Supplementary Material (ESI) for Chemical Communications
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Supplementary Information

Approach to Bio-degradable Star Polymeric Architectures Using Disulfide Coupling

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1. Materials

Trimethylolpropane tris[3-mercaptopropionate] (TMPMP) (Aldrich) 2,2'-dithiodipyridine (DTDP) (Sigma), N,N-dimethylacetamide (DMAc), DL-Dithiothereitol (DTT) (>99.8%, Sigma-Aldrich), reduced glutathione (γ-Glu-Cys-Gly) (GSH) (99%, Sigma-Aldrich), carbon disulfide (>99%, Aldrich), chloroform (>99.8%, Univar), acetone (>99.5%, Univar), tetrabutylammonium hydrogen sulfate (99.5%, Aldrich), NaOH (97%, Univar), concentrated HCl (32%, Univar), triethylamine (TEA) (99%, Aldrich), thionyl chloride (>98%, Riedel-DeHaen), acetonitrile, (99%, Aldrich), ethyl acetate(>99.5%, Univar), 2,2'-dithiodipyridine (97%, Fluka), *n*-hexane (95%, Ajax), dichloromethane (99.5%, Univar), dimethyl acetamide (DMAc), diethyl ether (>99%, Univar), ethyl acetate (>99.5%, Univar), 2,2'-azobis(isobutyronitrile) (AIBN), 98%, Sigma-Aldrich), hydroxyethyl acrylate (HEA), N-isopropylacrylamide (NIPAAm) (97%, Aldrich) and silica gel (Fluka)， styrene (>99%, Aldrich).

2. **Measurement**

2.1 Gel permeation chromatography (GPC)

Gel permeation chromatography (GPC) analyses of polymers was performed in N,N-dimethylacetamide (DMAc) (0.03% w/v LiBr, 0.05% BHT stabilizer) at 50 °C (flow rate: 0.85 mL.min^{-1}) using a Shimadzu modular system comprising a DGU-12A solvent degasser, an LC-10AT pump, a CTO-10A column oven, and an RID-10A refractive index detector. The system was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.8 mm^2) followed by four 300 × 7.8 mm^2 linear PL columns (10^5, 10^4, 10^3, and 500). Calibration was performed with narrow polydisperse polystyrene standards ranging from 500 to 10^6 gmol^{-1}.

2.2 UV-vis Spectrophotoscopy

The UV-vis absorption spectra were recorded on a Cary 300 Scan spectrophotometer (Varian).

