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Synthesis of Functionalized and Biodegradable Hyperbranched Polymers from Novel AB_2 Macromonomers Prepared by RAFT Polymerization

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ABSTRACT: A straightforward synthetic approach to the preparation of disulfide linked hyperbranched polymers with peripheral pyridyl disulfide functionalities is described. The hyperbranched polymers were obtained by the condensation of novel AB_2 macromonomers bearing α-dithobenzoate and ω-double pyridyl disulfide end-groups. These AB_2 macromonomers were prepared by reversible addition−fragmentation chain transfer (RAFT) polymerization of styrene or *N*-(2-hydroxypropyl) methacrylamide (HPMA) using a novel RAFT agent, *N*,*N*′-bis(2-(4-(2-pyridyldisulfide) ethyl butyric-1-carbonyloxo)ethyl) cyanopentanoic amide dithiobenzoate. After polymerization the α-dithobenzoate functionality was aminolyzed to yield thiol that was simultaneously subjected to an exchange reaction with pyridyl disulfide, resulting in the formation of hyperbranched structures. The primary chains of the hyperbranched polymers possessed well-defined molecular weights and low polydispersities. The linkages between primary chains consisted of biodegradable disulfide bonds, as confirmed by their reduction in the presence of DL-dithiothereitol (DTT), resulting in the destruction of the hyperbranched structure. The hyperbranched architectures were designed as carriers bearing excess pyridyl disulfide groups for potential reactions with any thiol-bearing biomolecule (e.g., cysteine residue in proteins, or −SH terminal nucleotides).

Introduction

Over the past decade, hyperbranched polymers have received considerable attention because of the expectation that their highly branched structures will impart unique physical and chemical properties.[1] Hyperbranched polymers may be considered as irregular analogues of dendrimers that have well-defined and perfectly branched structures.[1b] However, the synthetic approach to hyperbranched polymers tends to be much simpler than the multistep procedures required for the synthesis of many dendrimers. There are two main methodologies widely employed to prepare hyperbranched polymers: (1) polymerization of monomers containing a double bond and either a latent initiating site (*inimers*),[2−8] a chain transfer functionality[9,10] or a cross-linking agent;[11,12] (2) polycondensation of AB_x macromonomers.[13−15] The first methodology has been used to prepare branched vinyl polymers by conventional or living radical polymerization. Fréchet and co-workers[2] developed a self-condensing vinyl polymerization (SCVP) method using *inimers* and conventional comonomers to control the degree of branching in hyperbranched polyacrylates and polystyrene. Hawker and co-workers[8] found that an *inimer* bearing a nitroxyl moiety (for living radical polymerization control) was able to control the length and distribution of primary chains in hyperbranched structures. He[10] and co-workers synthesized novel polymerizable monomers containing either nitroxyl stable radicals,[10a,10b] or reversible addition−fragmentation transfer (RAFT) functionality,[10c] for copolymerization with comonomers to prepare branched polymers by living radical polymerization approaches. The second methodology using AB_x macromonomers has some advantages as it can be applied to a wide range of monomers and reaction conditions. Hedrick[13] and co-workers prepared hyperbranched and dendrimer-like star polymers by the co-condensation (esterification) of different AB_2 macromonomers with α-carboxylic acid and ω-hydroxyl functionalities, where the macromonomers were previously prepared by ring-opening polymerization (ROP) of a variety of substituted lactones using the benzyl ester of 2,2′-bis(hydroxymethyl)propionic acid as an initiator.

In the present paper, a novel strategy for the preparation of functionalized and biodegradable hyperbranched polymers from AB_2 macromonomers via the RAFT process is described. RAFT polymerization is a powerful method for synthesizing a wide variety of polymers with controlled molecular weights, narrow molecular weight distributions and end-group functionalities.[16−19] Recently synthetic strategies have been reported on using RAFT-based polymerizations to make biodegradable star, hyperbranched and higher architectures.[20] Herein, a newly synthesized RAFT agent carrying an R group containing double pyridyl disulfide functionality was employed to mediate the polymerizations of various monomers, yielding AB_2 macromonomers bearing α-dithobenzoate and ω-double pyridyl disulfides end-functionalities. Thiocarbonylthio functionality in the polymer chains can be converted easily to yield thiol in the presence of primary amines. In earlier work it has been shown that the aminolysis can be done in the presence of either an ene-containing compound or dipyridyl disulfide, yielding thioether or disulfide bonds (pyridyl disulfide).[21,22] In the present work thiol-disulfide exchange chemistry is exploited to generate biodegradable, disulfide linked, hyperbranched polymer chains. To demonstrate the versatility of the synthetic process, two common monomers were polymerized: styrene as a commonly used model system and *N*-(2-hydroxypropyl) methacrylamide (HPMA) as a building block of biocompatible, nontoxic and nonimmunogenic polymers.[23]

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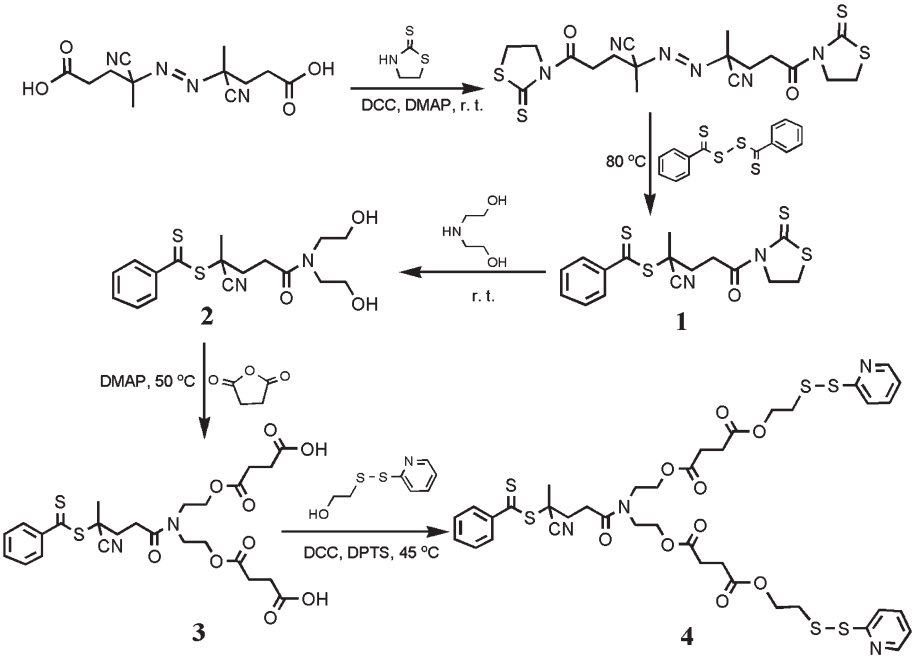
Scheme 1. Synthesis of RAFT Agent *N,N′*-Bis(2-(4-(2-pyridyldisulfide) Ethyl Butyric-1-carbonyloxo)ethyl) Cyanopentanoic Amide Dithiobenzoate, 4



