Self-Assembly of Amphiphilic Polymeric Dendrimers Synthesized with Selective Degradable Linkages

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Received September 4, 2007; Revised Manuscript Received November 6, 2007

ABSTRACT: Enhancing the structural complexity and functionality of building blocks allows the design and synthesis of complex macromolecular architectures. In this work, we use a combination of atom transfer radical polymerization to produce polymers with well-defined chain length and telechelic end group functionality and "click" reactions to quantitatively couple these polymer chains together to form functional second- and third-generation dendrimers. Importantly, this methodology provides starting polymers in combination with linkers and end group protecting chemistries to design dendrimers with degradable linkages between the desired generations and incorporates functionality at the polymer chain ends of each generation. We have synthesized second- and third-generation homo- and amphiphilic diblock copolymer dendrimers and specifically designed third-generation dendrimers in pure form such that the peripheral generational layer could be selectively cleaved off from the second-generation. The degradation of the peripheral polymer layer is a useful feature in biomedical delivery devices for slow and controlled release of its payload. These dendrimers also have either "free" or protected hydroxyl groups on the peripheral ends, which are useful for further chemical modification or chemical coupling to important biomolecules. The amphiphilic dendrimers self-assemble in water to form well-defined micelles of near identical size (18.2 nm, PDI = 1.04), each consisting of approximately 19 individual dendrimers. The dense core of the spherical micelles found from sizing measurements supports the postulate that these amphiphilic dendrimers have no mutual interpenetration and thus pack uniformly to form the micelles.

Introduction

Amphiphilic block polymers can self-organize into micelles, vesicles, or rods depending on the type of solvent and weight fraction of the polymer.[1−4] When these blocks are dispersed in water, they self-assemble to form micelles with a core−shell morphology, in which the core consists of the hydrophobic block and the shell consists of the hydrophilic block. Nanoscale micelles of this type have a potential for drug and vaccine delivery devices and can deliver and release water insoluble drugs or molecules in a controlled way.[5] However, control of their size and size distribution is difficult and is affected by the size and number of hydrophilic arms, temperature, concentration, and mechanical shear. Newkome et al.[6] proposed that single amphiphilic macromolecules could form "unimolecular micelles", in which each macromolecule becomes an individual and well-defined micelle. Dendrimers have been proposed as the optimum design to form unimolecular micelles due to the high number of hydrophilic branch sites per molecule. Ideally, these dendrimers should consist of layers in which the internal polymers are hydrophobic in nature and the outer polymers are hydrophilic.

The revolution of 'living' radical polymerization has led to the design and synthesis of complex dendrimer-like polymer architectures.[7,8] Asymmetric stars and miktoarm stars have been synthesized by atom transfer radical polymerization (ATRP).[9,10] The same methodology was used to produce polystyrene (PSTY) second- and third-generation dendrimers, and it was found that the intrinsic viscosities of these polymers were similar to those of regular dendrimers.[11] Dendritic copolymers of PSTY in the core and poly(ethylene oxide)[12] or poly(acrylic acid) (PAA)[13] at the corona were also synthesized using this methodology. However, "living" radical polymerizations using metal-catalyzed or ATRP methods in the production of complex architectures lead to a high concentration of side products formed through bimolecular radical termination, which increases with the increasing number of arms (or potential propagating chains) on the dendrimer, and is unavoidable. Therefore, new strategies need to be developed to make the next generation of complex polymer architectures.

Recently, a new methodology using a combination of ATRP and "click" reactions[14−21] was introduced and was found successful in the synthesis of complex polymer architectures, with the added advantage that star−star radical termination found in ATRP is avoided. There are reports using this methodology to synthesize 3- and 4-arm homopolymers[21,22] and miktoarm stars[20,23] and even second-generation polymeric dendrimers.[20,21] The click chemistry[24] approach of joining polymers together has several advantages: (i) click reactions can take place in the presence of other functional groups (e.g., alcohols, ketones, amines, etc.), (ii) it can be carried out in aqueous environments, (iii) the yields are close to quantitative, and (iv) the reactions are highly specific. The resulting triazole ring can also bind to biomolecules via hydrogen bonding or act as a ligand for metals. The first successful use of click chemistry was to make dendritic materials,[19] and it has since been used to make a wide range of materials[20,22,25−31] from dendronized linear polymers to comb polymers.[32,33]

The aim of this work is to use the ATRP−click methodology to design and synthesize complex macromolecular architectures of second- and third-generation polymeric dendrimers. Linear polymers with well-defined chain length and telechelic end group functionality (Scheme 1) will be synthesized by ATRP

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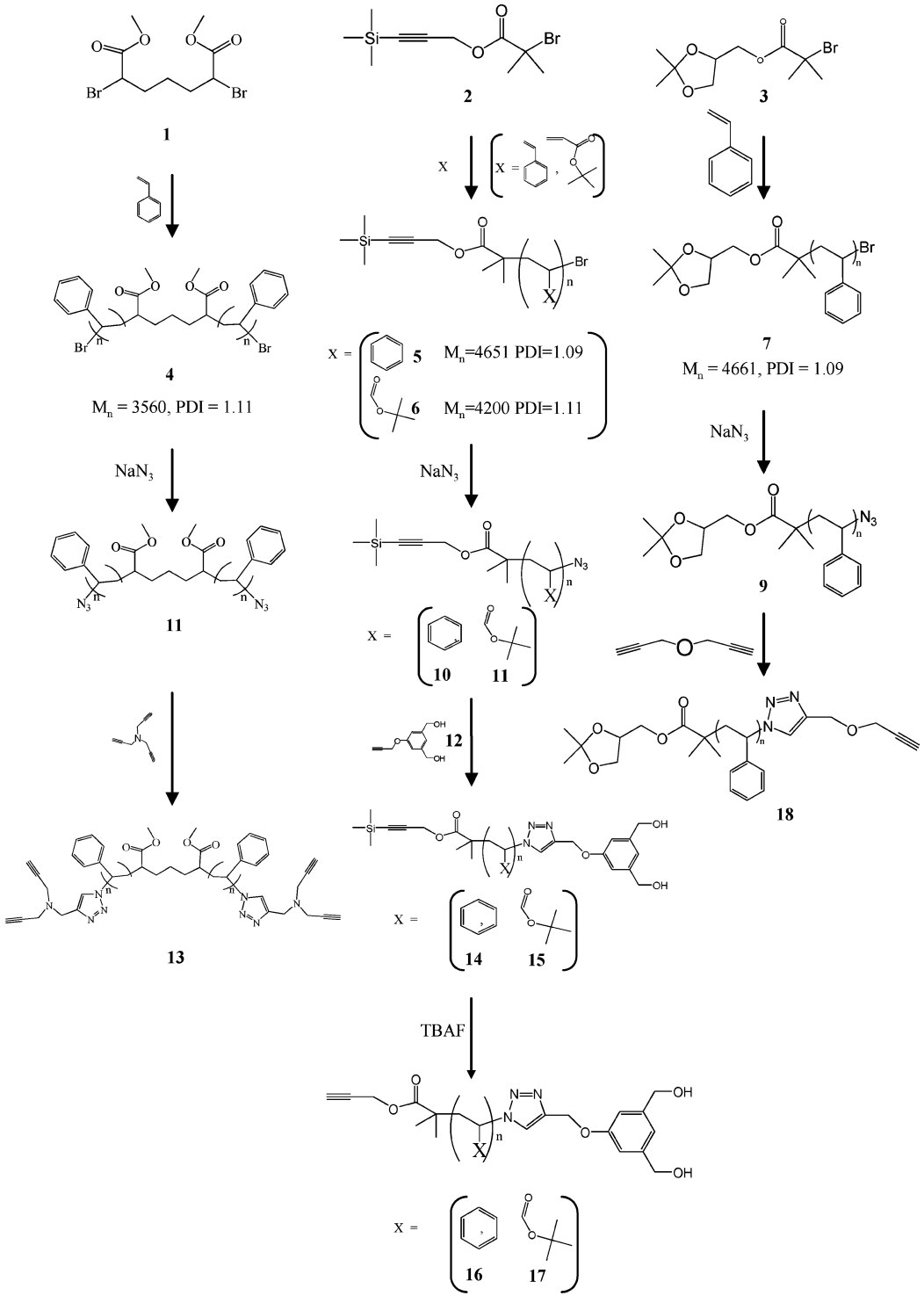
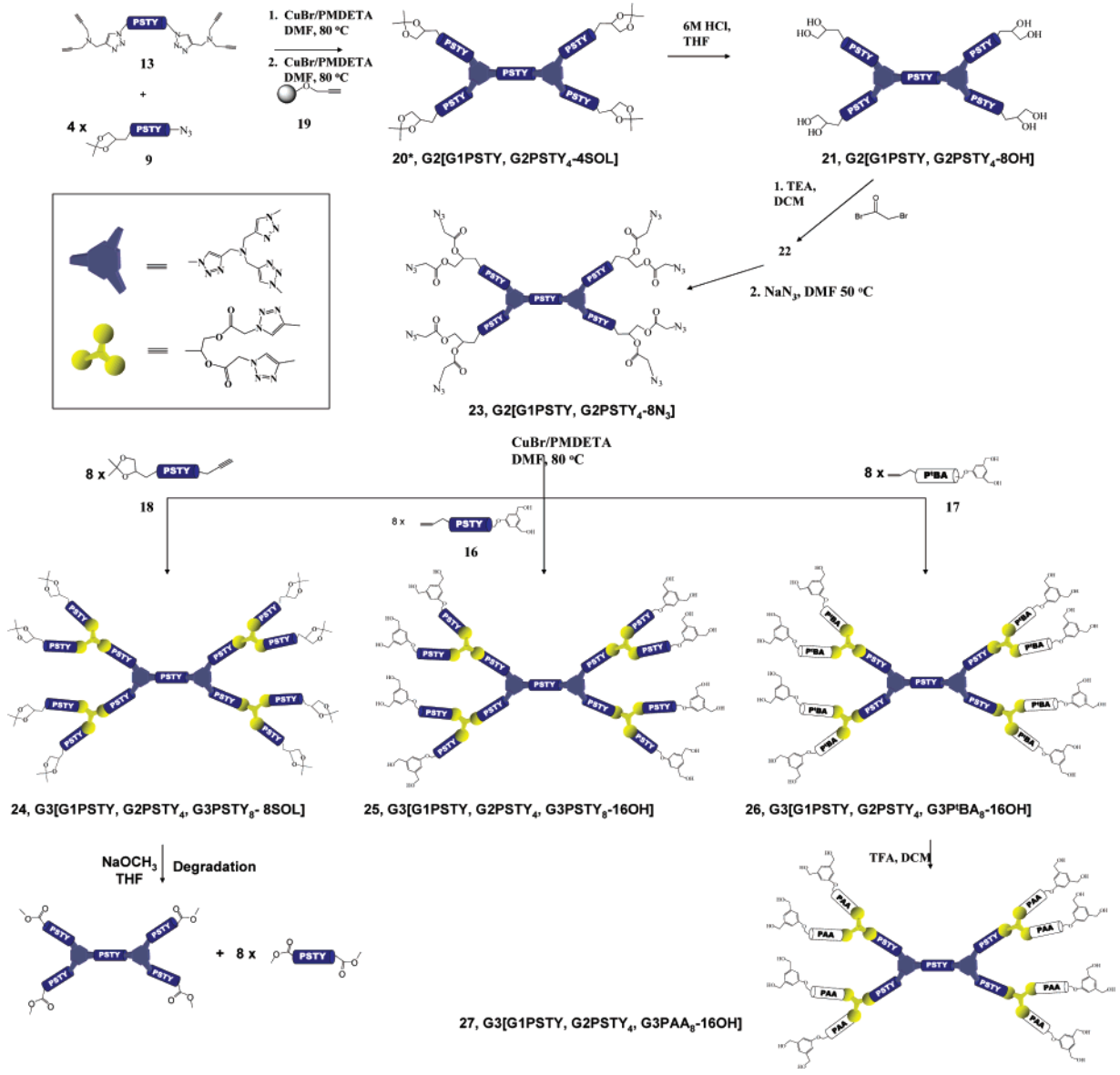
10.1021/ma701993w CCC: $40.75
Published on Web 12/12/2007

