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Controlled MegaDalton Assembly with Locally Stiff but Globally Flexible Polyphenylene Dendrimers

Christopher G. Clark, Jr.,† Ryan J. Wenzel,‡ Ekaterina V. Andreitchenko,†
Werner Steffen,† Renato Zenobi,‡ and Klaus Müllen*,†

*Contribution from the Max-Planck-Institute for Polymer Research, Ackermannweg 10, D-55128
Mainz, Germany, and the Department of Chemistry and Applied Biosciences, ETH Zurich,
CH-8093 Zurich, Switzerland*

Received October 26, 2006; E-mail: muellen@mpip-mainz.mpg.de

Abstract: The divergent polyphenylene dendrimer synthesis of the largest chemically monodisperse molecules to date, up to 28 nm at 271.6 kDa for the sixth generation, is presented. Monodispersity, conformational flexibility, and an assembly behavior reminiscent of multimeric proteins for the locally stiff, macroporous dendrimers were evaluated with a combination of molecular and polymer characterization tools, namely size exclusion chromatography, atomic force microscopy, ultrahigh-mass MALDI-TOF mass spectrometry, and dynamic light scattering. Remarkably, the high-precision MegaDalton assembly of shape-adaptable dendrimers occurs in the absence of electrostatic or hydrogen-bonding interactions and is the product of Lilliputian solvophobic interactions, mediated by the dendrimer arm size, shape, and stiffness. This covalent/noncovalent approach offers a general molecular shaping motif that is completely different than what has been previously accessible with conventional self-assembly.

Introduction

Dendrimers,[1] iteratively branched macromolecules, have been proposed for building blocks in the construction of nanoscale objects.[2] Polyphenylene dendrimers[3] are monodisperse, shape-persistent[4] macromolecules consisting of propeller-like pentaphenylbenzene repeat units with mostly radial branching directions. Linear extension of the branching unit with oligo(*p*-phenylene) spacers translates directly into a larger 3D macromolecule[5] and gives rise to solvent-filled intramolecular cavities when in solution. Aggregation is an intrinsic property for conjugated π-systems[6] such as 1-D oligo(phenylene ethynylene)s[7] and oligo-*p*-phenylenes[8,9] in solution. In this context, the solubility and stiffness of the oligo(*p*-phenylene)s within each dendron arm play major roles in determining the 3D aggregation behavior.

Described herein is the extension of the synthesis to a sixth generation "exploded"-type polyphenylene dendrimer (Scheme 1), which is, to the best of our knowledge, the largest diameter, molecularly monodisperse material, chemically synthesized to date. Assessment of structural identification and purity was complicated due to the magnitude of the molecular weight and prevalent self-assembly, even during analysis, and pushes the limits of conventional analytical approaches. The case for monodispersity was made with evidence from size exclusion chromatography (SEC) coupled with multiangle-laser-light scattering (MALLS), atomic force microscopy (AFM), and ultrahigh mass MALDI-TOF mass spectrometry. The conformational flexibility of the increasingly long, highly rigid polyphenylene dendritic backbone under external forces and its influence on the overall conformation accessible to the dendrimer were evaluated with AFM. While there are many examples of extended organizations of dendrimers using the phase separation of the chain ends and branching units,[10−18] few examples have been reported of the discrete aggregation of dendrimers,[19−22] In this study, leveraging the monodisperse nature of the dendrimers, ultrahigh mass MALDI-TOF mass

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† Max-Planck-Institute for Polymer Research.

‡ ETH Zurich.

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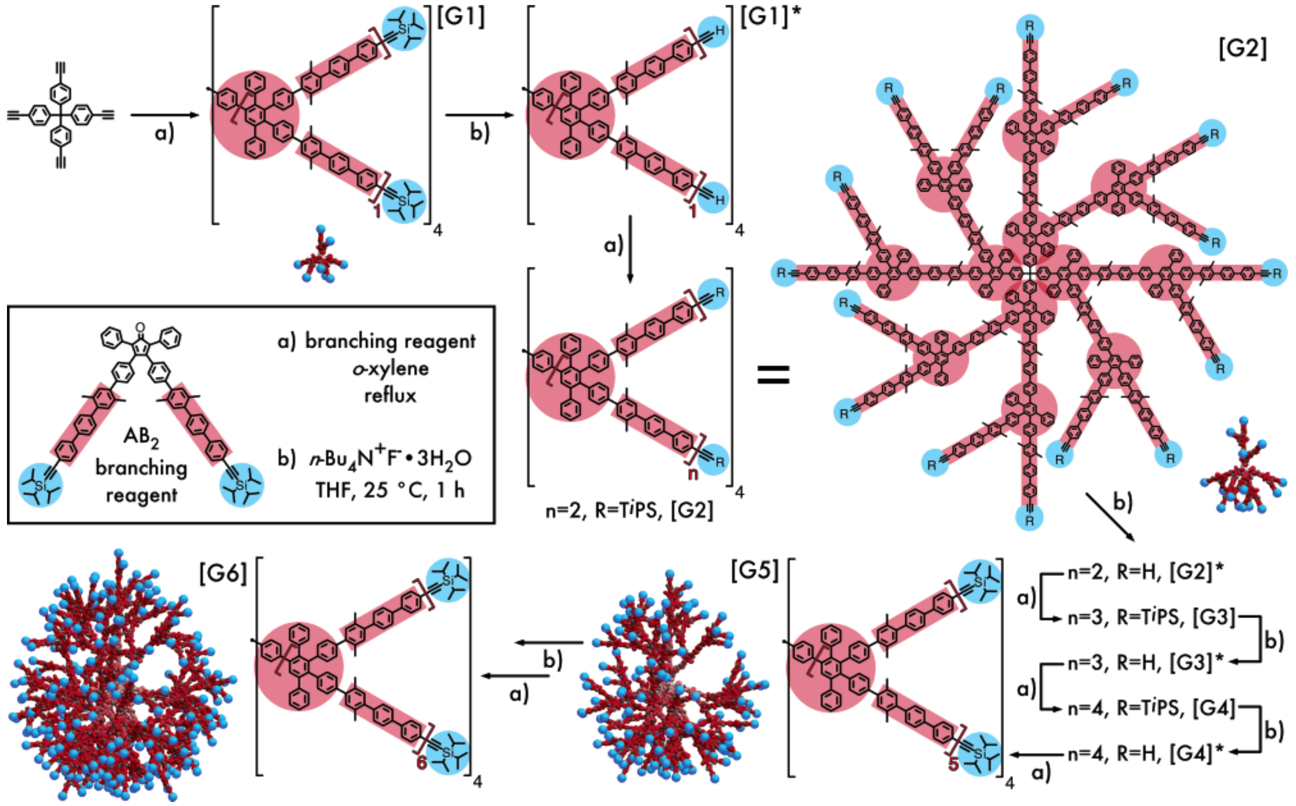
Scheme 1. Iterative Synthesis of a Sixth Generation, [G6], Polyphenylene Dendrimer with Oligo(*p*-phenylene) Spacers and Triisopropylsilylethynyl (TiPS) Chain Ends[a]



[a] Diels−Alder cycloadditions and cleavage of TiPS protecting groups are quantitative at each stage. Depicted are the simplified chemical structures and the 3-D, fully extended, roughly spherical geometries, resulting from the "mostly radial" branching geometry. The full repeat unit is highlighted by the one open and two closed red brackets. In the full [G6] dendrimer, six iterations, schematically connecting an open red bracket to each of the closed red brackets starting from each of the four connections of the tetraphenylmethane core, are achieved. Large cavities are readily apparent in the 3-D model, which should be solvent filled when in solution or can be partially collapsed if solvent is excluded, due to aggregation within and between the locally stiff arms.

spectrometry was combined with dynamic light scattering (DLS) and AFM to elucidate different aspects of a remarkable solution-phase assembly behavior, reminiscent of multimeric proteins. Self-assembly is inextricably linked with the synthesis as well as analysis but gives hitherto access to tremendous 3D nanostructural control.

