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Synthesis and Unimolecular Micelles of Amphiphilic Dendrimer-like Star Polymer with Various Functional Surface Groups

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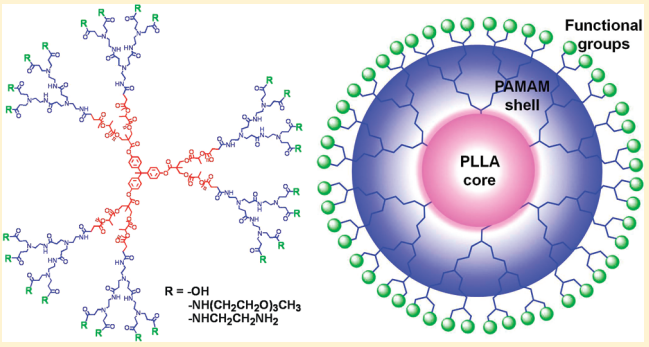
Supporting Information

ABSTRACT: We report the synthesis and functionalization of amphiphilic dendrimer-like star polymers (DLSPs) with a hydrophobic star-shaped poly(L-lactide) (PLLA) core and a hydrophilic poly(amidoamine) (PAMAM) dendron shell. First, carboxylic acid-functionalized PLLA star polymer was synthesized by ring-opening polymerization of L-lactide followed by functionalization with succinic anhydride. Second, 1-, 2-, and 3-generation PAMAM dendrons with a primary amine at the dendron root and benzyl ester protections at the periphery were prepared via a divergent method. By amide coupling between the carboxylic acid-terminated PLLA star polymer and six PAMAM dendrons, amphiphilic DLSPs were successfully synthesized. To enhance bioactivity and bioconjugation capability, the benzyl ester surface groups in these DLSPs were converted to carboxylic acid, primary amine, and triethylene glycol functional groups, respectively. Nuclear magnetic resonance spectroscopy and size-exclusion chromatography were used to confirm quantitative functionalization. These functional DLSPs exhibited a unique unimolecular micelle (14−28 nm) behavior in aqueous solution with a small amount of aggregation (205−344 nm), as studied by dynamic light scattering. In addition, they also exhibited large differences in thermal behaviors depending on the nature of different surface groups. Experimental results showed that these DLSPs had good solubility in aqueous solutions (ca. 10−25 mg/mL) and could greatly enhance the water solubility of hydrophobic drugs. Therefore, these amphiphilic DLSPs are promising candidates for controlled hydrophobic drug delivery.



INTRODUCTION

Even though many new anticancer drugs have been developed by the pharmaceutical industry, the clinical use of new chemotherapeutical drugs is still limited, and more than 6 million people die from cancer around the world every year.[1] New experimental approaches are being actively sought and researched to enhance delivery efficacy and reduce side effects and systemic damage. One of many research tasks is to enhance water solubility for anticancer drugs such as most alkylating agents and plant alkaloids and terpenoids (e.g., paclitaxel and docetaxel), while remaining anticancer potency. For example, conjunction with surfactants or covalent conjugation with a hydrophilic tail such as poly(ethylene glycol) (PEG) have been attempted to improve water solubility.[2] It is noted that a decrease in efficacy and harmful side effects potentially exist for this conjugation methodology.

Amphiphilic block or graft copolymers consisting of hydrophilic and hydrophobic segments are considered as good candidates for drug delivery because they may decrease the unwanted side effects, prolong the circulation time, and reduce the uptake by the reticuloendothelial system (RES).[3,4] Typically, poly(lactic acid) or polylactides (PLA), poly(lactic-*co*-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL) are copolymerized with PEG to form either AB or ABA block copolymers and are the most frequently used as biomaterials for drug delivery because of their outstanding biodegradability and biocompatibility.[5−10] These amphiphilic biodegradable (at least partially) copolymers can easily self-assemble in aqueous solution to form nanoparticles with an average size ranging from 50 to 500 nm, and the self-assembled hydrophobic cores are used to encapsulate hydrophobic drugs. In certain practical applications, however, the size of nanoparticles is important to the biodistribution in vivo.[11] Previous studies using liposomes as drug carriers have shown that the amount of sequestrated liposome

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nanoparticles in spleen decreased with decreasing the particle size[12,13] and smaller nanoparticles (e.g., 8−20 nm) may deliver drugs more effectively.[14−16]

To achieve smaller particle sizes, dendrimer, star, and hyperbranched polymers are attractive as potential drug carriers. Compared to linear polymers, dendrimers possess many unique advantages, such as small particle size (e.g., only several nanometers), large number of tunable surface functional groups, high surface area-to-volume ratios, and small polydispersity indices (PDI) with well-defined structures.[17,18] Therefore, dendrimer-based supermolecules are of widespread interest for many applications including drug delivery,[19−21] gene delivery,[22−25] tissue sealants,[26−28] catalysis,[29−31] and light harvesting.[32−34] In particular, the tunable surface functional groups allow a great opportunity for further chemical conjugation with other molecules, such as targeting molecules and polymers, fluorescence dye, or even drugs. Research on biodegradable and amphiphilic dendrimers for drug delivery has attracted substantial attention.[35−41] Nevertheless, synthesis of these dendrimers, which involves either a divergent[42,43] or a convergent[44−47] method, is nontrivial, and especially, high generations are necessary to obtain large enough unimolecular particles for drug encapsulation.

Alternatively, segment- and surface-block dendrimers and dendritic−linear hybrids have been pursued to simplify the synthetic steps.[48−51] The amphiphilicity of these dendrimer-based supermolecules can be easily tuned by introducing either a hydrophobic or a hydrophilic linear chain. Depending on the composition and hydrophobicity/hydrophilicity of the linear polymer and the dendron, these supermolecules may still self-assemble to form large aggregates in aqueous solution. Another seemingly good candidate is the amphiphilic dendrimer-like star polymer (DLSP), which has a similar structure to dendrimers but the hydrophobic core is replaced by a star polymer.[52−56] In addition to the advantages of dendrimers, amphiphilic DLSPs consisting of a hydrophobic core and a hydrophilic dendron shell have several advantages for drug delivery. (1) Unimolecualr micelles can be achieved and the particle size can be easily adjusted by controlling the molecular weight of the star polymer. (2) The amphiphilicity can be tuned by changing the ratio of the hydrophobic core to the hydrophilic shell in order to achieve good water solubility. (3) The synthesis can be easier because high-generation dendrons are not necessary to obtain large enough unimolecular micelles for hydrophobic drug encapsulation. (4) In contrast to the rigid inner structure of dendrimers, the flexible hydrophobic core in DLSP favors more encapsulation of hydrophobic drugs.

Most amphiphilic DLSPs have so far been developed as new materials for microelectronic industry.[57,58] In this study, we designed a novel DLSP comprised of a hydrophobic PLLA star polymer and six hydrophilic PAMAM dendrons with different generations. The surface of the DLSP could be quantitatively functionalized with various groups such as carboxylic acid, primary amine, and triethylene glycol. Their unimolecular micelles in aqueous solution were studied by dynamic laser light scattering (DLS). Intriguingly, these DLSPs had good solubility in aqueous solutions and could greatly enhance the water solubility of hydrophobic drugs.

EXPERIMENTAL SECTION

Materials. L-Lactide (Purac Inc.) was recrystallized from toluene twice and dried in a vacuum oven at room temperature for 3 days before use. Anhydrous toluene was dried over Na and distilled and stored under a dry N_2 atmosphere. Stannous(II) 2-ethylhexanoate [$Sn(Oct)_2$], ethylenediamine, methyl acrylate, and all other chemicals were purchased from Sigma-Aldrich, Fluka, or Alfa Aesar and used without further purification. The PLLA star polymer was prepared according to procedures developed before.[59] Its number-average molecular weight was 9200 g/mol as determined by end-group analysis from proton nuclear magnetic resonance (^{1}H NMR) and molecular weight distribution was 1.09 as determined by size-exclusion chromatography (SEC).

Measurements and Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-400 and DRX-500 (400 and 500 MHz, respectively), using either $CDCl_3$ or DMSO-d_6 as the solvent depending on the solubility of compounds. Mass spectrometer (MS) analysis of PAMAM dendrons with various generations was performed on either a VG Quattro II mass spectrometer equipped with an electrospray ionization source or a QSTAR Elite mass spectrometer. Methanol/water (50/50 vol/vol) with 0.1% formic acid was used as the solvent and eluent. The molecular weight distribution was determined by SEC using a Waters 150C Plus gel permeation chromatograph equipped with a Waters 410 differential refractometer. Dimethylacetamide (DMAc) with 0.05 M LiBr was used as the mobile phase at a flow rate of 1.0 mL/min, and poly(methyl methacrylate) standards were used for conventional calibration. Differential scanning calorimetry (DSC) was carried out on a TA Instruments DSC Q-20 with a scanning rate of 10 °C/min. The hydrodynamic sizes for micelles and aggregates in aqueous solutions were determined by DLS using a Malvern Zetasizer Nano S equipped with a 633 nm laser. The measurement was performed at 20 °C with a detection angle of 90°. All polymer solutions (0.5 mg/mL) were filtered through Millipore membranes with a pore size of 0.45 μm prior to measurements. The hydrodynamic diameters were calculated using the Zetasizer Nano Series software provided by the manufacturer. The morphology of the micelles was studied using transmission electron microscopy (TEM) on a Tecnai T12 operating at an accelerating voltage of 120 kV. One drop of the aqueous solution (0.2 mg/mL) of micelles was placed on a carbon-coated copper grid, which was irradiated with UV light to increase the hydrophilicity of the carbon film for 1 min. Excess solution was blotted away with a filter paper and dried in a vacuum oven overnight before being stained with RuO_4 for 15 min at ambient temperature.

Synthesis of Carboxylic Acid-Functionalized PLLA Star Polymer [G1-(COOH)$_6$] 1. Succinic anhydride (1.304 g, 13.04 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.08 g, 0.65 mmol) were dissolved in a mixture solvent of 20 mL of dichloromethane and 5.0 mL of pyridine. After the reaction flask was flushed with dry N_2, 15 mL of dichloromethane solution containing 1.0 g (0.11 mmol) of G1-$(OH)_6$ was added dropwise into the above solution at 0 °C. The mixture was reacted for 2 days at room temperature. Afterward, the solvent was removed under reduced pressure. The crude sample was redissolved in dichloromethane and precipitated into methanol twice. The precipitate was filtered and dried in a vacuum oven for 2 days to give 0.95 g of final product, [G1-$(COOH)_6$] **1** (yield: 89%). ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-CCH_3(CH_2O)_2-$), 1.49−1.60 (m, poly, $-CH_3$), 2.12−2.15 (br, 3H, $-C(CH_3)(Ar)_3$), 2.63−2.71 (m, 24H, $-COCH_2-$), 4.36−4.52 (m, 12H, $-CCH_3(CH_2O-)_2$), 5.14−5.20 (m, poly, $-CH-$), 6.91−7.06 (dd, 12H, $Ar-$). ^{13}C NMR ($CDCl_3$, δ): 16.76, 16.94, 17.78, 20.62, 28.74, 46.90, 65.86, 68.78, 69.13, 120.85, 129.85, 146.49, 148.66, 169.72, 170.24, 170.88, 171.70, 176.61. DSC results: a single melting temperature at 107 °C upon the first heating and only a glass transition temperature (T_g) at 54 °C upon the second heating. SEC results: PDI = 1.09.

