322, 3062, 3030, 2999, 2950, 1728, 1619, 1529, 1496, 1453, 1432, 1373, 1333, 1280, 1209, 1152, 1070, 1004, 922, 900, 799, 727, 694, 664. Anal. Calcd for $C_{15}H_{17}NO_5S_3$: C, 46.49%; H, 4.42%; N, 3.61%; S, 24.83%. Found: C, 46.55%; H, 4.43%; N, 3.60%; S, 24.78%. ESI-MS: *m/z* = 386.0201 [M − H]$^-$, theoretical value = 386.0196 (Calcd for $C_{15}H_{16}NO_5S_3$).

2-Acetamido-*S*-(methoxycarbonylphenylmethylsulfanylthiocarbonyl)glutathione (AMSG): purified by phase separation between water and DCM, filtration, and washed with water and DCM to afford a yellow solid product in 98.2% of isolated yield. ^{1}H NMR (DMSO-d_6), δ: 8.40 (d, *J*8.4, 1H, NH), 8.35 (m, 1H, NH), 8.16 (d, *J*7.7, 1H, NH), 7.3-7.5 (m, 5H, PhH), 5.86 (d, *J*2.5, 1H, CH), 4.62 (m, 1H, CH, $H_{\alpha\text{-cys}}$), 4.16 (m, 1H, CH, $H_{\alpha\text{-glu}}$), 3.84 and 3.49 (m, each 1H, CH_2, $H_{\beta\text{-cys}}$), 3.75 (m, 2H, CH_2, H_{gly}), 3.68 (s, 3H, CH_3O), 2.19 (t, *J*8.0, 2H, CH_2, $H_{\gamma\text{-glu}}$), 1.95 and 1.76 (m, each 1H, CH_2, $H_{\beta\text{-glu}}$), 1.84 (s, 3H, CH_3CO). ^{13}C NMR (DMSO-d_6), δ: 221.2 (C=S), 173.6, 171.7, 170.9, 169.7, 169.5, 168.7 (C=O), 132.5, 129.3, 128.7, 128.4 (PhC), 57.3, 53.3, 51.5, 50.5, 40.9, 38.9, 31.7, 27.0, 22.4. FT-IR (cm^{-1}): 3288, 3062, 3031, 2953, 2923, 2856, 1729, 1645, 1524, 1452, 1433, 1412, 1375, 1336, 1279, 1255, 1208, 1162, 1118, 1071, 1046, 1006, 923, 889, 870, 801, 729, 695. Anal. Calcd for $C_{22}H_{27}N_3O_9S_3$: C, 46.06%; H, 4.74%; N, 7.32%; S, 16.77%. Found: C, 46.02%; H, 4.74%; N, 7.30%; S, 16.75%. ESI-MS: *m/z* = 572.0850 [M − H]$^-$, theoretical value = 572.0837 (Calcd for $C_{22}H_{26}N_3O_9S_3$).

General Procedure for RAFT Polymerization. Polymerizations were conducted in dioxane using AIBN as the primary radical source and ABSPA, AMSPA, ABSG, AMSG or GSH-PA macro CTA as the chain transfer agent. In a typical polymerization, DMA (1.983 g, 20.0 mmol), ABSG (0.103 g, 0.20 mmol), and AIBN (6.6 mg, 0.040 mmol) was added to a glass tube with a magnetic stirring bar, and dioxane was added until the total volume was 12.5 mL. The tube was sealed with a rubber septum, and the contents were degassed with nitrogen for 10 min.

The tube was subsequently immersed into an oil bath preheated to 60 °C. After 45 min, the polymerization was quenched by ice water. The monomer conversion was determined to be 20.5% by ^{1}H NMR. The polymer was recovered by precipitating into a large amount of cold hexane thrice from its dioxane solution, and molecular weight and polydispersity of GSH-PNIPAM were obtained by GPC: M_n = 2910, PDI = 1.11. Other polymers were synthesized according to a similar approach, and obtained by precipitation into cold hexane, diethyl ether or methanol.