Results and Discussion

Synthesis. The perfect divergent synthesis hinges upon iteration of two flawless chemical reactions: in this case (Scheme 1), the Diels−Alder cycloaddition of 3,4-bis-{2′,5′-dimethyl-4‴-triisopropylsilylethynyl-1,1′:4′,1″:4″,1‴-quaterphenyl-4-yl}-2,5-diphenyl-cyclopentadienone[5] (AB$_2$ branching reagent) with terminal alkynes for generational growth and the fluoride-promoted unmasking of triisopropylsilyl (TiPS)-protected terminal alkynes. This approach was previously demonstrated up to the fifth generation,[5] starting with 4-fold initial cycloadditions to tetrakis(4-ethynylphenyl)methane. The 128 TiPS chain ends of the same fifth generation, [G5], dendrimer (Scheme 1) were now unmasked in THF at room temperature and reacted subsequently in *o*-xylene at reflux with an excess of the AB$_2$ branching reagent to give the sixth generation, [G6], macromolecule with 271.6 kDa in molecular weight. ^{1}H NMR is completely consistent with the formation of the product (Supporting Information). Mass spectrometry and purity will be discussed in a later section. Synthetic details are given in the Supporting Information. In addition, 256 chain ends are present at the surface of the macromolecule whose composition is chemically distinct from that of the phenylene backbone and potentially amphiphilic.[23]

Evaluation of the Dendrimer Chemical Identity. Starting with the fifth generation,[5] with convincing evidence of chemical purity, the presence of a small (less than 5 wt %) high molecular weight shoulder was detected by SEC in THF (Figure 1), which was not reduced by prolonged heating of the dendrimer sample in THF. High-temperature SEC in trichlorobenzene gave similar results. Attempts to decrease the shoulder by decreasing the dendrimer−dendrimer interactions during synthesis were not successful. Dilution (10-fold) of the parent dendrimer in the reaction mixture had little effect on the presence and size of the shoulder. Dendrimer solutions up to 10 mg/mL in THF, a good solvent, did not show any aggregates beyond the shoulder once equilibrated by heating overnight. That the dendrimer is not in equilibrium with its shoulder suggests two possibilities: physical crosslinking or a chemical side reaction during synthesis. Similar shoulders in the SECs have been reported previously for other dendrimers and either have not been

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(23) Amphiphilicity refers to solubility differences among two components and a solvent. In this context, only the solubilities of aryl (backbone) versus alkyl (chain ends) substituents in nonpolar solvents are different.

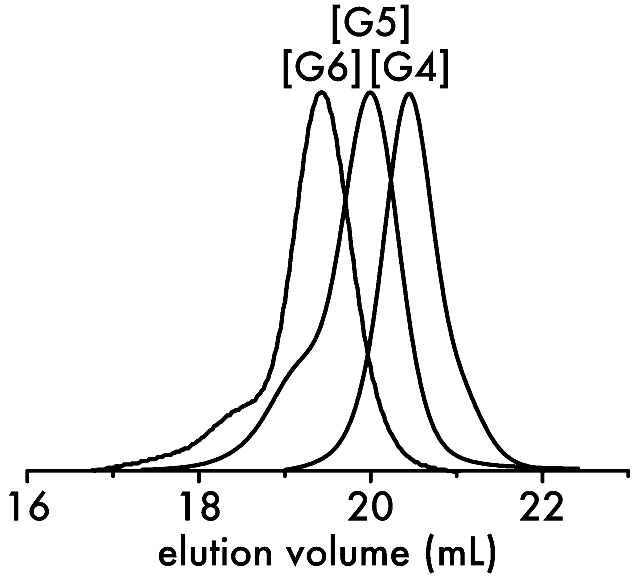
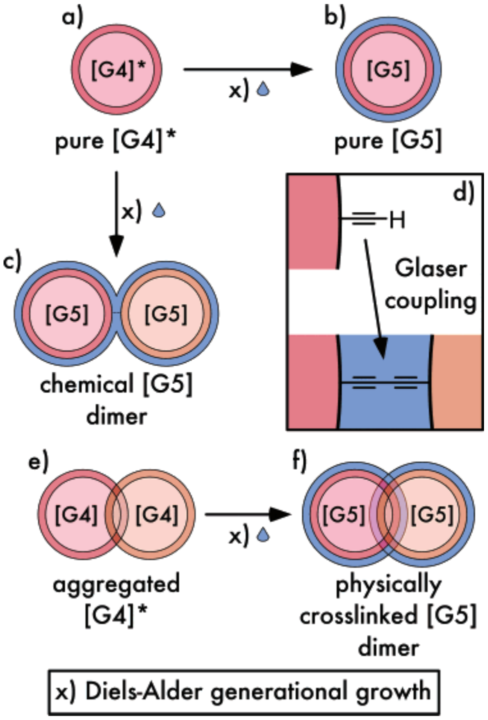


Figure 1. Size exclusion chromatography (SEC) traces for [G4], [G5], and [G6] dendrimers in THF. Beginning with [G5], a high molecular weight shoulder is present in each SEC trace.

discussed[24] because of lack of evidence or have been discounted as supramolecular dimers or larger aggregates and not a product of chemical imperfection.[25] The clarification of dendrimer aggregates is indeed nontrivial. A side reaction involving only one of the large number of chain end functionalities is required for chemical dimerization. In the case of [G5], if the shoulder were a 5% chemical dimer, the conversion would correspond to 99.92%, rendering chemical analysis limited. Furthermore, flexible dendrimers may reveal different conformations depending on the extent and mode of aggregation, making interpretation difficult. The size and highly porous nature of the polyphenylene dendrimers would support the hypothesis of aggregation by interdigitation during synthesis, while a Glaser-type oxidative chemical coupling of the terminal alkyne chain ends (Scheme 2d) is also possible; although, precautions were taken to avoid the presence of transition metals. Physical methods are more sensitive than chemical methods for evaluation of aggregation/chemical crosslinking processes. The elution volume of the shoulder in the [G5] sample is slightly lower than that of the main [G6] peak, which is suggestive of a species in the size range of a dimer. The [G5] sample was additionally fractionated by SEC (Figure 2) and analyzed with an SEC equipped with both refractive index (RI) and multiangle laser light scattering (MALLS) detectors. The scattering intensity for the shoulder is a bit less than twice that of the main peak with normalized RI signals (Figure 2c), again consistent with the formation of a few percent dimer. It should also be stated that the pure dendrimer fraction (Figure 2b) did not further show the presence of aggregates upon prolonged standing in a solution of THF.