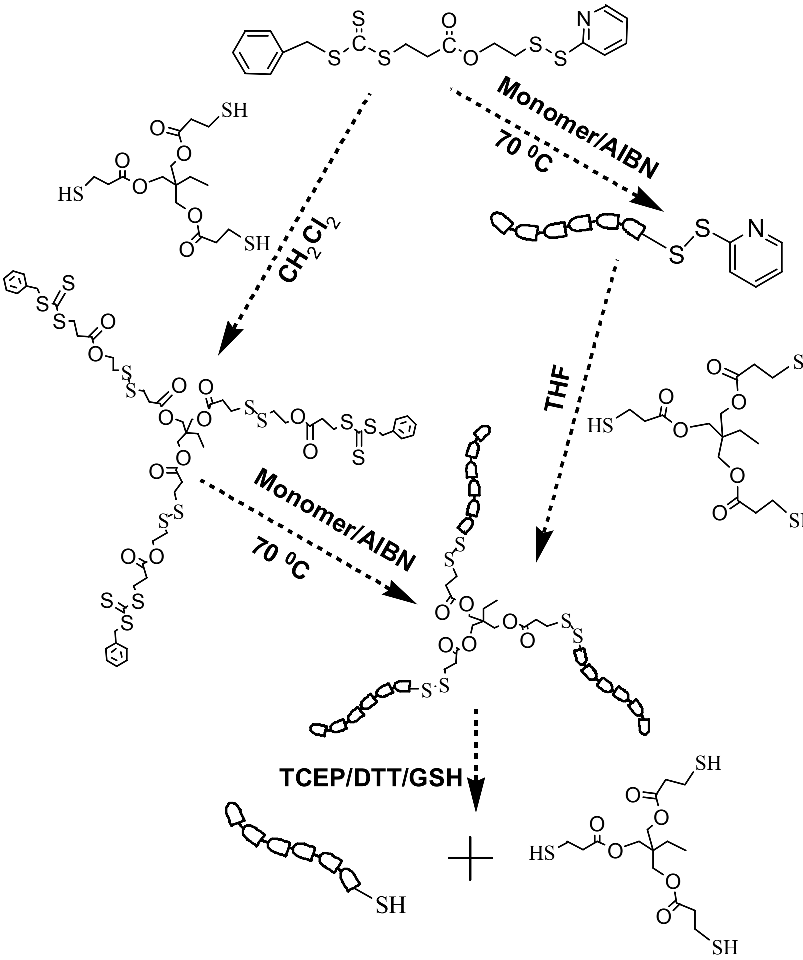
2.3 NMR Spectroscopy

^{1}H NMR spectra were obtained using a Bruker AC300F (300 MHz) Spectrometer or a Bruker DPX300 (300 MHz) Spectrometer. Data were reported by the chemical shifts (δ) measured in parts per million (ppm) downfield from TMS, multiplicity and proton count. Multiplicities were reported as singlet (s), broad singlet (bs), doublet (d), triplet (t), and multiplet (m). ^{13}C spectra were obtained on Bruker AC300F (300 MHz) spectrometer. ^{13}C chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS and identifiable signals were given. The protons and carbons relevant to the response in NMR spectra are highlighted in bold and italic character.

2.4 ESI mass spectroscopy

Mass spectra were obtained on a Finnigan LCQ Deca mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with an atmospheric pressure ionization source operating in the nebulizer-assisted electrospray mode. The instrument was calibrated in the *m*/*z* range 195-1822 Da using a standard containing caffeine, Met-Arg-Phe-Ala acetate salt (MRFA), and a mixture of fluorinated phosphazenes (Ultramark 1621) (all from Aldrich).

3. Synthesis



Scheme 1. The schematic of 'core first' and 'arm first' methodologies to generate identical three-armed polymers and the subsequent cleavage into single-armed linear chain.

As shown in Scheme 1, the synthesis of three armed polymers could be achieved via two protocols. In the first protocol a three armed RAFT agent was first synthesized by attaching a pyridyl disulfide (PDS) terminated trithiocarbonate RAFT agent to a trithiol precursor, TMPMP by disulfide linkage, followed by the in-situ polymerization in the presence of AIBN as initiator to generate the three armed branch polymer. In the second protocol the three armed polymer was generated by the synthesis of linear polymeric chains terminated with thiol reactive PDS groups, followed by the postpolymerization conjugation to TMPMP to form three armed polymers.

3.1 Synthesis of three armed RAFT agent

The synthesis of 3-benzylsulfanylthiocarbonylsulfanyl propionic acid (BSTP) and its derivative with terminal pyridyldisulfide functionality, 3-[pyridyldisulfide]ethyl, 3-benzyltrithiocarbonate propionate (PDEBP) were carried out following the method reported elsewhere.[1-3] The ^{1}H NMR of PDEPT is shown in **Figure S1** below.

