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Novel Water-Soluble Carbosilane Dendrimers: Synthesis and Biocompatibility

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A synthetic strategy has been developed for the preparation of new peripheral amine- or ammonium-terminated carbosilane dendrimers of type *n*G-[Si($OCH_2CH_2NMe_2$)$_y$]$_x$ or *n*G-[Si($OCH_2CH_2NMe_3^+I^-$)$_y$]$_x$, respectively. It consists of the alcoholysis of well-known chlorosilane-terminated dendrimers with *N*,*N*-dimethylethanolamine and the subsequent quaternization with MeI. All these systems are susceptible to hydrolysis, although the decomposition depends on concentration and dendrimer generation. Evaluation of dendrimer toxicities by phase-contrast light microscopy and MTT assay were carried out, and evidence of dendrimer/oligonucleotide complex formation was carried out by gel electrophoresis.

Introduction

There is currently significant interest in dendrimers as a result of their potential applications, including light harvesting and energy transfer, nanoscale catalysis, chemical sensors, unimolecular micelles, enzyme mimics, encapsulation of guest molecules, molecular recognition, diagnostic agents, and gene and drug delivery.[1]

The two common drug delivery systems are liposomes and polymers, although both present some problems.[2] Liposome-based systems have poor stability, difficulty targeting specific tissues, side effects like lung-inflammatory reactions, and in transfection, processes may fail in the presence of serum. The main drawbacks of the use of conventional degradable polymers as delivery agents are their polydispersity and thermodynamic instability that result in a short in vivo lifetime of the active species. Dendrimers represent an alternative approach to liposomes and polymeric systems for drug delivery. Their major advantages are the uniform structure, multiple sites of attachment, and the versatility to modify their skeletons and surfaces, allowing a precise characterization of the dendrimer/drug interaction. There are two general methods for the use of dendrimers as drug carriers, that is (i) encapsulation of drugs inside the dendritic skeleton[3] or (ii) formation of dendrimer-drug conjugates in which the drug is attached preferentially to its surface. For the latter method, two methodologies have been developed. The first technique consists of a covalent link between peripheral units of the dendrimer and the drug. For instance, methotrexate or folic acid have been attached to the exterior of polyarylether[4] dendrimers or conjugated in poly(amidoimine) (PAMAM)[5] dendrimers. The second approach is based on electrostatic interactions and has been mainly focused on the delivery of DNA drugs into the cell nucleus for gene or antisense therapy. Numerous reports have been published on the use of amino-terminated PAMAM dendrimers,[6] phosphorus-based dendrimers,[7] polypropylenimine (PPI)[8] or poly(lysine)[9] dendrimers as nonviral gene-transfer agents.

However, to the best of our knowledge, no studies concerning the use of water-soluble carbosilane-based dendrimers as potential drug carriers have been published, although an in vitro biocompatibility report was recently published on poly(ethylene oxide)-grafted carbosilane dendrimers.[10] Besides, a scarce number of polyionic silane dendrimers have been reported so far.[11–13]

Here we describe the synthesis and characterization of novel water-soluble carbosilane dendrimers up to the third generation, and the analysis of their biocompatibility in primary cell cultures of peripheral blood mononuclear cells (PBMCs).

Results and Discussion

Amine-Terminated Carbosilane Dendrimers

We have studied the synthesis of dendrimers containing peripheral amine groups. For this purpose, first-, second-,

