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β-Cyclodextrin-Based Polycationic Amphiphilic "Click" Clusters: Effect of Structural Modifications in Their DNA Complexing and Delivery Properties

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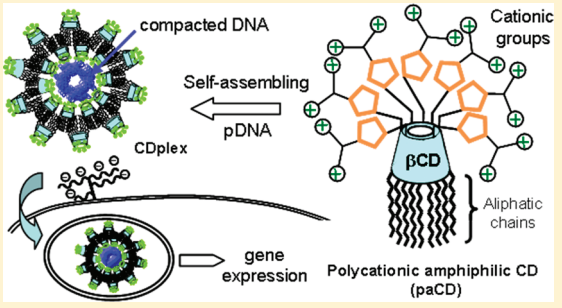
Supporting Information

ABSTRACT: Monodisperse facial amphiphiles consisting of a β-cyclodextrin (βCD) platform exposing a multivalent display of cationic groups at the primary rim and bearing hydrophobic chains at the secondary oxygens have been prepared by implementing two very robust "click" methodologies, namely cuprous cation-catalyzed azide−alkyne cycloaddition (CuAAC) and thiourea-forming reaction. Most interestingly, the use of solid-supported Cu(I) catalysts was found to be very well suited for multiple CuAAC while facilitating purification of the C_7-symmetric macromolecular triazole adducts. The strategy is compatible with molecular diversity-oriented approaches, which has been exploited to generate a small library of click polycationic amphiphilic CDs (paCDs) for assessing the influence of structural modifications in the ability to complex, compact, and protect pDNA and the efficiency of the resulting paCD:pDNA nanocomplexes (CDplexes) to deliver DNA into cells and promote transfection. The results indicate that fine-tuning the hydrophilic/hydrophobic balance is critical to achieve optimal self-assembling properties and stability of the resulting CDplexes in saline- and serum-containing media. Triazole-type paCDs were, in general, less efficient in promoting gene transfection than thiourea-type derivatives. Nevertheless, the current body of results support that the "dual click" approach implying sequential CuAAC and thiourea-forming reactions represents a versatile strategy to optimize the gene delivery capabilities of cyclodextrin-based facial amphiphiles.



INTRODUCTION

Gene therapy is a promising strategy for the treatment of a broad range of ailments including various types of cancer and cardiovascular, monogenic, and infectious diseases. The success of this approach is strongly dependent on the development of safe and efficient gene delivery systems that can provide protection of the therapeutic gene and effectively deliver exogenous genetic material into specific cell types. Much attention has been focused initially on viral-based delivery systems. However, their limited DNA carrying capacity, expensive cost, and safety concerns such as immunogenic response, toxicity or oncogenicity are major limitations inherent to these vectors.[1] During the last two decades, nonviral gene delivery systems have emerged as an attractive alternative.[2] A large diversity of (poly)cationic lipids, polymers, and dendrimers has been explored for gene transfer applications, and their use has moved from in vitro transfection to clinical gene therapy trials. Unfortunately, low efficiency and poor selectivity compared to their viral counterparts severely limit their application range.[3]

Understanding the mechanisms involved in cell and systemic traffic of vector:plasmid DNA (pDNA) complexes and elucidation of the relationships between vector structure and transfection efficiency is essential for the rational design of efficient nonviral gene delivery systems. Facial amphiphiles consisting of a nanometric platform such as fullerenes, calix[4]resorcinarenes (resorcarenes), calixarenes, or cyclodextrins (CD), onto which a variety of cationic groups and lipophilic moieties can be installed with a precise spatial orientation, have been shown to be particularly well-suited for this purpose.[4] As for classical cationic amphiphiles, facial cationic amphiphiles are able to form organized supramolecular structures with DNA. The resulting complexes can display further fusogenic,[5] permeation,[6] molecular recognition,[7] and/or transfection properties.[8] Most interestingly, homogeneity can be preserved at the molecular level in structurally related series of compounds by implementing selective

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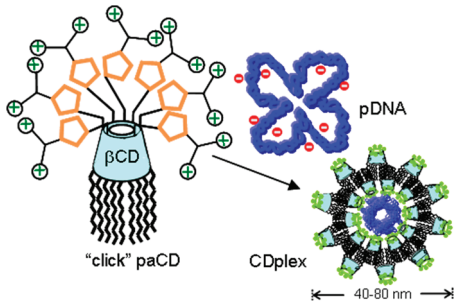


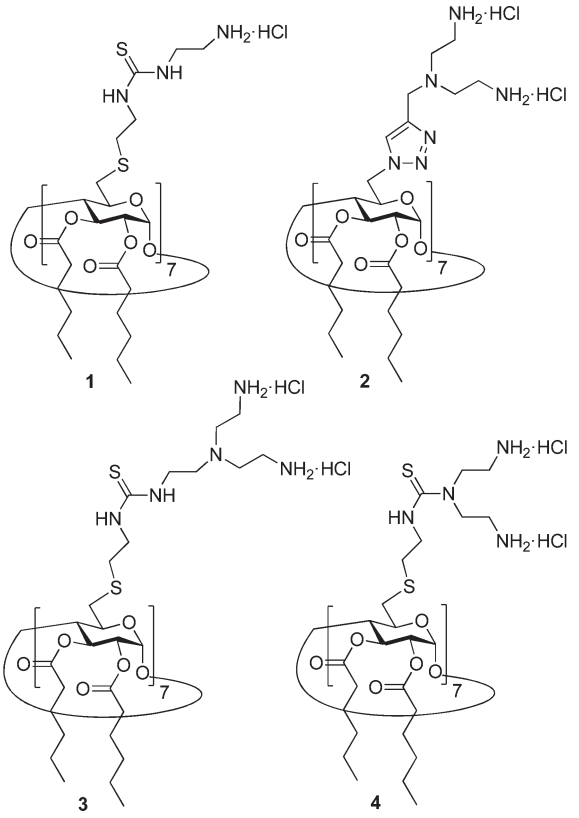
Figure 1. Schematic representation of CDplex formation from "click" paCDs (for a more realistic representation of CDplexes, see Figure 4).

chemical functionalization methodologies, offering unprecedented opportunities for structure−activity relationship studies.

Cyclodextrins (CDs), a family of cyclic oligosaccharides composed of α-(1→4)-linked D-glucopyranose units featuring a basket-like shape, represent a privileged scaffold for the above channels.[9] CDs have been classically used in drug delivery and controlled drug release systems taking advantage of their unique molecular inclusion abilities.[10] Their role as transfection enhancers in formulations containing cationic lipids, cationic polymers, or even viral vectors has been known for a long time.[11] However, the real potential of CDs in gene therapy has only been revealed by exploiting selective chemical functionalization tools.[12] Various CD-based polymeric,[13] dendritic,[14] or supramolecular materials[15] have been reported to promote gene delivery and expression. It is noteworthy that CD-based polycationic clusters were shown to self-organize in the presence of DNA to promote compaction and safe delivery to cells.[16] Amphiphilic versions of these polycationic cyclodextrins, namely polycationic amphiphilic cyclodextrins (paCDs), have been shown to exhibit better self-assembly properties and enhanced cell-membrane crossing capabilities.[4,17]

We have previously reported several paCDs constructs for gene delivery where the density and arrangement of the cationic groups, flexibility of linkers, and the presence or absence of hydrogen-bond-donating groups can be modified in a controlled manner compatible with molecular diversity-oriented approaches.[4,18] The key synthetic step implies the multiple coupling of the cyclodextrin core with the cationic arm, which requires very efficient procedures to warrant monodispersity. Two very robust "click-type" methodologies have been privileged, namely the thiourea-forming reaction between amine and isothiocyanate counterparts[19] and the Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction to give 1,4-substituted 1,2,3-triazole derivatives.[20] In both cases, the C_n-symmetric conjugates can be obtained with high efficiency. Yet, the choice of the clicking reaction may have strong consequences in the DNA complexing aptitude of the resulting paCDs as well as on the gene delivery capabilities of the corresponding paCD:DNA nanocomplexes (CDplexes; Figure 1). In principle, amine and thiourea groups can act in a cooperative manner in the reversible complexation of the phosphate groups in the DNA backbone through electrostatic and hydrogen-bonding interactions.[21] The triazole moiety on his side, similarly to other nitrogen heterocycles, could also act as a hydrogen bonding acceptor, as a DNA intercalating moiety, or as an endosomal buffering unit (pK_a of triazole = 6),[22] thereby affecting stability, endosomal release and transfection efficiency of the resulting CDplexes. Moreover, thiourea and triazole segments impose intrinsic conformational constrains that can affect the supramolecular interactions with biomolecule partners in different ways.[23]

The ensemble of results already accumulated on paCD vectors let us identify the *N*-(2-aminoethyl)thioureido moiety and the *N*,*N*-bis(2-aminoethyl)amino subunit as very favorable structural elements to promote DNA complexation and delivery. Thus, compounds **1** and compounds **2** and **3**, incorporating 7 copies of either the mentioned linear or branched motif onto the primary face of a cyclomaltoheptaose (β-cyclodextrin; βCD) core and 14 hexanoyl chains in the opposite rim, formed with pDNA nanoparticles (40−80 nm) that exhibited transfection capabilities comparable to that of commercial vectors of reference such as Jet-PEI or LipofectAmine in the absence of serum (Figure 1).[18] However, they failed to render efficient transfection in the presence of serum, which makes difficult their utility for in vivo applications. The *N*,*N*-disubstituted thiourea derivative **4** was found to be much more efficient than **1**−**3** in the presence of serum and has actually demonstrated its utility for gene delivery in vivo,[24] but its synthesis involves a CD-derived heptaisothiocyanate intermediate that handicaps the overall yield and makes its preparation significantly more costly.



From a practical point of view, the synthesis of the triazole prototype **2** presents advantages for library generation and optimization approaches. Particularly appealing is the possibility of combining triazole and thiourea motifs to adjust the architectural requirements for efficient CDplex formation and gene delivery. Broadening the spectrum of paCD structures available by combining both functional elements through a "dual" click strategy may open new opportunities for vector optimization.[25] In order to fully explore the potential of this approach, we have now examined the possibility to generate the basic multivalent triazole core using solid-supported Cu(I) catalysts, thereby simplifying the purification

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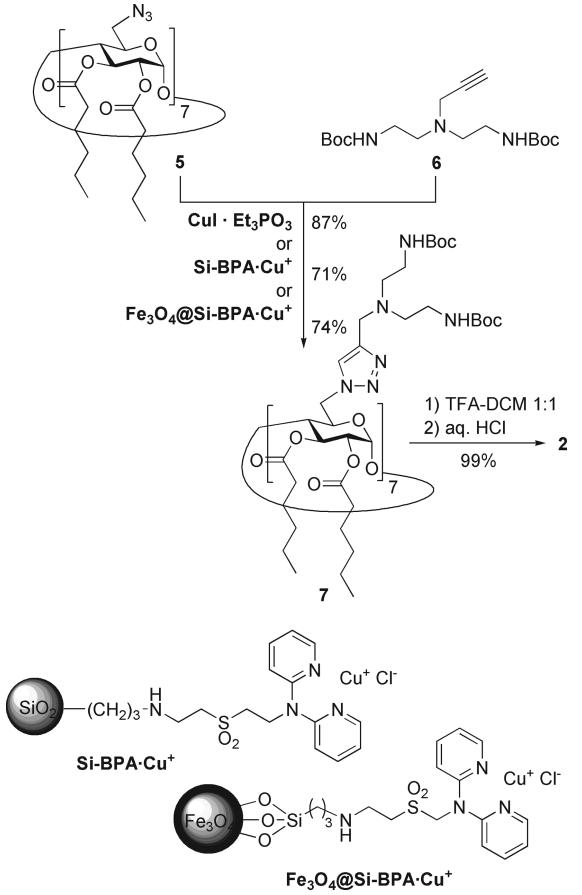
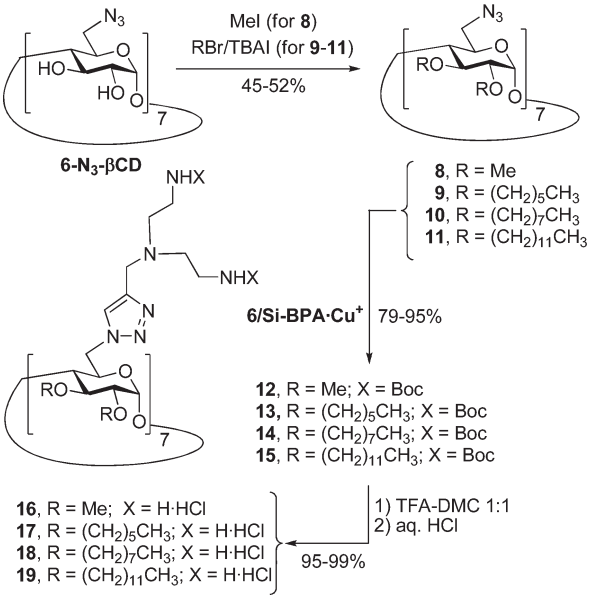
Scheme 1. Synthesis of the "Click" paCD 2 by Cu(I)-Promoted Azide−Alkyne Cycloaddition Reaction Using Homogeneous and Heterogeneous Catalysts



Scheme 2. Synthesis of the "Click" paCDs 16-19 by CuAAC Reaction Using the Silica-Supported Heterogeneous Catalysts Si-BPA-Cu+



steps and allowing access to higher quantities of material. The optimal conditions have been used to prepare a series of click paCD derivatives aimed at evaluating the impact of different displays of aminothiourea−triazole segments at the primary rim as well as of the length of the hydrophobic chains at the secondary face of βCD on their ability to complex, compact and deliver DNA. A comparative study of the size, surface potential and stability in saline and serum media of the resulting nanoparticles as well as of their gene delivery and transfection capabilities in BNL-CL2 (murine hepatocytes) and COS-7 (African green monkey kidney) cells is also included.

