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Acyclic Cucurbit[*n*]uril Dendrimers

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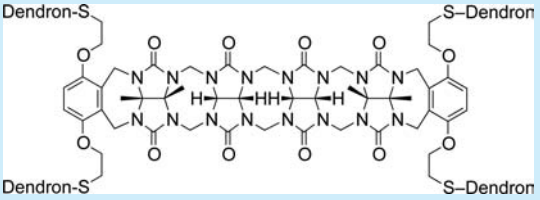
Supporting Information

ABSTRACT: The synthesis of acyclic cucurbit[*n*]uril dendrimers **G1**−**G3** that bear four dendrons on their aromatic sidewalls via thiolate S_N2 chemistry is reported. **G1**−**G3** are polycationic and can bind to pEGFP plasmid DNA as shown by dynamic light scattering (DLS), gel electrophoresis, and scanning electron microscopy (SEM). The gene delivery ability of **G1**−**G3** is presented.



The cucurbit[*n*]uril family of molecular containers (CB[*n*], *n* = 5, 6, 7, 8, 10, 14; Figure 1) features a hydrophobic

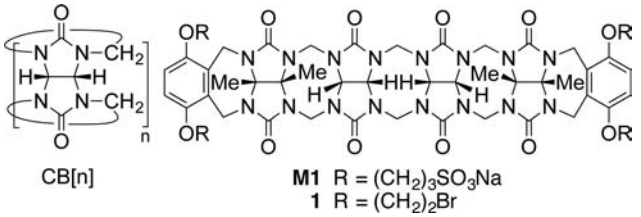


Figure 1. Chemical structures of CB[*n*] (*n* = 5, 6, 7, 8, 10, 14) molecular containers and acyclic CB[*n*]-type molecular containers **M1** and **1**.

cavity that is shaped by *n* glycoluril rings and guarded by two symmetry equivalent ureidyl C═O portals.[1] CB[*n*] compounds have attracted substantial interest from the supramolecular community in the past 15 years[2] because of the ready availability of a homologous series of hosts that display high affinity and high selectivity toward hydrophobic cations and even neutral species in water.[3] Even more importantly, CB[*n*] host−guest chemistry is highly stimuli responsive in that photochemistry, electrochemistry, pH changes, and chemical stimuli can be used to dictate changes in CB[*n*] host−guest constitutions.[4] Accordingly, CB[*n*] compounds have been used to construct a variety of functional supramolecular systems, including molecular machines, chemical sensors, drug delivery systems, and materials for gas sorption and purification.[4] Of high relevance to the work reported in this paper are previous reports of the use of CB[*n*] compounds to complex to the focal point of dendrons and the periphery of dendrimers in a noncovalent fashion. For example, Kim's group constructed ternary complexes between poly(propyleneimine) (PPI) dendrimers, CB[6], and DNA and explored gene delivery efficiency.[5] In contrast, Kaifer's group used CB[*n*] host−guest chemistry to bind the focal points of dendritic wedges and electrochemically trigger dendrimer assembly.[6] More recently, CB[7] noncovalent complexes of poly(amidoamine) (PAMAM) dendrimers formed the basis for a light harvesting system and a system capable of controlled release under redox or supramolecular control.[7] Despite significant interest in the noncovalent CB[*n*] dendrimer chemistry, there have been no examples to date of CB[*n*] compounds serving as a covalent core for dendrimers. The main reason for the absence of covalent CB[*n*] dendrimer chemistry is that controlled functionalization of CB[*n*] remains challenging with synthetic schemes for the preparation of monofunctionalized CB[*n*] derivatives appearing only in the past few years.[8] Dendrimers have broadly impacted science, especially the fields of drug and gene delivery.[9] Polycationic dendrimers bind DNA efficiently by multivalent electrostatical interactions and deliver it inside cells. Accordingly, we sought to prepare covalent CB[*n*] dendrimers bearing cationic arms for transfection studies.

