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Solid-phase construction: high efficiency dendrimer synthesis using AB_3 isocyanate-type monomers

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Abstract—Solid-phase synthesis is an ideal tool for reactions that require high concentrations and excess reagents and forcing chemical conditions. One such chemistry is that required for dendrimer construction. In this paper the synthesis of a series of symmetrical AB_3 isocyanate-type monomers is reported and used for the preparation of tri-branched dendrimers on the solid-phase. This method not only allows isolable dendrimer but can generate high-loading supports and devices for multivalent presentation.

1. Introduction

Dendrimers are macromolecules whose synthesis is generally based on a repetitive sequence of reaction steps, with each complete sequence creating a new generation of dendrimer.[1] This class of polymer are highly symmetrical monodisperse molecules consisting of three distinct regions: a core, from which they generally emanate, successive branching units and a peripheral multivalent surface. It is a combination of all these three regions that determine the physical and chemical properties of the molecule.[2] Since the early work of Vögtle[3] and Tomalia,[4] research in the area has evolved to enable the development of engineered dendritic macromolecules with unique chemical and physical properties for applications in the fields of medicinal chemistry, supramolecular chemistry, and catalysis among others.[5]

Divergent synthesis, based on the growth of the dendrimer from the central core to the periphery, is probably the most efficient and rapid procedure to construct dendrimers of high generation. This method is characterized by a rapid increase in the number of functional groups on the periphery However, due to the exponential increase in the number of terminal functional groups as well as the increasing steric congestion at the periphery during dendrimer growth, incomplete reactions can occur, especially at higher generations, which can produce structural defects.[2] In addition, excess reagents needed to drive reactions to completion may render purification difficult. Solid-phase synthesis has proven to be a powerful tool in making dendrimers, as excess reagents can be used to ensure complete reaction of the surface functional groups and can be removed easily by simple washing of the resin-bound dendrimer.[6] This methodology can be employed to prepare dendrimers which can be cleaved from the support via the use of a linker, or non-cleavable dendrimers which are covalently bound to a number of polymeric based supports. The former also allows the preparation of multivalent constructs which can be used to probe weak non-bonding interactions ('cluster effect')[7] while the latter is useful for increasing single-bead loading capacities. Both of these approaches have been widely investigated within our group since we reported the first solid-phase synthesis of PAMAM dendrimer in 1997.[8] The strategy was found to be an efficient method for enhancing resin loading by at least one order of magnitude and allowed structure characterization for compounds cleaved from a single bead.[9] However, some limitations were found to arise due to the formation of structural defects, especially at high generation, caused by retro-Michael addition or intramolecular lactam formation.[10] These side-reactions, which accumulate throughout dendrimer growth result in a decrease of terminal functional groups and the loss of symmetry. The AB_3-type monomers do not suffer from many of these potential limitations, and thus they became an attractive target for allowing solid-phase dendrimer synthesis, and allowing a much more rapid increase in functionality. In this paper, the synthesis of these monomers and their use in solid-phase dendrimer synthesis for the preparation of homogeneous dendrimers is described.

2. Results and discussion

Isocyanate monomers of the AB_3-type are advantageous

Keywords: solid-phase chemistry; dendrimers; high-loading; multivalency; isocyanate.

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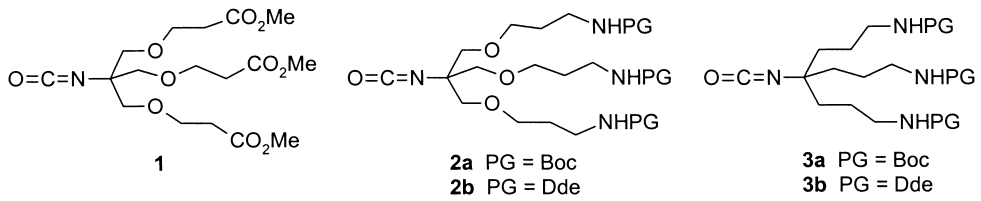


Figure 1. AB_3 Isocyanate-type monomers.

building blocks both for the solid-phase synthesis of dendrimers due to their high branching multiplicity and the presence of a reactive isocyanate group, which allows fast dendrimer construction without using additional coupling reagents. Our research focused on the use of AB_3-type monomers **1** and **3a** (Fig. 1) developed by Newkome et al.[11] and a series of novel monomers developed in our group (**3b**, **2a**–**b**).

The terminal amino groups of **2** and **3** were protected either with the Boc (*tert*-butoxycarbonyl) or the Dde-group (4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl), which is a highly selective protecting group for primary amines that can be cleaved under very mild conditions (2% H_2NNH_2 in DMF).[12]

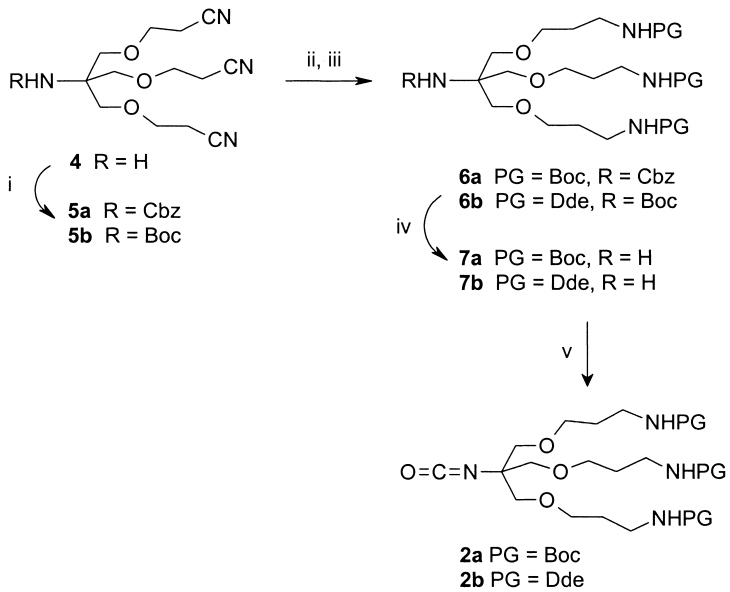
2.1. Monomer synthesis

The synthesis of monomers **2a**[13] and **2b** started from the tris-nitrile **4**, which was easily prepared by the Michael addition[14] of acrylonitrile to 1,1,1-tris-(hydroxymethyl) aminomethane (Scheme 1). The amine was protected either by the Boc or Cbz (benzyloxycarbonyl) group. Reduction of the nitrile group with the borane–tetrahydrofuran complex afforded the tris-amines, which were treated crude with Boc_2O (di-*tert*-butyl dicarbonate) or DdeOH (2-acetyl-dimedone) to afford the protected tris-amines **6a** and **6b**. After removal of the protecting groups the isocyanates **2a** and **2b** were prepared using stoichiometric amounts of DMAP (4-dimethylaminopyridine) and Boc_2O.[15] This method led to high yields while avoiding the use of triphosgene.

