Peptide Dendrimers from Natural Amino Acids

Yoonkyung Kim, Fanwen Zeng, and Steven C. Zimmerman*[a]

Abstract: The high-yielding cyanoethylation–hydrogenation strategy was used to prepare simple AB_2 monomers from natural amino acids. Third-generation peptide dendrimers were assembled from these monomers on a polyethylene glycol (PEG) resin by standard Boc peptide coupling methods. Preliminary conformational studies were conducted on these chiral peptide dendrimers by size-exclusion chromatography, optical activity measurements, and investigation of the solid-state properties. These peptide dendrimers have potential applications as drug-delivery agents, asymmetric catalysts, peptido- and protein mimetics, and new biomaterials.

Keywords: amino acids · dendrimers · peptides · solid-phase synthesis

Introduction

Recent advances in dendrimer syntheses have made available dendrimers with a wide range of sizes, functionalities, and properties.[1] Of the many monomer structures possible, those that contain natural or unnatural amino acids are particularly appealing because they are chiral and have the potential to produce dendrimers with enhanced biocompatibility and diversity. These properties may render such dendrimers suitable for use as drug-delivery agents, asymmetric catalysts, and peptido- and protein mimetics. Furthermore, defined three-dimensional structures might be attained through specific folding of the constituent amino acid units. The construction of a combinatorial library[2] of these dendrimers by variation of the amino acid monomers and dendrimer generation number might create a pool of proteinlike synthetic macromolecules which could be screened for desired properties.

Since Denkewalter's report of a polylysine dendrimer in 1983,[3] only a few dendrimers have been reported that are made from natural amino acid based building blocks.[4] We disclose herein an efficient and general method to prepare monomers containing natural amino acids and the corresponding third-generation peptide dendrimers. Preliminary studies of the chiroptical[5] and solid-state properties of these dendrimers are reported.

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Results and Discussion

Our goal in designing a monomeric unit for the natural amino acid based dendrimers was to minimally modify the natural amino acid structures in an effort to maximize the structural resemblance to natural proteins. Thus, the high-yielding cyanoethylation[6]–hydrogenation procedure of Meijer and co-workers[7] was adopted to make branched monomers from natural amino acids. To obviate the tedious purifications involved in many dendrimer syntheses, a liquid-phase peptide synthesis method using polyethylene glycol (PEG) as a support was employed.[8] Chapman and co-workers recently reported the iterative divergent synthesis of an eighth-generation polylysine dendrimer using this approach.[9]

The synthetic utility of our approach was demonstrated using L-valine and L-leucine, amino acids containing relatively bulky side chains, which provide a stringent test of the synthetic methodology. Double Michael addition of acrylonitrile to amino acid **1** in a refluxing aqueous sodium hydroxide (NaOH) solution gave bis(cyanoethyl)amino acid **2** (Scheme 1).[10] Hydrogenation of **2** with Raney Co catalyst afforded the bis(aminopropyl)amino acid **3** as a crude product in quantitative yield. Diamino acids **3** were easily obtained on a 100 g scale through the above two-step process.[11] *Tert*-butoxycarbonyl (Boc) protection of the amino groups, precipitation with dicyclohexylamine (DCHA), and removal of DCHA gave Boc-protected diamino acids **4**. Subsequently, esterification of **4** with pentafluorophenol afforded analytically pure activated monomers **5** for the peptide dendrimer synthesis.

The peptide dendrimers were synthesized on a poly(ethylene glycol) monomethyl ether (MeO-PEG-OH) resin (**6**)[12] derivatized with glycine to form **7**. Each generation of dendrimer growth comprises a deprotection, coupling, and capping reaction (i.e., **7**→**8**→**9**→**10**,

