Amphiphilic Poly(L-lactide)-*b*-dendritic Poly(L-lysine)s Synthesized with A Metal-Free Catalyst and New Dendron Initiators: Chemical Preparation and Characterization

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This study presents investigations on new approaches to novel biodegradable amphiphilic poly(L-lactide)-*b*-dendritic poly(L-lysine)s bearing well-defined structures. First, two new Boc-protected poly(L-lysine) dendron initiators G_2OH **4** (generation = 2) and G_3OH **6** (generation = 3) with hydroxyl end functional groups were efficiently derived from corresponding precursors **3** and **5** via methyl ester substitution with ethanolamine. Subsequently, two series of new diblock copolymers of poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s (**S1**−**S2**, **S3**−**S4**) were prepared in chloroform through ring-opening copolymerization of poly(L-lactide)s with a metal-free catalyst of organic 4-(dimethylamino) pyridine (DMAP) in the presence of a corresponding new poly(L-lysine) dendron initiator. Further, molecular structures of the prepared new dendron initiators as well as those of poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s bearing different dendron blocks and PLLA lengths were examined by means of nuclear magnetic resonance spectroscopy (NMR), gel permeation chromatography (GPC), mass spectrometry (ESI-MS, MALDI-FTMS), and thermal gravimetric analysis (TGA). The results demonstrated successful formation of the synthetic precursors, functional dendron initiators, and new diblock copolymers. In addition, the very narrow molecular weight distributions (PDI = 1.10−1.14) of these poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s further indicated their well-defined molecular structures. After the efficient Boc-deprotection for the dendron amino groups with TFA/CH_2Cl_2, new diblock poly(L-lactide)-*b*-dendritic poly(L-lysine)s bearing lipophilic PLLA and hydrophilic dendritic PLL were finally prepared. It was noteworthy that the MALDI-FTMS result showed that no appreciable intermolecular chain transesterification happened during the ROP of L-lactide catalyzed by the DMAP. Moreover, self-assembly of these new biodegradable amphiphilic copolymers in diverse solvents were also preliminarily studied.

Introduction

In the past decades, amphiphilic copolymers have been attracting much attention from both academic researchers and industrial scientists for their important realized and potential applications in biomimetic studies, drug delivery systems, membranes, functional vectors, and so forth. Diverse methodologies for synthesizing this kind of functional polymer have thus been developed.[1] With respect to the physical behavior of the amphiphilic block copolymers, up to date, it also remains an interesting field particularly concerning their self-assemblies to functional structural hierarchies.[2] On the other hand, as compared to the small molecular surfactants, synthetic macromolecular amphiphiles have been known to be commonly structurally less well defined, and many important parameters would practically influence their self-assembly and aggregation to organize functional microscopic particles.[3−5] Recently, dendritic structural macromolecules have been recognized to bear favorable precise structures, and this paves a possible route to better understand the structure dependence of properties for the synthesized amphiphiles. With the structurally well-defined dendritic macromolecules bearing abundant functional reactive end groups such as amino, hydroxyl, etc., new symmetric star-shaped diblock/multicblock copolymers have been developed via sequentially initiating the copolymerization with functional dendritic cores.[6] Furthermore, several linear-dendritic asymmetric diblock structural copolymers have recently been developed,[7−11] and the shapes of the self-assembled aggregates were found to be able to be readily manipulated for new amphiphilic linear-dendritic block copolymers via varying the size (generation of dendron) of the dendritic block.[12] Depending on the sizes of the hydrophilic head and hydrophobic tail, traditional low molecular weight surfactants could organize micellar, vesicular, and bilayer type functional structures,[12,13] and the amphiphilic linear-dendritic block macromolecules seem very interesting in self-assembly to new functional supramolecular structures.[7−13]

Regarding the asymmetric linear-dendritic block copolymers, up to date, most of the reported works were based on the hydrophilic poly(ethylene oxide) (PEO)[7,8,10] or lipophilic polystyrene (PS)[9] as the linear block, and the dendritic blocks were routinely comprised of benzyl ether,[7] L-lysine,[8] propylene imine,[9] amido amine,[11] and carbosilane.[10] Recently, Klok et al.[13] reported a new kind of linear-dendritic block copolymer consisting of poly(l-lysine) dendron and cholesteryl-substituted poly(L-lactic acid) (PLLA) via chemical coupling the Boc-protected dendrons to the PLLA blocks, and this new type of