Scheme 1: Synthetic Methodology to Make Starting Polymers with Desired End Group Functionality



and then coupled together quantitatively using click chemistry to form functional third-generation dendrimers (Scheme 2). A range of dendrimers are synthesized with different functionalities using a toolkit of starting polymers and linkers, ranging from second- and third-generation homo- and amphiphilic diblock copolymer dendrimers (Scheme 2) and specifically designed third-generation dendrimers such that the peripheral generational layer could be selectively cleaved off from the second-generation. The degradation of the peripheral polymer layer is a useful feature in biomedical delivery devices that could be used to make important biomolecules available in targeted cells especially should slow release be required, as the lifetime of many cleavable groups are dependent on the chemical environment. These dendrimers also have either free or protected hydroxyl groups on the peripheral ends, which are useful for further chemical modification or chemical coupling, thus providing a strategy to custom design the dendrimer for a specific application. The novel amphiphilic dendrimer is micellized in water, and its size, shape, and aggregation number are determined using dynamic light scattering (DLS), transmission electron microscopy (TEM), and asymmetric field flow fractionation (AFFF).

Scheme 2: Synthesis of Second- and Third-Generation Polymeric Dendrimers with Selectively Cleavable Linkages



Experimental Section

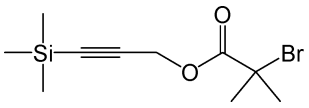
Materials. The inhibitor was removed from the following monomers prior to use by passing through a basic alumina column. 4-vinylbenzyl chloride (VBC, Aldrich, 97%), divinylbenzene (DVB, Aldrich, 80%, mixture of isomers, tech grade), styrene (STY, Aldrich, >99%), and *tert*-butyl acrylate (ᵗBA, Aldrich, >99%).

The following chemicals were used as received. 18-crown-6 (Aldrich, 99%), alumina, activated basic alumina (Aldrich, Brockmann I, standard grade, ~150 mesh, 58 Å), lithium aluminum hydride, powder ($LiAlH_4$, Aldrich, 95%), anhydrous magnesium sulfate ($MgSO_4$, Scharlau, extra pure), potassium carbonate (K_2CO_3, AnalaR, 99.9%), silica gel 60 (230−400 mesh, ATM (SDS)), sodium chloride (NaCl, Univar, 99.9%), sodium hydroxide (NaOH, Univar, AR grade), sodium methoxide ($NaOCH_3$, Aldrich, 95%), tetrabutyl ammonium fluoride hydrate (TBAF, Aldrich, 98%), triethylamine (TEA, Fluka, 98%), 2-bromoisobutyryl bromide (BIB, Aldrich, 98%), 3-(trimethylsilyl)-2-propyn-1-ol (Aldrich, 99%), dimethyl 5-hydroxyisophthalate (Aldrich, 98%), DL-1,2-isopropylideneglycerol (Solketal, Aldrich, 98%), bromoacetyl bromide (BAB, Fluka, 98%), propargyl alcohol (Aldrich, 99%), propargyl bromide solution (80 wt % in xylene, Aldrich), propargyl ether (Aldrich, 99%), sodium azide (NaN_3, Aldrich, ≥99.5%), and tripropargylamine (TPA, Aldrich, 98%).

The following solvents were used as received. Acetone (ChemSupply, AR), anisole (Fluka, 98%), chloroform ($CHCl_3$, Univar, AR grade), dichloromethane (DCM, Labscan, AR grade), diethylether (Univar, AR grade), ethanol (EtOH, ChemSupply, AR), ethyl acetate (EtOAc, Univar, AR grade), hexane (Wacol, technical grade, distilled), hydrochloric acid (HCl, Univar, 32%), methanol, anhydrous (MeOH, Mallinckrodt, 99.9%, HPLC grade), Milli-Q water (Biolab, 18.2 MΩm), N,N-dimethylformamide (DMF, Labscan, AR grade), and tetrahydrofuran (THF, Labscan, HPLC grade).

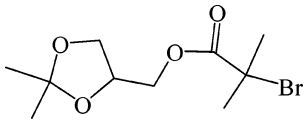
The following initiators, ligands, and metals for the various polymerizations were used as received unless otherwise stated. *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%), copper(I) bromide (CuBr, Aldrich, 99.999%), copper(II) bromide ($CuBr_2$, Aldrich, 99%) and dimethyl 2,6-dibromoheptanedioate (**1**, DMDBHD, Aldrich, 97%). 2,2-azobis(isobutyrylnitrile) (AIBN, Riedel-de Haën, 98%) was recrystallized from methanol before use.