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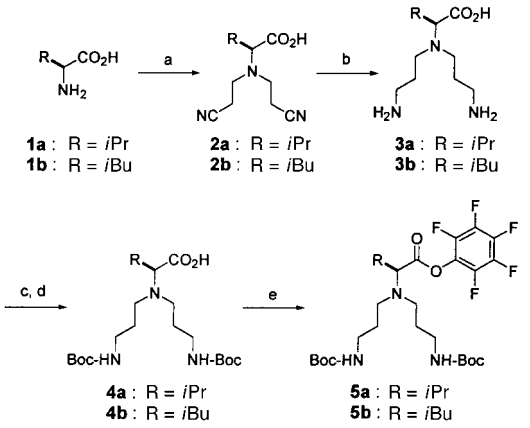
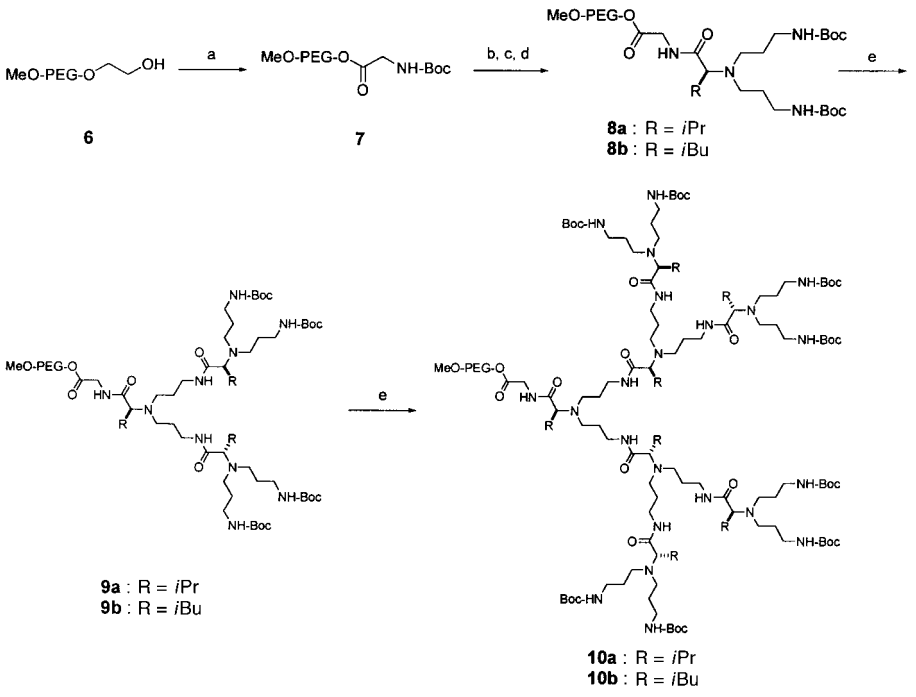
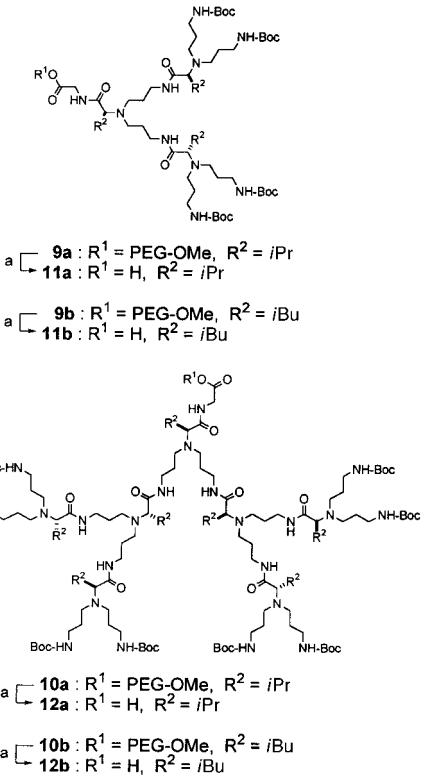


Scheme 1. Synthesis of activated amino acid monomers **5**: a) 1N NaOH (1.00 equiv), 0 °C, then add acrylonitrile (3.00 equiv), 25 °C, 6 h; 100 °C, 24 h (**2a**, 100 %; **2b**, 89 %); b) H_2, Raney Co, MeOH, 1000 psi, 70 °C, 7 h; c) 2N NaOH (2.10 equiv), $(Boc)_2O$ (2.20 equiv), *t*BuOH, 0 °C, 16 h; DCHA (1.02 equiv), 1:10 Et_2O/hexane, 25 °C, 5 min; d) 2N H_2SO_4 (1.20 equiv), EtOAc, 0 °C, 10 min (**4a**, 41 % for three steps; **4b**, 46 % for three steps); e) pentafluorophenol (1.09 equiv), DCC (1.03 equiv), EtOAc, 0 → 25 °C, 17 h (**5a**, 95 %; **5b**, 53 %).

Scheme 2). The reactions were monitored by ^{1}H NMR spectroscopy; the average molecular weights were obtained from MALDI-TOF mass spectrometry. The Boc groups were removed by treatment with 1:1 trifluoroacetic acid (TFA)/methylene chloride (CH_2Cl_2). The coupling reaction[13] was performed in a 4:1 mixture of *N*,*N*-dimethylformamide (DMF) and CH_2Cl_2 by preactivation of monomer **5** with 1-hydroxybenzotriazole (HOBt) and diisopropylethylamine (DIEA) introduced in the in situ neutralization protocol.[14] Any uncoupled free amino groups were capped with acetic anhydride (Ac_2O). Third-generation resin-bound peptide dendrimers **10a** and **10b** obtained through three such iterations were purified by size-exclusion chromatography (SEC).



Scheme 2. Peptide dendrimer synthesis on PEG support: a) Boc-Gly-OH (3.00 equiv), DCC (3.00 equiv), DMAP (0.25 equiv), CH_2Cl_2, 25 °C, 15 h (99 %); b) 1:1 TFA/CH_2Cl_2, 25 °C, 3 h; c) **5** (3.00 equiv), HOBt (3.00 equiv), DIEA (4.00 equiv), 4:1 DMF/CH_2Cl_2, 25 °C, 12 h; d) Ac_2O (4.00 equiv), DIEA (1.00 equiv), CH_2Cl_2, 25 °C, 20 h; e) see Experimental Section for details.



Scheme 3. Cleavage of dendrimers from PEG resin: a) 1N NaOH, MeOH, 25 °C, 6 h (**11b**, 51 %; **12a**, 84 %; **12b**, 75 %).

Cleavage from the resin with a dilute methanolic sodium hydroxide solution (Scheme 3) produced dendrimers **12a** and **12b**, which were characterized by ^{1}H NMR spectroscopy, including COSY experiments, ^{13}C-APT, MALDI-TOF mass spectrometry, and SEC. The third-generation dendrimers were pure as judged by ^{1}H NMR (> 95 %). Compound **10b** was converted in a final iteration to the fourth-generation resin-bound dendrimer **13**. Despite attempts to optimize the yield of this last cycle, **13** contained defects (^{1}H NMR).