RESULTS AND DISCUSSION

Synthesis. The reaction between heptakis(6-azido-6-deoxy-2,3-di-*O*-hexanoyl)cyclomaltoheptaose (**5**) and bis[2-(*N*-*tert*-butoxycarbonylamino)ethyl]propargylamine (**6**) was chosen as a model system to evaluate the suitability of solid-supported Cu(I) catalysts for the preparation of amphiphilic "click clusters" because of the promising pDNA complexing properties exhibited by compound **2** in a preliminary study[18c] Thus, the efficiency of the recently reported[26] silica-based nonmagnetic and magnetic nanoparticles incorporating bis(pyridyl)amine (BPA) Cu(I) chelating adsorbents **Si-BPA·Cu**$^+$ and **Fe$_3$O$_4$@Si-BPA·Cu**$^+$ to catalyze the 7-fold cycloaddition was compared to that of the homogeneous reaction using copper(I) iodide/triethyl phosphite complex. The reaction conditions and yields are collected in Scheme 1.

Both heterogeneous catalysts had been shown previously to afford yields in the 88−100% range for single-point CuAAC reactions.[26] Our results indicated that they were also very well suited to be used in multiple CuAAC couplings, a strategy that is becoming increasingly popular for the synthesis of multivalent conjugates.[27] The yield of pure **7**, for which no trace of copper ions was detected in the corresponding mass spectra, was above 70%, meaning that the yield of every single coupling step was above 95%. The suitability of this solid-supported Cu(I) catalyst in multiple click reactions was further reinforced by its extremely easy manipulation and recovery, which can be efficiently performed by centrifugation-decantation (**Si-BPA·Cu**$^+$) or magnetic decantation (**Fe$_3$O$_4$@Si-BPA·Cu**$^+$) in accordance with the pursued goal of gaining in simplicity. This is particularly important when dealing with macromolecular systems. Recycling efficiency was also investigated for both catalysts, and no discernible loss of catalytic activity was detected after three cycles.

The above heterogeneous CuAAC reaction conditions, using the more readily available **Si-BPA·Cu**$^+$ catalyst, were further applied to the preparation of a small library of analogues of compound **2** in which the acyl groups at the secondary hydroxyls have been replaced by alkyl chains of increasing length. We kept in mind that pDNA compaction by paCDs is driven to a certain extent by hydrophobic contacts between the apolar CD faces in the paCD/pDNA complex.[18d] Consequently, structural modifications at this region were expected to have an impact in the self-assembling process leading to CDplex formation. On the other hand, the much higher chemical and enzymatic stability of ether as compared to ester functionalities might increase the lifetime of the CDplexes in biological media. The synthesis of the tetradeca-*O*-methyl (**8**),[28] -hexyl (**9**), -octyl (**10**), and -docecyl per-(C-6)-azido βCD (**11**) precursors was accomplished in a straightforward manner by exhaustive alkylation of the known hepta-(C-6)-azide (**6-N$_3$-βCD**)[29] with methyl iodide (for the

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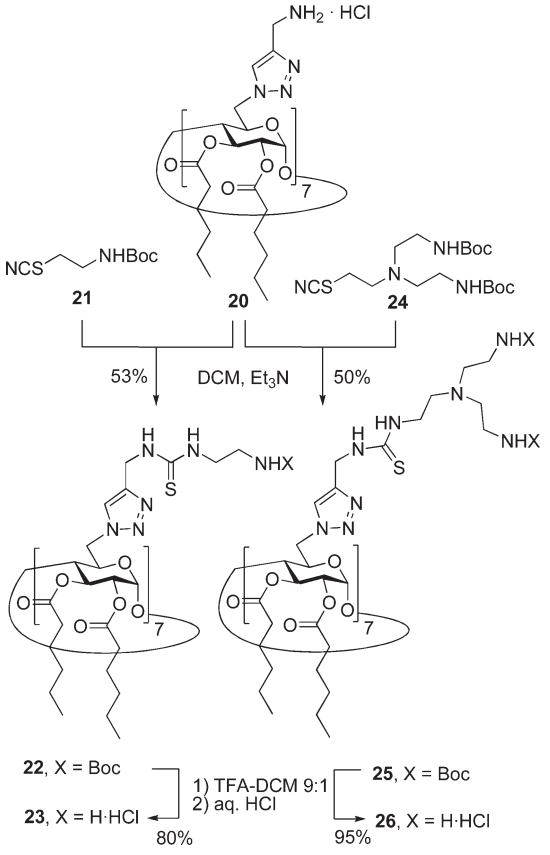
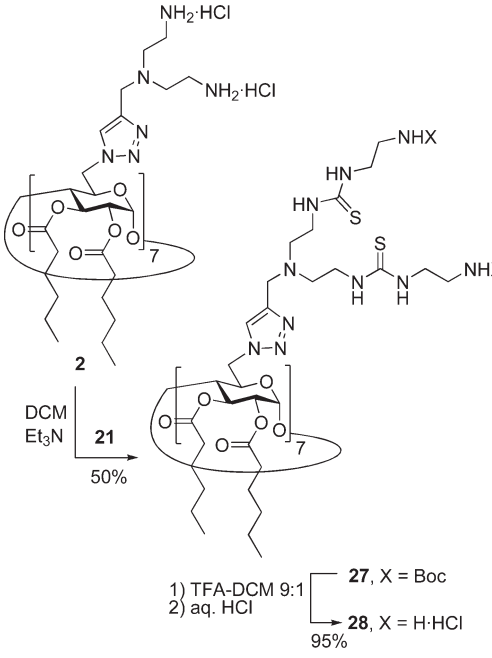
Scheme 3. Synthesis of the "Click" Triazole−Thiourea paCDs 23 and 26



Scheme 4. Synthesis of the "Click" Triazole−Thiourea paCD 28



known methylated derivative **8**) or with the corresponding alkyl bromide (for **9**−**11**) in the presence of tetrabutylammonium iodide. Subsequent heterogeneous CuAAC with **6** afforded the 1,4-substituted triazole clusters **12**−**15** in yields ranging from 79 to 95%. Final trifluoroacetic acid-promoted hydrolysis of the carbamate groups provided the corresponding polycationic click clusters **16**−**19**, which were characterized as the tetradecahydrochloride salts (Scheme 2).

The ^{1}H and ^{13}C NMR spectra of compounds **12**−**15** showed the typical line broadening associated to slow rotation about the C—N carbamate bonds. After hydrolysis (→**16**−**19**), the signals narrowed and were consistent with a C_7-symmetric arrangement, indicative of homogeneous substitution of the βCD core. The purity of all compounds was further confirmed by mass spectrometry and combustion analysis.

The heterogeneous CuAAC protocol significantly simplifies the synthesis of aminotriazole-type paCDs, making them attractive scaffolds for further structural modifications and library generation. Our next synthetic goal was the preparation of a series of analogues incorporating additional aminothiourea moieties at the primary rim while keeping unmodified the secondary face. Compound **23**, bearing a single aminoethylthiourea segment per arm, was obtained by coupling reaction of the known heptaamine click cluster **20**[18c] with 2-(*N*-*tert*-butoxyaminocarbonyl)ethyl isothiocyanate (**21**)[30] and hydrolysis of the carbamate protecting groups in the resulting heptathiourea adduct **22**. A similar reaction sequence involving coupling of **20** with the branched isothiocyanate **24**[18d] (→**25**) or multinucleophilic addition of the tetradecaamine click cluster **2** with the linear isothiocyanate **21** (→**27**), followed by acid-promoted carbamate cleavage, afforded the dendritic polycationic thiourea−click conjugates **26** and **28**, respectively. In both cases the structure features a cluster of 14 primary amino groups, located at a 10 C−C/C−N bond distance from the rigid triazole rings, and 7 tertiary amino groups in a C_7-symmetrical arrangement, as confirmed by NMR, MS, and analytical data. Compound **26** incorporates in addition seven thiourea groups, whereas in the case of **28** the number of thiourea segments is double (Schemes 3 and 4).

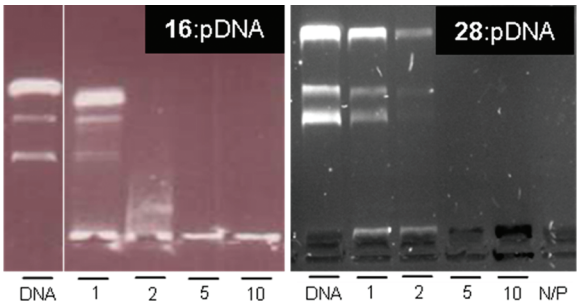


Figure 2. Gel electrophoresis shift assay showing binding of the nonamphiphilic and amphiphilic polycationic CDs **16** and **28**, respectively, to pDNA at different N/P ratios, as well as accessibility of the corresponding CDplexes to ethidium bromide.

pDNA Complexation and Nanoparticle Characterization. Agarose gel electrophoresis retardation experiments (Figure 2), using the fluorescent intercalating compound ethidium bromide (EB) as staining system, demonstrated that the whole set of paCDs prepared in this work **2**, **17**−**19**, **23**, **26**, and **28** were able to fully complex pDNA (pTG11236, a luciferase-encoding reporter gene of 5739 base pairs used also for the biological assays, see the following text) at protonable nitrogen/phosphate group ratios

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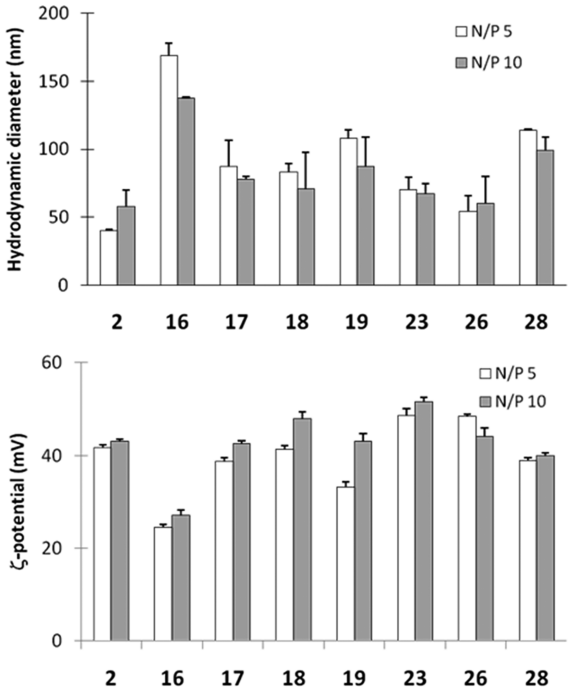

Figure 3. Hydrodynamic diameter (top) and ζ potential (down) of CDplexes prepared from click paCDs **2**, **16**−**19**, **23**, **26**, and **28** at N/P 5 and 10, determined by dynamic light scattering and M3-PALS analysis, respectively. Data represent mean standard deviation (n = 3).