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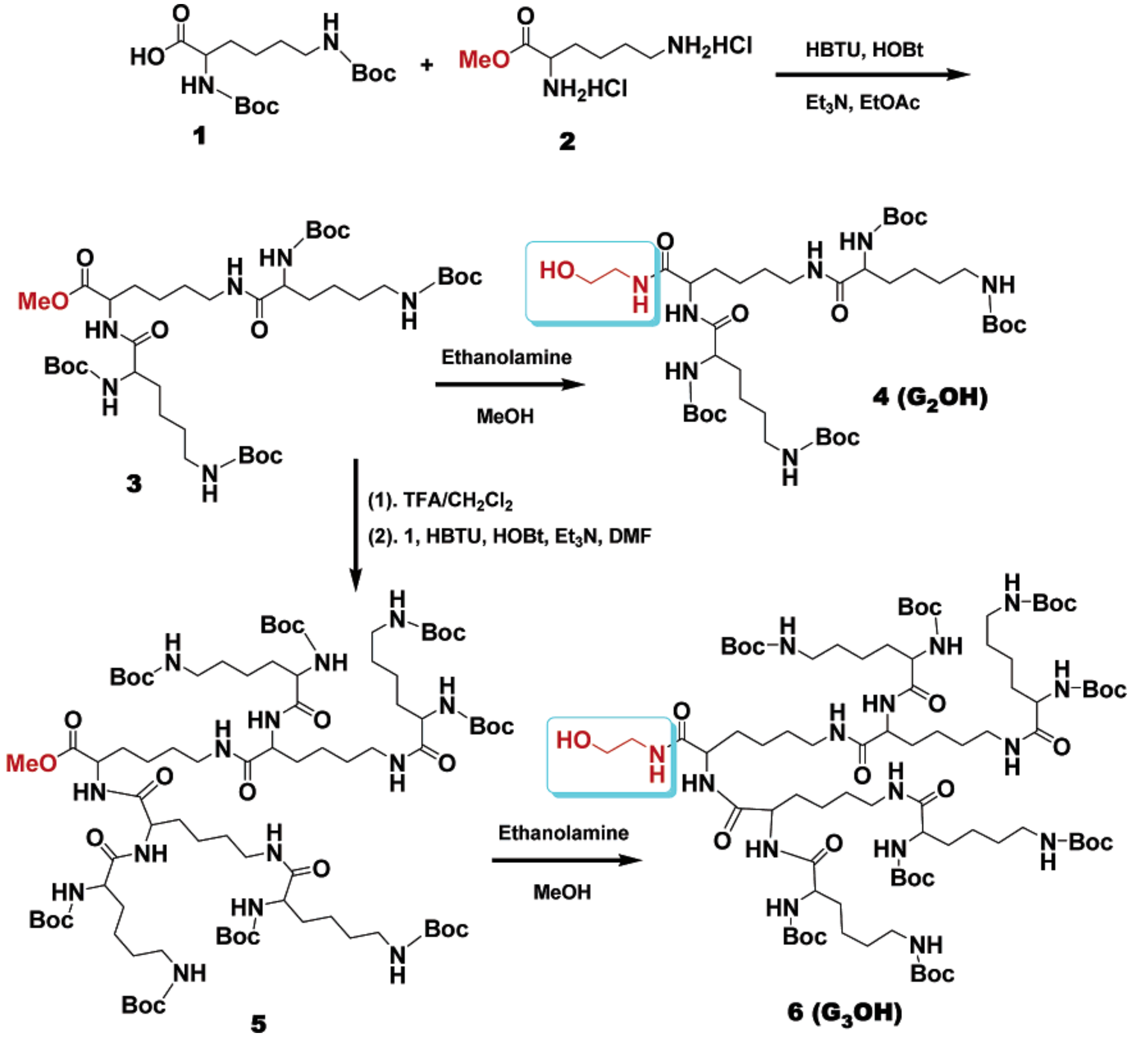
Scheme 1. Synthesis of New Hydroxyl End-Capped Functional Dendritic Boc-Protected Poly(L-lysine) Initiators G_2OH and G_3OH



copolymer was thereby found to have potential applications as temporary scaffolds for cell growth and tissue engineering due to its good biodegradability and biocompatibility.

Routinely, the linear-dendritic type of asymmetric diblock copolymers were reported to be synthesized via a site-specific coupling between a linear and a functional dendritic block[13] or an alternative convergent approach to couple the dendritic monomers to a linear block.[8,9] In practice, the diblock copolymers bearing a linear polyester block were usually prepared by the former strategy due to the lack of enough chain stability for the polyester.[13] Recently, Wursch et al.[14] reported an approach that dendritic polyesters were thereby employed as the initiator to synthesize asymmetric block copolymers with poly(ϵ-caprolactone) as the linear block in the presence of $SnOct_2$ as catalyst. However, there indeed existed few reported works concerning the preparation of new structurally well-defined linear-dendritic diblock polyester-poly(amino acid)s.

In this work, we will present a new approach to synthesize asymmetric biodegradable linear-dendritic structural poly(L-lactide)-*b*-dendritic poly(L-lysine)s bearing the hydrophilic poly(L-lysine) dendron block which has been known to be able to act as functional vectors for DNA transfection.[8,15] For this purpose, two new kinds of hydroxyl end-capped dendritic Boc-protected poly(L-lysine) dendrons, G_2OH **4** and G_3OH **6**, were rationally designed and efficiently prepared. Subsequently, these new dendritic initiators bearing a hydroxyl group were employed to prepare new linear-dendritic poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s with a metal-free organic catalyst of 4-(dimethylamino) pyridine (DMAP)[22] in chloroform. After the amino deprotection, new biodegradable diblock copolymers comprised of lipophilic PLLA and hydrophilic poly(L-lysine) dendron were finally synthesized. In addition, their molecular structures and preliminary self-assembly in diverse solvents have been examined by means of NMR, GPC, TGA, and MALDI-FTMS and are discussed.