Some of the peptide dendrimers were further examined by SEC and chiroptical methods. Interestingly, in the SEC, **12a** (lower molecular weight, MW) eluted earlier than **12b** (higher MW) (Figure 1), a fact suggesting that **12b** has a more compact structure, possibly as a result of folding. Alternatively, the valine-based dendrimer may exhibit a larger hydrody-

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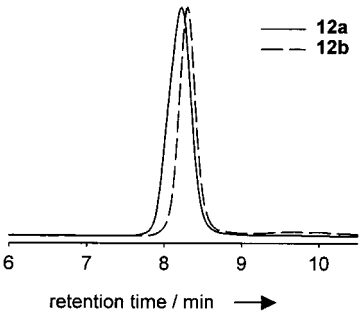

Figure 1. SEC traces in THF for the third-generation dendrimers **12a** and **12b**. SEC was performed on a Waters Styragel HR3 column (MW range 500–30000; flow rate: 1 mL min^{-1}).

namic radius because it is better solvated. Chiroptical studies of the valine- and leucine-derived dendrimers were performed (Table 1). In contrast to the extensive studies of chiral dendrimers by McGrath, Meijer, Seebach, and others,[5] in this case there are a limited number of comparisons possible, but the overall molar chirality increased from second- to third-generation in the leucine dendrimer, while the value per chiral subunit actually decreased.

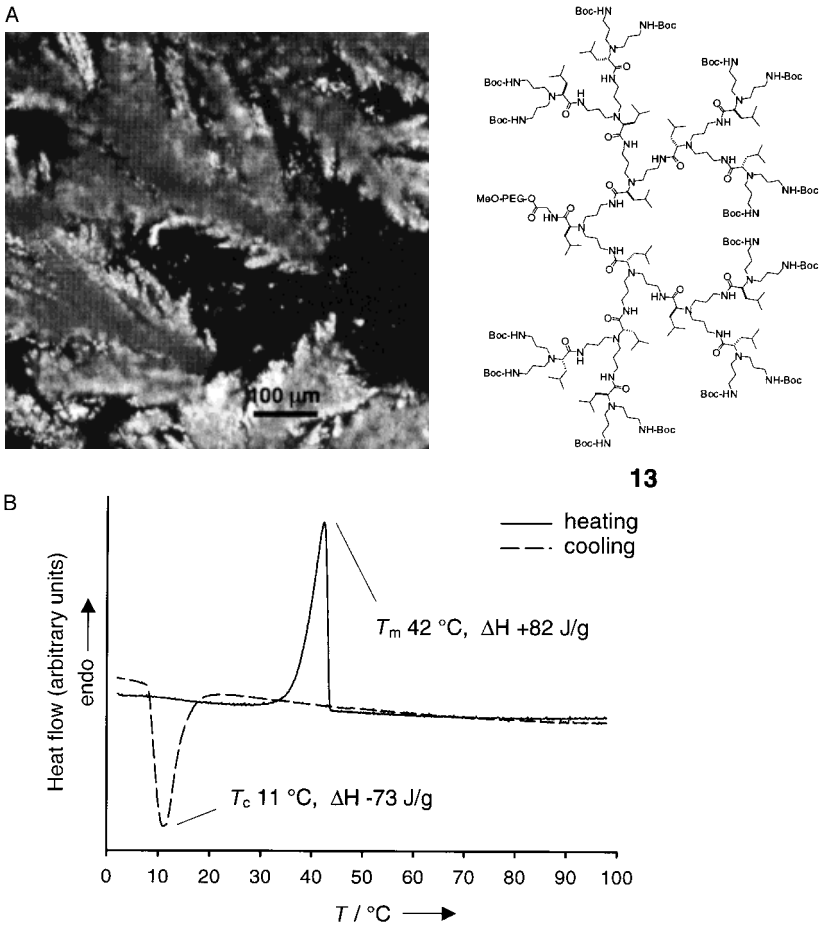

Figure 2. A) Cross-polarized optical micrograph at 25 °C and B) DSC trace measured on a Perkin–Elmer DSC7 at a heating rate of 5 °C min^{-1} for the fourth-generation leucine-based dendrimer attached to PEG, **13**.

Table 1. Chiroptical data for peptide dendrimers in CH_2Cl_2.

Compound	MW	Generation	c[a]	$[\alpha]_D^{23}$[b]	$[\Phi]_D$[c]	$[\Phi]_D/n$[d]
4a[e]	431.57	1	1.8	−28.7	−124	−124
11a	1115.51	2	n.d.	n.d.	n.d.	n.d.
12a	2369.27	3	1.5	−44.1	−1044	−149
4b[e]	445.60	1	2.0	+10.5	+47	+47
11b	1157.59	2	1.4	+14.9	+172	+58
12b	2467.46	3	1.5	+10.5	+259	+37

[a] Concentration in g 100 cm^{-3}. [b] Specific rotation in 10^{-1} ° cm^2 g^{-1}. [c] Molar rotation in 10 ° cm^2 mol^{-1}. [d] Molar rotation per chiral center n. [e] Unlike the second- and third-generation dendrimers, first-generation dendrimers did not contain the additional glycine unit in the molecule.

The third- and fourth-generation PEG-bound dendrimers slowly crystallized at room temperature from a 1:1 mixture of dimethyl sulfoxide (DMSO) and chloroform ($CHCl_3$).[15] A polarized optical micrograph of a representative crystalline sample of **13** is shown in Figure 2A. Supporting evidence for the crystalline behavior was obtained by differential scanning calorimetry (DSC, Figure 2B). Although the crystalline nature of these compounds might suggest a discrete folded structure for the peptide dendrimer, Fréchet reported crystallinity in a polybenzyl ether dendrimer attached to a PEG group; suggesting that the PEG group may be responsible for the crystallinity.[16] Preliminary small-angle X-ray scattering studies indicate overall crystallinity of the PEG-bound dendrimers, but do not show evidence of structural order in the peptide dendrimer segment alone. Efforts to enhance the crystallinity of peptide dendrimers through the modification of dendrimer structures are currently under investigation.

