Synthesis of Large Dendrimers with the Dimensions of Small Viruses

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Supporting Information

ABSTRACT: The dendrimer chemistry reported offers a route to synthetic target molecules with spherical shape, well-defined surface chemistries, and dimensions that match the size of virus particles. The largest target, a generation-13 dendrimer comprising triazines linked by diamines, is stable across ranges of concentration, pH, temperature, solvent polarity and in the presence of additives. This dendrimer theoretically presents 16 384 surface groups and has a molecular weight exceeding 8.4 MDa. Transmission electron and atomic force microscopies, dynamic light scattering, and computations reveal a diameter of ~30 nm. The target was synthesized through an iterative divergent approach using a monochlorotriazine macromonomer providing two generations of growth per synthetic cycle. Fidelity in the synthesis is supported by evidence from NMR spectroscopy, mass spectrometry, and high-pressure liquid chromatography.

Viruses—with diameters ranging from 20 to 400 nm—occupy a length scale that represents the heart of all that is nano.1 The lower bounds of this length scale are a convergent point for both top-down and bottom-up synthesis. Existing organic building blocks providing access to this size regime derive from all disciplines, including chemistry, molecular biology, and virology, and include micelles, liposomes, and viral capsids, respectively. These materials rely on self-assembly. Self-assembly reduces the burden of synthesis, but typically at the cost of stability, as reflected in sensitivity to concentration, pH, temperature, solvents, and additives such as denaturants and detergents. Covalent synthesis offers an alternative to self-assembly that provides significant opportunities for compositional control. Virus-sized, covalent organic molecules have remained elusive targets.

First described by Tomalia,§ Newkome,3 and Vögtle in the late 1970s and early 1980s, dendrimers are highly branched, globular macromolecules that present multiple surface groups on the periphery. These architectures have been the focus of experimental and theoretical investigations in areas including materials science and medicinal chemistry.3 While a myriad of platforms have been described, a handful have been the focus of intense scrutiny because of their ease of synthesis and/or commercial availability. These materials include the polylysines introduced by Denkewalter,6 the poly(propylene imine) (PPI) dendrimers of Vögtle4 and Meijer,7 the polyamidoamine (PAMAM) dendrimers of Tomalia,9 the polyaryl ethers of Fréchet,8 the polyesters advanced by Gillies and Fréchet,§ and the phosphorus-containing dendrimers of Majoral and Caminade.10 Still, the synthesis of large-generation dendrimers is rare. Reports of large dendrimers include PAMAM11 and phosphorus-containing dendrimers,10,12 both of which are commercially available up to generation 10. Phosphorus-based dendrimers have long occupied the benchmark for large molecules, but at generation 12 they encounter a solubility challenge that precludes additional pursuits.12b At these generations, however, both this platform and PAMAM dendrimers reach diameters of only 14–15 nm. As such, these materials populate the length scale of proteins but fall short of the length scales of viruses.

For many years, we have been interested in triazine dendrimers.13 Synthetically, these materials are derived from cyanuric chloride and diamines that can be elaborated into dendrimer cores and monomers. As a platform, this class of dendrimers offers a number of benefits, including (i) low cost of reagents, (ii) compositional diversity stemming from the wealth of available diamines and the stepwise substitution of the triazine nucleus, (iii) ease of large-scale synthesis, (iv) stability in highly acidic (pH 0) or basic (pH 14) solutions, and (v) long shelf life. These materials can be prepared using either convergent or divergent approaches, although the only latter provides access to large molecules.

To reach the target generation-13 dendrimer, G13, the synthesis started with a generation-1 amine core, G1, that was reacted with macromonomer M (Figure 1). Reaction of G1 with M provides the protected G3 dendrimer. Upon deprotection, the process is repeated iteratively. By design, macromonomer M had a single reactive site, a monochlorotriazine, which precludes the formation of covalent dimers, thereby limiting side products. Previously, in order to synthesize large dendrimers, we employed two different diamines as linkers: piperazine and an oligoether diamine.15c This synthetic approach was successful up to generation 9, but poor solubility was encountered at generation 11. The former diamine, piperazine, provides a more reactive, rigid linking group without hydrogen-bond donors. The latter sacrifices

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reactivity for flexibility and length and introduces hydrogen-bond donors and acceptors. Here, only the latter flexible diamine was adopted. Dendrimers comprising this diamine showed excellent solubility (>100 mg/mL) at all generations explored. The protected dendrimers are readily soluble in organic solvents. The amine-terminated dendrimers show good solubility in both water and organic solvents.

