A divergent route towards single-chemical entity triazine dendrimers with opportunities for structural diversity†‡

Hannah Crampton, Emily Hollink, Lisa M. Perez and Eric E. Simanek*

Received (in Montpellier, France) 7th December 2006, Accepted 24th January 2007
First published as an Advance Article on the web 2nd March 2007
DOI: 10.1039/b617875h

This manuscript describes the successful synthesis and characterization of five generations of dendrimers based on melamine. Early generations of these materials appear to be single chemical entities: upon purification, no detectable impurities are observed using NMR spectroscopy, mass spectrometry, and HPLC and GPC analysis. The analysis of larger generation materials precludes an unambiguous statement of purity. The synthetic route to these targets is divergent, relying on dichlorotriazine monomers that react with a polyamine dendrimer core of generation *n* to produce a poly(monochlorotriazine) dendrimer of generation *n* + 1. Subsequently, the poly(monochlorotriazine) is derivatized through nucleophilic aromatic substitution before additional nucleophilic amines are unmasked and the process iterated.

Introduction

Dendrimers have received widespread attention for potential applications ranging from medicine to materials science.[1–4] The field benefits greatly from the diversity of scientists and engineers engaged in these studies. This diversity is predicated on the commercial availability of these materials, notably PAMAM, PMMH and PPI.[5–7] Availability relies on synthetic routes to these materials that are efficient in terms of yield, costs of materials, and process conditions. To date, each dendrimer displays only one type of surface group, although the identity of this group is dependent on architecture. Our own interests include contributing a dendrimer scaffold with groups on the surface that can be orthogonally manipulated.

Recently, we reported on a divergent synthetic strategy that allowed access to a variety of macromolecular architectures including stars, dendrimers and hybrid molecules by exploiting the differential reactivity of dichlorotriazines.[8] To us, this represents a significant advance over our previous convergent routes. While both routes rely on the stepwise nucleophilic aromatic substitution of chlorotriazines, the convergent approach had practical limitations.[9] Of lesser issue, the macromolecule's constitution was dictated at the commencement of synthesis with the choice of surface groups. This limitation was offset by carrying latent functionality (in the form of orthogonal protecting groups) through the synthesis that could be manipulated.[10,11] Of greater issue, the repetitious coupling of smaller fragments into larger ones required that most of the mass be carried through the entire synthesis. Regardless, reasonable quantities of materials of high purity up to generation three could be obtained for evaluation.

Department of Chemistry, Texas A&M University, College Station, TX 77845, USA. E-mail: simanek@tamu.edu
† This paper was published as part of the special issue on Dendrimers and Dendritic Polymers: Design, Properties and Applications.
‡ Electronic supplementary information (ESI) available: NMR and mass spectra. See DOI: 10.1039/b617875h

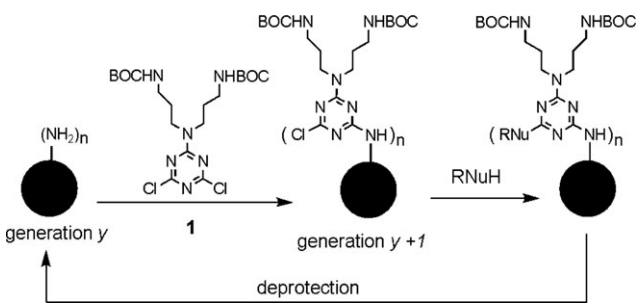
The divergent approach employed here overcomes the limitations of our convergent route. This route relies on the iterative incorporation of dichlorotriazine **1** bearing two BOC-protected nucleophilic amines onto a poly(amine) dendrimer of generation *n* (Scheme 1). Upon installation of the nucleophilic group (RNuH) and deprotection, the cycle can be repeated. Here, we report the successful synthesis and characterization of these architectures through generation five. The structural diversity referred to is reflected in the surface BOC and piperidine groups of each dendrimer, though clearly, the latter cannot be manipulated further.

Results and discussion

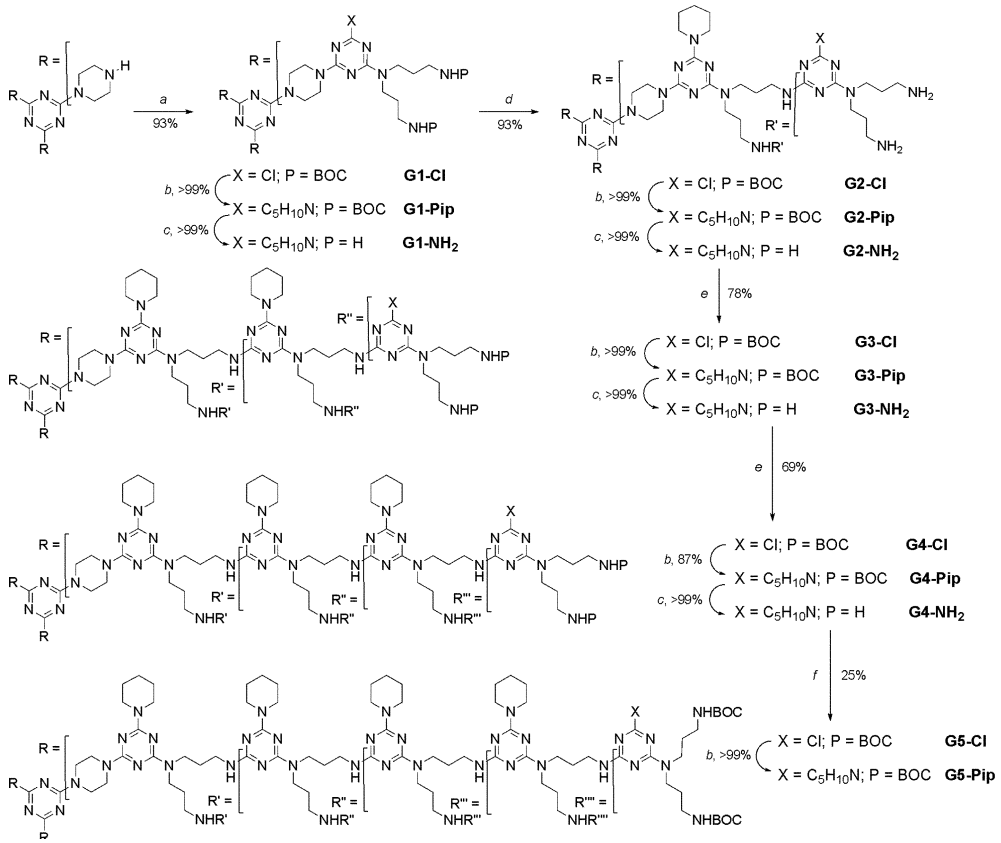
Synthesis

The dichlorotriazine monomer, **1**, used iteratively throughout this synthesis satisfies many demands: it is prepared in high yields in two steps, it is readily purified, and it is a stable crystalline solid. Indeed, methods for the kilogram-scale preparation of this material will be described in due course. Monomer **1** undergoes nucleophilic aromatic substitution cleanly to the poly(monochlorotriazine). Scheme 2 outlines the synthetic route adopted for these materials.