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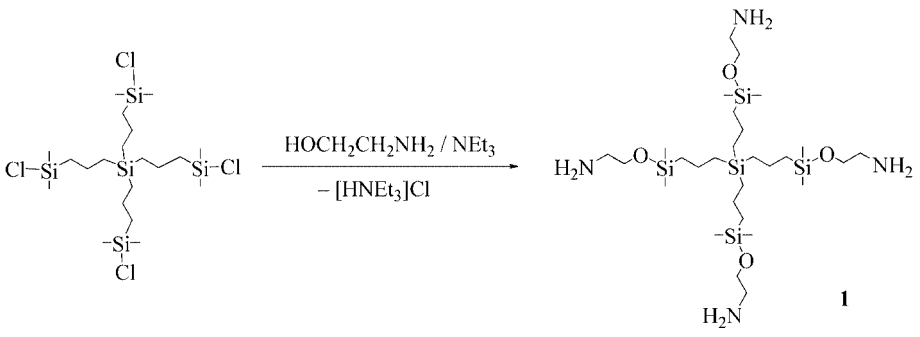
and third-generation chlorosilane-terminated dendrimers were synthesized as previously reported.[14] Typically, we used tetraallylsilane or other allylsilane-terminated dendrimers as the initiator core and chlorodimethylsilane or dichloromethylsilane for the hydrosilylation step in the presence of a platinum catalyst (Karstedt catalyst),[15] to afford the well-known Cl–Si-terminated dendrimers 1G-$(SiCl)_4$, 1G-$(SiCl_2)_4$, 2G-$(SiCl)_8$, 2G-$(SiCl_2)_8$, 3G-$(SiCl)_{16}$, and 3G-$(SiCl_2)_{16}$. These formed the starting materials for the preparation of new dendrimers by alcoholysis reactions.

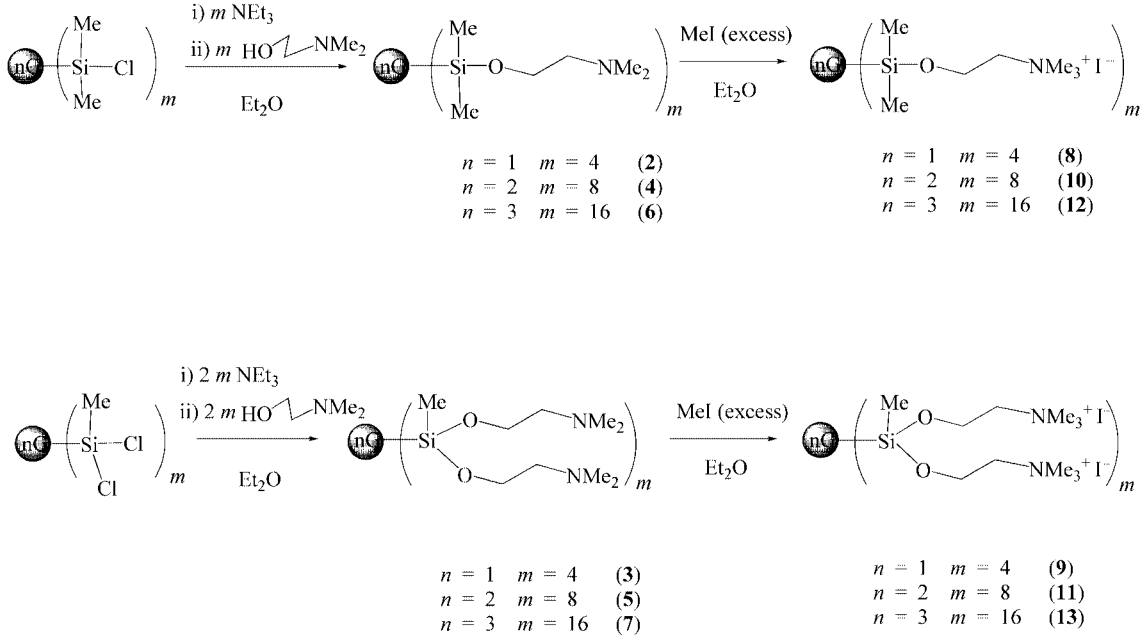
Treatment of dendrimer 1G-$(SiCl)_4$ with 4 equiv. of ethanolamine at room temperature in diethyl ether and in the presence of NEt_3 led to the formation of the corresponding amine-terminated dendrimer 1G-[$Si(OCH_2CH_2NH_2)]_4$ (**1**), which was isolated as a brown oil after separation from the precipitated ammonium salt $[HNEt_3]Cl$ and evaporation of the solvent (Scheme 1). In the absence of NEt_3, the dendrimer precipitated as a white solid material that we initially assigned to the self-quaternization of the amine groups in **1** because addition of NEt_3 to this white material in DMSO dissolved it to afford **1** again as the sole spectroscopic compound. However, when we tried to dissolve this material in water, an insoluble residue of polysiloxanes and the soluble ammonium salt of ethanolamine were separated. The data of the ^1H NMR spectrum performed in D_2O are consistent with the hydrolysis of the Si–O bonds under these slight acidic conditions. Derivative **1** exhibits a multiplet in the ^1H NMR spectrum located at δ = 1.32 ppm, attributed to the middle methylene group of the branch –$SiCH_2CH_2CH_2Si$–, and the two signals at δ = 0.63 and 0.53 ppm are assigned to the methylene groups directly bonded to silicon. The outer branch gives two triplets at δ = 3.55 and 2.74 ppm, corresponding to –CH_2O– and –CH_2N–. The ^{13}C NMR spectroscopic data are consistent with this assignment. Water-solubilization of dendrimer **1** was attempted by quaternization of the terminal amine groups. Reaction of **1** with a diethyl ether solution of HCl produced the hydrolytic breaking of the Si–O bonds, in accordance with the reactivity mentioned earlier. The attempt to quaternize derivative **1** using MeI also failed because of the hydrolysis of the Si–O bonds caused by the HI generated by Me/H exchanges with the amine protons.[16]