Recently, the groups of Isaacs[10] and Sindelar[11] have been studying the acyclic CB[*n*]-type receptors that consist of a central glycoluril oligomer capped with two aromatic walls as typified by **M1** (Figure 1). Acyclic CB[*n*]-type receptors retain the essential molecular recognition features of the CB[*n*] family of molecular containers. For example, **M1** and its derivatives have been shown to function as components of sensor arrays, as receptors for carbon nanotubes, as solubilizing agents for insoluble drugs, and as an in vivo reversal agent for the neuromuscular blocking agent rocuronium.[10] Because acyclic CB[*n*] compounds are synthesized by a building block methodology, they are amenable to a more straightforward synthetic modification which allows diversification. In this paper we report the preparation of three acyclic CB[*n*] cored dendrimers (**G1**−**G3**) with peripheral ammonium functionality, their self-assembly properties with DNA, and investigation of their gene delivery ability. We envisioned that acyclic CB[*n*] compounds would enhance transfection efficiency because their

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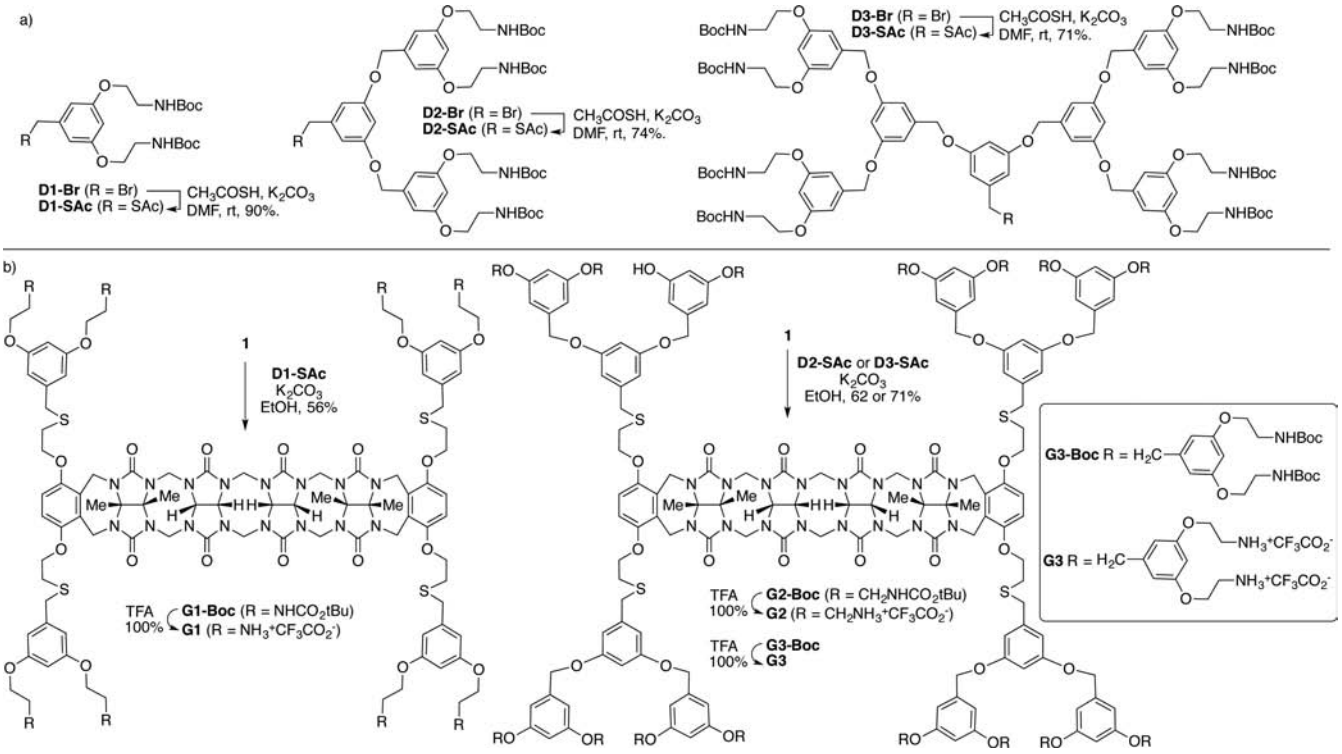
Scheme 1. Synthesis of (a) Dendrons D1-SAc–D3-SAc and (b) First, Second, and Third Generation Dendrimers G1–G3



C=O portals should promote intermolecular aggregation by ion–dipole interactions with the terminal NH_3^+ groups.