(N/P) ≥5, as indicated by the absence of free mobile or even partially complexed plasmid (no fluorescent staining in the corresponding lanes). Facial amphiphilicity seems to be a critical requirement to achieve a well-ordered arrangement of the paCDs and provide efficient protection of the pDNA material from the environment. Thus, the nonamphiphilic per-(*O*-2,*O*-3)-methyl βCD derivative **16** did also form a complex with pDNA at N/P 5 and 10 (no electrophoresis mobility at the corresponding lane), but the double-helix remained accessible to EB intercalation (fluorescent staining observed at the bottom spot).

In the absence of pDNA, all the new paCDs formed relatively large aggregates (hydrodynamic diameters 195−210 nm) with high polydispersity indexes (0.2−0.5) and positive ζ-potentials (60−65 mV) in aqueous solution, as determined by dynamic light-scattering (DLS) measurements. In stark contrast, nanoparticle size determinations after pDNA-paCD self-assembling showed quasi-unimodal size distributions with average hydrodynamic diameters in the range 40−110 nm (Figure 3), much smaller than the 150−200 nm nanoparticles obtained with polycationic lipids or polymers such as JetPEI, a polycationic polymer which ranks among the most powerful non viral pDNA delivery systems.[31] All these formulations displayed highly positive ζ-potentials (about +40 mV). In contrast to classical liposome systems, no extrusion process prior to mixing with DNA is needed to homogenize particle distribution (Figure 3).

The low polydispersities and small sizes of the CDplexes obtained with the new triazole (**17**−**19**) and triazole−thiourea (**23**, **26**, and **28**) paCDs are remarkable. Such a behavior has only been observed previously in the case of monomolecular condensation processes occurring upon mixing of DNA with dimerizable polycationic detergents.[32] Once again, facial amphiphilicity seems to be a critical architectural requirement; the tetradeca-*O*-methyl

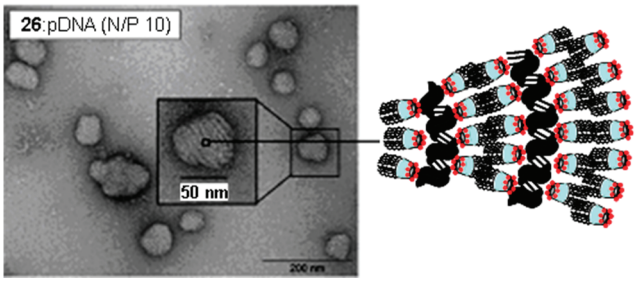

Figure 4. TEM micrograph of the thiourea−triazole click paCD **26**:pDNA CDplexes with amplification of the ultrafine structure of the particles and an schematic representation of the proposed arrangement of paCDs and the DNA double helix.

derivative **16** afforded much larger CDplexes (average hydrodynamic diameter about 150 nm) with a much lower ζ-potential (about 25 mV). Transmission electron microscopy (TEM) of the pDNA:paCD CDplexes evidenced spheroid-like nanoparticles with a snake-like ultrastructure, compatible with a lamellar and condensed L_α^C type arrangement,[33] probably made of alternating paCD bilayers and pDNA portions corresponding to low (lighter) and high electron dense (darker) regions, respectively (Figure 4).

The CDplexes obtained from the new triazole-containing paCDs proved to be rather stable in the presence of increasing concentrations of sodium chloride. Only moderate increases in the average hydrodynamic diameters were observed at 150 mM NaCl, a concentration that mimics physiological conditions, and they remained in general below 200 nm at 250 mM NaCl. The exceptions are the nonamphiphilic polycationic CD **16** and the dodecyl paCD **19**, probably due to less favorable hydrophobic−hydrophilic balances. The presence of the triazole moiety seems to impart stability to the corresponding CDplexes. Thus, the nanoparticles prepared from the reference aminothiourea-based polycationic clusters **1** or **3** and from the structurally related triazole (**2**) or hybrid triazole−thiourea click counterparts **23**, **26**, or **28** exhibited huge differences when exposed to saline media; while at 150 mM NaCl the pDNA:**1** or pDNA:**3** CDplexes experienced strong aggregation, the CDplexes prepared from **2**, **23**, **26**, or **28** kept an average hydrodynamic diameter below 100 nm (Figure 5). The stabilizing effect of the triazole moiety in the CDplexes toward salt-induced dissociation was further evidenced by a significantly lower accessibility of DNA to the intercalating agent ethidium bromide as compared with data for pDNA:**1** or pDNA:**3** CDplexes (see the Supporting Information).

Differences in the aggregation tendency were mitigated in 10% serum-containing media (Figure 6). Indeed, a size increase to approximately 150 nm was systematically observed for all formulations, excepting for CDplexes prepared from the non amphiphilic derivative **16** (250 nm). Neither precipitates nor larger aggregates were noticed. This observation is consistent with the formation of new species resulting from association between the positively charged CDplexes and negatively charged serum proteins.[34] The ternary protein:paCD:pDNA complexes remained further quite stable, since an EB exclusion assay indicated no significant increase of DNA accessibility under such conditions (data not shown).

pDNA Delivery and Transfection Efficiency. The transfection efficiency and cell viability of the new "click" CDplexes formulated at N/P 5 and 10 were evaluated in vitro using the luciferase-encoding reporter gene used for the pDNA complexation

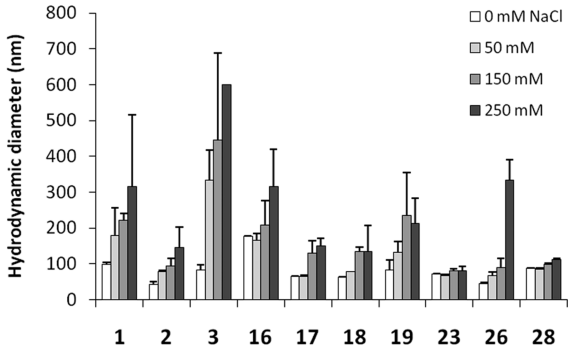

Figure 5. Hydrodynamic diameter (nm) of CDplexes prepared from click paCDs **2**, **16**−**19**, **23**, **26**, and **28** at N/P 10 determined by DLS with gradual increment of the NaCl concentration, in comparison with results obtained for CDplexes prepared from the reference thiourea-paCDs **1** and **3**. Data represent mean standard deviation (n = 3).

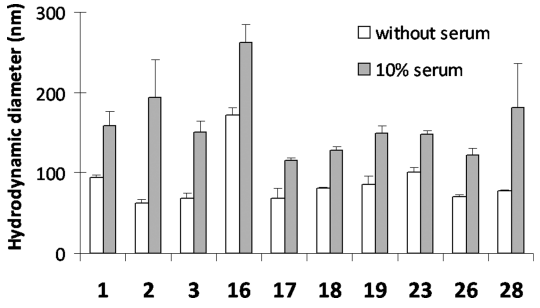

Figure 6. Hydrodynamic diameter (DLS) of CDplexes prepared from click paCDs **1**−**3**, **16**−**19**, **23**, **26**, and **28** at N/P 10 in the absence and in the presence of 10% serum. Data represent mean standard deviation (n = 3).

and nanoparticle characterization study (see above) on adherent BNL-CL2 and COS-7 cells in the absence or presence of 10% serum (Figure 7). Jet-PEI (22 kDa) N/P 10 polyplexes and naked pDNA were used as positive and negative controls, respectively. Data for CDplexes prepared from paCD **4**, the best performing paCD developed up to date in our laboratories, are also indicated as an additional reference. For comparative purposes, the CD-plexes obtained from the structurally related thiourea-based paCDs **1** and **3** were further included in this study.

In the absence of serum, all triazole-paCDs were found to mediate gene transfer and expression in BNL-CL2 with much higher efficiency than naked DNA (10^3−10^6-fold) with no cell toxicity implications, the performance being generally better at N/P 10 than at N/P 5. Replacing the hexanoyl chains in **2** into hexyl chains (**17**) had no impact on the luciferase expression results under these conditions. However, while **2** lost transfection ability in the presence of 10% serum, **17** was still 100-fold more efficient than naked pDNA. Further increasing the length chain to octyl (**18**) and, especially, dodecyl (**19**) was detrimental for transfection. Actually, the later vector behaved similarly to the nonamphiphilic derivative **16**, stressing the need for finely tuning the facial amphiphilicity of the system to optimize gene delivery.

The consequences of structural modifications at the cationic cluster of click paCDs on their transfection properties did not follow a clear trend and were more delicate to rationalize. The combination of triazole and thiourea moieties in **26** turned to be

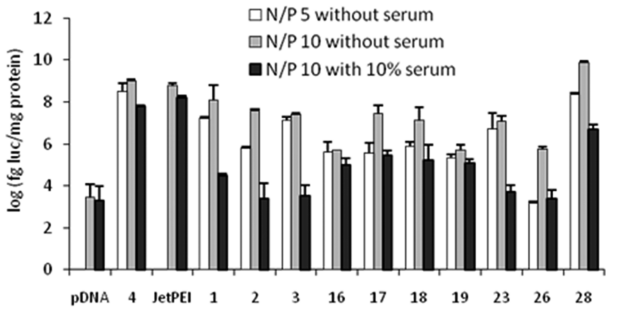

Figure 7. In vitro transfection efficiency in BNL-CL2 cells of CDplexes formulated with the βCD derivatives **1**−**3**, **16**−**19**, **23**, **26**, or **28** in comparison with data for naked DNA (= pTG11236) and Jet-PEI -based polyplexes. As an additional reference, data for CDplexes prepared from the thiourea-type paCD **4** are also included.

negative as compared to the conjugates that share the same type of bis(2-aminoethyl)amino coating but with only triazole (**2**) or only thiourea segments (**3**). In other words, the ensemble of data reflect that the triazole/thiourea combination cannot compensate for the decrease in transfection efficiency resulting from replacement of a flexible 2-thioureidoethylthio connecting unit (e.g., in **1** or **3**) by a rigid triazole moiety (e.g., in **23** or **26**) directly linked to the CD platform. Noteworthy, this detrimental effect was more than compensated by a higher valency display of aminoethylthioureido segments at the CD cluster. Thus, the dentritic triazole-thiourea derivative **28**, bearing 14 aminoethylthioureido motifs and 21 cationic charges, was up to 1000-fold more efficient than the heptacationic heptaaminoethylthioureidotriazole paCD **23** or heptaaminoethylthioureidothioethyl paCD **1** in promoting transfection, even surpassing the reference tetradecacationic paCD **4** and JetPEI by 10-fold in the absence of serum. This result is all the more remarkable since, for a given N/P ratio, much lower paCD equivalents are used for **28** (1/3 equiv) than for **1** and **23** (1 equiv) or **4** (2/3 equiv). The transfection performance of **28** remained 10^3−10^4-fold better as compared with the reference compounds **1**−**3** and **23** in 10% serum-containing medium. Although **28** appears 10- and 100-fold less efficient than **4** and JetPEI, respectively, under these conditions, it must be stressed that the data on luciferase expression were normalized to the milligrams of protein and, consequently, **4** and mostly JetPEI appear artificially high due to its higher cell toxicity. Hence, cell viability in the presence of **28**:pDNA CDplexes formulated at N/P 10 was 100%, while it was 80% for **4**:pDNA CDplexes and only 55% for JetPEI-based polyplexes (see the Supporting Information).

The promising results obtained with **28** might reflect a very favorable reversible complexation of the polyphosphate chain by aminothiourea segments through cooperative hydrogen bonding and electrostatic interactions, a biomimetic mechanism.[35] Variations in transfection activity could also arise from differences in the supramolecular structure of the corresponding CDplexes. It is worth mentioning that neutral amphiphiles bearing thiourea groups have also shown to exhibit DNA complexing and delivery capabilities.[36] Further investigation of the transfection properties of CDplexes formulated from thiourea, triazole and triazole-thiourea click paCDs in COS-7 cells were fully consistent with the above commented observations in BNL-CL2 cells, i.e., multiplication of aminoethylthioureido branches onto the βCD amphiphilic scaffold results in a dramatic increase of the transfection

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efficiency with no associated cytotoxicity (see the Supporting Information).

