Facile Synthesis of Polyester Dendrimer via Combining Thio-bromo “Click” Chemistry and ATNRC

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ABSTRACT: This article reports on developing an efficient synthesis approach to aliphatic polyester dendrimer, poly(thioglyceryl-2-propionate) (PTP), by combination of thio-bromo “Click” chemistry with atom transfer nitroxide radical coupling (ATNRC). Through the one-pot two-step method, linear poly(styrene with hydroxyl end groups (l-PS-2OH) was obtained by first atom transfer radical polymerization of styrene and following termination using 4-(2,3-dihydroxypropoxy)-TEMPO (DHP-TEMPO) to capture the PS macroradicals via ATNRC method. Using l-PS-2OH as support, the dendritic repeating units divergently were grown from the hydroxyl end groups via esterification and thio-bromo “Click” reaction two-step process. In every generation, the resulting intermediates l-PS-d-PTP (G1-G4) can be easily isolated from the excessive unreacted monomers by simple precipitation in ethanol without help of time, labor and solvent consuming column chromatographic purification. At last, cleavage of the alkoxyamine group between the PS support and dendrimer at elevated temperature (125 °C) provided the targeted polyester dendrimer PTP up to the fourth generation.

KEYWORDS: “ATNRC” coupling; emulsion polymerization; polyester dendrimer; thio-bromo “click” reaction; rheology; swelling; synthesis and processing

INTRODUCTION Dendrimers, a class of highly branched macromolecules characterized by precise molecular weight, high degree of surface functionality and well-defined three-dimensional architecture emanating from a central core, have attracted considerable attention in the past few decades. Due to their unique properties, dendrimers are of widespread interest for numerous applications including drug delivery, gene carriers, tissue engineering, catalysis, and light-harvesting. Up to now, dendrimers with different kinds of compositions have been prepared via the divergent or convergent approach, of which poly(propyleneimine) (PPI) and poly(amine) (PAMAM) are the most studied. However, PPI and PAMAM dendrimers are not biodegradable in vivo, and this drawback may hinder their applications in biomedical area. In contrast, polyester dendrimers can be degraded or hydrolyzed in vivo into small molecules easily being excluded from the body. The biocompatibility and biodegradability features endow polyester dendrimers with promising applications in biomedical area, especially as drug or gene carriers.

Polyester dendrimers were traditionally synthesized using 2,2-bis(hydroxymethyl) propionic acid as the growth unit through the divergent approach. In these synthesis strategies, hydroxyl groups or carboxylic acid groups of monomers needed tedious protection/deprotection, and defects may be introduced due to incomplete protection or deprotection reaction. Meanwhile, column chromatographic purification had to be used to remove the excessive unreacted monomers. Ma et al. demonstrated an efficient approach to polyester dendrimers by the combination of Michael addition reaction of acrylates with amines and methacrylates with thiols. Intermediate protection/deprotection steps were avoided due to the chemoselectivity between the functional groups in monomers. However, several days were required to allow the reaction of acrylates with amines to proceed completely. Recently, Rosen et al. developed another efficient approach to polyester dendrimer by the combination of thio-bromo “Click” reaction of thioglycerol with β-bromoester and acylation of surface hydroxyl groups with 2-bromopropionyl bromide. It should be noted that thio-bromo “Click” and acylation growth steps can be completed in 45 min, respectively. Meanwhile, protection/deprotection was avoided because thio-bromo “Click” reaction can tolerate presence of hydroxyl group. However, complicated
chromatographic purification had to be used to isolate the dendrimer. Therefore, it is greatly desirable to develop new and more efficient strategies for synthesis of polyester dendrimers.

In their early work, Ihre et al. reported the synthesis of novel linear-dendritic copolymers up to G4 via the 2,2-bis(hydroxy-methyl) propionic acid repeating units divergently growing from linear or star-shaped poly(ethylene glycol) (PEG). It is noteworthy that purification of the resulting copolymers only required a simple precipitation utilizing the difference in solubility between PEG conjugated copolymers and the growing units. In present study, a novel efficient approach to rapid synthesis of polyester dendrimer was developed via combination of thio-bromo “Click” chemistry with ATNRC coupling, as shown in Scheme 1. Using PS-2OH as support, dendrimer PTP was divergently grown from the end hydroxyl groups through the acylation and thio-bromo “Click” reaction two-step process. Due to the effect of PS part, the formed intermediates, linear-dendritic copolymers l-PS-d-PTP, were isolated from the excessive monomers by a simple precipitation utilizing the difference in solubility between PEG conjugated copolymers and the growing units. In present study, a novel efficient approach to rapid synthesis of polyester dendrimer was developed via combination of thio-bromo “Click” chemistry with ATNRC coupling, as shown in Scheme 1. Using PS-2OH as support, dendrimer PTP was divergently grown from the end hydroxyl groups through the acylation and thio-bromo “Click” reaction two-step process. Due to the effect of PS part, the formed intermediates, linear-dendritic copolymers l-PS-d-PTP, were isolated from the excessive monomers by a simple precipitation utilizing the difference in solubility between PEG conjugated copolymers and the growing units.