Target G13 is the largest dendrimer reported to date with 13 branching points (generations) between the core and the periphery (Figure 1). This target theoretically presents 16 384 amine groups on the surface and has a molecular weight (MW) exceeding 8.4 MDa. However, because of the size of G13, the extent to which the actual target(s) of this synthesis represent the idealized structure is unknown. Evidence for success is derived from multiple techniques, including the behavior of smaller-generation intermediates as well as the characterization of the final product. That is, the iterative synthesis revealed trends that matched expectations with regard to both differences in polarity of the protected and deprotected materials and the appearance and disappearance of characteristic lines derived from the peripheral groups in the $^1$H NMR spectra (Figure 2a). Specifically, removing the tert-butylxycarbonyl (BOC) protecting group led to a change in the chemical shift of the vicinal −CH$_2$− group from 3.2 to 2.75 ppm. While the spectra suggest a clean reaction, confidence in this interpretation is compromised by the limits of detection afforded by $^1$H NMR spectroscopy. In view of the large number of protons in the final product (737240 in theory), $^1$H NMR spectroscopy cannot provide evidence of complete success in conversion at the final product stage or even for intermediates beyond G3 because of inherent limits in the signal-to-noise ratio.

High-pressure liquid chromatography (HPLC) showed that larger-generation materials elute later than smaller-generation ones (Figure 2b). On the basis of the amount of readily discerned impurities in the traces, we can assign the purity of the G13 target and other smaller-generation intermediates to be ~95%. This purity label reflects purity in size and not atomic composition of the theoretical structure depicted. We infer that these impurities are lower-MW species that may arise from incomplete deprotection or incomplete reactions of M with the deprotected dendrimer.

The most compelling evidence for success in the preparation of virus-sized particles was provided by cryogenic transmission electron microscopy (cryo-TEM). Figure 3 shows cryo-TEM images of G13 and cowpea chlorotic mottle virus (CCMV), an icosahedral RNA plant virus. X-ray crystallography and TEM revealed that the virus has a diameter of ~28 nm. The microscopy images show similar sizes and shapes for the virus and G13. Both appear spherical when imaged either as dried samples stained to provide high contrast or as vitrified aqueous solutions. Because the staining is imperfect, a range of sizes rather than a single value is obtained. If the stain does not penetrate the dendrimer significantly (the standard assumption), the diameter of G13 is 24.4 ± 2.3 nm. If the dye layer is included with the belief that partial penetration occurs because of the nature of the periphery, the dimension increases to 31.9 ± 1.1 nm. Unstained samples derived from vitrified solutions provided a diameter of 25.2 ± 2.3 nm, although this may slightly underrepresent the size because the image contrast at the edges of the G13 particle is low.

Atomic force microscopy (AFM) corroborated the measurements of size and shape and suggested a hydration-state-dependent size. Figure 4 shows the AFM analysis of G13 on atomically flat hydrophilic mica in water. The average height of
G13 \( (\text{Z}_{\text{max}}) \), which corresponds to the dendrimer diameter assuming the ideal spherical shape, was \( 31.5 \pm 1.9 \) nm. AFM analysis of dry samples suggested that the dendrimers collapse significantly in the Z dimension \( [\text{Z}_{\text{max}} = 9.8 \pm 1.9 \text{ nm} \); see the Supporting Information (SI)]. This collapse suggests a high degree of hydration of the internal void space of dendrimer, or at least a higher sensitivity to the force at the AFM tip when the dendrimers are dehydrated. The results are similar to those observed by Haag with hydrogels that collapsed from \( \sim 100 \) to \( \sim 20 \) nm on drying.\(^{14} \) The AFM size distribution also provides insights into the dispersity of the samples. The histogram in Figure 4c shows evidence for the presence of two smaller materials \( (\sim 20 \text{ and } \sim 25 \text{ nm diameter}) \) along with G13. Representing almost 35% of the sample, these entities appear to be discrete populations of macromolecules and may correspond to materials with sizes similar to those of G7 and G9/G11, as suggested by the HPLC traces. Their appearance is consistent with failures of purification using conventional chromatographic methods and incomplete reactions throughout the synthesis. Clearly, room for improvement exists, but this has not proven trivial to date. Filtration during sample preparation and aggregation of G13 appeared to enrich the populations of smaller particles. Accordingly, the amount of smaller particles is likely to be closer to the HPLC-based estimates of \( \sim 5\% \) rather than the AFM-based estimates of \( \sim 35\% \). The HPLC traces suggest that these impurities are elaborated throughout the synthesis, perhaps as a result of failed deprotection or macromonomer addition early in the process. Their existence, however, suggests opportunities for finer control over the size of particles by manipulating the valency of the dendrimer core.

