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Kilogram-Scale Synthesis of a Second-Generation Dendrimer Based on 1,3,5-Triazine Using Green and Industrially Compatible Methods with a Single Chromatographic Step

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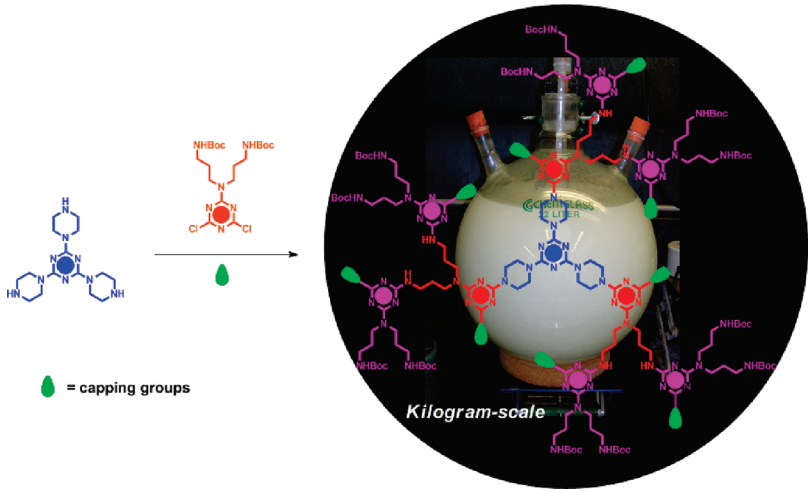

A kilogram scale, divergent and iterative synthesis of a second generation, triazine dendrimer with 12 protected amines on the periphery using common laboratory equipment is reported. The route benefits from common reaction conditions, inexpensive reagents, and aqueous solvents. From the monomers, the desired product dendrimer—the last uncommitted intermediate that leads to a range of committed, generation three targets—can be obtained in 70% overall yield. Of critical importance in the execution of this divergent synthesis is the differential reactivity of chlorine atoms of trichlorotriazine. The stepwise, nucleophilic aromatic substitution of these atoms with amine nucleophiles is both the basis for the dendrimer growth as well as incorporation of solubilizing piperidine groups. Intermediates are obtained and purified through precipitation and/or extraction protocols with the exception of the final product. Isolation of the target dendrimer requires a single silica gel plug filtration. The purity of this material is assessed at >93%, a level consistent with and/or exceeding other commercially available targets.

Introduction

The extensive literature surrounding dendrimers and their applications in areas ranging from materials science to medicine are largely predicated on the commercial availability of these compounds. The efforts of Tomalia, Newkome, Vögtle and Meijer, Majoral, Denkewalter, and Fréchet to make dendrimers based on poly(amidoamine),[1] Newkome poly(amides),[2] poly(propyleneimine),[3] phosphorus-based dendrimers,[4] polylysine,[5] and polyesters[6] available to scientists and engineers are rewarded with a rich literature and their use in products or methodologies. These areas include drug delivery,[7] gene transfection,[8] magnetic

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resonance imaging,[9] materials and coatings,[10] sensors and detectors,[11] catalysis,[12] and separations.[13] The allure of these materials is apparent in their depiction: dendrimers are monodisperse, polyfunctional polymers whose shape, size, topology, flexibility, and molecular weight can be controlled during preparation.[14]

Still, dendrimer synthesis remains a challenge. Obstacles to producing large quantities of pure compounds can be magnified by the need for sophisticated and/or sensitive building blocks, nontrivial isolation strategies, and the requirement for expensive and/or excess reagents. In the extreme, either convergent or divergent approaches can be applied.[1,3,14,19] In the divergent approach, the dendrimer is assembled from the central core. Growth of successive generations requires that more reactions be executed as the synthesis progresses. This approach—when executed with perfect efficiency—leads to mole conservation. That is, the number of moles of product is reflected in the number of moles of core that are initially employed. Mass is incrementally added throughout the course of the synthesis. The convergent method relies on building the dendrimer from the surface inward toward the core. Commonly, surface groups are repeatedly dimerized leading to larger dendrons that finally attached to a central core. The number of moles of product are a fraction (indeed, an exponentially unfavorable fraction) of the moles of surface groups initially available. A majority of the mass is carried throughout the synthesis. Yet, this strategy has as a primary advantage that only a limited number of reactions are performed at each generation. A variety of new dendrimers have been reported using both divergent and convergent methods that incorporate a wide range of functionalities such as ethers,[20] amides,[21] esters,[22] alkynes,[23] carbonates,[24] saccharides,[25] glycopeptides,[26] and siloxides.[27]

Our interest in dendrimers based on 1,3,5-triazine derives in part from the availability and low cost of 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride), the core reagent. The ease of displacement of chlorine atoms of cyanuric chloride by different amine nucleophiles to generate mono-, di-, and trisubstituted 1,3,5-triazines makes its adoption even more attractive.[15] The nucleophilic substitution of chlorine atoms with primary or secondary amines in presence of a hydrochloric acid acceptor (an organic or inorganic base) can be a controlled with temperature and proceeds as a one-pot procedure. The first substitution on cyanuric chloride occurs in minutes at 0 °C, while the second substitution occurs in 12−24 h at ambient temperature, and

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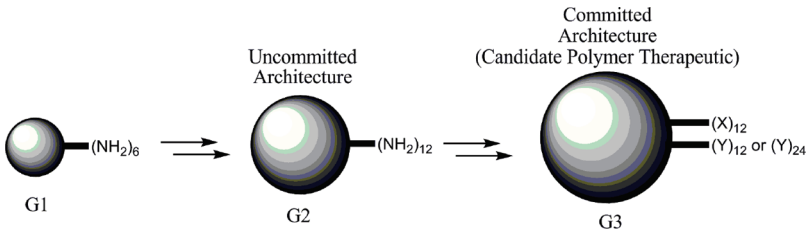
SCHEME 1. Target 1 is the Last Uncommitted Intermediate in the Preparation of Candidate Diagnostics or Therapeutics



finally the third substitution typically occurs in 12−24 h and requires temperatures above 60 °C. These characteristics allow for the preparation of triazine dendrimers with significant structural diversity (based both on the number of diamines available and the ability to manipulate their placement within the structure).[16] Indeed, without this level of control, neither the dichlorotriazine monomer, nor iterative generations of dendrimers, could be produced with any fidelity. Moreover, this reactivity offers an opportunity to control composition through the inclusion of groups (here, piperidine to convey solubility) at each generation[17] and to enable structure−property relationship studies in targets that have been described as well as those forthcoming.[18]

Our research is aimed at the development of practical, environmentally benign, and scaleable syntheses of triazine dendrimers that circumvent many of the barriers mentioned above. Here, we report the kilogram-scale synthesis of a generation two dendrimer. This material represents an important endpoint for synthesis: this compound presents 12 amines that can be functionalized (Scheme 1). The penultimate material affords additional opportunities for diversity with 6 chlorotriazines and 12 masked amines. This target represents the final uncommitted intermediate that can be elaborated to a wealth of diagnostic and therapeutic candidates/models, some of which are currently being explored in our laboratory. The value of such polymer therapeutics have been established.[28] The committed architectures result from elaboration of the target molecule to generation three dendrimers displaying groups numbering 12 and 12 or 12 and 24.

