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RAFT controlled synthesis of six-armed biodegradable star polymeric architectures via a 'core-first' methodology

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ABSTRACT

Six-armed biodegradable star polymers made from polystyrene (polySt), poly(polyethylene glycol) acrylate (polyPEG-A) and the block copolymer, polySt-b-polyPEG-A were synthesized using a 'core-first' methodology *via* RAFT polymerization. Disulfide linkages between the core and the arms conferred biodegradability on the stars. The star architectures were found to degrade rapidly on treatment with DL-dithiothreitol (DTT) and degrade more slowly in the presence of glutathione (GSH), the most abundant intracellular thiol tethered peptide. These star polymers were well characterized using gel permeation chromatography (GPC), nuclear magnetic resonance (NMR), electrospray ionization mass spectroscopy (ESI-MS) and dynamic light scattering (DLS).

1. Introduction

Star polymers are branched polymers consisting of several linear chains linked to a central core. In the past decade star polymers have attracted increasing interest due to their potential applications in a number of areas, e.g. encapsulation, sensing, catalysis, electronics, optics, biological engineering, coatings, additives, drug and gene delivery [1–4]. Generally speaking, these complicated architectures can be attained by 'arm-first' [5–12] or 'core-first' methodologies. The 'arm-first' methodology is often used to synthesize lower-armed simple star structures of lower molecular weight. As the architectures get more complex (more arms) or as higher molecular weights are targeted this 'arm-first' approach becomes less favorable as steric hindrance can inhibit the attachment process resulting in structural defects. Therefore more complicated star architectures are usually designed via a 'core-first' strategy [13–23]. Star polymers consisted of miktoarms have also been reported, often by combining these two methodologies [24–30].

A number of polymerization methods have been successfully utilized for the generation of multi-armed polymer architectures. In addition to ionic, coordination ring-opening and catalytic condensation polymerizations [31–33], the mostly exploited methods are living radical polymerizations (LRPs) e.g. atom transfer radical polymerization (ATRP) [7,21,24,34–38], nitroxide mediated radical polymerization (NMRP) [39,40] and reversible addition fragmentation chain transfer (RAFT) polymerization [9,10,14,15,17,19,41–44] to generate multi-armed structures with predetermined molecular weights and narrow molecular weight distributions. Most star polymers consist of homopolymer arms, however they can also be tailored with miktoarms [25,28–30,45]. A combination of different LRP methods can be also used for the generation of more complicated polymeric architectures [5,26].

Complex star polymeric architectures that can be cleaved into their component parts are promising candidates for applications in drug delivery and biotherapeutics. A number of covalent linkages are biodegradable, e.g. the disulfide linkage is cleavable in the presence of glutathione (GSH) [46–48], the acetal linkage is acid labile [49] and the ester linkage is degradable upon hydrolysis [33,50]. As the most abundant intracellular thiol (0.2–10 mM) in most mammalian and many prokaryotic cells GSH itself or with the aid of enzyme can *in vivo* cleave disulfide linkages that exist in proteins and enzymes [46]. Therefore a polymeric structure intra-linked by disulfide bonding could be easily cleaved into smaller fragments *in vivo* and subsequently excreted.

Matyjaszewski and coworkers have done elegant research on creating biodegradable linear polymers [48] and hydrogels containing internal disulfide links using ATRP polymerization. Biodegradable hydrogels cross-linked by disulfide linkages were also successfully synthesized by Armes et al. [47] Linear polymers with pyridyldisulfide (PDS) end groups have been extensively studied and utilized for the synthesis of functional polymers and biomolecule–polymer conjugates [51–54]. Recently we published

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a communication on the synthesis of biodegradable three-armed star architectures with disulfide intra-linkages using both 'arm-first' and 'core-first' methodologies [44]. In the current study we have extended our research to synthesize a more complex six-armed architecture containing biodegradable disulfide intra-linkages using a 'core-first' methodology. Six-armed star architectures of polystyrene, polyPEG-A and their amphiphilic block copolymers were generated using a novel six-armed multi-functional RAFT agent. The cleavability and biodegradability of these star structures were also tested using both DTT and GSH.

2. Experimental section

2.1. Materials

1,3,5-Benzenetricarbonyl trichloride (Aldrich, 98%), 2-amino-2-methyl-1,3-propanediol (Aldrich, 99%), succinic anhydride (Lancaster, 99%), 4-dimethylamino pyridine (DMAP) (Aldrich, 99%), 2,2′-dithiodipyridine (DTDP) (Sigma, >97%), 2-hydroxylethyl disulfide (Aldrich, technical grade), *N*,*N*-dimethyl acetamide (DMAc) (Aldrich, 99%), *N*,*N*-dicyclohexyl carbodiimide (DCC) (Fluka, >99.8%), DL-dithiothreitol (DTT) (Sigma–Aldrich, >99.8%), reduced glutathione (γ-Glu-Cys-Gly) (GSH) (Sigma–Aldrich, 99%), carbon disulfide (Aldrich, >99%), chloroform (Univar, >99.8%), acetone (Univar, >99.5%), tetrabutylammonium hydrogen sulfate (Aldrich, 99.5%), NaOH (Univar, 97%), concentrated HCl (Univar, 32%), triethylamine (TEA) (Aldrich, 99%), thionyl chloride (Riedel-DeHaen, >98%), acetonitrile, (Aldrich, 99%), ethyl acetate (Univar, >99.5%), *n*-hexane (Ajax, 95%), dichloromethane (Univar, 99.5%), diethyl ether (Univar, >99%), ethyl acetate (Univar, >99.5%), 2,2′-azobis(isobutyronitrile) (AIBN) (Sigma–Aldrich, 98%), poly(ethylene glycol) (PEG-A) (Aldrich, 97%) and styrene (Aldrich, >99%). The synthesis of 3-benzylsulfanylthiocarbonylsulfanyl propionic acid (BSTP) was carried out following the method reported elsewhere [55,56].

2.2. Synthesis of hexa-ol functionalized core [2]

A solution of 1,3,5-benzenetricarbonyl trichloride [1] (0.50 g, 1.88 mmol) in dry THF (1 ml) was added dropwise into a solution of 2-amino-2-methyl-1,3-propanediol (1.39 g, 13.2 mmol) in dry methanol (9 ml). The expected hexa-ol star molecule [2], a colorless solid, slowly precipitated out during the 4 h reaction. The hexa-ol [2] was then collected and dried under vacuum and used for the next step reaction without further purification (0.53 g, 59.8% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.21 (s, 3H, C***H***=C), 7.64 (s, 3H, CON***H***), 4.80 (t, 6H, O***H***), 3.60 (m, 9H, *C**H***$_2$OH), 1.28 (s, 9H, *C**H***$_3$CH$_2$). ^{13}C NMR (75 MHz): 166.5 (***C***O), 135.9 (***C***–CO), 129.0 (***C***H=C), 63.88 (***C***–OH), 59.57 (***C***–NH), 18.82 (***C***H$_3$).