Synthesis of Functional ATRP Initiators. *Synthesis of 3-(1,1,1-Trimethylsilyl)-2-propynyl 2-bromo-2-methylpropanoate* **2**. This compound was prepared according to the literature procedure.[34]



3-(Trimethylsilyl)-2-propyn-1-ol (2.0024 g, 0.0156 mol), TEA (2.2858 g, 0.0226 mol), and THF (20 mL) were added to a round-bottom flask and stirred at 0 °C under N_2. A solution of 2-bromoisobutyryl bromide (7.0308 g, 0.0306 mol) in THF (50 mL) was added dropwise over 1 h to the reaction mixture, and the contents were then stirred for 3 h further. The solvent was removed under rotary evaporation, and the residue taken up into diethylether (50 mL). The solution was filtered, and the organic layer washed with 10% HCl solution, brine, and Milli-Q water. The organic layer was then dried over $MgSO_4$, the solvent removed under rotary evaporation, and the resulting product was dried in vacuo. Purification was achieved with flash column chromatography (distilled hexane/ethyl acetate = 19:1). ^{1}H NMR ($CDCl_3$, δ): 0.164 (Si-(CH_3)$_3$), 1.935 (OC(=O)C(CH_3)$_2$), 4.745 (CH_2OC(=O)C). ^{13}C NMR ($CDCl_3$, δ): −0.38 (Si(CH_3)$_3$), 30.64 (OC(=O)C(CH_3)$_2$), 54.19 (CH_2OC(=O)C), 55.06 (OC(=O)C(CH_3)$_2$), 92.71 (SiC≡CCH$_2$OC(=O)), 98.15 (SiC≡CCH$_2$OC(=O)), 170.81 (OC(=O)C-(CH_3)$_2$). Anal. Calcd for $C_{10}H_{17}O_2SiBr$: C, 43.32; H, 6.18. Found: C, 43.29; H, 6.25.

Synthesis of 2,2-Dimethyl-1,3-dioxolane-4-methoxy-(2-bromo-2-methylpropionyl) **3**. This compound was prepared according to the literature procedure.[35]



DL-1,2-Isopropylideneglycerol (10.63 g, 0.080 mol), TEA (9.77 g, 0.097 mol), and THF (50 mL) were added to a round-bottom flask and stirred at 0 °C under N_2. A solution of 2-bromoisobutyryl bromide (23.064 g, 0.10 mol) in THF (100 mL) was added dropwise to the stirred solution over a 1 h period. The reaction mixture was stirred at room temperature for 3 h further. The solvent was removed under rotary evaporation and the residue taken up into diethylether (50 mL) and filtered. The organic layer was washed with a 10% HCl solution, brine, and Milli-Q water. The organic layer was dried over $MgSO_4$, the solvent removed under rotary evaporation, and the resulting product dried in vacuo. The product was used without further purification. ^{1}H NMR ($CDCl_3$, δ): 0.196 (Si(CH_3)$_3$), 1.350 (3H, s, CHCH$_2$OCCH_2), 1.425 (3H, s, CH$_2$CHOCCH_2), 1.930 (6H, s, OC(=O)C(CH_3)$_2$), 3.8−4.3 (5H, m).

Synthesis of Polymers using ATRP. *Synthesis of Br−PSTY−Br* **4**. Styrene (16.236 g, 0.156 mol), PMDETA (0.332 mL, 1.59 × 10^{-3} mol), and DMDBHD (**1**, 0.279 g, 8.1 × 10^{-4} mol) were added to a 50 mL Schlenk flask equipped with a magnetic stirrer. The solution was degassed by four freeze−pump−thaw cycles under high vacuum. The Schlenk flask was then flushed with high purity argon, and CuBr (0.114 g, 7.9 × 10^{-4} mol) was added carefully to the flask under a flow of argon. The polymerization was carried out in a temperature-controlled oil bath at 100 °C for 20 min. The polymerization was terminated by quenching in liquid nitrogen and then exposure to air. The mixture was diluted with THF, and the copper salts were removed by passage through an activated basic alumina column. The solution was concentrated by airflow, and the polymer recovered by precipitation into methanol. The recovered polymer was dried for 48 h under high vacuum at 25 °C. The polymer was characterized by SEC (M_n = 3560, PDI = 1.11).

Synthesis of TMS−≡−PSTY−Br **5**. Styrene (27.09 g, 0.26 mol), anisole (5 mL), **2** (0.60 g, 2.16 × 10^{-3} mol), $CuBr_2$/PMDETA complex (0.210 g, 5.3 × 10^{-4} mol), and PMDETA (0.45 mL, 2.15 × 10^{-3} mol) were added to a 50 mL Schlenk flask and degassed by purging with argon for 20 min. CuBr (0.373 g, 2.6 × 10^{-3} mol) was then added under an argon flow, and the polymerization was carried out at 80 °C for 2 h. The polymerization was terminated by quenching in liquid nitrogen and then exposure to air. The excess styrene was evaporated, and the residue was taken up into 200 mL $CHCl_3$. The solution was washed three times with water to remove the copper salts. The organic layer was dried with $MgSO_4$ and reduced in volume under a N_2 stream, and the polymer recovered by precipitation into methanol and collected by vacuum filtration. The molecular weight distribution was measured by SEC (M_n = 4650, PDI = 1.08).

Synthesis of TMS−≡−P^{t}BA−Br **6:** *tert*-butyl acrylate (8.83 g, 0.07 mol), PMDETA (0.12 g, 7.16 × 10^{-4} mol), $CuBr_2$ (0.02 g, 6.72 × 10^{-5} mol), **2** (0.32 g, 1.16 × 10^{-3} mol), and acetone (2.5 mL) were added to a 50 mL Schlenk flask equipped with a magnetic stirrer and was purged with N_2 for 15 min. CuBr (0.1 g, 6.81 × 10^{-4} mol) was then added under positive N_2 flow and the mixture purged with N_2 for another 10 min. The polymerization was carried out at 60 °C for 220 min. The polymerization was terminated by quenching in liquid nitrogen and then exposure to air. The polymerization mixture was taken up into 200 mL of $CHCl_3$ and washed three times with water to remove the copper salts. The organic layer was dried over $MgSO_4$, and the polymer recovered by removal of chloroform under vacuum. The polymer was dried for 24 h under vacuum at 25 °C. (SEC analysis: M_n = 4200, PDI = 1.11).

Synthesis of SOL−PSTY−Br **7**. Styrene (30.0 g, 0.288 mol), PMDETA (0.262 g, 1.5 × 10^{-3} mol), **3** (0.427 g, 1.5 × 10^{-3} mol), and preformed $CuBr_2$/PMDETA complex (0.061 g, 1.5 × 10^{-4} mol) were added to a 50 mL Schlenk flask equipped with a magnetic stirrer and was purged with N_2 for 20 min. CuBr (0.216 g, 1.5 × 10^{-3} mol) was then added under N_2 flow, and the flask sealed and purged with N_2 for another 5 min. The flask was placed in an oil bath at 80 °C for 2 h after which the polymerization was terminated by quenching in liquid N_2 and then exposure to air. The polymerization mixture was diluted with THF, and the copper salts were removed by passage through an activated basic alumina column. The solution was concentrated by airflow, and the polymer recovered by precipitation into methanol. The recovered polymer was dried for 48 h under high vacuum at 25 °C. The polymer was analyzed by SEC (M_n = 4660, PDI = 1.09).

Synthesis of Polymers with Azide Functionality. *Synthesis of N_3−PSTY−N_3* **8**. A typical azidation procedure was carried out as follows. Br−PSTY−Br (**4**, 2.0 g, 0.56 mmol) was dissolved in 20 mL of DMF. NaN_3 (0.365 g, 5.6 mmol) was added, and the mixture stirred for 24 h at 50 °C. The polymer was precipitated into MeOH, recovered by vacuum filtration, and washed exhaustively with water and MeOH. The polymer was dried under vacuum for 48 h at 25 °C.

Similarly SOL−PSTY−N_3 (**9**) was prepared from SOL−PSTY−Br (**7**) using the same procedure.

Synthesis of TMS−≡−PSTY−N_3 **10:** A typical azidation procedure was carried out as follows. TMS−≡− PSTY−Br (**5**, 2.0 g, 4.00 × 10^{-4} mol) was dissolved in DMF (15 mL). NaN_3 (0.109 mg, 8.68 × 10^{-4} mol) was added and the mixture stirred for 24 h at room temperature. The polymer was precipitated in MeOH, then recovered by vacuum filtration, and washed exhaustively with water and MeOH. The polymer was dried for 48 h under vacuum at 25 °C.

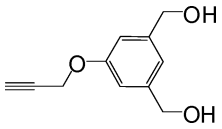
TMS−≡−P^{t}BA−Br (**6**) was azidated using the same procedure as above but was purified by dilution into chloroform and washing three times with water. The chloroform was dried over anhydrous $MgSO_4$, and the organic layer removed under vacuum. The polymer was allowed to dry for 24 h at 25 °C under vacuum to give the azidated polymer TMS−≡−P^{t}BA−N_3 (**11**).