Conclusion

In summary, we developed a general method to prepare simple branched amino acid monomers in a highly efficient fashion and synthesized the corresponding optically active peptide dendrimers using an established liquid-phase peptide synthesis strategy. The fact that the approach works well with valine and leucine bodes well for the construction of peptide dendrimer libraries using additional amino acids. The structural resemblance of our dendrimers to proteins and the ability to construct libraries may provide a novel approach to the discovery of new biomaterials.

Experimental Section

Materials: Glassware was flame-dried and cooled to room temperature in a nitrogen atmosphere before use. All reactions were carried out under a dry nitrogen atmosphere. CH_2Cl_2 was freshly distilled from calcium hydride.

DMF was dried over calcium sulfate, then distilled under reduced pressure, and stored over 4 Å molecular sieves. Ethyl acetate (EtOAc) was stored over 4 Å molecular sieves before use. DIEA was distilled from ninhydrin, then from potassium hydroxide, and stored over 4 Å molecular sieves. DCHA was distilled over NaOH and stored over 4 Å molecular sieves. TFA was purchased from Aldrich as HPLC grade and redistilled. Ac_2O was stored over phosphorous pentoxide (P_2O_5) for 3 h, then stirred over dry K_2CO_3 for 3 h, distilled and stored over 4 Å molecular sieves. Raney Co was provided by Grace Davison Chemical and rinsed three times with methanol before use. Polyethylene glycol monomethyl ether (MeO-PEG-OH) was purchased from Aldrich as M_n 5000 and dried over P_2O_5 in vacuo before use. All other reagents and solvents were of commercial grade and were used without further purification.

Methods: Hydrogenation was performed with a large rocker equipped with a thermocouple at the high-pressure laboratory in the School of Chemical Sciences, University of Illinois. Nuclear magnetic resonance (NMR) spectra were recorded on Varian Unity 400 or 500NB spectrometer. Chemical shifts are reported in parts per million (δ). When neat [D$_6$]DMSO or 1:1 [D$_6$]DMSO/$CDCl_3$ was used as a solvent, chemical shifts were measured relative to the center of the residual protio multiplet (^{1}H $\delta = 2.50$; ^{13}C $\delta = 39.51$). When D_2O was used as a solvent, chemical shifts were measured relative to the center of the residual water peak (^{1}H $\delta = 4.80$), and chemical shifts of ^{13}C NMR spectra were uncorrected. Chemical shifts of ^{19}F NMR spectra were uncorrected. COSY experiments were performed for complete spectral assignments, if needed. Analytical thin-layer chromatography (TLC) was performed on 0.2 mm silica glass coated sheets (E. Merck) with F_{254} indicator. The products were visualized on TLC plates with UV light and ninhydrin. Flash column chromatography was performed on Merck 40–63 μm silica gel. Analytical SEC was performed on a Waters Styragel® HR3 column (MW range 500–30000) coupled with Waters 410 differential refractometer and PD2000 dual-angle laser light scattering detectors, by means of a Hitachi L-6000 pump, with THF as eluent at a flow rate of 1.0 mL min^{-1}. Molecular weights were determined by SEC against polystyrene standards. Preparative SEC was performed on Bio-Beads® S-X1 beads (BIO-RAD), 200–400 mesh, with toluene as eluent. Melting points were recorded on a Thomas–Hoover melting point apparatus and were uncorrected. Mass spectra were measured by either fast atom bombardment (FAB) on a 70-SE-4F spectrometer or matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) on a PerSeptive Biosystems Voyager DE-STR spectrometer. *trans*-3-Indoleacrylic acid was used as the matrix for MALDI-TOF mass spectra. Elemental analyses were performed at the School of Chemical Sciences, University of Illinois.

Dendrimers are designated using the notation [G-*n*(aa^1, aa^2, aa^3)]-x$_m$, where *n* refers to the generation number, aa^1, aa^2, and aa^3 refer to the nature of the modified diamino acid monomer **4** for each layer starting from the core to the periphery of the dendrimer, and x refers to the functional group at the periphery of dendrimers.

General procedure for reaction of amino acids and acrylonitrile: An aqueous solution of NaOH (2 N, 250 mL, 0.50 mol) was at 0 °C slowly added to a suspension of each amino acid (0.50 mol) in water (250 mL). Subsequently, acrylonitrile (99 mL, 1.50 mol) was added at 0 °C and the mixture was stirred overnight while it warmed slowly to room temperature. The mixture was heated at reflux temperature for 24 h to complete the reaction; it was subsequently cooled to 0 °C, and then carefully neutralized with an aqueous solution of HCl (2 N) to pH 5–6. The excess of acrylonitrile was removed under reduced pressure at 40 °C. Each compound was further purified as described below.

[*N*,*N*-Bis-(2-cyanoethyl)]-L-valine (2a): The reaction was performed following the general procedure described above with L-valine (58.6 g, 0.50 mol). After removal of acrylonitrile, the mixture in water was extracted with ether (3 × 150 mL), and the combined organic extracts were washed with brine (200 mL), then dried over Na_2SO_4, and concentrated to give **2a** (112 g, 0.50 mol) as a fairly pure oil. (The product can be recrystallized by adding hexane to the ether solution at −78 °C, then filtering quickly when cold.) Yield: 100%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 2.95$ (m, 2H, NCH_2), 2.80 (d, $J = 10.5$ Hz, 1H, C*H*CH(CH$_3$)$_2$), 2.66 (m, 2H, NCH_2), 2.59 (m, 4H, CH_2CN), 1.85 (d-septet, $J = 10.6$, 6.5 Hz, 1H, CHC*H*(CH$_3$)$_2$), 0.97 (d, $J = 6.7$ Hz, 3H, CH(C*H*$_3$)$_2$), 0.85 (d, $J = 6.5$ Hz, 3H, CH(C*H*$_3$)$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 173.1$, 119.9, 69.9, 46.5, 27.2, 19.8, 19.6, 17.0; HRMS (FAB) calcd for $C_{11}H_{18}N_3O_2$ [*M*H$^+$]: 224.1399, found: 224.1400.

