Facile Synthesis of Dumbbell-Shaped Dendritic-Linear-Dendritic Triblock Copolymer via Reversible Addition-Fragmentation Chain Transfer Polymerization

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ABSTRACT: We report the first instance of facile synthesis of dumbbell-shaped dendritic-linear-dendritic triblock copolymer, [G-3]-PNIPAM-[G-3], consisting of third generation poly(benzyl ether) monodendrons ([G-3]) and linear poly(*N*-isopropylacrylamide) (PNIPAM), via reversible addition-fragmentation chain transfer (RAFT) polymerization. The key step was the preparation of novel [G-3]-based RAFT agent, [G-3]-$CH_2SCSSCH_2$-[G-3] (1), from third-generation dendritic poly(benzyl ether) bromide, [G-3]-CH_2Br. Due to the bulky nature of [G-3]-CH_2Br, its transformation into trithiocarbonate **1** cannot go to completion, a mixture containing ~80 mol % of **1** and 20 mol % [G-3]-CH_2Br was obtained. Dumbbell-shaped [G-3]-$PNIPAM_{310}$-[G-3] triblock copolymer was then successfully obtained by the RAFT polymerization of *N*-isopropylacylamide (NIPAM) using **1** as the mediating agent, and trace amount of unreacted [G-3]-CH_2Br was conveniently removed during purification by precipitating the polymer into diethyl ether. The dendritic-linear-dendritic triblock structure was further confirmed by aminolysis, and fully characterized by gel permeation chromatography (GPC) and ^{1}H-NMR. The amphiphilic dumbbell-shaped triblock copolymer contains a thermoresponsive PNIPAM middle block, in aqueous solution it self-assembles into spherical nanoparticles with the core consisting of hydrophobic [G-3] dendritic block and stabilized by the PNIPAM central block, forming loops surrounding the insoluble core. The micellar properties of [G-3]-$PNIPAM_{310}$-[G-3] were then fully characterized.
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Keywords: block copolymers; dendrimers; reversible addition-fragmentation chain transfer (RAFT); self-assembly

INTRODUCTION

In the past decade, great attention has been paid to amphiphilic dendritic-linear block copolymers because of their potential applications ranging from drug delivery, coatings, chemical sensors, molecular therapeutics to molecular templating.[1–8] Compared with conventional amphiphilic block copolymers, they possess unique solution and bulk properties due to the non-entangled, but densely packed structure of the dendritic segment.[9,10] In aqueous solution, amphiphilic dendritic-linear block copolymers with hydrophobic dendritic block and hydrophilic linear block typi-

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cally self-assemble into multimolecular micelles with the dendritic blocks forming the core, stabilized by the well-solvated flexible block. The micelle core will be capable of accommodating a large number of "guest" molecules not only in the internal voids of the dendrons, but also in the void space between individual monodendrons. Thus, the self-assembled supramolecular species provide a wide range of potential carrier systems for drugs, genes, and vaccines.[10]

Various amphiphilic dendritic-linear block copolymers have been synthesized and studied. Most of these systems included a linear poly(ethylene oxide) (PEO) block attached at one or both ends to hydrophobic dendritic block of either poly (benzyl ether),[2,9,11–17] poly(benzyl ester),[18–21] aliphatic polyester,[22–24] poly(α,ε-L-lysine),[25,26] carbosilane,[27,28] or triazine.[29,30] Thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM)[31–34] and pH-responsive poly(acrylic acid)[35] linear chains were also attached to poly(benzyl ether) dendrons. Hydrophilic dendritic segments such as poly(L-lysine) and poly(amidoamine) (PAMAM) have also been attached to the chain ends of PEO,[36–39] poly(propylene oxide) (PPO),[40] and poly(L-lactide) (PCL)[41] to obtain double hydrophilic[42] or amphiphilic dendritic-linear block copolymers.[43,44]

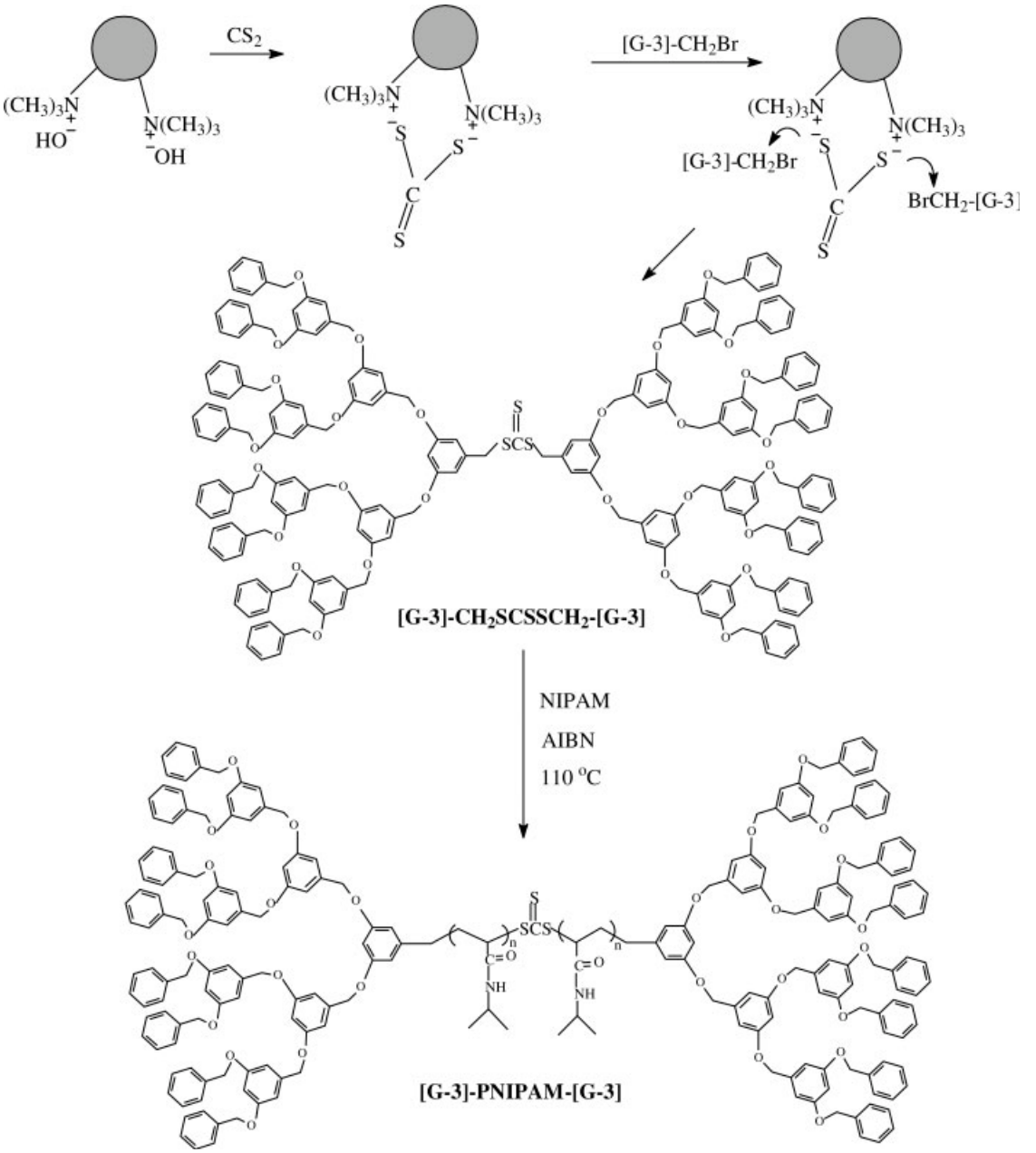
The synthesis of dendritic-linear block copolymers typically relies on coupling preformed dendrons of desired generation to the chain end of linear polymer chains,[2,9,12–16,45] or divergently growing dendrons from the linear chain ends,[38,40] or employing reactive dendrons as macromolecular initiators in the anionic polymerization of ε-caprolactone.[21,46] With the advent of controlled/living free radical polymerizations, such as nitroxide-mediated polymerization (NMP),[47] reversible addition-fragmentation chain transfer (RAFT),[48–50] and atom transfer radical polymerization (ATRP),[51,52] the syntheses of dendritic-linear block copolymers using the latter approach become more facile and convenient, especially for diblock copolymers. Poly (benzyl ether) dendrons containing either a single benzylic TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) or halide functionality at their focal point have been used for the nitroxide mediated polymerization of styrene[53] or ATRP polymerization of styrene,[54] *t*-butyl acrylate,[35] and *N*-isopropylacrylamide (NIPAM).[33]

