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Synthesis of Poly(amino)ester Dendrimers via Active Cyanomethyl Ester Intermediates

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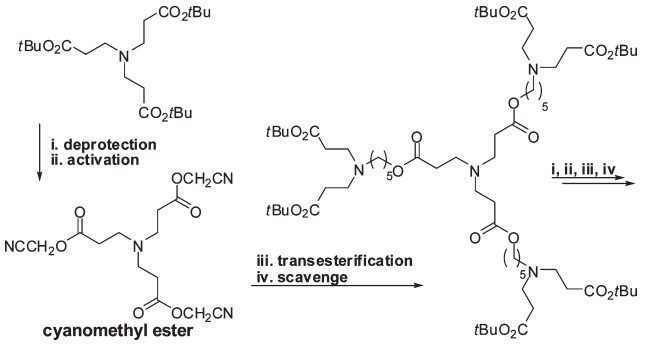

A novel strategy for the synthesis of poly(amino)ester dendrimers was developed on the basis of active cyanomethyl ester intermediates and an iteration of four consecutive steps of deprotection, activation, transesterification, and scavenging.

Dendrimers have attracted particular attention as drug delivery vehicles because of their high drug payload confined within a small nanosized volume.[1−3] This special feature is the result of their unique molecular architecture with cascade-branched units emanating from a focal point and numerous end groups on the surface.[4−6] Among the various dendrimers, poly(amino)ester dendrimers are particularly attractive as drug delivery systems because of their multiple advantageous features. First, they contain labile ester groups and are thus expected to be biodegradable via ester hydrolysis. Second, the amine functionalities present in the dendrimers can serve as buffers to neutralize the acids generated from the ester hydrolysis, allowing a benign and nonaggressive environment during dendrimer degradation. Finally, both the amine and the ester terminals are ready to undergo a large variety of chemical modifications which can offer various drug conjugations and/or confer specific properties such as solubility, dendrimer coating, and surface shielding.

Despite these appealing advantages, only a few examples of such dendrimers have been reported so far, mainly due to their complicated synthesis and limited available synthetic methods.[7−10] The synthesis of poly(amino)ester dendrimers can be envisaged either via Michael addition of amines yielding amine-bearing dendrimers, via ester formation giving ester-bearing dendrimers, or by a combination of both.[4,5,11−14] However, dendrimer growth via Michael addition of amines has a fatal drawback in that the ester groups present in the poly(amino)ester dendrimer may themselves be affected by the nucleophilic attack of amines, requiring special growing units containing amine and/or ester functionalities. Meanwhile, the conventional ester formation methods are not favorable for the synthesis of poly(amino)ester dendrimers due to the presence of amine functionalities, which often lead to incomplete ester formation and difficulties with purification. This is a general problem encountered during the synthesis of various amine-containing esters, which can be mainly ascribed to the easy protonation of the amines forming zwitterions or H-bonds in the reaction medium, resulting in reduced reactivity and impeded reaction progress as well as increased difficulties in product isolation.[15−17]

We have recently developed a strategy to synthesize amine-bearing esters via active cyanomethyl ester intermediates, which leads to a high product yield and easy purification.[17] Cyanomethyl esters are reported to be not only stable but also reactive enough to undergo transesterification with