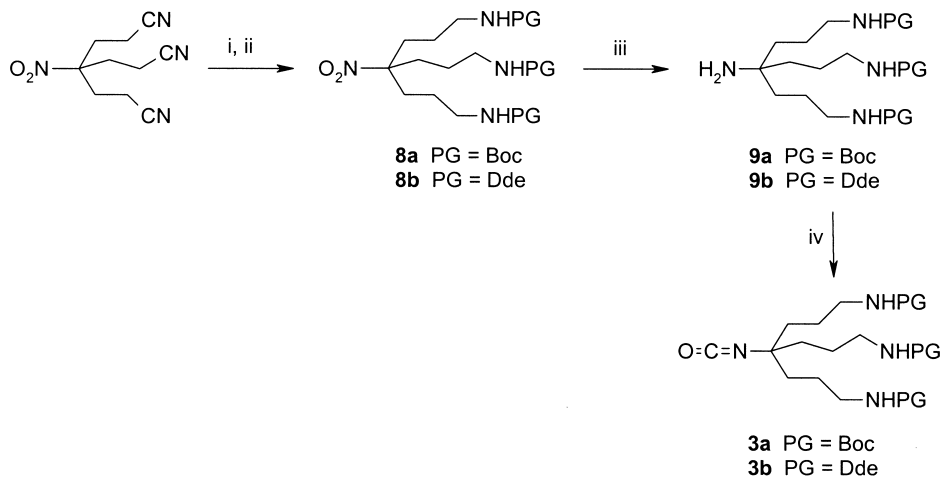
The Boc- and Dde-protected monomers **3a** and **3b** were prepared by a similar sequence using a modification of the procedure reported by Newkome for the synthesis of **3a** (Scheme 2).[16] The synthesis started from commercially available tris-(2-cyanoethyl)nitromethane. After reduction of the nitrile groups and protection of the resulting amines the nitro group was reduced by hydrogenation using commercial Raney Nickel as the catalyst. Treatment of the amines **9a** and **9b** with DMAP and Boc_2O gave the isocyanates **3a** and **3b** in good yields.

2.2. Non-cleavable dendrimers

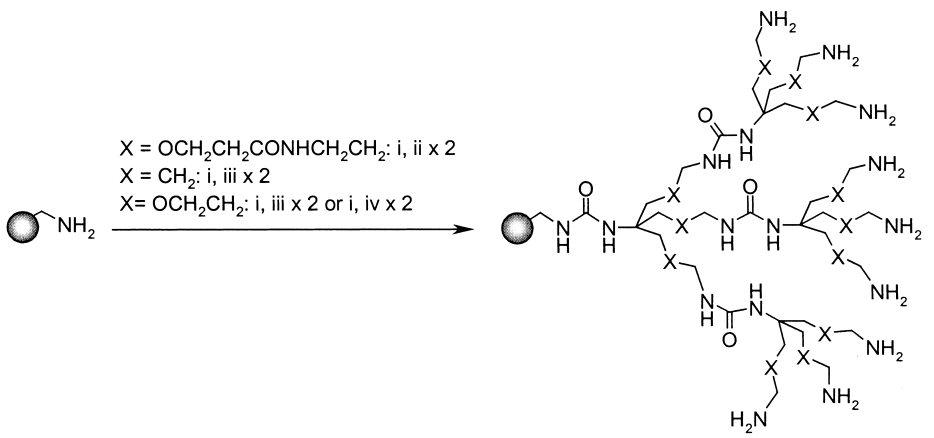
In 2000 the use of monomer **1** for the preparation of high-loading polystyrene resin beads was reported by our group.[17] Coupling of monomer **1** onto 250–300 μm aminomethyl polystyrene resin beads was followed by displacement of the methyl ester groups with propane-1,3-diamine to afford the first generation dendrimer (Scheme 3). Repetition of the cycle gave Generation 2.0 dendrimer functionalized resin with a loading of 36 nmol/bead (85% of the theoretical maximum loading). These beads were found to swell to a much greater degree in more polar solvents than the starting aminomethyl polystyrene resin, this is due to the polar nature of the dendrimer portion. Dendrimer synthesis was also carried out on 400–500 μm aminomethyl



Scheme 1. Synthesis of 1→3 C-branched tris-based isocyanate monomers. (i) CbzCl, sat. $NaHCO_3$ (91%) or Boc_2O, Et_3N (quant.); (ii) BH_3·THF, dioxane, 55°C; (iii) Boc_2O, Et_3N (37% for the two steps) or DdeOH, DIPEA (42% for the two steps); (iv) 10% Pd/C, H_2 (**7a** 92%) or 20% TFA in CH_2Cl_2 (**7b**, 87%); (v) Boc_2O, DMAP (**2a** 91%, **2b** 94%).



Scheme 2. Synthesis of 1→3 C-branched isocyanate monomers. (i) BH_3·THF, dioxane or THF, reflux; (ii) Boc_2O, NEt_3, MeOH, reflux (71% for the two steps) or DdeOH, MeOH (25% for the two steps); (iii) H_2, Raney-nickel, EtOH, 80°C (**9a** 81%, **9b** 75%); (iv) Boc_2O, DMAP, CH_2Cl_2 (**3a** 93%, **3b** 69%).