Dendritic-linear-dendritic triblock copolymers take a dumbbell-shape, which are structurally similar to star-*b*-linear-*b*-star triblock copolymers,[55–58] A_nBA_n, where n indicates the number of arms in the star. The differences in chemical composition and chain packing density between the dendritic and flexible linear block render dendritic-linear-dendritic triblock copolymers to possess unique properties compared with conventional star-*b*-linear-*b*-star copolymers and dendritic-linear diblock copolymers.[1]

However, the preparation of dendritic-linear-dendritic ABA triblock copolymers employing the controlled/living free radical polymerization techniques is not straightforward and still remains a challenge. We can postulate that the classic ATRP techniques could not be used as a one-step process in the formation of dendritic-linear-dendritic triblock copolymers.

Up to now, the only example of preparation of dendritic-linear-dendritic triblock copolymers employing the controlled/living free radical polymerization techniques was reported by Emrick et al.[59] They synthesized a bisdendritic unimolecular initiator containing TEMPO moieties. Under standard TEMPO-mediated polymerization conditions, polystyrene central block was grown into the poly (benzyl ether) dendron based bisdendritic unimolecular initiator. However, they also reported that the final product was largely contaminated by dendritic-linear diblock copolymer. After purification by chromatography, they managed to obtain ABA triblock copolymer in a slightly better yield than the AB diblock copolymer.

PNIPAM undergoes a phase transition at its lower critical solution temperature (LCST) of 32 °C and it has been widely studied as a polymer potentially useful for targeted drug delivery.[31] Recently we have reported the first preparation of dendritic-linear diblock copolymer composed poly(benzyl ether) dendrons and PNIPAM via the RAFT technique,[32] employing poly (benzyl ether) dendron-based dithioester as the RAFT agent. It is well known that trithiocarbonates with two good leaving groups can also serve as effective RAFT agents and polymer chains can grow in two directions, thus ABA triblock copolymers can be obtained in only two sequential monomer addition steps.[50,60] We then figured out that if we prepare a trithiocarbonate (**1**) with two poly(benzyl ether) dendrons as the leaving group, dendritic-linear-dendritic triblock copolymers should be obtained in one single step. Because of that, the RAFT technique is compatible with almost all of the conventional free radical polymerization monomers;[50] the central block can be either hydrophobic, hydrophilic, or even stimuli-responsive.



Scheme 1. Schematic illustration for the preparation of [G-3]-$CH_2SCSSCH_2$-[G-3] (1) and dumbbell-shaped [G-3]-PNIPAM-[G-3].

Herein, we report a facile approach to the preparation of dumbbell-shaped dendritic-linear-dendritic triblock copolymers via RAFT polymerization. Firstly, *S*,*S*′-bisdendritic trithiocarbonate (**1**) was prepared from third generation poly(benzyl ether) dendrons in the presence of anionic exchange resin (OH^- form) and carbon disulfide (Scheme 1). The dumbbell-shaped dendritic-linear-dendritic triblock copolymer, [G-3]-PNIPAM-[G-3], was then obtained by the RAFT polymerization of NIPAM using **1** as the RAFT agent. The triblock copolymer was fully characterized to confirm its dumbbell structure. The self-assembly behavior and solution properties of [G-3]-PNIPAM-[G-3] were then characterized by a combination of dynamic and static laser light scattering (LLS), fluorescence spectroscopy, transmission electron microscopy (TEM), and temperature-dependent transmittance measurements.

EXPERIMENTAL

Materials

The third-generation dendritic poly(benzyl ether) bromide, [G-3]-CH_2Br, was prepared using a known procedure;[61] GPC analysis revealed an M_n of ~1700 and an M_w/M_n of ~1.05. *N*-isopropylacrylamide (NIPAM) (97%, Tokyo Kasei Kagyo) was purified by recrystallization from a benzene/*n*-hexane mixture.

2,2′-azobis(isobutyronitrile) (AIBN) was recrystallized from 95% ethanol. Carbon disulfide (CS_2) was dried over calcium hydride and distilled just prior to use. Butyl acrylate (BA), *n*-butylamine, diethyl ether, tetrahydrofuran (THF), 1,4-dioxane, *N*,*N*-dimethylformamide (DMF), sodium bisulfite ($Na_2S_2O_4$), and anionic exchange resin 717 were purchased from Shanghai Chemical Reagent and used as received.

Characterization

All ^{1}H-NMR spectra were recorded using a Bruker 300 MHz spectrometer. [G-3]-CH_2Br, **1**, and [G-3]-PNIPAM-[G-3] were analyzed in $CDCl_3$.

Molecular weights and molecular weight distributions were determined by gel permeation chromatography (GPC) line equipped with Waters 1515 pump and Waters 2414 differential refractive index detector (set at 30 °C). It used a series of three linear Styragel columns HT2, HT4, and HT5 at an oven temperature of 45 °C. The eluent was THF at a flow rate of 1.0 mL/min. A series of low polydispersity polystyrene standards were employed for the calibration.

