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Tailoring *β*-Cyclodextrin for DNA Complexation and Delivery by Homogeneous Functionalization at the Secondary Face

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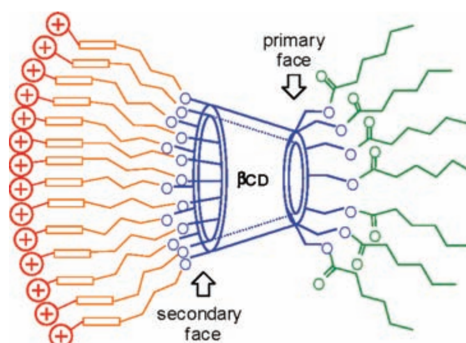
ABSTRACT



An efficient general strategy for the incorporation of functional elements onto the secondary hydroxyl rim of *β*-cyclodextrin has been developed and applied to the synthesis of a novel series of C_7-symmetric homogeneous macromolecular polycations with improved DNA complexing and delivery properties.

Controlled attachment of functional elements onto the bucket-shaped structure of cyclodextrins (cyclomaltooligosaccharides, CDs) allows engineering nanometric constructs capable of performing specific tasks far beyond the distinctive formation of inclusion complexes with hydrophobic molecules.[1] Thus, the development of efficient methods to manipulate the CD topology and recognition features has been translated into applications in fields such as site-specific drug delivery,[2] artificial enzymes,[3] catalysis,[4] molecular

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machines,[5] or supramolecular sensing,[6] among others. The intrinsic limitations associated with the dimensions of their internal cavity can be further overcome by inserting programmed CD building blocks into macromolecular architectures through covalent or supramolecular ligation, thereby widening the range of potential guest partners. Notably, chemically modified CDs have been incorporated into polycationic polymer[7] and polyrotaxane systems[8] that can effectively complex and deliver plasmid DNA (pDNA) with exceptional biocompatibility and efficacy. Unfortunately, these materials are polydisperse in nature, which represents a serious drawback for structure−activity studies and, ultimately, approval for therapeutic applications.

The promising properties of CDs as gene transfection enhancers have stimulated the design of a new generation of artificial DNA delivery vehicles with discrete, monodisperse, and symmetric geometries.[9] The general strategy is inspired in previous work on the synthesis of CD-centered glycoclusters[10] and takes advantage of the easy preparation of multivalent CD scaffolds selectively and homogeneously functionalized at the primary hydroxyl rim (Figure 1a). Highly dense polycationic clusters with a well-defined spatial orientation (e.g., **1** and **2**) have been built up following this concept and were shown to condense pDNA into stable

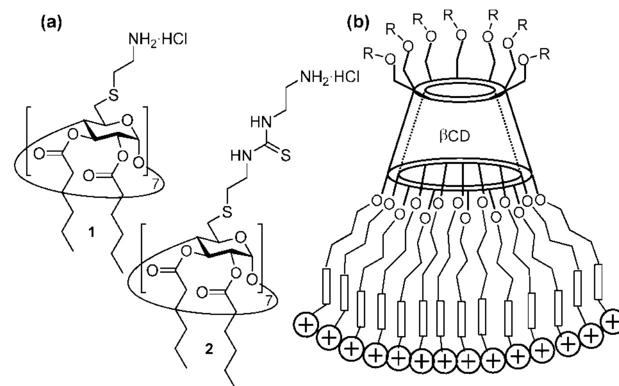

Figure 1. Examples of primary face polycationic βCD derivatives (a) and schematic representation of secondary face βCD-scaffolded polycationic clusters (b). The R substituents represent H or acyl chains. The rectangular boxes account for additional H-bond donor groups.