2.3. Synthesis of hexa-carboxylic acid functionalized core [3]

Hexa-ol functionalized star core [2] (0.18 g, 0.39 mmol), succinic anhydride (0.47 g, 4.68 mmol) and 4-dimethylamino pyridine (DMAP) (0.03 g, 0.25 mmol) were dissolved in *N*,*N*′-dimethyl acetamide (DMAc) (5 ml) in a 50 ml round bottom flask. Upon stirring for 24 h at 45 °C the reaction mixture was cooled to ambient temperature and precipitated in diethyl ether for two times. A colorless oil precipitate was collected and dried under vacuum to obtain the hexa-carboxylic acid functionalized core [3] (0.38 g, 89.9% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.16 (s, 6H, C***H***=C and CON***H***), 4.37 (s, 12H, *C**H***$_2$O), 2.41–2.50 (m, 24H, COC***H***$_2$*C**H***$_2$CO), 1.38 (s, 9H, *C**H***$_3$CH$_2$). ^{13}C NMR (75 MHz): 173.98 (***C***OOH), 172.19 (***C***O), 169.95 (***C***O), 125.78 (***C***=CH), 107.20 (C=***C***H), 65.15 (***C***H$_2$–O), 56.11 (***C***–CH$_3$), 29.31 (***C***H$_2$), 29.24 (***C***H$_2$), 21.78 (***C***H$_3$). The calculated mass with Na, 494.22; mass from ESI-MS spectrum, 494.27.

2.4. Synthesis of 3-[hydroxylethyl disulfide]ethyl, 3-benzyltrithiocarbonate propionate (HDEBP)

BSTP (2.17 g, 7.98×10^{-3} mol), 2-hydroxylethyl disulfide (3.70 g, 2.4×10^{-2} mol), DMAP (0.05 g, 4.1×10^{-4} mol) and DCC (2.00 g, 9.7×10^{-3} mol) were dissolved in THF (25 ml). The resulting mixture was stirred at room temperature for 12 h, followed by filtration of the white precipitate to afford a yellow solution. After removal of the volatiles under evaporation the crude product was purified by silica gel chromatography using ethyl acetate/hexane (50/50) as eluent to obtain the expected product (2.75 g, yield, 84.4% based on the BSTP precursor). 300 MHz ^{1}H NMR ($CDCl_3$) δ (ppm): 7.29–7.33 (m, 5H, phenyl group), 4.61 (s, 2H, 2 × C_6H_5–C***H***$_2$), 4.36–4.41 (t, 2H, C***H***$_2$O), 3.87–3.91 (t, 2H, HO–C***H***$_2$), 3.61–3.66 (t, 2H, C–S–C***H***$_2$), 2.86–2.95 (m, 4 H, S–S–C***H***$_2$) and 2.78–2.83 (t, 2H, CO–C***H***$_2$). ^{13}C NMR (75 MHz) ($CDCl_3$): 31.16 (CH_2), 33.00 (***C***H$_2$), 36.75 (***C***H$_2$), 41.50 (***C***H$_2$S), 60.15 (***C***H$_2$O), 62.52 (CH$_2$***C***O), 127.70 (***C***H=CH), 127.72 (***C***H=CH), 128.63 (***C***H=CH), 129.18 (***C***H=CH), 134.72 (CH$_2$–***C***H=CH), 171.23 (***C***O), 222.77 (***C***=S). The ^{1}H NMR spectrum is shown in Fig. 1. Calculated mass with Na, 1194.31; mass from ESI-MS spectrum, 1194.26.

2.5. Synthesis of hexa-functional RAFT agent [4]

Hexa-carboxylic acid functionalized core [3] (0.056 g, 5.22×10^{-5} mol), HDEBP (0.23 g, 5.66×10^{-4} mol), DMAP (2 mg, 1.56×10^{-5} mol) and DCC (0.086 g, 4.2×10^{-4} mol) were dissolved in THF (5 ml). The resulting mixture was stirred at room temperature for 12 h, followed by filtration to remove the solid by-product. The filtrate was then dried under evaporator and purified using a silica gel chromatography to afford the expected product (82 mg, 50.3%). 300 MHz ^{1}H NMR ($CDCl_3$) δ (ppm): 8.28 (s, 3H, phenyl core), 7.29–7.33 (m, 25H, phenyl group), 4.57 (s, 12 H, 2 × C_6H_5–C***H***$_2$), 4.24–4.51 (t, 36H, C***H***$_2$O), 3.59–3.64 (t, 12H, S–C***H***$_2$), 2.86–2.89 (m, 24 H, S–S–C***H***$_2$) and 2.76–2.81 (t, 12H, CO–C***H***$_2$), 2.65–2.70 (t, 12H, CO–C***H***$_2$), 1.53 (S, 9H, CH_3). 75 MHz ^{13}C NMR ($CDCl_3$): 20.96 (***C***H$_3$), 24.75 (***C***H$_2$), 28.87 (***C***H$_2$), 31.16 (***C***H$_2$S–S), 33.00 (***C***H$_2$S–S), 36.75 (***C***H$_2$S–S), 41.50 (***C***H$_2$S–CO), 60.15 (***C***H$_2$O), 62.62 (CH$_2$***C***O), 127.70 (***C***H=CH), 127.72 (***C***H=CH), 128.63 (***C***H=CH), 129.16 (***C***H=CH), 134.73 (CH$_2$–***C***H=CH), 171.08 (***C***O), 172.00, 222.74 (***C***=S). The ^{1}H NMR spectrum is shown in Fig. 2a. The calculated mass with Na ion, 3434.25; mass from ESI-MS spectrum, 3434.20.

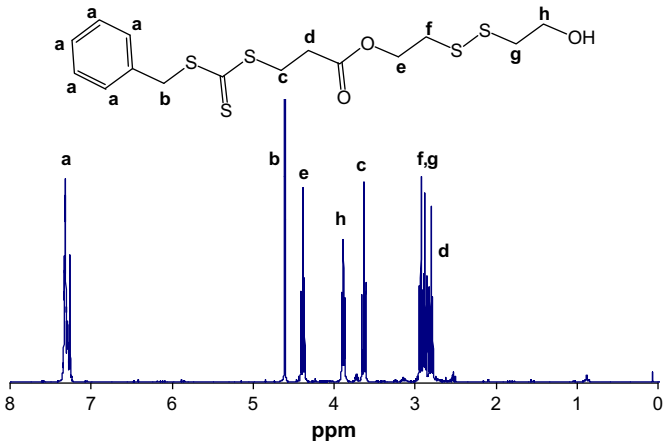


Fig. 1. ^{1}H NMR spectrum of 3-[hydroxylethyl disulfide]ethyl, 3-benzyltrithiocarbonate propionate (HDEBP) using $CDCl_3$ as deuterated solvent.