Synthesis of *N*-Boc-ethylenediamine 2. A dichloromethane solution (100 mL) of di-*tert*-butyl dicarbonate (4.0 g, 18.33 mmol) was added dropwise into 50 mL of dichloromethane solution of ethylenediamine (6.6 g, 110 mmol) at 0 °C. The reaction mixture was then stirred at room temperature overnight. The solvent was removed using a rotary

evaporator, and the crude product was dissolved in 50 mL of doubly distilled water, filtered, and then extracted with 100 mL of dichloromethane twice. The final product was obtained as a viscous and colorless oil (2.5 g, yield: 85%). ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.76−2.79 (br, 2H, $-CH_2NH_2$), 3.14−3.27 (br, 2H, $-NHCH_2-$), 5.28 (br, 1H, BocN*H*−). ^{13}C NMR ($CDCl_3$, δ): 28.48, 41.84, 43.30, 79.15, 156.37. MS results for $C_7H_{16}N_2O_2$: calculated 160.2 and found 160.9.

Synthesis of g0-[Boc, $(COOCH_3)_2$] 3 and General Synthesis Procedure for Boc-Protected and Methyl Ester-Terminated PAMAM Dendrons 4 and 5. A solution of 2.0 g (12.48 mmol) *N*-Boc-ethylenediamine **2** in 7.0 mL of methanol was added dropwise to a stirred solution of 4.33 g of methyl acrylate (49.92 mmol) in 8.0 mL of methanol at 0 °C. The resulting solution was then allowed to warm up to room temperature and stirred for 2 days. The solvent and the majority of the excess methyl acrylate were removed under reduced pressure using a rotary evaporator. The residue was further purified in a vacuum oven for 2 days to give the final product as a viscous yellowish oil **3** (4.11 g, yield: 99%). Similar procedures were used for the preparation of Boc-protected and methyl ester-terminated PAMAM dendrons **4** and **5**.

g0-[Boc, $(COOCH_3)_2$] **3**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.29−2.41 (t, 4H, $-CH_2CO_2-$), 2.41−2.50 (t, 2H, $-CH_2N(CH_2)_2$), 2.61−2.77 (t, 4H, $-CH_2N(CH_2)_2$), 3.14−3.16 (br, 2H, BocNHCH_2-), 3.64 (s, 6H, $-OCH_3$), 5.28 (br, 1H, BocN*H*CH_2-). ^{13}C NMR ($CDCl_3$, δ): 28.58, 32.79, 38.22, 49.34, 51.70, 53.24, 78.96, 156.22, 173.07. MS results for $C_{15}H_{28}N_2O_6$: calculated 332.4 and found: 332.5.

g1-[Boc, $(COOCH_3)_4$] **4**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.31−2.37 (t, 4H, $-CH_2CONH-$), 2.37−2.43 (t, 8H, $-CH_2CO_2-$), 2.47−2.59 (t, 6H, $-CH_2N(CH_2)_2$), 2.68−2.81 (m, 12H, $-CH_2N(CH_2)_2$), 3.09−3.21 (br, 2H, BocNHCH_2-), 3.25−3.33 (q, 4H, $-CONHCH_2-$), 3.64 (s, 12H, $-OCH_3$), 5.43 (br, 1H, BocN*H*CH_2-), 6.96 (br, 2H, $-CONHCH_2-$). ^{13}C NMR ($CDCl_3$, δ): 28.30, 32.48, 33.63, 36.92, 38.25, 49.05, 49.68, 51.45, 52.42, 52.83, 78.60, 155.93, 172.08, 172.85. MS results for $C_{33}H_{60}N_6O_{12}$: calculated 732.9 and found 732.8.

g2-[Boc, $(COOCH_3)_8$] **5**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.28−2.42 (m, 12H, $-CH_2CONH-$), 2.42−2.51 (t, 16H, $-CH_2CO_2-$), 2.51−2.65 (m, 14H, $-CH_2N(CH_2)_2$), 2.70−2.84 (m, 28H, $-CH_2N(CH_2)_2$), 3.09−3.17 (br, 2H, BocNHCH_2-), 3.19−3.34 (q, 12H, $-CONHCH_2-$), 3.64 (s, 24H, $-OCH_3$), 5.63 (br, 1H, BocN*H*CH_2-), 6.98 (br, 4H, $-CONHCH_2-$), 7.64 (br, 2H, $-CONHCH_2-$). ^{13}C NMR ($CDCl_3$, δ): 28.52, 32.72, 33.82, 37.25, 37.51, 38.50, 49.29, 49.84, 50.03, 51.68, 52.58, 52.63, 52.96, 78.87, 156.26, 172.48, 172.56, 173.11. MS results for $C_{69}H_{124}N_{14}O_{24}$: calculated 1533.8 and found 1534.2.

Synthesis of g0-[Boc, $(NH_2)_2$] 6 and General Synthesis Procedure for Boc-Protected and Amine-Terminated PAMAM Dendrons 7 and 8. Methyl ester-terminated PAMAM dendron g0-[Boc, $(COOCH_3)_8$] **3** (4.0 g, 12.03 mmol) was dissolved in 20 mL of methanol and was then added dropwise to a cold (0 °C) 30 mL methanol solution of 43.4 g (0.722 mol) of ethylenediamine over a period of 1 h. The reaction mixture was allowed to warm up to room temperature and stirred for 5 days. Afterward, the solvent was removed under reduced pressure using a rotary evaporator. The excess ethylenediamine was distilled off as an azeotrope with toluene/methanol, and the remaining toluene was removed by vacuum distillation. The residue was dried in a vacuum oven for 2 days to give the amino-terminated product as a honey-like oil **6** (4.58 g, yield: 98%). Similar procedures were used for the preparation of Boc-protected and amine-terminated PAMAM dendrons **7** and **8**.

g0-[Boc, $(NH_2)_2$] **6**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.25−2.40 (t, 4H, $-CH_2CONH-$), 2.41−2.55 (t, 2H, $-CH_2N(CH_2)_2$), 2.57−2.72 (t, 4H, $-CH_2N(CH_2)_2$), 2.72−2.88 (t, 4H, $-CH_2CH_2NH_2$), 3.06−3.20 (br, 2H, BocNHCH_2-), 3.25−3.31 (q, 4H, $-CH_2CH_2NH_2$), 6.38 (br, 1H, BocN*H*CH_2-), 7.23 (br, 2H, $-CONHCH_2-$). ^{13}C NMR ($CDCl_3$, δ): 28.60, 33.98, 38.48, 41.37, 41.97, 50.40, 53.28, 79.09, 156.51, 172.81. MS results for $C_{17}H_{36}N_6O_4$: calculated 388.5 and found: 388.8.

g1-[Boc, $(NH_2)_4$] **7**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.25−2.40 (m, 12H, $-CH_2CONH-$), 2.42−2.57 (t, 6H, $-CH_2N(CH_2)_2$), 2.60−2.78 (t, 12H, $-CH_2N(CH_2)_2$), 2.78−2.88 (t, 8H, $-CH_2CH_2NH_2$), 3.09−3.17 (br, 2H, BocNHCH_2-), 3.17−3.32 (q, 12H, $-CONHCH_2-$), 5.61 (br, 1H, BocN*H*CH_2-), 7.45 (br, 4H, $-CONHCH_2-$), 7.86 (br, 2H, $-CONHCH_2-$). ^{13}C NMR ($CDCl_3$, δ): 28.57, 33.95, 34.32, 37.86, 38.56, 41.53, 42.25, 50.11, 52.93, 78.14, 156.48, 172.95, 173.26. MS results for $C_{37}H_{76}N_{14}O_8$: calculated 845.1 and found: 845.2.

g2-[Boc, $(NH_2)_8$] **8**. ^{1}H NMR (DMSO-d_6, δ): 1.36 (s, 9H, $-C(CH_3)_3$), 2.14−2.26 (m, 28H, $-CH_2CONH-$), 2.39−2.46 (m, 14H, $-CH_2N(CH_2)_2$), 2.53−2.59 (m, 16H, $-CH_2CH_2NH_2$), 2.60−2.70 (m, 28H, $-CH_2N(CH_2)_2$), 2.90−2.99 (br, 2H, BocNHCH_2-), 3.00−3.14 (m, 28H, $-CONHCH_2-$), 6.55 (br, 1H, BocN*H*CH_2-), 7.88 (br, 14H, $-CONHCH_2-$). ^{13}C NMR (DMSO-d_6, δ): 28.24, 33.25, 33.32, 36.92, 41.28, 42.15, 49.58, 49.68, 52.24, 77.52, 155.56, 171.26, 171.48. MS results for $C_{77}H_{156}N_{30}O_{16}$: calculated 1758.3 and found: 1758.6.

Synthesis of g1-[Boc, $(COOBn)_4$] 9 and General Synthesis Procedure for Boc-Protected and Benzyl Ester-Terminated PAMAM Dendrons 10 and 11. Amine-terminated PAMAM dendron, g0-[Boc, $(NH_2)_2$] **6** (1.0 g, 2.57 mmol), and benzyl acrylate (3.34 g, 20.59 mmol) were flushed with dry N_2 and stirred at 80 °C for 5 days. The reaction mixture was then cooled to room temperature. The crude product was purified by column chromatography on silica gel with the eluting solvent changing from ethyl acetate to methanol, yielding 2.32 g (87%) of **9** in the form of a viscous and yellowish oil. Similar procedures were used for the preparation of Boc-protected and benzyl ester-terminated PAMAM dendrons **10** and **11**.

g1-[Boc, $(COOBn)_4$] **9**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.31−2.37 (m, 4H, $-CH_2CONH-$), 2.37−2.43 (m, 8H, $-CH_2CO_2-$), 2.47−2.59 (m, 6H, $-CH_2N(CH_2)_2$), 2.68−2.81 (m, 12H, $-CH_2N(CH_2)_2$), 3.09−3.21 (br, 2H, BocNHCH_2-), 3.25−3.33 (m, 4H, $-CONHCH_2-$), 5.10 (s, 8H, $-CH_2Ph$), 5.42 (br, 1H, BocN*H*CH_2-), 6.93 (br, 2H, $-CONHCH_2-$), 7.25−7.38 (m, 20H, $-CH_2Ph$). ^{13}C NMR ($CDCl_3$, δ):28.58, 32.78, 33.79, 37.24, 38.52, 49.26, 49.90, 52.64, 53.06, 66.47, 78.97, 128.37, 128.67, 135.90, 156.18, 172.29, 172.50. MS results for $C_{57}H_{76}N_6O_{12}$: calculated 1037.2 and found: 1037.2.