To a deoxygenated solution of TMPMP (0.035 g, 9 x 10^{-5} mol) in 10 ml CH_2Cl_2 was added slowly PDEBP (0.147 g, 3.6 x 10^{-4} mol) solution in 5 ml CH_2Cl_2. The resulting mixture was stirred for 3 h under nitrogen atmosphere, followed by the removal of the volatile. Slica gel chromatography using hexane/EtOAc (50/50) as eluent afforded 0.116 g pure three armed RAFT agent (yield, 93%). 300 MHz ^{1}H NMR ($CDCl_3$) δ: 7.28-7.31 (m, 15H, C***H***=C***H***), 4.61 (s, 6H, C***H2***-CH=***C***H), 4.33-4.38 (t, 6H, C***H***$_2$O-CO), 4.06 (s, 6H, C***H***$_2$O), 3.60-3.65 (t, 6H, C***H***$_2$-S), 2.90 (m, 12H, C***H***$_2$-S), 2.75-2.81 (m, 12H, C***H***$_2$-CO), 1.48-1.50 (m, 2H, C***H***$_2$-CH$_3$), 0.88-0.90 (t, 3H, C***H***$_3$-CH$_2$). 300MHz ^{13}C NMR ($CDCl_3$) δ: 31.18 (***C***H$_3$) 32.97 (***C***H$_2$), 33.81 (***C***H$_2$), 37.77 (***C***H$_2$), 41.44 (***C***H$_2$S), 62.68 (***C***H$_2$CO), 63.92 (***C***H$_2$CO), 127.73 (***C***H=CH), 128.63 (***C***H=CH), 129.16 (***C***H=CH), 134.75 (CH$_2$-***C***H=CH), 171.14 (***C***O), 222.79 (***C***=S). The ^{1}H NMR of three-armed RAFT agent is shown in **Figure 1** in the context of the paper.

3.2 Polymerization of N-isopropylacrylamide (NIPAAm) using three armed RAFT Agent.

A solution of NIPAAm (0.32 g, 2.85 x 10^{-3} mol), three armed RAFT agent (13.2 mg, 9.5 x 10^{-6} mol), and AIBN (0.80 mg, 4.75 x 10^{-6} mol) were dissolved in doxane (4.0 ml) to obtain a homogeneous solution. Aliquots were transferred to six different vials, which were then sealed with rubber septa. Each vial was deoxygenated for 30 min, followed by the placement in a preheated water bath at 60 ºC. The vials were removed at 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 h. Immediate cooling with ice and exposure to air halted the polymerization. The monomer conversion for each polymerization sample was determined by ^{1}H NMR after the removal of dioxane from polymerization mixtures under vacuum. The polymers were collected after precipitation in diethyl ether and then dried under vacuum.

The monomer conversion against the polymerization time, the molecular weight (MW) and PDI against the monomer conversion, ^{1}HNMR spectrum of purified polyNIPAAm (M_n 4,000 g/mol from GPC, PDI 1.28 in $CDCl_3$) are shown in Figure 3S below.

3.3 Polymerization of styrene (St) using three armed RAFT Agent.

A solution of styrene (0.31 g, 2.74 x 10^{-3} mol), three armed RAFT agent (12 mg, 8.64 x 10^{-6} mol), and AIBN (1.0 mg, 6.1 x 10^{-6} mol) were dissolved in doxane (4.0 ml) to obtain a homogeneous solution. Aliquots were transferred to five different vials, which were then sealed with rubber septa. Each vial was deoxygenated for 30 min prior, followed by the placement in a preheated water bath at 75 ºC. The vials were removed at 5, 9, 21, 29 and 45 h. Immediate cooling with ice and exposure to air halted the polymerization. The monomer conversion for each polymerization sample was determined by ^{1}H NMR after the removal of dioxane from polymerization mixtures under vacuum. The polymers were collected after precipitation in diethyl ether and then dried under vacuum.

The monomer conversion against the polymerization times, the molecular weight (MW) and PDI against the monomer conversion, ^{1}H NMR spectrum of purified PSt (M_n 4,000 g/mol from GPC, PDI 1.24 in $CDCl_3$) are summarized in Figure 4S below.

3.4 Cleavage of three-armed PNIPAAm using DTT

To the three armed PNIPAAm (10 mg, 1.0 x 10^{-5} mol) solution in 1 ml phosphate buffer (pH 6.5) was added DTT (15.4 mg, 1.0 x 10^{-4} mol). The resulting mixture was sealed and kept shaking for 2 hours prior to GPC analysis to monitor the MW change. The GPC chromatograms before and after cleavage is shown in Figure 2a in the paper context.