Scheme 3. Solid-phase dendrimer synthesis up to Generation 2.0. (i) Monomer **1**, **2a**,**b**, or **3a**, DIPEA, DMAP, CH_2Cl_2 and/or DMF; (ii) propane-1,3-diamine, MeOH or DMSO; (iii) 10–40% TFA/CH_2Cl_2, then 5% DIPEA/DMF; (iv) 5% H_2NNH_2/DMF.

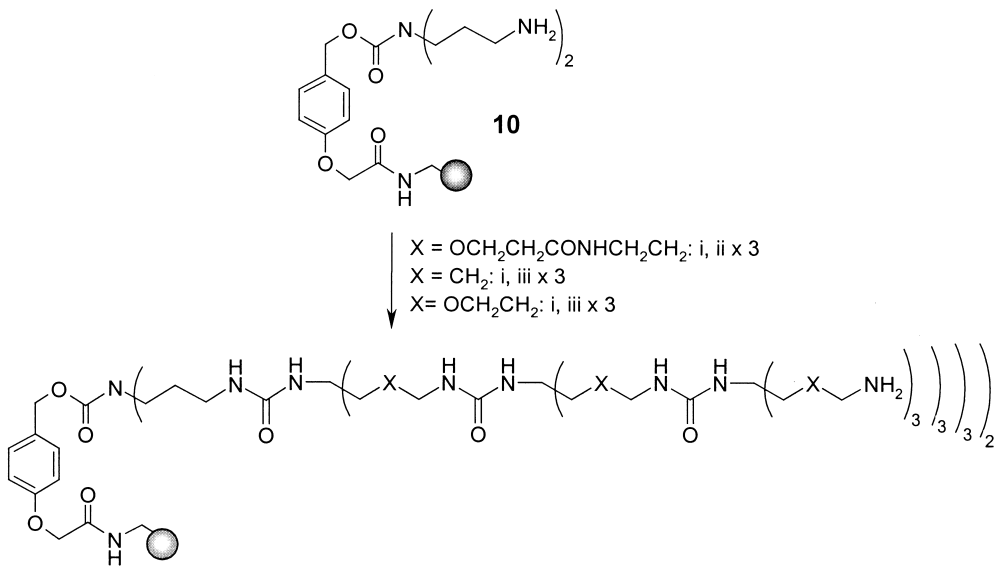
polystyrene beads, giving a loading of 116 and 230 nmol/bead for Generation 1.0 and 2.0, respectively.

Although this monomer afforded high-loading beads, one limitation came from possible macrocyclisation during ester displacement. The use of AB_3-type monomer **3a** was therefore investigated, as cross-coupling side-reactions with this monomer are prevented. Solid-phase dendrimer synthesis was carried out on polystyrene aminomethyl resins (250–300 μm, 1.38 mmol/g and 75–150 μm, 0.92 mmol/g) as well as on TentaGel™ resin (160 μm, 0.46 mmol/g).[16] The supports were reacted with the monomer in the presence of DIPEA and a catalytic amount of DMAP to afford Generation 0.5 resin-bound dendrimer. Treatment with TFA afforded Generation 1.0 (Scheme 3). This process was repeated to form higher generations. Each coupling was monitored by a qualitative ninhydrin test[18] and repeated if necessary until a satisfactory test was obtained. A portion of each full generation was then coupled to FmocGlyOH for loading determination via a quantitative Fmoc test.[19] The results are summarized in Table 1.

Table 1. Loading measurements on resin-bound dendrimers

Monomer	Support	Initial loading	Gen. 1.0	Gen. 2.0	Gen. 3.0	Overall loading increase
3a	PS (250–300 μm)	18	41 (76%)[a]	96 (78%)	Broken	×5.3
	PS (75–150 μm)	0.7	1.5 (77%)	3.6 (79%)	6.6 (61%)	×10.0
	TG (160 μm)	0.7	1.4 (68%)	3.2 (77%)	6.9 (72%)	×10.2
2a	PS (250–300 μm)	18	46 (85%)	120 (87%)	Broken	×6.7
	PS (75–150 μm)	0.7	1.7 (87%)	4.5 (86%)	12.5 (94%)	×18.9
	TG (160 μm)	0.7	1.4 (68%)	3.5 (84%)	9.0 (72%)	×13.2
2b	PS (200–250 μm)	13	36 (92%)	90 (83%)		×6.9

Loadings are in nmol/bead.
[a] Yields based on theoretical maximum loading.



Scheme 4. Solid-phase dendrimer synthesis up to Generation 3.0.

A 10-fold increase in loading for standard polystyrene resin and TentaGel™ was observed, however, a major problem was encountered with the larger polystyrene beads. Treatment of Generation 1.5 dendrimer-beads with 40% TFA in DCM, while attempting to form Generation 2.0, resulted in breakage of the majority of the beads. Observations under the microscope revealed that the damage was caused by the fast release of carbon dioxide and isobutylene bubbles formed during the Boc deprotection with TFA. It was found that progressively increasing the TFA concentration could overcome bead breakage, allowing the formation of Generation 2.0 dendrimer with a loading of nearly 100 nmol/bead. However, these resin beads were not physically robust enough to accommodate formation of Generation 3.0 when using the same deprotection procedure. Kinetic studies based on Fmoc release of Fmoc-Glycine derivatised resin-bound dendrimers with 20% piperidine in DMF were carried out on the large polystyrene beads (250–300 μm). Full deprotection required 22 and 57 min for Generations 1.0 and 2.0, respectively, compared to 5 min for the aminomethyl resin alone. This significant drop in reaction kinetics is the consequence of steric congestion interfering with site accessibility.

In order to improve reaction kinetics on the dendrimer functionalized resins, a new isocyanate monomer was designed. It was anticipated that increasing the flexibility of the dendritic assembly should help to maximize site accessibility, which in turn should result in speeding up the reaction rates, as well as allowing higher loadings to be obtained. Therefore monomers **2a**[13] and **2b** (where the monomer chain length was increased from three to five atoms) were investigated.