Here, we describe a divergent approach that benefits from ease of execution, simplicity in purification, and amenability to large-scale industrial synthesis. This divergent approach is largely universal; most commercially available dendrimers are synthesized via the divergent approach. The target molecule **1** (Figure 1) can be obtained in high yield and purity requiring only one chromatographic silica-plug filtration after the final reaction.

Results and Discussion

The synthesis requires iterative reactions of a dichlorotriazine intermediate on the dendrimer core followed by capping of the

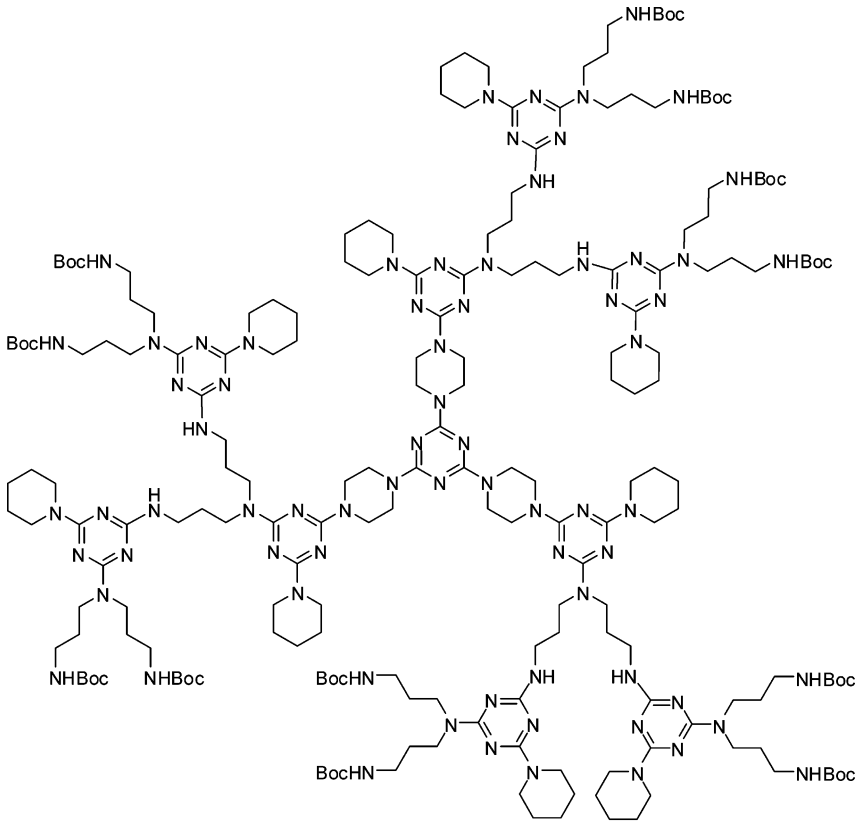


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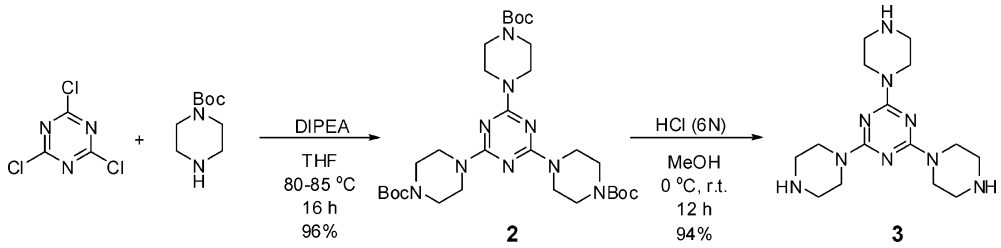
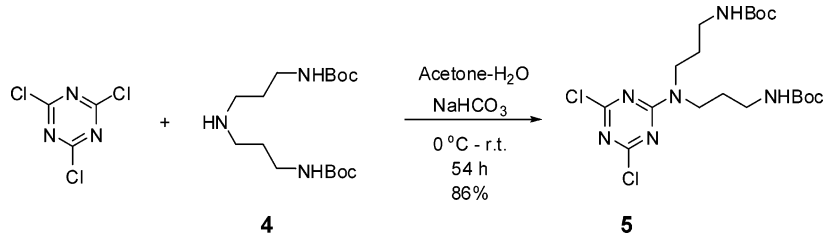
FIGURE 1. Target **1** comprising 12 Boc-protected amine groups.

SCHEME 2. Synthesis of Core 3



SCHEME 3. Synthesis of the Monomer



resulting poly(monochlorotriazine) dendrimer with piperidine and deprotection. This strategy can be affected up to the fifth generation at laboratory scale.[29] The synthesis of **1** proceeds without isolation of chlorinated dendrimers. Other intermediates are isolated through precipitation, recrystallization, and solvent extraction.

The core of target **1** is obtained in two steps. The reaction of cyanuric chloride with Boc-piperazine in acetone *or* THF at 80−85 °C in presence of *N*,*N*-diisopropylethylamine (DIPEA) for 16 h under nitrogen atmosphere affords **2** in 82% and 96% yield, respectively. Compound **2** was recrystallized from dichloromethane−hexane and characterized by ^{1}H NMR, ^{13}C NMR, MS spectroscopy, and elemental analysis. Cleavage of the Boc group using 6 N hydrochloric acid in methanol at 0 °C for 3 h and then at room temperature for 12 h affords the desired product **3** in 94% yield (Scheme 2). Core **3** was recrystallized from chloroform−hexane and characterized by ^{1}H NMR, ^{13}C NMR, MS spectroscopy, and elemental analysis.

The monomer used throughout the synthesis, **5**, comprises a dichloro-1,3,5-triazine with a branching triamine bearing two BOC-protected amines (Scheme 3). This triamine is prepared using Boc-ON (2-(Boc-oxyimino)-2-phenylacetonitrile)) on a 2.5 kg scale. The primary amines of 3,3′-iminobispropylamine are selectively protected using THF as solvent and without base to yield **4** in 84%. Using this procedure, no amount of Boc-protected secondary amine was detected. In contrast to previously reported procedures, we have eliminated the need for organic amine bases and, as a result, the need for unnecessary extraction steps.[29,30] The solvent, THF, was recovered and subsequently used again without further purification. Reaction of **4** with cyanuric chloride in acetone−water in the presence of inexpensive inorganic base $NaHCO_3$ produces **5** in 86% yield (Scheme 3). Monomer **5** was isolated by precipitation from acetone−water and characterized by ^{1}H NMR, ^{13}C NMR, MS spectroscopy, and elemental analysis. Monomer **5** can be regarded as an AB_2B' building unit where A is the first chloride displaced via nucleophilic aromatic substitution, B′ is the second chloride to be displaced, and B represents the Boc-protected amines. This monomer undergoes clean nucleophilic aromatic substitution reactions to afford monochlorotriazine decorated macromolecules.

We adopt a nomenclature for subsequent intermediates that reveals both generation and surface groups. The synthesis of G1-Cl (**6**), G1-Pip (**7**), and G1-NH_2 (**8**) is shown in Scheme 4.