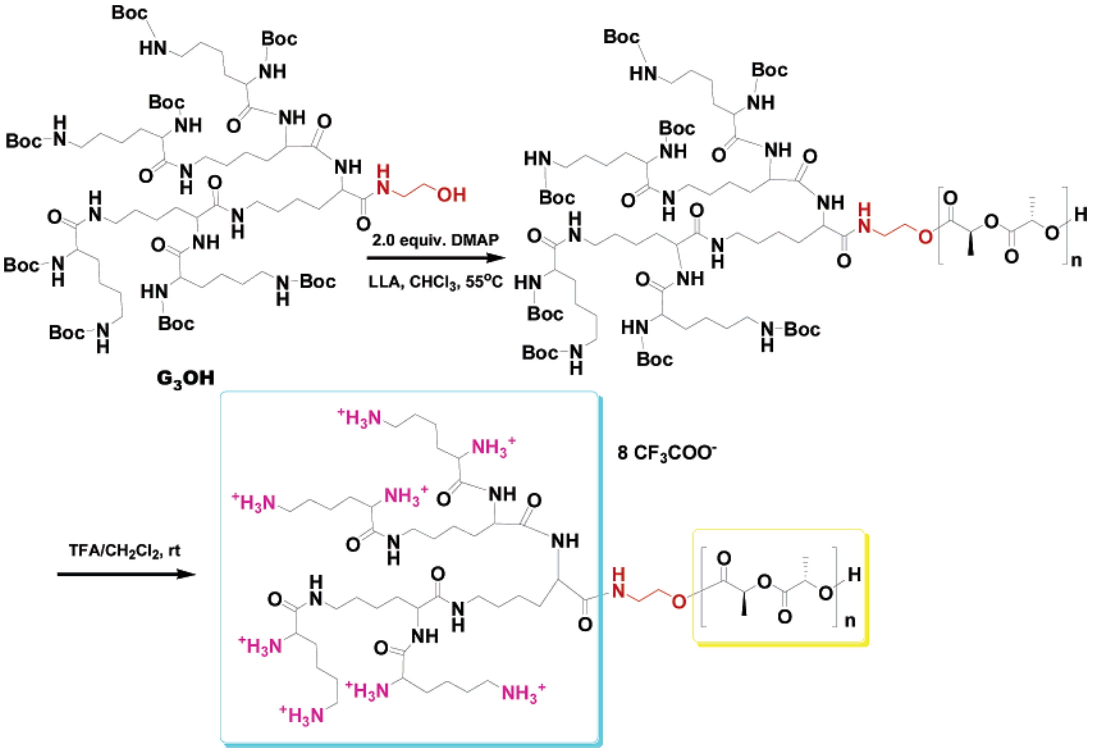
Experimental Section

Materials. N^{α},N^{ϵ}-di-Boc-L-lysine was prepared as partially referred to in the literature.[16] The DMF solvent was dehydrated over molecular sieves (4 Å), and chloroform of analytical grade was in turn washed by sulfuric acid and distilled water and was then dried over CaH_2 for 24 h and distilled prior to use. The monomer of L-lactide was purified via recrystallization in ethyl acetate. 4-(Dimethylamino) pyridine (DMAP) was purchased from Tokyo Kasei Co. Ltd. All other solvents and starting chemicals were purchased from commercial suppliers and used as received.

Syntheses of New Dendritic Poly(L-lysine) G_nOH Initiators and Linear-Dendric Diblock Biodegradable Copolymers. In this study, new dendritic poly(L-lysine) initiators G_nOH and novel linear-dendritic amphiphilic diblock biodegradable copolymers were successfully synthesized with our original synthetic strategies as shown in Schemes1 and 2.

Preparation of G_2COOCH_3 3. L-Lysine methyl ester dihydrochloride **2** (1.68 g, 7.2 mmol) was first well suspended in 80 mL of DMF

Scheme 2. Chemical Synthesis of New Structurally Well-Defined Amphiphilic PLLA-*b*-dendritic Poly(L-lysine)s



at ambient temperature, and then 4.56 g of triethylamine (45.0 mmol) was added; further 5.29 g of N^{α},N^{ϵ}-di-Boc-L-lysine (15.0 mmol) **1** was placed into the reaction mixture. Afterward, this reaction system was stirred under nitrogen atmosphere for 2 min and was cooled to 0 °C. Then 2.04 g (15.0 mmol) of hydroxylbenzotriazole (HOBt) and 5.69 g (15.0 mmol) of *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluromium hexafluorophosphate (HBTU) were added into the reaction system as a solid mixture. Subsequently, the reaction mixture was allowed to warm to the room temperature and stirred for 16 h, and then the reaction mixture was carefully dropped into distilled water. The crude product **3** was extracted by ethyl acetate and was further purified with column chromatography with a mixed elution solvent (silica, ethyl acetate/hexane = 1:1 v/v) to give the final white-colored solid G$_2$COOCH$_3$ **3** product with a good yield of 84%.

Molecular Characteristics of G$_2$COOCH$_3$ ***3***. ^{1}H NMR (in CDCl$_3$, δ in ppm): 7.58 (brs, 1H, CON*H*), 7.15 (brs, 1H, CON*H*), 5.99 (brs, 1H, CON*H*), 5.67 (brs, 1H, CON*H*), 4.94 (brs, 1H, CON*H*), 4.80 (brs, 1H, CON*H*), 4.38 (brm, 2H, COC*H*(R)NH), 4.17 (bm, 1H; COC*H*(R)-NH), 3.71 (s, 3H; COOC*H$_3$*), 3.10 (m, 6H, C*H$_2$*NH), 1.19−1.85 (m, 54 H, C*H$_2$* and C*H$_3$*). ^{13}C NMR (in CDCl$_3$, δ in ppm): 173.5, 172.4 (all *C*O$_2$Me, *C*ONH × 2), 156.2−156.4 (*C*OOC(CH$_3$)$_3$ × 4), 79.6 (O*C*(CH$_3$)$_3$ × 2), 78.5 (O*C*(CH$_3$)$_3$ × 2), 54.0(CO*C*H(R)NH), 53.5 (CO*C*H(R)NH), 52.3 (CO*C*H(R)NH), 52.1 (CO$_2$*C*H$_3$), 40.1 (*C*H$_2$NH × 2), 38.3 (*C*H$_2$NH), 32.9, 32.4, 31.0, 29.3, 29.2, 28.6, 28.3 (all *C*H$_2$ and *C*H$_3$). Mass spectrum (ESI-MS) $C_{39}H_{72}N_6O_{12}$(M_r = 816.5) *m*/*z* = 817.5 (M + H$^+$), 839.5 (M + Na$^+$), 855.5 (M + K$^+$).

Preparation of G$_3$COOCH$_3$ 5. An amount of 1.50 g of G$_2$COOCH$_3$ (1.79 mmol) was first well dissolved in 10 mL of dichloromethane, and then 10 mL of trifluoroacetic acid (TFA) was added, and this mixture solution was stirred at ambient temperature for 1 h. After evaporation of the solvent, the reaction system was dried under vacuum for 1 h. Subsequently, 80 mL of DMF was again placed into the mixture, and then 2.17 g of triethylamine (21.5 mmol) and 2.49 g of N^{α},N^{ϵ}-di-Boc-L-lysine (7.2 mmol) were further added. Afterward, a solid mixture comprising 0.97 g of HOBt (7.2 mmol) and 2.73 g of HBTU (7.2 mmol) was again added. This reaction mixture was stirred at ambient temperature for 1 day. After complete evaporation of the solvent under vacuum, the residual mass was dropped into distilled water. As a result, the precipitated crude product was collected by filtration and was further purified by column chromatography with a mixed solvent (silica, dichloromethane/methanol = 95/5 v/v) to obtain the final white-colored solid G$_3$COOMe product **5** with a synthetic yield of 85%.