The procedure for solid phase dendrimer synthesis remained identical (Scheme 3). As anticipated increasing the length of the dendritic branches greatly facilitated the formation of resin beads with higher loading due to the greater degree of flexibility that minimized peripheral steric congestion (Table 1). The length of the spacer between the branching center and the reactive groups dictated the successful addition of subsequent monomer units onto the dendritic assembly, although this effect was less pronounced in the case of TentaGel™ resin. A 19-fold increase in loading was possible on standard polystyrene resin after the third generation. The same trend was obtained with 250–300 μm polystyrene resin, with Generation 2.0 exhibiting an average of 120 nmol/bead. Kinetic studies showed that the longer dendritic branches significantly increased reaction kinetics. However, the mechanical instability of the big polystyrene beads towards Boc deprotection remained, with the formation of generation 3.0 resulting in broken beads. Monomer **2b**, where the peripheral amino groups were protected with the Dde protecting group rather than Boc, was then investigated as a means of loading enhancement. One advantage of this monomer is that Dde deprotection can be carried out under mild condition without the release of any gas. The solid-phase dendrimer synthesis strategy remained the same (Scheme 3) only the deprotection of the Dde protected amines was achieved with a 5% hydrazine in DMF overnight. Slightly smaller resin beads (200–250 μm) were used. Generation 2.0 exhibited a loading capacity of 90 nmol/bead, which corresponded to the same overall loading increase as that with monomer **2a** on 250–300 μm polystyrene resin beads (Table 1).

2.3. Cleavable dendrimers

Solid phase dendrimer synthesis was also carried out by attaching an acid-cleavable polyamine scaffold on polystyrene aminomethyl resin (75–150 μm, 0.61 mmol/g).[17,20] The resulted amino derivatised resins **10** were then reacted with the isocyanate monomer **1** and Dde-protected monomers **2b** and **3b** in CH_2Cl_2/DMF (1:1) in the presence of DIPEA and DMAP with microwave assisted heating (100°C, 60 min) to afford the respective Generation 0.5 resin-bound dendrimers, which were further reacted with diaminoethane in DMSO and 5% hydrazine in DMF, respectively, to give Generation 1.0 resin-bound dendrimers. The processes were repeated to afford higher dendrimer generations as outlined in Scheme 4. Each coupling was monitored by a qualitative ninhydrin test and

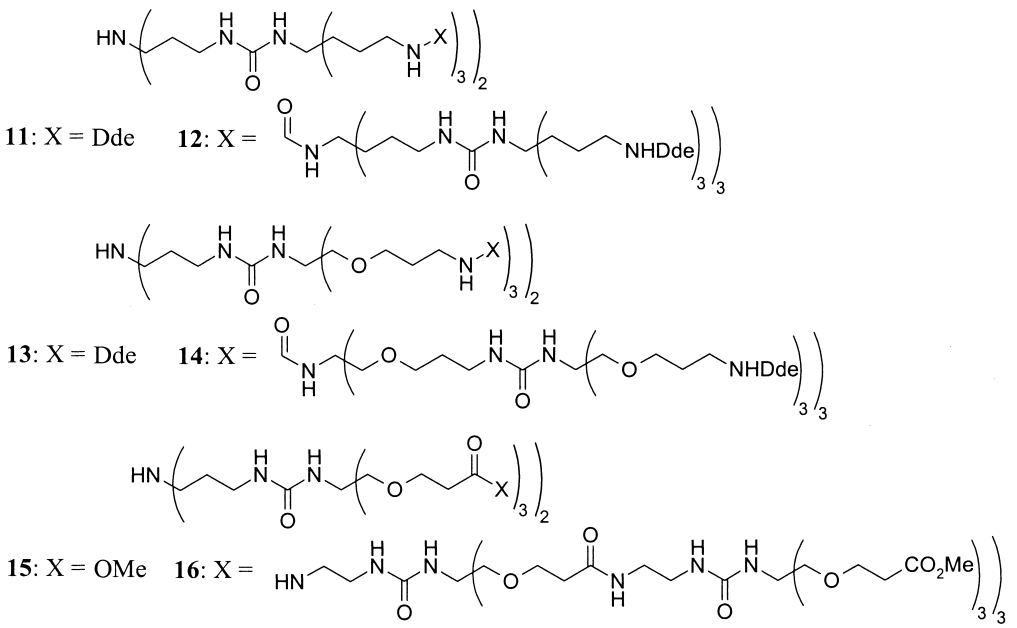


Figure 2. Generation 0.5 and 2.5 dendrimers cleaved as TFA salts. (i) Monomer **1**, **2b**, or **3b**, DIPEA, DMAP, CH_2Cl_2/DMF (1:1); (ii) ethane-1,2-diamine, DMSO; (iii) 5% H_2NNH_2/DMF.

repeated if necessary to drive the reactions to completion. Cleavage of the Generation 0.5 and 2.5 dendrimers with TFA/DCM/H_2O (9.5:0.25:0.25) gave the respective dendrimers (**11**–**16**) as TFA salts (Fig. 2). Attempts to obtain molecular ion of the Generation 2.5 products by ESI and MALDI-TOF were unsuccessful.

Various ligands were also successfully coupled to the constructed dendrimer prior to cleavage (results will be published elsewhere). The respective conjugates displayed multiple copies of ligands are excellent candidates for further biological evaluation[21] due to their high degree of define structure and multivalency.

3. Conclusion

AB_3-type monomers have proved to be very efficient for rapid dendrimer synthesis on solid supports. Non-cleavable dendrimers constructed on resins allow significant loading amplification. These resins have been successfully tested in a number of syntheses in our group and are used extensively in the area of single-bead screening. Synthesis and cleavage of these dendrimeric architectures showed that the reactions had proceeded as anticipated. The strategy allows multivalent constructs to be synthesized which subsequently can be screened for biological activity.