A commercial spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-tau digital time correlation (ALV5000) and a cylindrical 22 mW UNIPHASE He-Ne laser (λ_0 = 632 nm) as the light source was used. In static LLS, we can obtain the weight-average molar mass (M_w) and the *z*-average root-mean square radius of gyration ($\langle R_g^2\rangle^{1/2}$ or written as $\langle R_g\rangle$) of polymer chains in a dilute solution from the angular dependence of the excess absolute scattering intensity, known as Rayleigh ratio $R_{vv}(q)$, as

$$\frac{KC}{R_{vv}(q)} = \frac{1}{M_w}\left(1 + \frac{1}{3}\langle R_g^2\rangle q^2\right) + 2A_2C \qquad (1)$$

where $K = 4\pi^2 n^2 (dn/dC)^2/(N_A\lambda_0^4)$ and $q = (4\pi n/\lambda_0)\sin(\theta/2)$ with N_A, dn/dC, n, and λ_0 being the Avogadro number, the specific refractive index increment, the solvent refractive index, and the wavelength of the laser light in a vacuum, respectively; A_2 is the second virial coefficient. The specific refractive index increment was determined by a precise differential refractometer at the same wavelength of 632 nm as in LLS measurements. Strictly speaking, here $R_{vv}(q)$ should be $R_{vu}(q)$ because there is no analyzer before the detector. However, the depolarized scattering of the solution studied is insignificant so that $R_{vu}(q) \sim R_{vv}(q)$. Also note that in this study, the sample solution was so dilute (0.036 g/L) that the extrapolation of $C \to 0$ was not necessary, and the term $2A_2C$ in eq 1 can be neglected. Thus, the obtained M_w should be considered as apparent values, denoted as $M_{w,app}$.

In dynamic LLS, the Laplace inversion of each measured intensity-intensity-time correlation function $G^{(2)}(q,t)$ in the self-beating mode can lead to a line-width distribution $G(\Gamma)$. For a pure diffusive relaxation, Γ is related to the translational diffusion coefficient D by $(\Gamma/q^2)_{C\to 0,q\to 0} \to D$, or further to the hydrodynamic radius R_h via the Stokes-Einstein equation, $R_h = (k_BT/6\pi\eta_0)/D$, where k_B, T, and η_0 are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively.

Fluorescence spectra were recorded using a Shimadzu 5301PC spectrofluorometer. The temperature of the water-jacketed cell holder was controlled by a programmable circulation bath at 20 °C. The critical micellization concentration (CMC) was determined by fluorescence technique. Calculated volume of pyrene solution in acetone was added into a series of volumetric flasks, acetone was removed under reduced pressure, polymer solutions at different concentrations were then added into volumetric flasks, pyrene concentration was fixed at 5×10^{-7} mol/L. All the samples were sonicated for 0.5 h and then allowed to stand overnight before fluorescence measurements. The slit widths were set at 10 nm and 2.5 nm for the excitation and the emission light, respectively. The excitation spectra were acquired by monitoring the emission at a wavelength of 390 nm.

The solution transmittance was acquired on a Unico UV/vis 2802PCS spectrophotometer. The transmittance of the solution was measured at a wavelength of 500 nm using a thermostatically controlled cuvette.

TEM observations were conducted on a Philips CM 120 electron microscope at an acceleration voltage of 100 kV. The sample for TEM

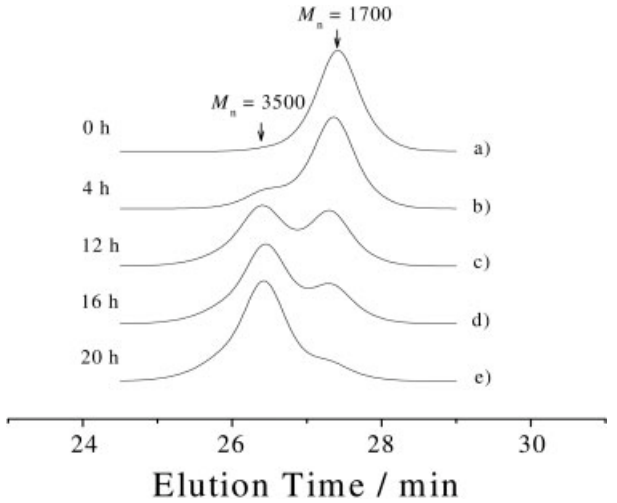


Figure 1. Evolution of GPC traces obtained at different reaction times during the preparation of trithiocarbonate [G-3]-$CH_2SCSSCH_2$-[G-3](1): a) 0 h, b) 4 h, c) 12 h, d) 16 h, and e) 20 h.

observations was prepared by placing 10 μL micellar solution at a concentration of 0.1 g/L on copper grids coated with thin films of Formvar and carbon successively. No staining was required.

Synthesis of *S,S'*-Bis-dendritic Trithiocarbonate (1)

Scheme 1 shows the general scheme used for the preparation of **1** and [G-3]-PNIPAM-[G-3]. The typical procedure is as follows. Anionic exchange resin 717 in the Cl^- form (2.0 mmol/g) was packed in a column and then continuously washed with 5% NaOH solution at a rate of 5 mL/min for 6 h. It was then washed with deionized water until the eluent was neutral. The resin was dried at 60 °C in a vacuum oven to a constant weight.[62] The dried resin (1 g) was added into the mixture of carbon disulfide (2.5 mL, 42 mmol) and dry THF (5 mL); carbon disulfide acts both as solvent and reactants. The solution mixture was stirred at room temperature for ~5 min. The color of resin turned from yellow to deep red due to the formation of CS_3^{2-} on the polymeric support. Into this suspension, [G-3]-CH_2Br (1.657 g, 1.0 mmol) was added. The mixture was stirred under reflux for 20 h, and the reaction progress was monitored by GPC. The mixture was filtered and washed with CS_2. The combined filtrate was dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure to yield 1.2 g red solids. According to the GPC [Fig. 1 (plot e)] and ^{1}H-NMR [Fig. 2 (plot ***b***)] results, the final product contains ~80 mol % of **1** and ~20 mol % of [G-3]-CH_2Br. We did not attempt to further purify it, considering that [G-3]-CH_2Br did not participate in the RAFT polymerization of NIPAM.

Synthesis of Dumbbell-Shaped [G-3]-PNIPAM-[G-3]

For the preparation of dendritic-linear-dendritic triblock copolymer via RAFT technique (Scheme 1), the red solids obtained above were employed as the RAFT agent (Scheme 1). Into a 10-mL polymerization tube equipped with a magnetic stirring bar, 0.365 g of red solids obtained above (containing 0.324 g, 9.93×10^{-2} mmol, of trithiocarbonate **1**), NIPAM (4.526 g, 40 mmol), AIBN (1.6 mg, 0.01 mmol), and 5 mL 1,4-dioxane were charged. After being degassed by three freeze-thaw cycles, the tube was sealed under vacuum and then immersed in an oil bath thermostated at 110 °C. After stirring for 12 h, the polymerization tube was quenched into liquid nitrogen to stop the polymerization. More 1,4-dioxane was added and the product was precipitated into a mixture of 1,4-dioxane/ethyl ether (1/6, v/v). The dissolution/precipitation cycle was repeated for 3 times. After drying in a vacuum oven overnight at room temperature, 3.72 g slightly pink solids was obtained with a yield of 76.7%. GPC analysis in THF revealed a monomodal peak with an M_n of ~41,600 and a polydispersity, M_w/M_n, of ~1.15 (Fig. 3). ^{1}H-NMR indicated a degree of polymerization (DP) of 310 for the PNIPAM middle block (Fig. 4). The obtained dendritic-linear-dendritic triblock copolymer was designated as [G-3]-$PNIPAM_{310}$-[G-3].