3.5 Cleavage of three-armed PNIPAAm using GSH

To the three armed PNIPAAm (5 mg, 5 x 10^{-6} mol) solution in 1 ml phosphate buffer (pH 5.0) was added GSH (40 mg, 1.4 x 10^{-4} mol). The resulting mixture was sealed and kept shaking for 48 hours prior to GPC analysis to monitor the MW variation. The GPC chromatograms of PNIPAAm before and after cleavage is shown in Figure 5S in the paper context.

3.5 Synthesis of three armed polystyrene via post-polymerization conjugation

To a deoxygenated solution of linear PSt (10 mg, 1 x 10^{-6} mol, MW, 10,000, PDI, 1.08) in THF (10 ml) was slowly added deoxygenated solution of TMPMP (0.13 mg, 3.33 x 10^{-7} mol) in 2 ml THF. The resulting mixture was kept stirring for three hours, followed by the DMAc GPC and UV-vis spectroscopic analysis. The mixture was then added 10 mg of DTT, and kept stirring for another 2 hours prior to GPC analysis. The GPC traces before and after post-polymerization conjugation and cleavage again using DTT are summarized in Figure 2b in the paper context. The UV-vis spectrum is shown in Figure 6S below.

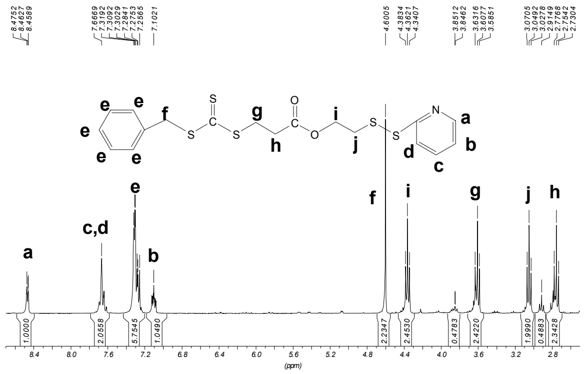


Figure S1. ^{1}H NMR of PDEBP

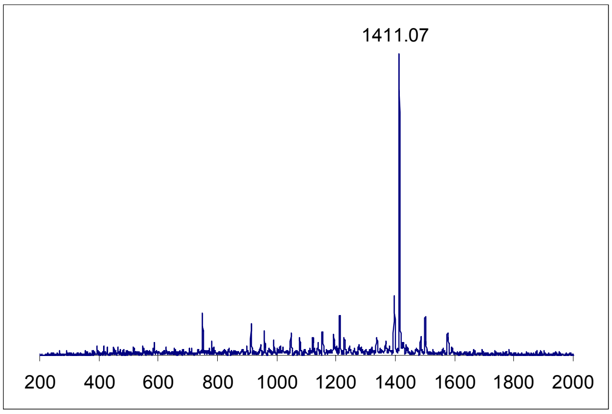
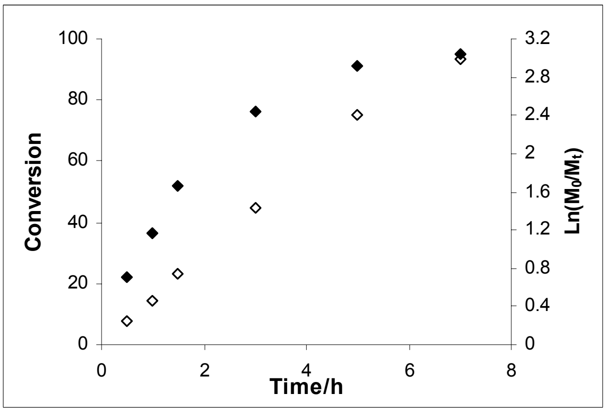
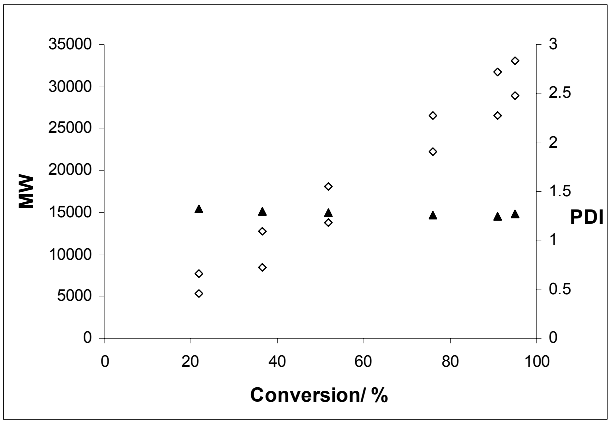