Core **3** is reacted with 3.0 equiv of monomer **5** in acetone−water at 0 °C and then room temperature in the presence of K_2CO_3 to give the G1-Cl dendron **6**, which precipitates out of solution as a white solid in quantitative yields. Monitoring the reaction mixtures with thin layer chromatography (TLC) and MALDI-TOF mass spectrometry revealed a small quantity of unreacted monomer **5**. To avoid standard flash chromatography on silica gel, we carried the crude product into the next step using a laboratory scale experiment and found that these impurities could be easily separated from the product by precipitation of the latter using acetone, followed by filtration. Nevertheless, a small amount of the crude material from this was subjected to chromatography in order to unambigously characterize **6** using ^{1}H NMR, ^{13}C NMR, MALDI-TOF mass spectrometry, HPLC, and elemental analysis. Batches up to 750 g of the G1-Cl dendron **6** in a 22 L reactor were reacted with 15 equiv of piperidine as a capping group in acetone at 0 °C and then warmed to room temperature and stirred for 48 h, at which point the reaction was judged to be complete (TLC and MALDI-TOF MS monitoring). Intermediate **7** is afforded in 85% yield as a white solid after workup. The excess piperidine used to ensure complete reaction could be removed with an acid−base wash. The low yield of 85% is attributed to losses resulting from handling/discarding emulsions that formed during the wash process. The side product that results from substitution of both chlorine atoms of monomer **5** with piperidine is highly soluble in acetone−water, whereas the product **7** is essentially insoluble in acetone−water and thus can be completely removed by filtration. The result is a procedure that is simple, efficient, scalable, and free of chromatography. Characterization of these materials was performed using NMR spectroscopy, mass spectrometry, HPLC, and elemental analysis.

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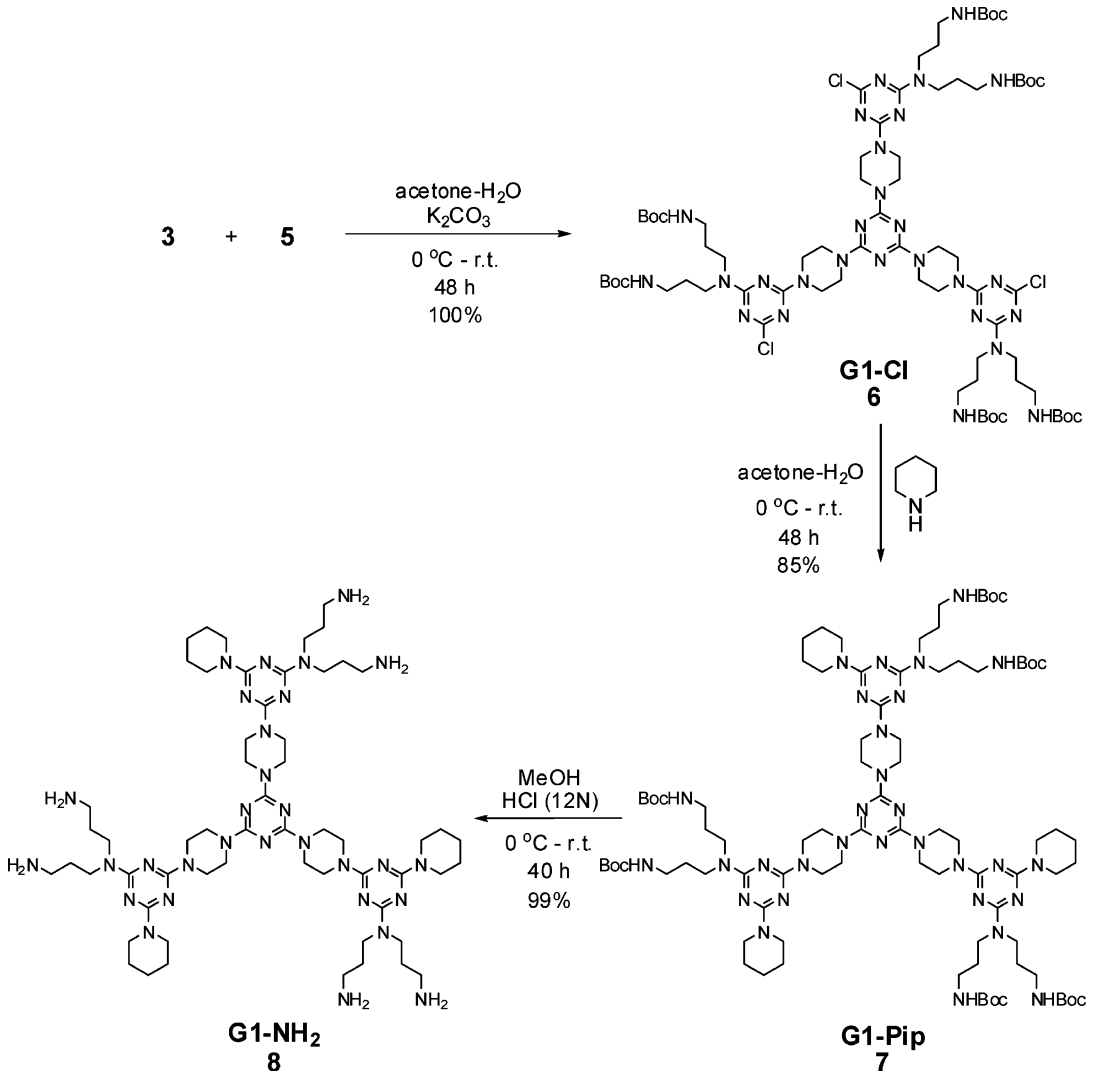
SCHEME 4. Synthesis of Dendrimers 6−8



Cleavage of the BOC groups of **7** employing 12 N hydrochloric acid in methanol (1:2) resulted, after 40 h at 0 °C then at room temperature, in amine-terminated dendrimer G1-NH_2 (**8**) in quantitative yield. The workup consisted of concentrating the reaction mixture to minimum volume, followed by addition of a 5 M NaOH aqueous solution to the acidic solution. Dendrimer **8** precipitated from the alkaline solution as a white solid. Because filtration of this compound proceeded slowly and resulted in a wet product, we turned to extraction with chloroform. To avoid emulsions, a continuous liquid−liquid extraction was employed. Dendrimer **8** was characterized by ^{1}H NMR, ^{13}C NMR, MS spectroscopy, and elemental analysis. A single species was observed in the mass spectrum corresponding to the expected isotope ration of $[M + H]^+$ and $[M + Na]^+$ (Figure 2). These observations are completely in accord with elemental analysis data which show high purity of **8** with a water content of 2.0 H_2O/**8** (see the Supporting Information).

The synthesis of intermediate **1** is shown in Scheme 5. In two steps, dendrimer **8** is reacted with monomer **5** and then capped with piperidine. The first reaction requires 6 equiv of **5** in acetone−water at 0 °C and then ambient temperature in the presence of Na_2CO_3. Dendrimer **9** precipitates out of solution as a gum which upon extraction from the aqueous medium turns into a white solid. Both TLC and MALDI-TOF MS analysis of this product showed the presence of the desired product **9** along with a small amount of unreacted monomer **5**. In addition, HPLC analysis of the crude reaction mixture indicated also that the predominant species were product and minor impurities of **5** totaling only about 7% (see the Supporting Information). A small amount of the crude material of **9** was subjected to chromatographic separation and further characterized by ^{1}H NMR, ^{13}C NMR, MALDI-TOF mass spectrometry, HPLC, and elemental analysis. This mixture was advanced to the next reaction. In an experiment analogous to **7**, batches up to 1250 g of crude **9** were reacted with 18 equiv of piperidine in acetone−water at room temperature. Monitoring the progress of the reaction by TLC and MALDI-TOF MS, the nucleophilic substitution of the six chlorine atoms with the secondary amine to give the G2-Pip (**1**) was complete within 48 h as shown in Figure 3. Unlike **7**, which could be isolated by precipitation in acetone−water, both **1** and the side product derived from reaction of monomer **5** with piperidine (**5-Pip**) were soluble under a variety of conditions. After evaluating a range of techniques including recrystallization and selective precipitation, we turned to chromatographic purification of **1** which could be executed in a plug-filtration fashion and provided material that shows a