[*N*,*N*-Bis-(2-cyanoethyl)]-L-leucine (2b): The reaction was performed following the general procedure described above with L-leucine (65.6 g, 0.50 mol). After removal of acrylonitrile, the mixture in water was extracted with ether (3 × 150 mL), and the combined organic extracts were washed with brine (200 mL), then dried over Na_2SO_4, and concentrated to ca. 150 mL. Solid precipitated from the viscous oil upon standing 3–4 days at room temperature. The product **2b** (105.6 g, 0.45 mol) was filtered and collected as a pure solid. Yield: 89%; m.p. 64–66 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 12.50$ (s, 1H, CO_2H), 3.38 (m, 1H, C*H*CH$_2$CH(CH$_3$)$_2$), 2.88 (m, 4H, NCH_2), 2.57 (m, 4H, CH_2CN), 1.80 (m, 1H, CHCH$_2$C*H*(CH$_3$)$_2$), 1.47 (m, 2H, CHC*H*$_2$CH(CH$_3$)$_2$), 0.90 (d, $J = 6.9$ Hz, 3H, CH(C*H*$_3$)$_2$), 0.87 (d, $J = 6.5$ Hz, 3H, CH(C*H*$_3$)$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 174.6$, 119.8, 60.9, 47.0, 38.8, 24.0, 23.1, 21.8, 17.6; HRMS (FAB) calcd for $C_{12}H_{20}N_3O_2$ [*M*H$^+$]: 238.1556, found: 238.1556.

General procedure for hydrogenation: Compound **3** was synthesized by a modified procedure of de Brabander-van den Berg et al.[7] Raney Co (cat) was added to a solution of **2** (0.50 mol) in methanol (500 mL). The mixture was saturated with anhydrous gaseous ammonia at 0 °C for 20 min. Hydrogenation was performed in a large rocker. The reaction mixture was rocked under 1000 psi of H_2 at 70 °C for 7 h, then slowly cooled to room temperature. The mixture was saturated with N_2, then filtered through a plug of Celite, and concentrated under reduced pressure to give a thick red oil contaminated with Co ions. The crude product was highly hygroscopic and was used for the next step without further purification.

[*N*,*N*-Bis-(3-aminopropyl)]-L-valine (3a): Reaction of **2a** (105 g, 0.47 mol) gave **3a** (110 g, 0.47 mol) as a red oil contaminated with a trace amount of cobalt. Yield of crude product: 100%; ^{1}H NMR (400 MHz, D_2O): $\delta = 2.81$ (d, $J = 9.8$ Hz, 1H, C*H*CH(CH$_3$)$_2$), 2.77 (m, 2H, NCH_2), 2.76 (t, $J = 7.1$ Hz, 4H, C*H*$_2$NH$_2$), 2.38 (m, 2H, NCH_2), 1.93 (d-septet, $J = 9.5$, 6.5 Hz, 1H, CHC*H*(CH$_3$)$_2$), 1.69 (m, 4H, NCH$_2$C*H*$_2$), 0.89 (d, $J = 6.7$ Hz, 3H, CH(C*H*$_3$)$_2$), 0.85 (d, $J = 6.3$ Hz, 3H, CH(C*H*$_3$)$_2$); ^{13}C NMR (100 MHz, D_2O): $\delta = 180.3$, 73.8, 48.7, 39.3, 27.6, 27.3, 19.7 (19.715), 19.7 (19.689).

[*N*,*N*-Bis-(3-aminopropyl)]-L-leucine (3b): Reaction of **2b** (102 g, 0.43 mol) gave **3b** (105.5 g, 0.43 mol) as a red oil contaminated with a trace amount of cobalt. Yield of crude product: 100%; ^{1}H NMR (400 MHz, D_2O): $\delta = 3.23$ (m, 1H, C*H*CH$_2$CH(CH$_3$)$_2$), 2.74 (m, 4H, C*H*$_2$NH$_2$), 2.71 (m, 2H, NCH_2), 2.47 (m, 2H, NCH_2), 1.68 (m, 4H, NCH$_2$C*H*$_2$), 1.62 (m, 1H, CHC*H*$_2$CH(CH$_3$)$_2$), 1.47 (m, 1H, CHCH$_2$C*H*(CH$_3$)$_2$), 1.24 (m, 1H, CHC*H*$_2$CH(CH$_3$)$_2$), 0.87 (d, $J = 6.3$ Hz, 6H, CH(C*H*$_3$)$_2$); ^{13}C NMR (100 MHz, D_2O): $\delta = 180.7$, 65.7, 48.8, 39.1 (39.109), 39.1 (39.050), 27.5, 25.6, 23.3, 21.6.