Characterization. The number-average molecular weight (M_n) and polydispersity index (PDI) of polymer samples were determined by GPC at ambient temperature using THF as an eluent at a flow rate of 1.0 mL/min and toluene as a flow rate marker. PSt samples were calibrated with PSt standard samples with M_n value in the range of 7500000-580 g/mol, other samples were calibrated using PMMA standard samples with M_n value in the range of 1944000-1020 g/mol. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker 400 UltraShield spectrometer in $CDCl_3$ or DMSO-d_6. C, H and N contents were determined by combustion followed by chromatographic separation and thermal conductivity detection using a Carlo Erba 1108 Elemental Analyzer. Sulfur analyses were conducted using the Schoniger Oxygen Flask combustion method followed by the relevant titration. Fourier Transform Infrared (FT-IR) spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer using a single reflection horizontal ATR accessory. The electrospray ionization mass spectrometry (ESI-MS) was performed on a Bruker Daltonics micrOTOF mass spectrometer.

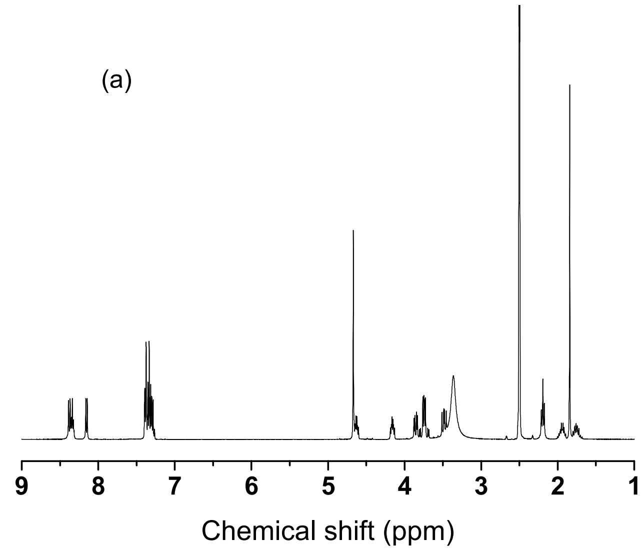


Scheme S1. Conjugates and side products obtained from living radical polymerisation initiated by polypeptide sequences.

Table S1. RAFT Polymerization of Various Monomers Mediated by ABSPA, AMSPA, ABSG and AMSG.[a]

run	CTA	M	$[M]_0/[CTA]_0$	t (h)	C%[b]	M_n(th)[c]	M_n(GPC)[d]	PDI[d]
1	ABSPA	NIPAM	200	3	86.1	19800	23600	1.15
2	ABSPA	DMA	200	3	92.4	18600	22200	1.11
3	ABSPA	MA	200	14	91.1	16000	21600	1.17
4	ABSPA	BA	200	14	88.3	23000	25900	1.21
5	ABSPA	St	200	14	16.7	3810	4160	1.20
6	ABSPA	MMA	200	14	59.9	12300	134000	1.59
7	AMSPA	NIPAM	200	15	97.7	22500	27300	1.12
8	AMSPA	DMA	200	15	97.8	19800	21100	1.09
9	AMSPA	MA	200	15	94.4	16600	20400	1.15
10	AMSPA	BA	200	15	93.2	24300	25900	1.18
11	AMSPA	St	200	15	10.4	2550	2870	1.25
12	AMSPA	MMA	200	15	35.2	7440	11400	1.35
13	ABSG	NIPAM	300	4	89.4	30900	38500	1.13
14	ABSG	DMA	300	4	92.8	28100	30200	1.18
15	ABSG	MA	300	4	82.4	21800	28800	1.16
16	ABSG	BA	300	4	75.1	29400	32000	1.18
17	ABSG	St	300	15	11.2	4010	4220	1.21
18	ABSG	MMA	300	15	47.4	14800	200000	1.68
19	AMSG	NIPAM	300	15	96.4	33300	39200	1.14
20	AMSG	DMA	300	15	97.8	29700	30800	1.21
21	AMSG	MA	300	15	90.7	24000	29500	1.18
22	AMSG	BA	300	15	89.1	34800	35600	1.17
23	AMSG	St	300	15	9.72	3610	4250	1.22
24	AMSG	MMA	300	15	28.5	9130	16300	1.37