The shape persistence of polyphenylene dendrimers up to the fifth generation in solution has already been demonstrated.[5] The lack of backfolding of the stiff polyphenylene backbone permits the quantitative hydrodynamic size analysis of the solvated dendrimer and higher order species since the lengths of the arms are largely unperturbed. As the chain ends are surface-bound and the dendrimer dimensions are known (Scheme 2, Table 1, Supporting Information) from the theoretical oligo(*p*-phenylene) arms lengths and dynamic light scattering,[5] the pure (Figure 2b) and "dimer"[26] enriched (Figure 2c) fractions were further subjected to inspection with atomic force microscopy (AFM). In the pure fraction, particles with a single diameter, 24 nm, consistent with the dendrimer theoretical diameter were observed (Figure 2d, Table 1). In the "dimer" enriched fraction (Figure 2c), particles with two different sizes were observed, one with the same diameter as that of the pure dendrimer (Figure 2e), and the other with 32 nm (Figure 2f). There is little error in the measurement of particle diameters from AFM images since convolution with the AFM tip is small with the low heights observed (discussed in a later section). Antiparallel chemical coupling of the chain ends would give rise to a 42 nm polar diameter (Scheme 2c, Table 1), significantly longer than that observed with AFM. This strongly suggests that the shoulder in the unfractionated sample (Figure 2a) is a physically crosslinked, interdigitated dimer (Scheme 2f), not a dimer from a chemical side reaction. Aggregation during dendrimer synthesis (Scheme 2e) could have resulted in a small fraction of interlocked dimeric aggregates (Scheme 2f), which were not readily pulled apart. As the SEC of the sixth generation sample contains a similarly sized shoulder, it is assumed that the interlocked dimers formed in the fifth generation would be

Scheme 2. Synthesis of Dendrimeric Products if Either Physical or Chemical Crosslinking, by a Side Reaction of Two Alkynes Instead of Diels−Alder Reactions, Occurs during Generational Growth[a]



[a] Two types of dimers may be formed during the growth reaction from (a) the fourth to (b) the fifth generation: (c) a chemically crosslinked dimer missing two branching units would be formed if a side reaction such as (d) a Glaser-type coupling of two terminal alkynes occurred, whereas (e) aggregation of dendrimers during synthesis would give rise to (f) a physically crosslinked dimer of two complete [G5] molecules. A more elaborate scheme including [G6] products can be found in the Supporting Information.

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[26] "Dimer" refers to that observed experimentally. Its nature, e.g., chemical (covalent) dimer or physical (noncovalent) dimer, is not a priori known.

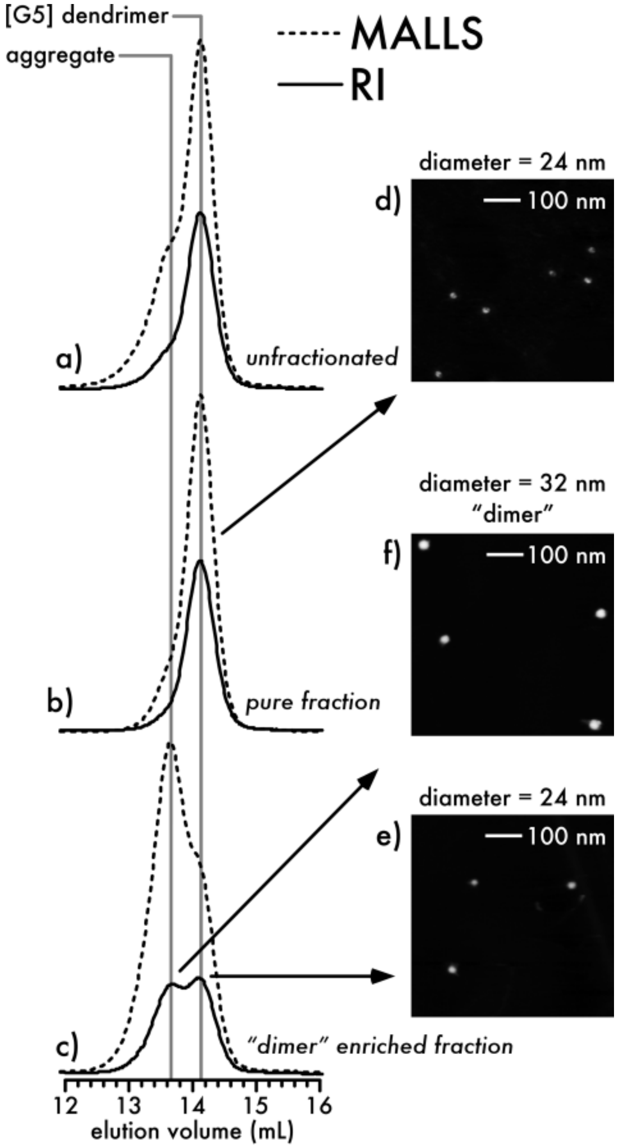

Figure 2. Multiangle laser light scattering (MALLS) size exclusion chromatography (SEC) results of fractionation of (a) a sample of the [G5] dendrimer. The (b) pure and (c) "dimer" enriched fractions were further subjected to (d, and e, f, respectively) inspection with atomic force microscopy, indicating that the shoulder in the SEC of (a) the sample generated during synthesis is a 32-nm-diameter dimer. THF was used for all analyses. All AFM images are on the same scale.

carried on to the sixth generation. [G6] dendrimers demonstrated pronounced aggregation by AFM as will be discussed in a later section; thus solution-phase analyses were more suitable for this task.

In order to perform a more complete analysis of the SEC shoulders, the [G5] and [G6] dendrimers and their respective "dimers" were completely separated with analytical SEC (Figure 3a,b) and each was subjected to dynamic light scattering (DLS) (Figure 3c,d) in THF and analyzed with CONTIN[27,28] to obtain hydrodynamic radii, r_H (Figure 3e,f, Table 1). Pure dendrimer r_H values are slightly smaller than the theoretical radii, determined from AM1 calculations, indicating only a small collapse. Considering that the [G5] "dimer" and [G6] dendrimer have identical r_H values but different SEC elution volumes, this immediately suggests a nonspherical shape for the G5 "dimer". The effective hydrodynamic radii (Table 1) for the "dimers" were analyzed using the Stokes−Einstein equation and the prolate spheroid model, which enabled the calculation of effective diffusion coefficients based on the ratio between equatorial and polar radii,[29,30] combining hydrodynamic data for the pure dendrimers. The theoretical dimensions for all chemical dimers (Scheme 2, Supporting Information) were explicitly calculated (Table 1). This is a reasonable estimation of hydrodynamic dimensions since the pure dendrimer r_H values were close to their corresponding theoretical radii. It was found for the [G5] "dimer" that the polar radius is significantly shorter than that for the product of chemical crosslinking. The polar radius for the [G6] dimer, at first approximation, is smaller than but quite close to that of the [G6] chemical dimer. Considering that the hydrodynamic radii for the pure dendrimers are somewhat smaller than the theoretical radii (11.2 vs 11.8 nm for [G5]; 13.2 nm vs 14 nm for [G6], respectively), use of the theoretical radii in the prolate spheroid calculations yielded more satisfying results, giving prolate radii consistent with that of physical crosslinking. This would imply that the dimerization resulted in further rigidified structures. The prolate spheroid calculation for the [G5] "dimer" is now in agreement with the diameters from AFM. Second, the AFM heights of the [G5] "dimers" are 5% larger than those of the pure [G5] dendrimer. These results completely establish that the SEC shoulders resulted from the physical crosslinking of supramolecular dimers during synthesis. It cannot be stated from these data that the aggregation of pure [G5] dendrimers did not occur during [G6] synthesis, giving rise to [G6] interlocked dimers. That the size of the shoulder in the GPC is similar to that of [G5] suggests the majority of the physical dimer formation occurred during the synthesis of [G5], likely due to incomplete aggregate disruption during the first few hours of generational growth. Foremost, these data confirm that the most likely side reaction did not occur, even over 128 reactions per molecule.