General procedure for Boc protection: An aqueous solution of NaOH (2 N, 21 mL, 42 mmol) was added dropwise at 0 °C to a solution of **3** (20 mmol) in *tert*-butyl alcohol (30 mL). Subsequently, di-*tert*-butyl dicarbonate (9.6 g, 44 mmol) was added in one portion. The mixture was sonicated for 10 min and stirred at room temperature for 16 h. The reaction was neutralized by an aqueous solution of $KHSO_4$ (1.1 N) at 0 °C, *tert*-butyl alcohol was removed under reduced pressure, water (100 mL) was added, and the aqueous layer was extracted with EtOAc (5 × 100 mL). Combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, concentrated under reduced pressure, and dried in vacuo to give a hygroscopic foamy solid. The solid was dissolved in Et_2O (50 mL), DCHA (1.02 equiv of dried solid) was added with vigorous stirring, and precipitation occurred within 5 min to give a thick mixture. The mixture was diluted with hexane (500 mL), stirred vigorously for 30 min, then left at 4 °C overnight, and filtered to collect a white solid, which was further washed with hexane to remove any remaining DCHA. The solid was suspended in EtOAc (35 mL), and then an ice-cold aqueous solution of H_2SO_4 (2 N, 1.20 equiv of dried solid) was added. The mixture was shaken vigorously until it became a clear solution. The organic layer was separated. Cold water (17 mL) was added to the aqueous layer, and it was extracted with EtOAc (2 × 35 mL). The above DCHA removal process was completed within 15 min. Combined organic layers were washed with water (2 × 35 mL) and brine (35 mL), dried over Na_2SO_4, then concentrated under reduced pressure, and dried in vacuo to give **4** as a white solid.

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[*N*,*N*-Bis-(3-*N*-*tert*-Boc-aminopropyl)]-L-valine (4a): Reaction of **3a** (6.49 g, 28.1 mmol) gave **4a** (4.92 g, 11.4 mmol) as a white solid. Yield: 41%; m.p. 62–68°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.18 (s, 1H, CO_2H), 6.75 (t, J = 5.3 Hz, 2H, NH), 2.90 (m, 4H, CH_2NH), 2.69 (d, J = 10.5 Hz, 1H, CHCH(CH$_3$)$_2$), 2.58 (m, 2H, NCH$_2$), 2.23 (m, 2H, NCH$_2$), 1.87 (d-septet, J = 10.6, 6.6 Hz, 1H, CHCH(CH$_3$)$_2$), 1.49 (m, 4H, NCH$_2$CH_2), 1.36 (s, 18H, C(CH$_3$)$_3$), 0.89 (d, J = 6.6 Hz, 3H, CH(CH_3)$_2$), 0.83 (d, J = 6.5 Hz, 3H, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 172.7, 155.5, 77.3, 69.4, 48.0, 38.1, 28.3, 26.8, 19.9, 19.6, 17.0; HRMS (FAB) calcd for $C_{21}H_{42}N_3O_6$ [MH$^+$]: 432.3074, found: 432.3075; elemental analysis calcd for $C_{33}H_{64}N_4O_6$ (612.90, DCHA salt): C 64.67, H 10.53, N 9.14; found: C 64.20, H 10.78, N 9.05 (contains 0.3 mol H_2O).

[*N*,*N*-Bis-(3-*N*-*tert*-Boc-aminopropyl)]-L-leucine (4b): Reaction of **3b** (17.5 g, 71.4 mmol) gave **4b** (14.6 g, 32.8 mmol) as a white solid. Yield: 46%; m.p. 68–72°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.74 (t, J = 5.2 Hz, 2H, NH), 3.30 (t, J = 7.1 Hz, 1H, CHCH$_2$CH(CH$_3$)$_2$), 2.91 (td, J = 6.3, 5.8 Hz, 4H, CH_2NH), 2.56 (AB$_q$-t, J_{ab} = 12.2 Hz, J = 7.9 Hz, 4H, NCH$_2$), 1.67 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$), 1.52 (m, 4H, NCH$_2$CH_2), 1.42 (m, 2H, CHCH_2CH(CH$_3$)$_2$), 1.37 (s, 18H, C(CH$_3$)$_3$), 0.88 (d, J = 6.7 Hz, 3H, CH(CH_3)$_2$), 0.86 (d, J = 6.6 Hz, 3H, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 173.6, 155.5, 77.3, 60.9, 48.5, 39.3, 38.0, 28.2, 28.1, 24.4, 22.6, 22.2; HRMS (FAB) calcd for $C_{22}H_{44}N_3O_6$ [MH$^+$]: 446.3230, found: 446.3229; elemental analysis calcd for $C_{34}H_{66}N_4O_6$ (626.92, DCHA salt): C 65.14, H 10.61, N 8.94; found: C 64.76, H 10.67, N 8.72.

General procedure for preparation of pentafluorophenyl activated esters: A solution of DCC (2.13 g, 10.3 mmol) in EtOAc (10 mL) at 0°C under N$_2$ was cannulated into a solution of **4** (10 mmol) and pentafluorophenol (2.01 g, 10.9 mmol) in EtOAc (40 mL). The mixture was stirred for 17 h while it warmed slowly to room temperature. The reaction flask was cooled to 0°C, then quickly filtered through a plug of Celite, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on a deactivated silica gel (prewashed with 1% triethylamine in the chromatographic solvent mixture). Triethylamine was removed completely by dissolving the product in EtOAc (100 mL), washing with water (10 × 100 mL) and brine (100 mL), drying over Na_2SO_4, and concentrating under reduced pressure. The compound was dried in vacuo to afford **5** as a colorless oil.