[a] Polymerization conditions: $[CTA]_0:[AIBN]_0 = 5:1$, $[M]_0 = 1.60$ mol/L, in dioxane at 60 °C. [b] Monomer conversion determined by ^{1}H NMR. [c] Theoretical molecular weight, $M_n(\text{th}) = MW_m \times C\% \times [M]_0/[CTA]_0 + MW_{CTA}$, where MW_m and MW_{CTA} stands for molecular weight of monomer and CTA. [d] Molecular weight and polydispersity determined by GPC.



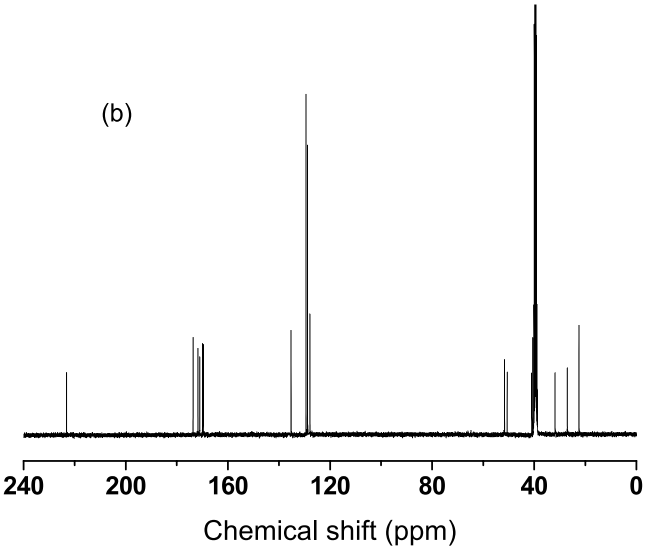
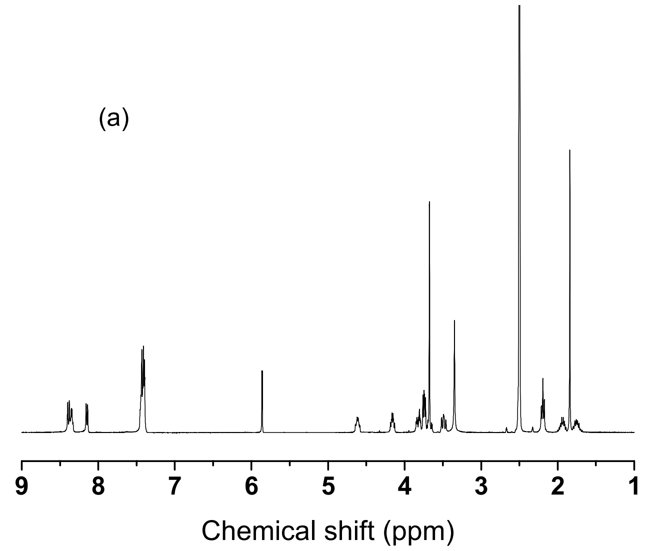


Figure S1. ^{1}H (a) and ^{13}C (b) NMR spectra of ABSG.