g2-[Boc, $(COOBn)_8$] **10**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.28−2.42 (m, 12H, $-CH_2CONH-$), 2.42−2.51 (m, 16H, $-CH_2CO_2-$), 2.51−2.65 (m, 14H, $-CH_2N(CH_2)_2$), 2.70−2.84 (m, 28H, $-CH_2N(CH_2)_2$), 3.09−3.17 (br, 2H, BocNHCH_2-), 3.19−3.34 (m, 12H, $-CONHCH_2-$), 5.10 (s, 16H, $-CH_2Ph$), 5.63 (br, 1H, BocN*H*CH_2-), 6.96 (br, 4H, $-CONHCH_2-$), 7.25−7.38 (m, 40H, $-CH_2Ph$), 7.65 (br, 2H, $-CONHCH_2-$). ^{13}C NMR ($CDCl_3$, δ): 28.65, 32.91, 33.87, 37.41, 37.59, 38.63, 49.37, 49.93, 50.12, 52.58, 52.73, 53.03, 66.48, 78.91, 128.41, 128.71, 135.97, 156.31, 172.45, 172.55. MS results for $C_{117}H_{156}N_{14}O_{24}$: calculated 2142.6 and found: 2143.2.

g3-[Boc, $(COOBn)_{16}$] **11**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, $-C(CH_3)_3$), 2.25−2.40 (m, 28H, $-CH_2CONH-$), 2.40−2.49 (m, 32H, $-CH_2CO_2-$), 2.49−2.62 (m, 30H, $-CH_2N(CH_2)_2$), 2.65−2.84 (m, 60H, $-CH_2N(CH_2)_2$), 3.09−3.19 (br, 2H, BocNHCH_2-), 3.19−3.34 (m, 28H, $-CONHCH_2-$), 5.10 (s, 32H, $-CH_2Ph$), 5.73 (br, 1H, BocN*H*CH_2-), 6.99 (br, 8H, $-CONHCH_2-$), 7.25−7.38 (m, 80H, $-CH_2Ph$), 7.59 (br, 4H, $-CONHCH_2-$), 7.75 (br, 2H, $-CONHCH_2-$). ^{13}C NMR ($CDCl_3$, δ): 28.4, 32.63, 33.68, 37.15, 37.40, 49.11, 49.70, 49.86, 52.35, 52.71, 66.16, 78.58, 128.11, 128.43, 135.72, 156.13, 172.28, 172.43. MS results for $C_{237}H_{316}N_{30}O_{48}$: calculated 4353.2 and found: 4354.0.

Synthesis of g1-[NH_2, $(COOBn)_4$] 12 and General Synthesis Procedure for Deprotection of Boc Groups for 13 and 14. Boc-protected and benzyl ester-terminated PAMAM dendron g1-[Boc, $(COOBn)_4$] **9** (1.0 g, 0.964 mmol) was dissolved in 3.0 mL of dichloromethane, and the solution was cooled to 0 °C. 1.5 mL of trifluoroacetic acid (TFA) was added, and the reaction mixture was stirred at room temperature for 12 h. The solvent and the majority of the excess TFA were removed under reduced pressure using a rotary evaporator. The dried solid was redissolved in 30 mL of dichloromethane and extracted with 10 mL of 25% aqueous solution of sodium carbonate. The organic phase was dried by anhydrous $MgSO_4$. The product was dried in a vacuum oven for 2 days to give a viscous and yellowish oil (0.81 g, yield: 90%). Similar deprotection procedures were used to prepare benzyl ester-terminated PAMAM dendrons **10** and **11** with primary amine at the root.

g1-[NH_2, $(COOBn)_4$] **12**. ^{1}H NMR ($CDCl_3$, δ): 2.23−2.35 (m, 4H, −CH_2CONH−), 2.36−2.43 (m, 8H, −CH_2CO_2−), 2.43−2.54 (m, 4H, −$CH_2N(CH_2)_2$), 2.54−2.67 (m, 6H, −$CH_2N(CH_2)_2$), 2.68−2.81 (m, 8H, −$CH_2N(CH_2)_2$), 2.96−3.16 (br, 2H, H_2NCH_2−), 3.13−3.26 (m, 4H, −CONHCH_2−), 5.10 (s, 8H, −CH_2Ph), 6.93 (br, 2H, −CONHCH_2−), 7.25−7.38 (m, 20H, −CH_2Ph). ^{13}C NMR ($CDCl_3$, δ): 32.90, 33.67, 37.38, 37.55, 48.95, 49.35, 51.21, 52.89, 66.57, 128.46, 128.76, 135.99, 172.78. MS results for $C_{52}H_{68}N_6O_{10}$: calculated 937.1 and found: 937.2.

g2-[NH_2, $(COOBn)_8$] **13**. ^{1}H NMR ($CDCl_3$, δ): 2.28−2.42 (m, 12H, −CH_2CONH−), 2.42−2.51 (m, 16H, −CH_2CO_2−), 2.51−2.65 (m, 14H, −$CH_2N(CH_2)_2$), 2.70−2.84 (m, 28H, −$CH_2N(CH_2)_2$), 3.09−3.19 (br, 2H, H_2NCH_2−), 3.19−3.34 (m, 12H, −CONHCH_2−), 5.10 (s, 16H, −CH_2Ph), 7.23 (br, 4H, −CONHCH_2−), 7.25−7.38 (m, 40H, −CH_2Ph), 7.54 (br, 2H, −CONHCH_2−). ^{13}C NMR ($CDCl_3$, δ): 32.87, 33.37, 34.20, 36.56, 37.27, 37.46, 49.37, 49.89, 50.11, 52.82, 53.54, 66.43, 128.34, 128.67, 135.94, 172.54, 172.94. MS results for $C_{112}H_{148}N_{14}O_{22}$: calculated 2042.4 and found: 2043.3.

g3-[NH_2, $(COOBn)_{16}$] **14.** ^{1}H NMR ($CDCl_3$, δ): 2.24−2.40 (m, 28H, −CH_2CONH−), 2.40−2.51 (m, 32H, −CH_2CO_2−), 2.51−2.64 (m, 30H, −$CH_2N(CH_2)_2$), 2.64−2.86 (m, 60H, −$CH_2N(CH_2)_2$), 3.09−3.18 (br, 2H, H_2NCH_2−), 3.19−3.34 (m, 28H, −CONHCH_2−), 5.10 (s, 32H, −CH_2Ph), 6.99 (br, 8H, −CONHCH_2−), 7.25−7.38 (m, 80H, −CH_2Ph), 7.59 (br, 4H, −CONHCH_2−), 7.75 (br, 2H, −CONHCH_2−). ^{13}C NMR ($CDCl_3$, δ): 32.8, 33.56, 36.75, 37.36, 48.57, 49.28, 49.86, 50.08, 52.33, 52.85, 66.37, 128.30, 128.62, 135.90, 172.39, 172.49, 172.89. MS results for $C_{232}H_{308}N_{30}O_{46}$: calculated 4253.1 and found: 4254.0.

Synthesis of G1-g1-$(COOBn)_{24}$ 15 and General Synthesis Procedure for Conjugation of the PLLA Core and Different PAMAM Dendrons. Carboxylic acid-functionalized PLLA core **1** (1.0 g, 0.102 mmol) was dissolved in 30 mL of dichloromethane, and then 0.25 g (2.45 mmol) of triethylenamine (TEA) and 0.64 g (1.23 mmol) of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) were added. The reaction solution was stirred at room temperature for 1 h, and the amine-functionalized PAMAM dendron **12** (0.86 g, 0.92 mmol) was added. The reaction mixture was stirred at room temperature for 20 h before precipitation in methanol. The crude product was redissolved in dichloromethane and precipitated in methanol again. The precipitated solid was collected and washed carefully with fresh methanol several times. The final product was obtained as a yellowish solid **15** (1.28 g, yield: 82%). Similar procedures were carried out for the synthesis of G1-g2-$(COOBn)_{48}$ **16** and G1-g3-$(COOBn)_{96}$ **17**.

G1-g1-$(COOBn)_{24}$ **15**. ^{1}H NMR ($CDCl_3$, δ): 1.41(s, 9H, −$CCH_3(CH_2O)_2$−), 1.49−1.60 (m, poly, −CH_3), 2.12−2.15 (br, 3H, −$CCH_3(Ar)_3$), 2.23−2.31 (m, 24H, −CH_2CONH−), 2.34−2.44 (m, 48H, −CH_2CO_2Bn), 2.44−2.52 (m, 48H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 2.61−2.81 (m, 84H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 3.13−3.33 (m, 36H, −CONHCH_2−), 4.36−4.52 (m, 12H, −$CCH_3(CH_2O)_2$−), 5.09 (s, 48H, −CH_2Ph), 5.14−5.20 (m, poly, −CH−), 6.80−6.89 (m, 12H, −CONHCH_2−), 6.92−7.05 (dd, 12H, *Ar*−), 7.16−7.23 (m, 6H, −CONHCH_2−), 7.25−7.35 (m, 120H, −CH_2Ph). ^{13}C NMR ($CDCl_3$, δ): 16.69, 16.84, 17.71, 20.60, 29.27, 30.49, 32.85, 33.90, 37.28, 37.70, 46.84, 49.28, 49.86, 52.83, 52.95, 65.81, 66.42, 68.37, 69.06, 120.78, 128.32, 128.64, 129.78, 135.87, 146.43, 148.60, 169.66, 170.38, 170.82, 171.24, 172.51. DSC results: T_g at 19 °C upon second heating. SEC results: PDI = 1.12.

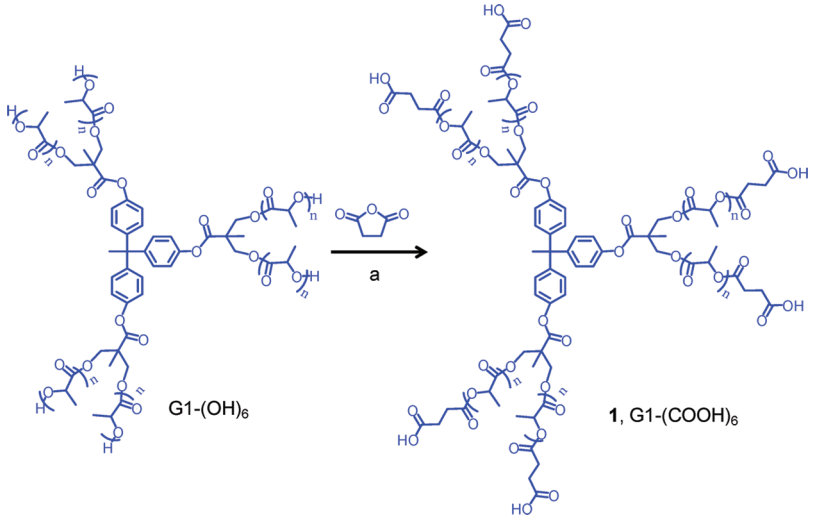
G1-g2-$(COOBn)_{48}$ **16**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, −$CCH_3(CH_2O)_2$−), 1.49−1.60 (m, poly, −CH_3), 2.12−2.15 (br, 3H, −$CCH_3(Ar)_3$), 2.23−2.31 (m, 72H, −CH_2CONH−), 2.34−2.44 (m, 96H, −CH_2CO_2Bn), 2.44−2.52 (m, 96H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 2.61−2.81 (m, 180H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 3.13−3.33 (m, 84H, −CONHCH_2−), 4.36−4.52 (m, 12H, −$CCH_3(CH_2O)_2$−), 5.09 (s, 96H, −CH_2Ph), 5.14−5.20 (m, poly, −CH−), 6.95−6.97 (m, 30H, −CONHCH_2−, *Ar*−), 7.08−7.10 (d, 6H, *Ar*−), 7.25−7.36 (m, 240H, −CH_2Ph), 7.46−7.49 (m, 6H, −CONHCH_2−), 7.55−7.57 (m, 12H, −CONHCH_2−). ^{13}C NMR ($CDCl_3$, δ): 16.72, 16.88, 17.73, 20.60, 29.31, 30.45, 32.85, 33.91, 34.03, 37.35, 37.60, 37.92, 46.86, 49.31, 49.87, 50.15, 52.67, 52.96, 53.52, 65.81, 66.42, 68.37, 69.08, 120.80, 128.34, 128.65, 129.80, 135.91, 146.44, 148.62, 169.68, 170.41, 170.84, 171.33, 172.50. DSC results: T_g at 12 °C upon second heating. SEC results: PDI = 1.15.