Ultrahigh-Mass MALDI-TOF Mass Spectrometry. For structural proof, only mass spectrometry can distinguish a pure dendrimer product from a mixture of the dendrimer with incompletely coupled products, but detection of molecules the size of G6 required more than routine MALDI-TOF mass spectrometry. The mass spectrometer was equipped with a superconducting cryodetector,[31] which, in addition to extending the sensitivity into the MDa range, permitted differentiation of singly versus multiply charged ions by directly measuring the kinetic energy of the detected ions[32] (Figure 4a, Supporting Information). In the singly charged ion mass spectrum (Figure 4b), instead of observing the signal for the [G6] molecular ion, stable clusters of [G6] dendrimers, above 1 MDa in molecular weight, were detected: tetramer (1.09 MDa), pentamer (1.36 MDa), and hexamer (1.63 MDa), with the tetramer being the most abundant. MALDI-TOF mass spectrometry has been used

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Table 1. Summary of Size Data for Fractionated G5 and G6 Dendrimers and Their Respective "Dimers"

	Diff. coeff, D ($x10^{14}$ m^2/sec)	$\frac{D_{eff}}{\text{dendrimer D}}$	hydrodynamic radius, r_H (nm)	$\frac{r_H}{\text{dendrimer } r_H}$	prolate radii (nm)	physical radii (nm)
[G5]	[a]5.00	-	[a]11.2	-	-	[c]12.0
"dimer"	[a]4.24	0.85	[a]13.2	1.18 [e]1.12	[d]17.4/11.2 [e]16.1/11.8	[c]16.0/12.0
chemical [G5] dimer[c]					21.2/11.2	-
[G6]	[a]4.24	-	[a]13.2	-	-	[c]14.0
"dimer"	[a]3.29	0.78	[a]17.0	1.29 [e]1.21	[d]25.2/13.2 [e]23.3/14.0	-
chemical [G6] dimer[b]					25.4/13.2	-
chemical [G5] dimer +1 gen[b]					23.3/13.2	-

[a] Results from DLS data in THF, CONTIN fit, and the Stokes−Einstein equation: $D = kT/6\pi\mu\, r_H$. [b] Results from dimensions (polar and equatorial radii) from AM1 semiempirical calculations for the product of chemical crosslinking during generational growth and the prolate spheroid model.[29,30] [c] Results from AFM data. [d] The calculated prolate radii for the "dimer" experimental data were calculated using the same model, the experimental hydrodynamic radii ratios, and the hydrodynamic radius for the pure dendrimer. [e] Calculated using the dendrimer theoretical radius instead of the hydrodynamic radius. Calculation details are found in the Supporting Information.

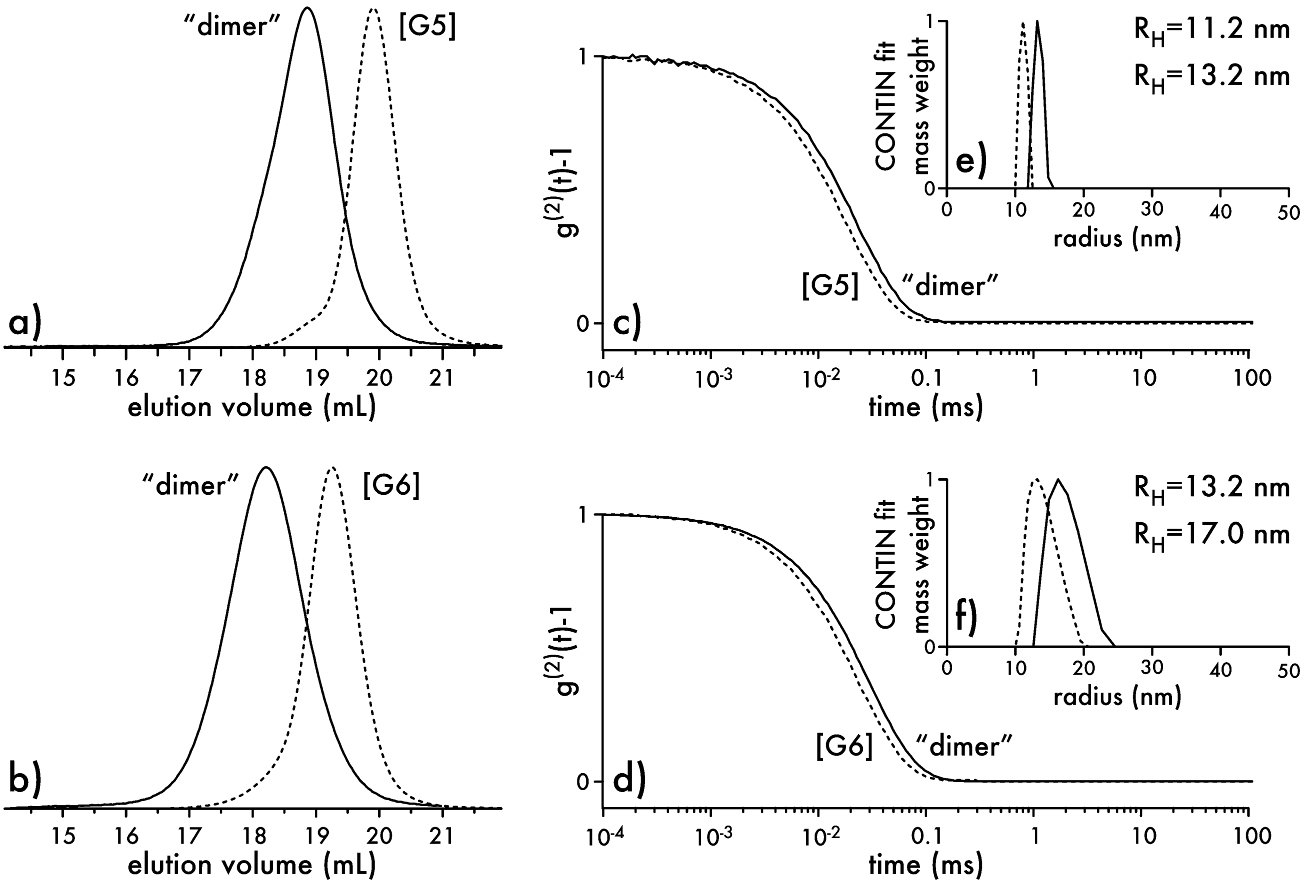

Figure 3. Results of complete GPC fractionation of [G5] and [G6] dendrimers (dashed lines) and their respective "dimers" (solid lines): (a, b) SEC elution profiles; (c, d) dynamic light scattering (DLS); and (e, f) CONTIN-generated size distributions for G5 and G6, respectively.