Here, we carry piperidine groups through the synthesis under the assumption that these groups (i) promote greater



Scheme 1 General synthetic route.



Scheme 2 Synthesis of **G1–G5** dendrimers. *Reagents and conditions*: (a) **1**, DIPEA, THF, 0–25 °C, 15 h; (b) piperidine, THF, 25 °C, 12–16 h; (c) 5 M HCl–MeOH, 25 °C, 15 h; (d) **1**, DIPEA, THF–water, 0–25 °C, 15 h; (e) **1**, DIPEA, EtOAc–THF–water, 25 °C, 16 h; (f) **1**, DIPEA, $CHCl_3$, 25 °C, 5 d.

solubility than the aminoalcohols used in the preliminary report, and (ii) undergo facile reactions with monochlorotriazines. The nomenclature adopted identifies the generation, **G*n***, and the functional group of greatest interest as Cl, Pip (piperidine) and NH_2. The iterative nature of the synthesis proceeds from the core, **2**, through **G1-Cl** upon reaction with monomer **1**, through **G1-Pip** upon capping the poly(monochlorotriazine) with piperidine, to **G1-NH_2** upon deprotection. Iterative reaction with **1** yields **G2-Cl**, and the cycle of reactions continues.

One advantage of this synthetic sequence is the set of universal conditions employed for both the piperidine step and deprotection. Throughout the course of the synthesis, both of these reactions proceed quantitatively. These conditions are described explicitly in the experimental section. The problematic step of this relatively straightforward synthesis is iteration with **1**. Under the conditions currently employed, the yields for this step decrease with each successive generation. In addition, solubility of the **G*n*-NH_2** intermediates require that the solvent be reconsidered at every generation. We find that moving from tetrahydrofuran to methylene chloride and ultimately to chloroform provides the best results and the cleanest reactions. Solvents that proved less useful include dimethylformamide, dimethylacetamide, dimethyl sulfoxide, methanol, water, tetrahydrofuran/water with and without sodium dodecyl sulfate, and the ionic liquid octylmethylimidazolium tetrafluoroborate.

Characterization of these materials rests on analysis using thin layer chromatography (TLC), NMR spectroscopy, mass spectrometry, HPLC and GPC. While exact estimates of purity for higher generation materials are imprecise, we are confident that these materials are as close (and in many cases closer) to single-chemical entity status as other dendrimers described in the literature. We believe that lower generation materials are pure (>97%) as defined by the conventional definition of synthetic organic chemistry.

Characterization

Characterization of the G*n*-NH_2 species. The polarity of the polyamine cores makes TLC analysis problematic. Both full and partially deprotected dendrimers adhere to silica and cannot be separated. ^{1}H NMR spectroscopy is quite diagnostic, especially for lower generations where solubility is not a problem: it reveals both the loss of the *tert*-butyl protons of the BOC groups, as well as the significant upfield shift of the CH_2NHBOC peak upon deprotection (from 3.07 to 2.68 ppm). Mass spectrometry confirms the purity of these dendrimers through G4. While **G5-NH_2** has been isolated, characterization of this material by mass spectrometry has proven unsuccessful to date.

Characterization of the G*n*-Cl species. TLC reveals a significant shift in polarity upon complete substitution of the polyamine core. Unfortunately, and presumably attributed to

the polarity of the amine intermediates, we cannot follow the reaction progress by TLC. That is, intermediates derived from a substoichiometric number of reactions with monomer **1** cannot be separated from starting polyamine. NMR spectroscopy is of limited use due to the broad and overlapping lines. However, the CH_2NHBOC peak stands alone at ~3 ppm in each generation, and can be integrated with respect to the area of peaks from ~3.8–3.2 ppm that correspond to methylenes α to nitrogen derived from cyclic and acyclic groups (Fig. 1). By defining the area of the former methylenes, the expected and observed integrations can suggest the extent of reaction. In Fig. 1, the expected value for the downfield region is shown in parentheses for the latter protons, while the observed integration is reported above.

Mass spectrometry confirms at isotopic resolution that substitution is complete in lower generations: a single parent line is seen for each dendrimer, and no lines corresponding to truncated intermediates are seen. The traces for **G4-Cl** and **G5-Cl** lack this level of resolution. Fig. 2 shows the trace for **G4-Cl** with a molecular ion peak that shows incremental losses of BOC as a "tailing" to lower m/z values. A similar pattern is evident from the doubly-charged M^{2+} ion. We believe that these loss-of-BOC species arise during the MALDI-MS ionization event: previous studies wherein the voltage was changed affected the relative populations of these species.

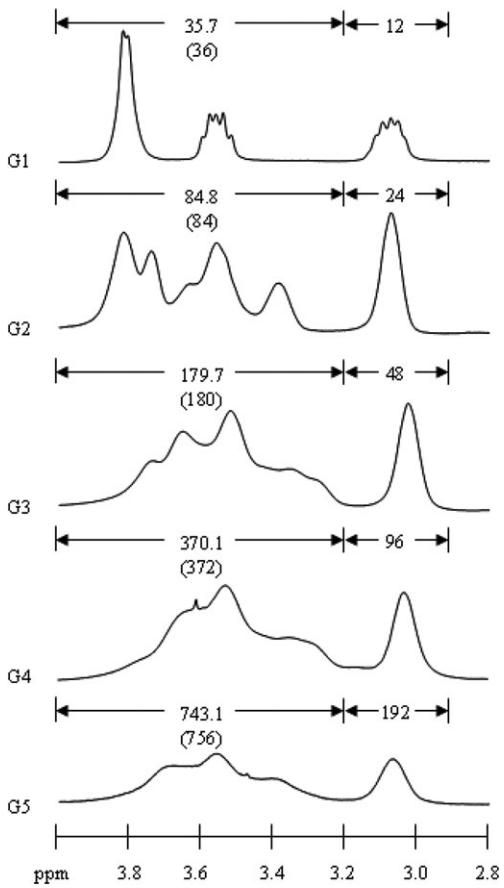


Fig. 1 ^{1}H NMR spectra of the aliphatic regions of the **G*n*-Cl** targets. The integration of the upfield peak is assigned the theoretically expected value and compared to the observed and (expected) values for the downfield signals. The integration region is indicated with arrows.

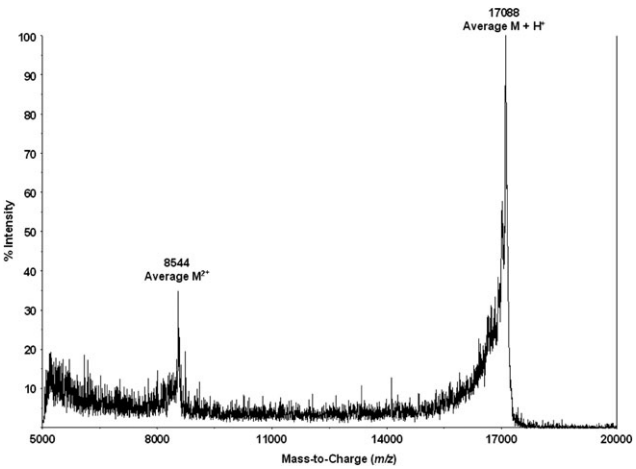


Fig. 2 The MALDI-TOF MS spectrum of **G4-Cl** reveals the limitations of characterization. The tailing towards lower m/z values reflects loss of BOC (presumably during ionization) and obscures evidence of incomplete reaction.