2.6. Polymerization of styrene using hexa-functional RAFT agent

Styrene (0.15 g, 1.41×10^{-3} mol), hexa-functional RAFT agent [4] (0.024 g, 7.03×10^{-6} mol), and AIBN (1.8 mg, 1.10×10^{-5} mol) were dissolved in dioxane (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 to the incubation in a preheated water bath at 75 °C. The vials were removed at 4, 7, 10, 15 and 20 h polymerization period. Immediate cooling with ice and exposure to air halted the polymerizations. The monomer conversion for each polymerization sample was determined by ^{1}H NMR of each sample mixture before purification. The pure polymers were obtained by precipitation of the reaction mixture in hexane three times and then dried under vacuum.

2.7. Polymerization of PEG-A using hexa-functional RAFT agent

PEG-A (0.27 g, 5.8×10^{-4} mol), hexa-functional RAFT agent (0.020 g, 5.8×10^{-6} mol), and AIBN (1.2×10^{-3} g, 7.3×10^{-6} mol) were dissolved in dioxane (5 ml) to obtain a homogeneous solution. Aliquots were transferred to five vials, which were then sealed with rubber septa. Each vial was deoxygenated for 30 min prior to the incubation in a preheated water bath at 70 °C. The vials were removed at 2, 4, 6, 10 and 15 h polymerization period. Immediate cooling with ice and exposure to air halted the polymerization. The monomer conversion for each polymerization sample was determined by the ^{1}H NMR of the reaction mixture before purification. The pure polymers were collected after precipitation in diethyl ether three times and then dried under vacuum.

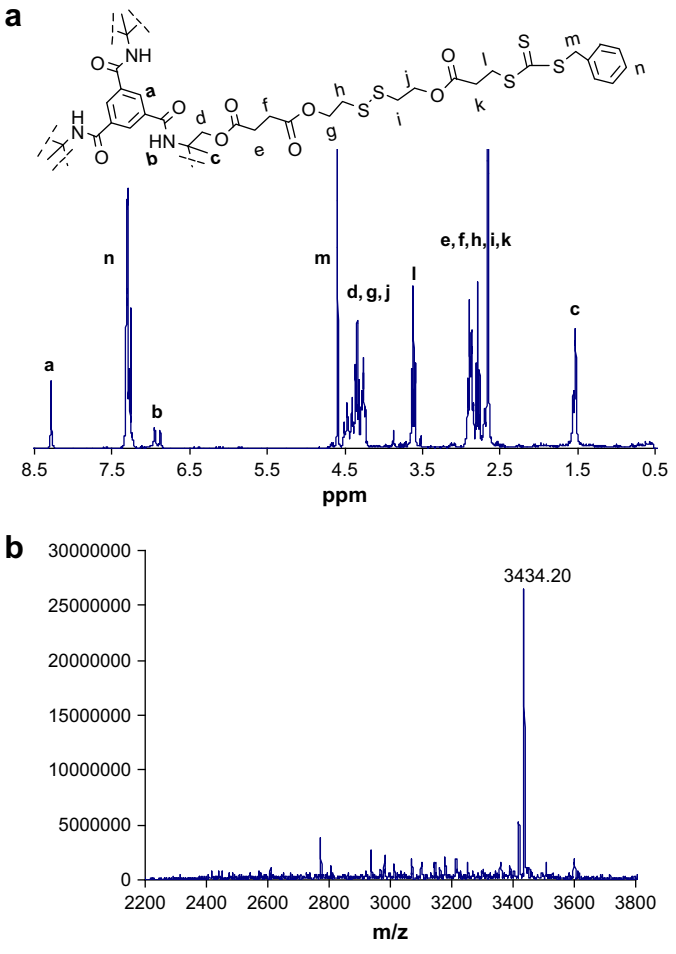


Fig. 2. ^{1}H NMR spectrum of six-armed RAFT initiator using $CDCl_3$ as deuterated solvent (a) and its ESI-mass spectrum (b).

2.8. Chain extension reaction of hexa-polystyrene with PEG-A

Six-armed polystyrene (0.12 g, 7.1×10^{-4} mol, MW 17,000 from DLS), PEG-A (0.32 g, 7.1×10^{-4} mol), and AIBN (1.4×10^{-3} g, 8.5×10^{-6} mol) were dissolved in dioxane (4 ml) to obtain a homogeneous solution. Aliquots were transferred to four 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 70 °C. The vials were removed at 4, 8, 12 and 20 h polymerization period. Immediate cooling with ice and exposure to air halted the polymerization. The monomer conversion for each polymerization sample was determined by ^{1}H NMR spectra of the polymerization mixtures before purification. The block copolymers were purified by precipitation in diethyl ether three times and then dried under vacuum.

2.9. Cleavage of six-armed polySt-b-polyPEG-A using DTT

Six-armed polySt-b-polyPEG-A (4 mg, 1.1×10^{-7} mol, MW 35,600 from ^{1}H NMR) was dissolved in DMAc (1 ml), followed by the addition of DTT (1 mg, 6.5×10^{-6} mol). The resulting mixture was sealed and shaken for 12 h at ambient temperature prior to GPC analysis to monitor the MW change. The GPC chromatograms before and after cleavage by DTT are shown in Fig. 6a.

2.10. Cleavage of six-armed polyPEG-A using glutathione (GSH) in pH 5 phosphate buffer

Six-armed polyPEG-A (1.8 mg, 1.2×10^{-7} mol, MW 159,00 from DLS) was dissolved in phosphate buffer (pH 5.0) solution (1 ml), followed by the addition of GSH (10.7 mg, 3.5×10^{-5} mol). The resulting mixture was sealed and shaken for 5 days prior to water GPC analysis to monitor the cleavage reaction. The GPC chromatograms of polyPEG-A before and after cleavage by GSH were obtained via aqueous GPC and are shown in Fig. 6b.

2.11. Gel permeation chromatography (GPC)

DMAc GPC: DMAc GPC analyses was performed in *N*,*N*-dimethyl acetamide (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 g mol^{-1}.