Dynamic light scattering (DLS) measurements were performed on dendrimers in aqueous salt solutions at neutral pH. With the exception of G13, results were obtained in phosphate-buffered saline (PBS) at pH 7.1. Aggregation of G13 in PBS required the use of 0.01 M NaCl(aq) at pH 7. With the exception of G3, the distribution of light-scattering intensities was found to be bimodal. The fast-diffusion mode was related to the dendrimer size using the Stokes–Einstein equation. The extracted hydrodynamic diameters \( (d_{\text{DLS}}) \) increased with dendrimer generation in going from G3 to G13 (Figure 1). The slow-diffusion mode can be related to the presence of large dendrimer aggregates with diameters of 50–150 nm. As hydration contributes to \( d_{\text{DLS}} \), the values are consistent with the ranges provided by TEM and AFM. DLS measurements performed on G13 in the absence of NaCl yielded dendrimer diffusion coefficients that were \( \sim 10\% \) higher. This effect can be related to the dendrimer charge and the absence of electrostatic screening.\(^{15} \)

Computational models provide additional structural insights and anchored our intuitive picture of these molecules. A fully atomistic simulation in explicit solvent was not possible for G13, as inclusion of explicit molecules of water would have exceeded the computational infrastructure necessary for this 1.3 million atom molecule (G13 theoretically has the formula \( C_{76583}H_{737240}N_{1164582}O_{98298} \)). Moreover, the use of coarse-grained simplified models for G13 was incompatible with the linear and extremely flexible character of the monomers constituting the dendritic scaffold. The construction of the G13 atomistic model was extremely challenging because of the high structural complexity emerging from the large number of monomer connections. Molecular dynamics (MD) simulations revealed that G13 reaches a “hard sphere” limit (Figure 5). The peripheral amine groups extend not only outward but also inward by back-folding into the dendrimer, resulting in high structural density (see the radial distribution function plots in the SI). The measured radius of gyration of this molecule is \( R_g \approx 11.0–11.4 \) nm. This value is in good agreement with the diameters measured using DLS, as \( R_g \) and the radius of hydration \( (R_h) \) are related by the equation \( R_h \approx 1.29R_g \) for spherical molecules of uniform density.\(^{16} \) Accordingly, these diameters of 28.6–29.4 nm match the estimates derived from TEM and AFM.

The success in generating virus-sized materials observed here and not yet seen in PAMAM or phosphorus platforms may derive from more than just the solubility of the linking diamine. Clearly, the generation unit of the triazene dendrimers is approximately twice as long as those for the other platforms: while PAMAM relies on \(-N(\text{-}(\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}))\text{CH}_2\text{CH}_2\text{N}-\) with seven atoms and phosphorus dendrimers rely on \(-P(\text{-}(\text{CH}_2\text{CH}_2\text{N}-(\text{CH}_2\text{CH}_2\text{CH}_2\text{N}))\text{O}-\text{CH}_2\text{CH}_2\text{N}-\) with nine atoms, the triazine platform utilizes 18 atoms per generation. In terms of the number of flexible atoms per monomer \( (P) \), de Gennes predicted the maximum size of a dendrimer with perfect branching before defects are necessitated by steric crowding of the periphery (eq 1).\(^{17} \)

\[
\text{limiting generation} = 2.88(\ln P + 1.5)
\]

With \( P = 18 \), the maximum generation achieved before defects is 12.6.
The nature of the branching unit may be less relevant. While branching in both PAMAM and phosphorus dendrimers (indicated by R in the above formulas) occurs from a single atom (a tertiary amine N or pentavalent P, respectively, shown in bold above), both triazines and cyclophosphazene dendrimers branch from a larger rigid ring.

The covalent single-molecule nanomaterial described herein constitutes a new platform for nanosynthesis. Its viral dimensions and spherical shape are complemented by a well-defined composition. Extending opportunities for functional group variation both within and on the periphery of these building materials is preceded in smaller-generation dendrimers of all classes. Polymerization on the periphery of these building materials is precedented in dendrisomes and other systems that can be subsequently cross-linked, although such materials are usually more sensitive to factors including temperature, detergents, and concentration. The primary disadvantage of the present system, the burden of synthesis, has been reduced significantly by using an iterative route with a common macromonomer. Accordingly, we believe that there materials further expand the nanoperiodic table, and offer viruses. These properties include (i) attachment to biological surfaces such as bacteria, cells, and bone; (ii) delivery of cargo, including nucleic acids or small molecules; and (iii) use as building blocks for hybrid materials.

ASSOCIATED CONTENT

Supporting Information
Details of synthesis, characterization, and computation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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