Experimental Section

Materials. 4,4′-Azobis(4-cyanovaleric acid) (ACVA, Fluka, 98%), diethanolamine (Aldrich, 99%), 2-mercaptothiazoline (Aldrich, 98%), *N,N′*-dicyclohexylcarbodiimide (DCC, Aldrich, 99%), 4-(dimethylamino)pyridine (DMAP, Aldrich, 99%), succinic anhydride (Lancaster, 99%), 2,2-dithiodipyridine (Aldrich, 98%), mercaptoethanol (Aldrich, 99%), DL-dithiothereitol (DTT) (Aldrich, 98%), and *N*-(2-hydroxypropyl) methacrylamide (HPMA, Polysciences Inc., 97%) were used as received. 2,2′-Azobis(isobutyronitrile) (AIBN, Aldrich, 99%) was recrystallized from methanol. 4-(Dimethylamino)-pyridinium-4-toluene sulfonate (DPTS) was prepared according to the procedure described in the literature.[24] Hydroxyethyl pyridyldisulfide (HPDS) was synthesized according to a known procedure.[25] All other chemicals were used as received.

Instrumental Analyses. Gel permeation chromatography (GPC) was performed using *N,N*-dimethylacetamide (DMAc) (0.03% w/v LiBr, 0.05% BHT stabilizer) as the continuous phase at 50 °C (flow rate: 1 mL min^{-1}). A Shimadzu modular system was employed 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 achieved using low polydispersity polystyrene standards ranging from 500 to 10^6 g mol^{-1}.

Nuclear magnetic resonance (NMR) spectroscopy was carried out on a Bruker DPX 300 spectrometer operating at 300.17 MHz for ^1H and 75.48 MHz for ^{13}C using $CDCl_3$ and D_2O as solvents and tetramethylsilane (TMS) as a reference. Data were reported as follows: chemical shift (δ) measured in ppm downfield from TMS; multiplicity; proton count. Multiplicities were reported as singlet (s), broad single (bs), doublet (d), triplet (t), and multiplet (m).

Electrospray ionization mass (ESI MS) spectra were obtained using 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. Positive ion spectra were obtained by direct infusion at a solvent flow rate of 3 μL min^{-1} and a spray voltage of 5 kV, with nitrogen as sheath gas. Xcalibur ver. 1.3 (Finnigan Co.) was used for spectral processing. UV−vis spectra were recorded on a Varian Cary 300scan spectroscope using wavelengths from 200 to 600 nm.

FT-IR spectra were obtained using a Bruker Spectrum BX FT-IR system using diffuse reflectance sampling accessories. High performance liquid chromatography (HPLC) was performed with a C18 column (150 × 4.6 mm^2, 5 μm, Phenomenex, Lane Cove, NSW, Australia) equipped with a UV detector at 254 nm. Liquid chromatography conditions: 20% 0.1 M phosphate buffer (pH 7.0) solution and 80% acetonitrile; flow rate: 1.0 mL/min; column temperature: 25 °C.

A Malvern BI-9000AT digital autocorrelator was used for static light scattering (SLS) measurements using a vertically polarized laser light source of wavelength 600 nm. The SLS, measurements were carried out at a temperature of 25 °C to determine the weight-average molecular weight (M_w) in THF (for polystyrene, $dn/dc = 0.187$) or water (for PHPMA, $dn/dc = 0.167$).

Synthesis of RAFT Agent, *N,N′*-Bis(2-(4-(2-pyridyldisulfide) ethyl butyric-1-carbonyloxo)ethyl) Cyanopentanoic Amide Dithiobenzoate, 4 (Scheme 1). Precursor **1** was synthesized according to a previous procedure.[21a] Yield: 53.2%. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.89 (d, 2H, phenyl group), 7.56 (t, 1H, phenyl group), 7.39 (t, 2H, phenyl group), 4.59 (t, 2H, N*C*$\mathbf{H}_2$$CH_2S$), 3.60−3.66 (m, 2H, (CN)C(CH_3)CH_2*C*$\mathbf{H}_2$CON), 3.31 (t, 2H, NCH_2*C*$\mathbf{H}_2$S), 2.50−2.78 (m, 2H, (CN)C(CH_3)*C*$\mathbf{H}_2$$CH_2$CON), 1.95 (s, 3H, (*C*$\mathbf{H}_3$)C(CN)S). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 222.5 (Ph**C**=S), 201.6 (N**C**=S), 172.3 (**C**=O), 144.5, 132.9, 128.5, 126.6, 118.6 (**C**N), 55.9, 45.7, 34.3, 33.3, 28.4, 24.2. IR:1696 (C=O), 1166 (PhC=S), 1046 cm^{-1} (NC=S).

A solution of diethanolamine (0.84 g, 8.01 mmol) dissolved in tetrahydrofuran (THF) (20 mL) was added dropwise into a solution of **1** (3.04 g, 8.01 mmol) dissolved in THF (30 mL) cooled in an ice/salt bath. After addition, within 30 min, the reaction mixture was warmed up to ambient temperature and stirred overnight. After removing THF under vacuum, the crude product was purified by column

