Articles

Synthesis and Conformations of Dendronized Poly(L-lysine)

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ABSTRACT: Dendronized polymers based on a poly(L-lysine) backbone have been synthesized up to the fourth generation. The hydroxyl-terminated polymers are completely water-soluble, which makes them good candidates for drug delivery applications. The dendronized polypeptide backbones are helical at lower generations, but undergo a dramatic conformational change from α-helical to disordered upon increasing the dendron size to the third generation. This conformational change, attributed to steric repulsions between dendrons, is supported by spectroscopic measurements, while chain extension upon dendronization is confirmed by scanning force microscopy.

Introduction

Dendronized linear polymers are linear polymers that bear pendant dendrons at every single repeat unit.[1] As the sizes and generations of the appended dendrons increase, the number of unfavorable repulsive interactions between adjacent dendrons also increase, and as the dendrons must pack around the polymer main chain, the latter stretches, resulting in shape-persistent macromolecules with extended, rigidified backbones. Because of their unique backbone conformations and the encapsulating effect of pendant dendrons on a polymer chain, dendronized polymers are the first known macromolecules that behave as single molecule glasses[2] and are therefore candidates for a variety of materials applications. For example, we have used them recently as catalysts in organic chemistry,[3] as "molecular pixels" in imaging applications,[4] and as carriers for drug delivery.[5] The influence that dendrons can have on a polymer backbone has been explored by a number of groups, and while dendron side chains most notably affect a main chain's persistence length and rigidity,[6−9] other properties such as helicity[10−15] and extended conjugation lengths[16,17] for conjugated polymers can be imparted upon a polymer backbone.

We have recently become interested in establishing the utility of rigid-rod dendronized linear polymers for drug delivery applications.[5] We believe that the usefulness of these polymers derives mainly from their multivalent and highly functionalizable structures that enable facile drug attachment, and from their rodlike conformation that may result in interesting pharmacokinetic properties.[18,19] Initial synthetic work toward hydrophilic dendronized polymers suitable for drug delivery applications focused on relatively hydrophobic poly(4-hydroxystyrene) and substituted poly(ϵ-caprolactone), which gave water-soluble materials at high generations but had limited water solubility at low generations.[20−22] Because the backbone of a dendronized polymer represents a larger proportion of the overall macromolecule than the core of a dendrimer, its effect on the overall solubility of the macromolecule should be greater, especially at low generation. Therefore, postulating that more polar backbone repeat units would enhance water solubility at lower generations, poly(α-amino acid)s were considered as suitable backbones for our purposes since they are easily prepared, allow for the presence of backbone functionalities, and have biocompatibility profiles for both the polymers and their degradation products that are well documented.[23,24] Since the preparation of dendronized polymers following a facile divergent dendronization route[20−22] requires an amino acid that contains a nucleophilic pendant moiety, polymers based on L-lysine were chosen for this work. Poly(L-lysine) and its derivatives have been thoroughly studied, often demonstrating good water solubility and ready functionalization.[25−29] In addition, poly(L-lysine) is known to adopt well-defined secondary structures,[26,30] and since the polymer is capable of forming α-helices we hypothesized that such a conformation might aid in the rigidification and straightening of the polymer backbone.

We now report the preparation of water-soluble dendronized linear polymers based on a poly(L-lysine) backbone, and discuss the surprisingly striking dependence of polymer conformation on the generation of the attached polyester dendrons.

Results and Discussion

Poly(L-lysine) samples of varying molecular weights were prepared as described in the literature[31,32] from ϵ-carbobenzyloxy-L-lysine *N*-carboxyanhydride. Dendronization of the poly(L-lysine) was subsequently carried out to the fourth generation via an iterative anhydride-coupling and deketalization procedure that utilized the anhydride of isopropylidene-2,2-bis(oxymethyl)propionic acid (**11**) (Scheme 1).[22,33,34] Monitoring the various coupling and deprotection steps by NMR spectroscopy and elemental analysis suggested that they were essentially quantitative, and SEC-MALLS data showed good agreement between the experimental and theoretical increases in molecular weights upon dendronization. The molecular characteristics of a representative polymer with an average degree of polymerization of ∼150 are presented in Table 1. All of the hydroxyl-terminated

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Scheme 1. Divergent Dendronization of Poly(L-lysine)