previously to identify dendrimer aggregation processes.[19,33] These results prompted the investigation of smaller dendrimers[5] [G4] and [G5] under the same conditions. For [G4], a broad distribution of aggregates was observed (Figure 5a). The MALDI sample preparation had a marked influence on the mass spectrum with the molecules and aggregates in solution ultimately trapped within the matrix. In order to allow more time for dendrimer−dendrimer interactions to occur by lowering the viscosity before crystallization of the matrix, a 10-fold dilution of the [G4] dendrimer−matrix solution (keeping the dendrimer−matrix ratio constant) was made prior to deposition. The aggregate population was found to have shifted to a higher mass range (Figure 5b), with the tetramer (261.8 kDa) as the major aggregate, as well as significant peaks for the pentamer (327.3 kDa) and hexamer (392.7 kDa), and no signal due to the individual dendrimer was detected. This confirms that the clusters observed in the gas phase were formed in solution. Similarly for [G5], tetramer (536.6 kDa), pentamer (670.8 kDa),

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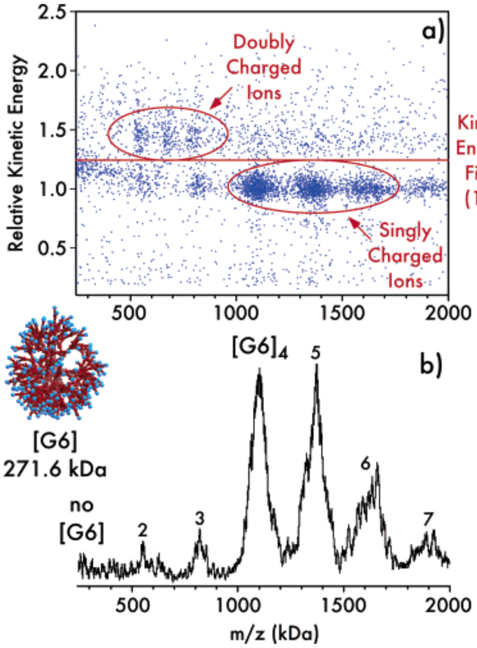

Figure 4. (a) Kinetic energy distribution of individual ion impact events upon the cryodetector for a MALDI-TOF analysis of sixth generation dendrimer. The signals at mass to charge values above 1000 kDa correspond to singly charged ions from the cluster formation of [G6]. Those below this mass are primarily of higher kinetic energy values confirming that they are comprised primarily of multiply charged ion signals from higher mass compounds. (b) MALDI-TOF mass spectrum using data from (a), filtered to remove (multiply charged) ions with relative kinetic energy values above 1.2: the singly charged ion MALDI-TOF mass spectrum of the sixth generation dendrimer, using dithranol as the matrix compound, mixed with the dendrimer in THF.

and hexamer (805.0 kDa) populations were increased (Figure 5c), and no individual dendrimers were observed in any mass spectrum.

The nature of the solvophobic interactions, which drives the aggregation of dendrimers into clusters, is mediated by the sample preparation. Evaporation of the solvent from a solution of the dendrimer containing the matrix compound necessitates that the solution take on more of the character of the matrix compound than the solvent. Dithranol, the matrix used, is more polar than THF, and an increase in the concentration of a polar component is known to induce aggregation in aromatic systems.[34] Change of the matrix to DCTB[35] produced similar results. Modification of the sample preparation such as the addition of salt, amount of matrix, and or the amount of solvent added before deposition produced either similar results or no signal at all. Beyond the fourth generation, previous characterization by MALDI[5] was difficult because isolated dendrimers embedded in the matrix were not present at sufficient concentration, the laser energy required to separate the clusters resulted in fragmentation of the dendrimers, or the instruments lacked the sensitivity to detect clusters of such high mass. That such large clusters, formed by self-complementary self-assembly in solution, are able to be identified by mass spectrometry is evidence that a large number of noncovalent interactions hold the individual dendrimers together, which has only been previously observed for biological macromolecules.[36]

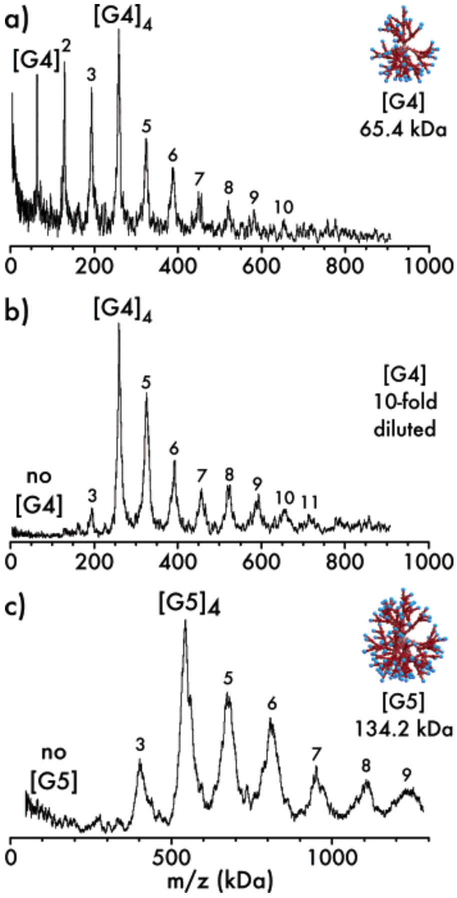

Figure 5. MALDI-TOF mass spectra of (a) [G4], (b) 10-fold dilution of the preparation for [G4], and (c) [G5] dendrimers, prepared by deposition from THF with dithranol as matrix.

Returning to chemical identification of [G6], first, the breadths of the individual peaks for the clusters are quite large compared to that of low molecular weight compounds. The peak breadth depends on the mass observed (Supporting Information). This is no more obvious than in the case of [G4] where significant broadening was observed with increasing aggregation number. The same trend is observed in [G5] and [G6]. One possible explanation is that the number of matrix adducts is increased at higher aggregation numbers, although, delayed extraction was used to minimize this effect. Second, while signals from neither the molecular ion (271.6 kDa) nor the dimer (543.2 kDa) were significant in the mass spectrum of [G6], it is clear that the dendrimers in the clusters are very narrowly disperse if not monodisperse. The peak widths are narrower than those for biomacromolecules investigated by the same technique.[31] The lack of Glaser-coupled reactions, demonstrated in the previous section, suggests that any polydispersity would only arise from incomplete reaction. It should be noted that a convergent dendrimer synthesis,[37,38] where incompletely coupled products can be removed, is conventionally required to generate dendrimers of this purity. Unfortunately, the resolution of MALDI-TOF mass spectrometry for this molecular weight range is not sufficiently high, and it cannot be stated that the dendrimer is unequivocally monodisperse without an advance in the tech-

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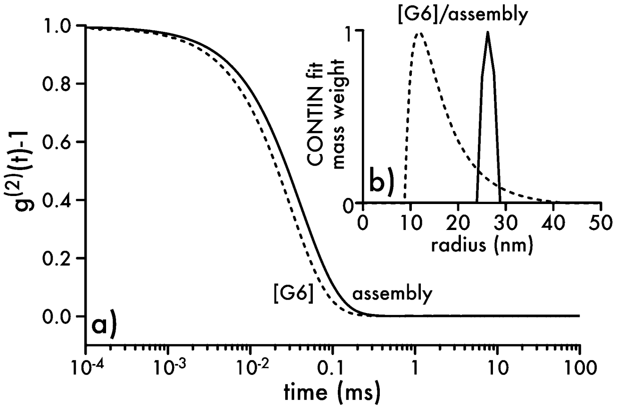

Figure 6. (a) Dynamic light scattering (DLS) results for the unfractionated [G6] dendrimer (dashed line) in THF (0.1 mg/mL) with trace aggregates (CONTIN, b) and in a mixture of THF/hexane (0.2 mg/mL, 40 mol % hexane) (solid line), which forms exclusively a narrowly disperse assembly with a 26 nm hydrodynamic radius. Both measurements were acquired at 35 °C.

nique. Furthermore, the “dimer” was not detected in the mass spectrum for either [G5] or [G6], independent of sample preparation. In addition to this further evidence of molecular perfection, the dimer has no apparent inhibition on the assembly behavior, having been incorporated into the larger clusters. The lack of dimers in all cases suggests allosteric binding of additional dendrimers. In each of the fourth through the sixth generation matrix-promoted solution assembly processes, the tetramer is the major aggregate, suggesting that the mechanism of assembly of the macromolecules may indeed be general, but it must be deduced by other means.