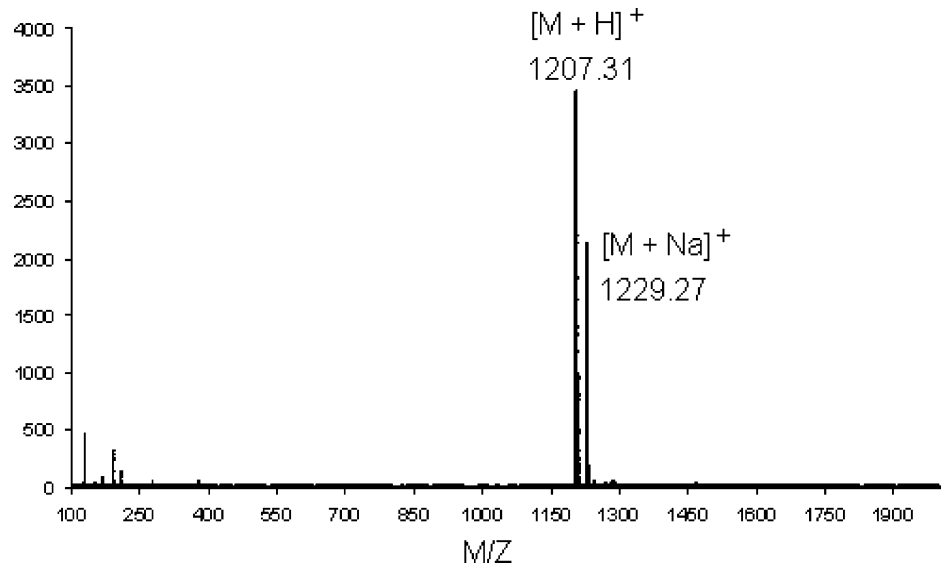
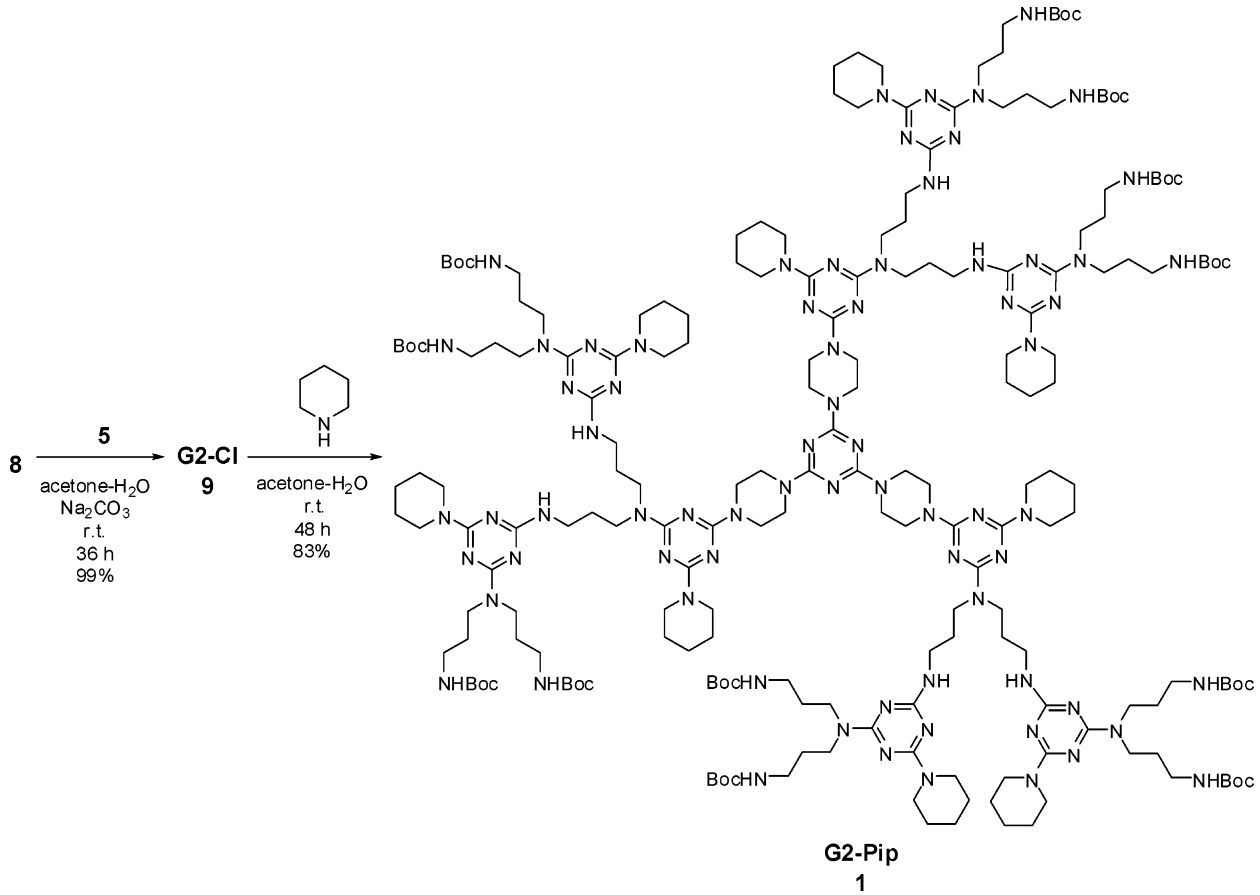


FIGURE 2. MALDI-TOF MS spectra of the G1-NH_2 dendrimer **8**.

SCHEME 5. Synthesis of Dendrimers 9 and 1



single ion by mass spectrometry and appears pure by NMR spectroscopy, and elemental analysis.

HPLC analysis allowed us to further assign purity and reaction efficiencies for many of these species including **1**, **3**, **5**, **6**, **7**, and **9** and quanitify side products including **5-Pip**. A gradient of CH_3CN and THF revealed the greatest extent of impurities. In Figure 4, both the first- and second-generation chloride dendrimers **6** and **9** eluted as single narrow peaks after column purification with a purity of 99 and 96%, respectively. The corresponding piperidine analogue **7** eluted as a single narrow peak with a purity of 95%. The core, **3**, appears to be 99% pure. The desired product **1** elutes as single broad peak with a purity of 88−97% dependent on the batch. This broadness is an artifact of loading; at this loading, the impurities are readily identifiable. At lessor loading even prior to chromatography (Supporting Information), not all species are resolved from the baseline. While alternative explanations for some of these impurities including aggregate species have not been ruled out,

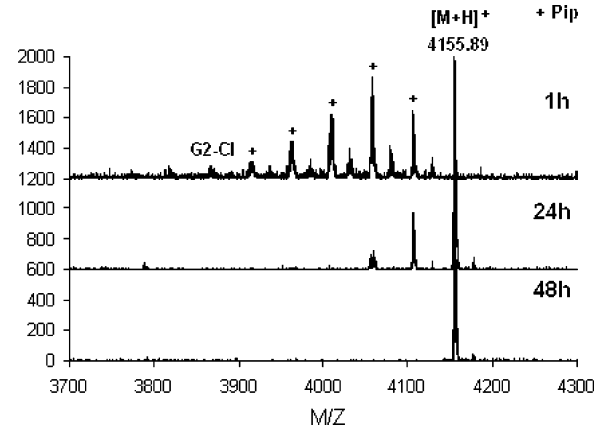


FIGURE 3. MALDI-TOF MS spectra of the G1-Pip dendrimer **1** at different reaction times of 1, 24, and 48 h. The starting material and product are shown. The "+" sign indicates substitution of a chlorine atome with a piperidine group. Six substitutions are required to provide product.