4. Experimental

4.1. General information

NMR spectra were recorded using a Bruker AC-300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, or a Bruker DPX 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported on the δ scale in ppm and are referenced to residual non-deuterated solvent resonances. Mass spectra were obtained on a VG Platform single quadrupole mass spectrometer in electrospray positive mode. IR spectra were obtained on a Biorad Golden gate FTS 135 spectrometer with neat compounds as oils or solids with stretches reported in cm^{-1}. Melting points are uncorrected. Microwave assisted heating was carried out by irradiating the mixture in a Smith Synthesizer at 2.45 GHz (available from Personal Chemistry). Commercially available reagents were used without further purification. THF was freshly distilled under nitrogen from a solution of sodium and benzophenone. Purifications by column chromatography were done on silica gel 60 (230–400 mesh) purchased from Merck. Compounds **1**, **3a**, and **4** were synthesized according to literature procedures.[11,14]

4.1.1. [2-(2-Cyanoethoxy)-1,1-bis-(2-cyanoethoxymethyl)ethyl]carbamic acid benzyl ester (5a). To a stirred solution of the amine **4** (2.00 g, 7.13 mmol) in ethyl acetate (15 mL) was added a saturated aqueous solution of $NaHCO_3$ (10 mL). Benzyl chloroformate (1.59 g, 9.32 mmol) was added and the reaction mixture was stirred for 2 h. Water (80 mL) was added and the reaction mixture was extracted with ethyl acetate (2×80 mL). The combined organic phases were washed with water (2×100 mL). The organic layer was dried over $MgSO_4$, concentrated in vacuo and purified by column chromatography (ethyl acetate/hexane 6:4) to give compound **5a** (1.69 g, 58%) as a colorless oil.

R_f=0.21 (ethyl acetate/hexane 6:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.58 (t, J=5.9 Hz, 6H), 3.67 (t, J=5.9 Hz, 6H), 3.79 (s, 6H), 5.06 (s, 2H), 5.14 (s, 1H), 7.30–7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.9, 58.9, 65.9, 66.7, 69.4, 118.1, 128.4, 128.7, 136.7, 155.2; IR (neat) ν 2251, 1716, 1236, 1105; HRMS (ES+) calcd for $C_{21}H_{26}O_5N_4Na$ $[M+Na]^+$: 437.1795, found: 437.1800.

4.1.2. [2-(2-Cyanoethoxy)-1,1-bis-(2-cyanoethoxymethyl)-ethyl]carbamic acid *tert*-butyl ester (5b). To a solution of amine **4** (1.32 g, 4.70 mmol) in CH_2Cl_2 (5 mL) and MeOH (5 mL) was added triethyl amine (5.64 mmol) and Boc_2O (1.23 g, 5.64 mmol). The reaction mixture was stirred overnight and poured into water (100 mL) and extracted with CH_2Cl_2 (2×100 mL). The combined organic layers were washed with water (200 mL), dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 98:2) to obtain **5b** (1.85 g, 100%) as a colorless oil.

R_f=0.62 (CH_2Cl_2/MeOH 14:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.65 (t, J=6.0 Hz, 6H), 3.74 (t, J=6.0 Hz, 6H), 3.82 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.0, 28.5, 58.7, 66.0, 69.6, 79.9, 118.1, 155.0; IR (neat) ν 2251, 1706, 1106; HRMS (ES+) calcd. for $C_{18}H_{28}N_4O_5Na$ $[M+Na]^+$: 403.1952, found: 403.1941.

4.2. General procedure A (reduction of tris-nitriles with BH_3·THF complex)

To a stirred solution of tris-nitrile was slowly added BH_3·THF complex. The solution was refluxed (times given below). After cooling, 2 M HCl was added until pH 1–2 was reached. The mixture was then neutralized with NaOH, and solvent was removed in vacuo. Yields and spectroscopic data are given below.

4.2.1. {3-[2-Benzyloxycarbonylamino-3-(3-*tert*-butoxycarbonylamino-propoxy)-2-(3-*tert*-butoxycarbonylaminopropoxymethyl)propoxy]propyl}carbamic acid *tert*-butyl ester (6a). According to general procedure A, carbamate **5a** (2.13 g, 5.14 mmol) and BH_3·THF (1.0 M solution in THF, 25.7 mL, 25.7 mmol) in dry dioxane (20 mL) were stirred for 3 h. After the work-up the crude tris-amine was dissolved in methanol (20 mL). Triethyl amine (0.323 g, 23.13 mmol) and Boc_2O (5.05 g, 23.13 mmol) were added and the reaction mixture was refluxed at 85°C for 4 h. The solvent was then removed in vacuo. The whitish gel obtained was extracted with ethyl acetate (2×100 mL). The combined organic phases were then washed with water (2×100 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo and the crude product was purified by column chromatography (hexane/ethyl acetate 1:1) to obtain **6a** (1.37 g, 37% for the two steps) as a colorless oil.

R_f=0.19 (hexane/ethyl acetate 6:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 27H), 1.72 (quint, J=5.9 Hz, 6H), 3.18 (dt, J=5.9 Hz, 6H), 3.49 (t, J=5.9 Hz, 6H), 3.66 (s, 6H), 4.93 (br s, 3H), 5.06 (s, 2H), 5.35 (s, 1H), 7.29–7.36 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 29.7, 38.4, 58.8, 66.4, 69.7, 70.1, 79.1, 128.2, 128.6, 136.5, 155.3, 156.1; IR (neat) ν 1689, 1247, 1166, 1102; HRMS (FAB+) calcd for $C_{36}H_{63}O_{11}N_4$ $[M+H]^+$: 727.4497, found: 727.4493.

4.2.2. [2-{3-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethylamino]propoxy}-1,1-bis-{3-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]propoxymethyl}-ethyl] carbamic acid *tert*-butyl ester (6b). According to general procedure A, compound **5b** (2.46 g, 6.47 mmol) and BH_3·THF complex (1 M solution in THF, 39 mL, 39 mmol) in dioxane (10 mL) was stirred at 55°C for 5 h. After work-up the crude product was dissolved in MeOH (15 mL) and DIPEA (7.76 mmol) was added. A solution of DdeOH (3.53 g, 19.41 mmol) in MeOH (2 mL) was added and the reaction mixture was stirred at 45°C overnight. The reaction mixture was poured into brine and extracted with CH_2Cl_2 (2×250 mL). The combined organic layers were washed with water (400 mL), dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 97:3) to give **6b** (2.41 g, 42% for two steps) as a colorless oil.

