

## Discrete Molecular-Sized Nanocages Derived from Disintegratable Dendrimer Templates

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The controlled synthesis of discrete hollow nanospheres have attracted tremendous attention because of their potential for diverse applications such as enzyme mimics, nanoreactors, and delivery of target molecules. Typically, such hollow spheres have been fabricated from templates with a core–shell architecture: a polymerizable shell to form a spherical wall and a core serving as the sacrificial template to form the cavity. For example, core–shell structures formed by self-assembly of block copolymers into micelles<sup>1–3</sup> are shell cross-linked (SCK), utilizing the reactive side chains built into the shell composition<sup>1,2</sup> or the inorganic polymerizable precursors sorbed into the shell domain.<sup>3</sup> A cavity is produced upon degradation of the core. Another common method involves forming an organic or inorganic polymer around a degradable template.<sup>4–8</sup> Recently, a new method to construct a nanocapsule without a templating core via thiol-ene photopolymerization of (allyloxy)12cucurbit[6]uril and dithiol was reported.<sup>9</sup>

For many applications, e.g., catalysis, other structural attributes besides a basic hollow sphere are required. A target structure will be a nanocage with molecular-sized cavity and position-specific interior functional groups attached to a thin porous wall that also permits molecular-size selectivity. However, many of the hollow spheres described above lack interior directing functionalities, often have thick walls, and are rather large, with their sizes ranging from at least 50 nm to micrometers. Equally important is the need of a methodology that permits precise control and easy tuning of the cavity size and interior functionalities. Many of the existing

synthesis protocols lack the required precision in structural control or ability to include internal functionalities in the cavity. Recently, we reported the synthesis of a molecular-sized nanocage with a 2 nm cavity and interior amine functional groups, using a micellar template formed with the surfactant (triethoxysilyl)propylcarbamate.<sup>10</sup> This custom-designed surfactant has ethoxy headgroups that can be polymerized to form an atom-layer-thick shell and carbamate bond that can be cleaved to generate an internal amine group and the cavity. Although successful, the method is unsatisfactory because it needs a micelle that is stable during SCK, which imposes severe restrictions on the choice of surfactant molecule and the workable concentration, and limits the range of cavity size and the synthesis yield.

Using disintegratable dendrimers instead of micelles as template would ease these limitations. Dendrimers are highly branched symmetrical macromolecules that can be synthesized with high monodispersity. Because of their modular construct, tuning of their architecture such as size and peripheral group density are easily attained, thus permitting unprecedented control of the cavity size and shell structure and are positioned to meet the needs of diverse applications. In addition, judicious choices of the nature and size of the linkages that built the branching units would permit, after SCK, degradation into small fragments that can diffuse through the porous shell to create a molecular-sized cavity in the nanocage.

Self-immolative dendrons<sup>11</sup> and photolabile dendrimers<sup>12</sup> have been described but have not been used as templates. Cored dendrimer<sup>13,14</sup> with cavities surrounded by a wall of dendrons in addition to the cross-linked shells have also been synthesized. We demonstrate our immolative dendrimer-templating method by the synthesis of ~10 nm diameter carbosilane nanocages with ~4.5 nm diameter cavities using a G4 (fourth-generation) dendrimer **3** that is built with immolative carbamate bonds. Each nanocage possesses 32 OH groups on the interior shell wall, and the shell porosity permits smaller molecules such as allylamine to access the cavity, but excludes larger ones such as tritylamine ((1,1,1-triphenylmethyl)amine).

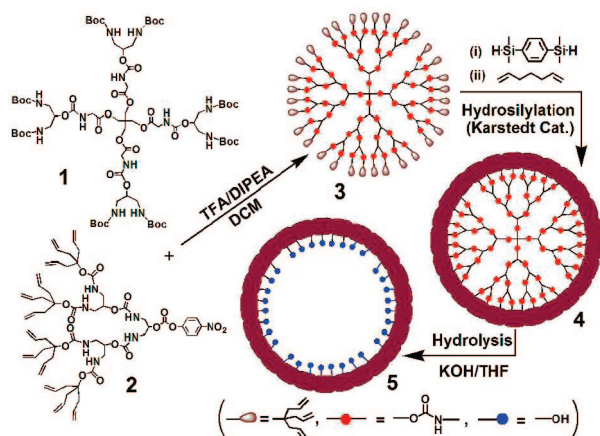
We used the reported method to synthesize **3**<sup>15</sup> except that 1,1,1-triallylmethyl alcohol was used instead of dimethylallylmethyl alcohol to achieve three times the density of allyl groups at the periphery (Scheme 1). Briefly, a tetrahedral G2-core **1** was prepared by condensing an ester, formed from Boc-protected glycine and pentaerythritol, with 1,3-diamino-2-propanol activated with 4-nitrophenyl (PNP) chloroformate. Separately, a G2 dendron **2** was prepared with a convergent