[*N*,*N*-Bis-(3-*N*-*tert*-Boc-aminopropyl)]-L-valine pentafluorophenyl ester (5a): Reaction of **4a** (1.23 g, 2.85 mmol) gave **5a** (1.62 g, 2.71 mmol) as a colorless oil. The crude product was chromatographed in 2:1 hexane/EtOAc. Yield: 95%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.78 (t, J = 5.4 Hz, 2H, NH), 3.30 (d, J = 10.7 Hz, 1H, CHCH(CH$_3$)$_2$), 2.94 (m, 4H, CH_2NH), 2.70 (m, 2H, NCH$_2$), 2.29 (m, 2H, NCH$_2$), 2.06 (d-septet, J = 10.8, 6.4 Hz, 1H, CHCH(CH$_3$)$_2$), 1.56 (m, 4H, NCH$_2$CH_2), 1.36 (s, 18H, C(CH$_3$)$_3$), 1.00 (d, J = 6.3 Hz, 3H, CH(CH_3)$_2$), 0.95 (d, J = 6.4 Hz, 3H, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 167.5, 155.6, 141.6, 139.1, 136.3, 124.3, 77.4, 69.2, 47.8, 37.9, 28.4, 28.2, 27.2, 19.2; ^{19}F NMR (376 MHz, [D$_6$]DMSO): δ = −153.5 (d, J = 20.3 Hz, 2F, F-2 of pentafluorophenol), −159.0 (t, J = 23.8 Hz, 1F, F-4 of pentafluorophenol), −163.7 (t, J = 21.8 Hz, 2F, F-3 of pentafluorophenol); HRMS (FAB) calcd for $C_{27}H_{41}F_5N_3O_6$ [MH$^+$]: 598.2916, found: 598.2918; elemental analysis calcd for $C_{27}H_{40}F_5N_3O_6$ (597.62): C 54.26, H 6.75, N 7.03; found: C 54.30, H 6.99, N 6.92.

[*N*,*N*-Bis-(3-*N*-*tert*-Boc-aminopropyl)]-L-leucine pentafluorophenyl ester (5b): Reaction of **4b** (3.13 g, 7.02 mmol) gave **5b** (2.27 g, 3.71 mmol) as a colorless oil. The crude product was chromatographed in 4:1 hexane/EtOAc. Yield: 53%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.75 (t, J = 5.1 Hz, 2H, NH), 3.81 (t, J = 7.4 Hz, 1H, CHCH$_2$CH(CH$_3$)$_2$), 2.93 (td, J = 6.3, 6.2 Hz, 4H, CH_2NH), 2.68 (m, 2H, NCH$_2$), 2.44 (m, 2H, NCH$_2$), 1.73 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$), 1.60 (m, 2H, CHCH_2CH(CH$_3$)$_2$), 1.50 (m, 4H, NCH$_2$CH_2), 1.36 (s, 18H, C(CH$_3$)$_3$), 0.95 (d, J = 6.6 Hz, 3H, CH(CH_3)$_2$), 0.92 (d, J = 6.5 Hz, 3H, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 168.8, 155.5, 141.7, 138.7, 136.2, 124.1, 77.3, 60.8, 48.2, 38.4, 37.8, 28.6, 28.2, 24.3, 22.6, 21.9; ^{19}F NMR (376 MHz, [D$_6$]DMSO): δ = −153.9 (d, J = 20.1 Hz, 2F, F-2 of pentafluorophenol), −159.2 (t, J = 23.6 Hz, 1F, F-4 of pentafluorophenol), −163.8 (t, J = 20.9 Hz, 2F, F-3 of pentafluorophenol); HRMS (FAB) calcd for $C_{28}H_{43}F_5N_3O_6$ [MH$^+$]: 612.3072, found: 612.3072; elemental analysis calcd for $C_{28}H_{42}F_5N_3O_6$ (611.64): C 54.98, H 6.92, N 6.87; found: C 55.02, H 7.01, N 6.80.

General procedure for peptide dendrimer synthesis on PEG: Poly(ethylene glycol) monomethyl ether (MeO-PEG-OH) was derivatized with Boc-protected glycine to give **7** following the procedure of Zalipsky et al.[17] Amino groups were deprotected by stirring the PEG-bound dendrimer in 1:1 TFA/CH_2Cl_2 (5–10 mL per 1 g resin) at room temperature for 3 h. Peptide coupling was carried out in 4:1 DMF/CH_2Cl_2 by preactivating monomer **5** with HOBt and DIEA for 5 min, then cannulating this mixture under N_2 into a solution of the peptide dendrimer in 4:1 DMF/CH_2Cl_2. Actual amounts of reagents used relative to the number of the coupling sites are the following: 3 equiv for the monomer **5**; 3 equiv for HOBt; 3 equiv plus the equivalent amount of TFA salt present for DIEA (4 equiv for the first-generation, 9 equiv for the second generation, and 19 equiv for the third-generation); 10 mL per gram resin for 4:1 DMF/CH_2Cl_2. The coupling reaction was completed in 12–24 h. Longer reaction times increased the amount of the unidentified side products. The uncoupled free amino groups were capped by stirring the PEG-bound dendrimer with Ac_2O (4 equiv) and DIEA (1 equiv) in CH_2Cl_2 at room temperature for 20 h. After each reaction, the mixture was poured into a 100-fold excess of cold anhydrous ether, filtered, and washed with ether to obtain the product. Additional washing with cold ethanol was necessary after each coupling step to remove any remaining HOBt. Before cleavage of PEG, third-generation dendrimers **10** were purified further, by preparative SEC in toluene. The mass balances for each step were essentially quantitative.

General procedure for the cleavage of PEG: The dendrimers were cleaved from the resin through the modified procedure of Chapman and Mahan.[9b] Third-generation PEG-bound dendrimer **10** (50 μmol) was dissolved in MeOH (250 mL), then an aqueous solution of NaOH (1N, 63 mL, 63 mmol) was added at 0°C. A white precipitate was detected instantly, but the reaction mixture became a clear, homogeneous solution upon stirring for 2–3 min. Additional MeOH (10 mL) was added and the mixture was stirred at room temperature for 6 h. The solution became cloudy by diluting with water (250 mL), then at 0°C was neutralized with citric acid powder to pH 6. The cloudy mixture was further diluted with water (300 mL) and left at 4°C overnight. The white solid was filtered off and washed with plenty of water to ensure the removal of PEG. The collected solid was dissolved in CH_2Cl_2, dried over Na_2SO_4, then concentrated under reduced pressure, and dried in vacuo to give **12** as a white solid.