CONCLUSION

In summary, we have implemented an efficient approach based on the use of solid-supported Cu(I) catalysts for the synthesis of well-defined and characterized "click" polycationic amphiphilic CDs with a "skirt"-type architecture.[37] These discrete macromolecules self-organized in the presence of plasmid DNA to form very small nanoparticles which are close to monomolecular in DNA and can cross biological membranes to mediate transfection. The methodology allows introducing structural modifications at either the hydrophobic or the hydrophilic (polycationic) face with a relatively low synthetic cost. Notably, the incorporation of a triazole−thiourea "belt" by a "dual click" approach, through sequential CuAAC—thiourea-forming reactions, can be used for adjusting the molecular topology to optimize pDNA complexation and delivery. The flexibility and selectivity provided by "click chemistry" is also compatible with specific ways to perform CD functionalization (e.g., for monitoring in vivo fate, cell uptake, cell targeting, intracellular trafficking, nuclear localization, etc.).[38] Moreover, the possibility to carry out structure−activity relationship studies with homogeneous molecular vectors should help to improve the current notions in the field of nonviral gene delivery and offers further opportunities for the development of synthetic viruses that will merit investigation. Work in that direction is currently sought in our laboratories.

EXPERIMENTAL PROCEDURES

General Methods. Reagents and solvents were purchased from commercial sources and used without further purification. Optical rotations were measured at 20 °C in 1-cm or 1-dm tubes. ^{1}H (and ^{13}C NMR) spectra were recorded at 500 (125.7) and 400 (100.6) MHz. 2D COSY and HMQC experiments were used to assist on NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminum sheets, with visualization by UV light and by charring with 10% H_2SO_4. Column chromatography was carried out on silica gel (230−400 mesh). Electrospray mass spectra were obtained for samples dissolved in MeCN, MeOH, or H_2O−MeOH mixtures at low μm concentrations.

Heptakis[6-(4-aminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose heptahydrochloride (**20**),[18c] 2-(*N*-*tert*-butoxyaminocarbonyl)ethyl isothiocyanate (**21**),[30] 2-[bis[2-(*tert*-butoxycarbonylamino)ethyl]amine]ethyl isothiocyanate (**24**),[18d] heptakis-(6-azido-6-deoxy)cyclomaltoheptaose (**6-N$_3$-βCD**),[29] and heptakis[6-azido-6-deoxy-2,3-di-*O*-methyl]cyclomaltoheptaose (**8**)[28] were prepared according to the literature.

Heptakis[6-(4-(2,2-bis-*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (7). *a. Synthesis Using Homogeneous Cu(I) Catalysis.* To a solution of heptazide **5** (156 mg, 58 μmol) and alkyne **6** (182 mg, 0.53 mmol, 1.3 equiv) in acetone (10 mL) was added CuI·(EtO)$_3$P (15 mg, 41 μmol, 0.1 equiv), and the reaction mixture was refluxed for 5 h. The solvent was evaporated under vacuum, and the residue was purified by column chromatography (30:1 → 9:1 DCM−MeOH): yield 256 mg (87%); R_f = 0.61 (9:1 DCM/MeOH); $[\alpha]_D$ = +25.9 (*c* 1.0, DCM); ^{1}H NMR (400 MHz, $CDCl_3$, 323 K) δ = 7.73 (s, 7 H, =CH), 5.44 (d, 7 H, $J_{1,2}$ = 3.2 Hz, H-1), 5.36 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 8.7 Hz, H-3), 5.21 (bs, 14 H, NH Boc) 4.90 (bd, 7 H, $J_{6a,6b}$ = 13.6 Hz, H-6a), 4.75 (dd, 14 H, H-2, H-6b), 4.51 (m, 7 H, H-5), 3.78, 3.74 (2 d, 14 H, $^2J_{H,H}$ = 15.6 Hz, CH_2N), 3.53 (t, 7 H, $J_{4,5}$ = 8.5 Hz, H-4), 3.16 (q, 28 H, $^3J_{H,H}$ = 5.6 Hz, CH_2NHBoc), 2.56 (t, 28 H, CH_2CH$_2$NHBoc), 2.45−2.13 (m, 28 H, CH_2CO), 1.60 (m, 28 H, CH_2CH$_2$CO), 1.44 (bs, 126 H, CMe_3), 1.30−1.20 (m, 56 H, CH_2CH$_3$, CH_2CH$_2$CH$_3$), 0.94, 0.93 (2 t, 42 H, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 6.8 Hz, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$, 323 K) δ = 172.7, 171.5 (CO ester), 156.0 (CO carbamate), 143.6 (C-4 triazole), 125.4 (C-5 triazole), 96.5 (C-1), 78.8 (C-4, C_q), 70.0 (C-3), 69.7 (C-5), 69.3 (C-2), 53.0 (*C*H$_2$CH$_2$NHBoc), 50.0 (C-6), 48.0 (CH_2N), 38.5 (CH_2NHBoc), 33.8, 33.5 (CH_2CO), 31.1, 31.0 (*C*H$_2$CH$_2$CH$_3$), 28.3 (CMe_3), 24.1 (CH_2-CH$_2$CO), 22.1 (CH_2CH$_3$), 13.6 (CH_3); ESIMS *m*/*z* 2558.2 $[M + 2\,Na]^{2+}$, 1713.6 $[M + 3\,Na]^{3+}$. Anal. Calcd for $C_{245}H_{420}N_{42}O_{70}$: C, 57.99; H, 8.34; N, 11.59. Found: C, 57.72; H, 8.20; N, 11.24.

b. Synthesis Using Heterogeneous Nonmagnetic Catalysis. To a solution of **5** (250 mg, 95 μmol) and **6** (245 mg, 0.70 mmol, 1.1 equiv) in ^{t}BuOH−H_2O (3:1, 3 mL) was added the Cu-supported catalyst **Si-BPA·Cu$^+$** (23 mg), the reaction mixture was refluxed 6 h, the solvent was removed, the residue was diluted with DCM, and the catalyst was filtered. After evaporation of the solvent, the residue was purified by column chromatography chromatography (30:1 → 9:1 DCM−MeOH): yield 335 mg (71%).

c. Synthesis Using Heterogeneous Magnetic Catalysis. To a solution of **5** (250 mg, 95 μmol) and **6** (245 mg, 0.7 mmol, 1.1 equiv) in ^{t}BuOH−H_2O (3:1, 15 mL) was added the magnetic Cu-supported catalyst **Fe$_3$O$_4$@Si-BPA·Cu$^+$** (7 mg), the reaction mixture was refluxed for 8 h, the solvent was removed, the residue was diluted with DCM, and the catalyst was decanted using a magnet. After evaporation of the solvent, the residue was purified by column chromatography chromatography (30:1 → 9:1 DCM−MeOH): yield 350 mg (74%).

Heptakis[6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (2). Treatment of **7** (33 μmol) with TFA−DCM (1:1, 2 mL) at rt for 2 h, followed by evaporation of the solvents and freeze-drying from a diluted HCl solution, gave pure **2**: yield 136.4 mg (99%); $[\alpha]_D$ = +18.5 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 313 K) δ = 8.11 (s, 7 H, =CH), 5.60 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.9 Hz, H-3), 5.59 (s, 7 H, H-1), 5.15 (dd, 7 H, $J_{6a,6b}$ = 15.2 Hz, $J_{5,6a}$ = 2.5 Hz, H-6a), 4.58 (dd, 7 H, $J_{5,6b}$ = 3.0 Hz, H-6b), 4.57 (m, 14 H, $J_{1,2}$ = 3.5 Hz, H-2, H-5), 3.91, 3.89 (2 d, 14 H, $^2J_{Ha,Hb}$ = 15.3 Hz, CH_2N), 3.54 (t, 7 H, $J_{4,5}$ = 8.9 Hz, H-4), 3.15 (t, 28 H, $^3J_{H,H}$ = 6.0 Hz, CH_2NH$_2$), 2.83 (t, 28 H, CH_2CH$_2$NH$_2$), 2.49−2.17 (m, 28 H, CH_2CO), 1.73−1.58 (m, 28 H, CH_2CH$_2$CO), 1.37 (m, 56 H, CH_2CH$_3$, CH_2-CH$_2$CH$_3$), 0.94, 0.92 (2 t, 42 H, $^3J_{H,H}$ = 7.1 Hz, $^3J_{H,H}$ = 6.9 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD, 313 K) δ 175.7, 174.6 (CO ester), 144.6 (C-4 triazole), 129.7 (C-5 triazole), 99.2 (C-1), 79.4 (C-4), 73.0 (C-2), 72.9 (C-5), 71.7 (C-3), 53.0 (*C*H$_2$CH$_2$NH$_2$), 52.6 (C-6), 48.3 (CH_2N), 39.4 (CH_2NH$_2$), 36.3, 36.2 (*C*H$_2$CO), 33.9, 33.7 (*C*H$_2$CH$_2$CH$_3$), 26.8 (*C*H$_2$CH$_2$CO), 24.7 (*C*H$_2$CH$_3$), 15.6 (CH_3); ESIMS *m*/*z* 1837 $[M + 2\,H]^{2+}$, 1225.0 $[M + 3\,H]^{3+}$, 919.0 $[M + 4\,H]^{4+}$, 735.4 $[M + 5\,H]^{5+}$. Anal. Calcd for $C_{175}H_{322}Cl_{14}N_{42}O_{42}$: C, 50.25; H, 7.76; N, 14.06. Found: C, 49.98; H, 7.65; N, 13.79.

Heptakis[6-azido-6-deoxy-2,3-di-*O*-hexyl]cyclomaltoheptaose (9). To a solution of heptakis(6-azido-6-deoxy)cyclomaltoheptaose (**7**, 470 mg, 0.36 mmol) in dry DMF (15 mL) was added NaH (808 mg, 20.2 mmol, 4 equiv) at 0 °C under N_2 atmosphere, and the reaction mixture was stirred at 0 °C for 30 min and at rt for 2 h. Then, 1-bromohexane (2.8 mL, 20.2 mmol, 4 equiv) and TBAI (1.8 g, 5.04 mmol) were added, and the mixture was stirred overnight at 60 °C, quenched with cold water (8 mL), and extracted with Et_2O (60 mL). The organic layers were washed with water (3 × 20 mL), dried ($MgSO_4$), concentrated, and purified by column chromatography (60:1→ 50:1 petroleum ether−acetone): yield 443 mg (49%); R_f = 0.43 (petroleum ether−acetone 9:1); $[\alpha]_D$ = +92.1 (*c* 1.0 in petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.10 (d, 7 H, $J_{1,2}$ = 3.6 Hz, H-1), 3.94−3.90 (dt, 7 H, $^2J_{H,H}$ = 8.4 Hz, $^3J_{H,H}$ = 5.85 Hz, OCHa), 3.77−3.74 (ddd, 7 H, $J_{4,5}$ = 8.8 Hz, $J_{5,6a}$ = 1.9 Hz, $J_{5,6b}$ = 4.7 Hz, H-5), 3.71−3.68 (dd, 7 H, $J_{6a,6b}$ = 13.3 Hz, H-6a), 3.67−3.62 (m, 21 H, OCH_b, OCH_2), 3.61−3.54 (m, 21 H, H-3, H-4, H-6b), 3.22 (dd, 7 H, $J_{2,3}$ = 9.4 Hz, H-2),

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1.62−1.56 (m, 28 H, OCH_2CH_2), 1.40−1.25 (m, 84 H, OCH_2CH_2-$(CH_2)_3$, 0.89 (t, 21 H, $^3J_{H,H}$ = 7 Hz, CH_3), 0.88 (t, 21 H, $^3J_{H,H}$ = 6.9 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 98.5 (C-1), 80.3−80.0 (C-2, C-3, C-4), 74.6 (OCH_2), 72.4 (OCH_2), 71.5 (C-5), 52.3 (C-6), 32.4, 32.1 ($CH_2CH_2CH_3$), 30.8, 30.5 (OCH_2CH_2), 26.2, 26.0 ($OCH_2CH_2CH_2$), 23.1, 23.0 (CH_2CH_3). 14.4 (CH_3); ESIMS m/z 1266.9 $[M + 2\ Na]^{2+}$. Anal. Calcd for $C_{126}H_{231}N_{21}O_{28}$: C, 60.82; H, 9.36; N, 11.82. Found: C, 60.85; H, 9.26; N, 11.66.