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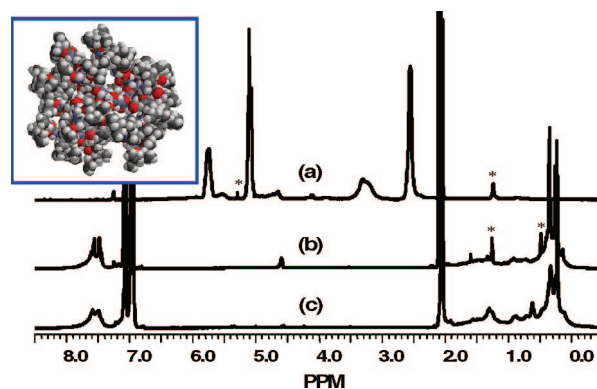
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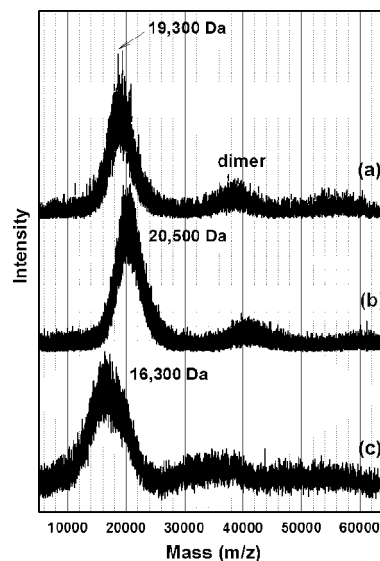
**Scheme 1. Immolative Dendrimer-Templated Synthesis of Nanocage with a Carbosilane Shell (not to scale)**


method, starting with 1,1,1-triallylmethyl alcohol and using 1,3-diamino-2-propanol for branching. **2** was then coupled to **1** to yield gram quantities of **3** with greater than 60% purified yield. The chemical structure of **3** was confirmed by  $^1\text{H}$  NMR spectroscopy (Figure 1a),<sup>16</sup> and MALDI-TOF MS spectrum showed a mass of 9325 Da (Figure S1 in the Supporting Information). Its structure, as derived from semiempirical equilibrium geometry calculation (AM1), is globular, roughly spherical in shape (see inset, Figure 1), and  $\sim 4.5$  nm in diameter, which is consistent with DLS measurement (Figure S2 in the Supporting Information). It possesses 96 peripheral allyl groups.

Because the carbamate bonds in **3** would be completely degraded after SCK to form the cavity, productive cross-linking of allyl across head groups and across dendrons is necessary to form an intact shell. This requirement was similar to those in the synthesis of porphyrin-imprinted dendrimer,<sup>17</sup> except that our aim of removing the whole dendrimer instead of just the core imposed a more stringent requirement for productive cross-linking. To maximize interheadgroup linkages, we chose 1,4-bis(dimethylsilyl)benzene (**K1**) as the linking agent, whose size and rigidity would reduce the probability of nonproductive cyclization of C=C of the headgroups, to react with the peripheral C=C groups by Karstedt's catalyst promoted hydrosilylation. From the relative  $^1\text{H}$  NMR peak areas of  $-\text{C}_6\text{H}_4-$  (7.4–7.6 ppm) to Si-H (4.6 ppm), or  $-\text{CH}_2-$  (0.5–2.0 ppm) to Si- $\text{CH}_3$  (0.2–0.5 ppm), it was estimated that 70–80% of the reacted **K1** formed dipodal linkages with the dendrimer, and 20–30% formed monopodal linkages. Practically all C=C bonds reacted with **K1** in one of these forms, as evident by the disappearance of allyl C-H peaks (5–6 ppm) (Figure 1b). **K1** attached to the shell in the monopodal form are further cross-linked with 1,6-heptadiene (**K2**). **K2** was also used to cap isolated **K1** to prevent undesirable hydrolysis of Si-H to silanol in strong base and subsequent oligomerization of the nanocages, which resulted in heptylsilylphenyl groups radiating from the nanocage. Si-H groups (4.6 ppm) associated with **K1** attached in the monopodal form disap-



**Figure 1.**  $^1\text{H}$  NMR spectra of: (a) **3** (in  $\text{CDCl}_3$ ), (b) after **K1** reaction with **3**, and (c) after additional **K2** reaction to form **4** (in  $\text{Tol}-D_8$ ). \*Solvent peaks. Inset is the 3D structure of dendrimer **3**.