To overcome these problems, chlorosilane-terminated dendrimers were treated with stoichiometric amounts of *N*,*N*-dimethylethanolamine in diethyl ether and in the presence of stoichiometric NEt_3, to afford the corresponding amine-terminated dendrimers 1G-[$Si(OCH_2CH_2NMe_2)]_4$ (**2**), 1G-[$Si(OCH_2CH_2NMe_2)_2]_4$ (**3**), 2G-[$Si(OCH_2CH_2NMe_2)]_8$ (**4**), 2G-[$Si(OCH_2CH_2NMe_2)_2]_8$ (**5**), 3G-[$Si(OCH_2CH_2NMe_2)]_{16}$ (**6**), and 3G-[$Si(OCH_2CH_2NMe_2)_2]_{16}$ (**7**) as brown oils in high yields (Scheme 2). All these derivatives are soluble in common organic solvents, but are insoluble in water.

The NMR spectroscopic and analytical data for derivatives **2**–**7** are consistent with their proposed structures (Scheme 2). The ^1H NMR spectra of the carbosilane framework for dendrimers **2**–**7** have almost identical chemical shifts for analogous nuclei in different generations, although broader and less structured resonances are present with increasing generation. These features have been ascribed to both a polymer-like structure with slightly different chemical environments for the nuclei in different generations and restricted mobility of the respective protons in the outer shells.[17,18] Five sets of signals attributed to the methylene groups are observed with the expected integration ratio. For the $SiCH_2CH_2CH_2Si$ branches, the middle methylenes are located at δ = 1.30 ppm, whilst the methylene groups bonded directly to silicon atoms are centered at δ = 0.61 and 0.51 ppm. We ascribed the resonances at δ = 0.61 ppm to the –CH_2SiO– groups and those at δ = 0.51 ppm to the rest of the methylene groups, based on the enlargement of the latter signal intensity with increasing dendrimer generation, and also on 1D ^1H TOCSY and NOESY experiments. Two triplets are observed for the $SiOCH_2CH_2N$ fragment, located at δ = 3.64 (for dendrimers **2**, **4**, and **6**) or 3.74 ppm (for dendrimers **3**, **5**, and **7**) attributed to the –CH_2O– groups, and δ = 2.43 ppm for the –CH_2N– groups; this observation is supported by data from NOESY experiments. Interestingly, the downfield shifts shown by dendrimers **3**, **5**, and **7** are consistent with the presence of two oxygen atoms bonded to the silicon atom, although this effect is negligible for the –CH_2N– groups. The ^{13}C NMR spectra for the methylene groups show two resonances located at δ = 61.4 (–CH_2N–) and 60.7 ppm (–CH_2O–) for the outer



Scheme 1.



Scheme 2.

$SiOCH_2CH_2N$ branches, and for the inner $SiCH_2CH_2CH_2Si$ branches, signals in the range of δ = 21.3 to 17.4 ppm are observed. HMQC experiments were required to locate their resonances. For the N–Me groups, the signals in the ^{1}H- and ^{13}C NMR spectra show unchanged resonances at δ = 2.23 and 46.1 ppm, respectively. Both the $–SiMe_2–$ and –SiMe– fragments can be easily distinguished in all derivatives and generations (see Exp. Sect.). It is worth noting that for the outer –SiMe– groups the resonance appears about δ = –1.7 ppm in dendrimers **2**, **4**, and **6**, whilst it is located around δ = –4.4 ppm for dendrimers **3**, **5**, and **7**, as a consequence, again, of the presence of one or two silicon-bonded oxygen atoms, respectively. This feature is clearly observed in the ^{29}Si NMR spectra, although the innermost silicon atom is only detected for the first generations.