nanoparticles (CDplexes).[11] As a general rule, the resulting transfection efficiencies improved with an increase in the number of cationic groups over the initial 6, 7, or 8 primary centers in the commercially available α, β, or γCD, respectively. In principle, reversing the facial polarity, by anchoring the charged functionalities at secondary positions, would provide compounds with double charge density, thereby favoring interactions with the polyanionic nucleic acid chain. However, the difficulties associated with secondary hydroxyl group reactivity, steric hindrance, and positional isomerism make homogeneous functionalization at this face a far more complicated challenge. Here we report the efficient synthesis of a "reverse" βCD core molecule, per-[(*O*-2,*O*-3)-3-methanesulfonyloxypropyl]-per-(*O*-6)-[*tert*-butyldimethylsilyl]-β-cyclodextrin (**5**, Scheme 1), and its transformation into a series of 7-fold symmetrical clusters bearing 14 primary amino groups at the wider secondary rim. The design motif allows further chemical tailoring to refine the pDNA complexing and delivery properties by (a) esterification of the primary hydroxyls with fatty acids to facilitate membrane crossing and (b) insertion of hydrogen-bonding segments for reversible, biomimetic complexation of polyphosphates (Figure 1b).

The pivotal intermediate **5** was synthesized from the known per-[(*O*-2,*O*-3)-allyl]-per-(*O*-6)-[*tert*-butyldimethylsilyl]-β-cyclodextrin derivative **3**[12] by a two-step transformation involving hydroboration−oxidation (→**4**) and subsequent methanesulfonylation. Nucleophilic displacement of the mesylate groups in **5** by azide anion or *N*-Boc-protected cysteamine afforded the tetradecaazide **6** or the aminothio-

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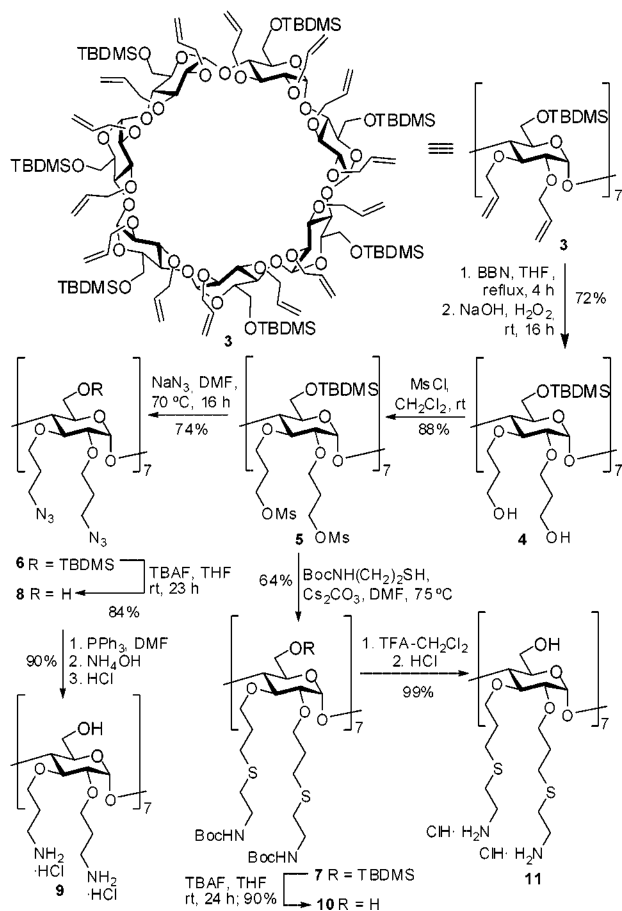
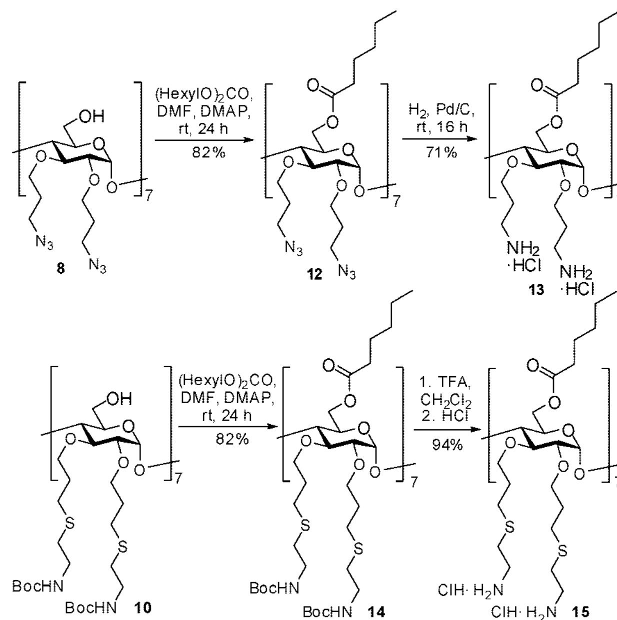