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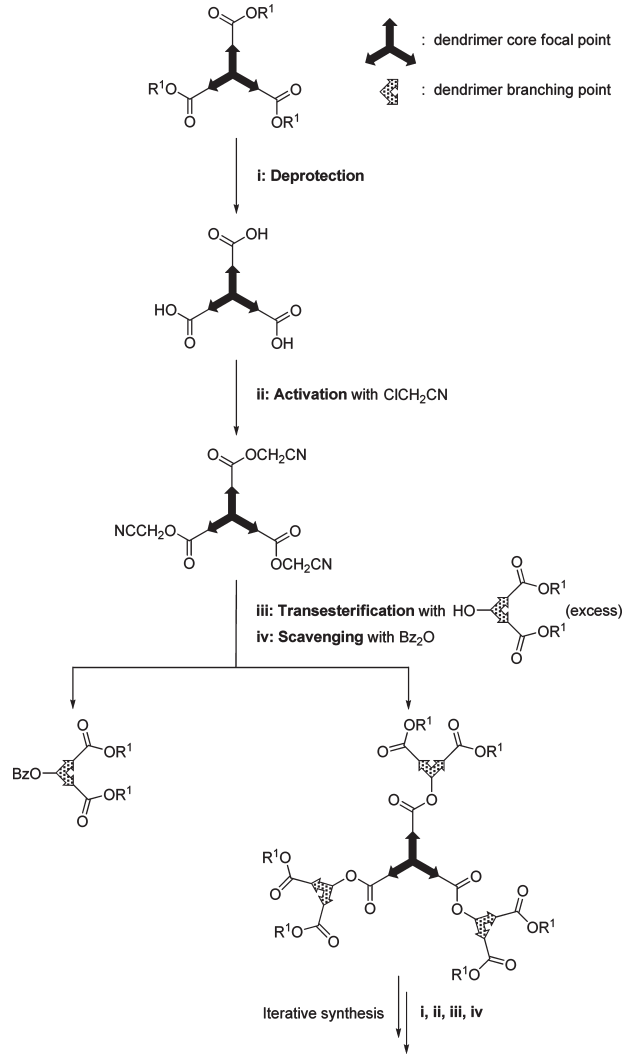
SCHEME 1. Synthetic Strategy to Construct Poly(amino)ester Dendrimers Based on Cyanomethyl Ester Intermediates and an Iteration of (i) Deprotection, (ii) Activation, (iii) Transesterification, and (iv) Scavenging



various alcohols.[18−21] We further explored this strategy for the preparation of poly(amino)ester dendrimers. Here, we report a novel and efficient method of synthesizing poly(amino)ester dendrimers (Scheme 1) based on the active cyanomethyl ester intermediates, including an original iteration of four consecutive reactions (Scheme 1): (i) deprotection of the ester terminals to generate acid functionalities, (ii) activation of the acid terminals as cyanomethyl esters, (iii) transesterification with an excess of alcohol bearing ester terminals and a tertiary amine as the branching point, and (iv) scavenging of the excess of alcohol. Further construction of higher generation dendrimers can be simply initiated *via*

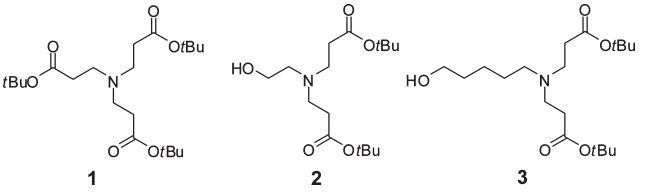
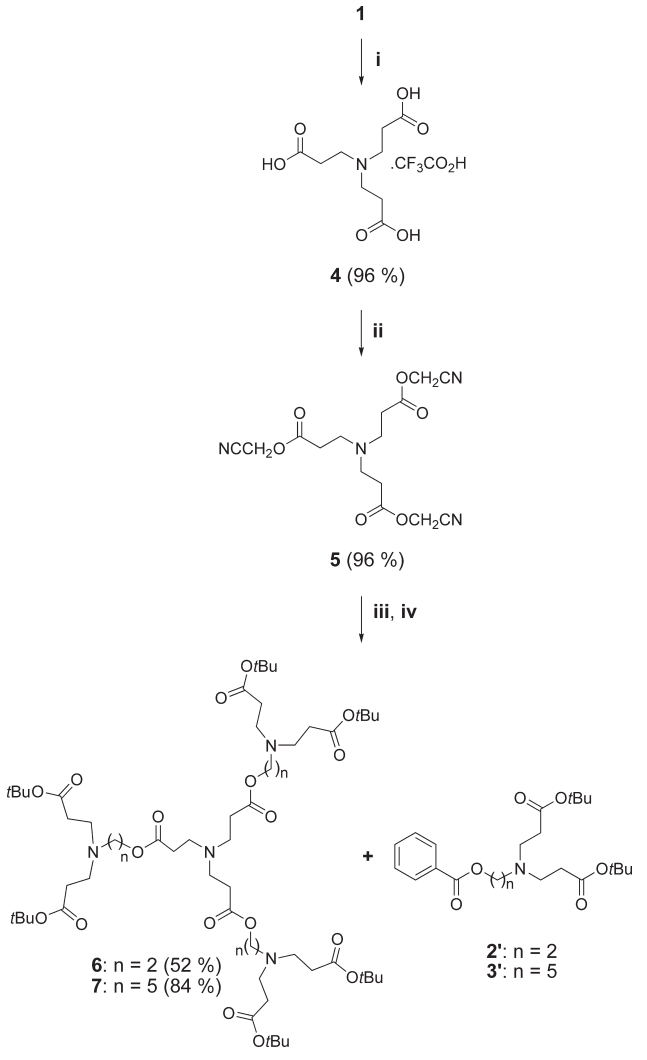