Molecular Characteristics of G$_3$COOCH$_3$ ***5***. ^{1}H NMR (in CDCl$_3$, δ in ppm): 4.05−4.25 (m, 7H, COCH(R)N*H*), 3.71 (s, 3H, COOC*H$_3$*), 2.99−3.19 (m, 14H, CH$_2$N*H*), 1.27−1.87 (m, 114H, C*H$_2$* and C*H$_3$*). ^{13}C NMR (in CDCl$_3$, δ in ppm): 173.2−172.3 (all *C*O$_2$Me, CONH × 6), 155.1−155.3 (*C*OOC(CH$_3$)$_3$ × 8), 79.2 (O*C*(CH$_3$)$_3$ × 4), 78.7 (O*C*(CH$_3$)$_3$ × 4), 54.1−53.5 (all CO*C*H(R)NH),52.3(CO$_2$*C*H$_3$), 40.1−38.3 (all *C*H$_2$NH), 32.9−28.3 (all *C*H$_2$ and *C*H$_3$). Mass spectrum (ESI-MS) $C_{83}H_{152}N_{14}O_{24}$ (M_r = 1729.11) *m*/*z* = 1730.0 (M + H$^+$).

Synthesis of New Dendritic Initiator G$_2$OH 4. An amount of 2.50 g of the above prepared G$_2$COOCH$_3$ **3** (2.98 mmol) was dissolved in 25 mL of methanol, and 5 mL of ethanolamine was again added. The reaction mixture was continuously stirred at 55 °C under argon atmosphere for 24 h, and then the solvent was evaporated under vacuum, and aqueous citric acid solution was added. The reaction product was extracted with ethyl acetate and further in turn washed with distilled water and brine. As a result, the obtained solution was dehydrated over anhydrous MgSO$_4$, and then, the residual solvents were evaporated and 10 mL of dichloromethane was added. After complete removal of the solvents under high vacuum, a white solid product of the new dendritic G$_2$OH **4** was finally attained with a good yield of 89%.

Molecular Characteristics of G$_2$OH ***4***. ^{1}H NMR (in CDCl$_3$, δ in ppm): 7.49 (brs, 1H, −CON*H*), 7.33 (brs, 1H, CON*H*), 7.12 (brs, 1H, CON*H*), 5.96 (brs, 1H, CON*H*), 5.71 (brs, 1H, CON*H*), 5.06 (brs, 1H, CON*H*), 4.80 (brs, 1H, CON*H*), 4.32 (brm, 2H, COCH(R)N*H*), 4.20-(bm, 1H, COCH(R)N*H*), 3.68 (m, 2H, HOC*H$_2$*), 3.42 (m, 2H, CH$_2$-CH$_2$N*H*CO), 3.10 (m, 6H, CH$_2$N*H*), 1.17−1.88 (m, 54H, C*H$_2$* and C*H$_3$*). ^{13}C NMR (in CDCl$_3$, δ in ppm): 172.3−173.6 (all *C*ONH), 156.2−156.4 (all NH*C*OOC(CH$_3$)$_3$) 80.1, 79.9(all O*C*(CH$_3$)$_3$), 79.1 (HO*C*H$_2$), 54.2−53.7 (all CO*C*H(R)NH), 42.1−38.5 (all *C*H$_2$NH), 32.4−22.5 (all *C*H$_2$ and *C*H$_3$). Mass spectrum (MALDI-FTMS) $C_{40}H_{75}N_7O_{12}$ (M_r = 845.6) *m*/*z* = 868.6 (M + Na$^+$), 884.5 (M + K$^+$). HRMS (MALDI-FTMS) *m*/*z* = 868.5400 (calculated 868.5366) for $C_{40}H_{75}N_7O_{12}$Na.

Chemical Preparation of New Dendritic Initiator G$_3$OH 6. An amount of 2.50 g (1.45 mmol) of the above-synthesized G$_3$COOCH$_3$ **5** was dissolved in 25 mL of methanol, and further 5 mL of ethanolamine was added into the solution. After stirring the reaction mixture at 55

°C under argon atmosphere for 48 h, the solvent was removed under vacuum, and citric acid aqueous solution was further added. The precipitated crude product was collected via filtration and was washed with distilled water. Finally, the white solid product was dried under high vacuum to give the final new G_3OH **6** with a good synthetic yield of 91%.

Molecular Characteristics of G_3OH ***6.*** ^{1}H NMR (in $CDCl_3$, δ in ppm): 3.92−4.59 (m, 7H, COCH(R)N*H*), 3.70 (m, 2H, $HOCH_2$), 2.97−3.58 (m, 14H, CH_2NH), 1.26−1.92 (m, 114H, CH_2 and CH_3). ^{13}C NMR (in $CDCl_3$, δ in ppm): 172.3−173.2 (all *C*ONH), 156.1−156.4 (all $NHCOOC(CH_3)_3$), 78.9−80.1(all $OC(CH_3)_3$ and $HOCH_2$), 53.6−54.2 (all COCH(R)NH), 38.6−42.4 (all CH_2NH), 21.8−31.8 (all CH_2 and CH_3). Mass spectrum (MALDI-FTMS) $C_{84}H_{155}N_{15}O_{24}$ ($M_r = 1758.1$) $m/z = 1781.3$ ($M + Na^+$), 1797.3 ($M + K^+$). HRMS (MALDI-FTMS) $m/z = 1781.1261$ (calculated 1781.1262) for $C_{84}H_{155}N_{15}O_{24}Na$.

General Synthetic Approaches to New Amphiphilic Linear-Dendritic Diblock Biodegradable Copolymers. Typically, the new hydroxyl end-capped dendric poly(L-lysine) initiator (G_2OH **4**, G_3OH **6**) and a predetermined amount of cyclic L-lactide monomer were first well dissolved in chloroform solution, and 2.0 equiv of 4-(dimethylamino) pyridine (DMAP) was applied as the ring-opening polymerization catalyst to prepare new diblock amphiphiles, and the reaction mixture was stirred at 55 °C under argon atmosphere. After an appropriate period of LLA polymerization, the resultant reaction solution was diluted with dichloromethane and was then in turn washed with dilute citric acid and brine, and the organic layer was concentrated. Finally, the crude product was further purified with column chromatography with a mixed solvent of dichloromethane and methanol (silica, $CH_2Cl_2/CH_3OH = 10/1$ v/v) to give new structural diblock copolymer products with good yields higher than 80%.