Water GPC: water GPC analyses were performed using a Shimadzu modular system comprising a DGU-12A solvent degasser, on LC-10AT pump, a CTO-10A column oven, and a RID-10A refractive index detector and SPD-10A Shimadzu UV vis spectrometers (flow rate: 1 ml min^{-1}). The column was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50×7.8 mm^2) followed by three PL aquagel-OH columns (50, 40, 30; 8 μm). Calibration was performed with PEG standards ranging from 500 to 500,000 g mol^{-1}.

2.12. NMR spectroscopy

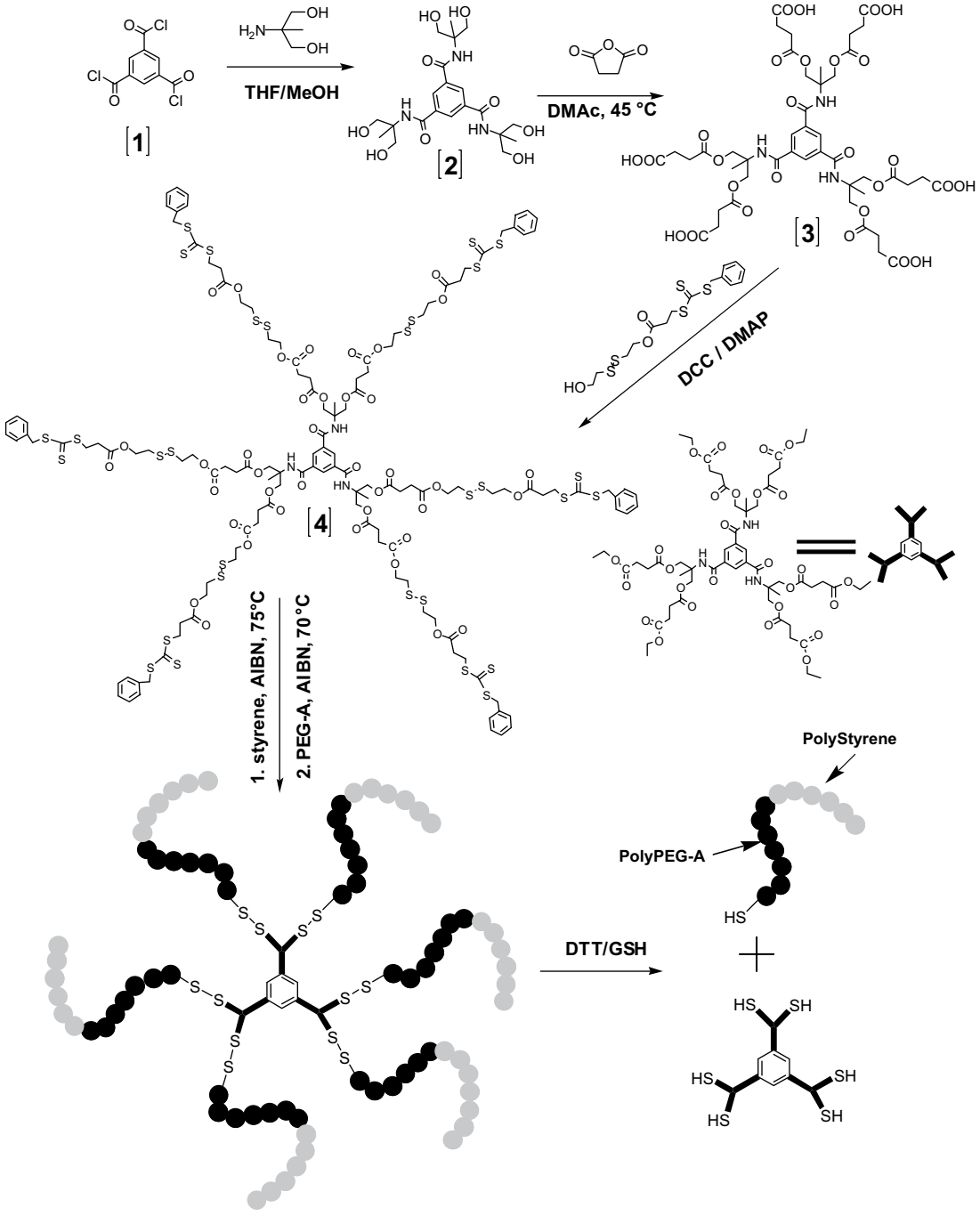
^{1}H NMR spectra were obtained using a Bruker AC300F (300 MHz) spectrometer or a Bruker DPX300 (300 MHz) spectrometer.

2.13. Electrospray ionization mass spectroscopy (ESI-MS)

ESI-MS 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).

2.14. Dynamic light scattering

The molecular weight of six-armed polystyrene and polyPEG-A were also analyzed at 25 °C on a Malvern dynamic light scattering analyzer (Laser type: HeNe gas laser; beam wavelength: 633 nm) using d*n*/d*c* of 0.185 for polystyrene and 0.134 for polyPEG-A in THF.



Scheme 1. Synthesis of a six-armed RAFT agent and the subsequent generation of biodegradable six-armed star polymers via 'core-first' methodology.

Table 1
Parameters of homopolymerization of PEG-A using six-armed star agent [4].

Time/h	Conversion[a]/%	M_n, from GPC/g mol^{-1} [b]	M_n, measured from ^{1}H NMR/g mol^{-1}	M_n, measured from DLS	M_{theo}, theoretical/g mol^{-1} [c]	PDI[b]
2	25.5	10,000	14,800	15,900	15,000	1.31
4	45.5	19,300	28,250	30,000	24,100	1.25
6	64.5	23,200	32,300	36,400	32,700	1.23
10	79.8	28,600	41,000	44,400	39,600	1.24
18	94.7	33,800	50,000	54,500	46,400	1.29

[a] The monomer conversion was calculated from ^{1}H NMR spectra of the polymerization mixtures in $CDCl_3$.
[b] The experimental number-average molecular weight, M_n and the polydispersity index, PDI, were measured by GPC using polystyrene standards and dimethyl acetamide (DMAc) (0.03% w/v LiBr, 0.05% BHT) as eluent.
[c] Theoretical value (M_{theo}) calculated using the following equation: M_{theo} = (mole ratio of PEG-A to six-armed RAFT[4]) × conversion × MW^{PEG-A} + $MW^{[4]}$, where MW^{PEG-A} represents MW of PEG-A and $MW^{[4]}$ represents MW of six-armed RAFT agent.