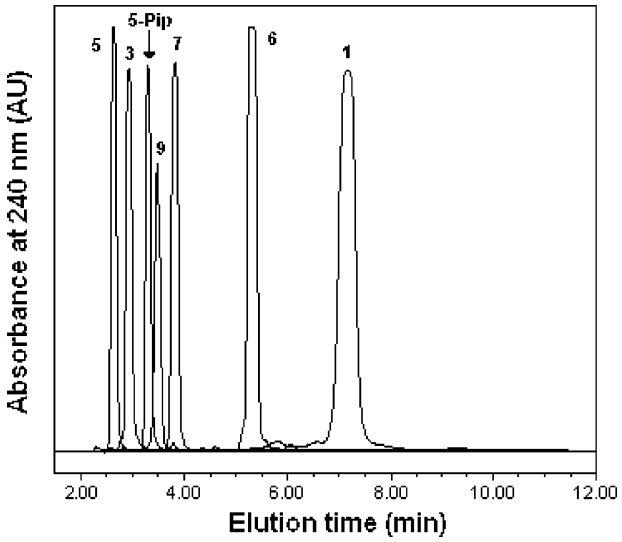


FIGURE 4. Traces obtained on an HPLC apparatus equipped with a C18 silica based reversed-phase column (4.6 × 250 mm, 5 μm, 100 Å pore size) using acetonitrile/THF (7:3) as solvent (1 mL/min). Injection: 40 μL. The detection wavelength of UV was 240 nm. Run time: 30 min.

GPC traces of the material in organic solvents do not readily support this hypothesis.[29]

The Boc-protecting group of **1** can be removed to afford the amino-terminated dendrimer G2-NH_2 (**10**) in quantitative yield (Scheme 6). The G2-NH_2 (**10**) was characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. A single species was observed in the mass spectrum corresponding to the expected isotope ratio of the expected cation $[M + H]^+$.

In summary, we have developed a kilogram scale process for the synthesis of generation two triazine dendrimers. This route can be executed in five steps in 70% overall yield from the monomers. We attribute these suboptimal yields to our existing physical infrastructure and isolation protocols, and not primarily to the existence of sideproducts. The purity of the final material (~93%) leads us to refer to it as "technical grade" although materials with similar purities are being considered for potential clinical applications. Presently, however, we do not know whether these impurities can be reproducibly produced in identical proportions. All reagents derive from commercially available materials, and with the exception of the BOC-reagent, are inexpensive. The reagent and solvent costs incurred are <$10/g of **1** at the scales performed. It is estimated that a single scientist could prepare 10−20 kg/yr using the simple laboratory equipment (22 L flasks, small volume rotory evaporators) available in our laboratory. Simple isolation and purification procedures could be used with the exception of a single chromatography step. The simplicity and reliability of this approach should permit an extension of this synthetic methodology to the synthesis of higher-generation dendrimers. With more than 1 kg of material in hand, a range of generation three materials, diagnostics and therapeutics are now accessible.

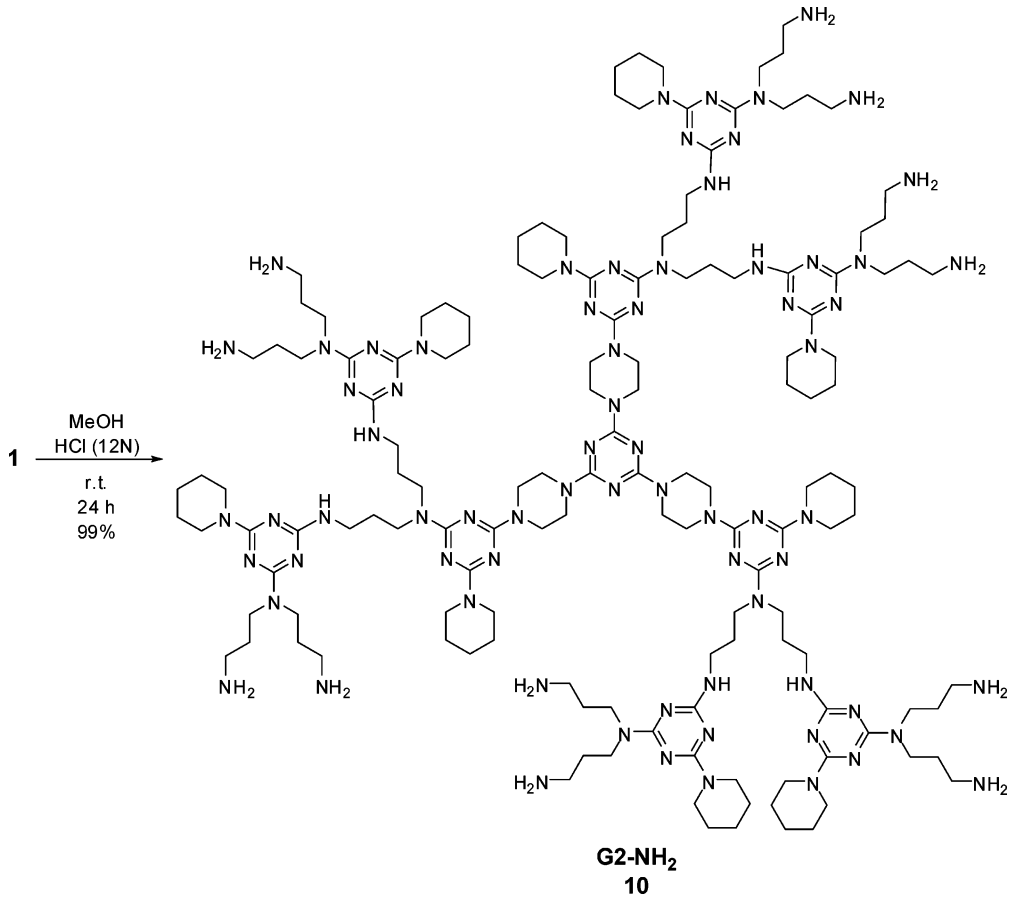
Experimental Methods

Intermediate 2. Method A. In a 3 L round-bottom flask (RBF) were mixed cyanuric chloride (30.0 g, 0.16 mol) and Boc-protected piperazine (100.0 g, 0.54 mol) in 1 L of THF and stirred at rt for 1 h. DIPEA (284.0 mL, 1.63 mol) was added, and the reaction was stirred at room temperature for 1 h and then at 80−85 °C for 16 h, at which point the reaction was deemed to be complete by TLC (silica gel, R_f = 0.6 using 5% CH_3OH/CH_2Cl_2). After being cooled to room temperature, the solvent was removed in vacuo, and then the white residue was dissolved in 600 mL of CH_2Cl_2. This solution was washed with water (3 × 250 mL) and brine (3 × 200 mL). The organic phase was dried with Na_2SO_4. Following filtration, the solvent was removed under reduced pressure to give **2** as white solid. The product was further recrystallized from CH_2Cl_2−hexanes to yield a white crystalline material (98.7 g, 96%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.74 (m, 12 H), 3.44 (m, 12 H), 1.47 (s, 27 H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ: 165.3, 154.8, 79.9, 42.9, 28.4. MS (ESI-TOF): calcd for $C_{30}H_{51}N_9O_6$ 633.40, found 634.40 $(M + H)^+$. Anal. Calcd for $C_{30}H_{51}N_9O_6$: C, 56.85; H, 8.11; N, 19.89. Found: C, 56.92; H, 8.18; N, 19.89.