Synthesis of Polymers with Alkyne Functionality. *Synthesis of the Propargyl Ether of Dimethyl 5-Hydroxyisophthalate*. This compound was prepared according to the literature procedure.[36]

A two-neck 1 L round-bottom flask was charged with dimethyl 5-hydroxyisophthalate (10.0 g, 47.6 × 10^{-3} mol), acetone (200 mL), K_2CO_3 (7.9 g, 57 × 10^{-3} mol), 18-crown-6 (0.13 g, 4.9 × 10^{-4} mol), and propargyl bromide (80 wt %) in xylene (6.3 mL, 57 × 10^{-3} mol). The reaction mixture was refluxed overnight under N_2 with stirring. After the reaction mixture was cooled to room temperature, it was filtered and the filter cake was washed with 50 mL of acetone. The filtrate was concentrated by rotary evaporation. The residue was recrystallized in ethanol and dried under vacuum. Isolated yield (10.45 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.56 (t, J = 2 Hz, 1H, CH$_2$CtCH), 3.95 (s, 6H,

COOCH_3), 4.79 (d, $J = 2$ Hz, 2H, CH_2Ct CH), 7.84 (d, $J = 2$ Hz, 2H, Ar*H*), 8.34 ppm (t, $J = 2$ Hz, 1H, Ar*H*).

Synthesis of 1-Propargylbenzene-3,5-dimethanol ***12***.



Prior to use, all glassware and the magnetic stir bar were dried in an oven (110 °C) for 1 h. A solution of the propargyl ether of dimethyl 5-hydroxyisophthalate (10.10 g, 0.04069 mol) in THF (100 mL) was added dropwise into a flame-dried two-neck 1 L round-bottom flask placed in an ice bath and containing a cold slurry of $LiAlH_4$ (5.80 g, 0.153 mol) in THF (400 mL). The reaction mixture was refluxed with stirring under N_2 for 18 h. A saturated aqueous solution of NH_4OH was added until no more H_2 gas was observed, and then the mixture was diluted with aqueous HCl (10%) until the pH reached 7. The reaction mixture was filtered, the filter cake was washed with THF, and the filtrate was concentrated under rotary evaporation. The resulting solid was recrystallized in EtOAc: hexane (1/1). Isolated yield was 5.82 g (75%). ^{1}H NMR (300 MHz, CD_3OD, δ): 2.92 (t, $J = 2$ Hz, 1H, $CH_2C{\equiv}CH$), 4.58 (s, 4H, CH_2-OH), 4.73 (d, $J = 2$ Hz, 2H, $CH_2C{\equiv}CH$), 6.89 (d, $J = 2$ Hz, 2H, ArH), 6.96 ppm (t, $J = 2$ Hz, 1H, ArH). Anal. Calcd for $C_{11}H_8O_3$: C, 68.74; H, 6.29. Found: C, 68.25; H, 6.21.

Synthesis of (≡—)$_2$PSTY(—≡)$_2$ ***13***. N_3—PSTY—N_3 (**8**, 0.5 g, 1.40 $\times 10^{-4}$ mol), PMDETA (0.587 mL, 2.81×10^{-3} mol), TPA (0.791 mL, 5.60×10^{-3} mol), and DMF (5 mL) were added to a 10 mL Schlenk flask equipped with a magnetic stirrer. The solution was purged with N_2 for 10 min and then CuBr (0.403 g, 2.81×10^{-3} mol) was carefully added under a positive flow of N_2 and purged with N_2 for another 5 min. The flask was placed in a temperature-controlled oil bath at 80 °C for 2 h. The reaction mixture was diluted with 5 mL of THF and passed through activated basic alumina to remove the copper salts. The polymer was precipitated in MeOH, filtered, and dried for 24 h under vacuum.

Synthesis of TMS—≡—PSTY(OH)$_2$ ***14***. TMS—≡—PSTY—N_3 (**10**, 1.0 g 2.00×10^{-4} mol), PMDETA (0.035 g, 2.00×10^{-4} mol), and **12** (0.156 g, 8×10^{-4} mol) in DMF (5 mL) were added to a 10 mL Schlenk flask equipped with a magnetic stirrer. The solution was purged with nitrogen for 10 min and CuBr (0.0286 g, 2.00×10^{-4} mol) was added under a positive N_2 flow. The mixture was purged with N_2 for another 10 min and stirred at 80 °C for 60 min. The flask was opened, the reaction mixture diluted with chloroform (100 mL), and the organic layer extracted three times with water. The organic layer was concentrated under airflow, and the polymer was precipitated into methanol, recovered by filtration, and washed with MeOH. The polymer was dried for 48 h under vacuum at 25 °C.

The polymer TMS—≡—P^{t}BA(OH)$_2$ (**15**) was prepared in a similar manner but using TMS—≡—P^{t}BA—N_3 (**11**) as the starting telechelic polymer.

Synthesis of ≡—PSTY(OH)$_2$ ***16***. TMS—≡—PSTY(OH)$_2$ (**14**, 0.5 g, 9.03×10^{-5} mol) was dissolved into THF (5 mL). Tetrabutyl ammonium fluoride hydrate (TBAF, 0.236 g, 9.03×10^{-4} mol) was added, and the solution was stirred overnight at 25 °C. The polymer was recovered by precipitation into MeOH and dried for 24 h under vacuum at 25 °C. The complete removal of the TMS protecting group was confirmed by ^{1}H NMR.

The polymer ≡—P^{t}BA(OH)$_2$ (**17**) was prepared in a similar manner but using TMS—≡—P^{t}BA(OH)$_2$ (**15**) as the starting polymer.

Synthesis of SOL—PSTY(—≡) ***18***. SOL—PSTY—N_3 (**9**, 0.501 g, 9.7×10^{-5} mol), propargyl ether (0.210 mL, 2.04×10^{-3} mol), PMDETA (0.035 mL, 1.67×10^{-4} mol), and DMF (5 mL) were added to a 10 mL Schlenk flask and purged with N_2 for 10 min. CuBr (0.0218 g, 1.52×10^{-4} mol) was added under a positive flow of N_2 and the flask was sealed and purged with N_2 for another 5 min. The flask was then placed in a temperature-controlled oil bath set at 80 °C for 2h. The polymer mixture was diluted with THF, and the copper salts were removed by passage through an activated basic alumina column. The solution was concentrated by airflow, and the polymer recovered by precipitation into methanol. The polymer was dried under vacuum at 25 °C.

Synthesis of Functionalized Cross-Linked Solid Supports[21]. *Synthesis of 4-Vinylbenzyl chloride Cross-Linked Solid Supports*. 4-Vinylbenzyl chloride (4 mL, 0.028 mol), styrene (3.2 mL, 0.028 mol), divinylbenzene (0.120 mL, 8.42×10^{-4} mol), and AIBN (6.9 mg, 4.19×10^{-5} mol) were added to a 20 mL glass vial equipped with a magnetic stirrer and sealed with rubber septa. The mixture was purged with N_2 for 10 min then heated in a temperature-controlled oil bath set at 50 °C for 24 h. The cross-linked polymer was ground to a fine powder with mortar and pestle then stirred in 50 mL of DMF at 50 °C for 1 h. The mixture was filtered hot, and this washing procedure was repeated twice. The polymer was then filtered and washed with DMF and then acetone. The functional cross-linked polymer was then dried under high vacuum for 16 h.

Synthesis of Alkyne Functionalized Cross-Linked Solid supports **(19)**. Propargyl alcohol (4.9 mL, 0.087 mol), NaOH (0.07 g, 0.017 mol), and DMF (40 mL) were added to a 50 mL round-bottom flask. The mixture was heated under nitrogen at 40 °C. After 20 min, 4-vinylbenzyl chloride cross-linked solid supports (4 g) was added, and the mixture was stirred for 24 h. The reaction mixture was filtered hot and washed with water and then acetone. The functionalized cross-linked polymer was then stirred in 50 mL of DMF at 90 °C for 30 min after which the mixture was filtered hot, and this washing procedure was repeated twice. Finally, the polymer was filtered and washed with DMF and then acetone. The polymer was then dried under high vacuum.