Heptakis[6-azido-6-deoxy-2,3-di-*O*-octyl]cyclomaltoheptaose (10). To a solution of heptakis(6-azido-6-deoxy)cyclomaltoheptaose (**7**, 100 mg, 0.076 mmol) in dry DMF (10 mL) was added NaH (255 mg, 6.38 mmol, 6 equiv) at 0 °C under N_2 atmosphere, and the reaction mixture was stirred at 0 °C for 30 min and at rt for 1 h. Then, 1-bromooctane (1.0 mL, 4.26 mmol, 4 equiv) and TBAI (400 mg, 0.53 mmol, 1 equiv) were added, and the reaction mixture was stirred for 3 days at 40 °C, quenched with cold water (8 mL), and extracted with Et_2O (30 mL). The organic layers were washed with water (3 × 10 mL), dried ($MgSO_4$), concentrated, and purified by column chromatography (1:20 EtOAc−petroleum ether): yield 113 mg (52%); R_f = 0.7 (1:5 EtOAc−petroleum ether−acetone); $[\alpha]_D$ = +91.6 (c 1.1 in DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.09 (d, 7 H, $J_{1,2}$ = 3.4 Hz, H-1), 3.94−3.90 (dt, 7 H, $^2J_{H,H}$ = 8.4 Hz, $^3J_{H,H}$ = 13.8 Hz, OCHa), 3.74 (ddd, 7 H, $J_{4,5}$ = 7.0 Hz, $J_{5,6a}$ = 2.0 Hz, $J_{5,6b}$ = 5.3 Hz, H-5), 3.70 (dd, 7 H, $J_{6a,6b}$ = 13.3 Hz, H-6a), 3.67−3.61 (m, 21 H, OCH_b, OCH_2), 3.60−3.53 (m, 21 H, H-3, H-4, H-6b), 3.22 (dd, 7 H, $J_{2,3}$ = 9.4 Hz, H-2), 1.63−1.55 (m, 28 H, OCH_2CH_2), 1.40−1.20 (m, 140 H, $OCH_2CH_2(CH_2)_5$, 0.88 (t, 21 H, $^3J_{H,H}$ = 6.7 Hz, CH_3), 0.87 (t, 21 H, $^3J_{H,H}$ = 6.8 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 98.4 (C-1), 80.1 (C-2, C-3), 80.0 (C-4), 74.6 (OCH_2), 72.3 (OCH_2), 71.4 (C-5), 52.1 (C-6), 32.2, 32.1 ($CH_2CH_2CH_3$), 30.8, 30.5 (OCH_2CH_2), 30.2, 29.9 ($OCH_2CH_2CH_2CH_2$), 29.8, 29.6 ($CH_2CH_2CH_2CH_3$) 26.6, 26.2 ($OCH_2CH_2CH_2$), 22.9 (CH_2CH_3). 14.3 (CH_3); ESIMS m/z 1463.1 $[M + 2\ Na]^{2+}$, 2904.2 $[M + Na]^+$. Anal. Calcd for $C_{154}H_{287}N_{21}O_{28}$: C, 64.20; H, 10.04; N, 10.21. Found: C, 64.32; H, 9.91; N, 10.11.

Heptakis[6-azido-6-deoxy-2,3-di-*O*-dodecyl]cyclomaltoheptaose (11). To a solution of heptakis(6-azido-6-deoxy)cyclomaltoheptaose (**7**, 200 mg, 0.15 mmol) in dry DMF (10 mL) was added NaH (0.5 g, 13 mmol, 6 equiv) at 0 °C under N_2 atmosphere, and the reaction mixture was stirred at 0 °C for 30 min and at rt for 1 h. Then, 1-bromododecane (2.1 mL, 8.7 mmol, 4 equiv) and TBAI (800 mg, 2.15 mmol) were added, and the reaction mixture was stirred for 3 days at 40 °C, quenched with water (6 mL), and extracted with Et_2O (40 mL). The organic layer was washed with water (3 × 10 mL), dried ($MgSO_4$), concentrated, and purified by column chromatography (1:20 EtOAc−petroleum ether): yield 248 mg (45%); R_f = 0.31 (1:10 EtOAc−petroleum ether); $[\alpha]_D$ = +68.9 (c 1.0 in DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.12 (d, 7 H, $J_{1,2}$ = 3.3 Hz, H-1), 3.97−3.92 (m, 7 H, OCHa), 3.77−3.74 (ddd, 7 H, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 5.8 Hz, $J_{5,6b}$ = 2.6 Hz, H-5), 3.72 (dd, 7 H, $J_{6a,6b}$ = 13.2 Hz, H-6a), 3.71−3.64 (m, 21 H, OCH_b, OCH_2), 3.63−3.56 (m, 21 H, H-3, H-4, H-6b), 3.24 (dd, 7 H, $J_{2,3}$ = 9.1 Hz, H-2), 1.7−1.50 (m, 28 H, OCH_2CH_2), 1.40−1.20 (m, 252 H, $OCH_2CH_2(CH_2)_9$), 0.90 (t, 42 H, $^3J_{H,H}$ = 6.2 Hz, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 98.2 (C-1), 79.9 (C-2, C-3) 79.7 (C-4), 74.3 (OCH_2), 72.1 (OCH_2), 71.2 (C-5), 52.0 (C-6), 32.0, 31.9, ($CH_2CH_2CH_3$), 30.6−29.4 ($OCH_2CH_2CH_2(CH_2)_6$), 26.4, 26.1 ($OCH_2CH_2CH_2$), 22.7 (CH_2CH_3), 14.1 (CH_3); ESIMS m/z 1856.0 $[M + 2\ Na]^{2+}$. Anal. Calcd for $C_{210}H_{399}N_{21}O_{28}$: C, 68.79; H, 10.97; N, 8.02. Found: C, 68.70; H, 10.83; N, 7.91.

Heptakis[6-(4-(2,2-bis-*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-methyl]cyclomaltoheptaose (12). To a solution of heptakis(6-azido-6-deoxy-2,3-di-*O*-methyl)cyclomaltoheptaose (**8**, 50 mg, 33 μmol) and 3-bis[2-(tert-butoxycarbonylamino)ethyl]aminopropine (**6**, 89 mg, 0.26 mmol, 1.1 equiv) in ^{t}BuOH−H_2O (2:1, 3 mL) was added the Cu-supported catalyst **Si-BPA·Cu**$^+$ (8 mg), the reaction mixture was refluxed overnight, the solvent was removed, the residue was diluted with DCM, and the catalyst was filtered. After evaporation of the solvent, the residue was purified by column chromatography (30:1→9:1 DCM−MeOH): yield 122 mg (95%); R_f = 0.59 (9:1 DCM−MeOH); $[\alpha]_D$ = +21.5 (c 0.9 in DCM); ^{1}H NMR (500 MHz, 333 K, CD_3OD) δ (s, 7 H, =CH), 5.52 (bs, 7 H, H-1), 4.77 (bs, 14 H, H-6a, H-6b), 4.35 (bs, 7 H, H-5), 3.80 (bs, 14 H, CH_2-triazole), 3.64 (s, 28 H, H-3, OCH_3), 3.51 (s, 21 H, OCH_3), 3.35 (m, 7 H, H-4), 3.13 (bt, 35 H, H-2, CH_2NHBoc), 2.59 (bs, 28 H, CH_2CH_2NHBoc), 1.46 (s, 126 H, CMe_3); ^{13}C NMR (125.7 MHz, 313 K, CD_3OD) δ 159.2 (CO carbamate), 146.3 (C-4 triazole), 128.6 (C-5 triazole), 100.2 (C-1), 83.7 (C-2, C-3), 82.3 (C-4), 80.9 (C_q), 72.2 (C-5), 62.8 (OCH_3), 60.1 (OCH_3), 55.6 (CH_2CH_2-NHBoc), 52.8 (C-6), 50.0 (CH_2-triazole), 40.5 (CH_2NHBoc), 29.9 (CMe_3); ESIMS m/z 1299.1 $[M + 3\ H]^{3+}$, 1949.1 $[M + 2\ H]^{2+}$. Anal. Calcd for $C_{175}H_{308}N_{42}O_{56}$: C, 53.94; H, 7.97; N, 15.10. Found: C, 53.61; H, 7.71; N, 14.89.

Heptakis[6-(4-(2,2-bis-*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexyl]cyclomaltoheptaose (13). To a solution of **9** (39 mg, 16 μmol) and **6** (41 mg, 0.12 mmol, 1.1 equiv) in ^{t}BuOH−H_2O (2:1, 3 mL) was added the Cu-supported catalyst **Si-BPA·Cu**$^+$ (3.6 mg), the reaction mixture was refluxed overnight, the solvent was removed, the residue was diluted with DCM, and the catalyst was filtered. After evaporation of the solvent, the residue was purified by column chromatography (30:1 →9:1 DCM−MeOH): yield 62 mg (79%); R_f = 0.64 (9:1 DCM−MeOH); $[\alpha]_D$ = +21.5 (c 0.9 in DCM); ^{1}H NMR (500 MHz, 315 K, CD_3OD) δ 7.95 (s, 7 H, =CH), 5.57 (d, 7 H, $J_{1,2}$ = 2.8 Hz, H-1), 4.77 (m, 14 H, H-6a, H-6b), 4.33 (m, 7 H, H-5), 3.94 (m, 7 H, OCHa), 3.83−3.70 (m, 28 H, OCH_b, CH_2-triazole, H-3), 3.65−3.61 (m, 14 H, OCH_2), 3.33 (m, 7 H, H-4), 3.12 (m, 35 H, CH_2NHBoc, H-2), 2.56 (t, 28 H, $^3J_{H,H}$ = 5.8 Hz, CH_2CH_2NHBoc), 1.70−1.60 (m, 28 H, OCH_2CH_2), 1.49 (s, 126 H, CMe_3), 1.50−1.30 (m, 84 H, $OCH_2CH_2(CH_2)_3$), 0.95 (t, 21 H, $^3J_{H,H}$ = 7.1 Hz), 0.94 (t, 21 H, $^3J_{H,H}$ = 6.8 Hz); ^{13}C NMR (125.7 MHz, 315 K, CD_3OD) δ 159.1 (CO carbamate), 146.0 (C-4 triazole), 128.6 (C-5 triazole), 99.9 (C-1), 82.1 (C-2, C-3), 81.0 (C-4), 80.9 (C_q), 76.1 (OCH_2) 73.8 (OCH_2), 70.1 (C-5), 55.5 (CH_2CH_2NHBoc), 53.0 (C-6), 50.0 (CH_2-triazole), 40.5 (CH_2NHBoc), 34.1, 33.8 ($CH_2CH_2CH_3$), 32.5, 32.3 (OCH_2CH_2), 28.0 ($OCH_2CH_2CH_2$), 24.7, 24.6 (CH_2CH_3). 15.4 (CH_3); ESIMS m/z 1648.8 $[M + 3\ Na]^{3+}$, 2461.7 $[M + 2\ Na]^{2+}$. Anal. Calcd for $C_{245}H_{455}N_{42}O_{56}$: C, 60.23; H, 9.39; N, 12.04. Found: C, 60.08; H, 9.11; N, 11.77.

Heptakis[6-(4-(2,2-bis-*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-octyl]cyclomaltoheptaose (14). To a solution of **10** (69 mg, 24 μmol) and **6** (63 mg, 0.18 mmol, 1.1 equiv) in ^{t}BuOH−H_2O (15:1, 3 mL) was added the Cu-supported catalyst **Si-BPA·Cu**$^+$ (5.4 mg), the reaction mixture was refluxed overnight, the solvent was removed, the residue was diluted with DCM, and the catalyst was filtered. After evaporation of the solvent, the residue was purified by column chromatography (30:1→9:1 DCM−MeOH): yield 109 mg (86%); R_f = 0.66 (9:1 DCM−MeOH); $[\alpha]_D$ = +19.6 (c 1.0 in DCM); ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (s, 7 H, =CH), 5.77 (d, 7 H, $J_{1,2}$ = 2.8 Hz, H-1), 4.99 (m, 14 H, H-6a, H-6b), 4.53 (m, 7 H, H-5), 4.18 (m, 7 H, OCHa), 4.03−3.90 (m, 28 H, OCH_b, CH_2-triazole, H-3), 3.87−3.81 (m, 14 H, OCH_2), 3.52 (t, 7 H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4), 3.34 (m, 35 H, CH_2NHBoc, H-2), 2.78 (t, 28 H, $^3J_{H,H}$ = 5.7 Hz, CH_2CH_2NHBoc), 1.88−1.81 (m, 28 H, OCH_2CH_2), 1.67 (s, 126 H, CMe_3), 1.65−1.50 (m, 168 H, $OCH_2CH_2(CH_2)_5$), 1.14 (t, 42 H, $^3J_{H,H}$ = 5.0 Hz); ^{13}C NMR (100.1 MHz, CD_3OD) δ 156.7 (CO carbamate), 143.5 (C-4 triazole), 126.8 (C-5 triazole), 97.6 (C-1), 79.6 (C-2, C-3), 79.3 (C-4), 78.6 (C_q), 74.0 (OCH_2), 71.6 (OCH_2), 69.9 (C-5), 53.0 (CH_2CH_2-NHBoc), 50.5 (C-6), 38.1 (CH_2NHBoc), 31.7, 31.6 ($CH_2CH_2CH_3$), 30.2, 30.0 (OCH_2CH_2), 29.7, 29.4 ($OCH_2CH_2CH_2CH_2$), 29.2, 29.2

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($CH_2CH_2CH_2CH_3$), 27.7 (CMe_3), 26.1, 25.9 ($och_2ch_2ch_2$), 22.5, 22.3 (CH_2CH_3). 13.2, 13.1 (CH_3); ESIMS m/z 1793.3 $[M + 2 K + Na]^{3+}$, 2658.4 $[M + 2 Na]^{2+}$. Anal. Calcd for $C_{273}H_{511}N_{42}O_{56}$: C, 62.12; H, 9.76; N, 11.15. Found C, 61.98; H, 9.70; N, 10.89.