FIGURE 1. Construction units for the synthesis of poly(amino)-ester dendrimers: **1** as dendrimer core and **2** and **3** as the branching units.

SCHEME 2. Synthesis of the First-Generation Dendrimers 6 and 7[a]



[a]Conditions: (i) CF_3COOH, CH_2Cl_2, rt; (ii) chloroacetonitrile, Et_3N, CH_2Cl_2, rt; (iii) **2** or **3**, DBU, CH_3CN, rt; (iv) benzoic anhydride, DMAP, CH_2Cl_2, rt.

the regeneration of the terminal acids from the resulting dendrimer followed by the above-mentioned iterative steps (Scheme 1). The overall synthesis can be performed under very mild conditions, allowing the corresponding products to be obtained in a pure state and with good yields after easy purification.

We present here the synthesis of our prototype poly(amino)-ester dendrimers based on this novel synthetic strategy by

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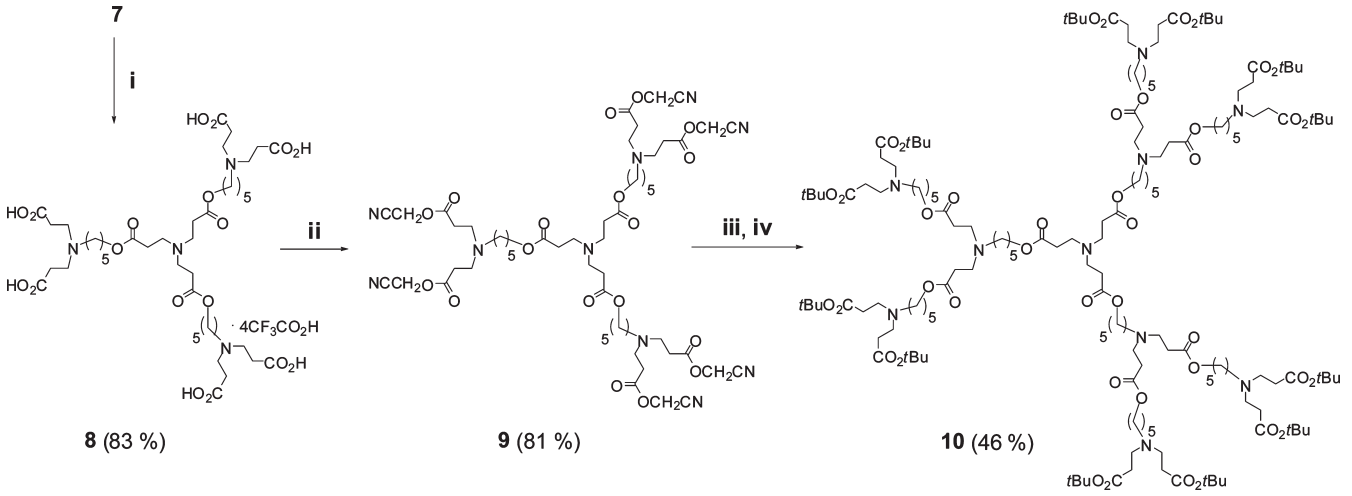
SCHEME 3. Synthesis of the Second-Generation Dendrimer 10[a]



[a]Conditions: (i) CF_3COOH, CH_2Cl_2, rt; (ii) chloroacetonitrile, Et_3N, CH_2Cl_2, rt; (iii) **3**, DBU, CH_3CN, rt; (iv) benzoic anhydride, DMAP, CH_2Cl_2, rt.

employing the triester **1** as the dendrimer core and the amine- and ester-bearing alcohols **2** and **3** as branching units for dendrimer growth (Figure 1).[17]