The **G*n*-Cl** species were subjected to HPLC and GPC analysis. In either case, **G5-Cl** did not elute from the columns. However, **G1-Cl** through **G3-Cl** showed sharp HPLC traces and symmetric GPC traces (Fig. 3). Both the HPLC and GPC traces of **G4-Cl** show tailing that could be attributed to impurities of similar polarity and dimension. Accordingly, we cannot confidently ascribe these materials as single-chemical entities using the accepted definitions of organic chemistry. However, this material does represent a purity that is rarely seen in synthetic macromolecules.

Characterization of the G*n*-pip species. The substitution of chloride by piperidine does not have an appreciable effect on the polarity of these dendrimers, particularly at higher generations, making it impossible to follow reaction progress by TLC. Analysis by NMR spectroscopy of dendrimers beyond generation one is hindered by broad and overlapping lines, but as with the **G*n*-Cl** dendrimers, the CH_2NHBOC peak can be

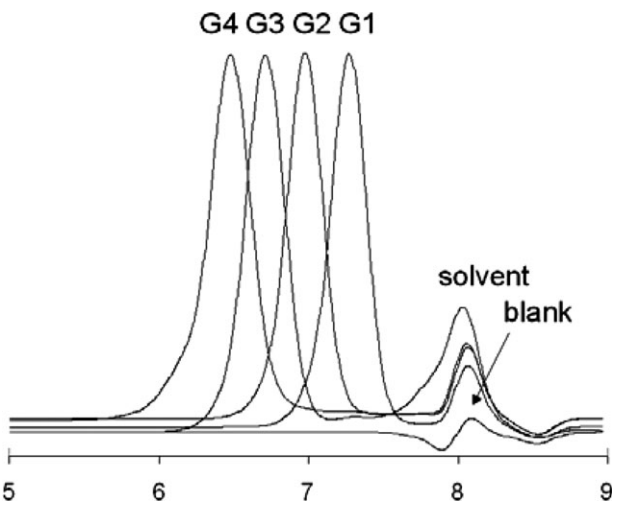


Fig. 3 GPC traces of **G*n*-Cl** (n = 1–4) and a blank.

integrated with respect to the group at 3.8–3.2 ppm, which contains the piperidine α-CH_2 peak. The mass spectra of **G1-Pip** through **G4-Pip** confirm that substitution is complete: the trace from **G5-Pip** is too broad to interpret.

Computation

Computational modeling of these architectures reveals the onset of globular structure at generation three as indicated by localization of the core, shown in maroon, to the interior of the dendrimer (Fig. 4). The three arms of each dendrimer that eminate from the core are colored red, green and blue. Distinct surface domains emerge at generation four and are pronounced in generation five. Smaller generations are not sufficiently encumbered sterically to completely allow segregation of end groups into specific regions of space. The dimensions reported in angstroms for these targets corroborate earlier investigations.

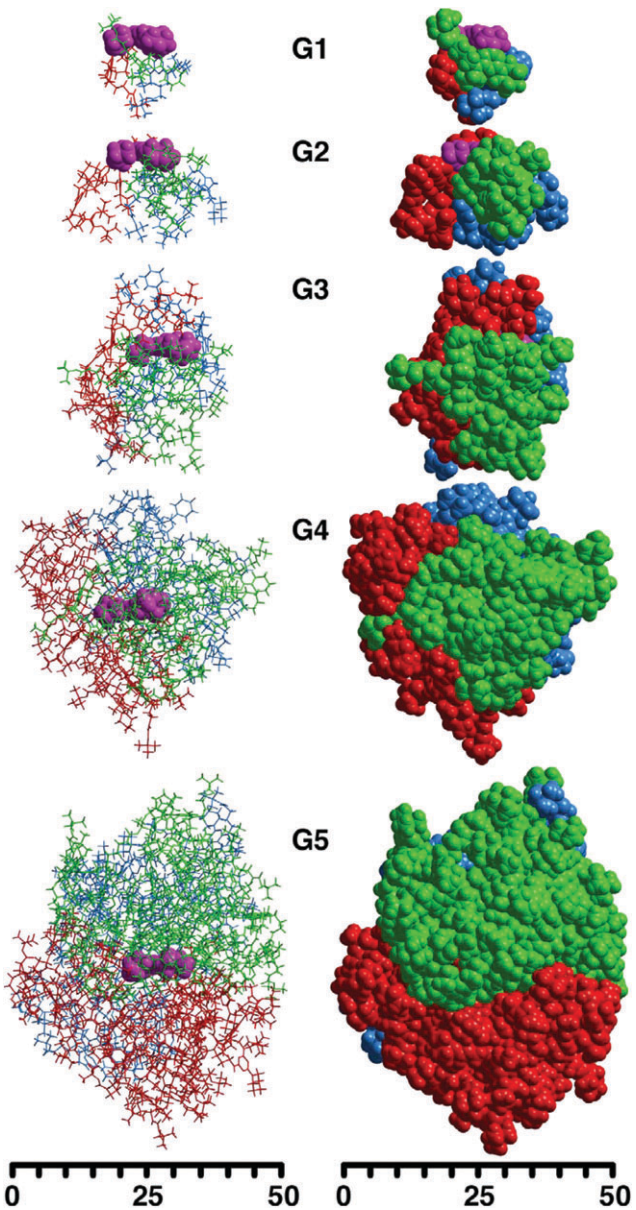


Fig. 4 Computation reveals the onset of globular structure at generation 3. The core is shown in maroon. The dimensions are in angstroms.

Conclusions

We report the synthesis and characterization of five generations of dendrimers based on melamine. The divergent route employed appears to yield single-chemical entities through generation four. Establishing the purity of fourth and fifth generation materials remains challenging due to the current reliance on NMR spectroscopy: mass spectrometry provides a broad peak centered appropriately and HPLC and GPC analysis have not been particularly successful, but corroborate the assertion that in many cases, the purity of these materials competes favorably with what is commercially available. These targets are available in excellent yields at lower generations and modest yield at higher generations. Purification has proven straightforward. Extending these reactions to larger generation dendrimers will require additional investigations of reaction conditions, or the substitution of piperidine with some other capping group.

The value of these investigations are in establishing synthetic routes that combine the purity usually associated with convergent syntheses and the efficiency associated with divergent routes. Of perhaps greatest importance is the potential for diversity in this system. One can imagine adding a different nucleophile at each generation during the capping step, where here we use piperidine. This potential for diversity, along with the purity of these materials, makes them particularly good candidates for a variety of uses, including drug delivery.