R_f=0.44 (CH_2Cl_2/MeOH 14:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (s, 18H), 1.33 (s, 9H), 1.85 (quint, J=6.0 Hz, 6H), 2.29 (s, 12H), 2.49 (s, 9H), 3.41 (t, J=6.0 Hz, 6H), 3.46 (t, J=6.0 Hz, 6H), 3.59 (s, 6H), 13.37 (br s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.3, 29.8, 29.9, 30.8, 31.4, 42.1, 54.4, 60.0, 69.8, 71.3, 80.6, 109.4, 156.4, 175.0, 199.3; IR (neat) ν 1710, 1636; HRMS (ES+) calcd for $C_{48}H_{77}N_4O_{11}$ $[M+H]^+$: 885.5583, found: 885.5586.

4.2.3. {3-[2-Amino-3-(3-*tert*-butoxycarbonylaminopropoxy)-2-(3-*tert*-butoxycarbonyl aminopropoxy-methyl)-propoxy]propyl} carbamic acid *tert*-butyl ester (7a). Tris-carbamate **6a** (1.31 g, 1.80 mmol) and 10% Pd/C (1.00 g) in EtOH (20 mL) was stirred under an atmosphere of hydrogen for 18 h. The product was filtrated over celite and washed thoroughly with EtOH before removal of the solvent in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 95:5) to give **7a** (0.98 g, 92%) as a colorless oil.

R_f=0.52 (CH_2Cl_2/MeOH/NH_3 9:1:0.1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 27H), 1.74 (quint, J=5.9 Hz, 6H), 1.92 (br s, 2H), 3.20 (dt, J=5.9, 5.9 Hz, 6H), 3.33 (s, 6H), 3.50 (t, J=5.9 Hz, 6H), 5.00 (br s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 29.7, 38.7, 56.2, 69.9, 73.1, 79.1, 156.1; IR (neat) ν 3347, 1688, 1167, 1103; HRMS (ES+) calcd. for $C_{28}H_{57}O_9N_4$ $[M+H]^+$: 593.4120, found: 593.4117.

4.2.4. [2-{3-1-(4,4,-Dimethyl-2,6-dioxocyclohexylidene)ethylamino]propoxy}-1,1-bis-{3-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]propoxymethyl}-ethyl]amine (7b). The protected amine **6b** (0.18 g, 0.20 mmol) in 20% TFA in CH_2Cl_2 (5 mL) was stirred for 30 min. The solvent was removed in vacuo and CH_2Cl_2 (100 mL) was added to the crude product and washed with saturated aqueous $NaHCO_3$ solution (100 mL) and water (100 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo and the crude product was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 94:6:1) to afford **7b** (0.14 g, 87%) as a colorless oil.

R_f=0.34 (CH_2Cl_2/MeOH 14:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (s, 18H), 2.00 (m, 6H), 2.37 (s, 12H), 2.59 (s, 9H), 3.58 (m, 6H), 3.64 (t, J=5.3 Hz, 6H), 3.76 (s, 6H), 13.29 (br s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.3, 28.6, 29.1, 30.5, 42.6, 53.1, 59.3, 69.9, 70.9, 108.4, 174.0, 198.3; IR (neat) ν 1676; HRMS (ES+) calcd for $C_{43}H_{69}N_4O_9$ $[M+H]^+$: 785.5059, found: 785.5045.

4.2.5. {3-[3-(3-*tert*-Butoxycarbonylaminopropoxy)-2-(3-*tert*-butoxycarbonylaminopropoxymethyl)-2-isocyanatopropoxy]propyl} carbamic acid *tert*-butyl ester (2a). The amine **7a** (4.54 g, 7.66 mmol) was stirred in THF (15 mL) at −13°C for 5 min. DMAP (1.03 g, 8.42 mmol) was added and the reaction mixture was stirred for another 5 min before a solution of Boc_2O (2.34 g, 10.72 mmol) in THF (2 mL) was added dropwise. The mixture was stirred at −13°C for 30 min, then concentrated in vacuo. The resulting residue was purified by column chromatography (SiO_2, ethyl acetate/hexane 1:1) to give **2a** (4.29 g, 91%) as a yellowish oil.

R_f=0.35 (ethyl acetate/hexane 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 27H), 1.75 (quint, J=5.9 Hz, 6H), 3.20 (dt, J=5.9, 5.9 Hz, 6H), 3.45 (s, 6H), 3.53 (t, J=5.9 Hz, 6H), 4.95 (br s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 29.7, 38.5, 64.1, 70.0, 71.6, 79.1, 127.8, 156.2; IR (neat) ν 2247, 1704, 1167, 1104; HRMS (ES+) calcd. for $C_{29}H_{54}O_{10}N_4Na$ $[M+Na]^+$: 641.3732, found: 641.3733.

4.2.6. [2-{3-{1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-ethylamino]-propoxy}-1,1-bis-{3-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]propoxymethyl}-ethyl]isocyanate (2b). Amine **7b** (1.41 g, 1.79 mmol) and DMAP (262 mg, 2.15 mmol) was dissolved in THF (20 mL) and stirred at −13°C for 5–10 min. To this solution was added dropwise a solution of Boc_2O (508 mg, 2.33 mmol) in THF (5 mL). The reaction mixture was stirred for 30 min. The solvent was removed in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 98:2) to obtain **2b** (1.37 g, 94%) as a colorless oil.

R_f=0.49 (CH_2Cl_2/MeOH 14:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 18H), 1.98 (m, 6H), 2.39 (s, 12H), 2.60 (s, 9H), 3.52 (s, 6H), 3.55 (m, 6H), 3.61 (s, 6H), 13.50 (br s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2, 28.7, 29.7, 30.5, 40.6, 53.3, 64.3, 68.7, 72.0, 108.3, 127.9, 174.1, 198.2; IR (neat) ν 2242, 1636; HRMS (ES+) calcd for $C_{44}H_{66}N_4O_{10}Na$ $[M+Na]^+$: 833.4671, found: 833.4659.