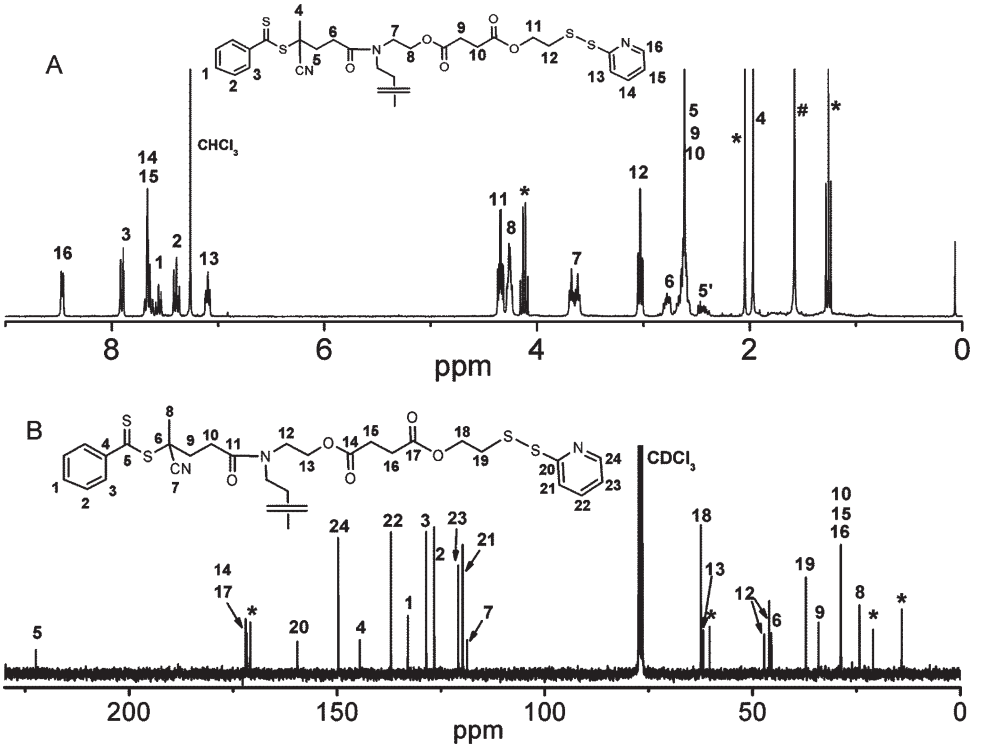


Figure 1. ^{1}H NMR (300 MHz) (A) and ^{13}C NMR (75 MHz) (B) spectra of RAFT agent **4** in $CDCl_3$ with the corresponding assignments. Notes: (*) ethyl acetate; (#) H_2O.

chromatography on silica gel, eluting with DCM and gradually increasing the polarity to methanol/DCM (1/19) to give compound **2** as a pink oil (2.61 g, 88.5% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.89 (d, 2H, phenyl group), 7.56 (t, 1H, phenyl group), 7.39 (t, 2H, phenyl group), 3.81−3.87 (m, 4H, $N(CH_2\mathit{C}\mathbf{H}_2OH)_2$), 3.55 (m, 4H, $N(\mathit{C}\mathbf{H}_2CH_2OH)_2$), 3.13 (bs, 2H, $N(CH_2CH_2\mathbf{O}\mathbf{H})_2$), 2.80 (t, 2H, $(CN)C(CH_3)CH_2\mathit{C}\mathbf{H}_2CON$), 2.40−2.70 (m, 2H, $(CN)C(CH_3)\mathbf{C}\mathbf{H}_2\mathit{C}H_2CON$), 1.97 (s, 3H, $(\mathit{C}\mathbf{H}_3)C(CN)S$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 222.5 (Ph**C**=S), 172.55 (**C**=O), 144.5, 132.9, 128.5, 126.6, 118.8 (**C**N), 61.3 ($N(CH_2\mathbf{C}H_2OH)_2$), 60.5 ($N(CH_2\mathbf{C}H_2OH)_2$), 51.9 ($N(\mathbf{C}H_2CH_2OH)_2$), 50.5 ($N(\mathbf{C}H_2CH_2OH)_2$), 46.0, 34.1, 29.1, 24.2. IR:3315 (O−H), 1685 (C=O), 1512 (N−C), 1160 (PhC=S), 1042 cm^{-1} (NC=S). ESI MS: 389.04 (MNa^+).

Compound **2** (2.32 g, 6.3 mmol), succinic anhydride (1.58 g, 15.8 mmol) and DMAP (0.08 g, 0.71 mmol) dissolved in tetrahydrofuran (THF) (50 mL) were stirred at 50 °C. After 10 h, the reaction mixture was cooled down. After removing THF under vacuum, the crude product was purified by column chromatography on silica gel, eluting with DCM and gradually increasing the polarity to methanol/DCM (1/39) to give compound **3** as a pink oil (3.01 g, 84% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.89 (d, 2H, phenyl group), 7.56 (t, 1H, phenyl group), 7.39 (t, 2H, phenyl group), 4.21−4.31 (m, 4H, $N(CH_2\mathit{C}\mathbf{H}_2OCO)_2$), 3.60−3.64 (m, 4H, $N(\mathit{C}\mathbf{H}_2CH_2OCO)_2$), 2.77 (t, 2H, $(CN)C(CH_3)CH_2\mathit{C}\mathbf{H}_2CON$), 2.57−2.73 (m, 9H, $(CN)C(CH_3)\mathbf{C}\mathbf{H}_2\mathit{C}H_2CON$, $OCO\mathit{C}\mathbf{H}_2\mathit{C}\mathbf{H}_2COOH$), 2.47 (m, 1H, $(CN)C(CH_3)\mathbf{C}\mathbf{H}_2\mathit{C}H_2CON$), 1.97 (s, 3H, $(\mathit{C}\mathbf{H}_3)C(CN)S$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 222.5 (Ph**C**=S), 177.2, 177.0 ($OCOCH_2CH_2\mathbf{C}OOH$), 172.0, 171.8 ($O\mathbf{C}OCH_2CH_2COOH$), 171.28 ($CH_2CH_2\mathbf{C}ON$), 144.5, 132.9, 128.5, 126.6, 118.8 ($(CH_3)C\mathbf{C}N$), 62.5, 61.9.3 ($N(CH_2\mathbf{C}H_2OCO)_2$), 47.36, 46.0, 45.9, 34.1, 28.9, 28.8, 28.6, 24.2. IR: 3452 (COO-H), 1752 (C=OOH), 1689 (C=O), 1526 (N−C), 1178 (PhC=S), 1056 cm^{-1} (NC=S). ESI MS: 589.23 (MNa^+).

Compound **3** (0.531 g, 0.94 mmol) and DCC (0.483 g, 2.34 mmol) were dissolved in DCM (6 mL). HPDS (0.438 g, 2.34 mmol) and DPTS (0.036 g, 0.117 mmol) dissolved in DCM (4 mL) were added slowly. The reaction mixture was warmed up to 45 °C and stirred overnight. After removal of the undissolved solid, the liquid phase was concentrated and purified by column chromatography on silica gel, eluting with *n*-hexane/ethyl acetate (20/80) (R_f = 0.3) to give the RAFT agent **4** as a pink viscous oil (0.65 g, 76.5% yield). Purity: 94.3% (HPLC). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.47 (d, 2H, pyridyl group), 7.92 (d, 2H, phenyl group), 7.67 (m, 4H, pyridyl group), 7.56 (t, 1H, phenyl group), 7.39 (t, 2H, phenyl group), 7.10 (q, 2H, pyridyl group), 4.35 (m, 4H, $COO\mathit{C}\mathbf{H}_2CH_2SS$), 4.27 (m, 4H, $N(CH_2\mathit{C}\mathbf{H}_2OCO)_2$), 3.67 (t, 2H, $N(\mathit{C}\mathbf{H}_2CH_2OCO)_2$), 3.62 (t, 2H, $N(\mathit{C}\mathbf{H}_2CH_2OCO)_2$), 3.03 (t, 4H, $COO\mathit{C}\mathbf{H}_2CH_2SS$), 2.77 (t, 2H, $(CN)C(CH_3)CH_2\mathit{C}\mathbf{H}_2CON$), 2.57−2.73 (m, 9H, $(CN)C(CH_3)\mathbf{C}\mathbf{H}_2\mathit{C}H_2CON$, $OCO\mathit{C}\mathbf{H}_2\mathit{C}\mathbf{H}_2COOH$), 2.47 (m, 1H, $(CN)C(CH_3)\mathbf{C}\mathbf{H}_2\mathit{C}H_2CON$), 1.97 (s, 3H, $(\mathit{C}\mathbf{H}_3)C(CN)S$). ^{13}C NMR (75 MHz, $CDCl_3$): Exact carbon assignments were shown in Figure 1. IR: 2108 (C=N, pyridyl), 1696 (C=O), 1540 (N−C), 1176 (PhC=S), 1051 cm^{-1} (NC=S). ESI MS: 927.31 (MNa^+).