EXPERIMENTAL

Materials
Styrene (≥99%, Sigma-Aldrich) was passed through a short column of neutral Al₂O₃ to remove the inhibitor: Copper(I) bromide (98%, Sigma-Aldrich), N,N,N',N"-pentamethyldiethylenetriamine (PMDETA) (>98%, Tokyo Chemical Industry Co., Ltd), 1-bromoethyl benzene (98%, Aldrich), 2-bromopropionyl bromide (97%, Aldrich), thioglycerol (≥97%, Sigma) were used as received. Triethylamine (TEA), pyridine, dichloromethane (DCM) were dried over calcium hydride (CaH₂) and distilled under argon atmosphere before use. 4-(2,3-dihydroxypropoxy)-TEMPO (DHP-TEMPO) was synthesized according to the literature procedure.

Measurement
Gel permeation chromatographic (GPC) analysis was performed on an Agilent 1100 equipped with a G1310A pump, a G1362A refractive index detector, and a G1314A variable wavelength detector. One 5 LP gel column (500 Å, molecular range 500–2 × 10⁴ g/mol) and two 5 mm LP gel mixed bed column (molecular range 200–3 × 10⁶ g/mol) were calibrated by PMMA standard samples. THF was used as eluent with the flow rate of 1.0 mL/min. ¹H nuclear magnetic resonance (¹H NMR) spectra were recorded on a Bruker Ultraschield 600 MHz/54 mm NMR spectrometer in CDCl₃ or DMSO-d₆ at room temperature. The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) measurement was performed using a Perseptive Biosystem Voyager-DESTRMALDI-TOFMS (PEApplied Biosystems, Framingham, MA). 2,5-Dihydroxybenzoic acid was used as the matrix and silver trifluoroacetate as cationizing salt. Fourier transform infrared (FT-IR) spectra were recorded on a NEXUS 470 FT-IR instrument.
Synthesis of Linear End Functional Polystyrene PS-20H via ATRP and ATNRC

Styrene (20.00 mL, 0.174 mol), CuBr (62.5 mg, 0.435 mmol), and PMDETA (0.09 mL, 0.435 mmol) were introduced into a Schlenk flask. The reaction mixture was degassed by bubbling with argon. After 30 min, the initiator 1-bromoethyl benzene (0.06 mL, 0.435 mmol) was added to the system followed by bubbling with argon for another 5 min. Then the flask was put into an oil bath preheated to 90 °C. The reaction lasted for 3.5 h. After taking out a small portion of the solution, 5 mL degassed toluene solution of DHP-Tempo (0.322 g, 1.305 mmol) was added to the system. After an additional 2 h, the polymer solution was diluted with THF and passed through a short neutral aluminum oxide column to remove the copper complex. The concentrated eluate was precipitated three times in ethanol to provide the linear end functional polystyrene support PS-20H, in which the end hydroxyl groups are connected with PS via a reversible alkoxyamine group. (M₅, NMR = 12,850 g/mol; M₅,GPC = 17,680 g/mol, M₅/M₄ = 1.13.)

1H NMR (CDCl₃, δ, ppm): 6.27–7.27 (br, −CH₂CH(C₆H₅)−), 5.13–5.32 (br, −CH(O−)CH₂−O−), 4.07–4.58 (br, −CH(O−)

H₂O−), 3.46–3.85 (m, −OC(=O)CH₂CH₂OH), 2.64–3.08 (m, −OC(=O)CH₂CH₂OH), 1.25–2.11 (br, −CH₂CH(C₆H₅)−), 1.04 (t, −CH₃ in TEMPO); FT-IR: 3021 cm⁻¹ (νc−c in phenyl ring), 2935 cm⁻¹ (νc−c in PS backbone), 1490 cm⁻¹ (νc−c in phenyl ring).

Synthesis of Linear-dendrimer Like Copolymer l-PS-d-PTP via Thio-bromo "Click" Reaction and Acylation l-PS-d-PTP-G₁

PS-20H (1.505 g, containing OH 0.234 mmol) and pyridine (0.19 mL, 2.34 mmol) were dissolved in 10 mL dried DCM. The reaction solution was cooled to 0 °C by ice/water bath and 2-bromopropionyl bromide (0.25 mL, 2.34 mmol) was added dropwise via syringe under argon atmosphere. The reaction lasted for 1 h at 0 °C, then another 3 h at room temperature. The reaction solution was concentrated on a rotary evaporator and precipitated in ethanol. The crude product and thioglycerol (0.20 mL, 2.34 mmol) were dissolved in 5 mL tetrahydrofuran (THF), then Et₃N (0.32 mL, 2.34 mmol) was added dropwise. After 2 h, the mixture was precipitated twice in ethanol to provide the copolymer l-PS-d-PTP-G₁. (M₅, NMR = 12,850 g/mol; M₅,GPC = 17,680 g/mol, M₅/M₄ = 1.13.)