4.2.7. 4-Nitro-1,7-bis{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}-4-{3-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}propylheptane (8b). According to general procedure A, tris-(2-cyanoethyl)nitromethane (5.00 g, 22.7 mmol) and BH_3·THF complex (1 M solution in THF, 120 mL, 120 mmol,) in THF (50 mL) were refluxed for 4 h. After work-up, the crude product was dissolved in MeOH (50 mL) and a solution of DdeOH (15.0 g, 82 mmol) in CH_2Cl_2 (10 mL) was added. The reaction mixture was stirred overnight and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (ethyl acetate/MeOH 9:1) to yield **8b** (4.03 g, 25%) as a white solid.

R_f=0.24 (CH_2Cl_2/MeOH 95:5); mp: 165–167°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 18H), 1.64–1.72 (m, 6H), 2.06–2.11 (m, 6H), 2.37 (s, 12H), 2.58 (s, 9H), 3.46 (td, J=5.7, 6.4 Hz, 6H), 13.59 (br s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2, 23.9, 28.6, 30.5, 33.1, 43.2, 53.2, 93.2, 108.4, 174.0, 197.8; IR (neat) ν 1629, 1560, 1535, 1335; HRMS (ES+) calcd for $C_{40}H_{60}N_4O_8Na$ $[M+Na]^+$: 747.4303, found: 747.4338.

4.2.8. 4-Amino-1,7-bis{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}-4-{3-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]propyl}heptane (9b). A mixture of nitro compound **8b** (3.44 g, 4.74 mmol) and Raney Nickel (1.0 g) in ethanol (100 mL) was heated at 60°C for 2 days. After cooling the reaction mixture was filtered over celite and the solvent was removed in vacuo. The residue was dissolved in ether (100 mL) and then washed with 20% aqueous AcOH (4×20 mL). The aqueous phase was basified to pH 13–14 and extracted with CH_2Cl_2 (4×50 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo to give the amine **9b** (2.47 g, 75%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (s, 18H), 1.43–1.49 (m, 6H), 1.76–1.68 (m, 8H), 2.36 (s, 12H), 2.57 (s, 9H), 3.40 (td, J=5.9, 6.1 Hz, 6H), 13.5 (br s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.2, 23.6, 28.4, 30.3, 37.2, 43.9, 53.0, 108.0, 173.6; IR (neat) ν 1630, 1565, 1460, 1330; HRMS (ES+) calcd. for $C_{40}H_{62}N_4O_6Na$ $[M+Na]^+$: 717.4562, found: 717.4588.

4.2.9. 4-Isocyanato-1,7-bis{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}-4-{3-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]propyl}heptane (3b). To a mixture of amine **9b** (1.22 g, 1.76 mmol) and DMAP (0.215 g, 1.76 mmol) in DCM (15 mL) was added dropwise a solution of Boc_2O (0.538 g, 2.46 mmol) in CH_2Cl_2 (7 mL). The resulting mixture was stirred for 1 h. The solvent was evaporated in vacuo and the crude product was purified by column chromatography (CH_2Cl_2/MeOH 95:5) to yield **3b** (0.876 g, 69%) as a colorless oil.

R_f=0.26 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (s, 18H), 1.65–1.85 (m, 12H), 2.34 (s, 12H), 2.55 (s, 9H), 3.38–3.42 (m, 6H), 13.5 (br s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.0, 23.6, 28.2, 30.1, 36.5, 43.2, 53.5, 62.5, 107.9, 122.8, 173.5; IR (neat) ν 2255, 1635, 1570; HRMS (ES+) calcd for $C_{41}H_{60}N_4O_7Na$ $[M+Na]^+$: 743.4354, found: 743.4390.

4.3. General procedure for the solid-phase synthesis of Generation n.5 dendrimers (n=0, 1 or 2)

Isocyanate monomers **1**, **2** or **3** (1.5 equiv.), DMAP (0.1 equiv.) and DIPEA (1.5 equiv.) were dissolved in CH_2Cl_2/DMF (1:1). The resulting solutions were added to preswollen aminomethyl polystyrene resin or resin-bound dendrimer generations n.0 (n=0, 1 or 2). The mixtures were shaken for 1–5 days or irradiated with microwaves at 100°C for 60 min and reaction completion monitored by a qualitative ninhydrin test and FTIR. Couplings were repeated until a satisfactory ninhydrin test was obtained. The resins were filtered and washed thoroughly with DMF, DMF/THF (1:1), THF, MeOH, and CH_2Cl_2 and dried in vacuo to yield the corresponding resin-bound dendrimers.

4.4. Coupling of resin-bound dendrimer Generation n.5 with diaminoethane (n=0 or 1)

Diaminoethane (20 equiv.) in DMSO was added to the resin-bound dendrimer Generation n.5 (n=0 or 1) synthesised from monomer **1** and the mixtures shaken for 3–7

days. Reaction completion was monitored by FT-IR. The resins were filtered and washed thoroughly with DMF, DMF/THF (1:1), THF, MeOH, and CH_2Cl_2 and dried in vacuo to yield the resin-bound dendrimers.

4.5. General procedure for the deprotection of Boc group

To the respective preswollen resin-bound dendrimer Generation n.5 (n=0, 1 or 2) in CH_2Cl_2 was added 10% TFA in CH_2Cl_2 (5×30 min) with occasional stirring and washed with CH_2Cl_2. The same procedure was repeated with 25% TFA in CH_2Cl_2 and finally 40% TFA in CH_2Cl_2 (2×15 min). The resin was washed with CH_2Cl_2, DMF, 4% DIPEA in DMF, DMF, THF, THF/CH_2Cl_2, and CH_2Cl_2, before being dried in vacuo at 40°C.

4.6. General procedure for the deprotection of Dde group

To the respective preswollen resin-bound dendrimer Generation n.5 (n=0 or 1) in DMF were added 5% $N_2H_4.H_2O$ in DMF and the mixtures shaken for 4 h. Progress of reactions was monitored by the ninhydrin test. The resins were filtered and washed thoroughly with DMF, DMF/THF (1:1), THF, MeOH, and CH_2Cl_2 and dried in vacuo to yield the corresponding resin-bound dendrimers.