Aminolysis of [G-3]-$PNIPAM_{310}$-[G-3] (Scheme 2)[63,64]

[G-3]-$PNIPAM_{310}$-[G-3] (0.2 g, 5.2×10^{-3} mmol) was dissolved in THF (5 mL); one drop of saturated aqueous sodium bisulfite ($Na_2S_2O_4$) solution was added. The reaction mixture was degassed by three successive freeze-pump-thaw cycles. *n*-Butylamine (4.0 mg, 5.5×10^{-2} mmol, ~10-fold molar excess with respect to the thiocarbonylthio moiety) was injected into the solution. The reaction mixture was stirred for 2 h under a nitrogen atmosphere. Then, degassed butyl acrylate (BA, 25-fold molar excess with respect to the thiocarbonylthio moiety) was added to transform the terminal thiol group into thioether via Michael addition. The reaction

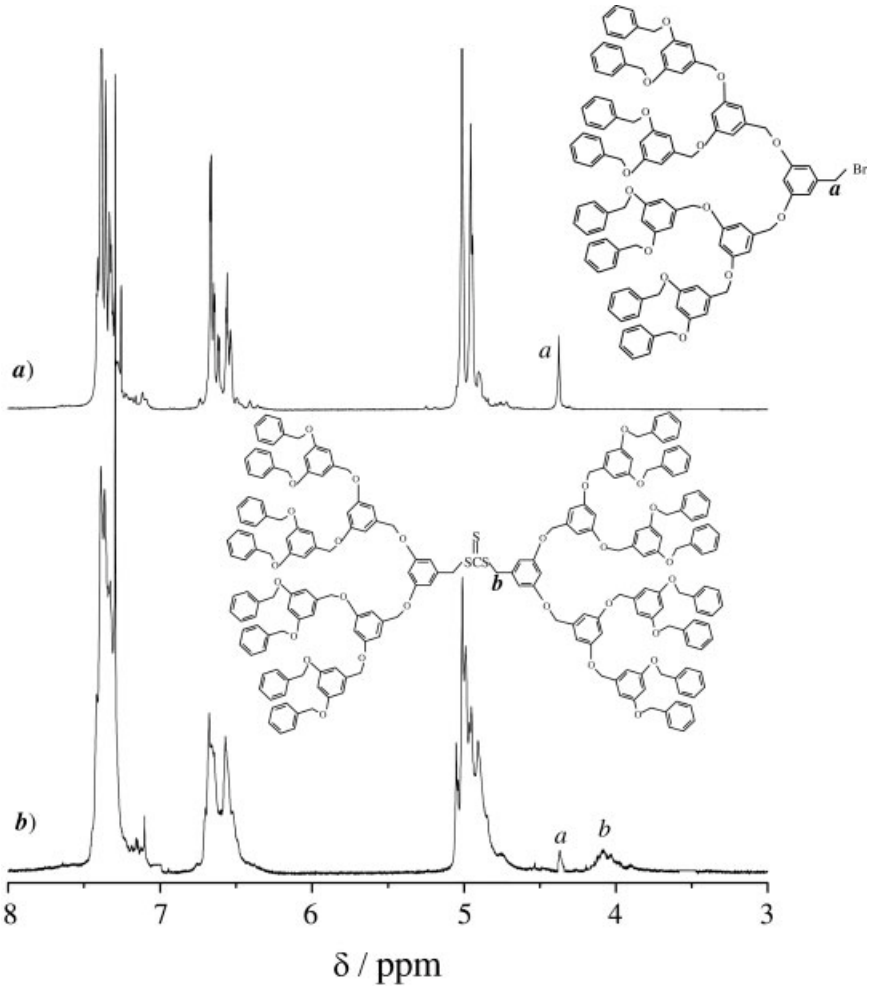


Figure 2. ^{1}H-NMR spectra of ***a***): [G-3]-CH_2Br and ***b***): [G-3]-$CH_2SCSSCH_2$-[G-3] (**1**, 20 h reaction time) in $CDCl_3$.

mixture was stirred overnight at room temperature. The polymer was recovered and purified by three repeated re-precipitations from THF to diethyl ether.

General Procedures for the Preparation of Micelles

[G-3]-$PNIPAM_{245}$-[G-3] was not directly soluble in water. During the preparation of micelles, a cosolvent approach was employed. [G-3]-$PNIPAM_{245}$-[G-3] was dissolved in DMF at a concentration of 10.0 g/L. Under vigorous stirring, water was added drop-wise into the polymer solution at a rate of 0.2 mL/min. The water/DMF ratio in the final solution was 9/1 v/v. After slowly stirring further for 10 h, DMF was thoroughly removed by dialysis against deionized water for 2 days. Fresh deionized water was replaced approximately every 6 h. The obtained micellar solution appears to be bluish, which is characteristic of micellar nanoparticles.

RESULTS AND DISCUSSION

Synthesis of *S,S′*-Bis-dendritic Trithiocarbonate 1

General schemes used for the preparation of [G-3]-based bisdendritic trithiocarbonate **1** and dendritic-linear-dendritic triblock copolymer was shown in Scheme 1. Tamami et al.[65,66] first reported that symmetrical dialkyl trithiocarbonates can be conveniently synthesized from carbon disulfide and alkyl halides using a commercially available hydroxide form of an anion-exchange resin. You et al.[62] reported the preparation of cyclic trithiocarbonate and polytrithiocarbonates from carbon disulfide and dimethyl

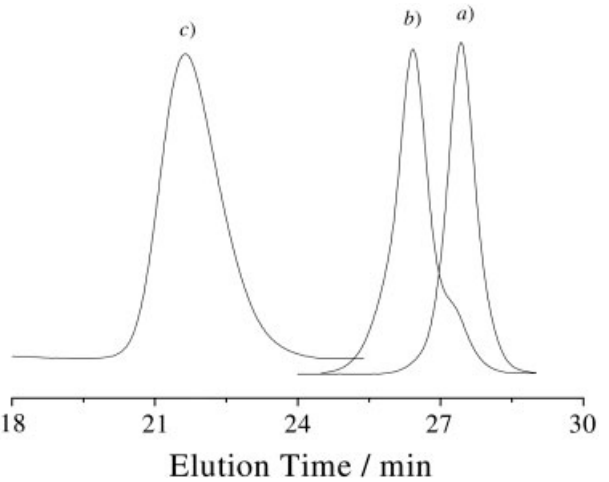


Figure 3. GPC traces obtained for a): [G-3]-CH_2Br (M_n = 1700, M_w/M_n = 1.05), b): [G-3]-$CH_2SCSSCH_2$-[G-3], and c): [G-3]-$PNIPAM_{310}$-[G-3] (M_n = 41,600, M_w/M_n = 1.15).

α,α′-dibromoalkylanedioates on a polymeric resin support. Initially, we were quite concerned that the bulky nature of [G-3] will prevent the effective formation of bisdendritic trithiocarbonates. Gitsov et al.[14] studied the reactivity of the functional group at the focal point of benzylic dendritic polyethers of different generations toward the end group of linear PEO chains. They found that the reactivity increased with increasing generations of the dendrons. Even for the fourth generation dendrimers, the functional group at the focal point still preserves its accessibility and reactivity.