Solvent Induced Self-Assembly − Dynamic Light Scattering. Unlike mass spectrometry, DLS offers the opportunity for in situ and equilibrium measurements of dendrimer aggregation. DLS was performed with the [G6] dendrimer (Figure 6a,b dashed line), which was found to be nearly fully swollen in THF. The presence of a small quantity of the dimeric interlocked aggregates in the unfractionated sample also gives rise to a high mass tail in the DLS. Increasing the polarity of the solvent by the addition of methanol induced aggregation, and particles were formed as large as 1 μm (not shown), with the particle growth rate to that size tuned according to the amount of the incompatible solvent used, similar to large compound micelles.[39] Alternatively, hexane, a solvent which is incompatible with the oligo(phenylene) backbone, but more compatible with the chain ends of the dendrimer, was chosen. THF/hexane mixtures induced aggregation, but in 40 mol % hexane in THF (Figure 6a,b solid line), the assembly size strikingly stabilized at a hydrodynamic radius of 26 nm. This is noticeably larger than the [G6] “dimer”. This comparison of solvent quality proves that the chain ends play a role in the aggregation and that the assemblies are driven by the solvophobic interactions with the oligo(phenylene) backbone. Furthermore, the dendrimer assembly distribution is narrower than that of the unfractionated dendrimer, suggesting that the “dimer” does not inhibit the discrete aggregate formation. Addition of 50% more THF to the solution resulted in the disruption of most of the assemblies almost immediately, indicating the reversibility of the assembly mechanism. An open issue is how the globular dendrimer with its surface-bound chain ends makes discrete assemblies, as in the solutions containing hexane, rather than extended assemblies, as in the case of solutions containing methanol.

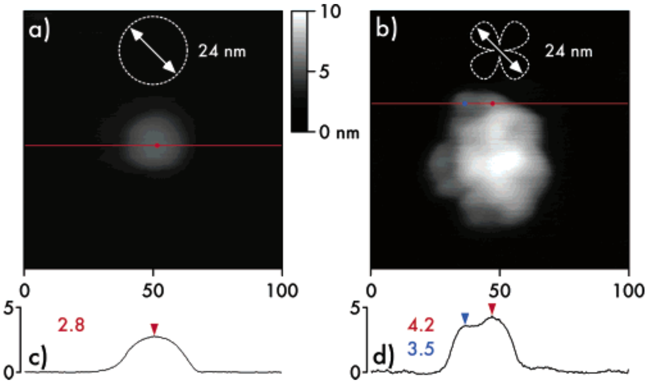

Figure 7. AFM height images of the [G5] dendrimer and its aggregates: spun-cast on mica from (a) tetrahydrofuran (single molecule) and (b) dichloromethane (six molecules) with (c,d) respective cross sections.

Conformational Flexibility − AFM. The question arose as to why the dendrimers display special aggregation numbers. The expectation was that a rigid dendrimer of this size and chain end density would not be able to dock into large clusters efficiently and would instead resemble a sphere. Atomic force microscopy has been used to visualize[40,41] as well as to measure the flexibility of large macromolecules.[42,43] AFM of shape-persistent polyphenylene dendrimers[22] has revealed heights comparable with the theoretical dendrimer geometry. Spun cast from THF onto mica and imaged using far-off resonance tapping mode AFM,[44] the [G5] dendrimer was heavily collapsed against the substrate with a diameter (Figure 7a,c) comparable to the solution-state diameter.[5] At first glance, the dendrimer appeared to be collapsed by more than 75% due to surface adsorption forces. Investigation of the same dendrimer spun cast from dichloromethane gave the answer. The tetra-lobed structure was bent into a single plane through van der Waals contacts with the substrate (Figure 7b,d). Dichloromethane is a worse solvent than THF for the dendrimer, as indicated by the greater extent of aggregation and the shape of the dendrimer by AFM. In dichloromethane, the branching units were contracted along the longest axis of each dendron. As a result, the dendrimer was actually taller and hence more rigid than the dendrimer processed from THF. The height measured in AFM was the breadth, not the length, of a dendron. The solvent-dependent height differential in the AFM images of [G5] dendrimers is a result of either good solvation or solvatophobic collapse within the dendrons prior to collapse against the surface. For the dichloromethane case, most of the conformational freedom within a single dendron has already been used prior to the surface deposition, so the collapse within the dendrons is minimized. Orienting two dendrons of the tetrahedral dendrimer (Figure 8a) toward the AFM substrate during deposition (Figure 8b), followed by collapse of these dendrons, and downward bending

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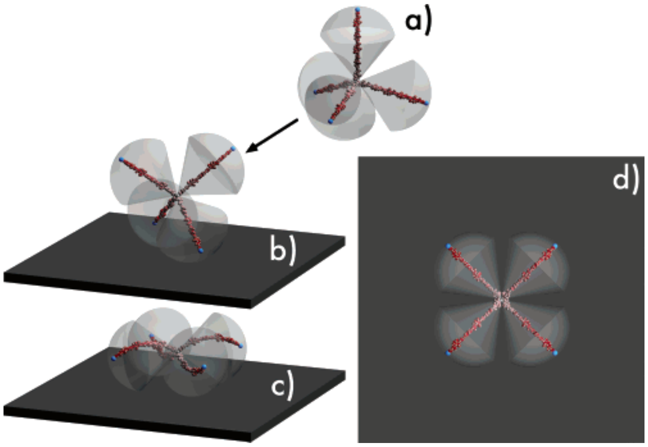

Figure 8. AFM image simulation, assuming the bending mode for each longest oligo-*p*-phenylene arm in the dendrimer. Deposition of (a, b) the tetrahedral dendrimer was made such that the dendrons were bent pairwise with respect to the surface, ultimately resulting in (c) the flattened conformation with (d) top view. All of the atoms except for those of the phenyl rings connected to the longest oligo(*p*-phenylene) arm have been omitted for clarity.