1H NMR (CDCl₃, δ, ppm): 6.27–7.27 (br, −CH₂CH(C₆H₅)−), 5.20 (s, −CH(O−)CH₂−O−), 4.40 (s, −CH(O−)CH₂−O−), 3.50–3.80 (m, −OC(=O)CH₂CH₂OH), 2.80 (d, −OC(=O)CH₂CH₂OH), 1.25–2.11 (br, −CH₂CH(C₆H₅)−), −SCH₂CH(OH)CH₂OH); FT-IR: 3475 cm⁻¹ (νOH), 3020 cm⁻¹ (νc−c in PS backbone), 2935 cm⁻¹ (νc−c in phenyl ring), 1704 cm⁻¹ (νc−c in phenyl ring).

l-PS-d-PTP-G₂

l-PS-d-PTP-G₁ (1.360 g, containing OH 0.413 mmol) and pyridine (0.17 mL, 2.07 mmol) were dissolved in 8 mL dried DCM. The reaction solution was cooled to 0 °C and 2-bromopropionyl bromide (0.22 mL, 2.07 mmol) was added dropwise into the system via syringe under argon atmosphere. The mixture was allowed to react for 1 h at 0 °C and an additional 3 h at room temperature. The polymer solution was precipitated in excess ethanol. The crude product and thioglycerol (0.18 mL, 2.07 mmol) were dissolved in 4 mL THF, then Et₃N (0.29 mL, 2.07 mmol) was added dropwise. The reaction mixture was stirred for 2 h. The copolymer l-PS-d-PTP-G₂ was purified following the procedure above.

1H NMR (CDCl₃, δ, ppm): 6.27–7.27 (br, −CH₂CH(C₆H₅)−), 5.15–5.36 (br, −CH(O−)CH₂−O−), 4.01–4.58 (br, −CH(O−)

H₂O−), 3.47–3.89 (m, −OC(=O)CH₂CH₂OH), 2.67–3.10 (m, −OC(=O)CH₂CH₂OH), 1.25–2.11 (br, −CH₂CH(C₆H₅)−), −SCH₂CH(OH)CH₂OH); FT-IR:
Preparation of Dendrimer G4-PTP from the Copolymer l-PS-d-PTP-G4

The copolymer l-PS-d-PTP-G4 (0.524 g) and excessive TEMPO (20 mg, 0.116 mmol) were dissolved in toluene (3 mL) and the reaction mixture was stirred for 3 h at 125 °C. After toluene was removed under reduced pressure, the residue was redissolved with a small amount of THF. The solution was poured into ethanol to precipitate PS support free of dendrimer, while the soluble dendrimers remained in the solvent. The solution containing dendrimer was concentrated on rotary evaporator and precipitated in hexane. The obtained dendrimer was dried under vacuum to constant weight at 35 °C (yield: 0.112 g).

The end functional polystyrene PS-2OH was synthesized by combining ATRP with high efficient coupling reaction ATNRC via one-pot two-step method. In the presence of CuBr/PMDETA, the 1-bromoethyl benzene first initiated ATRP of styrene. After a predetermined period of time, excessive excess unreacted monomers through a simple precipitation into ethanol by employing the difference in solubility between the insoluble copolymers conjugated with PS support and soluble excessive unreacted monomers. At last, the targeted polyester dendrimer was obtained by cleavage of the alkxoyamine at elevated temperature (125 °C).

RESULT AND DISCUSSION

Polyester dendrimer up to G4 was successfully synthesized according to the procedure shown in Scheme 1. First, the dendrimer was divergently grown from the end hydroxyl groups of PS-2OH support to form the intermediate linear-dendrimer like copolymer l-PS-d-PTP-G4, in which PTP-G4 was connected with PS through the thermally reversible alkxoyamine bond. In every growth step, the resulting linear-dendritic copolymers can be efficiently separated from the excessive unreacted monomers through a simple precipitation into ethanol by employing the difference in solubility between the insoluble copolymers conjugated with PS support and soluble excessive unreacted monomers. At last, the targeted polyester dendrimer was obtained by cleavage of the alkxoyamine at elevated temperature (125 °C).
DHP-TEMPO was added to stop the polymerization and PS-2OH was formed as TEMPO groups capturing the PS macro-radicals via ATNRC. It has been proven that ATNRC can rapidly proceed with quantitative conversion, possessing the attributes of “Click” reaction.30–37 From Figure 1, it can be seen that the molecular weight of the PS support did not increase during the 2 h after adding DHP-TEMPO. This result means that PS macro-radicals generated under ATRP conditions were completely captured and PS-2OH support was successfully obtained. The 1H NMR spectrum of PS-2OH is shown in Figure 2, in which the resonance signals (d) at 1.04 ppm and (e, f, g) between 3.42 and 3.84 ppm correspond to the characteristic protons of end group of DHP-TEMPO. The molecular weight of PS-2OH was calculated by the integration ratio of peaks (c) at 6.2 and 7.1 ppm and (d) at 1.04 ppm.