Polymerization of Styrene Mediated by RAFT Agent 4. A typical procedure is described as follows: RAFT agent **4** (78 mg, 0.086 mmol), styrene (2.24 g, 0.022 mol), and initiator AIBN (4.7 mg, 0.028 mmol) were dissolved in toluene (6 mL). The stock solution was transferred to five glass ampules and each was purged with nitrogen. The polymerizations were carried out at 70 ° C. Samples were withdrawn at different intervals for molecular weight (GPC) and conversion analyses (^{1}H NMR). Conversion was calculated by comparing the integration of the signals at δ 5.7 and δ 5.18 ppm originating from the double bond of styrene in ^{1}H NMR spectra (in $CDCl_3$) to that of two of phenyl groups of the polymer at δ 6.5 ppm. The polymer was purified by (3-fold) precipitation in methanol.

Polymerization of *N*-(2-Hydroxypropyl)methacrylamide (HPMA) Mediated by RAFT Agent 4. A typical procedure is described as follows: RAFT agent **4** (50 mg, 0.055 mmol), HPMA (1.7 g, 11.8 mmol), and initiator AIBN (3 mg, 0.018 mmol) were dissolved in DMAc (6 mL The stock solution was transferred to five glass ampules and each was purged with nitrogen for 30 min. The polymerizations were carried out at 60 ^{o} C. Samples were withdrawn at different intervals for molecular weight (GPC and ^{1}H NMR) and conversion analyses (^{1}H NMR). Conversion was calculated by comparing the integration of the signals at δ 5.35 and δ 5.58 ppm originating from the double bond in ^{1}H NMR spectra (in D_2O) to that of the characteristic CH signal in the repeat unit of HPMA at δ 3.78 ppm. The polymer was purified by (3-fold) precipitation in diethyl ether. Molecular weights were calculated by comparing the integration of the proton of the chain-end functionalities CH_2 signal at δ 4.21 ppm (or pyridyl disulfide signal at δ 8.30 ppm) and that of the characteristic CH signal in the repeat unit of HPMA at δ 3.78 ppm in ^{1}H NMR (in D_2O).

Aminolysis of Polymers (AB_2 Macromonomers) in the Presence of Hexylamine/TEA. Polystyrene AB_2 macromonomer: Pink powder ($M_{n,GPC}$ = 5960 Da, $M_{n,NMR}$ = 6420 Da, PDI = 1.10, 60 mg, 1×10^{-5} mol) was dissolved in DMAc (0.5 mL) followed by degassing with nitrogen for 30 min. Hexylamine (1.02 mg, 1×10^{-5} mol) and triethylamine (2.04 mg, 2×10^{-5} mol) were injected into the solution using a syringe. The reaction mixture was shaken at ambient temperature. Samples were withdrawn at predetermined times for GPC and UV−vis analysis. The final reaction mixture was precipitated in methanol (10 folds excess). After drying, the light pink precipitate was collected and characterized by NMR.

PHPMA AB_2 macromonomer: Pink powder ($M_{n,GPC}$ = 14650 Da, $M_{n,NMR}$ = 7385 Da, PDI = 1.08, 60 mg, 8.1×10^{-6} mol) was dissolved in DMAc (0.5 mL), followed by degassing with nitrogen for 30 min. Hexylamine (0.83 mg, 8.1×10^{-6} mol) and triethylamine (1.66 mg, 1.62×10^{-5} mol) were injected into the solution using a syringe. The reaction mixture was shaken at ambient temperature. Samples were withdrawn at predetermined times for GPC and UV−vis analyses. The final reaction mixture was precipitated in diethyl ether (10 folds excess). After drying, the light pink precipitate was collected and measured by NMR.

Cleavage of Hyperbranched Polymer in the Presence of DTT. Hyperbranched polystyrene (10 mg, 1.67×10^{-6} mol, based on the molecular weight of corresponding macromonomer) and DTT (6.2 mg, 4×10^{-5} mol) were dissolved in DMAc (0.5 mL). After 2 h, a portion of the solution was withdrawn and subjected to GPC analysis. Cleavage of hyperbranched PHPMA followed an identical procedure.

Results and Discussion

Synthesis of RAFT Agent 4. The strategy for the synthesis of RAFT agent **4** is highlighted in Scheme 1. Precursor **1,** bearing a 2-mercaptothiazolidine active ester in the *Z* group can react readily with primary amine in a one-step reaction without any degradation of the thiocarbonylthio moiety via a carefully optimized reaction with a molar ratio of one or less.[21a,26] A secondary amine, commercial diethanolamine, was employed to react with the active ester yielding product **2** with two functionalizable hydroxyl groups in high yield (88.5%), without any degradation of the thiocarbonylthio moiety. The reaction conditions and procedure were optimized and found to be almost the same when using a primary amine, implying that amidation of the active ester and amine (both primary and secondary amine) was heavily favored over the thioamidation of thiocarbonylthio moieties. Subsequently, the two hydroxyl groups were converted to carboxylic groups (**3**) by esterification with succinic anhydride in the presence of DMAP. Then, hydroxyethyl pyridyldisulfide (HPDS) was attached to the scaffold **3** by esterification between the hydroxyl and carboxylic groups using coupling reagents DCC and DPTS yielding RAFT agent **4**. After purification by column chromatography, RAFT agent **4** was fully characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry (Figure 1—see the Experimental Section for exact proton and carbon assignments). The pink color of the products **1**, **2**, **3**, and **4** made their purification by column chromatography much easier.

Pyridyl disulfide (PDS) groups can be used to react with any free thiol present in biomolecules or polymers. Moreover, a long spacer between the two functional PDS groups can minimize the steric hindrance involved in further reactions.