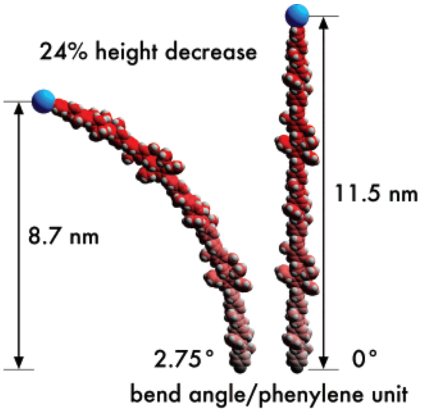

Figure 9. Analysis of the bending of the longest oligo(*p*-phenylene) arm required for deformation of the [G5] dendrimer.

of the other two dendrons furnishes a pseudo-square planar shape (Figure 8c,d) with maximum contact to the substrate. This conformation gives an extent of bending (Figure 9) with only 24% height decrease per arm. For the 26 phenylene rings of the longest oligo(*p*-phenylene) arm, this corresponds to 2.75° of bending between each ring, which has little energetic penalty at room temperature.[45] Calculation of a persistence length[46] from this bending mode gave a value of ca. 25 phenyl rings or 10.5 nm, which is smaller than the reported values, 13−15 nm for poly(*p*-phenylene)s in solution,[47,48] but similar to that measured in a monolayer of a poly(*p*-phenylene) by AFM.[49] As the dendron is directional, there is clearly not sufficient conformational flexibility to backfold a single oligo(phenylene) to the point where the chain end reaches somewhere other than the surface or near the surface of the dendrimer. Investigation of a single [G6] molecule on highly oriented pyrolytic graphite (HOPG) by AFM showed a tripodal geometry with the topmost dendron off-center (Figure 10), where the four dendron lobes are too bulky to fit in a single plane. Further, the topmost dendron is bent through interaction with the other dendrons. It is this topology, which first implied that the dendrimers could adopt an open conformation with which it could dock into an assembly. The role of an amphiphile's shape on the supramolecular assembly is well established[50,51] and furthermore can be changed in response to a stimulus.[52,53] This forms the hypothesis that the dendrimer's slight shape-adaptability and amphiphilic/solvophobic character dictate its self-assembly behavior.

Assembly - AFM. While the sample preparation for AFM is vastly different from that for DLS, where dendrimers or aggregates are separated by solvent and at equilibrium, or even that for MALDI, where aggregates are isolated and trapped in the drying matrix, insights into the limited aggregation numbers, assembly motifs, and binding topologies of individual aggregates may be extracted from the AFM images. A solution of [G6] dendrimers in THF was spun cast onto highly oriented pyrolytic graphite (HOPG) in order to trap the assemblies by minimizing the contact and reorganization time with the substrate before being imaged using far-off resonance tapping-mode imaging.[44] The aggregates, whose shapes and features were large enough to resolve, were not heavily disrupted by the casting procedure. Assemblies were found to have diameters of ca. 50 nm (Figure 11a,b), similar to that obtained from DLS with dendrimer center-to-center distances significantly less than two dendrimer radii. Striking are the presence of 90° angles in the aggregates and the occurrence of tetramers (Figure 11a), which have a shape more like a triangular pyramid than a tetrahedron. With increasing dendrimer generation and increasing branching unit density, the likelihood that the dendrons are able to interdigitate through several layers of branching units is decreased. The DLS experiments showed that the assembly process is rapidly reversible. The interlocked aggregates in the syntheses of G5 and G6 have been shown to be resistant to pull apart even at elevated temperature. These observations suggest that the time scale for separation of a multigeneration-deep interdigitated species is much larger than the time scale for assembly/disassembly in the DLS experiment. Thus, in order to reach the center-to-center distances measured in the AFM images, phenylene residues must be shielded from solvent by interdigitation of a complete axially oriented dendron (Figure 11h) into the pocket created by three folded (completely open conformation, Figure 11i) dendrimers, whereby the internal sides of the dendrons are in direct contact (Figure 11j). An increase in the number of equatorial (folded) or axial dendrimers by one then gives a pentamer, and a further increase in the number of axial dendrimers to two gives the hexamer (Figure 11b,k). Upon exclusion of solvent, the dendrimers “glue” together, which, remarkably enough, under these experimental conditions requires no additional chemical fixation for stability. To further accentuate the solvophobic effect, the solvent composition for deposition was changed to 40 mol % hexane in THF as in the case for the

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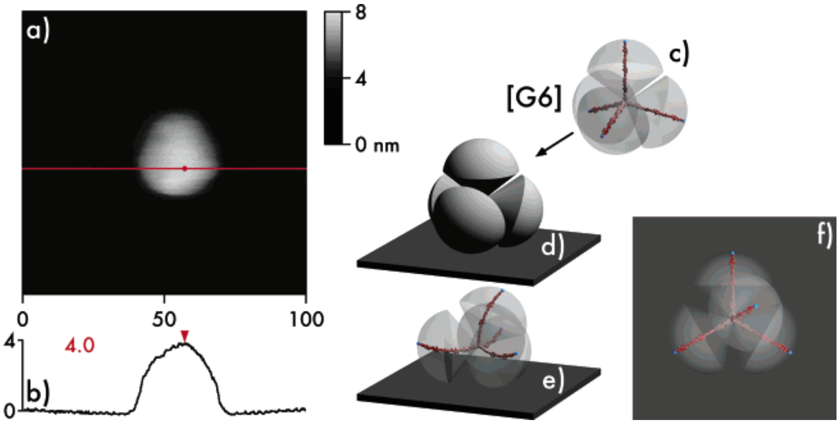

Figure 10. AFM height image of the [G6] dendrimer spun-cast onto highly oriented pyrolytic graphite (HOPG) from (a) tetrahydrofuran with (b) cross section. Simulation of the AFM image of the [G6] dendrimer, assuming the bending mode for each longest oligo(*p*-phenylene) arm in the dendrimer. Deposition of (c) the dendrimer was made such that there was (d) contact of three dendrons with the surface, which ultimately results in (e) the flattened conformation with (f) top view.