Heptakis[6-(4-(2,2-bis-*tert*-butoxycarbonylamino)ethyl-aminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-dodecyl]-cyclomaltoheptaose (15). To a solution of **11** (40 mg, 11 μmol) and **6** (29 mg, 0.08 mmol, 1.1 equiv) in ^{t}BuOH−H_2O (7:1, 3 mL) was added the Cu-supported catalyst **Si-BPA·Cu**$^+$ (3 mg) , and the reaction mixture was refluxed overnight, the solvent was removed, the residue was diluted with DCM, and the catalyst was filtered. After evaporation of the solvent, the residue was purified by column chromatography (30:1→9:1 DCM−MeOH): yield 37.3 mg (56%); R_f = 0.53 (9:1 DCM−MeOH); $[\alpha]_D$ = +24.7 (c 0.9 in DCM); ^{1}H NMR (500 MHz, $CDCl_3$, 313 K) δ 7.98 (bs, 7 H, =CH), 5.42 (bs, 7 H, H-1), 5.12 (bm, 12 H, H-6a, H-6b), 4.26 (bm, 14 H, OCHa, H-5), 4.00−3.50 (bm, 28 H, OCHb, OCH_2, H-3), 3.40 (bm, 7 H, H-4), 3.14 (bs, 35 H, CH_2NHBoc, H-2), 2.60 (bs, 14 H CH_2CH_2NHBoc), 1.63 (bm, 28 H, OCH_2CH_2), 1.56−1.10 (bm, 252 H, $OCH_2CH_2(CH_2)_9$), 1.45 (s, 126 H, OMe_3), 0.91 (t, 42 H, $^3J_{H,H}$ = 6.6 Hz); ESIMS m/z 2041.2 $[M + 3 Na]^{3+}$, 3050.4 $[M + 2 Na]^{2+}$. Anal. Calcd for $C_{329}H_{616}N_{42}O_{56}$: C, 65.24; H, 10.25; N, 9.71. Found: C, 64.98; H, 10.04; N, 9.36.

Heptakis[6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-methyl]cyclomaltoheptaose Tetradecahydrochloride (16). Treatment of **12** (107 mg, 27 μmol) with TFA−DCM (1:1, 2.5 mL) at rt for 2 h followed by evaporation of the solvent and freeze-drying from a solution of 0.1 N HCl gave **16**: yield 80 mg (99%); $[\alpha]_D$ = +20.9 (c 0.9 in H_2O); ^{1}H NMR (500 MHz, D_2O) δ 8.14 (s, 7 H, =CH), 5.46 (s, 7 H, H-1), 4.96 (d, 7 H, $J_{6a,6b}$ = 13.4 Hz, H-6a), 4.75 (s, 7 H, H-6b), 4.31 (m, 7 H, H-5), 4.10 (m, 14 H, CH_2-triazole), 3.71 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.7 Hz, H-3), 3.53 (s, 21 H, OCH_3), 3.45 (m, 7 H, H-4), 3.37 (s, 21 H, OCH_3), 3.22 (t, 28 H, $^3J_{H,H}$ = 6.2 Hz, CH_2NHBoc), 3.05 (bd, 7 H, H-2), 2.98 (bt, 28 H, CH_2CH_2NHBoc); ^{13}C NMR (125.7 MHz, D_2O) δ 139.3 (C-4 triazole), 128.4 (C-5 triazole), 97.3 (C-1), 50.5−48.5 (C-2, C-3, C-4), 69.7 (C-5), 60.6 (OCH_3), 58.2 (OCH_3), 50.5 (C-6), 49.7 ($CH_2CH_2NH_2$), 46.01 (CH_2-triazole), 35.6 (CH_2NH_2); ESIMS m/z 832.5 $[M + 3 H]^{3+}$, 1248.3 $[M + 2 H]^{2+}$. Anal. Calcd for $C_{105}H_{210}Cl_{14}N_{42}O_{28}$: C, 41.96; H, 7.04; N, 19.57. Found: C, 41.64; H, 6.77; N, 19.31.

Heptakis[6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexyl]cyclomaltoheptaose Tetradecahydrochloride (17). Treatment of **13** (51.3 mg, 11 μmol) with 1:1 TFA−DCM (1 mL) at rt for 2 h followed by evaporation of the solvents and freeze-drying from a solution of 0.1 N HCl gave **17**: yield 40 mg (95%); $[\alpha]_D$ = +20.6 (c 1.2 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 313 K) δ 8.11 (s, 7 H, =CH), 5.59 (d, 7 H, $J_{1,2}$ = 3.1 Hz, H-1), 4.98 (dd, 7 H, $J_{6a,6b}$ = 15.1 Hz, $J_{6a,5}$ = 3.4 Hz, H-6a), 4.76 (dd, 7 H, H-6b), 4.37 (m, 7 H, H-5), 3.95 (m, 7 H, OCHa), 3.94−3.7 (2d, 14 H, $^2J_{H,H}$ = 15.5 Hz, CH_2-triazole), 3.77 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.6 Hz, H-3) 3.80−3.70 (m, 7 H, OCHb), 3.63 (m, 14 H, OCH_2), 3.34 (m, 7 H, H-4), 3.15 (t, 28 H, $^3J_{H,H}$ = 5.9 Hz, CH_2NH_2), 3.10 (dd, 7 H, H-2), 2.81 (m, 14 H, $CH_2CH_2NH_2$), 1.70−1.56 (m, 28 H, OCH_2CH_2), 1.50−1.30 (m, 84 H, $OCH_2CH_2(CH_2)_3$), 0.94 (t, 21 H, $^3J_{H,H}$ = 6.9 Hz), 0.93 (t, 21 H, $^3J_{H,H}$ = 6.9 Hz); ^{13}C NMR (125.7 MHz, CD_3OD) δ 144.0 (C-4 triazole), 129.3 (C-5 triazole), 99.9 (C-1), 82.0 (C-2, C-3), 81.3 (C-4), 76.3 (OCH_2) 72.2 (OCH_2), 70.0 (C-5), 52.9 (C-6), 52.5 ($CH_2CH_2NH_2$), 47.9 (CH_2-triazole), 39.1 (CH_2NH_2), 34.1, 33.9 ($CH_2CH_2CH_3$), 32.5, 32.3 (OCH_2CH_2), 28.0, 27.8 ($OCH_2CH_2CH_2$), 24.9, 24.7 (CH_2CH_3). 15.4 (CH_3); ESIMS m/z 870.2 $[M + 4 H]^{4+}$, 1159.9 $[M + 3 H]^{3+}$, 1739.3 $[M + 2 H]^{2+}$. Anal. Calcd for $C_{175}H_{357}Cl_{14}N_{42}O_{28}$: C, 52.62; H, 9.01; N, 14.73. Found: C, 52.34; H, 8.76; N, 14.51.

Heptakis[6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-octyl]cyclomaltoheptaose Tetradecahydrochloride (18). Treatment of **14** (52 mg, 10 μmol) with 1:1 TFA−DCM (1 mL) at rt for 2 h followed by evaporation of the solvents and freeze-drying from a solution of 0.1 N HCl gave **18**: yield 42.2 mg (96%); $[\alpha]_D$ = +19.6 (c 1.1 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 323 K) δ 8.13 (s, 7 H, =CH), 5.57 (d, 7 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.99 (dd, 7 H, $J_{6a,6b}$ = 15.1 Hz, $J_{6a,5}$ = 3.8 Hz, H-6a), 4.78 (dd, 7 H, $J_{6b,5}$ = 2.1 Hz, H-6b), 4.38−4.34 (m, 7 H, H-5), 3.97−3.93 (m, 7 H, OCHa), 3.90 (2d, 14 H, $^2J_{H,H}$ = 15.6 Hz, CH_2-triazole), 3.80−3.70 (m, 14 H, H-3, OCH_b), 3.68−3.59 (m, 14 H, OCH_2), 3.38 (t, 7 H, $J_{3,4} = J_{4,5}$ = 8.5 Hz, H-4), 3.16 (t, 28 H, $^3J_{H,H}$ = 5.9 Hz, CH_2NH_2), 3.11 (dd, 7 H, $J_{2,3}$ = 9.6 Hz, H-2), 2.84 (t, 14 H, $CH_2CH_2NH_2$), 1.69−1.59 (m, 28 H, OCH_2CH_2), 1.50−1.26 (m, 140 H, $OCH_2CH_2(CH_2)_5$), 0.93 (t, 42 H, $^3J_{H,H}$ = 7.1 Hz); ^{13}C NMR (125.7 MHz, CD_3OD) δ 143.4 (C-4 triazole), 129.4 (C-5 triazole), 99.9 (C-1), 82.1 (C-2, C-3), 81.5 (C-4), 76.4 (OCH_2) 73.9 (OCH_2), 72.3 (C-5), 52.9 (C-6), 52.6 ($CH_2CH_2NH_2$), 48.1 (CH_2-triazole), 39.1 (CH_2NH_2), 34.1 ($CH_2CH_2CH_3$), 32.6, 32.4 (OCH_2CH_2), 32.1−31.6 ($OCH_2CH_2(CH_2)_3$), 28.4, 28.3 (CH_2CH_3), 24.8 (CH_3); ESIMS m/z 774.0 $[M + 5 H]^{5+}$, 967.5 $[M + 4 H]^{4+}$, 1289.2 $[M + 3 H]^{3+}$. Anal. Calcd for $C_{203}H_{406}Cl_{14}N_{42}O_{28}$: C, 55.67; H, 9.34; N, 13.43. Found: C, 55.27; H, 9.22; N, 13.09.

Heptakis[6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-dodecyl]cyclomaltoheptaose Tetradecahydrochloride (19). Treatment of **15** (32 mg, 5 μmol) with 1:1 TFA−DCM (0.5 mL) at rt for 2 h followed by evaporation of the solvents and freeze-drying from a solution of 0.1 N HCl gave **19**: yield 28 mg (97%); $[\alpha]_D$ = +18.1 (c 0.9 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 323 K) δ 8.10 (s, 7 H, =CH), 5.55 (d, 7 H, $J_{1,2}$ = 2.8 Hz, H-1), 4.89 (bd, 7 H, $J_{6a,6b}$ = 13.2 Hz, H-6a), 4.77 (bd, 7 H, H-6b), 4.34 (m, 7 H, H-5), 3.96−3.87 (m, 7 H, OCHa), 3.88 (2d, 14 H, $^2J_{H,H}$ = 15.5 Hz, CH_2-triazole), 3.76 (t, 7 H, H-3), 3.80−3.60 (m, 21 H, OCH_b, OCH_2), 3.36 (t, 7 H, $J_{3,4} = J_{4,5}$ = 8.2 Hz, H-4), 3.15 (t, 28 H, $^3J_{H,H}$ = 5.8 Hz, CH_2NH_2), 3.10 (dd, 7 H, $J_{2,3}$ = 9.7 Hz, H-2), 2.83 (t, 14 H, $CH_2CH_2NH_2$), 1.69−1.59 (m, 28 H, OCH_2CH_2), 1.45−1.26 (m, 252 H, $OCH_2CH_2(CH_2)_9$), 0.93 (t, 42 H, $^3J_{H,H}$ = 6.7 Hz); ^{13}C NMR (125.7 MHz, CD_3OD): δ 143.9 (C-4 triazole), 129.4 (C-5 triazole), 99.9 (C-1), 82.1 (C-2, C-3), 81.5 (C-4), 76.3 (OCH_2) 73.8 (OCH_2), 72.3 (C-5), 52.8 (C-6), 52.6 ($CH_2CH_2NH_2$), 48.0 (CH_2-triazole), 39.1 (CH_2NH_2), 34.2 ($CH_2CH_2CH_3$), 32.7−31.7 ($OCH_2CH_2CH_2(CH_2)_6$), 28.6, 28.5 ($OCH_2CH_2CH_2$), 24.8 (CH_2CH_3), 15.6 (CH_3) ESIMS m/z 930.8 $[M + 5 H]^{5+}$, 1163.4 $[M + 4 H]^{4+}$, 1550.5 $[M + 3 H]^{3+}$. Anal. Calcd for $C_{259}H_{518}Cl_{14}N_{42}O_{28}$: C, 60.22; H, 10.11; N, 11.39. Found C, 59.82; H, 9.87; N, 11.16.