Through the iterative two-step processes involving the first acylation of surface hydroxyl groups with 2-bromopropionyl bromide followed by thio-bromo “Click” reaction of thioglycerol with z-bromoster, the polyester dendrimer divergently grow up from the end of PS-2OH and the intermediate linear-dendrimer copolymers l-PS-d-PTP were formed. The GPC traces of the resulting copolymers from G1 to G4 are given in Figure 3. It should be noted that the traces of the resulting copolymers from G1 to G4 shift to the longer retention time region, which may attribute to that the affinity of the resulting copolymers with the column become stronger with increasing of the surface hydroxyl groups from G1 to G4. The 1H NMR spectra of l-PS-d-PTP from G1 to G4 are shown in Figure 4. As compared with the precursor PS-2OH, the newly appearing signals (d) at 5.16–5.38 ppm, (e) at 4.05–4.60 ppm, (f, i, j) at 3.49–3.90 ppm, and (h) at 2.71–3.10 ppm are ascribed to the methine protons (–CH(O–)CH2O–), methene protons (–CH(O–)CH2O–), methine and methene protons (–OC(=O)CH(CH3)SCH2CH(OH)CH2OH) and methene protons (–SCH2CH(O–)CH2(O–)–) of the growth units, respectively. Most importantly, the intensity of the signals corresponding to the growth units gradually increases from G1 to G4 in comparison with the phenyl rings of PS support. From the FT-IR spectra (Fig. 5), it is also observed that the intensity of the absorption peaks at 3745 cm−1 and 1704 cm−1 corresponding to the hydroxyl and carbonyl groups on dendritic PTP increases with increasing of the generation number. This
further confirms the successful formation of the intermediate copolymer I-PS-dPTP up to G4.

Cleavage of the alkoxyamine group connecting the linear PS and dendrimer at elevated temperature (125 °C) provided the targeted polyester dendrimer. In 1H NMR spectrum of dendrimer G4 (Fig. 6), it can be seen that the characteristic signals at 6.27 to 7.27 ppm belonging to phenyl-ring protons of PS support disappeared completely, indicating the PS was cleaved thoroughly from the dendrimer. The signals (a) at 1.04 ppm and (g, h) at 3.48 to 3.69 ppm are ascribed to the methyl protons of the TEMPO group and methine and methylene protons (–SCH2CH(OH)CH2OH) of the outer arms, respectively. Based on the integral ratio of the peaks (a) and (g, h), the molecular weight of the dendrimer G4 was obtained, which (4950 Da) is near to the theoretical value (5112 Da). The absolute molecular weight of G4-PTP was also determined by MALDI-TOF mass (Fig. 7). Unfortunately, the obtained spectrum is not a single peak, but a series of peaks with an interval value of 162 Da, which corresponds to the exact molecular weight of the thioglycerol-2-propionate repeat unit (–C(O)CH(CH3)SCH2CH(OH)CH2OH). The peak observed at 5265 corresponds to the structurally perfect dendrimer (C92H144NO94S30 + Ag: 5220). Other peaks correspond to dendrimers with the loss of one arm (162), two arms (2 × 162), three arms (3 × 162), etc. This phenomenon could be explained by this: the laser-mediated cleavage of ester bonds occurred during MALDI-TOF process. In one word, an efficient strategy for synthesis of polyester dendrimer was developed without help of tedious protection/dep-protection methods and column chromatographic purification.

CONCLUSIONS
In summary, we successfully developed an efficient synthesis approach to polyester dendrimer up to the fourth generation via combining thio-bromo “Click” chemistry with ATNRC. Unlike traditional methods, herein, the polyester dendrimer divergently grew up from the end groups of a linear polymer support. Due to the presence of the linear polymer support, the resulting intermediate, linear-dendritic copolymer, can be isolated from the excessive monomers by a simple precipitation, so it saves time, labour and solvent. The targeted dendrimer was separated from the linear polymer support just by cleavage of the alkoxyamine at elevated temperature. The good properties of biocompatibility and biodegradability will make this polyester dendrimer to be an attractive carrier for bioactive molecules. Meanwhile, the surface active hydroxyl groups can be further conjugated with some targeted molecules or hydrophilic polyethylene glycol (PEG) to facilitate its applications in biomedical areas.

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REFERENCES AND NOTES