**Figure 2.** MALDI-TOF mass spectra of (a) structure formed after **K1** reaction with **3**, (b) after additional **K2** reaction to form **4**, and (c) nanocage **5** (the mass in the figure were taken at the center of half-height in intensity).

peared after the addition of **K2** (Figure 1c). Using self-terminating hydrosilylation instead of uncontrolled polymerization reactions for SCK ensures the formation of thin, near-atomic-layer-thick shells. Furthermore, slow addition of excess of **K1** and **K2** at  $80^\circ\text{C}$  to a dilute dendrimer solution ( $<1 \times 10^{-4}$  M) helps discriminate against the intermolecular hydrosilylation that forms dendrimer oligomers.

The formation of **4** from **3** by SCK increased the mass by about 10 000 Da after reaction with **K1**, and another 1200 Da after **K2** addition (Figure 2). The mass spectrum of **4** showed a broad distribution (about 5000 Da at half-maximum) due to the statistical nature of the hydrosilylation reaction with **K1**. The mass increase is reasonably close to the distribution of dipodal and monopodal linkages measured by NMR, which would be 11 664 Da for a distribution of 75 and 25%, respectively.

To generate the nanocage **5** from **4**, we cleaved the internal carbamate bonds by hydrolysis using excess aq. KOH in THF at  $50^\circ\text{C}$  followed by purification with dialysis. Bond cleavage was monitored using FT-IR and MALDI-TOF. The FT-IR spectrum of **4** shows intense bands at  $1700\text{ cm}^{-1}$

(16) The major peak assignment of  $^1\text{H}$  NMR:  $\delta$  5.77 ( $-\text{CH}=\text{CH}_2$ ), 5.11 ( $-\text{CH}=\text{CH}_2$ ), 3.31 ( $-\text{CH}_2\text{NH}-$ ), 2.56 ( $-\text{CH}_2\text{CH}=\text{CH}_2$ ).

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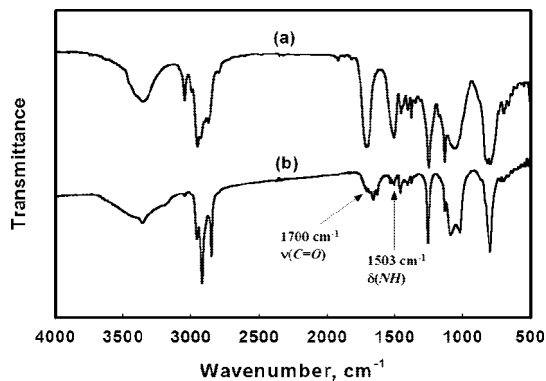


Figure 3. FT-IR spectra of (a) SCK dendrimer **4** and (b) nanocage **5**.

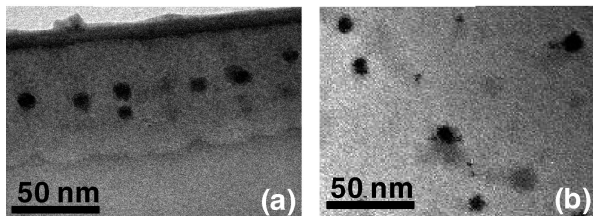
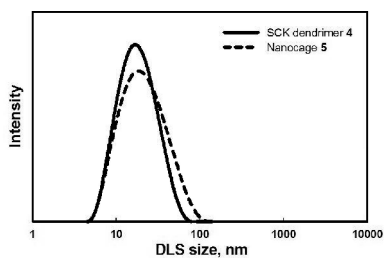


Figure 4. Hydrodynamic size distribution determined by DLS (top), and HRTEM pictures of (a) **4** and (b) **5** stained with  $\text{OsO}_4$ .