3. Result and discussion

3.1. Synthesis of six-armed RAFT agent [4]

The synthesis of hexa-functional star RAFT agent [4] is summarized in Scheme 1. A condensation reaction of 1,3,5-benzenetricarbonyl trichloride [1] with 2-amino-2-methyl-1,3-propanediol yielded a six-armed star precursor with six hydroxy group terminated arms [2]. The condensation reaction of carboxyl chloride groups on the phenyl ring is selective with the secondary amine groups on 2-amino-2-methyl-1,3-propanediol when excess secondary amine is used. The hexa-ol star precursor [2] was then reacted with excess succinic anhydride in the presence of 4-dimethylamino pyridine (DMAP), as a catalyst, to yield the star precursor with six arms terminated with carboxylic acid groups [3]. The esterification of the carboxylic acid end groups of the star precursor with excess hydroxyl terminated RAFT agent, HDEBP, afforded the six-armed hexa-functional RAFT agent [4], which was then used for controlling homo- and co-polymerizations. HDEBP was synthesized by the reaction of BSTP with three equivalents of 2-hydroxylethyl disulfide in order to increase the mono-addition yield, since an excess of BSTP will lead to the formation of biadduct. The protons of the hydroxyl terminated RAFT, HDEBP, can be assigned exactly by ^{1}H NMR as shown in Fig. 1. The hexa-functional RAFT agent [4] was also characterized by ^{1}H NMR (Fig. 2a). The signals at 8.29 ppm and 7.32 ppm originate from the protons attached to the phenyl core and from the six phenyl groups in HDEBP respectively. The ratio of the signals at 8.29 ppm to 7.33 ppm was found to be 1:10, consistent with complete RAFT functionalisation. Other peaks in the ^{1}H NMR spectrum of the multi-functional RAFT agent can also be well assigned as shown in Fig. 2a. The successful synthesis of the six-armed RAFT agent was also supported by the presence of a parent sodium ion at m/z 3434.20 (cal. 3434.25) via electrospray ionization mass spectrometry(ESI-MS) (Fig. 2b).

3.2. Synthesis of six-armed star polyPEG-A using six-armed RAFT agent [4]

The synthesis of six-armed star polymer of PEG-A using the six-armed RAFT agent is summarized in Table 1 and Fig. 3. It is evident

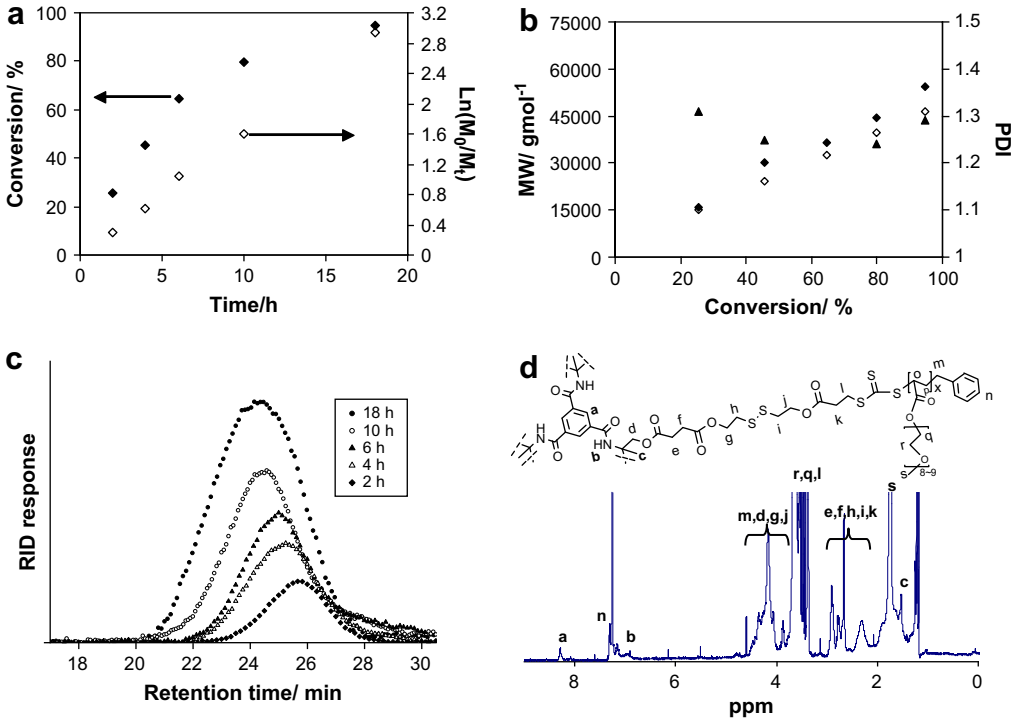


Fig. 3. Polymerization of PEG-A using six-armed RAFT agent in dioxane at 70 °C ([M]/[RAFT]/[AIBN] = 100:1:0.2). (a) Monomer conversion at varying polymerization times. (b) Molecular weight (MW) and PDI of the polyPEG-A against monomer conversion (filled and empty diamonds represent the experimental (obtained from DLS) and theoretical MW values, respectively, while filled triangles represent PDI). (c) GPC traces at different polymerization times (from DMAc GPC) and (d) ^{1}H NMR spectrum of the purified polyPEG-A (M_n 15,900 g mol^{-1} from DLS, PDI 1.31 in $CDCl_3$).

Table 2
Parameters of homopolymerization of styrene using six-armed RAFT agent [4].

Time/h	Conversion[a]/%	M_n, from GPC/g mol^{-1} [b]	M_n, measured from 1H NMR/g mol^{-1}	M_n, measured from DLS	$M_{theo,}$ theoretical/g mol^{-1} [c]	PDI[b]
5	15	3700	5900	5700	4680	1.18
9	22.5	5800	11,400	9300	7020	1.16
21	48.7	11,800	21,500	18,500	15,194	1.15
29	62.8	13,800	25,200	22,400	19,594	1.18
45	81	18,100	31,800	27,400	24,648	1.2

[a] The monomer conversion was calculated from ^{1}H NMR spectra of the polymerization mixtures in $CDCl_3$.
[b] The experimental number-average molecular weight, M_n and the polydispersity index, PDI, were measured by GPC using polystyrene standards and dimethyl acetamide (DMAc) (0.03% w/v LiBr, 0.05% BHT) as eluent.
[c] Theoretical value (M_{theo}) calculated using the following equation: M_{theo} = (mole ratio of styrene to six-armed RAFT$^{[4]}$) × conversion × MWSt + MW$^{[4]}$, where MWSt represents MW of styrene and MW$^{[4]}$ represents MW of six-armed RAFT agent.