Method B. In a 5 L, three-neck RBF was dissolved cyanuric chloride (75.0 g, 0.41 mol) in acetone (2 L) cooled to 0 °C. Boc-protected piperazine (250.0 g, 1.34 mol) in 1 L of acetone was added to the cyanuric chloride solution over a period of 1 h followed by DIPEA (708 mL, 4.06 mol). The white suspension was allowed to stir at 0 °C for 1 h, allowed to warm to rt, and then heated at 80−85 °C for 24 h. After the suspension was cooled to room temperature, the solvent was removed in vacuo, and then the white residue was dissolved in 1.2 L of CH_2Cl_2. This solution was washed with water (3 × 500 mL) and brine (3 × 500 mL). The organic phase was dried with Na_2SO_4 and the solvent evaporated under reduced pressure. The product was further recrystallized from CH_2Cl_2−hexanes to yield the desired product as a white crystalline material (213 g, 82%). TLC, NMR, and MS spectra analysis of this material showed the presence of the desired product.

Core 3. In a 3 L round-bottom flask (RBF) was dissolved intermediate **2** (177 g, 0.28 mol) in 1.8 L of CH_3OH, and 933 mL of 6 N HCl was added. The light yellow solution was allowed to stir at 0 °C for 3 h and then at room temperature for 12 h. The volatile components were removed by evaporation, and the resulting aqueous solution was made alkaline (pH = 14) by addition of a 10% NaOH solution. Using a 1 L liquid−liquid extractor, the milky alkaline solution (using ~500 mL each time) was extracted with $CHCl_3$ (4 L total) until it became clear (~4 h). The organic phase was dried over Na_2SO_4, and the solvent was evaporated to dryness to give the desired product **3** as a white solid (88 g, 94%). Subsequent crystallization from a $CHCl_3$−hexane solution produced a crystalline material. ^{1}H NMR (CD_3OD, 300 Hz) δ: 4.04 (t, 5.3 Hz, 12 H), 3.24 (t, 5.3 Hz, 12 H). ^{13}C NMR (CD_3OD, 75 Hz) δ: 166.6, 44.4, 41.2. MS (ESI-TOF): calcd for $C_{15}H_{27}N_9$ 333.24, found 334.25 $(M + H)^+$. Anal. Calcd for $C_{15}H_{27}N_9 \cdot 0.25H_2O$: C, 53.26; H, 8.06; N, 37.28. Found: C, 53.02; H, 8.01; N, 37.27.

SCHEME 6. Synthesis of Dendrimer 10



HN(CH$_2$CH$_2$CH$_2$NHBoc)$_2$ (4). Prior to starting the reaction, a solution of HN(CH$_2$CH$_2$CH$_2$NH$_2$)$_2$ (666.0 g, 5.07 mol) in 7.0 L of THF in a 22 L three-neck RBF fitted with a mechanical stirrer and BOC-ON (2.5 Kg, 10.15 mol) in 8.0 L of THF in two 4 L large erlenmeyers were cooled to 0 °C. The BOC-ON solution was added gradually in 500 mL portions to the triamine solution over a 2 h period. After complete addition, the mixture was allowed to stir at 0 °C for 5 h and then at 25 °C for 14 h. The solvent was removed under reduced pressure, and the recycled THF was subsequently used again without further purification in a scale-up process. The residue was dissolved in 4.0 L of CH_2Cl_2. This solution was washed with water and brine (3 × 1.5 L each). The organic phase was dried with Na_2SO_4. Following filtration, the solvent was removed under reduced pressure to afford an oily product. An off-white solid was precipitated after addition of hexane (1.5 L) to the oily compound and upon standing for 24 h in the freezer. The solid was filtered, washed thoroughly with hexane, and dried under high vacuum. This material was further recrystallized from CH_2Cl_2−hexanes (1:1) to yield a white crystalline material (1.41 kg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.17 (br, 2H), 3.19 (t, $^3J_{H-H}$ = 6 Hz, 2H), 3.17 (t, $^3J_{H-H}$ = 6 Hz, 2H), 2.62 (t, $^3J_{H-H}$ = 6 Hz, 4H), 1.62 (tt, $^3J_{H-H}$ = 6 Hz, $^3J_{H-H}$ = 6 Hz, 4H), 1.41 (s, 18H). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ: 156.1, 78.9, 47.2, 38.7, 29.6, 28.3. MS (ESI): calcd for $C_{16}H_{33}N_3O_4$ 331.2471 $(M)^+$, found 332.2490 $(M + H)^+$. Anal. Calcd for $C_{16}H_{33}N_3O_4$: C, 57.98; H, 10.04; N, 12.68. Found: C, 57.87; H, 10.07; N, 12.54.

Monomer 5. In a 22 L three neck RBF fitted with two 2 L additional funnels and a mechanical stirrer was dissolved cyanuric chloride (362.0 g, 1.96 mol) in acetone (2.5 L) and the mixture cooled to 0 °C. A solution of **4** (650 g, 1.96 mol) in acetone (9 L) was added dropwise to the cyanuric chloride solution over a period of 15 h. A white suspension formed during the course of addition. $NaHCO_3$ (165 g, 1.96 mol) in water (2.5 L) was added dropwise over a period of 3 h. The white mixture was allowed to stir at 0 °C for 12 h and then allowed to warm to rt and stirred for a further 24 h. The product was filtered, and the solvent was concentrated under reduced pressure to yield more material. The white solid was dissolved in 4 L of CH_2Cl_2, washed with H_2O (3 × 1.5 L) and brine (1 × 1 L), and dried with Na_2SO_4. Following filtration, the solvent was concentrated (∼1 L) in vacuo, and the product **5** was obtained as a pure white solid by reprecipitation with hexane. Yield: 810 g (86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.02 (br, 2H), 3.61 (t, $^3J_{H-H}$ = 7 Hz, 4H), 3.12 (t, $^3J_{H-H}$ = 6 Hz, 2H), 3.11 (t, $^3J_{H-H}$ = 6 Hz, 2H), 1.78 (tt, $^3J_{H-H}$ = 7 Hz, $^3J_{H-H}$ = 6 Hz, 4H), 1.43 (s, 18H). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ: 170.1, 164.7, 156.0, 79.3, 44.9, 37.3, 28.3, 27.7). MS (ESI): calcd 478.1862 $(M)^+$, found 479.1797 $(M + H)^+$. Anal. Calcd for $C_{19}H_{32}Cl_2N_6O_4$: C, 47.60; H, 6.73; N, 17.53. Found: C, 47.67; H, 6.79; N, 17.42.