G1-g3-$(COOBn)_{96}$ **17**. ^{1}H NMR ($CDCl_3$, δ): 1.41 (s, 9H, −$CCH_3(CH_2O)_2$−), 1.49−1.60 (m, poly, −CH_3), 2.12−2.15 (br, 3H, −$CCH_3(Ar)_3$), 2.23−2.31 (m, 168H, −CH_2CONH−), 2.34−2.44 (m, 192H, −CH_2CO_2Bn), 2.44−2.52 (m, 192H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 2.61−2.81 (m, 372H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 3.13−3.33 (m, 180H, −CONHCH_2−), 4.36−4.52 (m, 12H, −$CCH_3(CH_2O)_2$−), 5.09 (s, 192H, −CH_2Ph), 5.14−5.20 (m, poly, −CH−), 6.92−6.98 (m, 54H, −CONHCH_2−, *Ar*−), 7.04−7.06 (d, 6H, *Ar*−), 7.18−7.20 (m, 24H, −CONHCH_2−), 7.25−7.30 (m, 480H, −CH_2Ph), 7.50−7.55 (m, 12H, −CONHCH_2−), 7.64−7.66 (m, 6H, −CONHCH_2−). ^{13}C NMR ($CDCl_3$, δ): 16.83, 17.01, 17.85, 20.72, 29.38, 30.50, 32.96, 33.86, 37.47, 37.67, 37.98, 46.97, 49.42, 50.00, 50.18, 52.61, 53.05, 65.92, 66.52, 68.49, 69.19, 120.91, 128.45, 128.76, 129.92, 136.04, 146.56, 148.74, 169.79, 170.53, 170.95, 172.50, 172.61. DSC results: T_g at −4 °C upon second heating. SEC results: PDI = 1.16.

Synthesis of G1-g1-$(COOH)_{24}$ 18 and General Procedure for Deprotection of Benzyl Ester Terminal Groups. G1-g1-$(COOBn)_{24}$ **15** (0.5 g, 32.65 μmol) was dissolved in 15 mL of TFA, and the solution was cooled to 0 °C. 6.0 mL of methanesulfonic acid (MsOH) and 1.5 mL of anisole were added slowly, and the resulting solution was kept stirring at 0 °C for 2 h. Once the reaction was complete, the polymer was precipitated in diethyl ether and dried in a vacuum oven. The dried sample was redissolved in 10 mL of DMSO, and 0.5 mL of TEA was added to neutralize the excess acid. The sample was then purified by dialysis in DMSO using a dialysis tube with a molecular weight cutoff (MWCO) of 6000 g/mol (regenerated cellulose dialysis tube from Fisher Scientific). The final product was precipitated in diethyl ether and was obtained as a slightly yellow solid **18** (0.34 g, yield: 79%). Similar procedures were used to synthesize G1-g2-$(COOH)_{48}$ **19** and G1-g3-$(COOH)_{96}$ **20**.

G1-g1-$(COOH)_{24}$ **18**. ^{1}H NMR (DMSO-d_6, δ): 1.33 (s, 9H, −$CCH_3(CH_2O)_2$−), 1.41−1.47 (m, poly, −CH_3), 2.15−2.22 (m, 27H, −$CCH_3(Ar)_3$, −CH_2CONH−), 2.32−2.36 (m, 48H, −CH_2CO_2Bn), 2.48−2.52 (m, 48H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 2.67−2.76 (m, 84H, −OCOCH_2CH_2CO−, −$CH_2N(CH_2)_2$), 3.06−3.17 (m, 36H, −CONHCH_2−), 4.32−4.43 (m, 12H, −$CCH_3(CH_2O)_2$), 5.08−5.19 (m, poly, −CH−), 7.03−7.10 (dd, 12H, *Ar*−), 7.69−7.97 (m, 18H, −CONHCH_2−). ^{13}C NMR (DMSO-d_6, δ): 16.45, 16.54, 16.89, 20.32, 28.65, 29.67, 31.58, 32.96, 36.32, 36.82, 46.44, 48.81, 49.42, 51.93, 52.06, 65.71, 67.83, 68.67, 120.99, 129.33, 146.22, 148.31, 169.19, 169.81, 170.30, 171.29, 171.86, 173.59. DSC results: T_g at 45 °C upon second heating. SEC results: PDI = 1.10.

Scheme 1. Synthesis of Carboxylic Acid-Functionalized G1-$(COOH)_6$[a]



[a] Reagents and conditions: (a) DMAP, pyridine, CH_2Cl_2, 2 days.

G1-g2-$(COOH)_{48}$ **19**. ^{1}H NMR (DMSO-d_6, δ): 1.33 (s, 9H, $-CCH_3(CH_2O)_2-$), 1.41−1.47 (m, poly, $-CH_3$), 2.15−2.27 (m, 75H, $-CCH_3(Ar)_3$, $-CH_2CONH-$), 2.29−2.38 (s, 96H, $-CH_2CO_2H$), 2.45−2.52 (m, 96H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 2.62−2.78 (m, 180H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 3.06−3.18 (m, 84H, $-CONHCH_2-$), 4.32−4.43 (m, 12H, $-CCH_3(CH_2O-)_2$), 5.08−5.19 (m, poly, $-CH-$), 7.03−7.10 (dd, 12H, *Ar*−), 7.43−7.48 (m, 42H, $-CONHCH_2-$). ^{13}C NMR (DMSO-d_6, δ): 16.45, 16.55, 16.89, 20.32, 28.63, 29.65, 31.57, 32.93, 36.27, 36.66, 36.81, 46.43, 48.84, 49.41, 49.46, 51.88, 51.98, 52.10, 65.71, 67.82, 68.66, 120.98, 129.33, 146.22, 148.30, 169.18, 169.81, 170.31, 171.28, 171.86, 173.64. DSC results: T_g at 51 °C upon second heating. SEC results: PDI = 1.12.

G1-g3-$(COOH)_{96}$ **20**. ^{1}H NMR (DMSO-d_6, δ): 1.33 (s, 9H, $-CCH_3(CH_2O)_2-$), 1.41−1.57 (m, poly, $-CH_3$), 2.15−2.29 (m, 171H, $-CCH_3(Ar)_3$, $-CH_2CONH-$), 2.32−2.40 (m, 192H, $-CH_2CO_2Bn$), 2.45−2.52 (m, 192H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 2.64−2.80 (m, 372H, $-COCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 3.04−3.21 (m, 180H, $-CONHCH_2-$), 4.32−4.43 (m, 12H, $-CCH_3(CH_2O-)_2$), 5.13−5.23 (m, poly, $-CH-$), 7.03−7.10 (dd, 12H, *Ar*−), 7.74−8.01 (m, 90H, $-CONHCH_2-$). ^{13}C NMR (DMSO-d_6, δ): 16.46, 16.57, 16.90, 20.32, 28.63, 29.66, 31.51, 32.81, 36.19, 36.58, 36.87, 46.44, 48.89, 49.39, 51.85, 52.09, 65.71, 67.84, 68.67, 121.00, 129.34, 146.23, 148.32, 169.20, 169.83, 170.36, 171.30, 171.88, 173.69. DSC results: T_g at 61 °C upon second heating. SEC results: PDI = 1.13.

Synthesis of G1-g2-$(TEG)_{48}$ 21. G1-g2-$(COOH)_{48}$ **19** (50 mg, 2.82 μmol) and *N*-hydroxysulfosuccinimide (NHS, 47 mg, 408 μmol) were dissolved in 1.0 mL of dry DMSO, and then 279 mg (1.35 mmol) of *N,N*-dicyclohexylcarbodiimide (DCC) was added. The reaction solution was left stirring at room temperature overnight and then filtered to remove the white precipitate, *N,N*-dicyclohexylurea (DCU). Amine-functionalized TEG (44 mg, 0.27 mmol) was added to the activated solution and stirred at room temperature overnight. Once the reaction was complete, DCU was removed by filtration. The crude product was precipitated in diethyl ether, dried, redissolved in dichloromethane, and finally precipitated in diethyl ether. The final product was obtained as a yellowish solid **21** (64 mg, yield: 92%). ^{1}H NMR (DMSO-d_6, δ): 1.33 (s, 9H, $-CCH_3(CH_2O)_2-$), 1.41−1.47 (m, poly, $-CH_3$), 2.14−2.25 (m, 171H, $-CCH_3(Ar)_3$, $-CH_2CONH-$), 2.35−2.43 (m, 96H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 2.58−2.70 (m, 180H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 3.02−3.13 (m, 84H, $-CONHCH_2-$), 3.13−3.21 (m, 96H, $-CONHCH_2CH_2O-$), 3.23 (s, 144H, $-CH_2CH_2OCH_3$), 3.37−3.44 (m, 192H, $-CONHCH_2CH_2O-$, $-CH_2CH_2OCH_3$), 3.47−3.52 (m, 288H, $-CH_2OCH_2CH_2O-$), 4.32−4.43 (m, 12H, $-CCH_3(CH_2O-)_2$), 5.08−5.19 (m, poly, $-CH-$), 7.03−7.10 (dd, 12H, *Ar*−), 7.71−7.82 (m, 42H, $-CONHCH_2-$), 7.82−7.93 (m, 48H, $-CONHCH_2-$). DSC results: T_g at −8 °C upon second heating. SEC results: PDI = 1.27.