For the preparation of a series of acyclic CB[*n*] dendrimers, we decided to use a postfunctionalization strategy to graft Frechet-type dendrons[12] onto a central acyclic CB[*n*]-type receptor. As a starting material we selected the known acyclic CB[*n*]-type container **1** which contains four electrophilic primary alkyl bromide arms.[10f] Scheme 1 shows the structures of dendrons **D1-Br**, **D2-Br**, and **D3-Br** which were prepared according to the literature procedures.[13] To transform **D1-Br**, **D2-Br**, and **D3-Br** from their electrophilic alkyl bromide forms into nucleophilic forms that would react with acyclic CB[*n*] container **1**, we allowed them to react with thiolacetic acid under basic conditions to yield **D1-SAc**, **D2-SAc**, and **D3-SAc** in good yield (Scheme 1). The attachment of the dendrons to the core scaffold **1** was accomplished by the *in situ* deprotection of **D1-SAc–D3-SAc** using K_2CO_3 in EtOH to yield the thiolates which undergo efficient S_N2 reaction with **1** to yield the corresponding first–third generation dendrimers **G1-Boc–G3-Boc** in good yields (56–71%) after purification by precipitation. Deprotection of **G1-Boc–G3-Boc** into **G1–G3** was accomplished in quantitative yield by treatment with CF_3CO_2H at room temperature. Compounds **G1–G3** were characterized by ^1H and ^{13}C NMR spectroscopy and by electrospray mass spectrometry; the data were fully consistent with the depicted structures (Supporting Information). For example, the ^1H NMR spectra recorded for **G1–G3** at 70 °C in DMSO (Figure 2) show the expected number of aromatic C–H resonances (**G1**: 3; **G2**: 5; **G3**: 7) in the 6–7 ppm region and the expected 5 resonances for the glycoluril CH_2 and CH groups in the 5.3–5.7 ppm region of the spectrum. ^1H NMR spectra recorded at lower temperatures in DMSO or in water are broadened which is indicative of aggregation phenomena. Similarly, the ^{13}C NMR spectra recorded for **G1-Boc–G3-Boc** in DMSO display the expected 3 C=O resonances along with

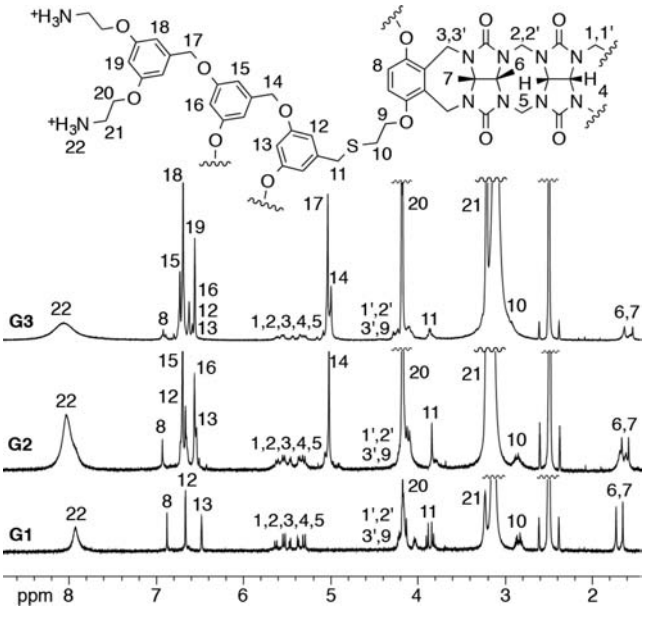


Figure 2. ^1H NMR spectra recorded (600 MHz, DMSO-d_6, 70 °C) for the **G1**, **G2**, and **G3** dendrimers.

aromatic resonances (**G1-Boc**: 7 expected, 7 found; **G2-Boc**: 11 expected, 11 found; **G3-Boc**: 15 expected, 14 found). The high resolution electrospray mass spectra recorded for **G1–G3** show multiply charged ions (+2 to +7) that match the calculated values derived from their free base molecular formulas.