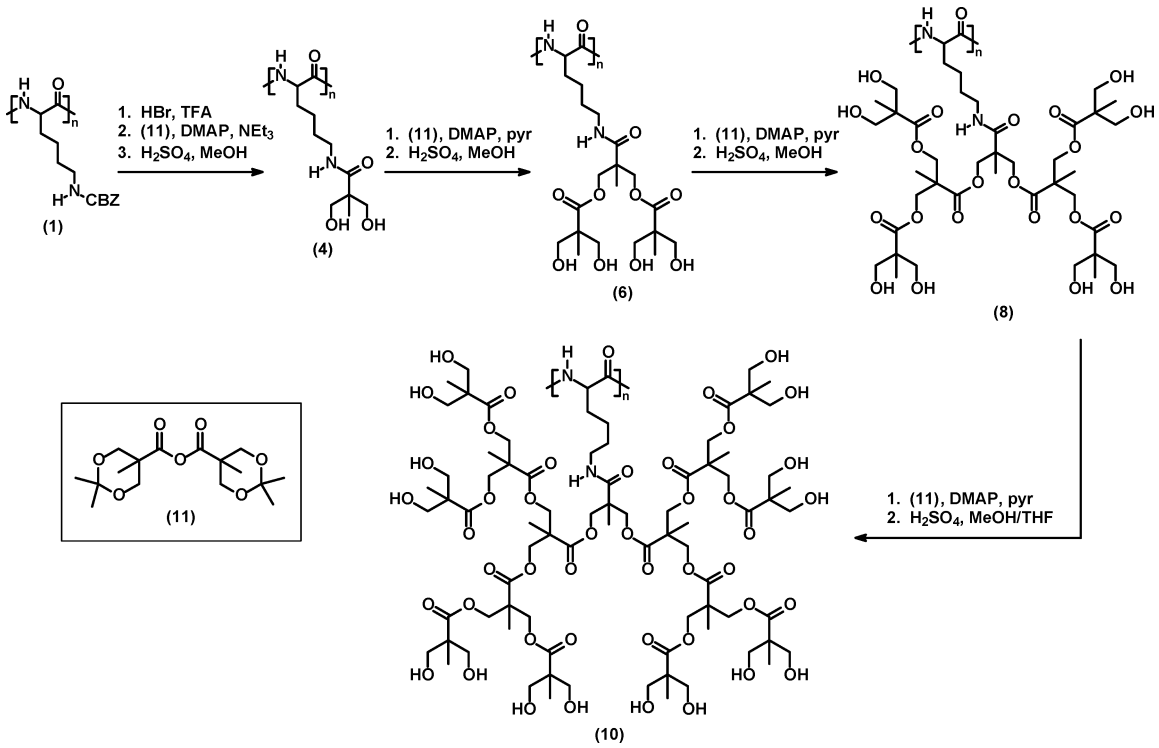

Table 1. Characterization of Dendronized Polymers 1−10

	SEC[a]			SEC-MALLS[b]		theor[c]
	M_n	M_w	PDI	M_w	PDI	M_w
poly(Z-L-lysine) (**1**)	15 900	32 500	2.04	50 500	1.12	
poly(L-lysine) (**2**)	28 300[a]	35 300[a]	1.25[a]			24 700
poly([G1]-(O_2Ac)-L-lysine) (**3**)	17 800	34 000	1.90	48 100	1.13	54 700
poly([G1]-(OH_2)-L-lysine) (**4**)	29 100	46 600	1.60			47 000
poly([G2]-(O_2Ac)2-L-lysine) (**5**)	37 100	54 000	1.45	92 200	1.12	107 200
poly([G2]-(OH_2)$_2$-L-lysine) (**6**)	51 400	63 300	1.23			91 700
poly([G3]-(O_2Ac)4-L-lysine) (**7**)	76 400	92 700	1.21	188 000	1.08	212 013
poly([G3]-(OH_2)4-L-lysine) (**8**)	101 400	119 500	1.18			181 200
poly([G4]-(O_2Ac)$_8$-L-lysine) (**9**)	108 200	129 900	1.20	372 000	1.17	421 700
poly([G4]-(OH_2)$_8$-L-lysine) (**10**)	113 600	139 400	1.23			360 000

[a] For all polymers except **2**, SEC measurements were in DMF with 0.2% LiBr, calibrated with PMMA standards. For **2**, SEC measurements were in 0.5 M acetic acid and 0.3 M Na_2SO_4 at pH 3, calibrated with polysaccharide standards. [b] Absolute molecular weight determined by SEC in DMF with multiangle laser light scattering detection (SEC-MALLS). [c] Calculated M_w based on the DP of **1** determined by SEC-MALLS and the assumption of quantitative reactions at each step.

polymers from generations one through four were freely soluble in water, an important attribute for their intended application as drug carriers.

The secondary structures of the polymers in pH 7.4 buffer were characterized using circular dichroism (CD) spectroscopy. The CD spectra for the first- and second-generation hydroxyl-terminated dendronized poly(L-lysine)s contained features in the 200−250 nm absorbance range suggestive of an α-helix (Figure 1).[30] It should be noted that while poly(L-lysine) itself possesses a disordered "random coil" structure in water at neutral pH because it is a polyelectrolyte, a helical conformation can be obtained by acylation of its ϵ-amino groups.[25,26,29,35] In contrast to the first- and second-generation materials, the third- and fourth-generation dendronized polymers exhibited CD spectra consistent with a disordered backbone conformation (Figure 1). Although the spectra presented here are for a polymer with an average degree of polymerization of ∼150, the spectral features of polymers of equivalent generations were also similar for shorter chain lengths.

Monitoring of the amide bands by infrared spectroscopy was not helpful in determining the polymers' secondary structure[36]

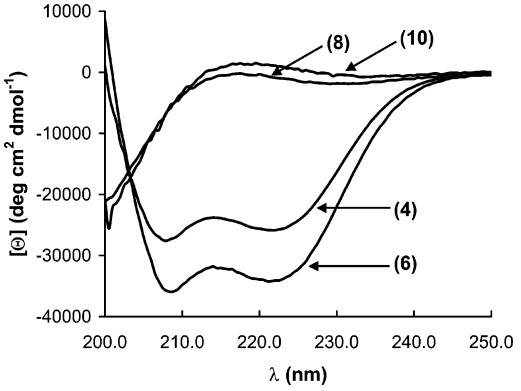

Figure 1. Circular dichroism spectra of dendronized linear polymers in aqueous buffer (pH 7.4).

due to the presence of overlapping side chain amide bands, but ^{1}H and ^{13}C NMR spectroscopies did provide evidence of a helix to coil transition. As shown in Table 2, a comparison of the observed chemical shifts for the polypeptide α-carbons and protons in the first- through third-generation polymers with the