We first deprotected **1** with trifluoacetic acid to yield the triacid **4** (Scheme 2), which was further transformed to the activated cyanomethyl ester **5** by treatment with chloroacetonitrile in the presence of Et_3N in CH_2Cl_2. The resulting ester **5** was stable enough to be isolated and fully characterized. It also turned out to be sufficiently reactive to undergo a straightforward transesterification reaction with an excess of either **2** or **3** in the presence of DBU in CH_3CN.[17] After completion of the reaction, the excess of alcohol was scavenged with benzoic anhydride in the presence of DMAP and converted into the corresponding benzoic esters **2′** and **3′**, respectively (Scheme 2).[17] Both **2′** and **3′** possess very different polarity compared to the corresponding products **6** and **7** (Scheme 2), thus allowing easy product purification. This first cycle of four iterative steps was performed with an overall yield of 77% for **7**, starting from the core **1** and branching unit **3**, and 48% for **6** with the branching unit **2**. It is worth noting that the use of traditional methods for ester formation did not allow us to identify or isolate either **6** or **7** due to a low product yield and the presence of many side products as well as a tedious and difficult purification process.[15] Therefore, the synthetic strategy using active cyanomethyl ester intermediates is clearly superior with respect to constructing our prototype poly(amino)ester dendrimers **6** and **7**. It should also be noted that a higher yield was obtained for **7** compared to **6**. This could be reasonably ascribed to the beneficial effect of the longer spacer between the hydroxyl group and amine functionality in **3** when compared with **2** (Figure 1), making this branching unit less congested in space and thus more active in reaction. Consequently, we focused our attention on the use of the branching unit **3** to construct higher generation dendrimers.

We then deprotected **7** using trifluoacetic acid (Scheme 3). The resulting **8** was then treated with chloroacetonitrile, and the obtained cyanomethyl ester **9** was further transesterified with **3** in the presence of DBU. After the excess **3** was scavenged with benzoic anhydride, the expected ester **10** (Scheme 3) was obtained with 46% isolated yield and ready for a further cycle of dendrimer growth after deprotection of its terminal *tert*-butyl groups.

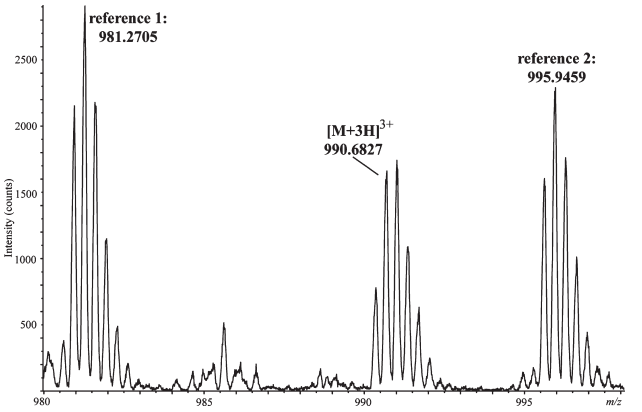


FIGURE 2. HRMS ESI analysis of poly(amino)ester dendrimer **10**: a triply protonated molecule at m/z 990.6827, corresponding to the molecular weight of **10** (2969.95 $g \cdot mol^{-1}$).

All the key intermediates and compounds described above were fully characterized by NMR analysis (^1H, ^{13}C, ^{13}C-HMQC) and high-resolution mass spectroscopic analysis for their structural assignment and purity control. Figure 2 shows the HRMS ESI spectral analysis of **10**, which displayed a characteristic triply charged peak at m/z 990.6827 (maximum of isotopic pattern) for $[M + 3H]^{3+}$, corresponding to the molecular weight of **10** (2969.95 $g \cdot mol^{-1}$) with an error of -0.3 ppm, therefore confirming the results observed by NMR analysis.