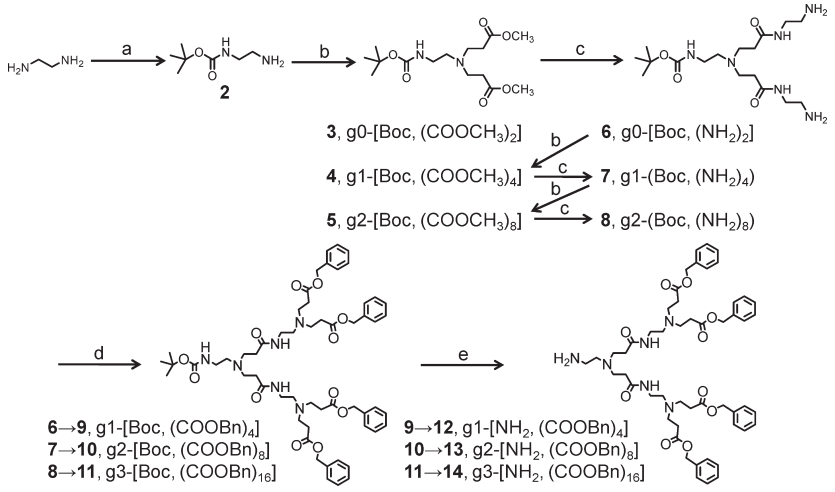
Synthesis of G1-g2-$(NHBoc)_{48}$ 22. According to the above procedure, G1-g2-$(COOH)_{48}$ **19** (50 mg, 2.82 μmol) was reacted with *N*-Boc-ethylenediamine (44 mg, 0.27 mmol) to give the final product as a yellowish solid (63 mg, yield: 91%). ^{1}H NMR (DMSO-d_6, δ): 1.33 (s, 9H, $-CCH_3(CH_2O)_2-$), 1.37 (s, 432H, $-C(CH_3)_3$), 1.41−1.47 (m, poly, $-CH_3$), 2.15−2.27 (m, 171H, $-CCH_3(Ar)_3$, $-CH_2CONH-$), 2.35−2.43 (m, 96H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 2.58−2.70 (m, 180H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 2.90−3.06 (m, 96H, $-CH_2CH_2NHBoc$), 3.06−3.18 (m, 180H, $-CONHCH_2-$, $-CH_2CH_2NHBoc$), 4.32−4.43 (m, 12H, $-CCH_3(CH_2O-)_2$), 5.08−5.19 (m, poly, $-CH-$), 6.70−6.78 (m, 48H, $-CH_2NHBoc$), 7.03−7.10 (dd, 12H, *Ar*−), 7.71−7.82 (m, 42H, $-CONHCH_2-$), 7.82−7.93 (m, 48H, $-CONHCH_2-$). DSC results: T_g at 43 °C upon second heating. SEC results: PDI = 1.21.

Synthesis of G1-g2-$(NH_2)_{48}$ 23. G1-g2-$(NHBoc)_{48}$ **22** (50 mg, 2.04 μmol) was dissolved in 1.0 mL of TFA and stirred at 0 °C for 3 h. TFA was evaporated by using a rotary evaporator, and the solid was redissolved in 1.0 mL of DMSO. TEA (25 mg) was added to neutralize the excess acid. The sample was then purified by precipitation in diethyl ether twice. The final product was obtained as a yellowish solid **23** (34 mg, yield: 85%). ^{1}H NMR (DMSO-d_6, δ): 1.33 (s, 9H, $-CCH_3(CH_2O)_2-$), 1.41−1.47 (m, poly, $-CH_3$), 2.15−2.27 (m, 171H, $-CCH_3(Ar)_3$, $-CH_2CONH-$), 2.35−2.43 (m, 96H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 2.58−2.70 (m, 180H, $-OCOCH_2CH_2CO-$, $-CH_2N(CH_2)_2$), 2.78−2.90 (m, 96H, $-CH_2CH_2NH_2$), 3.06−3.18 (m, 180H, $-CONHCH_2-$, $-CH_2CH_2NH_2$), 4.32−4.43 (m, 12H, $-CCH_3(CH_2O-)_2$), 5.08−5.19 (m, poly, $-CH-$), 7.03−7.10 (dd, 12H, *Ar*−), 7.71−7.82 (m, 42H, $-CONHCH_2-$), 7.82−7.93 (m, 48H, $-CONHCH_2-$). DSC results: T_g at 46 °C upon second heating.

RESULTS AND DISCUSSION

Synthesis of PLLA-Based DLSPs. The hexahydroxy-functionalized ring-opening polymerization (ROP) initiator based on

Scheme 2. Divergent Synthesis of Amine-Functionalized PAMAM Dendrons[a]



[a] Reagents and conditions: (a) $(Boc)_2O$, CH_2Cl_2, overnight; (b) methyl acrylate, MeOH, 2 days; (c) excess ethylenediamine, MeOH, 5 days; (d) benzyl acrylate, 80 °C, 5 days; (e) TFA, CH_2Cl_2, overnight.

2,2-bis(hydroxymethyl)propionic acid (bis-MPA) and 1,1,1-tris-(hydroxyphenyl)ethane (THPE) was prepared according to the literature.[55] Well-defined PLLA star polymer, G1-$(OH)_6$, was obtained by ROP of L-lactide from the initiator in bulk at 110 °C, using $Sn(Oct)_2$ as the catalyst, and the targeted degree of polymerization for each arm was 12. It was confirmed that the polymerization had been initiated from all six hydroxyl groups in the initiator by $Sn(Oct)_2$.[59] The six hydroxyl end groups of G1-$(OH)_6$ were then functionalized with a large excess of succinic anhydride in the presence of DMAP and pyridine to form carboxylic acid-terminated G1-$(COOH)_6$ **1** (Scheme 1) with a 89% yield.

The PAMAM dendrons with different generations were synthesized using a divergent method, i.e., the Michael addition of monoprotected ethylenediamine with methyl acrylate, followed by exhaustive amidation of the resulting esters with a large excess of ethylenediamine to give an amine-terminated dendron in a nearly quantitative yield.[60,61] Boc anhydride was selected as the protecting group for the starting ethylenediamine because it could be easily deprotected by TFA to form free amine. The Michael addition and amidation were allowed to repeat a couple times in methanol to form amine-terminated dendrons with various generations, denoted as g0-[Boc, $(NH_2)_2$] **6**, g1-[Boc, $(NH_2)_4$] **7**, and g2-[Boc, $(NH_2)_8$] **8** (Scheme 2). The resulting amine-terminated PAMAM dendrons were then reacted with excess benzyl acrylate at 80 °C for 5 days, and the products were isolated by column chromatography on fumed silica to give the benzyl ester-terminated PAMAM dendrons, denoted as g1-[Boc, $(COOBn)_4$] **9**, g2-[Boc, $(COOBn)_8$] **10**, and g3-[Boc, $(COOBn)_{16}$] **11**, respectively. Here, benzyl acrylate was selected as the final Michael acceptor because it allowed formation of carboxylic acid groups after deprotection. Compounds **9**, **10**, and **11** were thus orthogonally protected; the amine group at the root was Boc-protected, and the carboxylic acid terminal groups were protected by benzyl esters. These orthogonally protected PAMAM dendrons were then deprotected by TFA in dichloromethane at room temperature to provide amine-functionalized PAMAM dendrons in excellent yields, denoted as g1-[NH_2, $(COOBn)_4$] **12** (90% yield), g2-[NH_2, $(COOBn)_8$] **13** (93% yield), and g3-[NH_2, $(COOBn)_{16}$] **14** (95% yield). In this deprotection, the Boc group was selectively removed by acidolysis with TFA without cleavage of the benzyl ester groups. The reaction sequence is shown in Scheme 2.

These benzyl ester-protected PAMAM dendrons with a primary amine at the root allowed for conjugation to the carboxylic acid-functionalized PLLA core, G1-$(COOH)_6$ **1**, by the amide coupling to provide amphiphilic benzyl ester-protected DLSPs, G1-g1-$(COOBn)_{24}$ **15**, G1-g2-$(COOBn)_{48}$ **16**, and G1-g3-$(COOBn)_{96}$ **17** in good yields (82% for **15**, 89% for **16**, and 91% for **17**). PyBOP was found more efficient in the amidization reaction in organic solvents than 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC), DCC, and other commercially available peptide coupling reagents. To obtain carboxylic acid-functionalized DLSP, the benzyl ester groups were deprotected by acidolysis with TFA/MsOH/anisole at 0 °C for a short period of time followed by precipitation in diethyl ether (Scheme 3). Since the tertiary amines in the PAMAM dendrons could be easily protonated by excess MsOH or TFA to form salts, the precipitate was dissolved in a small amount of DMSO and treated with excess TEA to neutralize the protonated tertiary amines in the PAMAM dendrons. Finally, the pure carboxylic acid-terminated DLSPs, G1-g1-$(COOH)_{24}$ **18**, G1-g2-$(COOH)_{48}$ **19**, and G1-g3-$(COOH)_{96}$ **20** were obtained by dialysis in DMSO and subsequent precipitation in diethyl ether with good yields (79% for **18**, 84% for **19**, and 85% for **20**).

The terminal carboxylic acid groups in the PAMAM dendrons rendered us an opportunity to further functionalize the DLSP surface with other groups, including TEG and primary amine. Here, we chose G1-g2-$(COOH)_{48}$ as an example. First, G1-g2-$(COOH)_{48}$ was activated in dry DMSO in the presence of DCC and NHS. Excess DCC was used because G1-g2-$(COOH)_{48}$ was hygroscopic and could contain some moisture and excess DCC could scavenge possible moisture. This activated compound then reacted with amine-functionalized TEG or Boc-protected ethylenediamine in good yields, denoted as G1-g2-$(TEG)_{48}$ **21** and G1-g2-$(NHBoc)_{48}$ **22**, respectively. This amide coupling reaction was found to be much more efficient than the alternative method, where PyBOP was used

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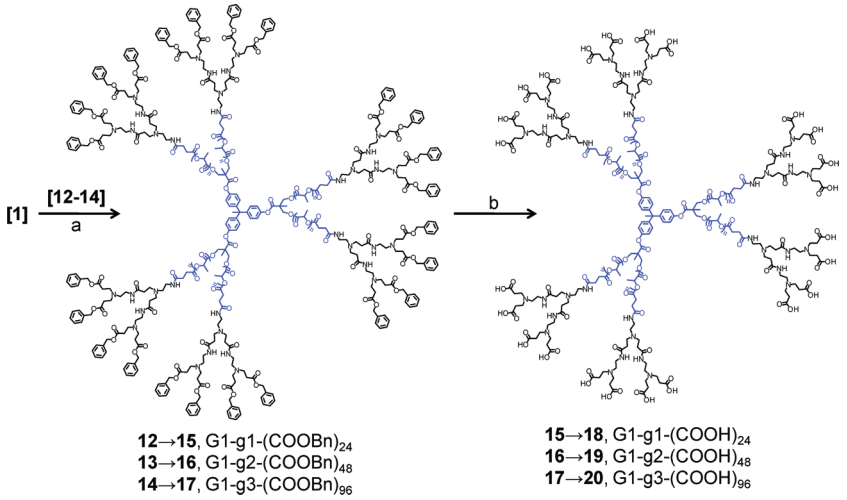
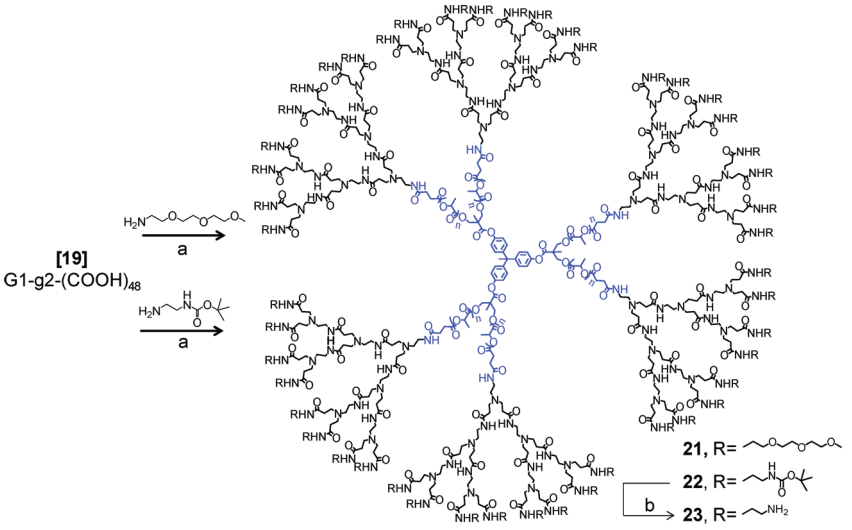
Scheme 3. Synthesis of Carboxylic Acid-Functionalized DLSPs[a]



[a] Reagents and conditions: (a) PyBOP, TEA, CH_2Cl_2, 24 h; (b) TFA/MsOH/anisole, 0 °C, 2 h.