Employing similar procedures as in the syntheses of trithiocarbonates,[65,66] **1** was prepared from [G-3]-CH_2Br in the presence of excess CS_2 and anion-exchange resin (OH^- form). The nucleophilic substitution reactions of CS_3^{2-} on polymeric supports with [G-3]-CH_2Br produced will presumably lead to the formation of *S*,*S*′-bisdendritic trithiocarbonate **1**. After the addition of [G-3]-CH_2Br, the reaction mixture were sampled from time to time for GPC analyses. Figure 1 shows typical GPC traces at different reaction times. The starting material, [G-3]-CH_2Br, exhibited a fairly monodisperse GPC trace, yielding a number average molecular weight, M_n, of 1700 and a polydispersity, M_w/M_n, of 1.05. With increasing reaction time, we can clearly see the appearance of a new peak at

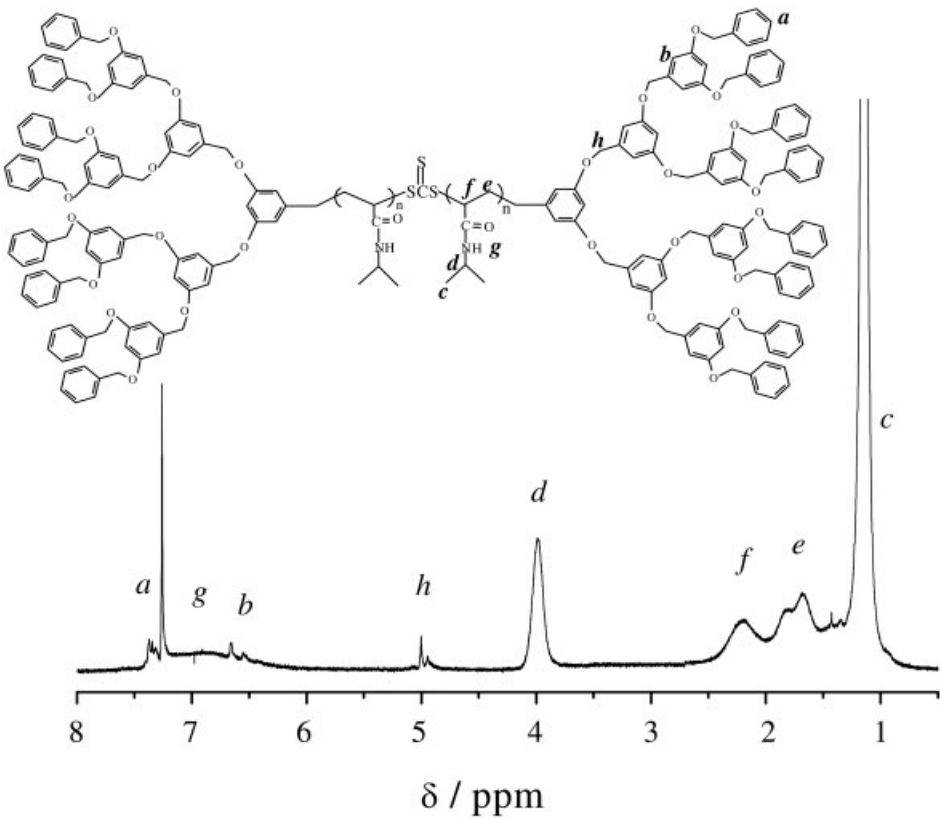
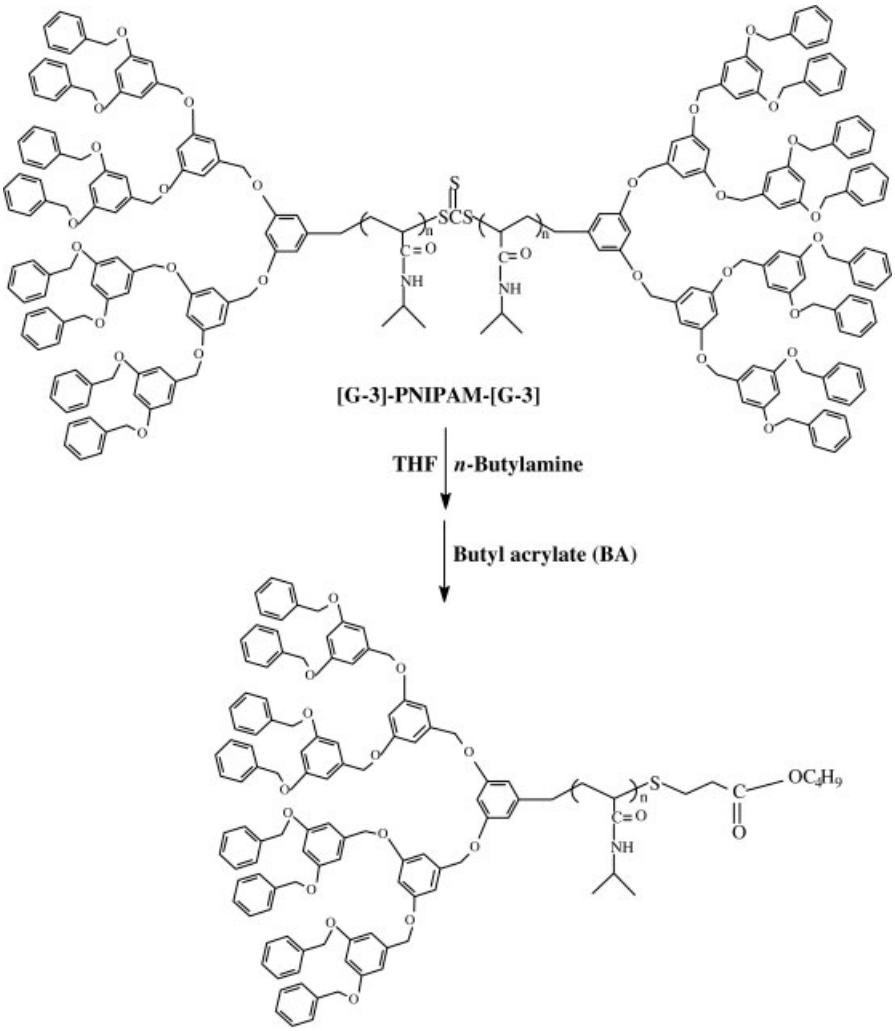


Figure 4. ^{1}H-NMR spectrum of purified [G-3]-$PNIPAM_{310}$-[G-3] in $CDCl_3$.



Scheme 2. Schematic illustration for the reaction pathways of aminolysis of [G-3]-PNIPAM-[G-3] with *n*-butylamine in the presence of BA.

~3500, the intensity of which increased with time. At the same time, the relative intensity of the original peak at 1700, which is due to [G-3]-CH_2Br, decreased with time. The doubling of molecular weight of the new peak can be unambiguously ascribed to the formation of **1**. After 20 h, GPC analysis still revealed the presence of [G-3]-CH_2Br. We further found that extending the reaction time to longer than 20 h did not improve the relative peak intensity of **1**.

Before the addition of [G-3]-CH_2Br, the premixing of CS_2 with anion-exchange resin (OH^- form) led to the formation of CS_3^{2-} and they immobilize onto the polymeric support. This can be judged by the appearance of the deep red color. After the addition of [G-3]-CH_2Br, the deep red color partially faded away, turning to slightly red. This indicated the occurrence of nucleophilic substitution reactions between [G-3]-CH_2Br and CS_3^{2-}. It should be noted that there exist the possibility that the immobilized CS_3^{2-} only react with one molecule of [G-3]-CH_2Br, the product will strongly adsorb onto the resin support through electrostatic interactions and is the possible reason for the observed low yield of **1** (65%). If CS_3^{2-} reacted with two molecules of [G-3]-CH_2Br, the product will become neutral and desorbed from the polymeric support.