To further investigate the aggregation of **G1–G3** in water which was inferred based on the NMR experiments described above, we initially performed DLS measurements. DLS measurements of aqueous solutions of **G1–G3** show size

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distributions with maxima at 118, 131, and 100 nm, respectively which correspond to aggregated forms of the dendrimers (Supporting Information). Similarly, SEM of deposited aqueous solutions of **G1**−**G3** showed particles in the 20 nm−1 μm range (Supporting Information). We posit that the amphiphilic nature of polycationic **G1**−**G3** with their large aromatic surfaces along with their ureidyl C═O portals which can bind to ammonium ions by ion−dipole interactions promotes the aggregation process.

Next, we sought to determine the ability of the polycationic **G1**−**G3** to interact with plasmid DNA (pEGFP). Figure 3a−c

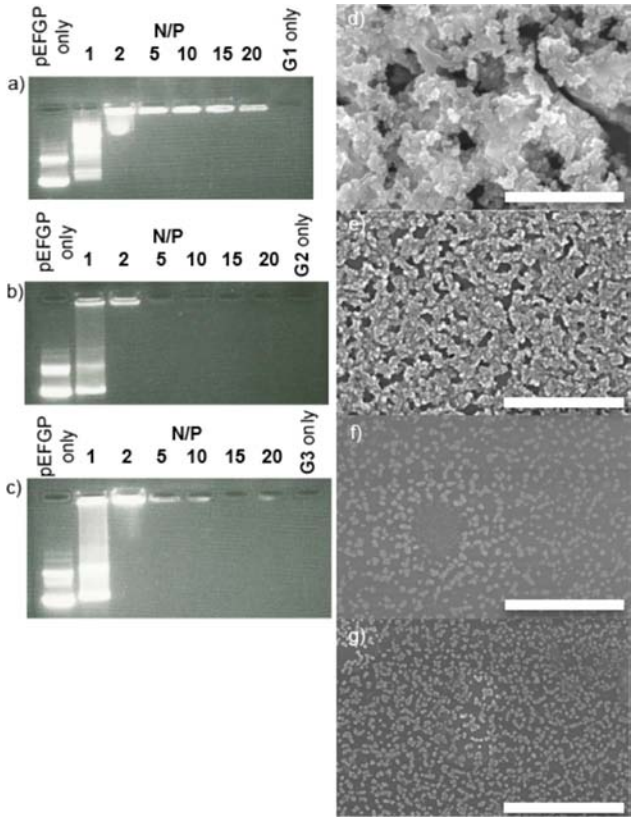

Figure 3. Agarose gel electrophoresis images of pEFGP (0.8 μg/well) and EtBr (0.5 μg mL^{-1}) in the presence of (a) **G1**, (b) **G2**, and (c) **G3** at different N/P ratios. SEM images of (d) pEGFP alone (3 μg/mL), (e) pEGFP and **G1**, (f) pEGFP and **G2**, and (g) pEGFP and **G3** at N/P 20. Scale bar: 5 μm.

show agarose gel electrophoresis images of pEGFP in the presence of ethidium bromide (EtBr) and an increasing concentration of **G1**, **G2**, or **G3** defined by the N/P ratio (number of amine/number of phosphate). For **G1**, **G2**, and **G3** free DNA remains visible at the electrically neutral ratio (N/P = 1); at higher N/P ratios (N/P ≥ 2) the bands migrate more slowly indicative of condensation of the plasmid. For **G1**, the fluorescence produced by the intercalation of EtBr into the DNA base pairs persists even at higher N/P ratios whereas for **G2** and **G3** the fluorescence becomes diminished (Figure 3a−c). This result suggests that the condensation of pEGFP is more efficient with **G2** and **G3** than with **G1** and that displacement of EtBr occurs readily for **G2** and **G3**. Our interpretation regarding the pEGFP condensing ability of **G1**−**G3** is supported by SEM measurements (Figure 3d−g). At N/P = 20, the plasmid−**G1** complexes (Figure 3e) are structurally similar to that of pEGFP alone (Figure 3d). In sharp contrast, however, for the **G2** and **G3** plasmid DNA complexes, individual particles with diameters in the 20 to 100 nm range are observed (Figure 3f,g). Further support for the condensation of the pEGFP in solution in the presence of **G1**−**G3** was obtained by DLS measurements. At N/P = 20, the DLS measurement of particles formed by **Gn**−plasmid DNA complexes show size distributions (75, 66, 81 nm) that are significantly smaller than aggregates formed by dendrimers alone (118, 131, 100 nM) or pEGFP alone (49 and 471 nm) (Supporting Information). Peaks are also observed at ∼2 nm in the DLS for **G2** and **G3** alone that are at the detection limit of the instrument which may correspond to small amounts of the unaggregated dendrimer. Finally, we measured the zeta potential of the **Gn**−plasmid DNA polyplexes (**G1**: +38 mV; **G2**: +46 mV; **G3**: +49 mV) which confirmed that the Gn−plasmid DNA polyplexes are strongly positively charged (Supporting Information) and provides an explanation of the condensation that occurs upon polyplex formation.