Synthesis of Dendrimers. *Synthesis of G2[G1PSTY, G2PSTY$_4$, 4SOL]* ***20****. (≡—)$_2$PSTY(—≡)$_2$ (**13**, 0.1 g, 2.5×10^{-5} mol), SOL—PSTY—N_3 (**9**, 0.512 g, 1.1×10^{-4} mol), PMDETA (0.209 mL, 1.0×10^{-3} mol), and 5 mL of DMF were added to a 10 mL Schlenk flask. The solution was purged with N_2 for 10 min after which CuBr (0.148 mg, 1.03×10^{-3} mol) was carefully added under a positive flow of N_2, and the contents were further purged with N_2 for 5 min. The flask was placed in a temperature-controlled oil bath at 80 °C for 2 h. The reaction was diluted with 5 mL of THF and then passed through activated basic alumina to remove the copper salts to give the dendrimer.

THF was removed by evaporation, and the polymer and residual DMF were added to a 10 mL Schlenk flask equipped with a magnetic stirrer. PMDETA (0.057 mL, 0.27×10^{-4} mol) and **19** (0.18 g) were added to the flask and the mixture purged with N_2 for 10 min. CuBr (0.036 g, 2.5×10^{-4} mol) was then carefully added under a positive flow of N_2 and the solution further purged with N_2 for 5 min. The flask was placed in a temperature-controlled oil bath set at 80 °C for 4 h. The reaction was filtered hot through a fine glass frit and the solid support washed with 10 mL of THF. The filtrate was passed through activated basic alumina to remove the copper salts, and the polymer **20*** was precipitated in MeOH and then filtered and dried for 24 h under vacuum.

Synthesis of G2[G1PSTY, G2PSTY$_4$, 8OH] **21**. **20*** (0.55 g, 2.4×10^{-5} mol) was dissolved in 50 mL of THF in a 100 mL conical flask equipped with a magnetic stirrer. A portion of 6 M HCl (1—2 mL) was added dropwise to the solution over a period of 5 min while maintaining the solubility of the polymer. The mixture was allowed to stir for 6 h at room temperature. The polymer was precipitated in MeOH, filtered, and dried for 24 h under vacuum.

Synthesis of G2[G1PSTY, G2PSTY$_4$, 8Br] **22**. **21** (0.5 g, 2.2×10^{-5} mol), TEA (0.026 mL, 1.9×10^{-4} mol), and 10 mL of dry DCM were added to a 50 mL round-bottom flask equipped with a stirrer bar and pressure equalizing dropping funnel under N_2. BAB (0.8 g, 8.8×10^{-4} mol) in 5 mL of dry DCM was added dropwise to the stirred mixture over 10 min at room temperature. After complete addition, the mixture was further stirred for 16 h at room temperature. The polymer was precipitated in MeOH, filtered, and washed 3 times with MeOH. The recovered polymer was dried for 24 h under vacuum.

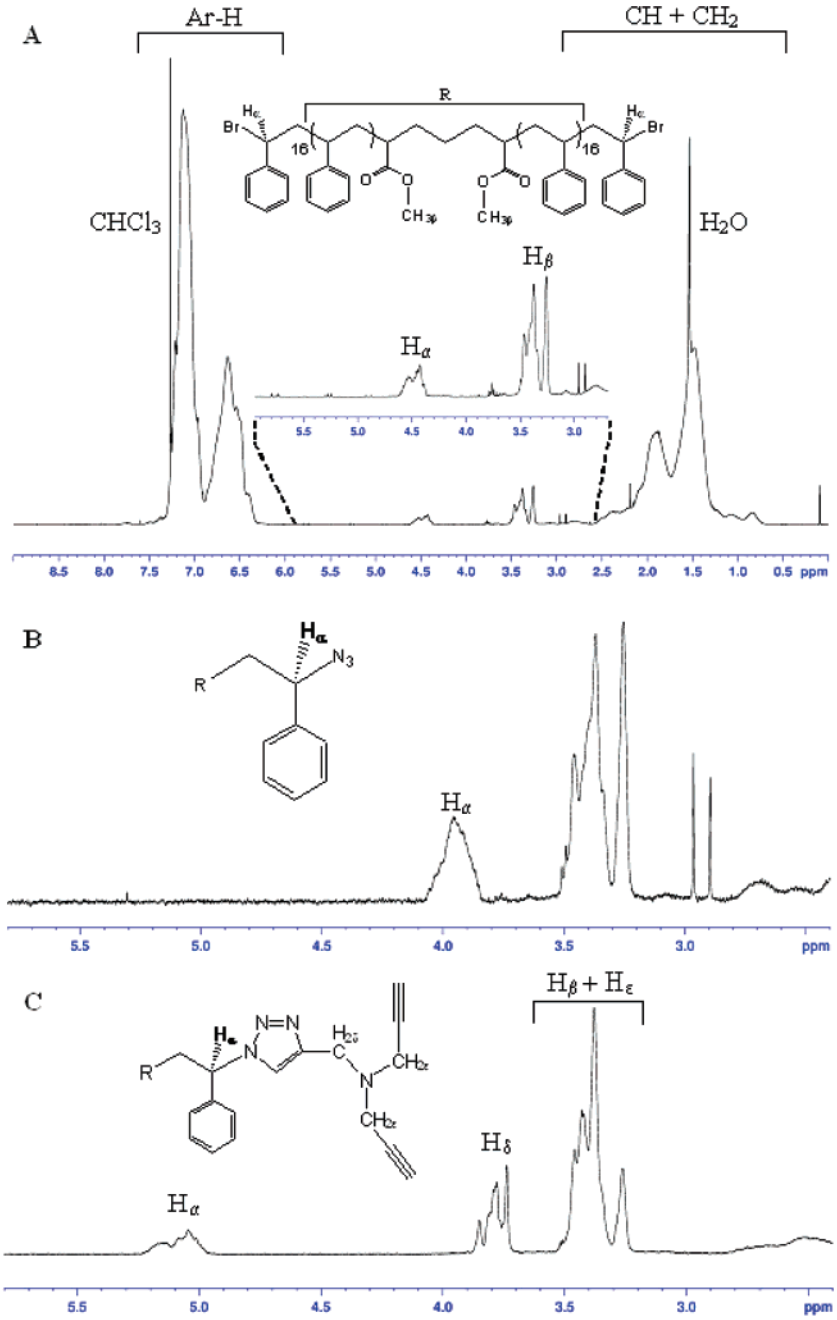


Figure 1. 500 MHz ^{1}H NMR of (A) Br−PSTY−Br **4** (H_α located at 4.5 ppm), (B) N_3−PSTY−N_3 **8** (H_α shifts to 4.0 ppm), and (C) $(\equiv\!-)_2$N−PSTY−N$(-\!\equiv)_2$ **13** (H_α shifts again to 5.1 ppm and additional peaks appear at 3.4 and 3.8 ppm).

Synthesis of G2[G1PSTY, G2PSTY$_4$, 8N$_3$] **23**. **22** (0.48 g, 2.1 × 10^{-5} mol) was dissolved in 5 mL of DMF. NaN_3 (0.108 g, 1.67 × 10^{-3} mol) was added and stirred for 24 h in a temperature-controlled oil bath at 50 °C. The polymer was precipitated in MeOH, filtered, and dried under vacuum for 48 h at 25 °C.

Synthesis of G3[G1PSTY, G2PSTY$_4$, G3PSTY$_8$, 8SOL] **24**. **23** (4.8 mg, 2.1 × 10^{-7} mol), SOL−PSTY(−≡) (**18**, 8.6 mg, 1.8 × 10^{-6} mol), PMDETA (3.5 μL, 1.7 × 10^{-5} mol), and 0.5 mL of DMF were added to a 10 mL Schlenk flask equipped with a magnetic stirrer and purged with N_2 for 10 min. CuBr (2.4 mg, 1.7 × 10^{-5} mol) was then carefully added while maintaining a positive flow of N_2. The solution was further purged with N_2 for 5 min. The flask was placed in a temperature-controlled oil bath at 80 °C for a period of 2 h. The reaction was diluted with 5 mL of THF then passed through activated basic alumina to remove the copper salts. THF was removed by evaporation, and the polymer and residual DMF were added to a 10 mL Schlenk flask equipped with magnetic stirrer.

This procedure was repeated for the synthesis of G3[G1PSTY, G2PSTY$_4$, G3PSTY$_8$, 16OH] (**25**) and G3[G1PSTY, G2PSTY$_4$, G3P^{t}BA$_8$, 16OH] (**26**) using ≡−PSTY(OH)$_2$ (**16**) and ≡−P^{t}BA-(OH)$_2$ (**17**), respectively.

Degradation of G3[G1PSTY, G2PSTY$_4$, G3PSTY$_8$, 8SOL] ***24***. To a 250 μL aliquot of the reaction mixture from the synthesis of G3[G1PSTY, G2PSTY$_4$, G3PSTY$_8$, 8SOL] (**24**) was added THF (1 mL) and $NaOCH_3$ (10 mg, 1.85 × 10^{-4} mol). The mixture was stirred at room temperature for 16 h, diluted, and analyzed by SEC.