[G-3(L-Val^1, L-Val^2, L-Val^3)]-(NH-Boc)$_8$ (12a): Reaction of **10a** (327.3 mg, 44.5 μmol) gave **12a** (88.7 mg, 37.4 μmol) as a white solid. Yield: 84%; R_f: 0.45 [silica gel, 1:20 AcOH/EtOAc]; ^{1}H NMR (500 MHz, 1:1 [D$_6$]DMSO/$CDCl_3$, 25°C): δ = 12.23 (br s, 1H, CO_2H), 7.85 (br s, 1H, NH of Gly), 7.70 (s, 6H, NH of Val^1 and Val^2), 6.35 (s, 8H, NH of Val^3), 3.72 (AB$_q$-d, J_{ab} = 17.6 Hz, J = 5.2 Hz, 2H, CH_2 of Gly), 3.20–2.99 (m, 12H, CH_2NH of Val^1 and Val^2), 2.95 (m, 16H, CH_2NH of Val^3), 2.65 (d, 1H, CHCH(CH$_3$)$_2$ of Val^1), 2.63 (m, 14H, NCH_2 of Val^1, Val^2, and Val^3), 2.56 (d, 6H, CHCH(CH$_3$)$_2$ of Val^2 and Val^3), 2.31 (m, 14H, NCH_2 of Val^1, Val^2, and Val^3), 1.93 (m, 7H, CHCH(CH$_3$)$_2$ of Val^1, Val^2 and Val^3), 1.51 (m, 28H, NCH$_2$CH_2 of Val^1, Val^2, and Val^3), 1.35 (s, 72H, C(CH$_3$)$_3$), 0.88 (m, 21H, CH(CH_3)$_2$ of Val^1, Val^2, and Val^3), 0.81–0.73 (m, 21H, CH(CH_3)$_2$ of Val^1, Val^2, and Val^3); ^{13}C NMR (125 MHz, 1:1 [D$_6$]DMSO/$CDCl_3$): δ = 171.1, 170.8, 155.5, 77.2, 70.0, 69.8, 47.8, 47.6, 38.2, 36.3, 28.1, 27.7, 26.7, 19.8, 19.7, 19.6; MS (MALDI-TOF): 2368.7 [M]$^+$, 2391.7 ([M+Na]$^+$), 2410.1 ([M+K]$^+$); elemental analysis calcd for $C_{119}H_{230}N_{22}O_{25}\cdot 2H_2O$ (2405.31): C 59.42, H 9.81, N 12.81; found: C 59.65, H 9.77, N 12.56; SEC: M_n = 2.52 kg mol^{-1} (T_R = 8.11 min).

[G-3(L-Leu^1, L-Leu^2, L-Leu^3)]-(NH-Boc)$_8$ (12b): Reaction of **10b** (186.8 mg, 25.1 μmol) gave **12b** (46.2 mg, 18.7 μmol) as a white solid. Yield: 75%; R_f: 0.5 [silica gel, 1:20 AcOH/EtOAc]; ^{1}H NMR (500 MHz, 1:1 [D$_6$]DMSO/$CDCl_3$, 25°C): δ = 7.96 (s, 1H, NH of Gly), 7.73 (s, 6H, NH of Leu^1 and Leu^2), 6.37 (s, 8H, NH of Leu^3), 3.74 (AB$_q$-d, J_{ab} = 17.5 Hz, J = 5.0 Hz, 2H, CH_2 of Gly), 3.20–3.08 (m, 23H, CH_2NH of Leu^3; CHCH$_2$CH(CH$_3$)$_2$ of Leu^1, Leu^2, and Leu^3), 3.08–2.88 (m, 12H, CH_2NH of Leu^1 and Leu^2), 2.50–2.36 (m, 28H, NCH_2 of Leu^1, Leu^2, and Leu^3), 1.52 (m, 35H, CHCH$_2$CH(CH$_3$)$_2$ of Leu^1, Leu^2, and Leu^3; NCH$_2$CH_2 of Leu^1, Leu^2, and Leu^3), 1.35 (s, 72H, C(CH$_3$)$_3$), 1.21 (m, 14H, CHCH_2CH(CH$_3$)$_2$ of Leu^1, Leu^2, and Leu^3), 0.84 (m, 42H, CH(CH_3)$_2$ of Leu^1, Leu^2, and Leu^3); ^{13}C NMR (125 MHz, 1:1 [D$_6$]DMSO/$CDCl_3$): δ = 172.4, 172.3, 172.1, 155.5, 77.3, 61.0, 60.7, 47.7, 47.5, 37.9, 36.4, 35.8, 28.1, 27.8, 24.7, 22.8, 22.0, 21.9; MS (MALDI-TOF): 2467.7 [M]$^+$, 2490.7 ([M+Na]$^+$), 2508.8 ([M+K]$^+$); SEC: M_n = 2.31 kg mol^{-1} (T_R = 8.19 min).

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[12] Supplied by Aldrich, M_n 5000.

[13] Although DMF was required as a major solvent for this step to disrupt any possible aggregation through hydrogen bonding, addition of a small amount of CH_2Cl_2 was necessary to get a homogeneous solution and thus drive the reaction to $>95\%$ (^{1}H NMR) for each generation.

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