Table 2. Chemical Shifts for α-Protons and α-Carbons of the Studied Polypeptides

	$^1H^b$	$^{13}C^b$
α-helix[a]	3.98 ± 0.26	59.11 ± 1.19
random coil[a]	4.28 ± 0.31	56.4 ± 1.80
4	3.94	59.1
6	3.93	59.1
8	4.0−4.7	54.5

[a] Published average chemical shifts and standard deviations for polypeptide lysine residues.[37] [b] Chemical shifts for **10** were of insufficient intensity to be observed by NMR.

published values for α-helical and random coil polypeptides supports the occurrence of such a conformational switch upon dendronization.[37]

The transition of a compact α-helical polypeptide backbone to a disordered but more extended state resulting from dendronization would be expected to lead to measurable differences in the dimensions of the second- and third-generation polymers in the solid state. This prediction could be confirmed visually through the use of scanning force microscopy (SFM). Using the acetonide-protected polymers **5** and **7**, individual chains with extended conformations could be identified on a mica surface. The heights of the second- and third-generation polymers were 0.5 and 0.8 nm, respectively, values that are consistent with the increasing dendron sizes. Inspection of the images presented in Figure 2 suggests that the backbone of the third-generation polymer is both straighter and more extended than that of the second-generation polymer, even though the degrees of polymerization of the two polymers are the same. Crude estimates of the mean end-to-end lengths of the second- and third-generation polymers of 20 ± 5 nm and 40 ± 10 nm, respectively, were obtained by measuring the longest dimension (in any direction) of individual chains. The actual contour lengths of the polymers would likely be longer due to the visible kinks in the polymer chains, however. As these polymers have an average degree of polymerization of ∼150, the corresponding completely α-helical polypeptide with a rise of 0.15 nm per residue would have an average end-to-end distance of ∼23 nm.[38] A completely extended, transoid polypeptide with a distance of 0.36 nm per residue would have an average end-to-end distance of ∼54 nm.[38] The dimensions of the individual chains visible by SFM are approximately in line with the calculations for the expected polymer chain lengths, and constitute additional evidence for a helix to coil transition.

Since interdendron steric repulsions are thought to be responsible for the chain extension seen for most dendronized polymers, we believe that the observed helix to coil transition is driven by steric factors. Similar steric arguments have been presented for helical polymers before[12] and, in a biological context, are often invoked to account for the highly extended conformations found in the class of *O*-glycosylated glycoproteins collectively known as mucins.[39,40] It is of interest to note, however, that most helical conformations of dendronized polymers are thought to be *stabilized* by their dendrons, and in at least one case helical stability has been found to *increase* with dendron size.[13]

Although steric strain at higher dendron generations seems to be the most likely cause for the observed loss of helicity, other factors may also be contributing. The conformation shift may also result from backbone racemization, hydrogen bond disruption of the polypeptide amides by the dendrons, or shielding of the hydrophobic backbone that would lessen the benefits of a compact helical conformation in an aqueous environment. Hydrolysis of the ester bonds of the third-generation polymer **8** under basic conditions to provide the first-generation polymer **4a** was used to show that racemization was not involved. If racemization had occurred, the polymer would be expected to lack a secondary structure or at least have a decreased helical content, but the CD spectrum of this polymer was found to be identical to that of the original polymer **4**. Further studies are required to determine to what extent, if any, factors other than steric may contribute to the conformational change observed upon dendronization.

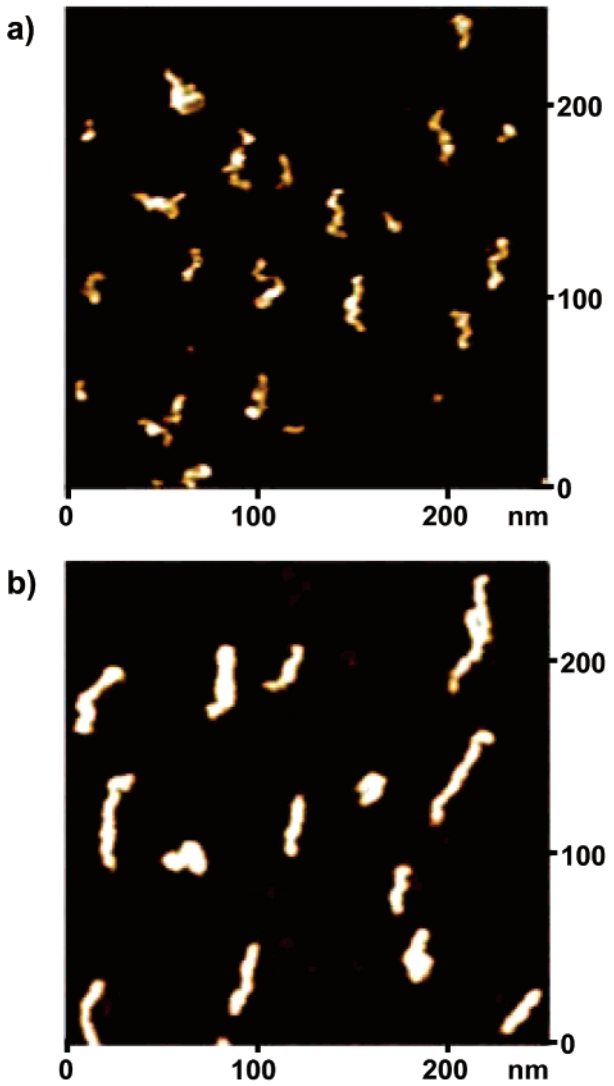

Figure 2. SFM images on mica of (a) acetonide-protected second-generation dendronized polymer **5** and (b) acetonide-protected third-generation dendronized polymer **7**.