Dendrimers were also analyzed by mass spectroscopy (electrospray or MALDI-TOF MS) using 1,8,9-trihydroxyanthracene (dithranol) as a matrix. However, the molecular peaks were not observed for the second and third generations, as described for many high-molecular-weight dendrimers.[19]

Ammonium-Terminated Carbosilane Dendrimers

Attempts to quaternize the amine-terminated carbosilane dendrimers **2**–**7** with stoichiometric amounts or an excess of HCl failed because of hydrolysis of the Si–O bonds. However, treatment of the dendrimers with an excess of MeI in diethyl ether led to quantitative quaternization of the amine groups within 24–48 h, causing the precipitation of the ammonium iodide salts 1G-$[Si(OCH_2CH_2NMe_3^+I^-)]_4$ (**8**), 1G-$[Si(OCH_2CH_2NMe_3^+I^-)_2]_4$ (**9**), 2G-$[Si(OCH_2CH_2NMe_3^+I^-)]_8$ (**10**), 2G-$[Si(OCH_2CH_2NMe_3^+I^-)_2]_8$ (**11**), 3G-$[Si(OCH_2CH_2NMe_3^+I^-)]_{16}$ (**12**), and 3G-$[Si(OCH_2CH_2NMe_3^+I^-)_2]_{16}$ (**13**) in high yields as white hygroscopic solids (Scheme 2). In the case of the amine-terminated dendrimer **7**, some dimethylamine groups remained unquaternized. The ^{1}H NMR spectrum revealed that roughly 29 of the 32 amine groups were quaternized, even if we used a large excess of MeI and prolonged reaction times. All the dendritic ionic derivatives are insoluble in normal organic solvents, but are soluble in DMSO, MeOH, and H_2O, although slow decomposition occurs in protic solvents (see later for decomposition behavior).

The NMR spectroscopic and analytical data of derivatives **8**–**13** are consistent with their proposed structures (Scheme 2 and Figure 1). The ^{1}H NMR spectra were recorded in DMSO at room temperature, although in this solvent the line widths of these spectra tended to be broader than those of derivatives soluble in common organic solvents. The ^{1}H- and ^{13}C NMR spectra of the quaternized dendrimers **8**–**13** exhibit identical resonance patterns to those observed in their counterparts **2**–**7** for the carbosilane framework, although broader signals are seen with increasing generation (see Exp. Sect. and Supporting Information). Two broad multiplets are observed for the outer $SiOCH_2CH_2N$ grouping, centered at δ = 3.94 (for dendrimers **8**, **10**, and **12**) or 4.12 ppm (for dendrimers **9**, **11**, and **13**) for the $–CH_2O–$ groups, and δ = 3.45 (for dendrimers **8**, **10**, and **12**) or 3.56 ppm (for dendrimers **9**, **11**, and **13**) for the $–CH_2N–$ fragment. The quaternization of the amine groups resulted in a deshielding of the chemical shifts of the $–CH_2O–$ groups by 0.3–0.4 ppm, whereas for the $–CH_2N–$