Experimental

All solvents and reagents were purchased from Aldrich Chemical Co. or Acros Organics and were used without purification. The reagent $HN(CH_2CH_2CH_2NHBoc)_2$ was prepared by a modification of previously reported method.[12] The core **2** was reported previously.[13]

Computation

Computational results were obtained using the software package Cerius^2 4.9 by Accelrys Inc. Minimization and dynamics calculations were performed with the Open Force Field (OFF) program, using the pcff second-generation force field.[14] The dendrimer was initially drawn and minimized in a fully extended conformation. Constant volume and temperature (*NVT*) molecular dynamics (MD) calculations were then performed on the minimized structure *via* simulated annealing. The simulated annealing was carried out for 560.0 ps, over a temperature range of 300–1000 K, with ΔT = 50 K, using the *T*-Damping temperature thermostat, a relaxation time of 0.1 ps, and a time step of 0.001 ps. The dendrimer was minimized after each annealing cycle, resulting in 200 structures.

Characterization

Thin-layer chromatography was performed using EMD silica gel 60 F_{254} pre-coated glass plates (0.25 mm). Preparative column chromatography was performed using EMD silica gel

60 (0.040 mm particle size). ^{1}H and $^{13}C\{^{1}H\}$ NMR data were acquired on a Varian 300 MHz spectrometer at 25 °C unless otherwise indicated. ^{1}H and $^{13}C\{^{1}H\}$ NMR chemical shifts are listed relative to tetramethylsilane in parts per million, and were referenced to the residual proton or carbon peak of the solvent. MS analyses were performed by the Laboratory for Biological Mass Spectrometry at Texas A&M University. MALDI-TOF mass spectra were obtained on an Applied Biosystems voyager-DE STR Biospectrometry workstation. Samples were diluted to 0.1 mg ml^{-1} and mixed with 2,4,6-trihydroxyacetophenone at 20 mg ml^{-1} in a 1 : 5 analyte : matrix ratio. HPLC analyses were performed on a Waters Delta 600 system with a Waters 2487 dual wavelength absorbance detector at 240 nm. A Waters Symmetry C_{18} silica-based RP-HPLC column (4.6 × 250 mm, 5 μm, 100 Å) was used with a mobile phase of 70 : 30 acetonitrile–THF at a flow rate of 1 mL min^{-1}. Injection volumes were 40 μL at a concentration of 0.5 mg mL^{-1}. Analyses were performed using Empower Pro software. GPC analyses were performed using a Viscotek VE3210 UV/Vis detector at 260 nm and 30 °C. A Viscogel mixed bed I-MBMMW-3078 GPC column (7.8 mm × 30 cm) was used with a mobile phase of THF at a flow rate of 1 mL min^{-1}. Injection volumes were 100 μL at a concentration of 1 mg mL^{-1}.

Syntheses

Monomer 1. Monomer **1** was prepared in two steps from a commercially available triamine. Protection of the triamine to yield $HN(CH_2CH_2CH_2NHBoc)_2$ commences with a solution of $HN(CH_2CH_2CH_2NH_2)_2$ (5.35 mL, 37.6 mmol) and DIPEA (17.9 mL, 105 mmol) dissolved in 80 mL of THF. BOC-ON (18.5 g, 75 mmol) was dissolved separately in 200 mL of THF. Both solutions were cooled to 0 °C and then combined. The mixture was warmed gradually to 25 °C and stirred for 4 h. The solvent was removed *in vacuo*, and then the residue was dissolved in 200 mL of CH_2Cl_2. This solution was washed with water, 1 M NaOH (aq.) solution and brine (3 × 150 mL each). The organic phase was dried with $MgSO_4$. Following filtration, the solvent was removed *in vacuo*. The product was purified by reprecipitation from a solution of hexanes and trace methanol to yield a white solid. Yield: 10.44 g (84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.19 (br, 2H, NH), 3.18 (t, $^{3}J_{H-H}$ = 6 Hz, 2H, CH_2NHBoc), 3.16 (t, $^{3}J_{H-H}$ = 6 Hz, 2H, CH_2NHBoc), 2.61 (t, $^{3}J_{H-H}$ = 6 Hz, 4H, NCH_2), 1.61 (qd, $^{4}J_{H-H}$ = 6 Hz, $^{2}J_{H-H}$ = 6 Hz, 4H, NCH_2CH_2), 1.41 (s, 18H, $C(CH_3)_3$). $^{13}C\{^{1}H\}$ NMR (75.5 MHz, $CDCl_3$) δ 156.1 (s, C(O)), 78.9 (s, $C(CH_3)_3$), 47.2 (s, NCH_2), 38.7 (s, CH_2NHBoc), 29.6 (s, NCH_2CH_2), 28.3 (s, $C(CH_3)_3$). MS (ESI): calc. 331.2471 (M^+); found 332.2490 ($M + H^+$).

To obtain **1**, a solution of cyanuric chloride (5.38 g, 29.2 mmol) in THF (200 mL) was cooled to 0 °C. A clear solution of $HN(CH_2CH_2CH_2NHBoc)_2$ (9.21 g, 27.8 mmol) in THF (100 mL) was added dropwise to the cyanuric chloride solution, followed by dropwise addition of a solution of DIPEA (4.49 mL, 27.8 mmol) in THF (150 mL). The solution was stirred at 0 °C for 1 h, then warmed gradually to 25 °C and stirred for an additional 12 h. The solvent was removed *in vacuo*, and then the residue was taken up in CH_2Cl_2 (200 mL). The solution was washed with water (3 × 300 mL), and then dried with $MgSO_4$. Following filtration, the solvent was removed *in vacuo*. The product was obtained as a pure white solid by reprecipitation with hexanes from a clear solution of EtOAc. Yield: 11.64 g (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.02 (br, 2H, NH), 3.61 (t, $^{3}J_{H-H}$ = 7 Hz, 4H, NCH_2), 3.12 (m, 4H, CH_2NHBoc), 1.77 (p, $^{3}J_{H-H}$ = 7 Hz, 4H, NCH_2CH_2), 1.43 (s, 18H, $C(CH_3)_3$). $^{13}C\{^{1}H\}$ NMR (75.5 MHz, $CDCl_3$) δ 170.1 (s, C_3N_3), 164.7 (s, C_3N_3), 156.0 (s, C(O)), 79.3 (s, $C(CH_3)_3$), 44.9 (s, NCH_2), 37.3 (s, CH_2NHBoc), 28.3 (s, $C(CH_3)_3$), 27.7 (s, NCH_2CH_2). MS (ESI): calc. 478.1862 (M^+); found 479.1992 ($M + H^+$).