Analytical Procedures. *GPC Characterization.* Molecular weights of the prepared new initiators and diblock amphiphiles were characterized at 40 °C on a PerkinElmer 200 series gel permeation chromatograph equipped with a refractive index detector (RI) and a network chromatograph interface NCI 900. Two PLgel 5μm mixed-D-type 300 mm × 7.5 mm columns (Polymer Laboratories Ltd., U.K.) were here employed in series with chloroform as the eluent at a flow rate of 1.0 mL/min, and a commercial polystyrene standard kit from Showa Denko Ltd., Japan was applied for GPC trace calibration. Thus molecular weights (M_w, M_n) and their polydispersity indexes (M_w/M_n) were evaluated.

NMR Characterization. NMR spectra for the dendritic synthetic precursors, new initiators, and diblock amphiphilic copolymers were characterized under ambient temperature on a Bruker AMX300 and Varian VXR 300 Fourier transform NMR spectrometer, operating at 300.0 and 75.5 MHz for the ^{1}H and ^{13}C nuclei, respectively, and tetramethylsilane (TMS) was applied as the internal chemical shift reference.

TGA Thermal Analysis. Thermal gravimetric analyses were conducted on a Perkin-Elmer TGA analyzer under a flowing nitrogen atmosphere (45 mL/min), and the TGA traces were recorded from 50 to 500 °C at 10 °C/min for the characterization of new structural polymers and copolymers.

Mass Spectra. For macromolecular structural characterization, mass spectra (ESI-MS, MALDI-FTMS) were obtained on a Varian SATURN 2000 or FTMS-7 spectrometer.

Results and Discussion

To prepare novel linear-dendritic diblock amphiphilic biodegradable poly(L-lactide)-*b*-dendritic poly(L-lysine)s, new synthetic approaches were rationally designed, and the hydroxyl end-capped dendritic Boc-protected poly(L-lysine) G_2OH **4** and G_3OH **6** with corresponding 2 and 3 generations were first molecularly designed and were thus synthesized according to the pathway as shown in Scheme 1. At first, new methyl ester and Boc-group-protected dendritic poly(L-lysine)s of G_2COOCH_3 **3** and G_3COOCH_3 **5** as the precursors of **4** and **6** were prepared via a divergent synthetic strategy as partially referred to the literature[17] and were further purified with column chromatography. Furthermore, the hydroxyl end-capped dendritic poly(L-lysine) initiators of G_2OH **4** and G_3OH **6** were efficiently prepared through a methyl ester substitution with ethanolamine, where an excess amount of ethanolamine was applied to achieve a complete methyl ester substitution and new formation of the desired hydroxyl end functional groups for further initiation of L-lactide ring-opening copolymerization.

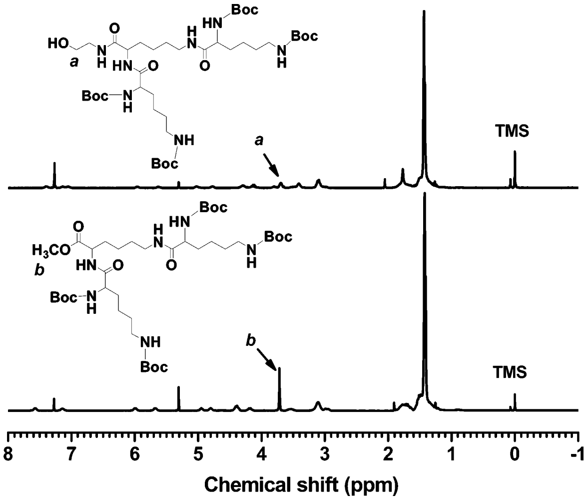


Figure 1. ^{1}H NMR spectra for the synthesized G_2COOCH_3 **3** (bottom) and new dendron initiator G_2OH **4** (top) in $CDCl_3$.

With regard to chemical structural characterization of the synthesized dendritic poly(L-lysine) precursors and new functional ring-opening polymerization initiators with different generations, in this study, ^{1}H and ^{13}C NMR and mass spectrometry (ESI-MS or MALDI-FTMS) were applied. It was found that each mass spectrum exhibited at most three spectral signals, which could be assigned to the corresponding proton-, sodium-, and potassium-ionized mass species, respectively, and that there was no detectable experimental evidence of structural defects in either the poly(L-lysine) dendrons (**3** and **5**) or the incomplete methyl ester substitution (**4** and **6**). Figure 1 depicts ^{1}H NMR spectra for the prepared dendritic G_2COOCH_3 **3** and the G_2OH product **4**. It seems that the proton resonance signal occurring at 3.72 ppm assignable to methyl proton origins of the G_2COOCH_3 **3** disappeared for the product of G_2OH **4**; in the meantime a new resonance signal occurring at 3.68 ppm could be detected for the hydroxyl neighboring methylene protons of $HO{-}CH_2{-}$ of the G_2OH **4**. Therefore, these evidences substantiated a complete methyl ester substitution with ethanolamine and new formation of the desired dendron initiators. In this way, new functional hydroxyl end-capped dendritic Boc-protected poly(L-lysine) initiators G_2OH **4** and G_3OH **6** with 2 and 3 generations were successfully synthesized with good yields close to 90%.

As for the ring-opening polymerization of lactone, up to date, it has been known that Lewis acid type catalysts with different central metal ions such as $SnOct_2$, $AlMe_3$, and so forth, were often applied to prepare favorable structurally well-defined biodegradable polyesters as well as functional block structural copolymers, and the monomer of L-lactide could be routinely polymerized in the bulk state or solution.[18−21] As compared to the bulk polymerization at a temperature higher than the melt point of the PLLA product, the lower reaction temperature of solution polymerization was practically needed for less occurrence of the unfavorable transesterification side reaction. Here,

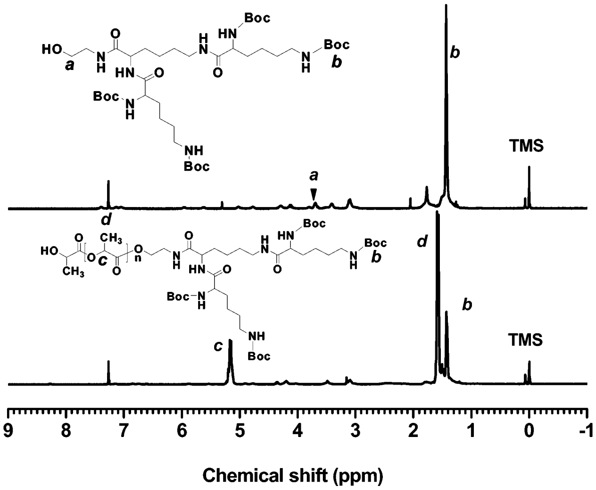


Figure 2. ^{1}H NMR spectra for the new G_2OH dendron initiator **4** (top) and the prepared PLLA-*b*-dendric Boc-protected poly(L-lysine) **S1** (bottom) in $CDCl_3$.