( $\delta_{\text{C=O}}$ ) and  $1503\text{ cm}^{-1}$  ( $\delta_{\text{NH}}$ ) (Figure 3a). After 24 h of hydrolysis, the intensities of these bands decreased by  $>80\%$  and the mass decreased by about 4200 Da (Figure 2c), which is about 94% of the theoretical maximum mass loss of 4480 Da. It should be noted that other peaks in the FT-IR spectra characteristic of the carbosilane shell, such as the  $\text{CH}_3$  deformation in  $\text{Si}-\text{CH}_3$  at  $1250\text{ cm}^{-1}$  (s), and the  $\text{Si}-\text{C}$  stretch,  $\text{Si}-\text{CH}_3$  rocking, and  $\text{CH}$  deformation (for *p*-substituted benzene) at  $750\text{--}870\text{ cm}^{-1}$  (s) did not change after hydrolysis, suggesting that the shell is stable. NMR evidence is also in agreement with this assessment. The single peak at  $-3.01\text{ ppm}$  in  $^{29}\text{Si}$  NMR for **4** due to the single form of Si in the SCK agent remained the same after hydrolysis to form **5** (Figure S3 in the Supporting Information), as well as the  $^1\text{H}$  NMR peaks (Figure S4 in the Supporting Information) at  $7.4\text{--}7.6\text{ ppm}$  ( $-\text{C}_6\text{H}_4-$ ),  $0.5\text{--}2.0\text{ ppm}$  ( $-\text{CH}_2-$ ), and  $0.2\text{--}0.5\text{ ppm}$  ( $\text{Si}-\text{CH}_3$ ).

TEM and DLS were conducted to determine the nanocage morphology. The TEM images (Figure 4) showed that the size of **4** was  $\sim 10\text{ nm}$  in diameter, consistent with a 4.5 nm dendrimer wall and a  $\sim 2\text{ nm}$  thick shell of a dimethylbenzyl layer and a heptylsilylphenyl layer. After hydrolysis, the size of the majority of the particles **5** remained the same as before hydrolysis, supporting the fact that the nanocages with carbosilane shell was successfully prepared by templating with the degradable carbamate dendrimers. The DLS hydrodynamic size distributions of both **4** and **5** showed a single

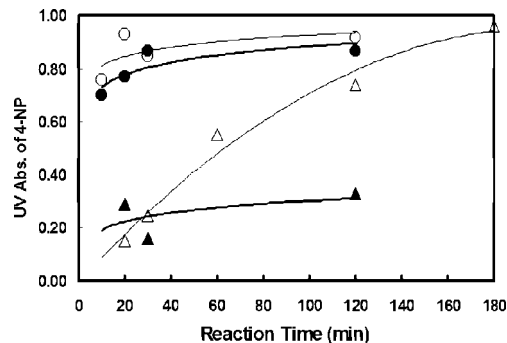


Figure 5. Formation of 4-nitrophenol from the reaction of allylamine (circles) or tritylamine (triangles) with PNP-activated interior OH groups in nanocages (filled points) and with a control PNP carbonate in solution (empty points), showing size-selectivity for the nanocage shell.

size distribution centered at about 15 nm (Figure 4), indicating that most of the particles existed as single discrete units. That this is larger than the expected size ( $\sim 10\text{ nm}$  at the dried state from TEM) may be due to the flexible nature of the shell and the stretched-out heptylsilylphenyl groups on them in solution that slow the nanocage movement.

To establish the presence of OH groups on the interior shell wall of the nanocage, we first activated them with PNP chloroformate to form PNP carbonate, and then their reaction with amines to form 4-nitrophenol was monitored with UV-vis (Scheme S5 in the Supporting Information). Interior PNP carbonate groups could react only with smaller amines that can diffuse through the shell wall, not larger ones. The activated OH groups of the nanocage reacted with allylamine ( $\sim 3.1 \times 5.3\text{ \AA}^2$ ) with an initial rate of  $0.048 \pm 0.01\text{ mol (min mol carbonate)}^{-1}$ , practically as rapidly as in the control with a PNP carbonate in solution (rate of  $0.053 \pm 0.01$ ), attesting to the high porosity of the shell (Figure 5). However, for the bulkier tritylamine ( $\sim 8.7 \times 9.5\text{ \AA}$ ), the reaction was 15 times slower for the carbonate in the nanocage ( $0.0004 \pm 0.0001$ ) than for the free carbonate ( $0.006 \pm 0.001$ ), demonstrating size selectivity. These results illustrate that the majority of the OH groups reside in the cage interior, are amenable to modifications, and readily accessible by smaller molecules.

Thus, we have succeeded in synthesizing a molecular-sized, internally functionalized, size-selective, and stable nanocage made with a carbosilane shell of a few atom layers thick. Very importantly, the synthesis technique permits variations in the uniform cavity size, and the density and type of internal functional groups, thus offering the possibility to tailor-make nanocages for applications in different environments, including *en vivo* applications.

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**Supporting Information Available:** Details for dendrimer and nanocage syntheses and characterization (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.