RAFT Polymerization of Styrene or *N*-(2-Hydroxypropyl) Methacrylamide Mediated by RAFT Agent 4 To Generate AB_2 Macromonomers. The new RAFT agent **4**, containing double-functional pyridyl disulfide (PDS) groups, was used to mediate the polymerizations of styrene and *N*-(2-hydroxypropyl) methacrylamide (HPMA). The control exerted by RAFT agent **4** was assessed by monitoring the kinetics and molecular weight development of the polymerizations. Polystyrene was characterized by GPC (Figure 2A) showing clear shifts of the polymer signal with increasing reaction times. In addition, linearity of the pseudofirst order kinetic plot (Figure 2B) was indicative of a constant radical concentration. However, high molecular weight tailing was evident at higher conversions (48%) as indicated by the GPC trace (from the 20 h polymerization time sample) and deviation from linearity of the plot of M_n versus conversion (Figure 2C). The polydispersities were slightly increasing with conversion, although the polydispersities remained below 1.2 over the entire polymerization. When high molar feed ratios of $[monomer]_0/[\mathbf{4}]_0$ were employed (entries 1 and 2 in Table 1), high PDIs (> 1.2) were obtained at high conversions. A hypothesis can be tendered that a small amount of transfer to the disulfide functionality is occurring causing the GPC molecular weight tailing at high conversions,[27] despite the fact that chain transfer has been studied previously and found to be insignificant.[28] Alternatively, the tailing could originate from bimolecular termination reactions. To avoid this problem, experiments were designed to produce polymers at short reaction times, maintaining low conversions. To verify the polymer structure, the product prepared at low conversion (18.6%) for subsequent aminolysis was characterized by GPC and ^{1}H NMR. The number average molecular weight M_n (5960 Da) measured by GPC agreed with the theoretical value (5740 Da) and the molecular weight measured by ^{1}H NMR (6420 Da, degree of polymerization about 53) (calculated by comparison of the integrations of the chain-end pyridyl disulfide signal at δ 8.47 and 7.66 ppm (proton 15 and 13, 14 in Figure 5A) to that of the characteristic styrene phenyl ring signal at δ 6.3 - 7.3 ppm). Moreover, the comparative integration of pyridyl disulfide signal at δ 8.47 and 7.66 ppm and dithiobenzoate signal atδ 7.85 and 7.46 ppm (proton 3 and 1 in Figure 5A) further proved the integrity of alpha and omega end-functionalities after polymerization.

HPMA polymerizations were well controlled, as indicated by clear shifts of the uniform polymer peaks and no obvious tailing observed (except at long reaction times (11 h)) in GPC traces (Figure 3A). A linear pseudofirst order kinetic plot (Figure 3B) was also obtained. The molecular weights,

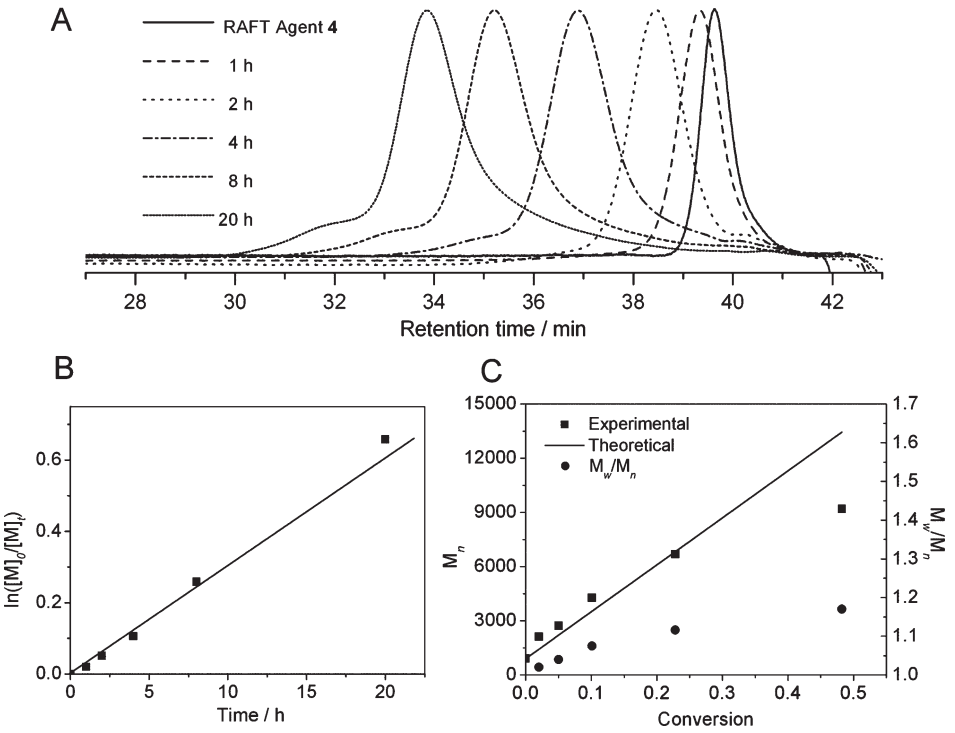


Figure 2. RAFT polymerization of styrene mediated by RAFT agent **4** in toluene at 70 °C. $[Styrene]_0/[\mathbf{4}]_0/[AIBN]_0 = 750/3/1$, $[styrene]_0 = 3.66$ mol/L. Key: (A) GPC traces; (B) kinetics plots; (C) evolution of M_n and polydispersities (PDI) versus conversion. The experimental molecular weight was measured by GPC calibrated with polystyrene standards using a RI detector.