Scheme 4. Synthesis of DLSPs with TEG and Amine Surface Groups[a]



[a] Reagents and conditions: (a) DCC, NHS, DMSO, 2 days; (b) TFA, overnight, and excess TEA.

as the coupling reagent, because of the lower steric hindrance of DCC. The Boc-protected DLSP G1-g2-$(NHBoc)_{48}$ was further deprotected by TFA at room temperature to provide the amine-functionalized PAMAM dendrons in an excellent yield (85%), denoted as G1-g2-$(NH_2)_{48}$ **23** (Scheme 4). Again, the primary and tertiary amines in the PAMAM dendrons could be easily protonated by TFA to form salts. These protonated amines were neutralized with excess TEA in DMSO to provide pure G1-g2-$(NH_2)_{48}$ **23**.

Molecular Characterization. The molecular structure of each compound was confirmed by ^1H and ^{13}C NMR spectroscopy in conjunction with SEC. Figures 1 and 2 show ^1H and ^{13}C NMR spectra for G1-g2-$(COOH)_{48}$ **19** and its intermediates, including g2-(NH_2, $(COOBn)_8$) **13**, G1-$(COOH)_6$ **1**, and G1-g2-$(COOBn)_{48}$ **16**. Figures 1A and 2A show ^1H and ^{13}C NMR spectra for g2-(NH_2, $(COOBn)_8$) **13** with corresponding peak assignments for the deprotected PAMAM dendron. No resonance peak at 1.4 ppm, which was assigned to the Boc protecting group, was observed (Figure 1A), indicating that the Boc group was successfully removed after treatment with TFA. This was also verified by ^{13}C NMR result in Figure 2A, where the Boc peaks at 28.6 and 156.3 ppm (data range not shown) completely disappeared.

Figures 1B and 2B show ^1H and ^{13}C NMR spectra for the succinic anhydride-functionalized PLLA star polymer, G1-$(COOH)_6$ **1**. New peaks (g/g′) appeared around 2.7 ppm, which could be assigned to the methylene proton from the succinic acid chain ends. In the ^{13}C NMR spectrum (Figure 2 B), a new peak (a, b) was found at 28.7 ppm, which could be assigned to the methylene carbons from succinic acid end groups. The complete disappearance of the resonance at 66.6 ppm (data range not shown), which is associated with the last methine carbon in the polymer chain, suggested the complete functionalization by succinic acid. SEC chromatograms in Figure 3 show

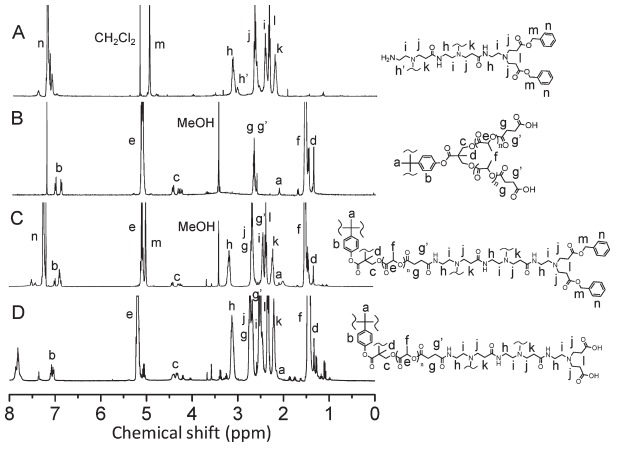


Figure 1. ^{1}H NMR spectra of (A) g2-[NH_2, $(COOBn)_8$] **13**, (B) G1-$(COOH)_6$ **1**, (C) G1-g2-$(COOBn)_{48}$ **16** in $CDCl_3$, and (D) G1-g2-$(COOH)_{48}$ **19** in DMSO-d_6.

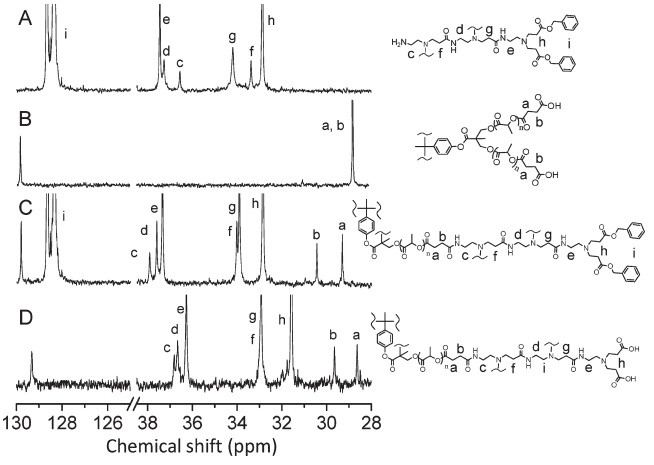


Figure 2. ^{13}C NMR spectra of (A) g2-[NH_2, $(COOBn)_8$] **13**, (B) G1-$(COOH)_6$ **1**, (C) G1-g2-$(COOBn)_{48}$ **16** in $CDCl_3$, and (D) G1-g2-$(COOH)_{48}$ **19** in DMSO-d_6.

that the molecular weight and its distribution (PDI = 1.09) did not change before and after functionalization of G1-$(OH)_6$ with succinic anhydride, indicating that no cross-link occurred during the reaction. Note that there were small shoulder peaks at 27.9 mL retention volume for both G1-$(OH)_6$ and G1-$(COOH)_6$ in Figure 3. By using both UV (wavelength at 254 nm) and differential refractive index (RI) detectors (see Figure S1A in the Supporting Information), we determined that these small shoulder peaks were attributed to the PLLA homopolymer contamination initiated from a trace amount of adventitious co-initiators present in the reaction system rather than the hexahydroxyl co-initiator purposely added.[62] These adventitious co-initiators could include any hydroxyl-containing compounds capable of forming −SnOR species from $Sn(Oct)_2$ in the carboxylate−alkoxide exchange reactions in solvent, monomer, or even $Sn(Oct)_2$ itself. Because the reaction kinetics for L-lactide initiated from the adventitious co-initiators was much slower than that for L-lactide initiated from the added hexahydroxyl co-initiator,[62] the molecular weight of the PLLA homopolymer contamination was lower than that of the G1-$(OH)_6$ star polymer.

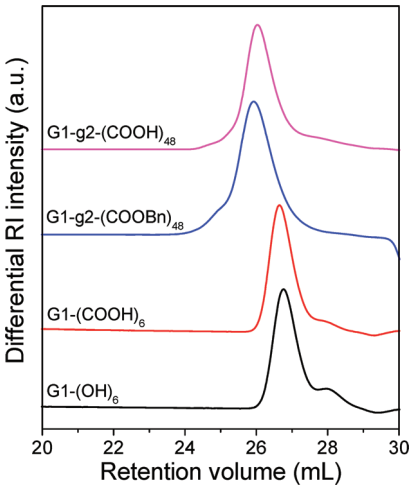


Figure 3. SEC curves of G1-$(OH)_6$, G1-$(COOH)_6$ **1**, G1-g2-$(COOBn)_{48}$ **16**, and G1-g2-$(COOH)_{48}$ **19**.

The benzyl ester-protected DLSP was synthesized by the amide coupling between the carboxylic acid-functionalized PLLA core and amine-terminated PAMAM dendron. Both ^{1}H and ^{13}C NMR were used to investigate whether all six carboxylic acid chain ends were reacted or not (Figures 1C and 2C). In the ^{1}H NMR (Figure 1C), the peak h′ at 3.1 ppm, which is assigned to the methylene protons adjacent to the amine group at the root (see Figure 1A), shifted downfield and overlapped with peak h after the coupling reaction. In the ^{13}C NMR spectra (Figure 2C), the peak at 28.7 ppm from succinic acid chain ends shifted downfield and split into two peaks, a and b, where peak a was the methylene carbon adjacent to ester group and peak b was the methylene carbon adjacent to amide group. Meanwhile, peaks c and d, which were assigned to the methylene carbons adjacent to the amine and amide groups in g2-[NH_2, $(COOBn)_8$] **13** (see Figure 1A), also shifted downfield. The SEC trace in Figure 3 shows that the overall molecular weight increased for the G1-g2-$(COOBn)_{48}$ **16**, but the molecular weight distribution was still fairly narrow with PDI = 1.15. The above results clearly indicated that the carboxylic acid chain ends in G1-$(COOH)_6$ **1** was fully substituted by six benzyl ester-protected PAMAM dendrons. The G1-g2-$(COOBn)_{48}$ **16** was soluble in most organic solvents, such as chloroform, THF, and DMSO, but not in hexane.

The deprotection of benzyl ester group was first attempted by hydrogenolysis using Pd/C as the catalyst, and the complete elimination of the benzyl ester protecting groups was not achieved. Instead, TFA/MsOH/anisole was used as the catalyst to remove the benzyl ester groups at 0 °C. ^{1}H and ^{13}C NMR were used to quantify the complete removal of the benzyl ester groups (see Figures 1D and 2D). The peaks at 5.1 and 7.3 ppm in ^{1}H NMR spectra (Figure 1D) and the peaks at 128.3 and 128.6 ppm in ^{13}C NMR spectra (Figure 2D) from benzyl ester groups disappeared after the deprotection reaction. The number-average molecular weight determined by ^{1}H NMR end-group analysis and the molecular weight distribution measured by SEC analysis (see Figure 3) were unaffected by the acidolysis, indicating that no degradation occurred under the reaction conditions. Ouchi also found that no degradation was observed on PLLA after acidolytic deprotection at 0−5 °C using TFA/MsOH/anisole.[63] The carboxylic acid-functionalized DLSP was

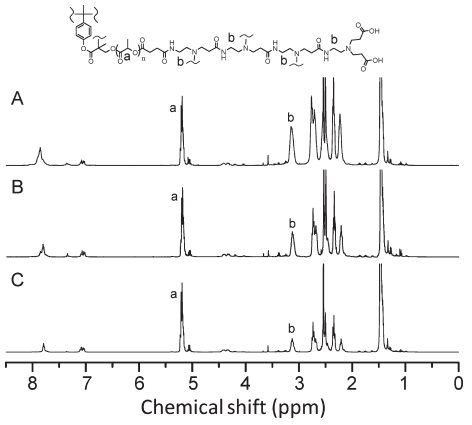


Figure 4. ^{1}H NMR spectra of (A) G1-g3-$(COOH)_{96}$ **20**, (B) G1-g2-$(COOH)_{48}$ **19**, and (C) G1-g1-$(COOH)_{24}$ **18** in DMSO-d_6.