Synthesis of G3[G1PSTY, G2PSTY$_4$, G3PAA$_8$, 16OH] ***27***. G3-[G1PSTY, G2PSTY$_4$, G3P^{t}BA$_8$, 16OH] (**26**) (15 mg, 7.8 × 10^{-5} mol ^{t}BA groups) was dissolved in 0.5 mL of DCM. TFA (44 mg, 3.9 × 10^{-4} mol) was added and the solution was stirred overnight at 25 °C after which the solution was dried under a nitrogen stream. The material was further dried for 48 h at 25 °C in a high vacuum oven. Hydrolysis of the ^{t}BA groups was confirmed by the loss of the *tert*-butyl groups in the ^{1}H spectrum at a chemical shift of 1.35 ppm.

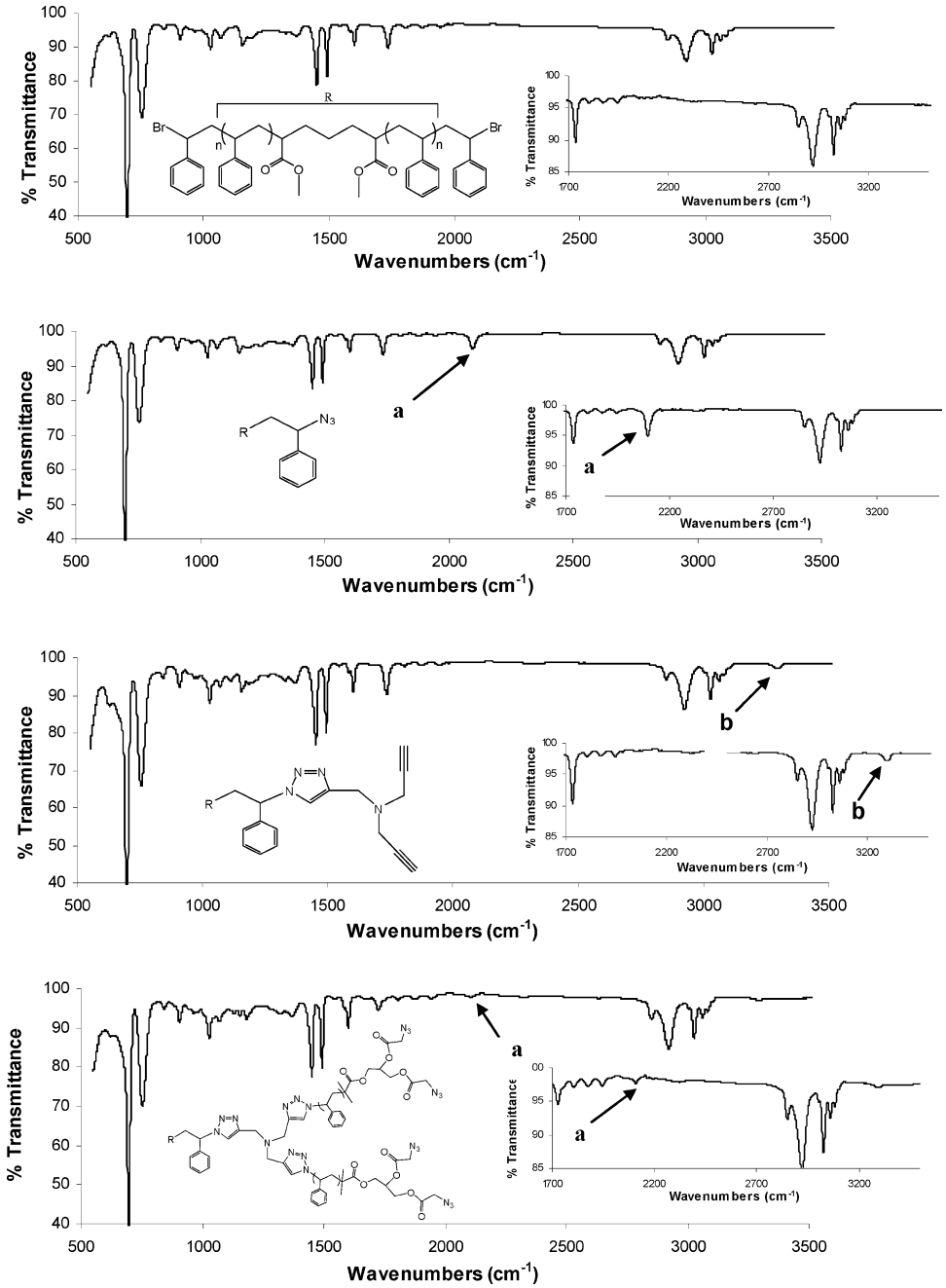


Figure 2. ATR-FTIR of the intermediate products to generate polymer chains with bromine, azido, and alkynyl end groups (a corresponds to the azide stretch and b corresponds to the alkyne stretch on the panels).

Table 1. SEC Data for the Synthesis of Third-Generation Dendrimers Using ATRP and Click Reactions

starting linear and dendritic polymers		polymeric dendrimer	M_n	PDI	M_p	$M_{p,theory}$[e]	yield[a] (%)	purity[b] (%)
13	**9**	**20**	10 582	1.64	22 200	23 460		72
(M_n = 3560, PDI = 1.11)	(M_n = 4660, PDI = 1.09)	**20***[c]	12 450	1.51	21 880	23 460		84
23	**18**	**24**	13 521	2.02	36 745	62 500	85	71
(M_n = 18 865, PDI = 1.09)	(M_n = 4660, PDI = 1.09)							
23	**16**	**25**	12 224	2.41	43 900	62 820	89	73
(M_n = 18 865, PDI = 1.09)	(M_n = 4650, PDI = 1.08)							
23	**17**	**26**	10 823	2.36	36 513	59 060	82	65
(M_n = 18 865, PDI = 1.09)	(M_n = 4200, PDI = 1.11)	**26**[f][d]	31 940	1.14	37 725	59 060		~100

[a] Yield is conversion of **23**. [b] Purity is the percentage of the product in the polymer mixture. [c] **20*** is product after reaction of **20** with reactive solid support **19**. [d] **26**[f] is after the purification of **26** by SEC. [e] $M_{p,theory}$ is greater than M_p from experiment due to hydrodynamic volume differences between linear PSTY standards and star dendrimers.

Analytical Methods. *^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR).* All NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer using an external lock ($CDCl_3$) and utilizing a standard internal reference (solvent reference). ^{13}C NMR spectra were recorded by decoupling the protons and all chemical shifts are given as positive downfield relative to these internal references.

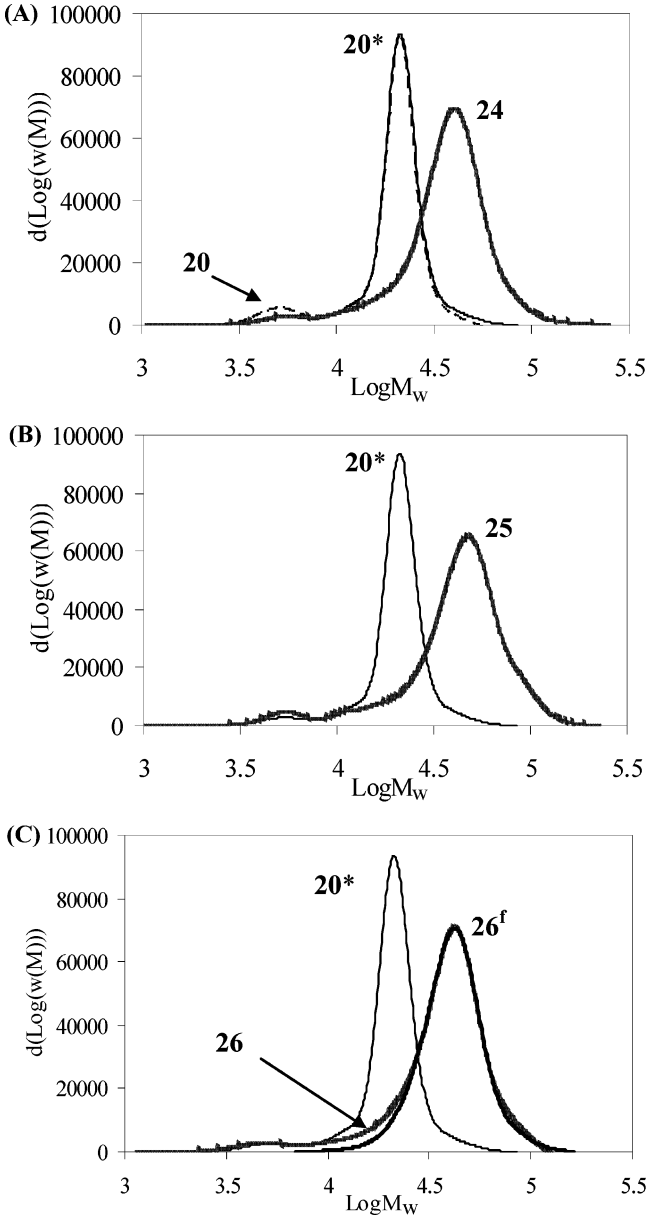


Figure 3. Size exclusion chromatograms using refractive index detection: (A) G2[G1PSTY, G2PSTY$_4$, 4SOL] (**20** and **20***) and G3-[G1PSTY, G2PSTY$_4$, G3PSTY$_8$, 8SOL] (**24**); (B) G3[G1PSTY, G2PSTY$_4$, G3PSTY$_8$, 16OH] (**25**), and (C) G3[G1PSTY, G2PSTY$_4$, G3P^{t}BA$_8$, 16OH] (**26**). (* represnets after reaction with alkyne functionalized cross-linked solid supports.)