Table 1. Ring-Opening Polymerization of L-Lactide in Chloroform with New Boc-Protected Poly(l-lysine) Dendritic Initiators Catalyzed by DMAP

entry	initiator	[LLA]/[I]	reaction time (h)	DP[a]	yield (%)[b]	PDI[c]
S1	G_2OH	15	20	14	85	1.10
S2	G_2OH	25	41	23	88	1.10
S3	G_3OH	15	40	14	84	1.14
S4	G_3OH	30	80	26	81	1.13

[a] Degree of polymerization (DP) for the poly(L-lactide) block was evaluated by ^{1}H NMR. [b] The values express the synthetic yield. [c] Molecular weight distributions denoted as PDI were evaluated by GPC in chloroform for the new synthesized PLLA-*b*-dendritic poly(L-lysine) diblock polymers.

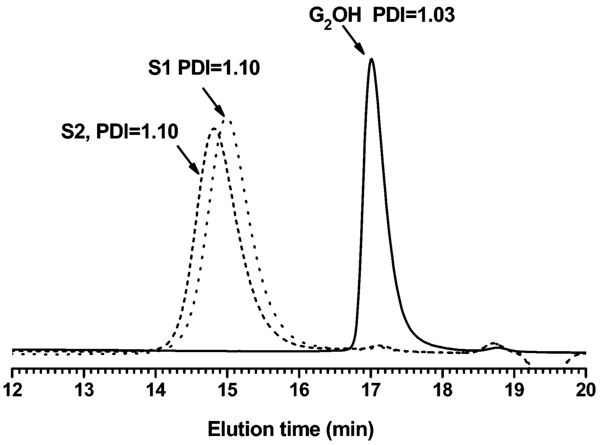


Figure 3. GPC elution traces for the synthesized new poly(L-lactide)-*b*-dendric Boc-protected poly(L-lysine)s **S1** and **S2** as well as that for the dendron initiator **4**.

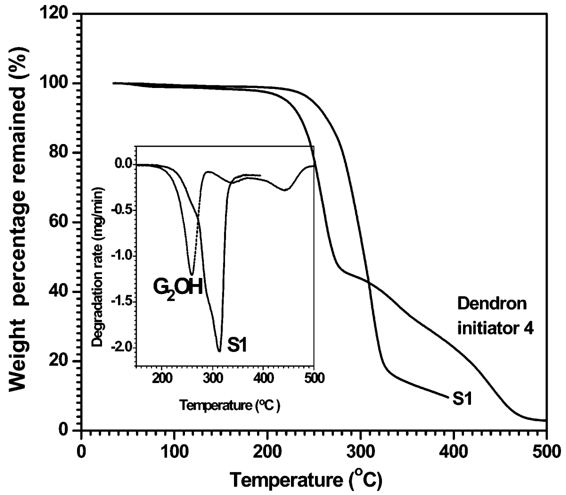


Figure 4. Thermal degradation rate profiles of the new diblock copolymer **S1** as well as that of its initiator.

it was found that the prepared new dendritic initiator G_3OH **6** formed an insoluble gel in a solvent of either toluene or THF even at a very low mass concentration. In contrast, these synthesized new poly(L-lysine) dendron initiators were found to be well dissolved in chloroform, and there were few literature reports on the polymerization of lactide in chloroform.

Recently, Nederberg et al.[22] reported a new interesting and efficient organic catalytic system for the synthesis of poly(lactide) in the presence of an alcohol initiator under mild condition, and 4-(dimethylamino) pyridine (DMAP) was thereby revealed to be a metal-free ring-opening polymerization catalyst with favorable less intermolecular transesterification and racemization. Hence, the organic DMAP catalytic system was hereby employed to prepared new linear-dendritic biodegradable amphiphilic diblock structural copolymers as shown in Scheme 2, and an optimized 2.0 equiv amount of DMAP catalyst was applied. It was found that the ring-opening copolymerization of poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s did not happen at 35 °C in dichloromethane with the above-prepared new dendron initiators (**4**, **6**), while good copolymerization results were achieved at 55 °C in chloroform.

Figure 2 shows the ^{1}H NMR spectrum in $CDCl_3$ for the prepared new diblock poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine) **S1** (bottom) as well as the corresponding dendron initiator G_2OH **4**. It could clearly be seen that the characteristic proton resonance signals attributed to both the poly(L-lactide) and Boc-protected poly(L-lysine) dendron blocks concurrently occurred as detected as the signal *c* of lactide methine protons and the signal *b* of the dendron Boc-groups. Meanwhile, the characteristic proton resonance signal *a* (upper) at 3.68 ppm attributable to the $HO-CH_2-CH_2-NH-$ (G_2OH **4**) entirely disappeared after the initiation of lactide ring-opening polymerization. Hence, these proton spectral evidences indicated the efficient initiation and new formation of the linear-dendritic diblock copolymers. In general, Table 1 summarizes the synthetic results of the new poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s at 55 °C under various L-lactide monomer to the new dendron initiator (G_2OH **4** or G_3OH **6**) molar ratios with 2.0 equiv amount of organic DMAP catalyst. For the **S1** and **S2** entries, it could be seen that the higher l-lactide to dendron initiator G_2OH molar ratio finally led to longer ROP reaction time to achieve a similar synthetic yield close to 85%, and a polymerization time of 41 h for **S2** was indicated, about 2 times that of the 20 h for the **S1** entry, and that GPC showed much low molecular weight distributions (PDI = 1.10) of the new diblock products, implying the formation of well-defined macromolecular architectures. With regard to the 3-generation dendron initiator of G_3OH **6**, the entries of **S3** and **S4** indicated a similar lactide to the G_3OH **6** molar ratio dependence of ring-opening copolymerization time. Moreover, as compared to those of the **S1** and **S2** entries initiated with the G_2OH **4**, much longer reaction times for the **S3** and **S4** entries were actually needed to achieve good yields. When taking the dendritic molecular structures of the individual G_2OH **4** and G_3OH **6** initiators into consideration, the lower reaction activities of the **S3** and **S4** entries could be reasonably interpreted due to