Materials and Methods

Materials. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. DMF was distilled from CaH_2 at room temperature under reduced pressure. NEt_3, CH_2Cl_2, and pyridine were distilled under an atmosphere of nitrogen from CaH_2. THF was distilled under an atmosphere of nitrogen from sodium/benzophenone immediately before use. Butylamine hydrochloride was prepared by the passage of HCl gas through a solution of butylamine in diethyl ether. Three rounds of precipitation from ethanol into diethyl ether provided a white powder with a melting point of 216−220 °C. ϵ-Carbobenzyloxy-L-lysine *N*-carboxyanhydride (Z-L-lysine NCA)[41] and isopropylidene-2,2-bis(oxymethyl)propionic anhydride (**11**)[34,42] were synthesized according to literature procedures. Solvents were

removed in vacuo with a rotary evaporator, and solid products were freed of volatile compounds by vacuum pump evacuation.

Characterization. A. General Characterization Methods. NMR spectra were recorded on Bruker AMX 300, AM 400, or DRX 500 MHz instruments with tetramethylsilane as the standard (0.00 ppm) when the solvent was $CDCl_3$, MeOH as the standard (4.87 ppm for ^1H and 49.0 for ^{13}C) when the solvent was MeOD, and DMSO as the standard (2.49 ppm for ^1H and 39.5 for ^{13}C). All chemical shifts are reported in parts per million (ppm), and coupling constants are reported in hertz (Hz). Fourier transform infrared (FTIR) spectroscopic analyses were performed using a thin film cast from an appropriate solvent on a NaCl plate. Elemental analyses were performed at the UC Berkeley Micro-Mass Facilities.

B. Size Exclusion Chromatography. Analytical size exclusion chromatography (SEC) in *N*,*N*-dimethylformamide (DMF) with 0.2% LiBr was performed at 70 °C at a nominal flow rate of 1.0 mL/min on a chromatography line calibrated with linear poly(methyl methacrylate) (PMMA) standards (620−910 500 g/mol) and fitted with two 7.5 × 300 mm PLgel mixed-bed C columns (5-μm particle size). Aqueous SEC was performed in a pH 3 eluent composed of 0.5 M acetic acid and 0.3 M Na_2SO_4 at 35 °C at a nominal flow rate of 0.8 mL/min on a chromatography line calibrated with linear polysaccharide standards (Polymer Laboratories, 738−788 000 g/mol) and fitted with three 7.8 × 300 mm TosoHaas columns ($GMPW_{XL}$, $G3000PW_{XL}$, and $G2500PW_{XL}$, in that order). The SEC system used when determining PMMA and polysaccharide-equivalent molecular weights consists of a Waters 510 pump, a Waters U6K or Rheodyne 7725i injector, and a Waters 410 differential refractive index detector thermostated at 35 °C. The SEC system for determining absolute molecular weights (SEC-MALLS) consists of a Waters 510 pump, a 7125 Rheodyne injector, a Wyatt DAWN-EOS multiangle laser light scattering detector (laser of $\lambda = 690$ nm), and a Wyatt Optilab differential refractive index detector. Light scattering data were analyzed using Astra software from Wyatt, and SEC data using the differential refractive index detector were analyzed using Millennium software from Waters.

C. CD Spectroscopy. Circular dichroism spectra were obtained using an AVIV 62DS circular dichroism spectrometer. Measurements were performed at 25 °C, sampling every 0.5 nm with a 5 s averaging time over the range of 200−250 nm (bandwidth = 1.5 nm). Solutions of the hydroxyl-terminated dendronized polymers were made in aqueous buffer (10 mM phosphate, pH 7.4). Polymer concentrations in all studies were between 50 and 350 μg/mL.

D. Scanning Force Microscopy. Imaging by scanning force microscopy was performed using a Nanoscope IIIa (Digital Instruments) in tapping mode. Acetonid-protected polymers were cast from a dilute chloroform solution (0.001 and 0.005 mg/mL for second- and third-generation dendronized polymers, respectively) onto a mica surface using spin-coating (4000 rpm). The silicon cantilevers used had a spring constant of ∼24 N/m, a tip radius of 5−10 nm, and a resonance frequency of 300 kHz.

Preparation of Poly(Z-L-lysine) (1). Method A. Lower molecular weight poly(Z-L-lysine) (**1**) was prepared by the method of Dimitrov and Schlaad,[32] using the hydrochloride salt of butylamine as initiator for the ring-opening polymerization of Z-L-lysine NCA at 40 °C in DMF. The polymer was precipitated into distilled water, collected by centrifugation, and dried in vacuo to yield a white solid. Spectroscopic characterization was consistent with the literature.[43]

Method B. Higher molecular weight poly(Z-L-lysine) (**1**) was prepared in a glovebox by the method of Deming,[31,44] using bpyNi(COD) as initiator for the ring-opening polymerization of Z-L-lysine NCA at room temperature in DMF. The polymer was precipitated into distilled water containing HCl (∼1 mM), collected by filtration, and dried in vacuo to yield a white solid. Spectroscopic characterization was consistent with the literature.[43] See Table 1 for SEC characterization data.