from Fig. 3a that the monomer conversion increased concomitantly with polymerization time and the radical concentration remained constant with conversion as indicated by the pseudo-first order plot. As shown in Fig. 3b, both the experimental (measured from DLS) and the theoretical molecular weights were proportional to the monomer conversion. The theoretical MW values were slightly smaller than the experimental ones and the polydispersity index (PDI) of the purified homo-polyPEG-A was less than 1.29, indicating a well-controlled polymerization consistent with the known traits of living radical polymerization. The GPC traces of polyPEG-A obtained at different conversions are shown in Fig. 3c. It is evident that the MW of star polyPEG-A increases with increasing conversion as shown by decreased retention times. Polymers were obtained by repeatedly precipitation of the reaction mixture using diethyl ether. A purified homopolymer (MW 15,900 g mol^{-1}; PDI 1.31) was analyzed by ^{1}H NMR using $CDCl_3$ as deuterated solvent (Fig. 2d). The peaks at 8.5, 7.75 and 7.48 ppm and other peaks labeled in Fig. 3d are consistent with the presence of residual multi-RAFT agent. It is well known that the hydrodynamic volume of branched polymers is smaller than the equivalent linear polymers. When comparing the MW using different analysis methods, we found that the MWs of the six-armed polystyrenes obtained from DLS is approximately 1.6 times of those obtained from GPC analysis. However the MWs obtained from ^{1}H NMR spectra are a. 10% higher than those obtained from DLS analysis.

3.3. Synthesis of six-armed star polystyrene using a six-armed RAFT agent [4]

As summarized in Table 2 and Fig. 4 the six-armed RAFT agent [4] was also used to synthesize six-armed star polymers with hydrophobic polystyrene arms. The multi-RAFT controlled

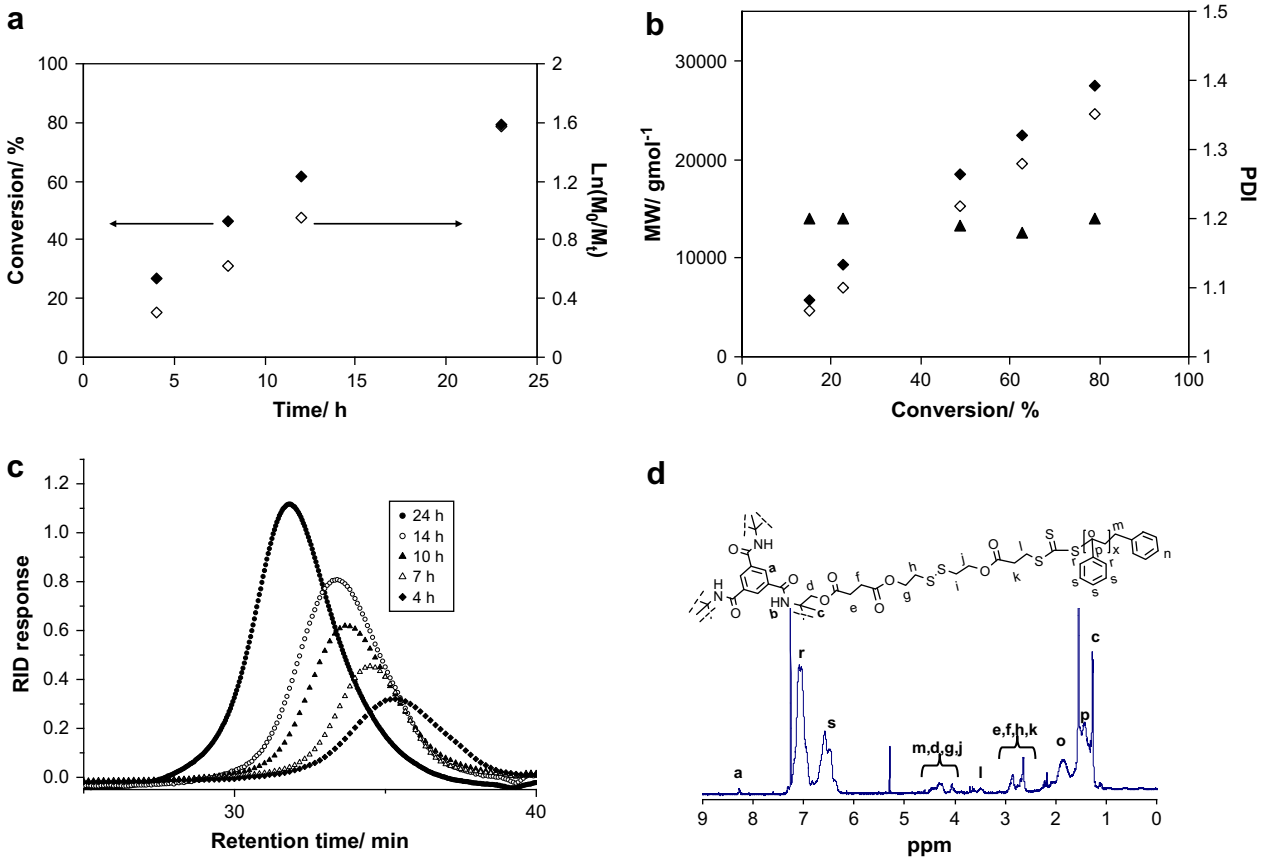


Fig. 4. Polymerization of styrene using six-armed RAFT agent in dioxane at 75 °C ([M]/[RAFT]/[AIBN] = 200:1:0.25). (a) Monomer conversion versus polymerization time. (b) Molecular weight and PDI of the purified polySt versus monomer conversion (filled and empty diamonds represent the experimental (obtained from DLS) and theoretical MW values, respectively, while filled triangles represent PDI). (c) GPC traces of purified polySt at different conversions and (d) ^{1}H NMR spectrum of purified polySt (MW 27,400 g mol^{-1} calculated from DLS, PDI 1.20) in $CDCl_3$.

polymerization of styrene was obviously much slower than that of PEG-A (Fig. 4a). However the PDIs of six-armed star polySt were less than 1.20. It should be emphasized that a lower PDI is not necessarily indicative of instantaneous arm growth from all thiocarbonate sites [57], as the fragmentation of the initial RAFT functionality may not favour the initiating group (R-group). This may be a noticeable problem at very low conversions but as conversion proceeds, and the main RAFT equilibrium is attained then this is unlikely to become a significant influence on the kinetics and/or architecture. The GPC traces obtained from polystyrene formed at different conversions are shown in Fig. 4c. A purified homo-polySt (MW 27,400 g mol^{-1}; PDI 1.20) was analyzed by ^{1}H NMR in $CDCl_3$ (Fig. 4d). The peaks labeled by a, m, d, g, j, e, f, h, k corresponded to the residue of the six-armed initiator evidencing its integrity after polymerization.