Figure 2 shows the ^{1}H-NMR spectra of [G-3]-CH_2Br and the obtained red solids after a reaction time of 20 h. From Figure 2 (plot ***a***), we can tell that the resonances for the exterior phenyl groups occur at 7.20–7.45 ppm, the resonances for the aromatic protons of the monomer units occur in the region 6.50–6.70 ppm, separate resonances are observed in the appropriate ratio

for each "layer" of monomer units. Resonances for the methylene protons of each monomer units occur in the region 4.80–5.10 ppm. The methylene resonances of —CH_2Br at the focal point is at 4.40 ppm. After a reaction time of 20 h, the ^{1}H-NMR spectrum of the collected red solids shows the appearance of a new peak at 4.10 ppm (peak *b*). We can still observe the presence of the resonance at 4.40 ppm (peak *a*), which is ascribed to the starting material, [G-3]-CH_2Br. This was in agreement with the GPC results shown in Figure 1, which indicated the presence of [G-3]-CH_2Br even after a reaction time of 20 h. In Figure 2 (plot ***b***), the integral ratio of peak *b* to *a* was ~8/1. This indicated that the collected product contained ~80 mol % of **1**. Preliminary thin layer chromatography (TLC) analysis indicated that column chromatography using a solvent mixture of CH_2Cl_2/hexane (2/1 v/v) could be used to obtain pure **1**. However, considering that unreacted [G-3]-CH_2Br will not participate in the RAFT polymerization of NIPAM, we did not attempt to further purify it.

Syntheses of Dumbbell-Shaped Dendritic-Linear-Dendritic Triblock Copolymer

Trithiocarbonate **1** was employed as RAFT agent to polymerize NIPAM, yielding dumbbell-shaped [G-3]-PNIPAM-[G-3] (Scheme 1). The molar ratio of [1[/[AIBN] was fixed to be 10/1 to decrease the contamination of PNIPAM oligomers from the polymerization of NIPAM by free radicals decomposed from AIBN.[48–50] Preliminary experiments revealed that triblock copolymers with shorter PNIPAM central block (DP ~200) cannot form stable micelles in aqueous solution. Thus, the target degree of polymerization (DP) of PNIPM central block was designed to be 400. Previously we have synthesized [G-3]-PNIPAM dendritic-linear diblock copolymers using [G-3]-based dithioester as RAFT agent, and the polymerization was conducted at 80 °C.[32] When trithiocarbonate **1** was employed as RAFT agent to polymerize NIPAM at 80 °C, the conversion was quite low (~20%) after 12 h. We then managed to discover that at a polymerization temperature of 110 °C, the conversion was ~75% after 12 h. This is perhaps due to the increased stability of *S*,*S*′-bisdendritic triothiocarbonate **1** relative to that of [G-3]-CH_2SSCPh or the bulky nature of two [G-3] based leaving groups.[59]

PNIPAM-[G-3]-PNIPAM was obtained after 3 cycles of dissolving in 1,4-dioxane and precipitation into a mixture of 1,4-dioxane/ethyl ether (1/6, v/v). Through this way, [G-3]-CH_2Br residues present in the RAFT agent **1** as impurities and PNIPAM oligomers can be efficiently removed. GPC analysis of the purified product revealed the presence of a mono-modal and symmetric peak (Fig. 3), yielding an M_n of 41,600 and an M_w/M_n of 1.15. The clear and complete shift of GPC traces to higher molecular weight for the product relative to that of RAFT agent **1** partially indicated the successful preparation of [G-3]-PNIPAM-[G-3], which was further confirmed by the following experiments.

If we assume that the final product is the desired dendritic-linear-dendritic triblock copolymer, it can be calculated from the GPC results that the PNIPAM central block has a DP of ~340. Based on the monomer conversion and considering the purity of RAFT agent used, the theoretical DP of PNIPAM block is calculated to be 309. It should be noted that GPC results were not accurate in determining the absolute molecular weight of [G-3]-PNIPAM-[G-3] because the GPC calibration was based on polystyrene standards. Figure 4 shows the ^{1}H-NMR spectrum of [G-3]-PNIPAM-[G-3], all resonances can be well ascribed to the presence of PNIPAM and [G-3] segments. Assuming a 100% triblock copolymer structure, the DP of the PNIPAM central block was calculated to be 310, which was in fairly good agreement with the theoretical DP.

Previously, Emrick et al.[59] reported the only example of preparation dendritic-linear-dendritic triblock copolymers via controlled/living free radical polymerization, employing a bisdendritic unimolecular initiator containing TEMPO moieties. Based on the large discrepancies between the DP of central polystyrene block calculated from GPC and that from ^{1}H-NMR, they concluded that their product is largely contaminated with dendritic-linear diblock copolymer. They ascribed the formation of diblock copolymer to that the large size of dendritic-TEMPO counter radicals hindered its effective mediation of the free radical polymerized as compared to TEMPO itself. In our case, although the GPC and ^{1}H-NMR results agree fairly well with the theoretical DP calculated assuming an ideal triblock structure, we still lack conclusive evidences.

Fortunately, the central trithiocarbonate moieties can be reduced into thiol groups via aminolysis, which will cleave one molecule of triblock copolymer into two molecules of dendritic-linear diblock copolymer chains and the molecular

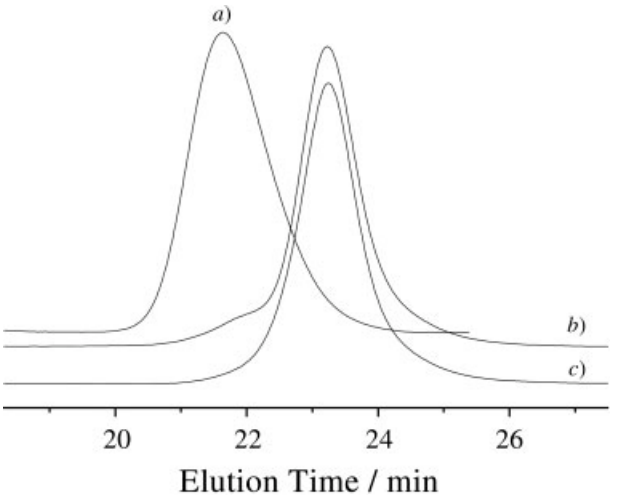


Figure 5. GPC traces of a): [G-3]-PNIPAM$_{310}$-[G-3] before aminolysis (M_n = 41,600, M_w/M_n = 1.15), b): Polymer obtained from aminolysis in the absence of BA, and c): Polymer obtained from aminolysis in the presence of BA (M_n = 19,800, M_w/M_n = 1.13).

weight will be subsequently halved.[50,63,64] The aminolysis reaction was conducted with the addition of *n*-butylamine into the copolymer solution in THF in the absence or the presence of BA (Scheme 2).[64] Figure 5 shows the GPC traces of [G-3]-PNIPAM-[G-3] and aminolysis products in the absence or presence of BA. Aminolysis in the presence of BA led to the clear shift of M_n from 41,600 [Fig. 5 (plot a)] to 19,800, almost half of that of the triblock precursor [Fig. 5 (plot c)]. The polydispersity of the aminolysis product remained fairly narrow (1.13), which was quite comparable to the precursors. BA can effectively cap the thiol end group resulting from the aminolysis reaction via Michael addition reaction and prevent its spontaneous oxidation into disulfide bonds,[64] that is, the re-formation of triblock structure. Indeed, when the aminolysis reaction was conducted in the absence of BA, we could observe a shoulder to the left of the main peak which corresponded to twice the molecular weight of the aminolysis product [Fig. 5 (plot b)]. The molecular weight of the aminolysis product was almost halved; this observation strongly confirmed the structure of dendritic-linear-dendritic triblock copolymers with central trithiocarbonate moieties.