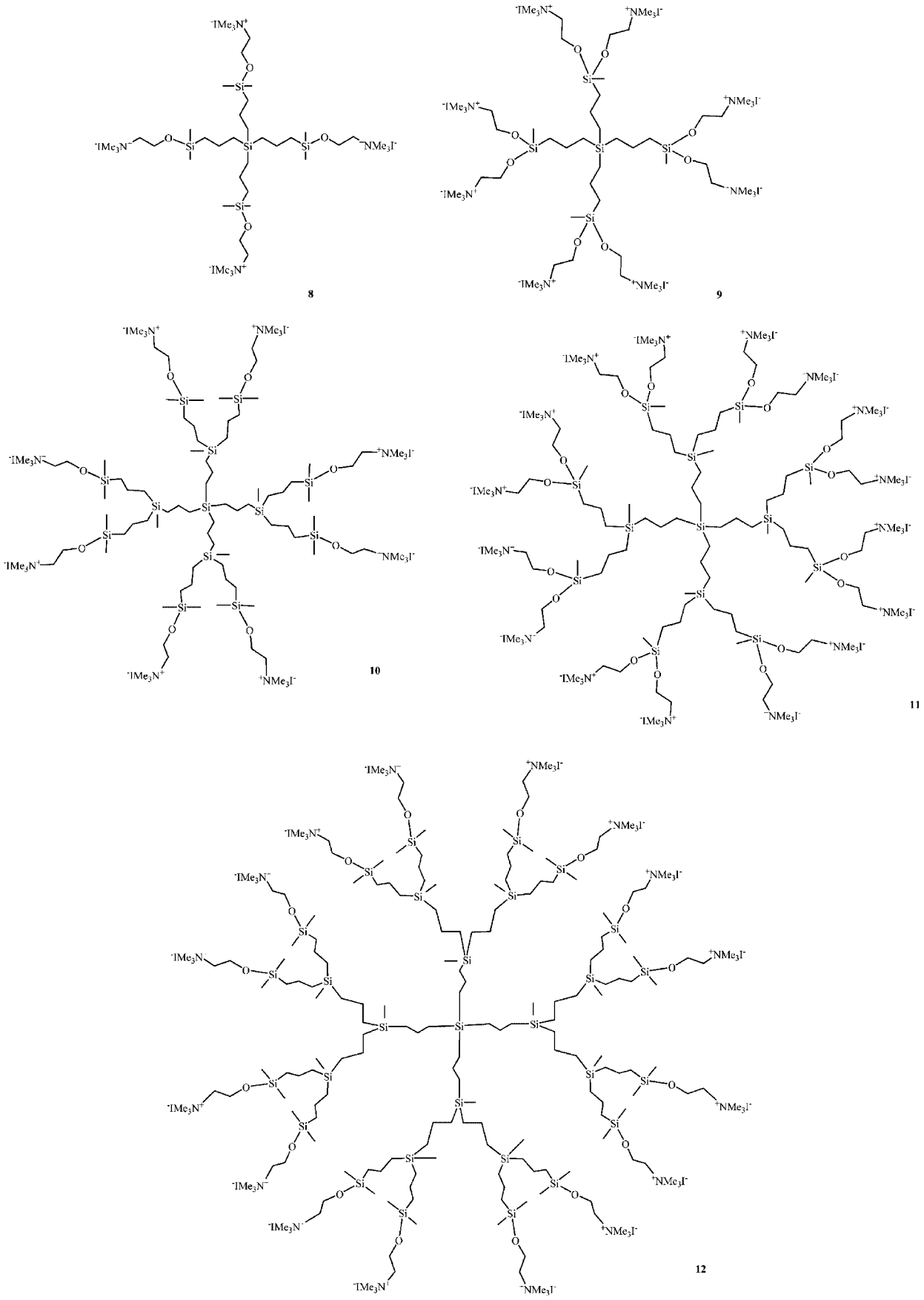


Figure 1. Molecular representations of the ammonium-terminated carbosilane dendrimers **8**–**12**.

methylene groups this shift was about 1 ppm, consistent with the presence of a positive charge on the nitrogen atom. Analogous shifts are observed for the carbon atoms in their ^{13}C NMR spectra. This behavior is also detected in the methyl groups, which appear in the ^{1}H- and ^{13}C NMR spectra centered at δ = 3.18 and 52.6 ppm, respectively, downfield of those observed in the amine-terminated dendrimers **2**–**7**.

Hydrolysis Behavior of Amine- and Ammonium-Terminated Dendrimers

As mentioned earlier, the amine-terminated dendrimers and the quaternized derivatives decompose in protic solvents. For instance, when dendrimers **3** or **9** are dissolved in methanol for several hours, after extraction with diethyl ether, a new dendrimer is formed, 1G-[Si(OMe)$_2$]$_4$, consistent with the alcoholysis of the Si–O bonds, the inclusion of methoxy groups,[20] and the respective formation of ethanolamine or its ammonium salt.