Preparation of Poly(L-lysine) (2). Poly(Z-L-lysine) was deprotected as reported by Klok and Rodriguez-Hernández.[43] Briefly, poly(Z-L-lysine) was dissolved in TFA to make a 33 mg/mL solution, and a 4-fold excess of HBr (33% in acetic acid) relative to monomer units was added. The reaction was capped and stirred at room temperature for 4 h. The reaction mixture was then poured into diethyl ether to precipitate the product as the TFA salt, which was recovered by filtration and dried in vacuo to yield a pale yellow solid. Spectroscopic characterization was consistent with the literature.[43] See Table 1 for SEC characterization data.

General Procedure for the Preparation of the First-Generation Acetonide-Protected Dendronized Poly(L-lysine), Poly([G1]-(O_2Ac)-L-lysine) (3). Compounds **11** (2.54 g, 7.68×10^{-3} mol), **2** (1.24 g, 5.12×10^{-3} mol of repeat unit), and DMAP (0.125 g, 1.02×10^{-3} mol) were dissolved in 20 mL of a 3:1 DMF/NEt_3 solution. After stirring for 40 h at room temperature, the reaction mixture was poured into ice-cooled diethyl ether to precipitate the product. The supernatant was decanted, and the precipitate was taken up in 80 mL of CH_2Cl_2 and washed with distilled water (2 × 30 mL) and brine (30 mL). The organic layer was dried with $MgSO_4$, concentrated, and dried in vacuo to yield the product as a brown solid: 1.11 g (76%). An analytical sample was prepared by precipitating the polymer from $CHCl_3$ into diethyl ether. ^1H NMR (500 MHz, $CDCl_3$): δ 1.07 (s, 3H, CH_3), 1.39 (s, 3H, C(CH_3)$_2$), 1.44 (s, 3H, C(CH_3)$_2$), 1.2−2.3 (b, 6H, CH_2), 3.23 (b, 2H, CH_2-NHCOR), 3.70 (d, 2H, CH_2O, J = 11.5 Hz), 3.84 (b, 1H, C*H*NHCOR), 3.97 (d, 2H, CH_2O, J = 10.5 Hz), 7.19 (b, 1H, N*H*), 8.25 (b, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.2, 20.5, 23.9, 26.9, 29.4, 30.1, 39.4, 40.4, 57.7, 66.9, 98.2, 174.5, 175.6. FTIR (cm^{-1}): ν 3294 (N−H), 1653 (C═O, amide I), 1540 (amide II). Anal. Calcd for $C_{14}H_{24}N_2O_4$: C, 59.14; H, 8.51; N, 9.85. Found: C, 58.78; H, 8.39; N, 9.74. See Table 1 for SEC characterization data.

General Procedure for the Preparation of the First-Generation Hydroxyl-Terminated Dendronized Poly(L-lysine), Poly-([G1]-(OH_2)-L-lysine) (4) and General Procedure for the Removal of the Acetonide Protecting Group. Compound **3** (1.08 g) was dissolved in 110 mL of methanol, followed by the addition of concentrated sulfuric acid (22 drops). The solution was stirred for 20 h at room temperature, and was then neutralized with NH_3 (7 M in MeOH). Insoluble NH_4SO_4 was removed by filtration, and the filtrate was concentrated. The residue was dissolved in 25 mL of distilled water, transferred into a Spectrum Spectra/Por regenerated cellulose dialysis membrane (MWCO = 1000), and dialyzed against water over a period of 24 h with frequent changing of the bath water. The retained solution was filtered through a 1-μm Acrodisc and lyophilized to an off-white solid: 0.745 g (80%). ^1H NMR (300 MHz, MeOD): δ 1.13 (s, 3H, CH_3), 1.2−2.6 (b, 6H, CH_2), 3.22 (b, 2H, CH_2NHCOR), 3.65 (AB, 4H, CH_2OH, J = 11.4 Hz), 3.94 (b, 1H, C*H*NHCOR), 8.34 (b, 1H, N*H*). ^{13}C NMR (125 MHz, MeOD): δ 18.0, 24.8, 30.2, 31.3, 40.2, 50.0, 59.1, 66.6, 177.3, 178.0. FTIR (cm^{-1}): ν 3293 (N−H, O−H), 1653 (C═O, amide I), 1541 (amide II). Anal. Calcd for $C_{11}H_{20}N_2O_4$: C, 54.08; H, 8.25; N, 11.47. Found: C, 53.69; H, 8.25; N, 11.30. See Table 1 for SEC characterization data.