Thus, all the results obtained from GPC, ^{1}H-NMR, and the aminolysis reactions conclusively confirmed the successful preparation of dumbbell-shaped [G-3]-PNIPAM-[G-3]. The excellent agreement of DP of the PNIPAM central block to that of the theoretical values also indicated that the RAFT polymerization of NIPAM using **1** as the mediating agent can be conducted in a quite controlled manner although we did not study the polymerization kinetics in detail. The actual DP of PNIPAM central block was calculated to be 310 from ^{1}H-NMR because the structure of [G-3] dendrons was well established. The prepared dumbbell-shaped triblock copolymer was designated as [G-3]-PNIPAM$_{310}$-[G-3].

Micellization Behavior of [G-3]-PNIPAM$_{310}$-[G-3] in Aqueous Media

The dendritic-linear-dendritic triblock copolymer consists of hydrophilic linear central PNIPAM block and highly hydrophobic [G-3] dendrons. In aqueous media, we expect that that they will self-assemble into supramolecular flower-like micellar aggregates with [G-3] dendrons in the micelle core, and the PNIPAM central block will form loops in the corona. The micellar properties of the dendritic-linear-dendritic triblock copolymer were then studied by a combination of fluorescence spectroscopy, LLS, optical transmittance, and TEM techniques. The dumbbell-shaped [G-3]-PNIPAM$_{310}$-[G-3] cannot be directly dissolved in water, so a cosolvent approach was employed to prepare stable micelles.[67]

The CMC of [G-3]-PNIPAM$_{310}$-[G-3] in aqueous solution was determined by fluorescence technique using pyrene as a probe. Changes in the pyrene fluorescence characteristics were frequently used to monitor the onset of micellization of various amphiphilic block copolymers. The characteristic feature of the pyrene excitation spectra, the pyrene low-energy (0,0) band undergoing a shift from 332 to 338 nm upon pyrene partition into a micellar hydrophobic core, was employed to determine the CMC. Figure 6 shows the concentration dependence of intensity ratios (I_{338}/I_{332}) of pyrene excitation spectra in the presence of [G-3]-PNIPAM$_{310}$-[G-3]. In the low concentration range, negligible changes in the intensity ratios can be detected. However, above a certain concentration the intensity ratios exhibited a dramatic increase, suggesting that pyrene molecules are incorporated into the hydrophobic core region above the CMC. Therefore, the CMC of [G-3]-PNIPAM$_{310}$-[G-3] was determined to be 2.45×10^{-5} g/L from the crossover point. Previously, we determined that the CMC of [G-3]-PNIPAM$_{220}$ was 9.5×10^{-3} g/L,[32] which is ~400 times higher than that of [G-3]-PNIPAM$_{310}$-[G-3]. This perhaps indicated that

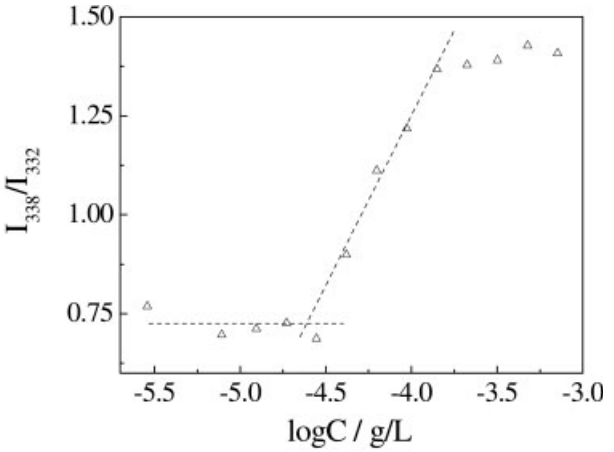


Figure 6. Plot of the intensity ratios, I_{338}/I_{332}, from pyrene excitation spectra as a function of the concentrations of [G-3]-PNIPAM$_{310}$-[G-3] in water at 20 °C. Pyrene concentration was fixed at 5.0×10^{-7} mol/L.

it is quite difficult for the PNIPAM central block to solubilize the two terminal [G-3] dendrons, that is, the conformation of the PNIPAM block is quite restrained after attaching with two highly hydrophobic and densely packed globules.

Inset in Figure 7 shows the hydrodynamic radius distribution, $f(R_h)$, of [G-3]-PNIPAM$_{310}$-[G-3] micelles at 20 °C and a concentration of 0.036 g/L. The micelles are quite monodisperse, the polydispersity index of the size distribution (μ_2/Γ^2) is ~0.08. The average hydrodynamic radius, $\langle R_h \rangle$, is determined to be 106 nm. Once the micelles were prepared, they are very stable upon dilution. In the concentration range (0.01–0.8 g/L), which is much higher than the CMC, $\langle R_h \rangle$ and μ_2/Γ^2 did not change with concentration. Figure 8 shows typical TEM images of micelles assembled from [G-3]-PNIPAM$_{310}$-[G-3] in water. The aggregates are typically spherical and relatively narrow-distributed, ranging from 80 to 120 nm in diameter. Since the poly(benzyl ether) dendron-based micelle cores absorb more of the electron beam, the dark region corresponds to the compact poly(benzyl ether) cores of the micelles, whereas the PNIPAM chains are "invisible." The discrepancy in the micellar sizes obtained from dynamic LLS and TEM should be due to that the former reflects the dimension of micelles in solution, which includes the contribution of PNIPAM coronas. Considering the molecular size of [G-3] dendrons (1.5–2.0 nm) and the diameter of the micelle core as determined by TEM (80–120 nm), some PNIPAM blocks must be buried inside the hydrophobic core.

Figure 7 also shows the angular dependence of the Rayleigh ratio, $R_{vv}(q)$, of [G-3]-PNIPAM$_{310}$-[G-3] micelle solutions determined by static LLS over a scattering angle range 15–90°. It is known that the slope of this curve is related to the radius of gyration, $\langle R_g \rangle$, and the reciprocal of the intercept is equivalent to the weight-average molar mass, $M_{w,app}$, of the nanoparticles. $\langle R_g \rangle$ and $M_{w,app}$ were then determined to be 87 nm and 3.7×10^7 g/mol, respectively. The average aggregation number, N_{agg}, of triblock copolymer chains inside each micellar aggregate is then calculated to be ~840. The density of micelles was calculated to be 0.012 g/cm^3, which is pretty low. This may reflect the fact that micelle core is loosely packed.