In conclusion, we have developed a novel and efficient method of synthesizing poly(amino)ester dendrimers via ester formation of active cyanomethyl ester intermediates. The synthetic sequence for dendrimer growth involves four iterative steps, namely (i) deprotection, (ii) activation, (iii) transesterification, and (iv) scavenging. All reactions can be carried out conveniently under very mild conditions and do not require any special manipulation and equipment. Moreover, the resulting dendrimers can be easily identified, purified, and obtained in a pure state and with good yields. Compared

to current methods of poly(amino)ester dendrimer synthesis based on both Michael addition via amine terminals and ester formation via activation of acid terminals using DCC or acid chlorides,[7−10] our method using cyanomethyl ester intermediates has proven to be much more simple and efficient with respect to its substrate scope, mild reaction conditions, easy purification procedure, and high product yields. In addition, the simple and convenient synthetic procedure developed here can be readily amended for the solid-phase synthesis of poly(amino)ester dendrimers and further applied to the synthesis of other ester-bearing dendrimers with diverse chemical structures. We are actively working in this direction with a view to developing efficient synthetic methods for the preparation of biologically relevant poly(amino)ester dendrimers for a variety of biological applications.

Experimental Section

Synthesis of 4. Compound **1** (6.3 g, 15.40 mmol) was treated with TFA (35.4 mL, 460 mmol) in CH_2Cl_2 (300 mL). After the mixture was stirred for 36 h at room temperature, the solvent and excess of TFA were removed. The oily crude residue was then triturated with Et_2O and filtered, affording **4** as a white solid of the TFA salt (5.10 g, yield 96%): ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.77 (t, 6 H, ^3J = 7.0 Hz), 3.33 (t, 6 H, ^3J = 7.0 Hz); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 28.4, 48.7, 171.9; HMRS (ESI) m/z calcd for the sodium adduct of **4** ($C_9H_{15}NO_6Na^+$) 256.0792, found 256.0792.

Synthesis of 5. To a solution of **4** (1.0 g, 2.88 mmol) in CH_2Cl_2 (5 mL) were added dropwise first Et_3N (3.2 mL, 23.10 mmol) and then chloroacetonitrile (2.2 mL, 34.60 mmol). The reaction solution was stirred overnight at room temperature. After removal of solvent, the obtained residue was diluted with EtOAc (30 mL), washed with water (2 × 20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting crude material was purified by flash chromatography (EtOAc/cyclohexane) to provide **5** as a pale amber oil (967 mg, yield 96%): R_f 0.14 (EtOAc/cyclohexane 1:1, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.49 (t, 6 H, ^3J = 6.3 Hz), 2.69 (t, 6 H, ^3J = 6.3 Hz), 4.69 (s, 6 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 32.0, 48.2, 48.7, 114.6, 170.5; HMRS (ESI) m/z calcd for the protonated molecule **5** ($C_{15}H_{19}N_4O_6^+$) 351.1299, found 351.1297.

Synthesis of 6. A solution of **5** (95 mg, 0.27 mmol), **2** (519 mg, 1.64 mmol), and DBU (245 μL, 1.64 mmol) in CH_3CN (4 mL) was stirred at room temperature for 48 h. The solvent was then removed, and the residue was diluted with EtOAc (20 mL), washed with water (2 × 15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, and concentrated. The resulting crude material was treated with benzoic anhydride (370 mg, 1.64 mmol) in the presence of DMAP (210 mg, 1.64 mmol) in CH_2Cl_2 (10 mL) for 1 h at room temperature. After removal of the solvent, the residue was diluted with EtOAc (15 mL), washed with saturated $NaHCO_3$ solution (2 × 15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, and concentrated. After purification by chromatography on silica gel (EtOAc/cyclohexane), **6** was obtained as a colorless oil (160 mg, yield 52%): R_f 0.77 (EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (s, 54 H), 2.34 (t, 12 H, ^3J = 7.0 Hz), 2.44 (t, 6 H, ^3J = 7.0 Hz), 2.67−2.81 (m, 24 H), 4.09 (t, 6 H, ^3J = 6.3 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 28.1, 34.0, 48.9, 49.9, 52.0, 62.5, 80.3, 171.6−172.1; HMRS (ESI) m/z calcd for the doubly protonated molecule **6** ($C_{57}H_{104}N_4O_{18}^{2+}$) 566.3667, found 566.3667.