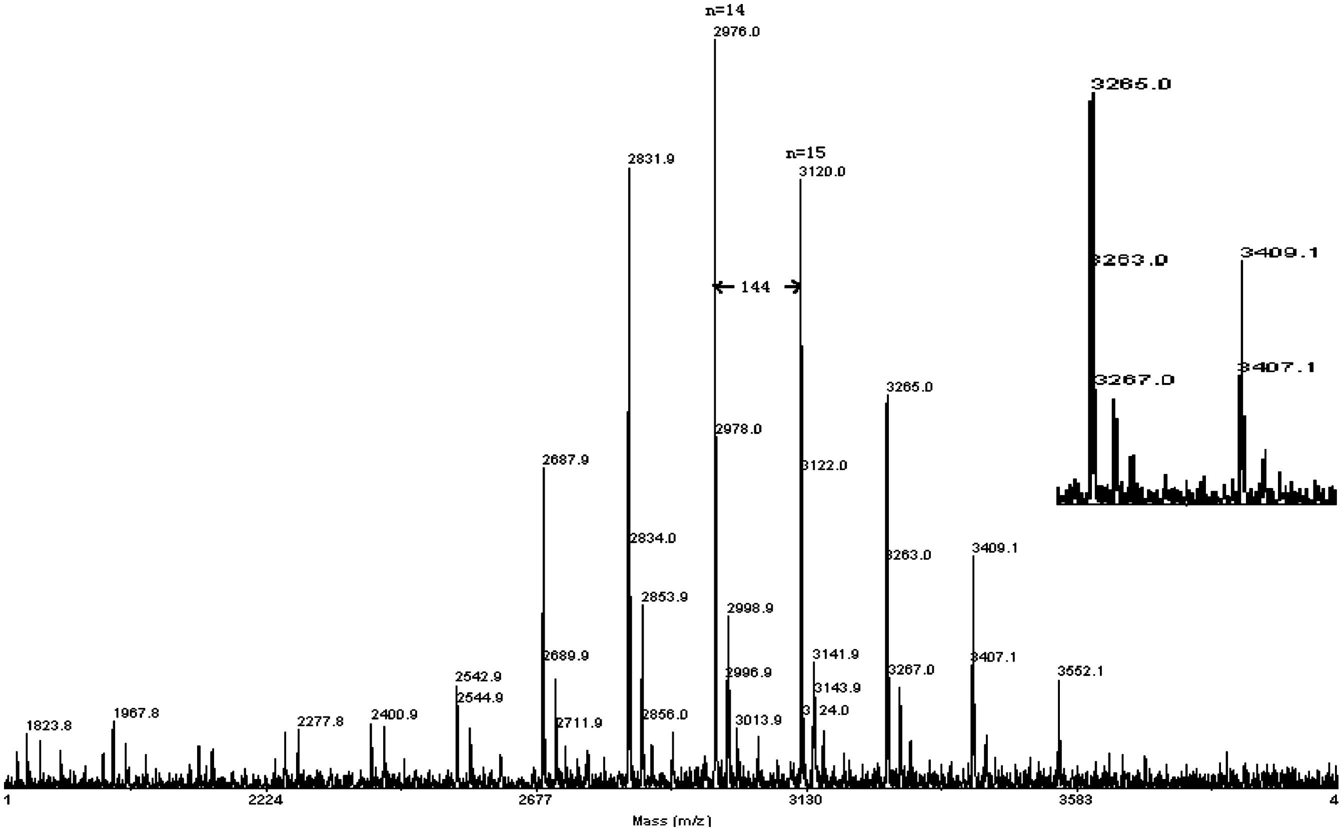


Figure 5. MALDI-FTMS spectra for the new amphiphilic diblock copolymer of the Boc-deprotected **S3**.

the steric effect around the hydroxyl groups of the true dendron initiators, and the higher generation of dendron initiator would finally lead to more remarkable steric hindrance around the hydroxyl initiation groups, thus decreasing the apparent reaction rates. In this way, two series of novel linear-dendritic poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s were efficiently prepared with very narrow molecular weight distributions and well-defined structures.

For further structural elucidation of the synthesized new diblock copolymers, Figure 3 depicts GPC elution traces for the diblock **S1** and **S2** bearing different poly(L-lactide) block lengths as well as their dendron initiator G_2OH **4**. It could be seen that only single symmetric narrow GPC elution peaks were detected for either the synthesized poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s (dotted line) or the dendron initiator G_2OH **4** (solid line) and that the copolymerization with L-lactide led to the shift of elution trace toward high molecular weight region. In addition, Figure 4 shows TGA thermal degradation profiles for the diblock **S1** and the G_2OH **4**, and corresponding thermal degradation rate curves are also shown as an inset. The Boc-protection groups were observed to decompose first for both the G_2OH **4** and diblock **S1**, indicating the lower thermal stabilities of the Boc moieties. On the other hand, as compared with the diblock **S1**, the weight loss percentage of the Boc moieties seemed much higher for the G_2OH **4** than those in the diblock **S1**, implying the decrease in Boc-moiety population due to the poly(L-lactide) copolymerization. Therefore, these new evidences further substantiated the well-defined structures of the novel diblock poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s.