3.4. Synthesis of amphiphilic star architecture using six-armed star polystyrene as a macroRAFT agent

It is known that radical-radical termination reactions will affect the molecular weight distributions of star polymers generated in RAFT polymerization, resulting in a broadening of the PDI [58]. However by judicious selection of the experimental conditions, termination reactions and their effects can be minimized [59]. In this study consistent results (in terms of PDI) were obtained for both star and linear polymers, suggesting that the reaction conditions used were sufficient to minimize the impact of termination by combination. The PDIs of the six-armed star polymers with amphiphilic copolymer arms of polySt-b-polyPEG-A were less than 1.31 for the copolymers up to 80% conversion, indicating a well-controlled mechanism by RAFT (Fig. 5a and b). The GPC traces of purified star polymers with polySt-b-polyPEG-A arms are shown in Fig. 5c, clearly demonstrating successful chain extension. The significant shift in retention times of macroRAFT agent (six-armed star polySt) and that of the block copolymer might be attributed to the dramatic change of the polarity of the copolymer after the addition of a hydrophilic PEG fragment. Since it is difficult to find suitable standards to measure the MW of copolymers, the MWs of the copolymers analyzed by GPC are inaccurate. DLS analysis is potentially a suitable method for measuring the MW. However, the difficulty in defining an exact value of dn/dc for polySt-b-polyPEG-A compromises the accuracy of the MW analysis. The data shown in Table 3 (^{1}H NMR analyses) allowed quantitative calculation of MWs by comparing the signals of protons from the RAFT residue with those from PEG-A units in the polymers. The ^{1}H NMR spectrum of a purified copolymer (M_n 35,600 g mol^{-1} by ^{1}H NMR, PDI 1.26) is shown in Fig. 5d. The peaks labeled as a, m, d, g, j, l, e, f, h, i and k in Fig. 5d are from the multi-armed RAFT initiator indicating the integrity of RAFT after copolymerization. GPC traces of star copolymers indicated the increase of MW with the polymerization

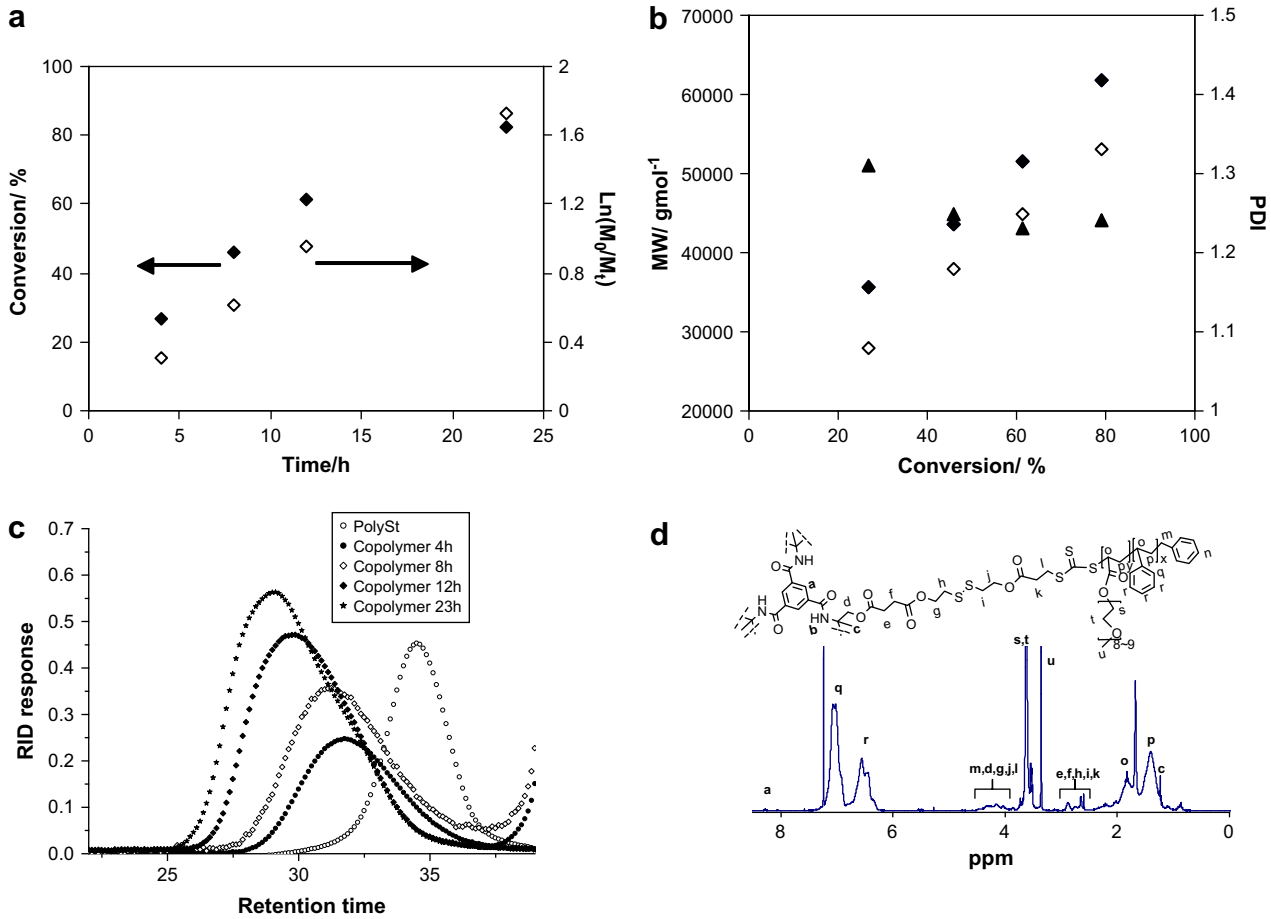


Fig. 5. Chain extension of hexa-polystyrene star polymer with polyPEG-A using six-armed polystyrene (MW 17,000 from DLS, PDI, 1.18) as macroRAFT agent in dioxane at 75 °C ([M]/[macroRAFT]/[AIBN] = 200:1:0.25). (a) PEG-A monomer conversion versus polymerization time. (b) Molecular weight of star block copolymers and their PDIs versus PEG-A monomer conversion (filled and empty diamonds represent the experimental (obtained from ^{1}H NMR) and theoretical MW values, respectively, while filled triangles represent PDI). (c) GPC traces of purified polySt-b-polyPEG-A at different conversions and (d) ^{1}H NMR spectrum of a purified star polymers with amphiphilic copolymer arms of polySt-b-polyPEG-A (M_n 35,600 g mol^{-1} by ^{1}H NMR, PDI 1.26) in $CDCl_3$.