Synthesis of 7. The ester **7** was synthesized according to a procedure similar to that described for **6**. Starting with **5** (100 mg, 0.29 mmol) and a large excess of **3** (569 mg, 1.74 mmol) in the presence of DBU (260 μL, 1.74 mmol) in CH_3CN (5 mL), followed by treatment with benzoic anhydride, **7** was obtained as a colorless oil (304 mg, yield 84%) after chromatography on silica gel (EtOAc/cyclohexane): R_f 0.50 (EtOAc/cyclohexane 7:3, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.19−1.28 (m, 12 H), 1.37 (s, 54 H), 1.51−1.62 (m, 6 H), 2.29−2.40 (m, 24 H), 2.63−2.70 (m, 18 H), 3.95−4.00 (m, 6 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.5, 26.7, 27.9, 28.3, 32.4, 33.4, 48.9, 49.1, 53.3, 64.3, 80.0, 171.8, 172.3; HMRS (ESI) m/z calcd for the doubly protonated molecule **7** ($C_{66}H_{122}N_4O_{18}^{2+}$) 629.4372, found 629.4392.

Synthesis of 8. The ester **7** (100 mg, 0.08 mmol) was treated by TFA (370 μL, 4.80 mmol) in CH_2Cl_2 (3.3 mL) for 3 days at room temperature. After removal of the solvent and the excess TFA, the oily crude residue was triturated with Et_2O and filtered, affording **8** as a white solid of TFA salt (91 mg, yield 83%): ^{1}H NMR (250 MHz, CD_3OD) δ 1.44−1.55 (m, 6 H), 1.70−1.83 (m, 12 H), 2.82−2.88 (m, 12 H), 2.95−3.00 (m, 6 H), 3.19−3.25 (m, 6 H), 3.42−3.51 (m, 18 H), 4.19 (t, 6 H, ^3J = 6.2 Hz); ^{13}C NMR (62.9 MHz, CD_3OD) δ 24.1, 24.4, 29.1, 50.6, 54.9, 66.2, 67.0, 172.7, 173.8; HMRS (ESI) m/z calcd for the protonated molecule **8** ($C_{42}H_{73}N_4O_{18}^+$) 921.4914, found 921.4916.

Synthesis of 9. The ester **9** was synthesized according to a procedure similar to that described for the synthesis of **5**, starting with **8** (740 mg, 0.54 mmol) and following treatment with chloroacetonitrile (1 mL, 16.10 mmol) in the presence of Et_3N (1.5 mL, 10.80 mmol) in CH_2Cl_2 (10 mL). Compound **9** was obtained as a pale amber oil (599 mg, yield 81%): R_f 0.78 (EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.16−1.45 (m, 12 H), 1.51−1.62 (m, 6 H), 2.32−2.55 (m, 24 H), 2.68−2.73 (m, 18 H), 4.00 (t, 6 H, ^3J = 7.1 Hz), 4.60 (s, 12 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.3, 26.6, 28.3, 32.1, 32.3, 48.2, 48.8, 48.9, 53.3, 64.2, 114.5, 170.7, 172.3; HMRS (ESI) m/z calcd for the protonated molecule **9** ($C_{54}H_{79}N_{10}O_{18}^+$) 1155.5568, found 1155.5571.

Synthesis of 10. The ester **10** was obtained in 46% yield (238 mg) according to a procedure similar to the one described for the synthesis of **7**, starting with **9** (200 mg, 0.17 mmol) and an excess of **3** (1.0 g, 3.12 mmol) in the presence of DBU (467 μL, 3.12 mmol) in CH_3CN (10 mL) for 48 h at room temperature. After workup, the crude reaction mixture was submitted to a solution of benzoic anhydride (1.8 g, 8.15 mmol) and DMAP (2.1 g, 16.3 mmol) in CH_2Cl_2 (15 mL) for 1 h at rt. After chromatography on silica gel (EtOAc/cyclohexane), **10** was isolated as a colorless oil: R_f 0.26 (EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.16−1.62 (m, 162 H), 2.27−2.36 (m, 60 H), 2.62−2.70 (m, 42 H), 3.95−4.00 (m, 18 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.7, 26.8, 27.0, 28.1, 28.5, 32.4, 33.6, 49.3, 53.5, 64.5, 80.2, 172.0, 172.5, 172.6; MALDI-MS m/z calcd for **10** ($C_{156}H_{285}N_{10}O_{42}$) 2969.95, found 2970.0; HMRS (ESI) m/z calcd for the triply protonated molecule **10** ($C_{156}H_{285}N_{10}O_{42}^{3+}$) 990.6830, found 990.6827.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and HRMS spectra of **4**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.