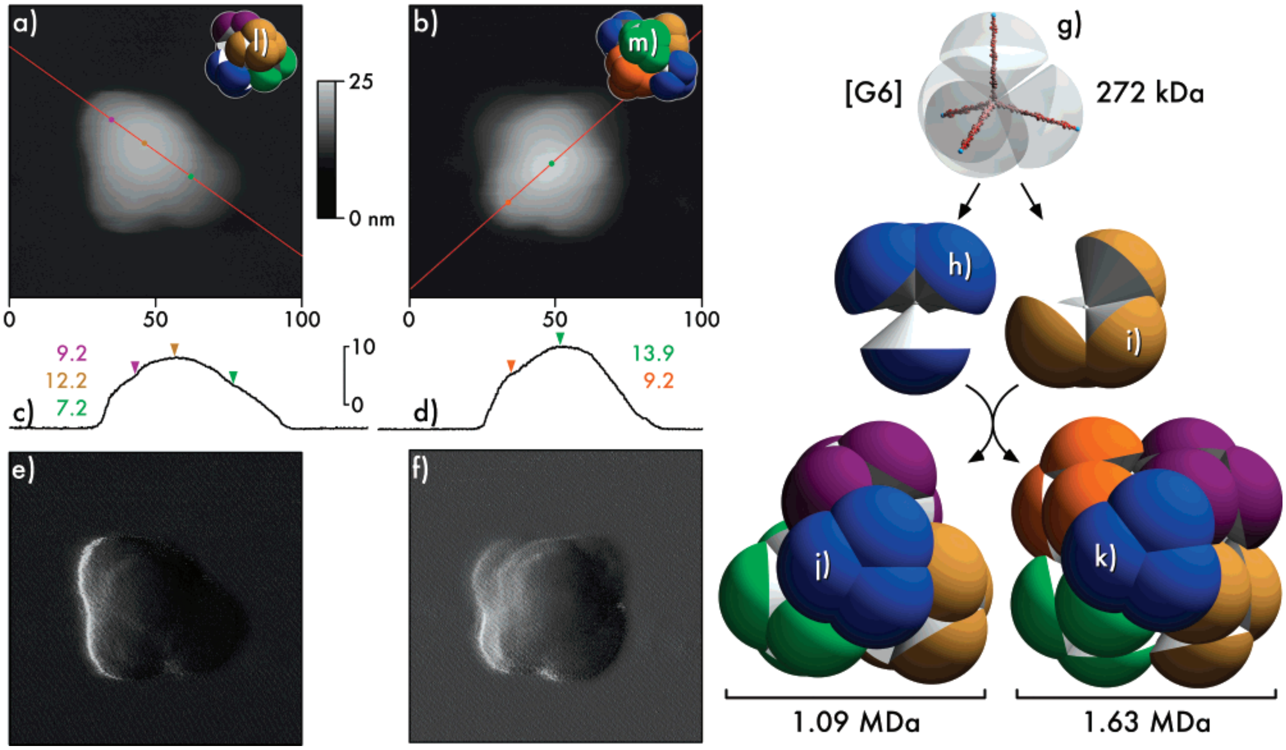

Figure 11. Elucidation of the aggregate geometries and the assembly mechanism. AFM height images of (a) a tetramer and (b) a hexamer of [G6] dendrimers spun-cast from THF on HOPG with (c, d) cross sections, and (e, f) amplitude images, respectively. The proposed mechanism for assembly with (g) the dendrimer is the directional collapse of dendrimers into two conformations, which exposes new solvent-incompatible surfaces (gray): (h) the partially collapsed conformer for the axial component (blue) and (i) the fully collapsed conformer for the equatorial component (beige and other colors). The matched orientation for (j, k) the proposed assembly in the AFM images is depicted for (l) the tetramer and (m) the hexamer.

DLS. Spin-casting resulted in clusters, which were comparable in diameter and with radially oriented dendrons on the surface (Figure 12). The increased amount of collapse of the dendrons along the longest oligo(*p*-phenylene) arms was obvious but complicated determination of exact aggregation numbers by inspection. The axially oriented dendrimer was apparent, however, (Figure 12, bottom right) which provides additional evidence for two different shapes of the dendrimers within the assemblies. The height of the cluster is somewhat taller than that of THF, consistent again with rigidification of the dendrimer from the geometrically oriented intradendron collapse. A valence-like assembly model fits well with the AFM data and further explains why the clusters are predominantly moderate in aggregation number. For anything larger, without room for more equatorial dendrimers, expansion of the cluster would result in a hollow center or nonspecific aggregation.

The distortion from the originally expected spherical shape of the dendrimer when forming clusters is foremost consistent with the DLS results that most of the solubilizing chain ends must be on the surface of the assembly. Otherwise, aggregation would not stop at a few molecules but would be extended in all directions. Full dendrons must be interdigitated at their sides, and due to the steric constraints of the outer generations and chain ends, this is possible only by bending of the longest arms. While the sample preparations are different, the similarity between the results of each experiment probing the assembly

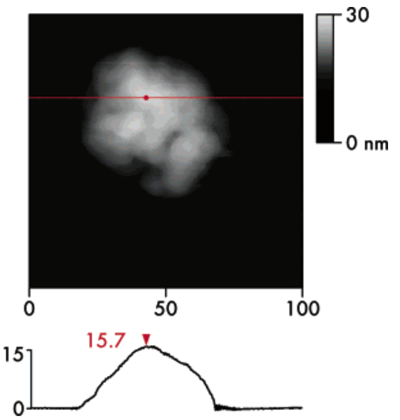

Figure 12. AFM height image of a cluster of [G6] dendrimers spun-cast from 40 mol % hexane in THF on HOPG with cross section. The axially oriented dendrimer in the assembly is observed on the bottom right.

behavior forms a cohesive picture of the assembly mechanism. In contrast to flexible linear polymeric amphiphiles, the overall diameter of each of the assemblies presented here is directly related to the length of the longest polymer arm. The length scale, over which individual polymer conformations are controlled, 14 nm, the length of each dendron in this case, is long even in biology. Some comparison to phase segregating monodendrons,[54,55] which also exhibit small aggregation number phenomena, can also be drawn here, but the distinct difference is that this much larger poly(phenylene) dendrimer amphiphile is globular, not cone shaped. The exact polyphenylene dendrimer aggregation numbers in the solution state were not elucidated but should depend on the shape or aspect ratio of the dendron, which in turn depends on the extent of swelling or collapse as a consequence of stiffness and solubility. The use of an open, but slightly flexible,[45,47−49] structural framework of branches with the propensity to aggregate demonstrates, for the first time, controlled globular self-assembly of dendrimers. The construction of discrete nonspherical objects has hitherto been a challenge.[56] The [G6] clusters are the most stable for the family of dendrimers and may be the best suited for 3D nanofabrication without chemical fixation.

Conclusions

In summary, a divergent dendrimer synthesis based on the Diels−Alder reaction, including the complete double chemical transformations of 128 functionalities on one molecule, was accomplished and has pushed the limits of confident synthetic chemistry. The radial geometry imparts an “openness” to the dendrimer and in turn translates directly into the availability of reactive sites (even those of aggregates). In the absence of side reactions, which would be present in the product, this enables the synthesis of chemically monodisperse dendrimers. The case for chemical perfection was made up to the limits of the reliability of the analytical methods employed, with aggregation during dendrimer synthesis resulting in a few percent of physically, not chemically, crosslinked dimers. Aggregation is generally regarded as a nuisance in polymer chemistry, but harnessing the large number of interactions between polymer chains gives remarkable control at high length scales. The consequence of the specific intramolecular packaging of a radially oriented, dendritic backbone with a flexibility that extends only to the longest oligo(*p*-phenylene) arms is an unprecedented valence-like assembly using only Lilliputian solvophobic interactions between shape-adaptable oligo(phenylene)s. The assembly geometry and surface topology are mediated by the solvent quality due to intradendron swelling or collapse. Furthermore, the stiff backbone was a priori believed to be inside a shell of surface-bound chain ends. The assembly results without requiring the chain ends to be the aggregating component, which leaves them available for hierarchical constructions or for a possible function. The tetramer is clearly a key intermediate and possibly the equilibrium structure in DLS, but determination of the exact aggregation number will require more elaborate experiments. This covalent/noncovalent approach offers a general molecular shaping motif that is completely different than what has been previously accessible with conventional self-assembly and is the first step of a new direction in 3D nanofabrication. There is plenty of room at the bottom and at the top. Future work will include efforts to extend even further the synthesis, to study the aggregation of the smaller generation dendrimers, to selectively suppress aggregation, and to stabilize the assemblies. In addition, the construction of objects with components near the persistence length for the backbone may provide a new mechanism for ultratough materials.

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Supporting Information Available: Procedures for the preparation and full characterization of the [G6] dendrimer, details of the size exclusion chromatography, atomic force microscopy, mass spectrometry, and dynamic light scattering, as well as calculations of hydrodynamic and molecular radii are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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