Heptakis[6-4-(*N*′-(2-(*N*-*tert*-butoxycarbonylaminoethyl)-thioureido)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (22). To a solution of **20** (70 mg, 21 μmol) and Et_3N (30 μL, 0.22 mmol, 1.5 equiv) in DCM (5 mL) was added *tert*-butyl *N*-(2-isothiocyanatoethyl)carbamate (**21**, 45 mg, 0.22 mmol, 1.5 equiv), and the reaction mixture was stirred at rt for 16 h. The reaction mixture was washed with aqueous diluted HCl (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (30:1→9:1 DCM−MeOH): yield 50 mg (53%); R_f = 0.62 (9:1 DCM−MeOH); $[\alpha]_D$ = +26.4 (c 1.0 in DCM); ^{1}H NMR (400 MHz, CD_3OD, 323 K) δ 7.96 (s, 7 H, =CH), 5.45 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.6 Hz, H-3), 5.40 (d, 7 H, $J_{1,2}$ = 2.9 Hz, H-1), 4.81 (dd, 14 H, H-2, H-6a), 4.71, 4.65 (2 d, 14 H, $^2J_{H,H}$ = 16.1 Hz, CH_2-triazole), 4.47 (m, 14 H, H-5, H-6b), 3.69 (t, 7 H, $J_{4,5}$ = 7.8 Hz, H-4), 3.53 (m, 14 H, CH_2CH_2NHBoc), 3.23 (t, 14 H, $^3J_{H,H}$ = 6.2 Hz, CH_2CH_2NHBoc), 2.48−2.20 (m, 28 H, CH_2CO), 1.67−1.59 (m, 28 H, CH_2CH_2CO), 1.45 (s, 63 H, CMe_3), 1.39−1.31 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.96−0.90 (t, 42 H, $^3J_{H,H}$ = 6.5 Hz); ^{13}C NMR (100.3 MHz, CD_3OD, 323 K) δ 184.4 (CS), 174.4, 173.4 (CO ester), 158.6 (CO carbamate), 146.1 (C-4 triazole), 127.2 (C-5 triazole), 98.1 (C-1), 80.3 (C_q), 78.9 (C-4), 71.2 (C-3, C-2, C-5), 51.8 (C-6), 45.3 (CH_2CH_2NHBoc), 41.0 (CH_2NHBoc), 40.5 (CH_2-triazole) 35.1, 34.9 (CH_2CO), 32.6, 32.4 ($CH_3CH_2CH_2$), 28.9 (CMe_3), 25.5 (CH_2CH_2CO), 23.5 (CH_3CH_2),

14.4 (CH_3); ESIMS m/z 1517.7 $[M + 3\ Na]^{3+}$, 2265.1 $[M + 2\ Na]^{2+}$. Anal. Calcd for $C_{203}H_{336}N_{42}O_{56}S_7$: C, 54.36; H, 7.55; N, 13.12; S, 5.00. Found C, 54.09; H, 7.31; N, 12.87; S, 4.56.

Heptakis[6-4-(*N*′-(2-aminoethylthioureido)methyl-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose Heptahydrochloride (23). Treatment of **22** (84 mg, 17 μmol) with 1:1 TFA−DCM (1 mL) at rt for 2 h followed by evaporation of the solvents and freeze-drying from a solution of 0.1 N HCl gave **23**: yield 55 mg (80%); $[\alpha]_D$ = +31.3 (*c* 0.8 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 323 K) δ 7.95 (s, 7 H, =CH), 5.49 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.5 Hz, H-3), 5.43 (d, 7 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.81 (dd, 14 H, H-2, H-6a), 4.78, 4.70 (2 d, 14 H, $^2J_{H,H}$ = 15.5 Hz, CH_2-triazole), 4.46 (m, 14 H, H-5, H-6b), 3.92−3.75 (m, 14 H, $CH_2CH_2NH_2$), 3.62 (t, 7 H, $J_{4,5}$ = 8.0 Hz, H-4), 3.22 (t, 14 H, $^3J_{H,H}$ = 6.1 Hz, $CH_2CH_2NH_2$), 2.48−2.20 (m, 28 H, CH_2CO), 1.74−1.59 (m, 28 H, CH_2CH_2CO), 1.41−1.30 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.95 (2 t, 42 H, $^3J_{H,H}$ = 7.0 Hz, $^3J_{H,H}$ = 6.4 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 323 K) δ 186.7 (CS), 175.9, 174.6 (CO ester), 147.4 (C-4 triazole), 128.3 (C-5 triazole), 99.2 (C-1), 79.9 (C-4), 72.7, 72.3 (C-3, C-2, C-5), 53.1 (C-6), 43.8 ($CH_2CH_2NH_2$), 42.1 (CH_2NH_2), 41.6 (CH_2-triazole), 36.4, 36.2 (CH_2CO), 33.9, 33.7 ($CH_3CH_2CH_2$), 26.8 (CH_2CH_2CO), 24.7 (CH_3CH_2), 15.6 (CH_3); ESIMS m/z 1262.2 $[M + 3\ H]^{3+}$ 1893.4 $[M + 2\ H]^{2+}$. Anal. Calcd for $C_{168}H_{287}Cl_7N_{42}O_{42}S_7$: C, 49.95; H, 7.16; N, 14.56; S, 5.56. Found C, 49.78; H, 7.0; N, 14.31; S, 5.24.

Heptakis[6-4-(1-(2-(bis(2-(*N*-*tert*-butoxycarbonyl)aminoethyl)amino)ethyl)thioureido)]methyl-1*H*-1,2,3-triazol-1yl-2,3-di-*O*-hexanoyl)-6-deoxy]cyclomaltoheptaose (25). To a solution of **20** (106 mg, 32 μmol) and Et_3N (45 μL, 0.33 mmol, 1.5 equiv) in DCM (5 mL) was added 2-[bis[2-(*tert*-butoxycarbonylamino)ethyl]-amine]ethylisothiocyanate (**24**, 130 mg, 0.33 mmol, 1.5 equiv), and the reaction mixture was stirred at rt for a week. The reaction mixture was washed with aqueous diluted HCl (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (30:1→9:1 DCM−MeOH): yield 94 mg (50%); R_f = 0.62 (9:1 DCM−MeOH); $[\alpha]_D$ = +29.0 (*c* 1.0 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 333 K) δ 7.96 (s, 7 H, =CH), 5.46 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.9 Hz, H-3), 5.41 (d, 7 H, $J_{1,2}$ = 2.8 Hz, H-1), 4.81 (dd, 14 H, H-2, H-6a), 4.74 (bs, 14 H, CH_2-triazole), 4.51 (m, 14 H, H-5, H-6b), 3.67 (t, 7 H, $J_{4,5}$ = 7.0 Hz, H-4), 3.51 (bs, 14 H, S=CNHCH_2), 3.14 (t, 28 H, $^3J_{H,H}$ = 6.1 Hz, CH_2NHBoc), 2.71 (t, 14 H, $^3J_{H,H}$ = 6.0 Hz, S=CNHCH$_2$CH_2), 2.63 (t, 28 H, CH_2CH_2NHBoc), 2.50−2.20 (m, 28 H, CH_2CO), 1.70−1.56 (m, 28 H, CH_2CH_2CO), 1.46 (s, 63 H, CMe_3), 1.42−1.30 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.96−0.93 (2 t, 42 H, $^3J_{H,H}$ = 6.9 Hz, $^3J_{H,H}$ = 6.8 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD, 323 K) δ 184.4 (CS), 173.0, 171.9 (CO ester), 157.0 (CO carbamate), 144.8 (C-4 triazole), 125.7 (C-5 triazole), 96.7 (C-1), 79.0 (C_q), 77.4 (C-4), 70.0 (C-3, C-2, C-5), 54.1 (CH_2CH_2NHBoc), 53.2 (S=CNHCH$_2$CH$_2$) 50.4 (C-6), 42.2 (S=CNHCH_2), 39.1 (CH_2-triazole), 38.7 (CH_2NHBoc), 33.7, 33.5 (CH_2CO), 31.2, 31.0 ($CH_3CH_2CH_2$), 27.6 (CMe_3), 24.1 (CH_2CH_2-CO), 22.0 (CH_3CH_2), 12.9 (CH_3); ESIMS m/z 2916.6 $[M + 2\ Na]^{2+}$. Anal. Calcd for $C_{266}H_{462}N_{56}O_{70}S_7$: C, 55.19; H, 8.04; N, 13.55; S, 3.88. Found: C, 55.28; H, 8.15; N, 13.19; S, 4.04.

Heptakis[6-4-(1-(2-(bis(2-aminoethyl)amino)ethyl)thioureido)]methyl-1*H*-1,2,3-triazol-1yl-2,3-di-*O*-hexanoyl)-6-deoxy]cyclomaltoheptaose Tetradecahydrochloride (26). Treatment of **25** (62 mg, 11 μmol) with 1:1 TFA−DCM (0.8 mL) at rt for 2 h followed by evaporation of the solvents and freeze-drying from a solution of 0.1 N HCl gave **26**: yield 60.5 mg (95%); $[\alpha]_D$ = +24.5 (*c* 0.7 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 333 K) δ 7.93 (s, 7 H, =CH), 5.47 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.6 Hz, H-3), 5.43 (bs, 7 H, H-1), 4.81 (bd, 14 H, H-2, H-6a), 4.74 (m, 14 H, CH_2-triazole), 4.40 (m, 14 H, H-5, H-6b), 3.73−3.52 (m, 21 H, H-4, S=CNHCH_2) 3.13 (t, 14 H, $^3J_{H,H}$ = 5.6 Hz, CH_2NH_2), 2.88 (t, 14 H, $CH_2CH_2NH_2$), 2.77 (bt, 14 H, $^3J_{H,H}$ = 5.9 Hz, S=CNHCH$_2$CH_2), 2.50−2.20 (m, 28 H, CH_2CO), 1.74−1.56 (m, 28 H, CH_2CH$_2$CO), 1.41−1.28 (m, 56 H, CH_2CH$_3$, CH_2CH$_2$CH$_3$), 0.94 (t, 42 H, $^3J_{H,H}$ = 6.7 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 333 K) δ 185.6 (C=S), 176.0, 174.5 (CO ester), 147.6 (C-4 triazole), 128.2 (C-5 triazole), 99.1 (C-1), 79.9 (C-4), 72.5 (C-3, C-2, C-5), 54.8 (S=CNH-CH_2CH_2), 53.8 ($CH_2CH_2NH_2$), 53.2 (C-6), 43.9 (S=CNHCH$_2$), 41.5 (CH_2-triazole), 39.8 (CH_2NH_2), 36.3, 36.2 (CH_2CO), 33.9, 33.7 (CH_3-CH_2CH_2), 26.8 (CH_2CH_2CO), 24.8 (CH_3CH_2), 15.6 (CH_3); ESIMS m/z 1097.6 $[M + 4\ H]^{4+}$ 1463.3 $[M + 3\ H]^{3+}$, 2194.6 $[M + 2\ H]^{2+}$. Anal. Calcd for $C_{196}H_{364}Cl_{14}N_{56}O_{42}S_7$: C, 54.36; H, 7.55; N, 13.12; S, 5.00. Found C, 47.75; H, 7.22; N, 15.74; S, 4.21.