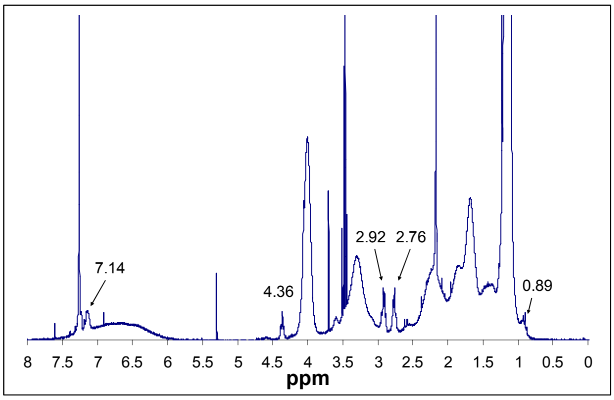
Figure S2. ESI mass spectrum of trifunctional RAFT initiator



(a)

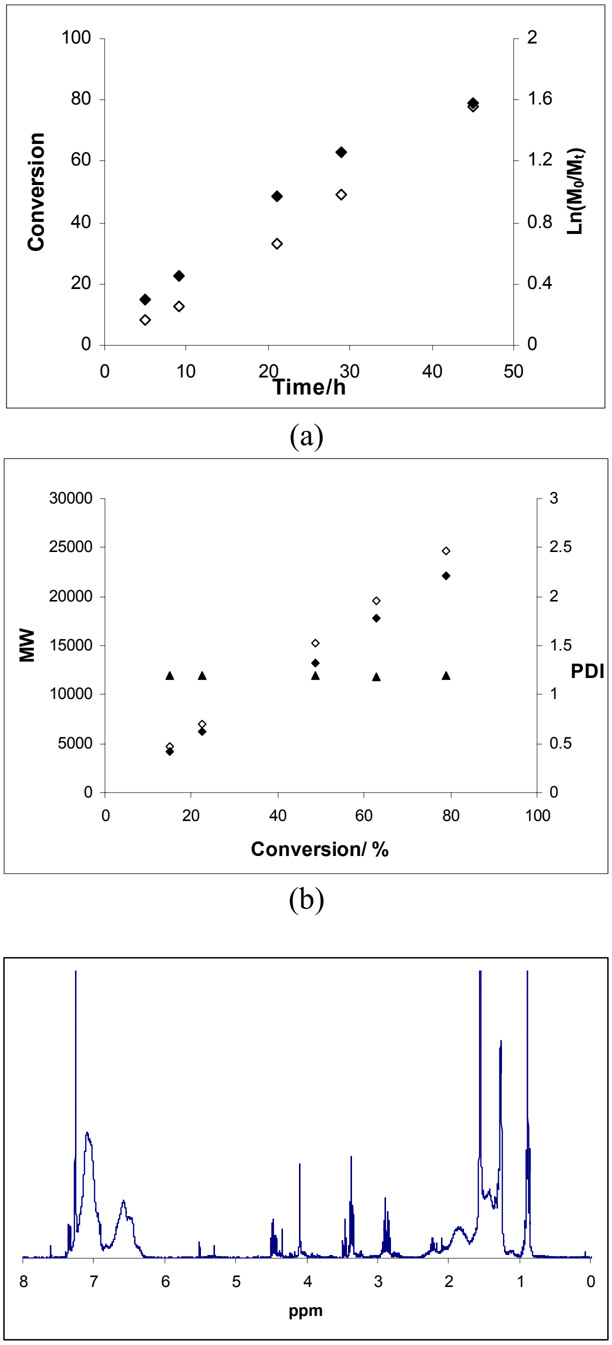


(b)