G1-Cl. Solutions of **2** (1.21 g, 3.63 mmol) and **1** (6.09 g, 12.7 mmol) were dissolved separately in THF (100 mL each) to give a slurry and a clear solution, respectively. DIPEA (6.5 mL, 36 mmol) was added to the solution of C_3N_3(piperazine)$_3$, and then both solutions were cooled to 0 °C. The mixture was added dropwise to the solution of **1**, and the slurry was stirred at 0 °C for 1 h. The solution was warmed gradually to 25 °C and stirred for an additional 15 h. The mixture was filtered through Celite, and then the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (150 mL), and this solution was washed with water (3 × 200 mL). The organic phase was dried with $MgSO_4$. Excess **1** was removed by filtration of the organic phase through a silica plug. The product was reprecipitated from CH_2Cl_2 with hexanes to afford a white solid. Yield: 5.63 g (93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.59 (br, 3H, NH), 4.83 (br, 3H, NH), 3.82 (br, 24H, CH_2, piperazine), 3.56 (m, 12H, pr-NCH_2), 3.08 (m, 12H, CH_2NHBoc), 1.73 (m, 12H, NCH_2CH_2), 1.424 (s, 27H, $C(CH_3)_3$), 1.420 (s, 27H, $C(CH_3)_3$). $^{13}C\{^{1}H\}$ NMR (75.5 MHz, $CDCl_3$) δ 169.2 (s, C_3N_3), 165.2 (s, C_3N_3), 164.9 (s, C_3N_3), 164.2 (s, C_3N_3), 156.1 (s, C(O)), 155.8 (s, C(O)), 79.2 (s, $C(CH_3)_3$), 78.8 (s, $C(CH_3)_3$), 43.8 (s, CH_2), 43.3 (s, CH_2, piperazine), 42.9 (s, CH_2), 42.6 (s, CH_2), 37.7 (s, CH_2NHBoc), 36.7 (s, CH_2NHBoc), 28.4 (s, $C(CH_3)_3$), 28.3 (s, $C(CH_3)_3$), 27.8 (s, NCH_2CH_2), 27.7 (s, NCH_2CH_2). MS (MALDI): calc. 1662.2559 (M^+); found 1663.0484 ($M + H^+$).

G1-Pip. A clear solution of **G1-Cl** (2.53 g, 1.52 mmol) and piperidine (1.52 mL, 15.2 mmol) was prepared in THF (50 mL). The mixture was stirred at 25 °C for 16 h, and the resulting slurry was filtered through silica to give a clear solution. The solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL), and this solution was washed with water (3 × 50 mL). The organic phase was dried with Na_2SO_4. Following filtration, the solvent was removed *in vacuo* to afford the product as a white powder (yield: 2.79 g, >99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.27 (br, 6H, NH), 3.80 (br, 24H, CH_2, piperazine), 3.72 (br, 12H, $C_5H_{10}N$, α-H), 3.96 (br, 12H, CH_2, pr-NCH_2), 3.07 (br, 12H, CH_2NHBoc), 1.70 (br, 12H, NCH_2CH_2), 1.62 (br, 6H, $C_5H_{10}N$, γ-H), 1.55 (br, 12H, $C_5H_{10}N$, β-H), 1.42 (s, 54H, $C(CH_3)_3$). $^{13}C\{^{1}H\}$ NMR (75.5 MHz, $CDCl_3$) δ 166.1 (s, C_3N_3), 165.5 (s, C_3N_3), 165.1 (s, C_3N_3), 156.2 (s, C(O)), 79.2 (s, $C(CH_3)_3$), 44.4 (s, CH_2, piperazine), 43.4 (s, pr-NCH_2), 43.3 (s, pr-NCH_2), 42.0 ($C_5H_{10}N$, α-C), 37.4 (s, CH_2NHBoc), 37.3 (s, CH_2NHBoc), 28.7 (s, $C(CH_3)_3$), 27.9 (s, NCH_2CH_2), 26.0 ($C_5H_{10}N$, β-C),

25.1 ($C_5H_{10}N$, γ-C). MS (MALDI): calc. 1807.2050 (M^+); found 1808.3271 ($M + H^+$).

G1-NH$_2$. A 5 M HCl solution (35 mL) was added to a clear solution of **G1-Pip** (2.75 g, 1.52 mmol) in CH_2Cl_2 (3 mL) and MeOH (70 mL), and the solution was stirred at 25 °C for 15 h. The volatile components were concentrated *in vacuo* until only *ca*. 15 mL of water remained. The residue was made basic (pH = 14) with 40 mL of 5 M NaOH (aq.) solution. The resulting milky suspension was extracted with $CHCl_3$ (5 × 250 mL). The combined organic phases were dried with Na_2SO_4. Following filtration, the solvent was removed *in vacuo* to afford the product as a white solid. Yield: 1.845 g, >99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (br, 24H, CH_2, piperazine), 3.71 (br, 12H, $C_5H_{10}N$, α-H), 3.63 (br t, $^3J_{H-H}$ = 7 Hz, 12H, pr-NC*H*$_2$), 2.68 (br t, $^3J_{H-H}$ = 7 Hz, 12H, C*H*$_2$NH$_2$), 1.73 (br m, 12H, NCH_2CH_2), 1.62 (br, 6H, $C_5H_{10}N$, γ-H), 1.54 (br, 24H, $C_5H_{10}N$, β-H and NH_2). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ 165.5 (s, C_3N_3), 165.2 (s, C_3N_3), 164.8 (s, C_3N_3), 44.0 ($C_5H_{10}N$, α-C), 43.0 (s, CH_2, piperazine), 42.4 (s, NH_2–NCH_2), 39.1 (s, CH_2NH_2), 39.0 (s, CH_2NH_2), 31.3 (s, NCH_2CH_2), 25.7 ($C_5H_{10}N$, β-C), 24.9 ($C_5H_{10}N$, γ-C). MS (MALDI): calc. 1206.8904 (M^+); found 1207.9496 ($M + H^+$).

G2-Cl. A solution of **G1-NH$_2$** (889 mg, 0.74 mmol), DIPEA (2.1 mL, 12.5 mmol) and **1** (2.99 g, 6.21 mmol) was prepared in a THF–water mixture (200 : 10 mL) to give a slurry. The solution was stirred at 25 °C for 16 h, and then filtered through Celite. The solvent was removed *in vacuo*. Purification was achieved using column chromatography on silica gel (40 : 1 CH_2Cl_2–MeOH; R_f = 0.19 using 20 : 1 CH_2Cl_2–MeOH as the developing solvent) to afford the product as a white solid. Yield: 2.66 g (93%). The excess/unreacted **1** may also be recovered from this purification (R_f = 0.50 using 20 : 1 CH_2Cl_2:MeOH as the developing solvent). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.08 (br, 2H, NH) 5.61 (br, 8H, NH), 5.44 (br, 2H, NH), 4.98 (br, 6H, NH), 3.82 (br, 24H, CH_2, piperazine), 3.74 (br, 12H, $C_5H_{10}N$, α-H), 3.64–3.56 (br, 36H, CH_2, NCH_2), 3.39 (br, 12H, CH_2, C*H*$_2$NH–C_3N_3), 3.08 (br, 24H, C*H*$_2$NHBoc), 1.85 (br, 12H, G1-NCH_2CH_2), 1.73 (br, 30H, G2-NCH_2CH_2, $C_5H_{10}N$, γ-H), 1.58 (br, 12H, $C_5H_{10}N$, β-H), 1.43 (s, 54H, $C(CH_3)_3$), 1.41 (s, 54H, $C(CH_3)_3$). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ 169.2 (s, C_3N_3), 168.4 (s, C_3N_3), 165.4 (s, C_3N_3), 165.1 (s, C_3N_3), 164.9 (s, C_3N_3), 164.6 (s, C_3N_3), 156.0 (s, C(O)), 155.7 (s, C(O)), 78.9 (s, *C*$(CH_3)_3$), 78.6 (s, *C*$(CH_3)_3$), 44.0 (br), 43.1 (br), 37.6 (s, *C*H$_2$NHBoc), 36.6 (s, *C*H$_2$NHBoc), 28.2 (s, C(*C*$H_3)_3$), 27.7 (s, NCH_2CH_2), 25.6 ($C_5H_{10}N$, β-C), 24.8 ($C_5H_{10}N$, γ-C). MS (MALDI): calc. 3865.2622 (M^+); found 3868.2945.