Dendritic βCD Derivative 27. To a solution of **2** (50 mg, 12 μmol) and Et_3N (35 μL, 0.25 mmol, 1.5 equiv) in DCM (3 mL) was added *tert*-butyl *N*-(2-isothiocyanatoethyl)carbamate (**21**, 50 mg, 0.25 mmol, 1.5 equiv), and the reaction mixture was stirred at rt overnight. The solvent was removed, and the residue was purified by column chromatography (50:1→20:1 DCM−MeOH): yield 39 mg (50%); R_f = 0.56 (9:1 DCM−MeOH); $[\alpha]_D$ = +19.7 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CD_3OD, 333 K) δ 8.08 (s, 7 H, =CH), 5.50 (s, 7 H, H-1), 5.45 (t, 7 H, $J_{2,3} = J_{3,4}$ = 7.8 Hz, H-3), 4.98 (bd, 7 H, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.81 (dd, 14 H, $J_{1,2}$ = 2.6 Hz, H-2, H-6b), 4.58 (m, 7 H, H-5), 3.85 (2 d, 14 H, $^2J_{H,H}$ = 15.1 Hz, CH_2-triazole), 3.70 (t, 7 H, $J_{4,5}$ = 8.3 Hz, H-4), 3.60 (t, 28 H, $^3J_{H,H}$ = 5.6 Hz, CH_2CH_2NHC=S), 3.55 (m, 28 H, CH_2CH_2NHBoc), 3.28 (t, 28 H, CH_2CH_2NHC=S), 2.71 (t, 28 H, $^3J_{H,H}$ = 6.0 Hz, CH_2NHBoc), 2.50−2.20 (m, 28 H, CH_2CO), 1.70−1.55 (m, 28 H, CH_2CH_2CO), 1.48 (s, 126 H, CMe_3), 1.40−1.25 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.95 (2 t, 42 H, $^3J_{H,H}$ = 7.0 Hz, $^3J_{H,H}$ = 6.7 Hz, CH_3); ^{13}C NMR (100.3 MHz, CD_3OD, 313 K) δ 183.1 (CS), 174.0, 172.9 (CO ester), 158.1 (CO carbamate), 144.5 (C-4 triazole), 127.7 (C-5 triazole), 97.9 (C-1), 79.9 (C_q, C-4), 70.8 (C-3, C-2, C-5), 53.1 (CH_2NHBoc), 51.3 (C-6), 47.5,(CH_2-triazole), 47.6 (CH_2NHC=S), 42.7 (CH_2CH_2NHBoc), 40.6 (CH_2CH_2NHC=S), 34.6, 34.4 (CH_2CO), 32.1, 31.9 ($CH_3CH_2CH_2$), 28.5 (CMe_3), 25.1 (CH_2CH_2CO), 23.0 (CH_3CH_2), 13.9 (CH_3); ESIMS m/z 2195.4 $[M + 3\ Na]^{3+}$ 3274.1 $[M + 2\ Na]^{2+}$. Anal. Calcd for $C_{287}H_{504}N_{70}O_{70}S_{14}$: C, 53.00; H, 7.81; N, 15.07; S, 6.90. Found: C, 52.71; H, 7.53; N, 14.74; S, 6.56.

Dendritic paCD 28. Treatment of **27** (35 mg, 5 μmol) with 1:1 TFA−DCM (0.6 mL) at rt for 2 h followed by evaporation of the solvents and freeze-frying from a solution of 0.1 N HCl gave **28**: yield 28 mg (99%); $[\alpha]_D$ = +19.1 (*c* 1.0 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 333 K) δ 8.57 (s, 7 H, =CH), 5.47 (m, 14 H, H-1, H-3), 5.03 (bd, 7 H, $J_{6a,6b}$ = 13.1 Hz, H-6a), 4.81 (m, 28 H, $J_{1,2}$ = 2.6 Hz, $J_{2,3}$ = 9.2 Hz, H-2, H-6b, CH_2-triazole), 4.59 (m, 7 H, H-5), 4.07 (bs, 28 H, $CH_2CH_2NH_2$), 3.93 (t, 28 H, $^3J_{H,H}$ = 5.0 Hz, CH_2NH_2), 3.79 (t, 7 H, $J_{3,4} = J_{4,5}$ = 8.5 Hz, H-4), 3.59 (bs, 28 H, CH_2CH_2NHC=S), 3.30 (t, 28 H, $^3J_{H,H}$ = 5.9 Hz, CH_2-CH_2NHC=S), 2.50−2.20 (m, 28 H, CH_2CO), 1.70−1.55 (m, 28 H, CH_2CH_2CO), 1.40−1.25 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.95 (2 t, 42 H, $^3J_{H,H}$ = 7.0 Hz, $^3J_{H,H}$ = 6.8 Hz, CH_3); ^{13}C NMR (100.3 MHz, CD_3OD, 333 K) δ 183.9 (CS), 173.1, 171.9 (CO ester), 135.7 (C-4 triazole), 130.2 (C-5 triazole), 96.8 (C-1), 77.5 (C-4), 69.9 (C-3, C-2, C-5), 53.3 (CH_2NH_2), 50.6 (C-6), 47.0 (CH_2-triazole), 41.2 (CH_2-NHC=S), 39.3 ($CH_2CH_2NH_2$), 39.0 (CH_2CH_2NHC=S), 33.6, 33.5 (CH_2CO), 31.1, 30.9 ($CH_3CH_2CH_2$), 24.1 (CH_2CH_2CO), 21.9 (CH_3-CH_2), 12.7 (CH_3); ESIMS m/z 1021.34 $[M + 5\ H]^{5+}$ 1276.6 $[M + 4\ H]^{4+}$, 1701.5 $[M + 3\ H]^{3+}$. Anal. Calcd for $C_{217}H_{406}Cl_{14}N_{70}O_{42}S_{14}$: C, 46.43; H, 7.29; N, 17.47; S, 8.00. Found: C, 46.07; H, 7.03; N, 17.12; S, 7.61.

Preparation of Complexes Formulated from CD Derivatives and Plasmid pTG11236. The plasmid pTG11236 (pCMV-SV40-luciferase-SV40pA) used for the preparation of the DNA complexes and for transfection assay is a plasmid of 5739 bp (base pairs). The quantities of compound used were calculated according to the desired DNA concentration of 0.02 mg·mL^{-1} or 0.07 mg·mL^{-1} for gel electrophoresis experiments (i.e., 60 μM or 200 μM phosphate, respectively), the N/P ratio, the molar weight, and the number of protonable nitrogen atoms in the selected CD derivative or cationic polymer (Jet-PEI). For

the preparation of the DNA complexes from CD derivatives and Jet-PEI, DNA was diluted in HEPES (20 mM, pH 7.4) to a final concentration of 60 μM, and then the desired amount of CD derivative was added from 10 or 20 mM stock solution (DMSO) and Jet-PEI was added from a 0.1 M stock solution (H_2O). For JetPEI polyplexes, DNA was diluted in a 150 mM NaCl solution to a final phosphate concentration of 60 μM, and then the desired amount of Jet-PEI was added from a 7.5 mM NaCl solution. The preparation was vortexed for 2 h and used for characterization or transfection experiments (see the Supporting Information).

Measurement of the Size of the Complexes by Dynamic Light Scattering (DLS) and of the ζ-Potential. The average sizes of the CDplexes were measured using a Zetasizer nano with the following specification: sampling time, automatic; number of measurements, 3 per sample; medium viscosity, 1.054 cP; refractive index, 1.33; scattering angle, 173°; λ = 633 nm; temperature, 25 °C. Data were analyzed using the multimodal number distribution software included in the instrument. Results are given as volume distribution of the major population by the mean diameter with its standard deviation. Zeta-potential measurements on the CDplexes were made using the same apparatus with "mixed-mode measurement" phase analysis light scattering (M3-PALS). M3-PALS consists of both slow field reversal and fast field reversal measurements, hence the name "mixed-mode measurement"; it improves accuracy and resolution. The following specifications were applied: sampling time, automatic; number of measurements, 3 per sample; medium viscosity, 1.054 cP; medium dielectric constant, 80; temperature, 25 °C. Before each series of experiments, the performance of the instruments was checked with either a 90 nm monodisperse latex beads (Coulter) for DLS or with DTS 50 standard solution (Malvern) for ζ potentials.

Transmission Electron Microscopy (TEM). Formvar-carbon-coated grids previously made hydrophilic by glow discharge were placed on top of small drops of the CDplex samples (HEPES 20 mM, pH 7.4, DNA 60 μM phosphate) prepared as described above. After 1−3 min of contact, grids were negatively stained with a few drops of 1% aqueous solution of uranyl acetate. The grids were then dried and observed using an electron microscope working under standard conditions. All these experiments were reproduced twice on each formulation.

Agarose Gel Electrophoresis. Each CD derivative/DNA sample (20 μL, 0.4 μg of plasmid) was submitted to electrophoresis for about 30 min under 150 V through a 0.8% agarose gel in 1:1:1 tris(hydroxymethyl)-aminomethane (tris)/acetate/ethylenediaminetetraacetic acid (EDTA) (TAE) buffer and stained by ethidium bromide (1 μL of a 10 mg·mL^{-1} EB solution for 20 mL of gel). DNA was then visualized after photographing using an UV transilluminator.

CDplex Colloidal Stability Assays. CDplexes were prepared as described above in order to obtain a final DNA concentration of 60 μM after adding a volume (representing 10% of total volume) of a NaCl solution in HEPES (in order to obtain a final NaCl concentration of 0, 50, 150, and 250 mM) or of serum (in order to obtain a final serum content of 0 to 10%), and a volume (representing 2% of total volume) of a 100 μM EB solution in DMSO. After addition, each preparation was vortexed for 15 min before size (see above) and fluorescence measurements. For the EB exclusion assay by fluorescence, each CDplex formulation was arrayed in triplicate into a white flat-bottom 96-well plate. The EB fluorescence of each sample was measured with a luminometer (excitation 485 nm, emission 590 nm). The dissociation index (% DNA accessibility to EB) was determined by the following relationship:

$$\text{dissociation index} = (F_{\text{sample}} - F_{\text{blank}})/(F_{\text{DNAonly}} - F_{\text{blank}}) \times 100$$

In Vitro Transfection and Cell Viabilily. Twenty-four hours before transfection, BNL-CL2 cells were grown at a density of 2×10^4 cells per well in 96-well plates in Dulbelcco modified Eagle culture medium (DMEM) containing 10% fetal calf serum (FCS), 10 mg mL^{-1} gentamycin in a wet (37 °C) and 5% CO_2/95% air atmosphere. The above-described CD:pDNA (= pTG11236) complexes and JetPEI:pDNA polyplexes were diluted to 100 μL in DMEM or in DMEM supplemented with 10% FCS so as to have 0.5 μg of pDNA in the well (15 μM phosphate). The culture medium was removed and replaced by these 100 μL of the complexes. After 4 and 24 h, DMEM (50 and 100 μL) supplemented with 30% and 10% FCS, respectively, were added. After 48 h, the transfection was stopped, the culture medium was discarded, and the cells were washed twice with PBS (100 μL) and lysed with lysis buffer (50 μL). The lysates were frozen at −32 °C before the analysis of luciferase activity. This measurement was performed using a luminometer in dynamic mode, for 10 s on the lysis mixture (20 mL) and using the "luciferase" determination system in 96-well plates. The total protein concentration per well was determined by the BCA test. Luciferase activity was calculated as femtograms (fg) of luciferase per mg of protein. The percentage of cell viability was calculated as the ratio of the total protein amount per well of the transfected cells relative to that measured for untreated cells × 100. The data were calculated from three or four repetitions in two fully independent experiments (formulation and transfection).

Statistical Analysis. Analysis of variance (Anova) was run on the logarithmic transformation of transfection levels (log 10(fg luciferase per mg protein)) and on the cell viability to fit normal distributions of the data. Two factors, that is, the nature of the complexing agent (CD derivative and PEI) and the N/P ratio, were analyzed as the source of the variation of logarithmic transformation of the transfection levels and of cell variability percentages using a multiple comparison procedure. Tukey's honestly significant difference (HSD) method was used to discriminate among the means of cell viability percentages and the logarithmic transformation of luciferase expression levels.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details for nanoparticle characterization and biological evaluation tests, dissociation index of CDplexes in the presence of NaCl, BNL-CL2 cell viability during transfection experiments using CDplexes, tranfection efficiency of CDplexes in COS-7 cells, cell viability data, and copies of the ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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