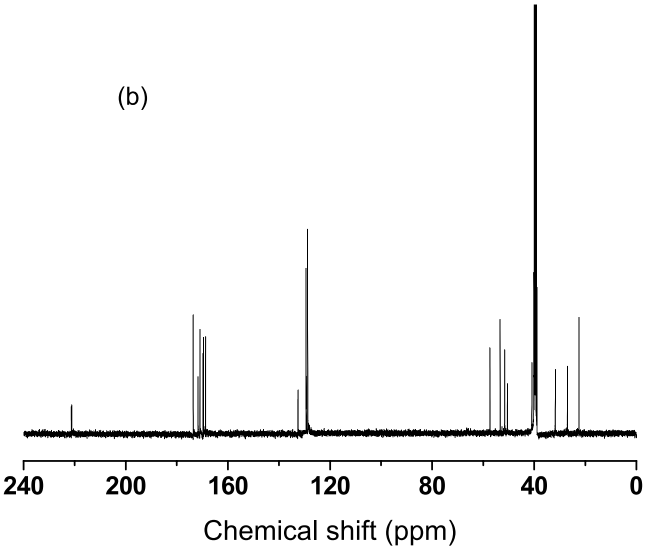


Figure S2. ^{1}H (a) and ^{13}C (b) NMR spectra of AMSG.

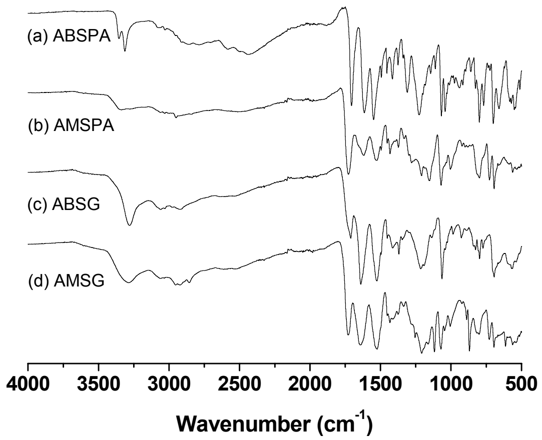


Figure S3. ATR FT-IR spectra of various RAFT agents.

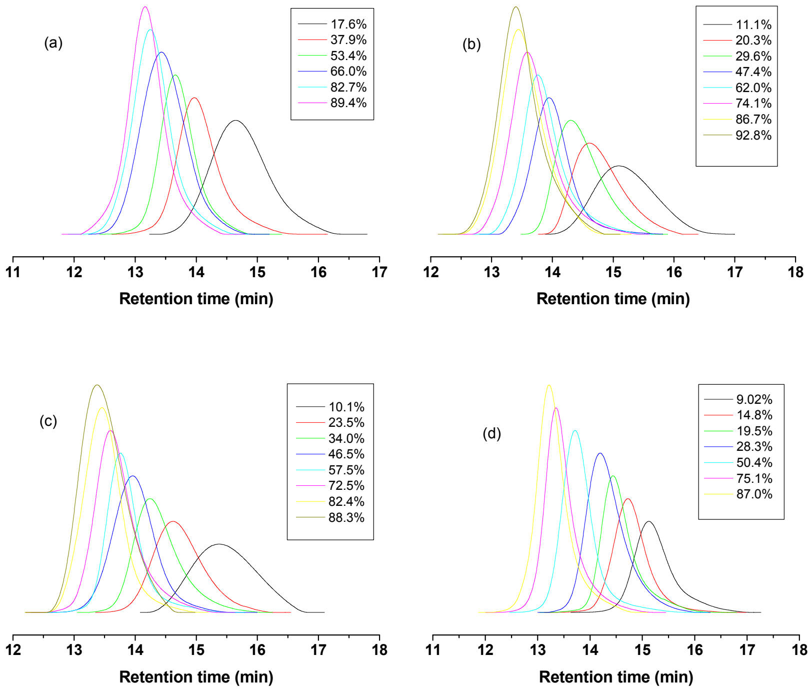
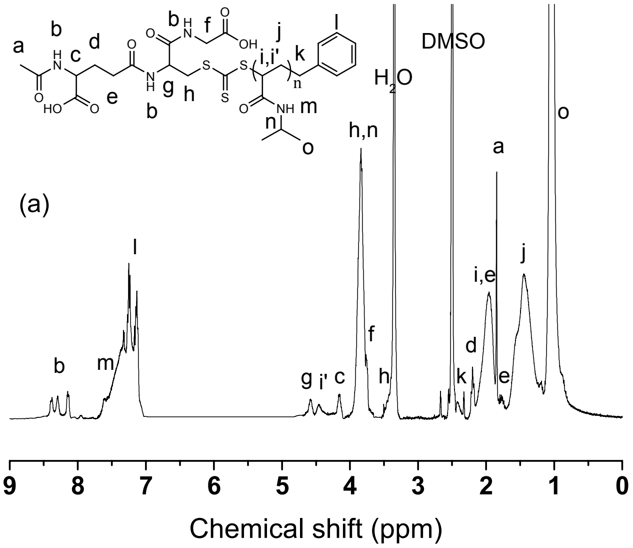