General Procedure for the Preparation of the Second-Generation Acetonide-Protected Dendronized Poly(L-lysine), Poly([G2]-(O_2Ac)$_2$-L-lysine) (5). Compounds **11** (2.76 g, 8.35×10^{-3} mol), **4** (0.680 g, 2.78×10^{-3} mol of repeat unit), and DMAP (0.068 g, 5.6×10^{-3} mol) were dissolved in 10 mL of a 3:2 pyridine/CH_2Cl_2 solution. After stirring for 18 h at room temperature, the reaction mixture was poured into ice-cooled diethyl ether to precipitate the product. The supernatant was decanted, and the precipitate was filtered, washed with ether, and dried in vacuo to yield the product as a yellow solid: 1.31 g (85%). ^1H NMR (500 MHz, $CDCl_3$): δ 1.13 (s, 6H, CH_3), 1.28 (s, 3H, CH_3), 1.32 (s, 6H, C(CH_3)$_2$), 1.40 (s, 6H, C(CH_3)$_2$), 1.2−2.6 (b, 6H, CH_2), 3.19 (b, 2H, CH_2NHCOR), 3.64 (d, 4H, CH_2O), 3.82 (b, 1H, C*H*NHCOR), 4.14 (b, 4H, CH_2O), 4.2−5.2 (b, 4H, CH_2OCOR) 7.13 (b, 1H, N*H*), 8.23 (b, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.9, 18.3, 21.8, 24.0, 25.4, 29.1, 37.0, 39.5, 41.9, 46.2, 58.1, 65.8, 97.9, 172.5, 173.4, 175.5. FTIR (cm^{-1}): ν 3291 (N−H), 1734 (C═O, ester), 1653 (C═O, amide I), 1540 (amide II). Anal. Calcd for $C_{27}H_{44}N_2O_{10}$: C, 58.26; H, 7.97; N, 5.03. Found: C, 58.03; H, 8.22; N, 4.99. See Table 1 for SEC characterization data.

General Procedure for the Preparation of the Second-Generation Hydroxyl-Terminated Dendronized Poly(L-lysine), Poly([G2]-$(OH_2)_2$-L-lysine) (6). Compound **5** (1.16 g) was suspended in 115 mL of methanol, followed by the addition of concentrated sulfuric acid (23 drops). The procedure described above for the removal of the acetonide protecting groups was repeated to yield an off-white solid: 0.833 g (84%). ^{1}H NMR (500 MHz, MeOD): δ 1.16 (s, 6H, CH_3), 1.30 (s, 3H, CH_3), 1.2−2.6 (b, 6H, CH_2), 3.21 (b, 2H, CH_2NHCOR), 3.65 (AB, 8H, CH_2OH, J = 10.0 Hz), 3.93 (b, 1H, C*H*NHCOR), 4.1−4.6 (b, 4H, CH_2-OCOR), 7.82 (b, 1H, N*H*), 8.31 (b, 1H, N*H*). ^{13}C NMR (100 MHz, MeOD): δ 17.7, 18.7, 25.1, 30.4, 31.6, 40.7, 47.5, 51.9, 59.1, 66.0, 67.1, 175.2, 176.0, 177.3. FTIR (cm^{-1}): ν 3292 (N−H, O−H), 1727 (C═O, ester), 1650 (C═O, amide I), 1548 (amide II). See Table 1 for SEC characterization data.

General Procedure for the Preparation of the Third-Generation Acetonide-Protected Dendronized Poly(L-lysine), Poly([G3]-$(O_2Ac)_4$-L-lysine) (7). Compounds **11** (3.08 g, 9.33 × 10^{-3} mol), **6** (0.727 g, 1.53 × 10^{-3} mol of repeat unit), and DMAP (0.150 g, 1.22 × 10^{-3} mol) were dissolved in 12 mL of a 1:1 pyridine/CH_2Cl_2 solution. After stirring for 48 h at room temperature, the reaction mixture was poured into ice-cooled methanol to precipitate the product. The precipitate was collected by centrifugation, washed with methanol, and dried in vacuo to yield the product as a white solid: 1.17 g (70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (s, 12H, CH_3), 1.28 (s, 9H, CH_3), 1.33 (s, 12H, C(CH_3)$_2$), 1.40 (s, 12H, C(CH_3)$_2$), 1.0−2.2 (b, 6H, CH_2), 3.16 (b, 2H, CH_2NHCOR), 3.61 (b, 8H, CH_2O), 3.8−4.0 (b, 1H, C*H*NHCOR), 4.12 (b, 8H, CH_2O), 4.2−5.0 (b, 12H, CH_2OCOR), 6.86 (b, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.7, 18.4, 21.9, 25.3, 29.2, 32.8, 39.7, 41.9, 46.1, 46.7, 54.2, 64.8, 65.8, 98.0, 171.9, 173.5. FTIR (cm^{-1}): ν 3298 (N−H), 1738 (C═O, ester), 1650 (C═O, amide I), 1547 (amide II). Anal. Calcd for $C_{53}H_{84}N_2O_{22}$: C, 57.81; H, 7.69; N, 2.54. Found: C, 57.61; H, 7.76; N, 2.71. See Table 1 for SEC characterization data.

General Procedure for the Preparation of the Third-Generation Hydroxyl-Terminated Dendronized Poly(L-lysine), Poly-([G3]-$(OH_2)_4$-L-lysine) (8). To a flask containing compound **7** (1.07 g) was added 110 mL of methanol, followed by the addition of concentrated sulfuric acid (22 drops). The procedure described above for the removal of the acetonide protecting groups was repeated to yield an off-white solid: 0.682 g (74%). ^{1}H NMR (400 MHz, MeOD): δ 1.14 (s, 12H, CH_3), 1.0−2.3 (b, 6H, CH_2), 1.29 (b, 9H, CH_3), 3.18 (b, 2H, CH_2NHCOR), 3.63 (AB, 16H, CH_2OH, J = 10.4 Hz), 4.0−4.7 (b, 13H, CH_2OCOR + C*H*NHCOR), 7.6−8.4 (b, 2H, N*H* + N*H*). ^{13}C NMR (100 MHz, MeOD): δ 17.5, 18.5, 24.5, 30.3, 33.3, 40.9, 47.7, 47.9, 51.8, 54.5, 65.9, 66.4, 68.0, 173.9, 174.4, 176.0. FTIR (cm^{-1}): ν 3296 (N−H, O−H), 1734 (C═O, ester), 1647 (C═O, amide I), 1540 (amide II). See Table 1 for SEC characterization data.