4.7. General procedure for the acidolytic liberation of dendrimers from the solid support

The dendrimers of Generation n.5 (n=0, 1 or 2) attached to the polyamine scaffold **10** were washed with CH_2Cl_2 before the addition of TFA/CH_2Cl_2/H_2O (9.5:0.25:0.25). The mixtures were shaken for 3 h. The solutions were filtered and the resins were washed with CH_2Cl_2 and dried in vacuo to yield dendrimers of Generation n.5 (n=0, 1 or 2) as TFA salts. The yield of products was calculated based on the molecular weight of fully protonated TFA salts relative to initial loading of aminomethyl resin (0.61 mmol/g).

4.7.1. Dendrimer 11. Cleavage from 50 mg of resin afforded **11** (26 mg, 36%) as a yellowish oil. ^{1}H NMR (400 MHz, CD_3CN) δ 0.98 (s, 36H), 1.59 (m, 12H), 1.68 (m, 12H), 1.77 (m, 4H), 2.34 (s, 24H), 2.50 (s, 18H), 2.91 (m, 4H), 3.18 (m, 4H), 3.42 (m, 12H), 13.32 (s, 6H); ^{13}C NMR (100 MHz, CD_3CN) δ 17.4, 22.4, 26.6, 27.0, 29.5, 32.0, 35.3, 43.3, 44.0, 51.4, 56.9, 106.9, 115.2, 158.4, 163.4, 174.0, 198.2; IR (neat) ν 1775,1564, 1152; MS (ES+) m/z 525.3 [$(M+3H)^{3+}$, 100%], 787.1 [$(M+2H)^{2+}$, 87%], 1572.7 [$(M+H)^+$, 5%].

4.7.2. Dendrimer 12. Cleavage from 50 mg of resin afforded **12** (32 mg, 5%) as a yellowish oil. ^{1}H NMR (300 MHz, CD_3CN) δ 0.98 (s, 324H), 1.59 (m, 156H), 1.68 (m, 160H), 2.34 (s, 216H), 2.50 (s, 162H), 2.91 (m, 4H), 3.18 (m, 4H), 3.42 (m, 156H), 13.22 (s, 54H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1, 22.5, 24.6, 27.2, 29.1,31.9, 43.5, 51.7, 57.5, 106.8, 164.0, 174.0, 197.0; IR (neat) ν 3329, 1775, 1562, 1152.

4.7.3. Dendrimer 13. Cleavage from 50 mg of resin afforded **13** (32 mg, quant.) as a yellowish oil. ^{1}H NMR (400 MHz, CD_3CN) δ 1.03 (s, 36H), 1.84 (t, J=6.0 Hz, 4H), 1.91 (quint, J=6.0 Hz, 6H), 2.41 (s, 24H), 2.56 (s, 18H), 2.95 (m, 4H), 3.27 (t, J=6.0 Hz, 4H), 3.52 (t, J=6.0 Hz, 12H), 3.58 (m, 12H), 3.64 (s, 12H), 13.26 (s, 6H); ^{13}C NMR (100 MHz, CD_3CN) δ 17.6, 26.9, 28.3, 29.6, 35.2, 41.0, 44.2, 51.0, 59.0, 68.3, 69.7, 106.9, 115.1, 158.4, 159.9, 175.5, 198.0; IR (neat) ν 1731, 1637, 1569, 1106; MS (ES+) m/z 585.4 [$(M+3H)^{3+}$, 30%], 877.4 [$(M+2H)^{2+}$, 60%].

4.7.4. Dendrimer 14. Cleavage from 50 mg of resin afforded **14** (36 mg, 5%) as a yellowish oil. ^{1}H NMR (400 MHz, CD_3CN) δ 1.04 (s, 324H), 1.70 (s, 52H), 1.93 (m, 108H), 2.45 (s, 216H), 2.56 (s, 162H), 2.97 (s, 4H), 3.20 (s, 4H), 3.54 (m, 156H), 3.60 (m, 156H), 3.65 (s, 156H), 13.22 (s, 54H); ^{13}C NMR (100 MHz, CD_3CN) δ 18.0, 26.9, 28.2, 29.7, 37.1, 41.3, 46.5, 50.4, 59.4, 68.3, 69.8, 106.9, 114.5, 158.0, 159.2, 175.1, 198.2; IR (neat) ν 3329, 1777, 1670, 1562, 1148.

4.7.5. Dendrimer 15. Cleavage from 50 mg of resin afforded **15** (20 mg, 62%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3CN) δ 1.79 (m, 4H), 2.52 (t, J=6.0 Hz, 12H), 2.91 (br s, 4H), 3.23 (t, J=6.0 Hz, 4H), 3.56 (s, 12H), 3.64 (s, 18H), 3.65 (t, J=6.0 Hz, 12H); ^{13}C NMR (100 MHz, CD_3CN) δ 26.8, 34.2, 34.9, 43.8, 50.9, 58.9, 66.5, 69.3, 159.5, 171.8; IR (neat) ν 3381, 1737, 1653, 1200; MS (ES+) m/z 471.8 [$(M+2H)^{2+}$, 65%], 942.4 [$(M+H)^+$, 100%].

4.7.6. Dendrimer 16. Cleavage from 50 mg of resin afforded **16** (54 mg, 15%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3CN) δ 1.85 (s, 4H), 2.50 (s, 48H), 2.57 (t, J=6.0 Hz, 108H), 3.04 (s, 4H), 3.21–3.35 (m, 100H), 3.59 (s, 108H), 3.62 (s, 48H), 3.67 (s, 162H), 3.69 (t, J=6.0 Hz, 156H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.0, 34.1, 35.7, 39.2, 39.7, 45.0, 51.0, 59.3, 66.6, 67.0, 69.4, 114.9, 158.0, 159.1, 171.8, 173.2; IR (neat) ν 3350, 1737, 1163.

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