G2-Pip. A clear solution of **G2-Cl** (3.18 g, 0.823 mmol) and piperidine (1.5 mL, 14.8 mmol) was prepared in THF (50 mL). The mixture was stirred at 25 °C for 12 h, and the resulting slurry was filtered through silica to give a clear solution. The solvent was removed *in vacuo*. The solid was washed with hexanes and dissolved in 50 mL CH_2Cl_2. The solution was washed with water (2 × 50 mL) and brine (1 × 50 mL). The organic phases were dried with Na_2SO_4 and the solvent was removed *in vacuo*. The residue was reprecipitated from CH_2Cl_2 with hexanes, then EtOAc with hexanes to afford the product as a white powder. Yield: 3.49 g (>99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.70–5.49 (br, 18H, NH), 3.79 (br, 24H, CH_2, piperazine), 3.68 (br, 36H, $C_5H_{10}N$, α-H), 3.53 (br, 36H, CH_2, NCH_2), 3.34 (br, 12H, CH_2, C*H*$_2$NH–C_3N_3), 3.02 (br, 24H, C*H*$_2$NHBoc), 1.80 (br, 12H, G1-NCH_2CH_2), 1.66 (br, 24H, G2-NCH_2CH_2), 1.58–1.52 (br, 54H, $C_5H_{10}N$, γ-$C_5H_{10}N$, β-H), 1.39 (s, 108H, $C(CH_3)_3$). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ 165.9 (s, C_3N_3), 165.6 (s, C_3N_3), 165.2 (s, C_3N_3), 165.0 (s, C_3N_3), 156.0 (s, C(O)), 79.0 (s, *C*$(CH_3)_3$), 44.3 (br), 43.3 (br), 42.2 (br), 37.3 (s, *C*H$_2$NHBoc), 28.7 (s, C(*C*$H_3)_3$), 27.9 (br, NCH_2CH_2), 26.0 (br, $C_5H_{10}N$, β-C), 25.1 (br, $C_5H_{10}N$, γ-C). MS (MALDI): calc. 4157.3818 (M^+); found 4158.6648 ($M + H^+$).

G2-NH$_2$. A concentrated aqueous HCl solution (15 mL) was added to a solution of **G2-Pip** (1.35 g, 0.32 mmol) in MeOH (40 mL), and the clear solution was stirred at 25 °C for 16 h. The volatile components were concentrated *in vacuo* until only *ca*. 5 mL of water remained. The residue was made basic (pH = 14) with 25 mL of 1 M NaOH (aq.) solution, and the resulting milky suspension was extracted with $CHCl_3$ (5 × 250 mL). The organic extractions were combined, and then the solvent was removed *in vacuo* to afford the product as a white solid. Yield: 959 mg, >99%. ^{1}H NMR (300 MHz, $CDCl_3$ with trace CD_3OD) δ 5.24 (br, 6H, NH), 3.80 (br, 24H, CH_2, piperazine), 3.73 (br, 12H, G1-$C_5H_{10}N$, α-H), 3.65 (br, 36H, G2-$C_5H_{10}N$, α-H, NCH_2), 3.57 (br, 24H, CH_2, NH_2NCH_2), 3.32 (br, 12H, C*H*$_2$NH–C_3N_3), 2.64 (br, 24H, C*H*$_2$NH$_2$), 1.81 (br, 12H, G1-NCH_2CH_2), 1.70 (br, 24H, G2-NCH_2CH_2), 1.58–1.51 (br, 54H, $C_5H_{10}N$, γ-H, $C_5H_{10}N$, β-H, and NH_2). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$ with trace CD_3OD) δ 165.8 (s, C_3N_3), 165.4 (s, C_3N_3), 165.2 (s, C_3N_3), 165.1 (s, C_3N_3), 165.0 (s, C_3N_3), 164.6 (s, C_3N_3), 164.3 (s, C_3N_3), 43.8 (s, NCH_2), 42.8 (s, NCH_2), 41.7 (s, NCH_2), 38.1 (s, G2-CH_2NH_2), 37.3 (br, G1-CH_2NH–C_3N_3), 30.3 (s, G2-NCH_2CH_2), 27.7 (br, G1-NCH_2CH_2), 25.5 ($C_5H_{10}N$, β-C), 24.6 ($C_5H_{10}N$, γ-C). MS (MALDI): calc. 2954.1932 (M^+); found 2955.0237 ($M + H^+$).

G3-Cl. A solution of **G3-NH$_2$** (0.234 g, 0.0794 mmol), DIPEA (0.43 mL, 2.86 mmol) and **1** (0.914 g, 1.9 mmol) was prepared in a CH_2Cl_2 (50 mL)–EtOAc (15 mL)–water (5 mL) mixture to give a slurry. The solution was stirred at 25 °C for 16 h, the solvents removed *in vacuo* and the residue taken up in 50 mL CH_2Cl_2. The solution was washed with water (3 × 100 mL) and brine (1 × 100 mL). The organic phases were filtered through silica to remove excess starting material. The solvent was removed *in vacuo* and any residual starting materials were removed by reprecipitation from CH_2Cl_2 with hexanes to afford the product as a white solid. Yield: 0.5118 g (78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.33, 5.62, 4.98 (br, 42H, NH), 3.75 (br, 24H, CH_2, piperazine), 3.65–3.52 (br, 120H, NCH_2 and $C_5H_{10}N$, α-H), 3.36–3.29 (br, 36H, C*H*$_2$NH–C_3N_3), 3.03 (br, 48H, C*H*$_2$NHBoc), 1.89 (br, 12H, G1-NCH_2CH_2), 1.79 (br, 24H, G2-NCH_2CH_2), 1.68 (br, 48H, G3-NCH_2CH_2), 1.49 (br, 54H, $C_5H_{10}N$, β-H and γ-H), 1.39 (s, 108H, $C(CH_3)_3$), 1.37 (s, 108H, $C(CH_3)_3$). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ 169.7 (s, C_3N_3), 168.8 (s, C_3N_3), 168.2 (s, C_3N_3), 165.9 (s, C_3N_3), 165.6 (s, C_3N_3), 165.3 (s, C_3N_3), 165.1 (s, C_3N_3),

164.8 (s, C_3N_3), 156.4 (s, C(O)), 156.1 (s, C(O)), 79.2 (s, $C(CH_3)_3$), 79.0 (s, $C(CH_3)_3$), 44.4 (br), 43.7 (br), 43.6 (br), 43.3 (br), 39.0 (br), 38.8 (br), 38.1 (br), 37.1 (br), 28.6 (s, $C(CH_3)_3$), 28.1 (br), 27.9 (br), 26.0 (br), 25.2 (br). MS (MALDI): calc. 8271.27 (average M^+); found 8272.27 (average $M + H^+$).