General Procedure for the Preparation of the Fourth-Generation Acetonide-Protected Dendronized Poly(L-lysine), Poly([G4]-$(O_2Ac)_8$-L-lysine) (9). Compounds **11** (0.271 g, 8.20 × 10^{-4} mol), **8** (64.0 mg, 6.80 × 10^{-5} mol of repeat unit), and DMAP (25.0 mg, 2.05 × 10^{-4} mol) were dissolved in 1 mL of a 1:1 pyridine/CH_2Cl_2 solution. After stirring for 48 h at room temperature, the reaction mixture was poured into a 10:1 hexanes/2-propanol solution to precipitate the product. The precipitate was collected by centrifugation, washed with 10:1 hexanes/2-propanol, and dried in vacuo to yield the product as a white solid: 0.121 g (81%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (s, 24H, CH_3), 1.27 (s, 21H, CH_3), 1.32 (s, 24H, C(CH_3)$_2$), 1.39 (s, 24H, C(CH_3)$_2$), 0.8−2.3 (b, 6H, CH_2), 2.7−3.4 (b, 2H, CH_2NHCOR), 3.61 (b, 16H, CH_2O), 3.8−5.0 (b, 1H, C*H*NHCOR), 4.12 (b, 16H, CH_2O), 4.2−5.0 (b, 28H, CH_2OCOR), 6.75 (b, 1H, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.6, 17.75, 18.5, 22.2, 25.2, 42.0, 46.8, 64.8, 65.9, 98.0, 171.9, 173.5. FTIR (cm^{-1}): ν 3370 (N−H), 1739 (C═O, ester), 1663 (C═O, amide I), 1531 (amide II). Anal. Calcd for $C_{53}H_{84}N_2O_{22}$: C, 57.58; H, 7.55; N, 1.28. Found: C, 57.25; H, 7.67; N, 1.44. See Table 1 for SEC characterization data.

General Procedure for the Preparation of the Fourth-Generation Hydroxyl-Terminated Dendronized Poly(L-lysine), Poly([G4]-$(OH_2)_8$-L-lysine) (10). To a flask containing compound **9** (47.1 mg) was added 4 mL of THF and 3 mL of methanol, followed by the addition of concentrated sulfuric acid (2 drops). The procedure described above for the removal of the acetonide protecting groups was repeated to yield an off-white solid: 39.8 mg (99%). ^{1}H NMR (500 MHz, MeOD): δ 1.17 (s, 24H, CH_3), 0.6−2.1 (b, 6H, CH_2), 1.33 (b, 21H, CH_3), 2.9−3.4 (b, 2H, CH_2-NHCOR), 3.67 (AB, 32H, CH_2OH, J = 8.5 Hz), 4.0−4.7 (b, 29H, CH_2OCOR + C*H*NHCOR), 7.4−8.6 (b, 2H, N*H* + N*H*). ^{13}C NMR (125 MHz, MeOD): δ 17.7, 18.7, 48.0, 48.1, 51.8, 65.9, 173.9, 176.0. FTIR (cm^{-1}): ν 3371 (N−H, O−H), 1730 (C═O, ester), 1652 (C═O, amide I), 1541 (amide II). See Table 1 for SEC characterization data.

Procedure for the Hydrolysis of Poly([G3]-$(OH_2)_4$-L-lysine) (8) to Poly([G1]-(OH_2)-L-lysine) (4a). Compound **8** (16.4 mg) was dissolved in 5 mL of methanol, and to this solution was added 280 μL of 0.5 M NaOMe in methanol. After stirring at room temperature for 48 h, the reaction was neutralized with glacial acetic acid and the solvent was removed in vacuo. The residue was dissolved in distilled water, transferred into a Spectrum Spectra/Por regenerated cellulose dialysis membrane (MWCO = 3500), and dialyzed against water over a period of 24 h with frequent changing of the bath water. The retained solution was filtered through a 1-μm Acrodisc and lyophilized to yield 4.5 mg of **4a** as a white solid. Characterization was identical to that of **4** reported above.