Figure S4. GPC traces of GSH-PA conjugates obtained by ABSG-mediated RAFT polymerization at different conversions: GSH-PNIPAM (a), GSH-PDMA (b), GSH-PMA (c) and GSH-PBA (d). Samples were synthesized as described in Figure 1.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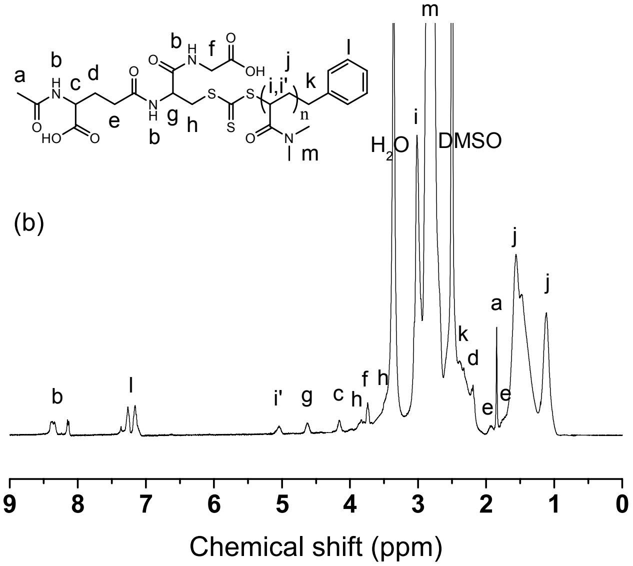
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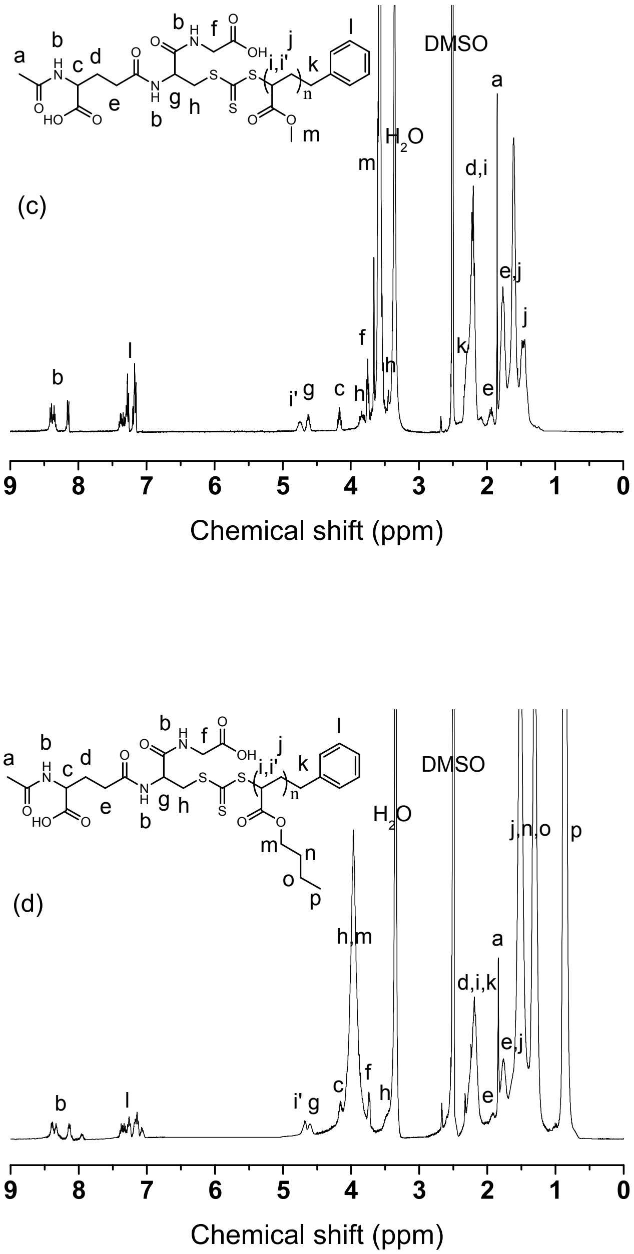