(c)

Figure 3S. Polymerization of NIPAAm using three armed RAFT agent in dioxane at 60 ºC ([M]/[RAFT]/[AIBN] = 300:1:0.25). (a) Monomer conversion at varying polymerization times. (b) Molecular weight (MW) and PDI of the PNIPAAm versus monomer conversion (filled and empty diamonds represent the experimental and theoretical MW values, respectively, while filled triangles represent PDI). (c) ^{1}HNMR spectrum of purified PNIPAAm (M_n 10,000 g/mol from GPC, PDI 1.24 in $CDCl_3$).



(a)

(b)

(c)

Figure S4. Polymerization of styrene using three armed RAFT agent in dioxane at 75 ºC ([M]/[RAFT]/[AIBN] = 300:1:0.25). (a) Monomer conversion against the polymerization time. (b) Molecular weight and PDI of the PSt versus monomer conversion (filled and empty diamonds represent the experimental and theoretical MW values, respectively, while filled triangles represent PDI). (c) ^{1}H NMR spectrum of purified PSt (M_n 10,000 g/mol by GPC, PDI 1.24) in $CDCl_3$.

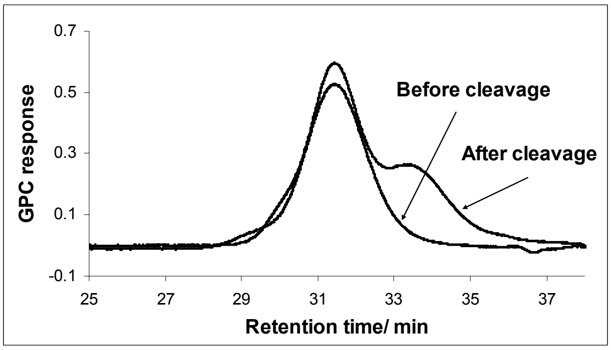


Figure S5. GPC trace of PNIPAAm after cleavage using GSH in phosphate buffer (pH 5.0) for 48 hours.

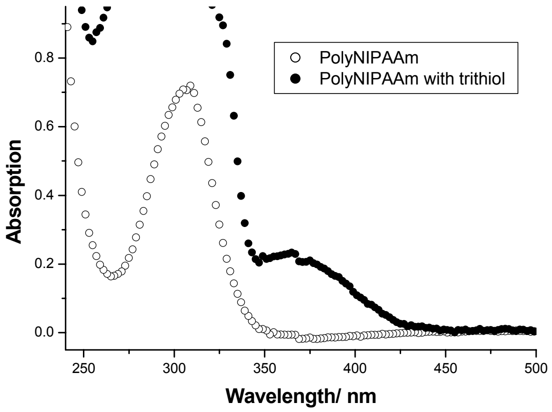


Figure S6. UV-vis monitoring of conjugation of PNIPAAm with trithiol, TMPMP. Empty circle represents the spectrum of PNIPAAm and the filled circle represents the reaction mixture of PNIPAAm with TMPMP.

(1) Stenzel, M. H.; Davis, T. P. *J Polym Sci Pol Chem* **2002**, *40*, 4498-4512.
(2) Liu, J.; Bulmus, V.; Barner-Kowollik, C.; Stenzel, M. H.; Davis, T. P. *Macromol Rapid Comm* **2007**, *28*, 305-314.

(3) Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754-6756.
(4) Klaikherd, A.; Ghosh, S.; Thayumanavan, S. *Macromolecules* **2007**, *40*, 8518-8520.
(5) Hermanson, G. T. *Bioconjugate Techniques* **1996**, *Academic Press: New York*.
(6) Wong, L. J.; Boyer, C.; Jia, Z. F.; Zareie, H. M.; Davis, T. P.; Bulmus, V. *Biomacromolecules* **2008**, *9*, 1934-1944.
(7) Fu, C. L.; Ouyang, W.; Sun, Z. Y.; An, L. *J Chem Phys* **2007**, *127*, -.