Table 3
Parameters of six-armed block copolymerization using six-armed polySt (MW 17,000 from DLS, PDI, 1.18) as a macroRAFT agent and PEG-A as co-monomer.

Time/h	Conversion[a]/%	M_n, from GPC/g mol^{-1} [b]	M_n, measured from ^{1}H NMR/g mol^{-1}	M_{theo}, theoretical/g mol^{-1} [c]	PDI[b]
4	26.6	23,500	35,600	28,000	1.26
8	46.1	28,800	43,500	38,000	1.28
12	61.4	35,900	51,600	44,900	1.30
23	82.2	42,500	61,800	53,000	1.31

[a] The monomer conversion was calculated from ^{1}H NMR spectra of the polymerization mixtures in $CDCl_3$.
[b] The experimental number-average molecular weight, M_n and the polydispersity index, PDI, were measured by GPC using polystyrene standards and dimethyl acetamide (DMAc) (0.03% w/v LiBr, 0.05% BHT) as eluent.
[c] Theoretical value (M_{theo}) calculated using the following equation: M_{theo} = (mole ratio of styrene to six-armed polySt macroRAFT) × conversion × $MW^{PEG\text{-}A} + MW^{macroRAFT}$, where $MW^{PEG\text{-}A}$ represents MW of PEG-A and $MW^{macroRAFT}$ represents MW of six-armed polystyrene.

time (Fig. 5d). A small shoulder appeared at the GPC trace of the copolymer at higher conversions (82.2%), consistent with the inevitable presence of side termination reactions.

3.5. Cleavage of six-armed star polymers in the presence of DL-dithiothreitol (DTT) and glutathione (GSH)

A cleavage test was first carried out on a six-armed star polymer with amphiphilic block copolymer arms, polySt-b-polyPEG-A in the presence of DTT in DMAc solution. The cleavage was complete within 4 h in 0.1 M DTT in phosphate buffer (pH 6.5). The MW of the cleaved single-armed mixture was found by GPC, to be approximately one sixth (MW: 6100 from DMAc GPC) of the MW of star precursor measured by ^{1}H NMR (MW: 35,600) and one fourth of that by GPC (MW: 23,500). (Fig. 6a) This observation is consistent with that observed with the star polySt and polyPEG-A (Tables 1 and 2) and that predicted by theoretical simulation [60]. After cleavage the PDI of the single-armed chains was found to be 1.20, in accord with successful living polymerization.

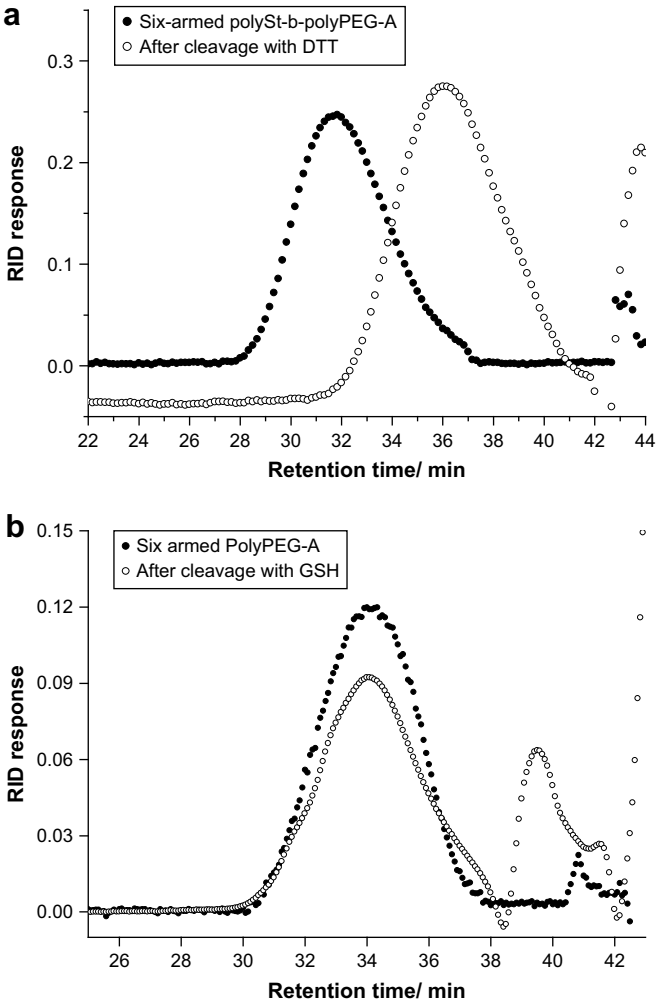


Fig. 6. (a) GPC traces of six-armed polymer with amphiphilic copolymer arms of polySt-b-polyPEG-A (MW: 44,900 from ^{1}H NMR) before and after cleavage by DTT in DMAc solution for 4 h and (b) Aqueous GPC traces of six-armed polymer with polyPEG-A (MW: 15,900 from DLS) before and after cleavage by GSH in phosphate buffer solution (pH 5.0) for 5 days.

The biodegradability of star polymer with six polyPEG-A arms was also tested by incubating the star polymer in GSH solution (50 molar equivalent amount of polyPEG-A star polymer) in pH 5.0 phosphate buffer. GPC analysis indicated that approximately 25% of six-armed precursor was cleaved by GSH in 5 days (Fig. 6b). In accordance with the earlier cleavage test of six-armed polySt-b-polyPEG-A, the MW of the single-armed polymer mixture (MW: 2300 from water GPC) was approximately one seventh of the star precursor (MW: 15,900 from DLS). From these results it is evident that the disulfide-cleavability of GSH was much weaker than that of DTT. The in vivo cleavability of disulfide linkages by GSH is likely to be much higher as co-factors may well play a role e.g. enzyme catalysis [46,61]. Lower pH will also influence the reduction potential of GSH as predicted by the Nernst equation [44,61].

4. Conclusion

We have successfully synthesized a six-armed RAFT agent and used it to synthesize star polymer architectures with identical arms of homo-polySt, homo-polyPEG-A and amphiphilic copolymers of polySt-b-polyPEG-A using a 'core-first' methodology. The radical polymerization of six-armed star polymers are shown to be mediated by the new hexa-functional RAFT agents. The study on the MWs of the six-armed polymers using different methods revealed the compromised dynamic volume of six-armed polymer structure relative to the linear precursors. These six-armed star polymers can be cleaved easily into single-armed linear polymers by DTT. They also proved to be slowly biodegradable in the presence of GSH, the most abundant intracellular thiol. We are currently studying these biodegradable star architectures as the basis of biomolecule conjugates, potentially biodegradable *in vivo*.

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