Figure S5. ^{1}H NMR spectra of GSH-PNIPAM (a), GSH-PDMA (b), GSH-PMA (c) and GSH-PBA (d). The GSH-PA samples were synthesized by runs 1-4 in Table S2.

Table S2. Chain extension polymerization mediated by GSH-PA macro CTAs in dioxane at 60 °C.[a]

run	macro CTA[b]					M	C%	M_n(th)[f]	M_n(GPC)[g]	PDI[g]
	C%	M_n(th)[c]	M_n(GPC)[d]	PDI[d]	M_n(NMR)[e]					
1	20.5	2840	2910	1.11	2780	DMA	93.5	21300	22200	1.11
2	36.3	4110	4200	1.12	4220	NIPAM	91.8	25000	29300	1.10
3	26.2	2770	3880	1.12	2700	NIPAM	92.7	23700	27500	1.09
4	21.9	3320	3480	1.13	3270	NIPAM	90.6	23800	25500	1.07

[a] Polymerization conditions: $[M]_0$:[macro CTA]$_0$ = 200:1, $[M]_0 = 1.00$ mol L^{-1}, $[AIBN]_0 = 1.20$ mmol L^{-1}, t = 6 h, macro-CTA = GSH-PNIPAM (run 1), GSH-PDMA (run 2), GSH-PMA (run 3) and GSH-PBA (run 4). [b] Macro CTAs synthesized by ABSG-mediated RAFT polymerization, $[M]_0:[ABSG]_0:[AIBN]_0 = 100:1:0.2$, $[M]_0 = 1.60$ mol L^{-1}, in dioxane at 60 °C. [c] Theoretically calculated molecular weight using equation $M_n(\mathrm{th}) = MW_M \times C\% \times [M]_0/[ABSG]_0 + MW_{ABSG}$, where MW_M and MW_{ABSG} mean the molecular weight of monomer and ABSG. [d] Molecular weight and polydispersity determined by GPC using PMMA standard. [e] Molecular weight determined by ^{1}H NMR by comparing the signal areas of protons in polymer chains with NH (7.9-8.5 ppm) or CH (about 4.6 ppm, $H_{\alpha\text{-cys}}$) protons in glutathione unit. [f] $M_n(\mathrm{th}) = MW_M \times C\% \times [M]_0/[\text{macro CTA}]_0 + MW_{\text{GSH-PA}}$, where MW_M and $MW_{\text{GSH-PA}}$ stand for the molecular weight of monomer and macro CTA. [g] Molecular weight and polydispersity of block copolymers determined by GPC using PMMA standard.