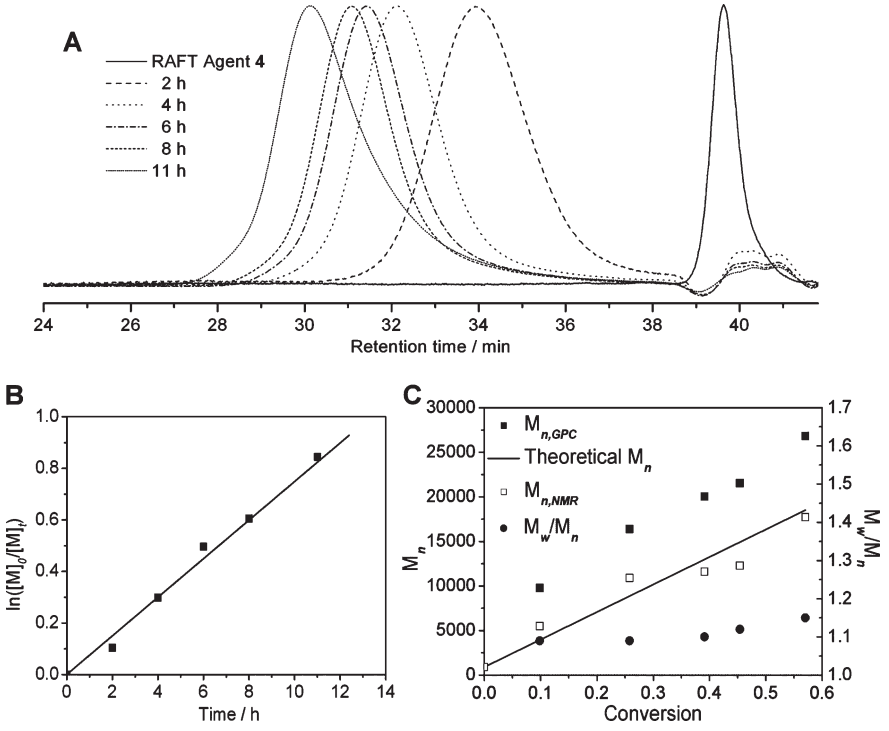


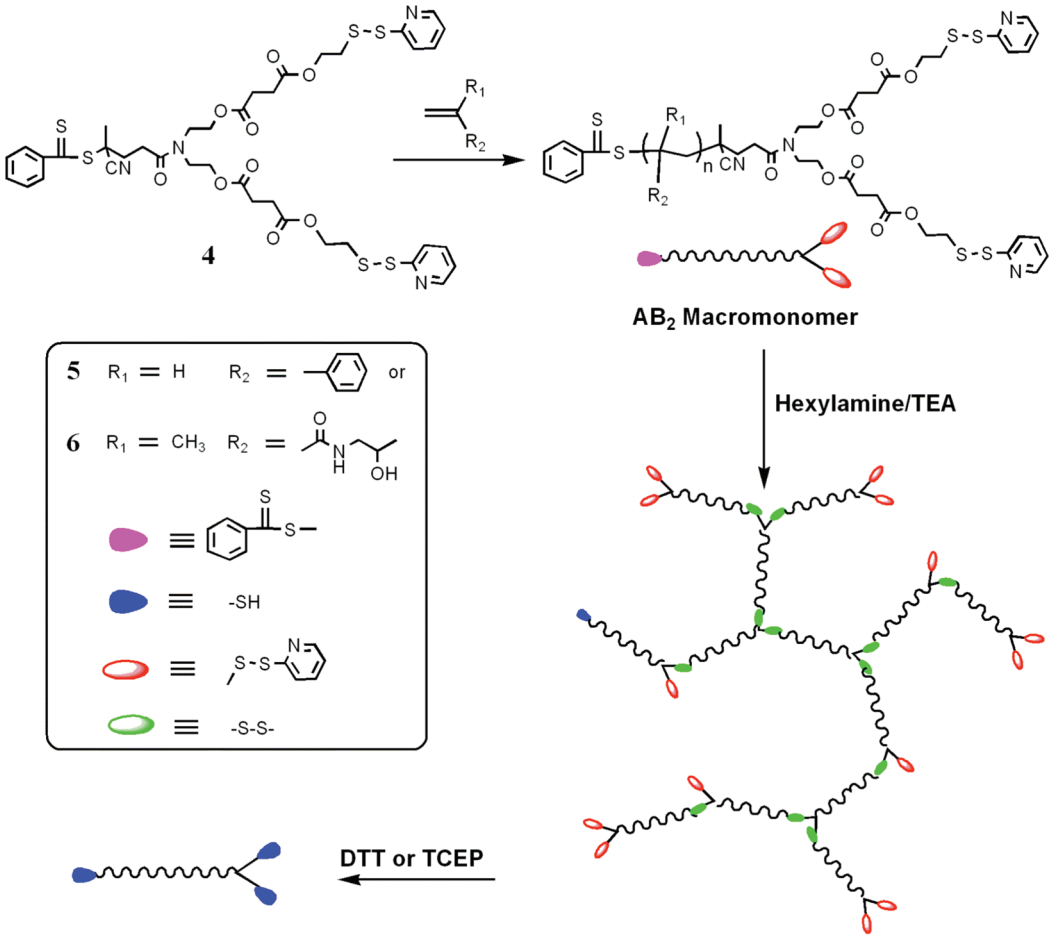
Figure 3. RAFT polymerization of *N*-(2-hydroxypropyl) methacrylamide (HPMA) mediated by RAFT agent **4** in DMAc at 60 °C. $[HPMA]_0/[\mathbf{4}]_0/[AIBN]_0 = 645/3/1$, $[HPMA]_0 = 1.97$ mol/L; (A) GPC traces; (B) Kinetics plots; (C) Evolution of M_n and polydispersities (PDI) versus conversion. The experimental molecular weight $M_{n,GPC}$ was measured by GPC calibrated with polystyrene standards using a RI detector.

measured by GPC (Figure 3C), were much higher than those measured by NMR (that were consistent with expected values, similar to previous results).[21] The polydispersities remained below 1.2. However, at long reaction time (11 h polymerization time entry 5 in Table 1) and high temperature (70 °C, entry 4) some molecular weight tailing and higher polydispersities were observed. The exact molecular weight calculated by ^{1}H NMR (by comparison of the integrations of the chain-end pyridyl disulfide signal at δ 8.30 ppm (CH in pyridyl ring or CH_2 adjacent to ester bonds, proton 15 or 7, 10, Figure S1A in Supporting Information) to that of the characteristic CH signal in the repeat unit at δ 3.78 ppm

Table 1. Polymerization of Styrene or HPMA Mediated by RAFT Agent 4 under Different Conditions

entry	monomer	temperature (°C)	$[monomer]_0$ (mol/L)	$[monomer]_0/[4]_0/[AIBN]_0$	time (h)	M_n	conversion (%)	PDI
1	styrene	70	2.85	300/3/1	20	8210	74.6	1.25
2			2.85	300/3/0.5	24	7150	62.1	1.20
3			3.66	750/3/1	8	6690	22.8	1.11
4	HPMA	70	1.23	300/3/1	7	8700	81.3	1.19
5		60	1.23	300/3/0.5	24	8810	76.4	1.21
6		60	1.97	645/3/1	4	10890	20.7	1.09

Scheme 2. One-Pot Preparation of Hyperbranched Polymers



(proton 5, Figure S1A in Supporting Information)) was in accord with the theoretical value, confirming that the pyridyl disulfide end-group maintained integrity during the polymerization. This provides evidence that any transfer occurring is not a significant kinetic event. In addition, the dithiobenzoate end-group signals (protons 1, 2, and 3, Figure S1A in Supporting Information) were retained, as expected.

Aminolysis and Hyperbranching of AB_2 Macromonomer. Aminolysis is a straightforward approach to the modification of thiocarbonylthio groups to thiols. The generated thiols can subsequently react with many functionalities, including maleimide, pyridyl disulfide (PDS), and ene. Polystyrene (M_n = 5960 Da, PDI = 1.10) with α-dithobenzoate and ω-double pyridyl disulfide end-functionalities, prepared by RAFT, was aminolyzed in the presence of hexylamine (equivalent) to yield thiol end-functionalized polystyrene. Simultaneously, thiol was reacted with the PDS group by thiol−disulfide exchange chemistry generating a hyperbranched structure linked by more stable disulfide bonds (Scheme 2). Aminolysis reaction conditions involved the use of equivalent hexylamine to polymer (feed molar ratio of polymer/hexylamine/triethylamine: 1/1/2) to prevent excess primary amine destroying the pyridyl disulfide groups. The polymer concentration used for aminolysis had a significant effect on reaction rates (including aminolysis and hyperbranching reactions) and on the resultant molecular weights of the final hyperbranched structures. High polymer concentrations were found to accelerate the aminolysis reaction and favor the formation of high molecular weights hyperbranched polymers (data not shown).