Scheme 1. Synthesis of Homogeneous Polyaminoclusters at the Secondary Rim of β-Cyclodextrin



Scheme 2. Synthesis of Amphiphilic βCD Polyaminocluster

ether **7**. Fluorolysis of the silyl ethers (→**8** and **10**) followed by Staudinger reduction of the azido groups with triphenylphosphine or acid-promoted hydrolysis of the carbamates yielded the target tetradecaamines, which were isolated as the corresponding perhydrochlorides **9** and **11**, respectively (Scheme 1). Amphiphilic versions[13] of **9** and **11** were next synthesized by hexanoylation of **8** and **10** (→**12** and **14**) followed by azide reduction or carbamate hydrolysis steps, to give the tetradecacationic heptahexanoates **13** and **15**, respectively (Scheme 2). Preliminary experiments to assess the DNA complexing capabilities by agarose gel electrophoresis (see Supporting Information) indicated that neither **9** nor **11** was able to stably condense and fully protect the plasmid from the environment.[14] The per-[(*O*-2,*O*-3)-3-aminopropyl]-per-[(*O*-6)-hexanoyl] derivative **13** likewise failed to efficiently complex and compact DNA at N/P[15] ratios <30, giving rise to rather large aggregates (>500 nm). In contrast, the cysteaminylpropyl homologue **15** formed stable nanoparticles with positive surface charge at N/P ratios ≥6 (62 ± 5 nm and ζ-potential[16] 28 ± 8 mV for N/P 6 to 10), underlining the strong influence of the spacer length in the interaction of polyaminoCDs with polyphosphates. Unexpectedly, the CDplexes thus obtained did not led in vitro to significant cell transfection.[17] These results contrast strongly with the powerful gene transfection properties observed for CDplexes obtained from amphiphilic βCD analogues bearing the positive charges at the primary face (e.g., **1** and **2**, Figure 1a).[11c]

The lack of cell transfection capabilities for the **15**:DNA CDplexes probably arises from inappropriate hydrophilic−hydrophobic balance of the βCD carrier. To test the suitability of both the molecular design and the synthetic strategy to tune the gene complexing and delivery potential, two alternative structural modifications were considered, namely: (i) increasing the length of the fatty acyl chains at the primary face of the βCD core (i.e., **17**) and (ii) inserting an alkylthioureido segment in the spacer arm at the secondary rim (i.e., **19**; Scheme 3).

Compound **17** was readily accessible from **10** through a reaction sequence similar to that previously discussed for the heptahexanoyl analogue **13**, using myristoyl anhydride as the acylating reagent (→**16**). For the preparation of **19**, the polythiourea **16** was first assembled by the coupling reaction of tetradecaamine **15** with 2-(*N*-*tert*-butoxycarbonyl)ethyl isothiocyanate. Final acid-catalyzed hydrolysis of

(13) For a recent review on amphiphilic cyclodextrin derivatives, see: Sallas, F.; Darcy, R. *Eur. J. Org. Chem.* **2008**, 957.

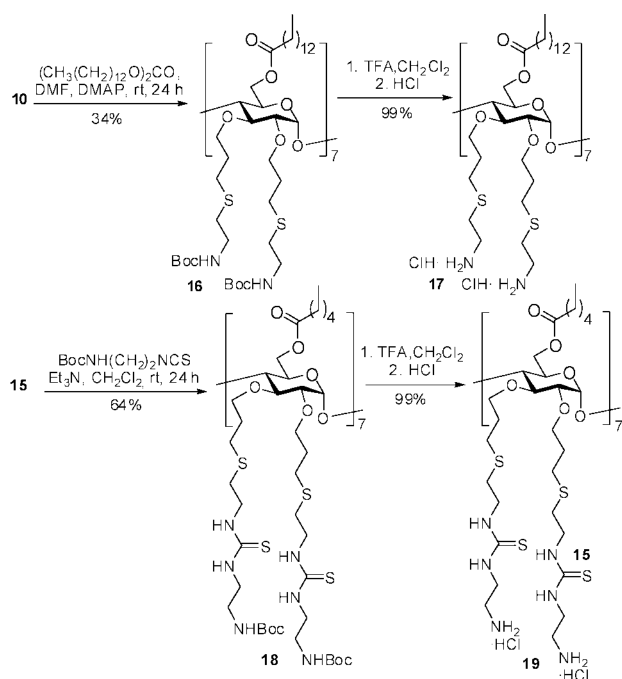
(14) The ability to protect pDNA was assessed by evaluating the accessibility to the intercalating agent ethidium bromide. See Supporting Information for experimental details.