G3-Pip. A clear solution of **G3-Cl** (0.66 g, 0.080 mmol) and piperidine (0.316 mL, 3.19 mmol) was prepared in THF (30 mL). The mixture was stirred at 25 °C for 12 h, and the resulting slurry was filtered through silica to give a clear solution. The solvent was removed *in vacuo*. The solid was washed with hexanes and dissolved in 50 mL CH_2Cl_2. The solution was washed with water (2 × 50 mL) and brine (1 × 50 mL). The organic phases were dried with Na_2SO_4 and the solvent was removed *in vacuo*. The residue was reprecipitated from CH_2Cl_2 with hexanes to afford the product as a white powder. Yield: 721 mg (>99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–5.00 (br, 42H, NH), 3.78 (br, 24H, CH_2, piperazine), 3.68 (br, 84H, $C_5H_{10}N$, α-H), 3.53 (br, 84H, NCH_2), 3.32 (br, 36H, CH_2NH–C_3N_3), 3.02 (br, 48H, CH_2NHBoc), 1.78 (br, 36H, NCH_2CH_2), 1.65 (br, 48H, NCH_2CH_2), 1.50 (br, 126H, $C_5H_{10}N$, γ-H and β-H), 1.40 (s, 108H, $C(CH_3)_3$), 1.39 (s, 108H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ 165.9 (s, C_3N_3), 165.6 (s, C_3N_3), 165.2 (s, C_3N_3), 164.6 (s, C_3N_3), 155.9 (s, C(O)), 78.7 (s, $C(CH_3)_3$), 43.9 (br), 42.9 (br), 41.7 (br), 33.9 (s, CH_2NHBoc), 28.3 (s, $C(CH_3)_3$), 27.5 (br, NCH_2CH_2), 25.7 (br, $C_5H_{10}N$, β-C), 24.8 (br, $C_5H_{10}N$, γ-C). MS (MALDI): calc. 8855.5141 (average M^+); found 8856.89 (average $M + H^+$).

G3-NH$_2$. 5 M HCl (25 mL) was added to a solution of **G3-Pip.** (706 mg, 0.0797 mmol) in MeOH (70 mL), and the clear solution was stirred at 25 °C for 16 h. The volatile components were concentrated *in vacuo* until only *ca.* 5 mL of water remained. The residue was made basic (pH = 14) with 26 mL of 5 M NaOH (aq.) solution, and the resulting milky suspension was extracted with $CHCl_3$ (5 × 150 mL). The organic extractions were combined, dried with Na_2SO_4, and then the solvent was removed *in vacuo* to afford the product as a white solid. Yield: 520 mg, >99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–5.00 (br, 18H, NH), 3.76 (br, 24H, CH_2, piperazine), 3.62 (br, 84H, NCH_2), 3.53 (br, 84H, NCH_2), 3.27 (br, 36H, CH_2NH–C_3N_3), 2.61 (br, 48H, CH_2NH_2), 1.99 (br, 48H, NH_2), 1.66 (br, 84H, NCH_2CH_2), 1.54 (br, 42H, $C_5H_{10}N$, γ-H), 1.47 (br, 84H, $C_5H_{10}N$, β-H). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ 166.0 (s, C_3N_3), 165.9 (s, C_3N_3), 165.3 (s, C_3N_3), 165.1 (s, C_3N_3), 164.8 (s, C_3N_3), 164.6 (s, C_3N_3), 164.5 (s, C_3N_3), 43.8 (s, NCH_2), 42.9 (s, NCH_2), 42.2 (s, NCH_2), 38.9 (s, CH_2NH_2), 37.4 (br, CH_2NH–C_3N_3), 31.1 (s, NCH_2CH_2), 27.9 (br, NCH_2CH_2), 25.6 ($C_5H_{10}N$, β-C), 24.8 ($C_5H_{10}N$, γ-C). MS (MALDI): calc. 6448.7990 (M^+); found 6471.9424 ($M + Na^+$).

G4-Cl. A clear solution of **G3-NH$_2$** (0.94 g, 0.146 mmol) in H_2O (15 mL) and EtOAc (80 mL) was added to a clear solution of **1** (5.03 g, 10.49 mmol) in CH_2Cl_2 (90 mL) with DIPEA (4.79 mL, 31.47 mmol) and stirred at room temperature for 16 h. The solvents were removed *in vacuo* and the residue taken up in 20 mL CH_2Cl_2. The solution was washed with water (3 × 100 mL) and brine (1 × 100 mL). The organic phases were filtered through silica to remove excess starting material. The solvent was removed *in vacuo* and any residual starting materials were removed by reprecipitation from CH_2Cl_2 with hexanes to afford the product as a white solid. Yield: 1.718 g, 69%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.39, 5.65, 5.03 (br, 90H, NH), 3.79–3.53 (br, 288H, CH_2, piperazine, NCH_2 and $C_5H_{10}N$, α-H), 3.35–3.30 (br, 84H, CH_2NH–C_3N_3), 3.04 (br, 96H, CH_2NHBoc), 1.88–1.69 (br, 180H, NCH_2CH_2), 1.58–1.47 (br, 126H, $C_5H_{10}N$, β-H and γ-H), 1.38 (bs, 432H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ 168.8 (br, C_3N_3), 168.2 (br, C_3N_3), 166.4 (br, C_3N_3), 165.9–165.8 (br, C_3N_3), 165.6 (s, C_3N_3), 165.4–165.3 (br, C_3N_3), 165.1 (br, C_3N_3), 164.9 (br, C_3N_3), 156.4 (s, C(O)), 156.1 (s, C(O)), 79.3 (s, $C(CH_3)_3$), 79.0 (s, $C(CH_3)_3$), 44.5–44.3 (br), 43.7–43.5 (br), 38.9 (br), 38.0–37.8 (br), 37.2–37.0 (br), 28.6 (s, $C(CH_3)_3$), 28.1 (br), 26.1 (br), 25.2 (br). MS (MALDI): calc. 17 083.3001 (average M^+); found 17 088.03 (~average $M + H^+$).

G4-Pip. A clear solution of **G4-Cl** (284 mg, 0.0166 mmol) and piperidine (0.14 mL, 1.394 mmol) was prepared in THF (16 mL). The mixture was stirred at 25 °C for 16 h, and the resulting slurry was filtered through silica to give a clear solution. The solvent was removed *in vacuo*. The solid was washed with hexanes and dissolved in 50 mL CH_2Cl_2. The solution was washed with water (2 × 50 mL) and brine (1 × 50 mL). The organic phases were dried with Na_2SO_4 and the solvent was removed *in vacuo*. The residue was reprecipitated from CH_2Cl_2 with hexanes to afford the product as a white powder. Yield: 265 mg (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12–5.08 (br, 90H, NH), 3.78–3.69 (br, 204H, CH_2, piperazine, $C_5H_{10}N$, α-H), 3.56 (br, 180H, CH_2, NCH_2), 3.35 (br, 84H, CH_2, CH_2NH–C_3N_3), 3.02 (br, 96H, CH_2NHBoc), 1.83 (br, 84H, NCH_2CH_2), 1.65 (br, 96H, NCH_2CH_2), 1.50 (br, 270H, $C_5H_{10}N$, γ-H and β-H), 1.41 (s, 432H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ 166.0–165.7 (br, C_3N_3), 164.9 (br, C_3N_3), 156.2 (s, C(O)), 79.1 (s, $C(CH_3)_3$), 44.3 (br), 43.3 (br), 42.2 (br), 38.9 (br), 37.3 (s, CH_2NHBoc), 28.7 (s, $C(CH_3)_3$), 27.9 (br, NCH_2CH_2), 26.0 (s, $C_5H_{10}N$, β-C), 25.1 (s, $C_5H_{10}N$, γ-C). MS (MALDI): calc. 18 251.78 (average M^+); found 18 279.01 (~average $M + Na^+$).