We were encouraged by the ability of **G1**−**G3** to condense pEGFP DNA and therefore proceeded to perform transfection experiments with HeLa cells. Experimentally, we treated HeLa cells (50,000 cells/well) with the **Gn**−plasmid DNA polyplexes for 24 h followed by cell lysis and measurement of EGFP fluorescence. Figure 4 shows a plot of fluorescence intensity as

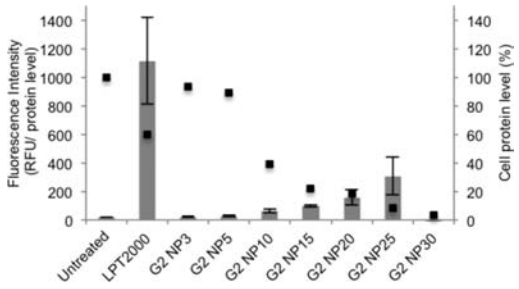

Figure 4. Gene delivery experiments of pEGFP on HeLa cells. Fluorescence intensity (bars) and percentage of total cellular proteins (squares) are given for various N/P ratios with **G2** (polyplexes prepared in 5% glucose solutions) and for negative (untreated HeLa cells) and positive (Lipofectamine 2000) controls. Means and standard deviation of triplicate measurements are given.

a function of N/P ratio for **G2** in comparison to untreated cells and Lipofectamine 2000 (Life Technologies) as a positive control (**G1** and **G3**, Supporting Information). Even at high N/P ratio (N/P = 20) only very modest increases in fluorescence intensity and therefore transfection were observed. Figure 4 also shows the cellular protein levels as measured using a commercial BCA protein assay kit (Pierce). At N/P > 5, the total amount of cellular protein decreases significantly which indicates that **G2** is cytotoxic at these concentrations. Related observations were made during the transfection experiments performed with **G1** and **G3** (Supporting Information). Dendrimers with similar arms and C_{60} or pillararene cores displayed better transfection efficiency and lower cytotoxicity which suggests the acyclic CB[*n*] core may require modification.[13,14] In addition to the observed cellular toxicity of **G1**−**G3**, additional barriers will need to be surmounted to improve the gene delivery ability of acyclic CB[*n*] dendrimers including cell culture medium stability, cell membrane transport, and endosomal release.[15]

In summary, we have prepared acyclic CB[*n*] dendrimers **G1**−**G3** which are the first examples of covalent dendrimers

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incorporating cucurbiturils. Dendrimers **G1**−**G3** are shown to aggregate in water and cause condensation of pEGFP as shown by DLS, SEM, and gel electrophoresis. Very modest enhancements of gene delivery in HeLa cells were observed for **G2**-pEGFP at high N/P ratios, although this was accompanied by increased cytotoxicity. This paper further demonstrates the synthetic versatility of the acyclic CB[*n*] scaffold and stimulates the design of complex acyclic CB[*n*] compounds for diverse applications including transfection and drug delivery.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03145.

Experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra for new compounds; DLS data, SEM data, and transfection protocol (PDF)

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Notes

The authors declare no competing financial interest.

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