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References and Notes

(1) Schlüter, A. D.; Rabe, J. P. *Angew. Chem., Int. Ed.* **2000**, *39*, 864−883.
(2) Das, J.; Yoshida, M.; Fresco, Z. M.; Choi, T. L.; Fréchet, J. M. J.; Chakraborty, A. K. *J. Phys. Chem. B* **2005**, *109*, 6535−6543.
(3) Liang, C. O.; Helms, B.; Hawker, C. J.; Fréchet, J. M. J. *Chem. Commun.* **2003**, *20*, 2524−2525.
(4) Fresco, Z. M.; Suez, I.; Backer, S. A.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 8374−8375.
(5) Lee, C. C.; Yoshida, M.; Fréchet, J. M. J.; Dy, E. E.; Szoka, F. C. *Bioconjugate Chem.* **2005**, *16*, 535−541. Gillies, E. R.; Fréchet, J. M. J. *Drug Discovery Today* **2005**, *10*, 35−43.
(6) Förster, S.; Neubert, I.; Schlüter, A. D.; Lindner, P. *Macromolecules* **1999**, *32*, 4043−4049.
(7) Ouali, N.; Méry, S.; Skoulios, A.; Noirez, L. *Macromolecules* **2000**, *33*, 6185−6193.
(8) Girbasova, N.; Aseyev, V.; Saratovsky, S.; Moukhina, I.; Tenhu, H.; Bilibin, A. *Macromol. Chem. Phys.* **2003**, *204*, 2258−2264.
(9) Helms, B.; Mynar, J. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15020−15021.
(10) Percec, V.; Ahn, C. H.; Cho, W. D.; Jamieson, A. M.; Kim, J.; Leman, T.; Schmidt, M.; Gerle, M.; Möller, M.; Prokhorova, S. A.; Sheiko, S. S.; Cheng, S. Z. D.; Zhang, A.; Ungar, G.; Yeardley, D. J. P. *J. Am. Chem. Soc.* **1998**, *120*, 8619−8631.
(11) Percec, V.; Ahn, C. H.; Ungar, G.; Yeardley, D. J. P.; Möller, M.; Sheiko, S. S. *Nature* **1998**, *391*, 161−164.
(12) Percec, V.; Obata, M.; Rudick, J. G.; De, B. B.; Glodde, M.; Bera, T. K.; Magonov, S. N.; Balagurusamy, V. S. K.; Heiney, P. A. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 3509−3533.
(13) Kamikawa, Y.; Kato, T.; Onouchi, H.; Kashiwagi, D.; Maeda, K.; Yashima, E. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 4580−4586.
(14) Zhuravel, M. A.; Davis, N. E.; Nguyen, S. T.; Koltover, I. *J. Am. Chem. Soc.* **2004**, *126*, 9882−9883.
(15) Ryu, J. H.; Bae, J.; Lee, M. *Macromolecules* **2005**, *38*, 2050−2052.

(16) Kaneko, T.; Horie, T.; Asano, M.; Aoki, T.; Oikawa, E. *Macromolecules* **1997**, *30*, 3118−3121.
(17) Li, W. S.; Jiang, D. L.; Aida, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 2943−2947.
(18) Uzgiris, E. *Invest. Radiol.* **2004**, *39*, 131−137.
(19) Uzgiris, E. E.; Cline, H.; Moasser, B.; Grimmond, B.; Amaratunga, M.; Smith, J. F.; Goddard, G. *Biomacromolecules* **2004**, *5*, 54−61.
(20) Grayson, S. M.; Fréchet, J. M. J. *Macromolecules* **2001**, *34*, 6542−6544.
(21) Lee, C. C.; Grayson, S. M.; Fréchet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 3563−3578.
(22) Yoshida, M.; Fresco, Z. M.; Ohnishi, S.; Fréchet, J. M. J. *Macromolecules* **2005**, *38*, 334−344.
(23) Kricheldorf, H. R. In *Models of Biopolymers by Ring-Opening Polymerization*; Penczek, S., Ed.; CRC Press: Boca Raton, FL, 1990; pp 1−132.
(24) Deming, T. J. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 3011−3018.
(25) Mezö, G.; Kajtár, J.; Nagy, I.; Szekerke, M.; Hudecz, F. *Biopolymers* **1997**, *42*, 719−730.
(26) Yu, M.; Nowak, A. P.; Deming, T. J.; Pochan, D. J. *J. Am. Chem. Soc.* **1999**, *121*, 12210−12211.
(27) Thoma, G.; Patton, J. T.; Magnani, J. L.; Ernst, B.; Öhrlein, R.; Duthaler, R. O. *J. Am. Chem. Soc.* **1999**, *121*, 5919−5929.
(28) Hudecz, F.; Pimm, M. V.; Rajnavolgyi, E.; Mezö, G.; Fabra, A.; Gaal, D.; Kovacs, A. L.; Horvath, A.; Szekerke, M. *Bioconjugate Chem.* **1999**, *10*, 781−790.
(29) Mezö, G.; Reményi, J.; Kajtár, J.; Barna, K.; Gaál, D.; Hudecz, F. *J. Controlled Release* **2000**, *63*, 81−95.
(30) Greenfield, N.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108−4116.
(31) Deming, T. J. *Nature* **1997**, *390*, 386−389.
(32) Dimitrov, I.; Schlaad, H. *Chem. Commun.* **2003**, *23*, 2944−2945.
(33) Gillies, E. R.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 14137−14146.
(34) Malkoch, M.; Malmström, E.; Hult, A. *Macromolecules* **2002**, *35*, 8307−8314.
(35) Anand, N.; Murthy, N. S. R.; Naider, F.; Goodman, M. *Macromolecules* **1971**, *4*, 564−569.
(36) Bandekar, J. *Biochim. Biophys. Acta* **1992**, *1120*, 123−143.
(37) Wang, Y.; Jardetzky, O. *Protein Sci.* **2002**, *11*, 852−861.
(38) Solomons, T. W. *Organic Chemistry*; John Wiley & Sons: New York, 1996.
(39) Jentoft, N. *Trends Biochem. Sci.* **1990**, *15*, 291−294.
(40) Butenhof, K. J.; Gerken, T. A. *Biochemistry* **1993**, *32*, 2650−2663.
(41) Bergmann, M.; Zervas, L.; Ross, W. F. *J. Biol. Chem.* **1935**, *111*, 245−260.
(42) Ihre, H.; Hult, A.; Fréchet, J. M. J.; Gitsov, I. *Macromolecules* **1998**, *31*, 4061−4068.
(43) Klok, H.-A.; Rodriguez-Hernández, J. *Macromolecules* **2002**, *35*, 8718−8723.
(44) Deming, T. J. *J. Am. Chem. Soc.* **1998**, *120*, 4240−4241.

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