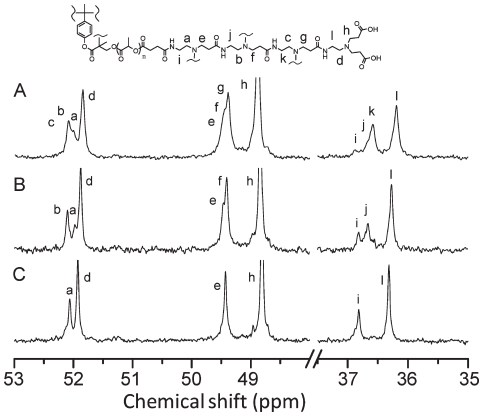


Figure 5. ^{13}C NMR spectra of (A) G1-g3-$(COOH)_{96}$ **20**, (B) G1-g2-$(COOH)_{48}$ **19**, and (C) G1-g1-$(COOH)_{24}$ **18** in DMSO-d_6.

soluble in DMSO, DMF, and DMAc, but not in most other organic solvents. In aqueous solution, their solubilities were around 10 mg/mL (below 10 mg/mL the solution was transparent and above 10 mg/mL the solution showed some turbidity). From SEC results in Figure 3, the small shoulder peaks at a higher retention volume disappeared for both G1-g2-$(COOBn)_{48}$ **16** and G1-g2-$(COOH)_{48}$ **19**. It was likely that the linear PLLA−dendron conjugates had different solubilities in methanol than the DLSP during repeated dissolution (in dichloromethane) and precipitation (in methanol) purification cycles. In other words, the linear PLLA−dendron conjugates were successfully fractionated out in the final products.

^{1}H and ^{13}C NMR spectra of the carboxylic acid-functionalized DLSPs with different generations of PAMAM dendrons are shown in Figures 4 and 5, respectively. Peak b from the PAMAM dendron increased with an increase in generation after normalizing peak a, which is assigned to the methine proton from PLLA in Figure 4. The significantly different chemical shifts from the methylene carbons in PAMAM dendrons clearly reflected the generation number in ^{13}C NMR spectra in Figure 5. The resonances from the methylene groups (a−l) were well separated up to the second generation. In the third-generation DLSP, the resonances from the first- and second-generation methylene groups (b and c, f and g, j and k) became inseparable.

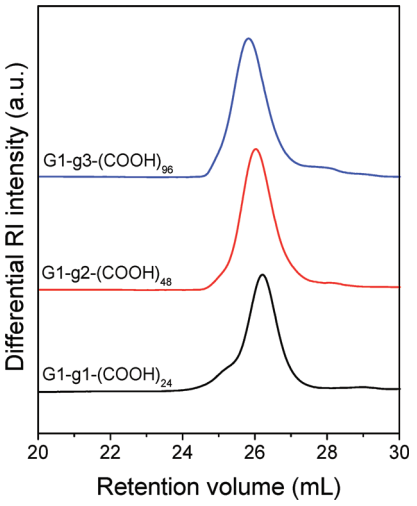


Figure 6. SEC curves of G1-g3-$(COOH)_{96}$ **20**, G1-g2-$(COOH)_{48}$ **19**, and G1-g1-$(COOH)_{24}$ **18**.

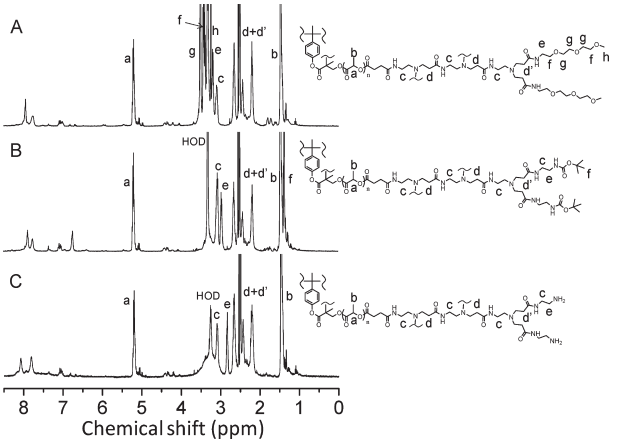


Figure 7. ^{1}H NMR spectra of (A) G1-g2-$(TEG)_{48}$ **21**, (B) G1-g2-$(NHBoc)_{48}$ **22**, and (C) G1-g2-$(NH_2)_{48}$ **23** in DMSO-d_6.

SEC curves of DLSPs with different generations of PAMAM dendrons are shown in Figure 6, where narrow and nearly symmetric peaks are shown [PDI = 1.10 for G1-g1-$(COOH)_{24}$, 1.12 for G1-g2-$(COOH)_{48}$, and 1.13 for G1-g3-$(COOH)_{96}$]. Meanwhile, the DLSP with a higher generation PAMAM dendrons had a smaller elution volume reflecting a higher molecular weight.

The carboxylic acid-terminated DLSP allowed other types of surface functionalization. As shown in Scheme 4, carboxylic acid end groups have been successfully and quantitatively converted to either TEG or primary amine groups. The chemical structures of G1-g2-$(TEG)_{48}$ **21**, G1-g2-$(NHBoc)_{48}$ **22**, and G1-g2-$(NH_2)_{48}$ **23** were confirmed by ^{1}H NMR (see Figure 7). Four new peaks from TEG, denoted as e−h, were observed in Figure 7A after coupling the G1-g2-$(COOH)_{48}$ **19** with amine-terminated methoxy-TEG. Furthermore, peak d′ originating from G1-g2-$(COOH)_{48}$ **19** shifted upfield and overlapped with peak d after terminal amidization. The peak around 3.3 ppm originated from HOD in DMSO-d_6. The coupling of Boc-protected ethylenediamine was verified by the appearance of peaks e and f in Figure 7B. After treating with TFA, peak f almost disappeared and peak e shifted upfield, indicating the removal of

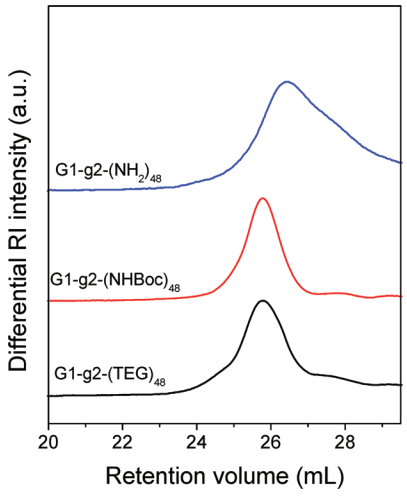


Figure 8. SEC curves of G1-g2-$(TEG)_{48}$ **21**, G1-g2-$(NHBoc)_{48}$ **22**, and G1-g2-$(NH_2)_{48}$ **23**.

Boc protection groups and the formation of primary amine surface groups.

Figure 8 shows SEC curves for three DLSPs with different surface groups. Apparently, no degradation or cross-linking occurred during the synthesis of G1-g2-$(TEG)_{48}$ **21** and G1-g2-$(NHBoc)_{48}$ **22**. Comparing with the SEC result for G1-g2-$(COOH)_{48}$ **19** in Figure 6, the retention volumes for G1-g2-$(TEG)_{48}$ **21** and G1-g2-$(NHBoc)_{48}$ **22** decreased from 26.0 mL for G1-g2-$(COOH)_{48}$ **19** to ca. 25.7 mL, indicating slight increases in hydrodynamic volume and thus molecular weight. After deprotecting the Boc groups in G1-g2-$(NHBoc)_{48}$ **22**, a broad peak at a higher retention volume was observed for G1-g2-$(NH_2)_{48}$ **23**, although no obvious degradation was observed based on ^{1}H NMR end-group analysis. From the previous discussion, we knew that TFA should not degrade the PLLA backbone at 0 °C. Combining the above evidence, we speculated that the terminal primary amine groups in G1-g2-$(NH_2)_{48}$ **23** stuck onto the SEC columns and resulted in a broad peak at a higher retention volume. To confirm this, SEC measurements were performed for two PAMAM dendron samples, g2-[Boc, $(COOCH_3)_8$] **5** and g2-[Boc, $(NH_2)_8$] **8**, to prove whether primary amine-terminated samples would adsorb onto the column or not. It was found that the primary amine-terminated PAMAM dendron, g2-[Boc,$(NH_2)_8$] **8**, eluted out at a much higher retention volume than that of the ester-terminated dendron, g2-[Boc, $(COOCH_3)_8$] **5**, even though the molecular weight increased after amidation with ethylenediamine (see Figure S1B in Supporting Information). Cao et al. also found that the molecular weight and molecular weight distribution of the primary amine-terminated PAMAM could not be determined by SEC due to its strong absorption to the column.[64] Therefore, we concluded that the interaction between the primary amine-terminated sample, G1-g2-$(NH_2)_{48}$ **8**, and the column led to the broadened SEC peak at a higher retention volume with a broad distribution. Note that both G1-g2-$(TEG)_{48}$ and G1-g2-$(NH_2)_{48}$ DLSPs were readily soluble in organic solvents such as chloroform, THF, DMSO, DMF, etc. In aqueous solution, their solubilities were around 25 and 15 mg/mL, respectively (again, below these values the solutions appeared transparent and above these values the solutions were slightly turbid).

Unimolecular Micelles and Aggregates in Aqueous Solutions. The dilute aqueous solution properties of the above amphiphilic DLSPs were studied by DLS. The dilute aqueous solution at 0.05% was prepared by slow addition of doubly distilled water, using a syringe pump, into a 0.05% DMF solution of the corresponding DLSP, followed by dialysis with a dialysis tube having a MWCO of 3000 g/mol (regenerated cellulose dialysis tube from Fisher Scientific) to completely remove DMF. The DLS results for G1-g2-$(COOH)_{48}$ **19**, G1-g2-$(TEG)_{48}$ **21**, and G1-g2-$(NH_2)_{48}$ **23** are summarized in Figure 9; Figure 9A shows the intensity distribution, and Figure 9B shows the volume distribution. From the intensity distribution in Figure 9A, two distinct peaks for the mean hydrodynamic diameter (D_h) were observed: (i) 28 and 205 nm for G1-g2-$(COOH)_{48}$ **19**, (ii) 15 and 295 nm for G1-g2-$(TEG)_{48}$ **21**, and (iii) 14 and 344 nm for G1-g2-$(NH_2)_{48}$ **23**. The smaller mean D_h around 15 nm for G1-g2-$(TGE)_{48}$ **21** and G1-g2-$(NH_2)_{48}$ **23** could be assigned to unimolecular micelles, while the larger mean D_h around 295−344 nm should result from their aggregates in the aqueous solution. We speculate that the hydrophilic dendron shell might not be enough to fully cover the hydrophobic DLSP core, and the interaction among the DLSP cores was the driving force for the large aggregates. Note that the scattered light intensity from the aggregates of G1-g2-$(TEG)_{48}$ **21** was weaker than that from the aggregates of G1-g2-$(NH_2)_{48}$ **23**, suggesting less aggregation for the G1-g2-$(TEG)_{48}$ **21** due to the repulsive TEG surface groups. For G1-g2-$(COOH)_{48}$ **19**, the smaller mean D_h was around 28 nm, almost twice the size of unimolecular micelles (~15 nm). Meanwhile, different from the cases for G1-g2-$(TEG)_{48}$ **21** and G1-g2-$(NH_2)_{48}$ **23**, the smaller D_h peak significantly overlapped with the larger D_h peak. We speculate that the strong hydrogen bonding among the surface carboxylic acid groups in G1-g2-$(COOH)_{48}$ **19** resulted in the formation of dimers or even trimers of the unimolecular micelles. Judging from the highest scattering light intensity for the aggregate at 295 nm for G1-g2-$(COOH)_{48}$ **19**, the amount of aggregates was the highest among three samples and might result from both hydrogen bonding in the dendron shells and hydrophobic interaction among the PLLA cores.