The presence of disulfide linkages among the polymer segments can be verified by following molecular weight development using GPC (Figure 4) and ^{1}H NMR (Figure 5) analyses. Multimodal GPC curves, consistent with a growth toward high molecular weight, were observed as aminolysis progressed (Figure 4). After 120 min, hyperbranched polystyrene with M_n = 15620, M_w = 31700, M_p =35540, and PDI = 2.03 (Figure 4A) and PHPMA with M_n = 30950, M_w = 53850, M_p =48270, and PDI = 1.74 (Figure 4B) measured by GPC were obtained, and high molecular weight polymers were the main components. Static light scattering was employed to measure the absolute molecular weight, yielding $M_w \sim 74800$ for the hyperbranched polystyrene, much higher than the value measured by GPC calibrated using narrow polystyrene

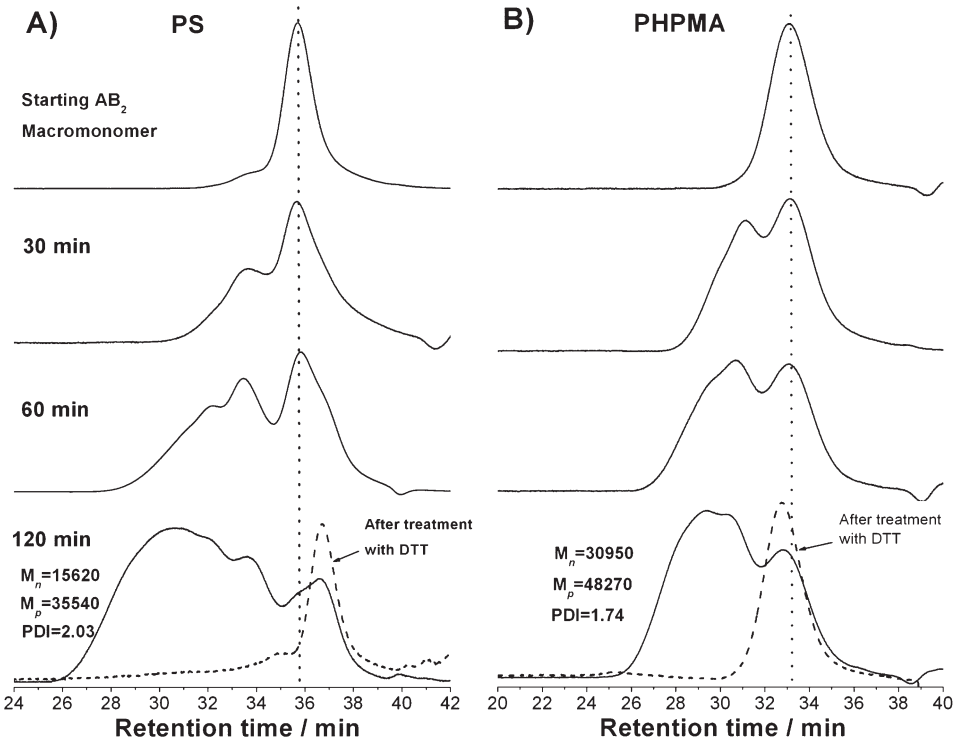


Figure 4. GPC traces monitoring aminolysis of AB_2 macromonomers: (A) PS (M_n = 5960 Da, PDI = 1.10); (B) PHPMA ($M_{n,GPC}$ = 14650 Da, $M_{n,NMR}$ = 7385 Da PDI = 1.08) at different time points and cleavage of final hyperbranched products in the presence of DTT (dashed line). Aminolysis conditions: $[macromonomers]_0/[hexylamine]_0/[triethylamine]_0$ = 1/1/2; room temperature; *N,N′*-dimethylacetamide (DMAc) solvent. Concentrations: PS, 0.019 mol/L; PHPMA, 0.016 mol/L.

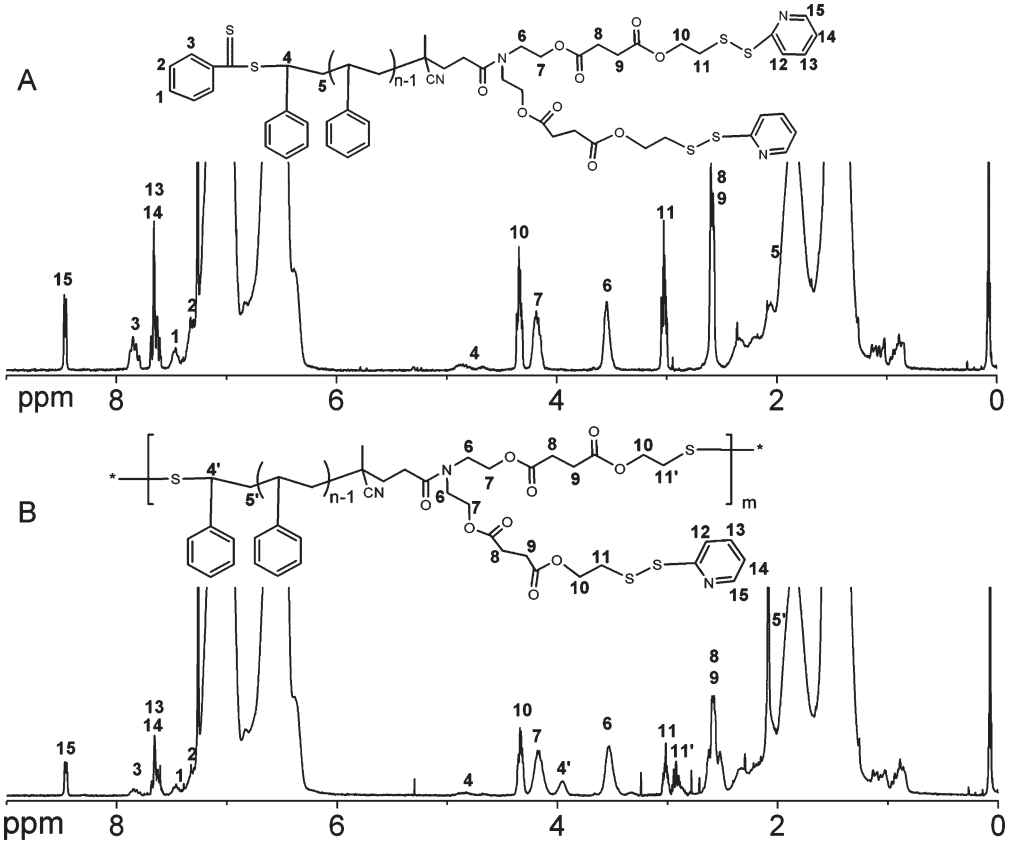
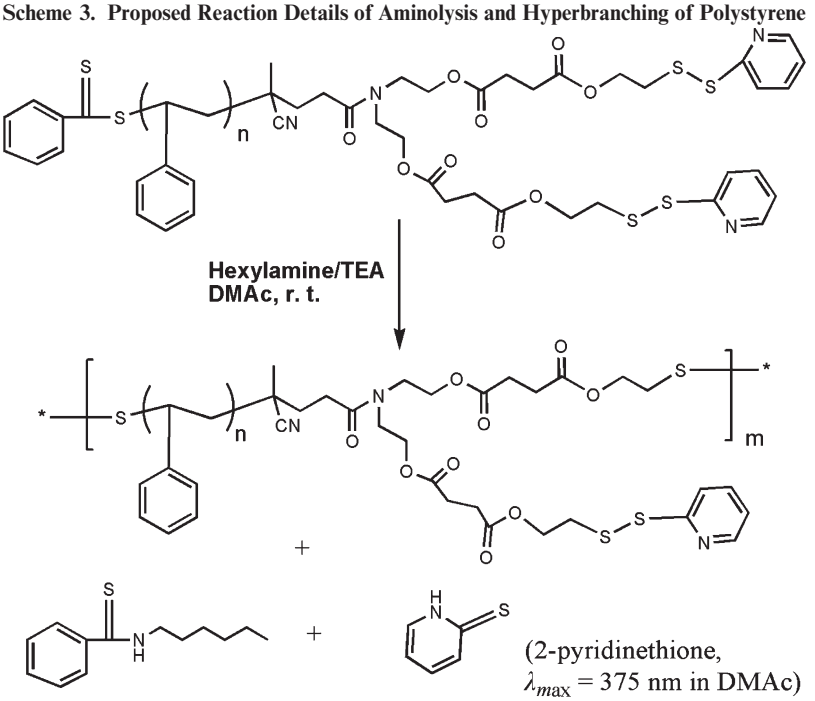