G1-Cl (6). In a 22 L three-neck RBF fitted with two 1 L additional funnels and a mechanical stirrer was dissolved monomer **5** (646 g, 1.35 mol) in acetone (9 L) and the mixture cooled to 0 °C. To a chilled solution of **3** (150 g, 0.45 mol) in H_2O (5 L) was added a solution of K_2CO_3 (621 g, 4.49 moL) in 3 L of H_2O. The clear aqueous solution was added dropwise to the acetone solution at 0 °C over a period of 15 h. A white suspension was obtained after complete addition which was left to stir at 0 °C for 5 h, warmed gradually to 25 °C, and allowed to stir for an additional 15 h. Monitoring by TLC (SiO_2, 2% MeOH/CH_2Cl_2) confirmed that the reaction was complete. The white solid was filtered and the solvent was concentrated under reduced pressure to yield more product (∼ 10 g). The wet solids were dissolved in CH_2Cl_2 (10 L), and the aqueous phase was separated. The organic phase was washed with water (3 × 1.5 L) and brine (3 × 1.5 L) and dried with Na_2SO_4. Following filtration, the solvent was removed under reduced pressure to yield a white crude material (748 g, 100%). Both TLC and MALDI-TOF MS analysis of this material showed

the presence of the desired product along with minute amount of unreacted **5**. This material was suitable for use without further purification; however, a small amount was purified for spectral characterization using column chromatography on silica gel (1:1 CH_2Cl_2/EtOAc; R_f = 0.10 using 20:1 CH_2Cl_2/CH_3OH) as the developing solvent) to afford the product as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.60 (br, 3H), 4.85 (br, 3H), 3.81 (br, 24H), 3.56 (m, 12H), 3.07 (m, 12H), 1.74 (m, 12H), 1.41 (s, 54H). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ: 169.2, 165.1, 164.8, 164.2, 156.1, 155.8, 79.1, 78.7, 43.8, 43.3, 42.9, 42.7, 37.7, 36.7, 28.3, 28.2, 27.8, 27.7. MS (MALDI): calcd 1659.8675 (M^+), found 1660.9742 $(M + H)^+$, 1684.2368 $(M + Na)^+$, 1688.2095 $(M + K)^+$. Anal. Calcd for $C_{72}H_{120}Cl_3N_{27}O_{12}$: C, 52.02; H, 7.28; N, 22.75; Cl, 6.40. Found: C, 51.95; H, 7.27; N, 22.58; Cl, 6.27.

G1-Pip (7). In a 22 L three neck RBF fitted with a mechanical stirrer, **6** (743 g, 0.45 mol) in acetone (18 L) was allowed to stir at 0 °C for 1 h. Once dissolution was almost complete, piperidine (633 mL, 6.71 mol) was added. The mixture was stirred at 0 °C for 24 h, warmed to rt, and further stirred for an additional 24 h, at which point the reaction was judged to be complete (TLC monitoring, silica gel, 2% CH_3OH/CH_2Cl_2). The resulting suspension was filtered, washed with acetone (6 L), and dried under vacuum to afford 903 g of a white crude material. TLC analysis of the crude compound showed one spot under UV lamp; however, after a ninhydrin stain a second spot was observed at the baseline which corresponds to the excess piperidine. The acetone filtrates (24 L) were concentrated to yield a light yellow residue. TLC analysis of this material showed that it corresponded to the reactive impurities **5** with piperidine (**5-Pip**) and it contained none of the desired product. It was therefore discarded. The white solid was dissolved in $CHCl_3$ (6 L), and this solution was washed with 5% aqueous HCl solution (3 × 1 L), 5% aqueous NaOH solution (3 × 1 L), and then brine (3 × 1 L). Monitoring by TLC showed the disappearance of the excess piperidine to be complete. The organic phase was dried with Na_2SO_4, and the solvent was removed in vacuo to afford an oily product. A white solid was precipitated after addition of hexane to the oily compound and upon standing for 48 h in the freezer. The solid was filtered, washed thoroughly with hexane, and dried under high vacuum. Yield: 690 g, 85%. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.33 (br, 6H), 3.78 (br, 24H), 3.70 (br, 12H), 3.56 (br, 12H), 3.02 (br, 12H), 1.67 (br, 12H), 1.59 (br, 6H), 1.52 (br, 12H), 1.37 (s, 54H). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ: 165.7, 165.1, 164.7, 155.8, 78.7, 44.0, 43.0, 42.8, 41.5, 37.0, 36.8, 28.3, 27.4, 25.6, 24.7. MS (MALDI): calcd 1807.2050 (M^+), found 1808.3271 ($M + H^+$). Anal. Calcd for $C_{87}H_{150}N_{30}O_{12}$: C, 57.78; H, 8.36; N, 23.24. Found: C, 57.78; H, 8.26; N, 23.18.

G1-NH$_2$ (8). Prior to starting the reaction, methanol and concentrated HCl were cooled to 0 °C for 3 h. In a 22 L three neck RBF fitted with a mechanical stirrer was dissolved **7** (315 g, 0.17 mol) in CH_3OH (9 L) and the mixture allowed to stir at 0 °C for 30 min. Concentrated HCl (12 N, 4.5 L) was added in 500 mL portions over a period of 2.5 h with a 15 min interval between each addition. The temperature slightly rose to 5 °C after addition. After the addition was complete, the resulting yellow solution was left to stir at 0 °C for 15 h and at 25 °C for 24 h. The volatile components were concentrated in vacuo until only ca. 800 mL of water remained. After cooling to 0 °C, the residue was made basic (pH = 14) with 1.5 L of 5 M NaOH (aq) solution. The resulting white suspension was filtered and attempt to dry this compound under vacuum and with low heating was unsuccessful. The solid was partially dissolved in $CHCl_3$ (4 L). The organic phase was separated by filtration. The remaining solid was dissolved in H_2O (3 L), and the resulting milky solution was extracted with $CHCl_3$ (1 L) using a 1 L size liquid−liquid extractor. The extraction was stopped when the aqueous solution turned clear (∼4−5 h). Fresh $CHCl_3$ was used after two successive extraction (500 mL each). The organic fractions were combined, dried with Na_2SO_4, and filtered, and the solvent was removed under reduced pressure. The desired material was isolated as a white solid by precipitation from the oily solution using hexane and upon standing for 48 h in the freezer (205 g, >99%). ^1H NMR (300 MHz, $CDCl_3$) δ: 3.74 (br, 24H), 3.66 (br, 12H), 3.58 (br t, $^3J_{H-H}$ = 7 Hz, 12H), 2.63 (br, 12H), 1.68 (br m, 12H), 1.57 (br, 6H), 1.49 (br, 12H), 1.38 (br, 12H). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ: 165.5, 165.2, 164.8, 44.0, 43.0, 42.4, 39.1, 39.0, 31.3, 25.7, 24.9. MS (MALDI): calcd 1206.8904 $(M)^+$, found 1207.9496 $(M + H)^+$. Anal. Calcd for $C_{57}H_{102}N_{30}\cdot 2H_2O$: C, 55.03; H, 8.52; N, 33.79. Found: C, 55.13; H, 8.16; N, 33.39.