Note that light scattering intensity is not a linear function of the concentration of different species in the solution, and large aggregates have much higher scattering power than individual molecules. Therefore, the intensity distribution in Figure 9A did not represent the population distribution of unimolecular micelles and their aggregates in the aqueous solution. Instead, the information on the population distribution could be obtained from the volume distribution results in Figure 9B. We can see that the amount of the large aggregates for three DLSP samples could nearly be ignored and most population was the unimolecular micelles of G1-g2-$(TEG)_{48}$ **21** and G1-g2-$(NH_2)_{48}$ **23** and dimers of G1-g2-$(COOH)_{48}$ **19**. The unimolecular micelle morphology for G1-g2-$(TEG)_{48}$ **21**, G1-g2-$(NH_2)_{48}$ **23**, and G1-g2-$(COOH)_{48}$ **19** was also studied by TEM, and results are shown in Figure S2 of the Supporting Information. From these TEM observations, the unimolecular micelles seen in DLS were further confirmed in real space.

Thermal Properties of DLSPs with Different Surface Groups. The nature of the surface groups played an important role in both solution and bulk properties of the corresponding DLSP. Figure 10A shows DSC thermograms for G1-g2-$(COOH)_{48}$ **19** and its intermediates. Although the precursor PLLA star polymers, G1-$(OH)_6$ and G1-$(COOH)_6$, could

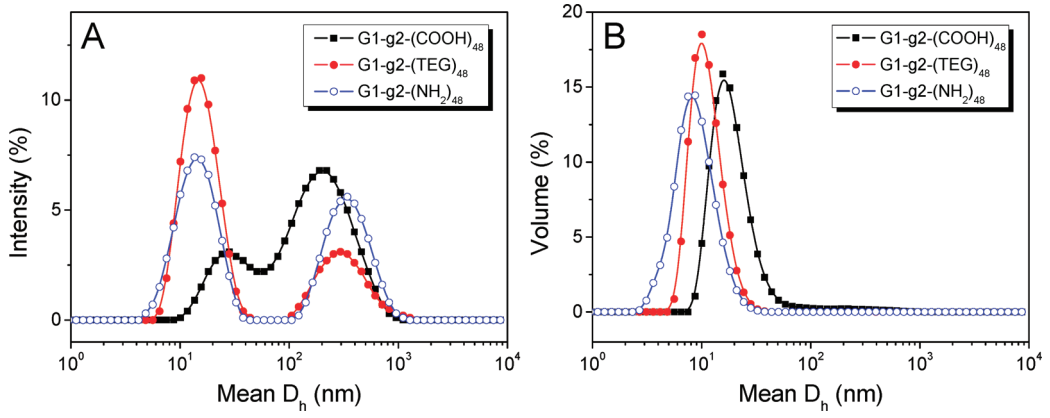


Figure 9. Dynamic light scattering (DLS) (A) intensity and (B) volume distributions for G1-g2-$(COOH)_{48}$ **19**, G1-g2-$(TEG)_{48}$ **21**, and G1-g2-$(NH_2)_{48}$ **23**.

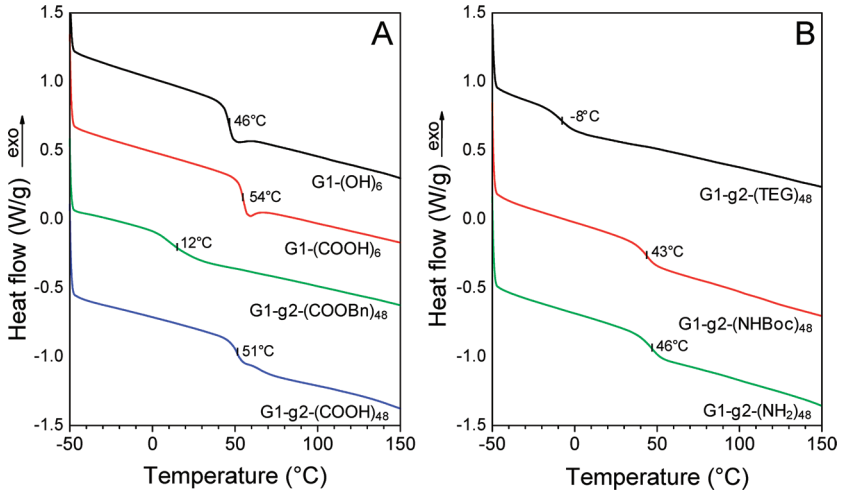


Figure 10. (A) Differential scanning calorimetry (DSC) thermograms of G1-g2-$(COOH)_{48}$ **19** and its intermediates, G1-$(OH)_6$, G1-$(COOH)_6$ **1**, and G1-g2-$(COOBn)_{48}$ **16**. (B) DSC thermograms of G1-g2-$(TEG)_{48}$ **21**, G1-g2-$(NHBoc)_{48}$ **22**, and G1-g2-$(NH_2)_{48}$ **23**.

crystallize after precipitation into methanol (see crystal melting peaks during the first heating process in Figure S3 of the Supporting Information), most samples were completely amorphous during the second heating process. Note that the amorphous state might be more beneficial for drug loading, delivery, and biodegradation than the crystalline state. It was reported that the T_g of dendritic polymers depended mainly on the internal monomer units, number of end groups, and the interaction between the main branch and end groups, such as hydrogen bonding.[46,65,66] This is also found in our DLSP samples. For example, G1-$(OH)_6$ had a T_g around 46 °C, while G1-$(COOH)_6$ **1** had a T_g of 54 °C, suggesting that hydrogen bonding among the end COOH groups promoted the T_g. After conjugated with the PAMAM dendron, g2-[NH_2, COOBn]$_{48}$ **13**, the T_g for G1-g2-$(COOBn)_{48}$ **16** decreased to 12 °C, indicating that the PAMAM dendron was miscible with the PLLA core. Finally, after deprotection G1-g2-$(COOH)_{48}$ **19** exhibited a T_g around 51 °C. Again, hydrogen bonding among the terminal COOH groups increased the T_g. Figure 10B shows the DSC thermograms for G1-g2-$(TEG)_{48}$ **21**, G1-g2-$(NHBoc)_{48}$ **22**, and G1-g2-$(NH_2)_{48}$ **23**. Apparently, attaching TEG to the G1-g2-$(COOH)_{48}$ **19**, the T_g decreased to − 8 °C, again indicating that TEG end groups were miscible with the DLSP and decreased the T_g. However, after converting the COOH end groups to NHBoc and NH_2 groups, the T_g increased to 43 °C for G1-g2-$(NHBoc)_{48}$ **22** and 46 °C for G1-g2-$(NH_2)_{48}$ **23**. This was possibly due to the hydrogen bonding among the terminal amide groups. Comparing the T_g values for DLSP samples with different end groups, the lower T_g of G1-g2-$(NH_2)_{48}$ **23** suggested that the hydrogen-bonding interaction was weaker than that in G1-g2-$(COOH)_{48}$ **19**. This was consistent with the DLS results discussed before, where G1-g2-$(COOH)_{48}$ **19** formed dimers and G1-g2-$(NH_2)_{48}$ **23** only formed unimolecular micelles.

Hydrophobic Drug Encapsulation. Although it is well-known that PLLA is hydrophobic and PAMAM is hydrophilic, to unambiguously determine the amphiphilicity of these DLSPs, a hydrophobic drug molecule, doxorubicin (DOX),[67] is encapsulated into G1-g2-$(TEG)_{48}$ unimolecular micelles. Our result showed that the maximum loading of DOX into the G1-g2-$(TEG)_{48}$ **21** was ca. 11.5 wt % without significant precipitation for a prolonged preriod. Therefore, the solubility of DOX in aqueous solution at pH 7.4 could increase from 0.0625 mg/mL without DLSP encapsulation to as much as 0.272 mg/mL with

DLSP encapsulation. Detailed drug encapsulation and an example release profile of DOX from the G1-g2-$(TEG)_{48}$ DLSP at 37 °C and pH = 7.4 are shown in Figure S5 of the Supporting Information.

CONCLUSIONS

A novel amphiphilic DLSP was synthesized by amide coupling between a carboxylic acid-functional PLLA star polymer and a benzyl ester-terminated PAMAM dendron with a primary amine at the root, using PyBOP as the catalyst. Deprotection of the benzyl ester groups by TFA/MsOH/anisole successfully rendered the corresponding carboxylic acid-terminated DLSP. By varying the generation of the PAMAM dendron, the number of surface functional groups was adjusted from 24 to 48 and finally to 96. The carboxylic acid-terminated DLSP could be further functionalized quantitatively with TEG and primary amine groups. These DLSPs exhibited a bimodal size distribution in aqueous solutions, as studied by DLS. The smaller size peaks in DLS (14−28 nm) were attributed to unimolecular micelles for TEG and amine-terminated DLSPs and dimers (and/or trimers) for the carboxylic acid-terminated DLSP. The large size peaks (205−344 nm) were attributed to the aggregates due to hydrogen-bonding and/or hydrophobic/hydrophilic interactions. Judging from the DLS volume distribution, most DLSPs existed as unimolecular micelles or dimers instead of aggregates. All the DLSPs were amorphous because of the low degree of polymerization (12 for each PLLA arm) and attachment to large PAMAM dendrons. The observation of the single T_g for all DLSP samples suggested that the PLLA core and PAMAM dendrons were miscible. The T_g values were largely dependent on the nature and specific interactions among the end groups; by increasing the hydrogen-bonding interactions among the surface functional groups, the T_g increased. Experimental results showed that these DLSPs had good solubility in aqueous solutions (ca. 10−25 mg/mL) and could greatly enhance the water solubility of a hydrophobic drug, DOX, from 0.0625 up to 0.272 mg/mL. Therefore, these amphiphilic DLSPs are promising candidates for controlled hydrophobic drug delivery.

ASSOCIATED CONTENT

Supporting Information. UV and RI SEC curves for G1-$(OH)_6$; RI SEC curves for g2-[Boc, $(COOCH_3)_8$] **5** and g2-[Boc, $(NH_2)_8$] **8**; TEM micrographs for G1-g2-$(COOH)_{48}$ **19**, G1-g2-$(TEG)_{48}$ **21**, and G1-g2-$(NH_2)_{48}$ **23**; DSC first heating and cooling cycles for G1-g2-$(COOH)_{48}$ **19** and its intermediates; DSC first heating and cooling cycles for G1-g2-$(TEG)_{48}$ **21**, G1-g2-$(NHBoc)_{48}$ **22**, and G1-g2-$(NH_2)_{48}$ **23**; hydrophobic drug (DOX) encapsulation and an example release profile at 37 °C and pH = 7.4. This material is available free of charge via the Internet at http://pubs.acs.org.

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