Figure 5. ^1H NMR (300 MHz, $CDCl_3$) spectra of polystyrene AB_2 macromonomer (M_n = 5960 Da, PDI = 1.10) before (A) and after (B) aminolysis with the corresponding assignments.

standards (M_w = 31700). This absolute MWT measurement strongly supports a hyperbranched architecture. Monteiro and co-workers[29] have reported an approach to prepare cyclic, multiblock and network polymers by coupling/cleavage of

Scheme 3. Proposed Reaction Details of Aminolysis and Hyperbranching of Polystyrene



thiol/disulfide groups. The approach described herein exploits AB_2 type macromonomers, widely used to synthesize hyperbranched structures, without any attendant byproducts of cyclic or network structures (provided optimized conditions are used). In this present work, no networks or cyclic structures could be detected.

Proof that the cause of molecular weight increase was the formation of disulfide bonds was accrued by subsequent treatment with excess DTT (the dashed line in Figure 4). The structure, post-DTT treatment, had a molecular weight profile similar to the starting macromonomers, although the molecular weights of post-treated polystyrene (M_n = 5370 Da, PDI = 1.11) and PHPMA (M_n = 16380 Da, PDI = 1.07) measured by GPC were a little lower or higher than starting macromonomers. These GPC results were complemented by data from ^{1}H NMR (Figure 5) showing a reduction in both dithiobenzoate and pyridyl disulfide groups after aminolysis. The characteristic protons for polystyrene before and after aminolysis are assigned in Figure 5. Before aminolysis, the signals 15, 14, 13 attributed to the corresponding protons in the pyridyl rings and 3, 1, 2 attributed to the phenyl group of dithiobenzoate, and 10, 7, 6, 11 attributed to the long spacer between polymer and PDS group were presented clearly in ^{1}H NMR spectrum. It is noted that the methine hydrogen adjacent to thiocarbonylthio moiety in terminal styrene unit (proton 4) can be seen as a broad "doublet" signal at δ 4.9−4.7 ppm, similar to results reported by Postma, etc.[30] After aminolysis, the characteristic signals were slightly broadened in accord with the higher molecular weight of the hyperbranched polystyrene. A new signal at δ 4.9−4.7 ppm appeared, consistent with the change of methine proton 4 adjacent to thiocarbonylthio group to methine proton 4′ adjacent to disulfide after aminolysis. The amount of dithiobenzoate converted to thiol was calculated as 81% by comparing the integration of proton 3 (phenyl group of dithiobenzoate) before and after aminolysis. The integration ratio of proton 15 (corresponding to signal 15 in the NMR spectra) before and after aminolysis was 46.6%, indicating that half of the PDS groups undergo exchange to disulfide during the aminolysis process. This result

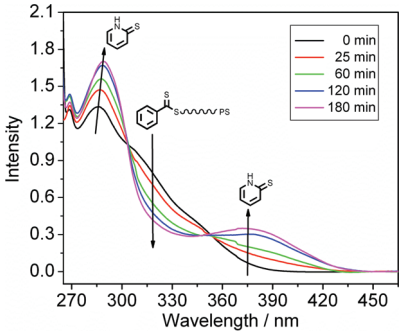


Figure 6. UV−vis spectrometric monitoring of the aminolysis and hyperbranching process of polystyrene (M_n = 5960 Da, PDI = 1.10) at different time points. Aminolysis conditions: $[macromonomers]_0/[hexylamine]_0/[triethylamine]_0$ = 1/1/2; room temperature; *N,N*′-dimethylacetamide (DMAc) solvent.

can be further confirmed by the integration of signal 4′ (methine proton 4′) in Figure 5B, accompanying with the splitting of signal 11 (before aminolysis) into two equal signals 11 and 11′ (after aminolysis).

The exchange reaction between thiol and PDS end-groups (Scheme 3) was monitored via UV−vis spectroscopy by detection of the byproduct, 2-pyridinethione, with absorbances at 290 and 375 nm wavelength (Figure 6). The absorbance intensities at 290 and 375 nm increased with reaction time, with an accompanying decrease of the absorbance intensity at 320 nm (dithiobenzoate end group), consistent with the expected reaction, as illustrated in Schemes 2 and 3.

Conclusions

A novel strategy for the preparation of functionalized and biodegradable hyperbranched polymers has been outlined. The approach utilizing AB_2 macromonomers prepared by RAFT polymerization is highly versatile. A wide-range of polymers (or copolymers) could be made using this approach. Excess pyridyl disulfide functionalities could be engineered easily on the periphery of these hyperbranched structures, providing anchoring points for bioconjugation chemistry.

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Supporting Information Available: Figures showing ^{1}H NMR and UV−vis spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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