For the sake of preparing new diblock structural amphiphiles, the Boc moieties for amino group protection in the poly(L-lysine) dendron block were efficiently removed with TFA/CH_2Cl_2 at the room temperature.[23] Figure 5 shows the MALDI-FTMS spectrum for the new amphiphilic poly(L-lactide)-*b*-dendritic poly(L-lysine)s after complete Boc-deprotection of the diblock **S3**. New three-homologous series of mass spectral signals were clearly observed, which could be assigned as the corresponding H^+-, Na^+-, and K^+-ionized macromolecular ions. In the meantime, it was interestingly found that the mass difference between two neighboring spectral peaks in Figure 5 was 144 Da, which is just equal to the mass of a repeating unit of the poly(L-lactide) block. Therefore, this mass spectral result unambiguously concluded that there was no appreciable intermolecular transesterification occurring during the DMAP-catalyzed copolymerization of poly(L-lactide). On the basis of the MALDI-FTMS spectrum, the degree of polymerization (DP) for the poly(L-lactide) block was calculated to be about 14, and this result agreed well with the value as evaluated by ^1H NMR.

Amphiphilic block structural copolymers have been known to assemble interesting supramolecular structures driven by solvophobic association in a selective solvent.[13] After the Boc-deprotection of the amino groups of the **S3**, the dendritic poly(L-lysine) block turned water-soluble, and the poly(L-lactide)-*b*-dendritic poly(L-lysine)s became a new amphiphilic copolymer comprised of biodegradable lipophilic PLLA and hydrophilic dendritic poly(L-lysine) blocks. Here, it was found that the new poly(L-lactide)-*b*-dendritic poly(L-lysine) could be partially or completely soluble in DMSO-d_6, D_2O, and $CDCl_3$. Recently, proton NMR spectroscopy has been known as a facile tool to reveal the assembled structures for an amphiphilic block copolymer, and only the segments of the solvent-soluble block with enough mobility could be detected with strong resonance intensities.[13] For the new poly(L-lactide)-*b*-dendritic poly(L-lysine), D_2O and $CDCl_3$ were experimentally found to be selective solvents for two blocks, while DMSO-d_6 was tested to be a common good solvent. Figure 6 depicts the ^1H NMR of the new amphiphilic biodegradable poly(L-lactide)-*b*-dendritic

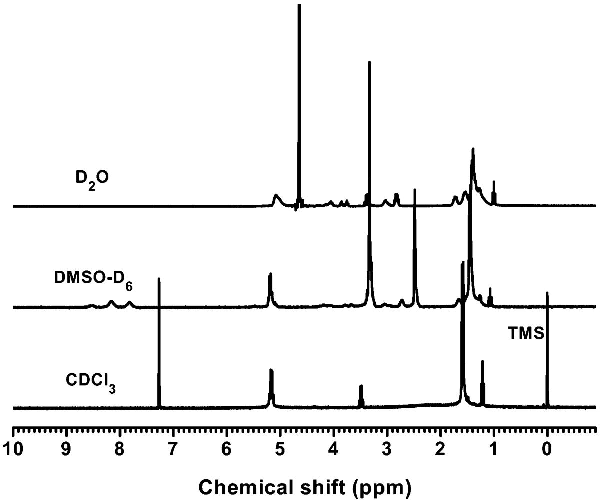


Figure 6. ^{1}H NMR spectra for the new amphiphilic diblock copolymer of the Boc-deprotected **S3** in diverse solvents.

poly(L-lysine) of the Boc-deprotected **S3** in diverse solvents. In $CDCl_3$ (bottom), only the resonance signals attributable to the solvent-soluble PLLA block and the remaining Et_2O impurities could obviously be detected, while the water-soluble dendritic poly(L-lysine) block became to be observed in the solvent of D_2O (upper). With regard to the common solvent of DMSO-d_6 (middle), the proton resonance signals for both the PLLA and dendritic poly(L-lysine) blocks could concurrently be observed. Therefore, these spectral results pave new possibilities for these novel amphiphilic biodegradable linear-dendritic diblock copolymers to assemble functional vectors for potential applications in gene transfection and construction of drug delivery systems.

Conclusions

In this study, novel amphiphilic biodegradable asymmetric poly(L-lactide)-*b*-dendritic poly(L-lysine)s bearing well-defined structures have been efficiently synthesized via our new original strategies. First, two hydroxyl end-capped functional dendritic poly(L-lysine) initiators of G_2OH **4** (generation 2) and G_3OH **6** (generation 3) were rationally designed and prepared with good yields from their corresponding precursors G_2COOCH_3 **3** and G_2COOCH_3 **5**. Furthermore, their molecular structures were characterized by NMR and mass spectrometry, clearly demonstrating the formation of structurally well-defined dendritic initiators. Instead of a routine Lewis acid type organometallic catalyst, new organic DMAP was hereby employed to catalyze the ring-opening copolymerization of linear poly(L-lactide) block to prepare two series of new linear-dendritic poly(L-lactide)-*b*-dendritic Boc-protected poly(L-lysine)s (**S1**−**S4**) in the presence of the prepared new initiators. NMR, GPC, and TGA experimental evidences confirmed efficient initiation of the dendritic initiators and the formation of new diblock structural copolymers bearing narrow molecular weight distributions (PDI = 1.10−1.14). After the amino-deprotection of the dendron block with TFA/CH_2Cl_2, new amphiphilic poly(L-lactide)-*b*-dendritic poly(L-lysine)s with a lipophilic polyester and a hydrophilic dendritic poly(L-lysine) block were finally prepared. The MALDI-FTMS spectrum of the new amphiphilic diblock copolymer after the Boc-deprotection of **S3** interestingly indicated that there were no appreciable intermolecular transesterification side reactions happening during the new organic DMAP-catalyzed ring-opening copolymerization of the linear poly(L-lactide) block under mild conditions. In addition, self-assemblies of the prepared new amphiphilic diblock copolymer were preliminarily investigated in diverse solvents, indicating the formation of "core−shell" type of functional supramolecular structures in a selective solvent like water and chloroform. Hence, due to the interesting positively charged amino functional groups of hydrophilic poly(L-lysine), dendron blocks have been reported to play as functional vectors for external DNA transfection; these novel biodegradable amphiphilic diblock copolymers would pave new possibilities as functional biomaterials for construction of new gene and drug delivery systems, and the related study is now ongoing in this lab.

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