G4-NH$_2$. A clear solution of **G4-Pip** (480 mg, 0.0263 mmol) in CH_2Cl_2 (15 mL) and MeOH (90 mL) was prepared. A 5 M HCl solution (24 mL) was added and the reaction was stirred at room temperature for 16 h. The volatile components were concentrated *in vacuo* until only *ca.* 5 mL of water remained. The residue was made basic (pH = 14) with 26 mL of 5 M NaOH (aq.) solution, and the resulting milky suspension was extracted with $CHCl_3$ (5 × 100 mL). The organic extractions were combined, dried with Na_2SO_4, and the solvent was removed *in vacuo* to afford the product as a white solid. Yield: 429 mg (>99%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.65, 3.04, 2.04, 1.56. MS (MALDI): calc. 13 446.22 (average M^+); found 13 453.87.

G5-Cl. A clear solution of **G4-NH$_2$** (0.175 g, 0.013 mmol) in 35 mL $CHCl_3$ with DIPEA (0.57 mL, 3.74 mmol) was prepared and concentrated *in vacuo* to *ca.* 10 mL. Monomer

1 was added and the solution was stirred at room temperature for 5 days. $CHCl_3$ (50 mL) was added and the solution was washed with water (3 × 75 mL) and brine (1 × 75 mL). The organic layers were filtered through silica to remove excess **1**. Portions containing the product and an impurity were filtered through silica again and the product was retrieved as a white solid. Yield: 0.111 g (25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70, 7.23, 5.67, 5.03 (NH), 3.65–3.36 (CH_2, piperazine, NCH_2, $C_5H_{10}N$, α-H, and CH_2NH–C_3N_3), 3.06 (CH_2NHBoc), 1.84–1.47 (br, 642H, NCH_2CH_2, $C_5H_{10}N$, β-H and γ-H), 1.40 (br, 864H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ 165.9–164.6 (br, C_3N_3), 163.5 (br, C_3N_3), 156.4 (s, C(O)), 156.2 (s, C(O)), 79.2 (s, $C(CH_3)_3$), 79.0 (s, $C(CH_3)_3$), 44.3 (br), 43.7 (br), 38.9 (br), 37.9 (br), 37.1 (s, CH_2NHBoc), 28.7 (s, $C(CH_3)_3$), 28.0, 27.9 (br, NCH_2CH_2), 26.0 (s, $C_5H_{10}N$, β-C), 25.6 (s, $C_5H_{10}N$, γ-C). MS (MALDI): calc. 34 707.36 (average M^+); found 32 932.41, 15 823.00.

G5-Pip. A clear solution of **G5-Cl** (0.0523 g, 0.0015 mmol) in THF (5 mL) was prepared. Piperidine (0.022 g, 0.216 mmol) was added and the solution was stirred at room temperature for 16 h. The cloudy solution was filtered through silica to remove excess piperidine. The solvents were removed *in vacuo* to yield a white solid. Yield: 0.059 g (>99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (CH_2, piperazine, $C_5H_{10}N$, α-H), 3.54 (br, 372H, NCH_2), 3.36 (CH_2, CH_2NH–C_3N_3), 3.04 (br, 192H, CH_2NHBoc), 1.68–1.52 (br, 930H, NCH_2CH_2, $C_5H_{10}N$, γ-H and β-H), 1.41 (s, 864H, $C(CH_3)_3$). MS (MALDI): calc. 37 044.33 (average M^+); found 33 010.74.

G5-NH$_2$. A clear solution of **G5-Pip** (0.0556 g, 0.0015 mmol) in CH_2Cl_2 (3 mL) and MeOH (9 mL) was prepared. 5 M HCl (aq.) (3 mL) was added and the solution was stirred at room temperature for 2 days. The volatile components were concentrated *in vacuo* until only *ca.* 1 mL of water remained. The residue was made basic (pH = 14) with 5 mL of 5 M NaOH (aq.) solution, and the resulting milky suspension was extracted with $CHCl_3$ (5 × 15 mL). The organic extractions were combined, dried with Na_2SO_4, and the solvent was removed *in vacuo* to afford the product as a white solid. Yield: mg (>99%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.73, 3.13, 2.08, 1.61.

Acknowledgements

This work is supported by the NIH (EES; R01 GM 064560). H. C. is an NSF Predoctoral Fellow. E. H. was a NSERC Postdoctoral Fellow. We thank Dr Shane Tichy and his staff in the Laboratory for Biological Mass Spectrometry for analytical services.

References

1 B. Helms and E. W. Meijer, *Science*, 2006, **313**, 929–930.
2 *Dendrimers in Medicine and Biotechnology: New Molecular Tools*, ed. U. Boas, J. B. Christensen and P. M. H. Heegaard, RSC Publishing, Cambridge, 1st edn, 2006, p. 182.
3 *Dendrimer Catalysis (Topics in Organometallic Chemistry)*, ed. L. H. Gade, Springer, Berlin, 1st edn, 2006, p. 189.
4 *Dendrimers V: Functional and Hyperbranched Building Blocks, Photophysical Properties, Applications in Materials and Life Sciences (Topics in Current Chemistry)*, ed. C. A. Schalley and F. Vögtle, Springer, Berlin, 1st edn, 2003, p. 273.
5 D. A. Tomalia and J. M. J. Frechet, *J. Polym. Sci., Part A: Polym. Chem.*, 2002, **40**, 2719–2728.
6 A.-M. Caminade, C.-O. Turrin, R. Laurent, C. Rebout and J.-P. Majoral, *Polym. Int.*, 2006, **55**, 1155–1160.
7 E. M. M. de Brabander-van den Berg and E. W. Meijer, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1308–11.
8 E. Hollink and E. E. Simanek, *Org. Lett.*, 2006, **8**, 2293–2295.
9 M. B. Steffensen, E. Hollink, F. Kuschel, M. Bauer and E. E. Simanek, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 3411–3433.
10 M. B. Steffensen and E. E. Simanek, *Angew. Chem., Int. Ed.*, 2004, **43**, 5178–5180.
11 J. Lim and E. E. Simanek, *Mol. Pharmacol.*, 2005, **2**, 273–277.
12 S. P. Rannard and N. J. Davis, *Org. Lett.*, 2000, **2**, 2117–2120.
13 H. T. Chen, M. F. Neerman, M. F. A. R. Parrish and E. E. Simanek, *J. Am. Chem. Soc.*, 2004, **126**, 10044–10048.
14 Cerius^2 4.9 Forcefield-Based Simulations, January 2003, Accelrys Inc., San Diego, CA, 2006.