(15) N and P represent the number of amine and phosphate equivalents of the polycationic cyclodextrins and pDNA, respectively.

(16) The electrokinetic or ζ-potential of a colloidal particle is defined as the potential at the plane where slip with respect to bulk solution is postulated to occur. See: Delgado, A. V.; González-Caballero, F.; Hunter, R. J.; Koopal, L. K.; Lyklema, J. *Pure Appl. Chem.* **2005**, *77*, 1753.

(17) The transfection efficiency of the self-assembled polycationic βCD−DNA complexes was assessed using the luciferase-encoding reporter gene as marker in BNL-CL2 murine embryonic cells. See Supporting Information for experimental details.

Scheme 3. Structural Modifications in the Per-[(*O*-2, *O*-3)-Cysteaminylpropyl] βCD Architecture



the carbamate groups afforded the target polyaminothiourea **19** in quantitative yield.

Noticeably, CDs **17** and **19** condensed pDNA into monodisperse populations of very small-sized cationic CDplexes (47 ± 15 nm, ζ-potential 39 ± 1 mV and 40 ± 3 nm, ζ-potential 44 ± 1 mV, respectively, at N/P 10). Figure 2a shows the luciferase expression mediated by **17** and **19** at N/P ratios 6 and 10 in comparison with naked pDNA and linear polyethyleneimine (Jet-PEI, N/P 10), one of the most efficient commercial gene delivery systems,[18] as negative and positive controls, respectively. Data for the previously reported CDplexes from derivative **2**,[11c] with the positive charges at the primary face, are also included for comparison. Remarkably, both compounds were similarly as efficient as the positive Jet-PEI control and compound **2**. The amazing increase in transfection efficiency for **17** and **19** as compared with **13** and **15** underlines the critical effect of incrementing hydrophobicity of the polycationic βCDs on the capability to deliver pDNA into a cell. The results also confirm the

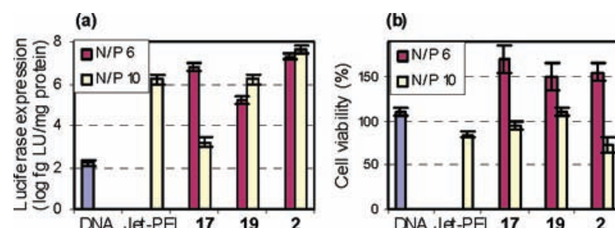


Figure 2. In vitro gene transfection efficiency (a) and cell viability (b) of the CDplexes from **17** and **19** in BNL-CL2 cells in comparison with Jet-PEI-based polyplexes, naked DNA, and data for compound **2**. Data represent mean standard deviation (*n* = 3). See Supporting Information for experimental details.

active role of the thiourea groups in promoting gene transfection. Moreover, the cell viability profiles, measured via protein content, demonstrated that the CDplexes from **17** or **19** have minimal or no cytotoxic effect on cultured cells (viability at or above 95%),[19] being superior in this respect to JetPEI (Figure 2b).

In summary, we have implemented an efficient, reliable, and highly versatile approach for the synthesis of secondary face CD-centered polyaminoclusters suitable for diversity-oriented strategies, structure−activity studies, and molecular tailoring. The above promising results show that the new discrete monodisperse macrocycles have high biocompatibility and efficacy in vitro and warrant further investigations as therapeutic DNA carriers.[20]

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Supporting Information Available: Experimental details and copies of the NMR spectra for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Cell viability profiles were measured via protein content and are normalized with respect to untreated cells (=100%). A cell viability >100% means that cell growth is enhanced in the presence of the tested formulation with respect to untreated cells. See Supporting Information for experimental details.

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