Attenuated Total Reflectance-Fourier Transform Spectroscopy (ATR-FTIR). ATR-FTIR spectra were recorded between 4000 and 550 cm^{-1} in a Perkin-Elmer FT-2000 FTIR spectrometer equipped with a single reflection diamond window. Each spectrum had a 32 scan accumulation using a spectral resolution of 8 cm^{-1}.

Size Exclusion Chromatography (SEC). The molecular weight distributions of the polymers were measured by SEC. All polymer samples were dried prior to analysis in a vacuum oven for 2 days at 40 °C. The dried polymer was dissolved in THF (Labscan, 99%) to a concentration of approximately 1 mg/mL. This solution was then filtered through a 0.45 μm PTFE syringe filter. Analysis of the molecular weight distributions was accomplished by using a Waters 2695 separations module fitted with two Ultrastyragel linear columns (7.8 × 300 mm) in series. These columns were held at a constant temperature of 35 °C for all analyses. The columns used separate polymers in the molecular weight range of 500−2 000 000 g/mol with high resolution. THF was used as the eluent under a flow rate of 1.0 mL/min. Calibration was carried out using narrow molecular weight PSTY standards (PDI < 1.1) ranging from 500−2 000 000 g/mol. Data acquisition was performed using Waters Millenium software (version 3.05.01) and molecular weights were calculated by using a fifth-order polynomial calibration curve.

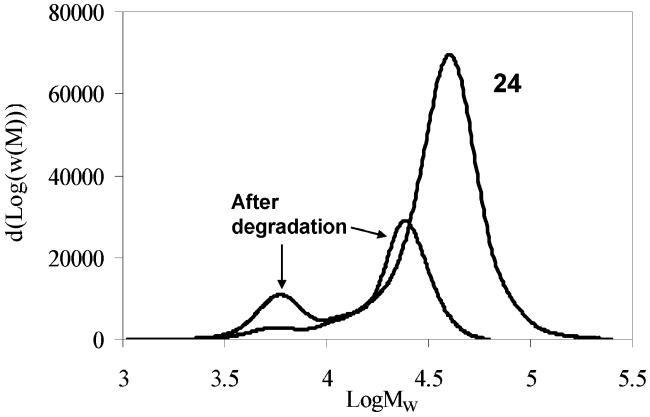


Figure 4. Size exclusion chromatograms using refractive index detection of G3[G1PSTY, G2PSTY$_4$, G3PSTY$_8$, 8SOL] (**24**) and the dendrimer after degradation with $NaOCH_3$.

Table 2. Characterization of G3[G1PSTY, G2PSTY$_4$, G3PAA$_8$, 16OH] (27) Amphiphilic Diblock Copolymer Micelles in DMF and Water

amphiphilic dendrimer (**27**, G3[G1PSTY, G2PSTY$_4$, G3PAA$_8$, 16OH)	
M_w from SEC	42 400
D_H of micelles in DMF [a]	4.2 nm
D_H of micelles in water [a]	18.2 nm
R_g of micelles in water [b]	11 nm
M_w of micelle in water [b]	7.90 × 10^5
PDI of micelles in water [b]	1.04
Z, aggregation number [b]	19

(a) determined by dynamic light scattering (DLS), and (b) determined by Asymmetric Field Flow Fractionation (AFFF).

Micellization of Polymer in Water. Hydrolyzed dendrimer (**27**, G3[G1PSTY,G2PSTY$_4$,G3PAA$_8$-16OH], 5 mg) was dissolved in DMF (1.05 mL), a common solvent for both PSTY and PAA blocks, resulting in 0.5% w/w polymer concentration. Sodium hydrogen carbonate (mol equiv to AA groups) was added to the solution and was stirred for 24 h. The solution was filtered through a 0.45 μL filter, and micelles were obtained by the gradual addition (0.013 mL/min) of the water (10 mL), a nonsolvent for the hydrophobic polystyrene blocks. The micelles were then transferred to a presoaked and rinsed dialysis bag (Pierce Snakeskin, MWCO 10 K) and dialyzed against a large volume of weak $NaHCO_3$ solution (pH 7.5) in MilliQ water for 3 days to remove the organic solvent.

Dynamic Light Scattering (DLS). Dynamic light scattering measurements were performed using a Malvern Zetasizer Nano Series running DTS software and by operating a 4 mW He−Ne laser at 633 nm. Analysis was performed at an angle of 90° and a constant temperature of 25 °C.

Asymmetric Field-Flow Fractionation (AFFF): Weight-average molecular weights (M_w) and the radius of gyration (R_g) of the polymer micelles were determined in Millipore water pH = 6.8 using an AFFF instrument equipped with a Wyatt DAWN EOSP multiangle laser light scattering (MALLS) detector, a Wyatt OPTILAB rEX refractive index detector, and Aligent Technologies G1314A UV detector. Flow control and sample injection were controlled with an Aligent Technologies G1310A pump and G1329A autoinjector. Separation was achieved using an AFFF membrane (Eclipse 2, Wyatt Technology). The light scattering data were analyzed using the Zimm method fitting a first-order polynomial with Astra version 5.1.9.1 software. Estimates of *dn*/*dc* used in the calculation of the apparent molecular weight of the micelles were determined via the Astra software using the quantitative mass recovery technique. Both the number average *R*g and M_w^{app} are reported.

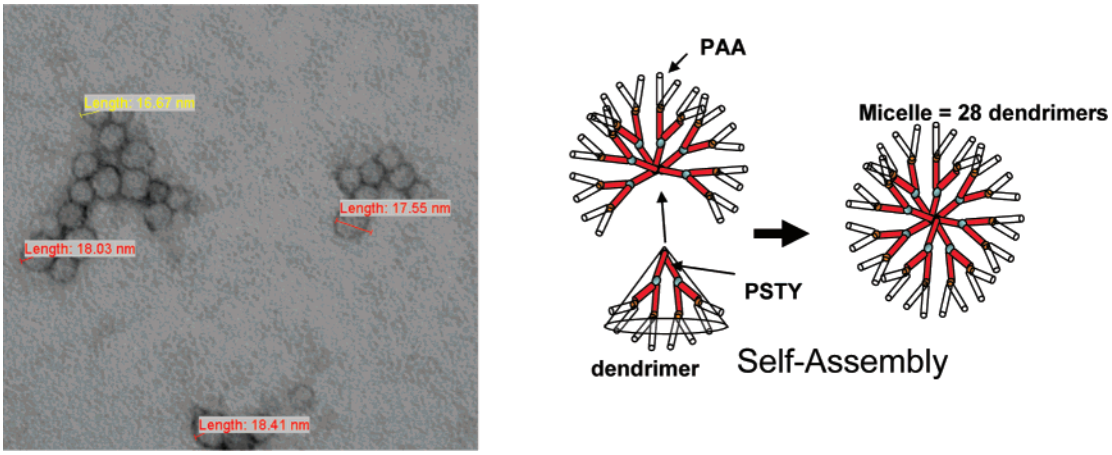


Figure 5. TEM of the self-assembly of the dendrimer **27** (G3[G1PSTY, G2PSTY$_4$, G3PAA$_8$,16OH]) in water, and representation of the self-assembly process of each dendrimer to form a spherical micelle.

Transmission Electron Microscopy (TEM): A drop of the micelle solution was allowed to air-dry onto a Formavar precoated copper TEM support grid. To obtain a negative stain, the samples were exposed to a drop of a 1% solution of uranyl acetate for 1 min after which excess staining solution was removed via careful blotting. The micelles were characterized on a Jeol-1010 instrument utilizing an accelerating voltage of 80 kV at ambient temperature.