As discussed previously, the PNIPAM central block in the micelle corona region will form loops surrounding the insoluble [G-3] dendron-core. Thus, the micelle corona should be thermoresponsive and exhibit thermotunable water solubility. Figure 9 shows the temperature dependence of transmittance of micellar solution prepared from [G-3]-PNIPAM$_{310}$-[G-3]. Starting from 30 °C, the transmittance started to decrease dramatically. The decrease of transmittance should be due to that the PNIPAM corona was getting hydrophobic and intermicellar aggregation occured. Since PNIPAM was attached with hydrophobic [G-3] dendrons, the decrease of its phase transition temper-

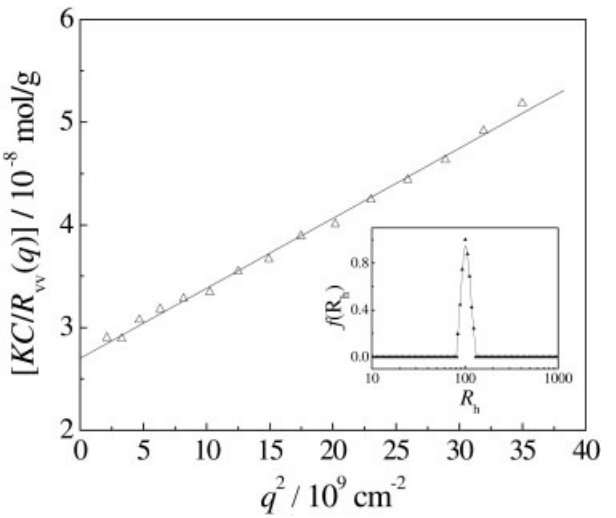


Figure 7. Angular dependence of the Rayleigh ratio, $R_{vv}(q)$, of [G-3]-PNIPAM$_{310}$-[G-3] in water as measured by static LLS over a scattering angle range 15–90°. The inset shows the hydrodynamic radius distribution, $f(R_h)$, as determined from dynamic LLS at a detection angle of 15°. The copolymer concentration is fixed at 0.036 g/L.

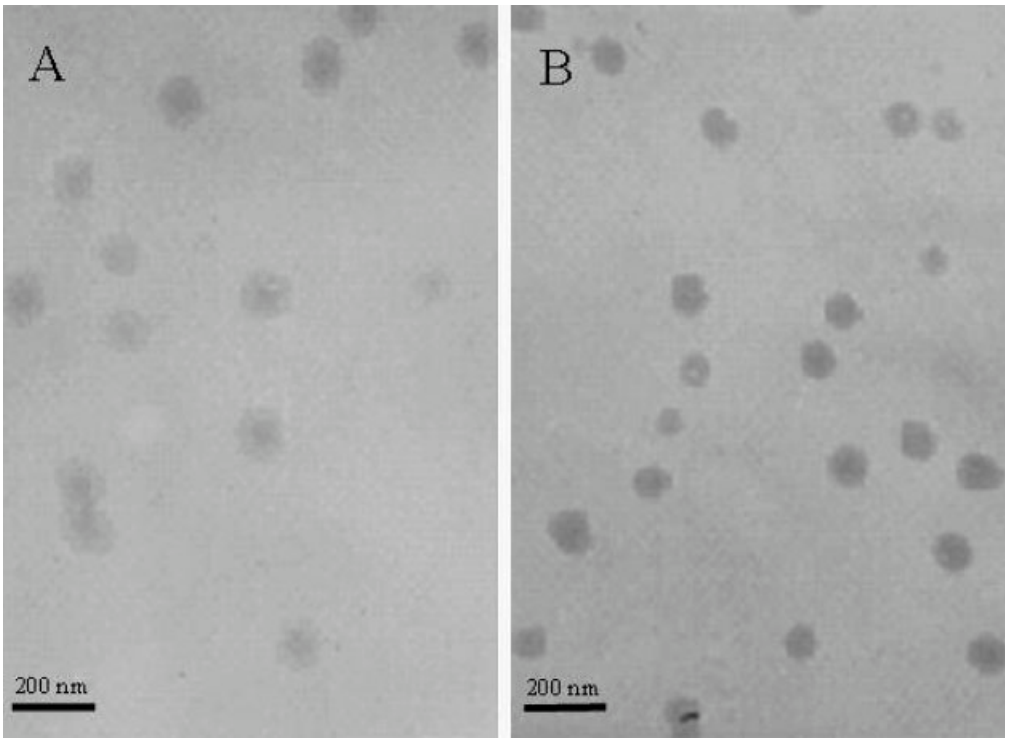


Figure 8. Typical TEM images of micelles assembled from [G-3]-PNIPAM$_{310}$-[G-3] in water at 20 °C at a final concentration of 0.1 g/L.

ature compared with that of free PNIPAM homopolymer (~32 °C) is quite reasonable.[31]

CONCLUSIONS

For the first time, dumbbell-shaped dendritic-linear-dendritic triblock copolymer, [G-3]-PNIPAM-[G-3], consisting of poly(benzyl ether) monodendrons of third generation ([G-3]) and linear poly(*N*-isopropylacrylamide) (PNIPAM), was synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization using a novel [G-3]-based RAFT agent, [G-3]-$CH_2SCSSCH_2$-[G-3] (**1**). The dendritic-linear-dendritic triblock structure was fully characterized and confirmed by gel permeation chromatography (GPC), ^{1}H-NMR, and aminolysis reactions. In aqueous solution, the amphiphilic dendritic-linear-dendritic triblock copolymer self-assembles into spherical nanoparticles with the core consisting of hydrophobic [G-3] dendritic block and stabilized by the PNIPAM central block, forming loops surrounding the insoluble core. The micellar properties of [G-3]-PNIPAM$_{310}$-[G-3] were then characterized by a combination of dynamic and static laser light scattering (LLS), fluorescence spectroscopy, transmission electron microscopy (TEM), and temperature-dependent turbidity measurements.

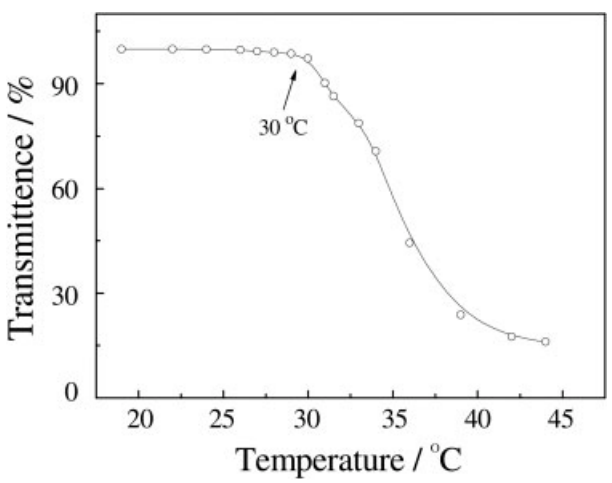


Figure 9. The temperature dependence of transmittance at 500 nm of an aqueous solution of [G-3]-PNIPAM$_{310}$-[G-3]. The triblock copolymer concentration is 0.8 g/L.

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