G2-Cl (9). A 22 L, three neck RBF equipped with a mechanical stirrer was charged with 130 g (0.11 mol) of **8** and 5 L of H_2O. A solution of Na_2CO_3 (205 g, 1.94 mol) in 1.5 L of H_2O was added, and the resulting slurry solution was stirred at 25 °C for 1 h. A solution of **5** (310 g, 0.65 mol) in acetone (13 L) was added, and the reaction mixture was stirred at 25 °C for 36 h. The reaction was monitored by MALDI-TOF MS and TLC (silica gel, 5% CH_3OH/CH_2Cl_2, R_f = 0.2) and deemed to be complete as a result a yellow gummy precipitated at the bottom of the flask during this time. The solvent was removed on rotary evaporator (∼13 L), and the resulting aqueous suspension was dissolved in CH_2Cl_2 (4 L). The aqueous layer was removed, and the organic phase was washed with H_2O (3 × 500 mL) and brine (3 × 500 mL) and dried with Na_2SO_4. Following filtration, the solvent was removed on rotary evaporator to afford a white crude material (420 g, 99%). Both TLC and MALDI-TOF MS analysis of this material showed the presence of the desired product along a small amount of unreacted **5**. This material was suitable for use without further purification; however, a small amount was purified for spectral characterization using column chromatography on silica gel (40:1 CH_2Cl_2/CH_3OH; R_f = 0.19 using 20:1 CH_2Cl_2/CH_3OH as developing solvent) to afford the product as a white solid. The excess/unreacted **5** may also recovered from this purification (R_f = 0.50 using 20:1 CH_2Cl_2/MeOH as the developing solvent). ^1H NMR (300 MHz, $CDCl_3$) δ: 6.60−4.80 (br, 18H), 3.80 (br, 24H), 3.72 (br, 12H), 3.56 (br, 36H), 3.36 (br, 12H), 3.05 (br, 24H), 1.83 (br, 12H), 1.70 (br, 30H), 1.60 (br, 12H), 1.42 (s, 54H), 1.39 (s, 54H). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$) δ: 169.2, 168.4, 165.4, 165.1, 164.9, 164.6, 156.0, 155.7, 78.9, 78.6, 44.0, 37.6, 36.6, 28.2, 27.7, 25.6, 24.8. MS (MALDI): calcd 3860.1476 (M^+), found 3860.5223. Anal. Calcd for $C_{171}H_{288}Cl_6N_{66}O_{24}$: C, 53.14; H, 7.51; N, 23.92; Cl, 5.50. Found: C, 53.31; H, 7.52; N, 23.79; Cl, 5.41.

G2-Pip (1). In a 22 L three-neck RBF fitted with a mechanical stirrer, G2-Cl (9) (1240 g, 0.32 mol) in acetone (16 L) was allowed to stir at 25 °C for 1 h. To the resulting solution was added piperidine (470 mL, 5.59 mol), and the mixture was allowed to stir at 25 °C for 48 h at which point the reaction was judged complete by MALDI-TOF MS and TLC (silica gel, R_f = 0.49 using 5% CH_3OH/CH_2Cl_2). The resulting suspension was filtered, washed with acetone (2 L), and dried to afford 238 g of a lightweight white material. Analysis (NMR, MS, and TLC) of this material showed the presence of the pyridinum chloride salt and none of the desired product, and it was therefore discarded. The filtrate was evaporated to dryness under reduced pressure to yield a yellowish white solid. TLC analysis (SiO_2, 5% MeOH−CH_2Cl_2) of the crude product showed two spots under UV lamp, one with an R_f value of 0.34 that corresponds to the desired product and the second with an R_f value of 0.48 and corresponds to **5-Pip**. Furthermore, a ninhydrin stain showed the presence of a third spot at the baseline which corresponds to the excess piperidine. The crude product was dissolved in $CHCl_3$ (8 L) and was washed with 5% aqueous HCl solution (3 × 2 L), 5% aqueous NaOH solution (3 × 2 L), and then brine (3 × 1 L). TLC monitoring confirmed the disappearance of the excess piperidine. The organic phase was dried with Na_2SO_4, and the solvent was removed in vacuo to afford a crude product which was passed through a column chromatography eluting with EtOAc−hexane (1:1) to isolate the reactive five impurities with piperidine (**5-Pip**), followed with 30% MeOH−CH_2Cl_2 to isolate the product. The appropriate fractions were collected, and the solvent was removed under reduced pressure. The resulting

yellowish white solid was dried under vaccum for 3 days. Yield: 1.105 kg, 83%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.71−4.85 (br, 18H), 3.80 (br, 24H), 3.70 (br, 36H), 3.55 (br, 36H), 3.36 (br, 12H), 3.04 (br, 24H), 1.83 (br, 12H), 1.68 (br, 24H), 1.58 (br, 18H), 1.53 (br, 36H), 1.38 (s, 108H). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ: 165.7, 165.3, 164.9, 164.5, 156.0, 78.8, 44.0, 43.0, 41.9, 37.0, 28.4, 27.6, 25.7, 24.8. MS (MALDI): calcd 4154.8224 (M^+), found 4155.2448 ($M + H^+$). Anal. Calcd for $C_{201}H_{348}N_{72}O_{24}$: C, 58.07; H, 8.44; N, 24.26. Found: C, 57.91; H, 8.33; N, 24.48. The sideproduct **5-Pip** was recovered during purification (92 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.35 (br, 2H), 3.69 (t, $^3J_{H-H} = 5$ Hz, 8H), 3.56 (t, $^3J_{H-H} = 6$ Hz, 4H), 3.02 (t, $^3J_{H-H} = 6$ Hz, 2H), 3.00 (t, $^3J_{H-H} = 6$ Hz, 2H), 1.66 (m, 4H), 1.60 (br, 4H), 1.54 (br, 8H), 1.40 (s, 18H). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$) δ: 166.0, 164.9, 156.0, 78.8, 44.1, 41.0, 36.8, 28.4, 27.4, 25.7, 24.9. MS (ESI): calcd 576.4112 (M^+), found 577.3884 ($M + H^+$). Anal. Calcd for $C_{29}H_{52}N_8O_4$: C, 59.41; H, 8.96; N, 19.12. Found: C, 59.20; H, 8.96; N, 19.69.

G2-NH$_2$ (10). Concentrated aqueous HCl (50 mL) was added to a solution of **1** (5.0 g, 0.32 mmol) in MeOH (100 mL), and the clear solution was stirred at 25 °C for 24 h. The volatile components were concentrated in vacuo until only ca. 40 mL of water remained. The residue was made basic (pH = 14) with 85 mL of 10% NaOH (aq) solution, and the resulting milky suspension was extracted with $CHCl_3$ (1 L) using a liquid−liquid extractor until the alkaline aqueous solution became clear. The organic phase was dried over Na_2SO_4, and then the solvent was removed in vacuo to afford the product as a white solid. Yield: 3.51 g, >99%. ^{1}H NMR (300 MHz, $CDCl_3$ with trace CD_3OD) δ: 5.22 (br, 6H), 3.68 (br, 24H), 3.60 (br, 12H), 3.52 (br, 36H), 3.45 (br, 24H), 3.19 (br, 12H), 2.48 (br, 24H), 1.69 (br, 12H), 1.57 (br, 24H), 1.48 (br, 18H), 1.39 (br, 36H). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$ with trace CD_3OD) δ: 165.8, 165.4, 165.2, 165.1, 165.0, 164.6, 164.3, 43.8, 42.8, 41.7, 38.1, 37.3, 30.3, 27.7, 25.5, 24.6. MS (MALDI): calcd 2954.1932 (M^+); found 2955.0237 ($M + H^+$). Anal. Calcd for $C_{141}H_{252}N_{72}\cdot 1.5CHCl_3$: C, 54.54; H, 8.08; N, 32.15. Found: C, 54.80; H, 8.08; N, 32.48.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra, mass spectroscopy data, and HPLC traces for the compounds reported herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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