Results and Discussion

Polymerization of **1** with styrene in the presence PMDETA and CuBr at 100 °C for 20 min produced well-defined linear polystyrene, **4** (Scheme 1). The number-average molecular weight (M_n) was kept low (~3500) with a narrow distribution (PDI of 1.11). The bromine end groups were converted to azides with NaN_3 to form **11** and functionalized through a click reaction with tripropagylamine to give the corresponding dialkynyl functional linear polymer **13**. Figure 1 shows the ^{1}H NMR spectra of the starting compound **4**, its conversion from bromine end groups to azide groups (**8**), and its subsequent reaction with tripropagylamine to form **13** (as shown in Scheme 1). Figure 1A shows the loss of protons (H_α) at 4.5 ppm upon conversion to azides (H_α at 3.9 ppm, Figure 1B). The loss of protons at 3.9 ppm was observed after the reaction of **8** with tripropagylamine to form **13** (H_α at 5.1 ppm, Figure 1C). All chemical shifts correspond to literature assignments.[22] These results suggest that all end groups have been near quantitatively converted to the corresponding desired functionality. The ATR FT-IR spectra of the same polymers (Figure 2) show the characteristic azide and alkyne stretches at 2094 and 3294 cm^{-1}, respectively, and although not quantitative this does support the conclusions based on the NMR results. Polymer **13** was used in all subsequent click reactions to make second- and third-generation dendrimers.

Dendrimers with desired end groups (i.e., protected or free hydroxyl groups) on the periphery and degradable linkages between the second- and third-generation were synthesized using linear polymers made from specialized ATRP initiators **2** (TMS protected alkyne) and **3** (solketal groups that can be hydrolyzed to alcohols). The resulting linear polymers were designed to have M_n's below 5000 and PDIs close to 1.1 and were used as the starting polymer building blocks to make dendrimers with diverse chemical composition and architecture (Scheme 2). A polystyrene second-generation dendrimer with four solketal groups on the chain ends was formed through the reaction of **13** with **9** to produce **20** (G2[G1PSTY, G2PSTY$_2$, 4SOL]) in 72% purity (Table 1), which is typical for such polymeric click reactions. The purity of **20** increased to 84% by carrying out the reaction in the presence of excess azide functional **9** and removal of unreacted **9** by a further click reaction with alkyne-functionalized solid support[21] **19** (Figure 3A, where **20*** represents **20** after purification from solid support through filtration). The remaining 16% impurity probably consists of dead starting polymer produced from bimolecular radical termination during ATRP or nonfunctionalized polymer chains.

The linkages between the first- and second-generation polymers were designed to be stable under acid or base hydrolysis. The solketals on **20** were ring opened with acid to form hydroxyl groups, which were converted to azides (**23**, G2-[G1PSTY, G2PSTY$_4$, 8N$_3$]) via a two step procedure (see Scheme 2). Figure 2D shows the ATR-FTIR spectrum of **23** with a distinct broad stretch at 2094 cm^{-1} corresponding to an azide moiety. It should be recognized that the end groups contain ester (base cleavable) groups, which will result in the desired degradable dendrimers between the second- and third-generation polymers.

Three G3 dendrimers consisting of two G3 dendrimers of polystyrene with either solketals or hydroxyl groups on the periphery and a dendrimer in which the G3 polymer consists of P^{t}BA were synthesized using the following procedure. Dendrimer **23** was used as the starting point to synthesize all G3 dendrimers via the click reaction with **16**, **17,** and **18** to yield **24**, **25,** and **26,** respectively. Telechelic polymers **16** and **17** have a dialcohol at one chain end and an alkyne group at the other and consist of either PSTY and P^{t}BA, respectively. The click reaction with **18** yields a G3 homopolymer (PSTY) dendrimer with solketals at the periphery. The yields for **24**−**26** range between 82 and 89% but have purities between 65 and 73% (Table 1 and Figure 3). It should be noted that the alkyne-functional linear polymer (**16**−**18**) was used in slight excess (1.05 equiv) to the azide groups on **23**. Removal of the excess **16**−**18** through a click reaction with azide functional solid supports proved difficult and is the focus of future work. An amphiphilic G3 dendrimer with poly(acrylic acid) consisting of the third-generation was synthesized by the hydrolysis of the *tert*-butyl (from **26**) to carboxylic acid groups, **27**. The results show that from a suite of well chosen starting polymers, linkers, and protecting chemistries a wide range of polymeric dendrimers can be synthesized. Importantly, pure forms of the dendrimers can be obtained through fractionation by SEC (see for example Figure 3C, in which **26** was fractionated giving an M_n of 39 140 and PDI of 1.14 (Table 1)).

An important design parameter in delivery devices is the degradability of linkages between the generations. Dendrimer

24 was designed to contain ester linkages between the second and third generations, which are degradable (Scheme 2). Therefore, to test that only the linkages between G2 and G3 degrade sodium methoxide in THF was added to a solution of **24** and stirred for 16 h. The SEC traces (Figure 4) show that after treatment with $NaOCH_3$ there was quantitative cleavage of the ester groups linking G2 to G3 resulting in a linear PSTY and a G2 PSTY. The degradation of these linkages is almost quantitative with little or no G3 remaining. Our strategy would also allow degradable linkages to be placed between any generation or all generations.

Self-Assembly of 27 (G3[G1PSTY, G2PSTY$_4$, G3PAA$_8$, 16OH]) in Water. The characterization of the amphiphilic polymeric dendrimer, **27**, in DMF and water was carried out using dynamic light scattering (DLS). The number-average hydrodynamic diameter (D_H) in DMF, a good solvent for both PSTY (214 STY units in total) and PAA (275 AA units in total), gave a size of approximately 4.2 nm (Table 2), which corresponds to the size of a nonaggregated (i.e., "unimolecular") structure. The size is smaller by approximately 2 nm than a linear diblock copolymer, PSTY-*b*-PAA, of similar length,[3,4] suggesting that due to the dendrimers architecture a more compact structure is observed.

The self-assembly of **27** in water was carried out by a slow addition of water to a concentrated solution of **27** in DMF over a 24 h period. The water solution was then dialyzed to remove the residual DMF. The hydrodynamic diameter increased from 4.2 nm in DMF to 18.2 nm in water and is similar in size to those observed by transmission electron micrographs, which was between 17 and 19 nm for the spherical micelles after negative staining (Figure 5). The aggregation number (Z), based on the number of dendrimers per micelle, was calculated from the weight-average molecular weight (M_w) of the micelles from AFFF divided by the M_w of **27** and was found to be 19, a value much lower than that found for linear diblocks ($Z \sim 176$)[3,4] but similar to that observed for 4-arm star diblocks.[3] The molecular weight distribution of the micelles is very narrow with a PDI of 1.04, suggesting that the self-assembly process produces micelles of near identical size. The radius of gyration (R_g) was found to be 10.2 nm, and by way of inference from the D_H value (18.2 nm), it is suggested that there is no mutual interpenetration between the dendrimers due to the large asymmetry between the hydrophilic and hydrophobic blocks and thus the amphiphilic dendrimers pack uniformly into the micelle (Figure 5).

Conclusion

In this work, we demonstrate a strategy for the design and synthesis of complex macromolecules with unprecedented architectural complexity in pure form. We use the ATRP to produce polymers with well-defined chain length and telechelic end group functionality in combination with the click reactions to quantitatively couple these polymer chains together to form functional second-and third-generation dendrimers. Importantly, this methodology also allows us to incorporate degradable linkages between the desired generations and to incorporate certain functionality at the polymer chain ends of each generation. We have demonstrated this through the synthesis of second- and third-generation homo- and amphiphilic diblock copolymer dendrimers and specifically designed third-generation dendrimers in pure form such that the peripheral generational layer could be selectively cleaved off from the second-generation dendrimer. The degradation of the peripheral polymer layer is a useful feature in biomedical delivery devices which could be used to make important biomolecules available in targeted cells via a slow release process. These dendrimers also have either free or protected hydroxyl groups on the peripheral ends, which are useful for further chemical modification or chemical coupling with important biomolecules. The synthetic strategy given here provides a toolkit to design and make a wide range of dendrimers using the same starting polymers, linkers, and protection groups. The amphiphilic dendrimers self-assemble in water to form micelles of near identical size with a size of 18 nm and consisting of 19 individual dendrimers. The dendrimers most probably have no mutual interpenetration and thus pack uniformly to form the micelles.

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MA701993W