The hydrolysis of the second- and third-generation ammonium-terminated dendrimers **10** and **12** was studied in more detail. Both dendrimers degrade in deuterated water to form [DOCH$_2$CH$_2$NMe$_3^+$I$^-$] and an insoluble polymeric carbosiloxane material. The changes in dendrimer concentration were measured by ^{1}H NMR spectroscopy on the basis of the integration of both –OCH$_2$CH$_2$N– methylene proton resonances, which were always at pD = 7.2. A plot of ln[dendrimer] versus time showed an apparently first-order decomposition (see Figure 2). However, when the plot was performed using different initial dendrimer concentrations (see Supporting Information) the results showed that the rate constant decreases with increasing dendrimer concentration. The same decrease is observed on going from the second to the third generation when the hydrolytic processes were carried out at the same pD and initial concentrations in [Si–O] bonds, suggesting the existence of a dendritic effect (Figure 2). A plausible explanation for all these features may be the presence of interactions between branches in a cooperative effect that may decrease the rate of the process, making the decomposition a result of complex behavior.

Evaluation of Dendrimer Toxicity

Quaternized second-generation carbosilane dendrimers **10** and **11** were tested on a primary cell culture of PBMCs (from healthy donors) as an initial screen for biocompatibility. Dendrimers of first generation were too water-sensitive for toxicity evaluation, while those of third generation were not tested because of solubility problems. As a primary cell culture, PBMCs are more sensitive to external challenge than immortalized cell lines; thus, they are good models in which to perform toxicity studies. Furthermore, there are a number of diseases that affect these cells (for example leukemia, viral infections such as HIV or HTLV, genetic disorders such as Severe Combined Immunodeficiency (SCID), and so on), and they are also a major target for immunomodulation. Additionally, PBMCs placed in the bloodstream are easily accessible to systemic drugs. In order to evaluate the range of biocompatibility of dendrimers **10** and **11**, PBMCs were incubated for 48 h with increased concentrations of free dendrimer (1, 5, 10, 20, and 100 μM). Untreated cells were used as a control for viability. After this period of incubation, cells were observed under a phase-contrast light microscope. In parallel, mitocondrial activity (MA) of cells challenged with dendrimers was evaluated by MTT test and compared with that shown by the control cells. MTT results demonstrated a similar MA in cells treated with both second-generation dendrimers **10** and **11** at the different concentrations tested (Figure 3). At the concentration of 1 μM, MA was around 80% of that shown by the control, while at 5 μM, MA decreased to 30%. Observation of cells under phase-contrast light microscopy showed that cells treated with concentrations from 1 to 5 μM of the dendrimers were alive, with no visible differences from control cells. Cells treated with concentrations of 10 μM or higher exhibited reduced membrane birefringence and a concentration-dependent increase in mortality, and even more, at these concentrations, formation of cell aggregates was observed with dendrimer **11**, probably caused by the presence of a higher cationic charge on its surface (not only dendrimers, but cationic macromolecules in general cause destabilization of the cell membrane).[1j–21] Overall, the results from the MTT test and the microscopic observa-

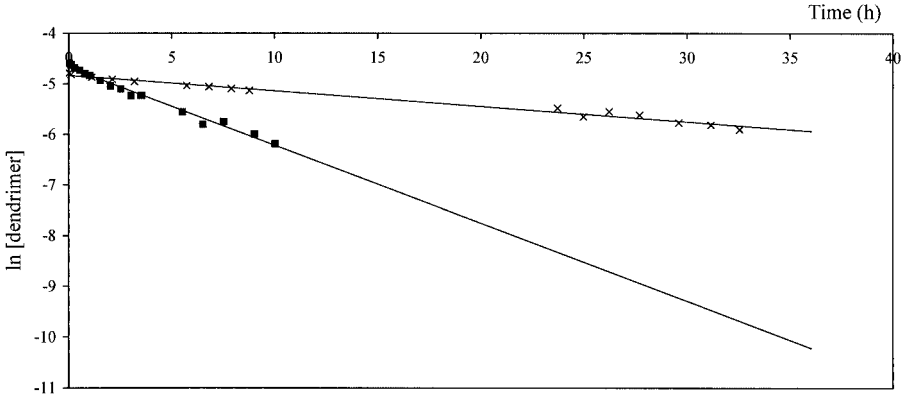


Figure 2. Plot of the hydrolysis process of the ammonium-terminated carbosilane dendrimers of second generation **10** (filled squares) and third generation **12** (crosses), at the same initial concentration.

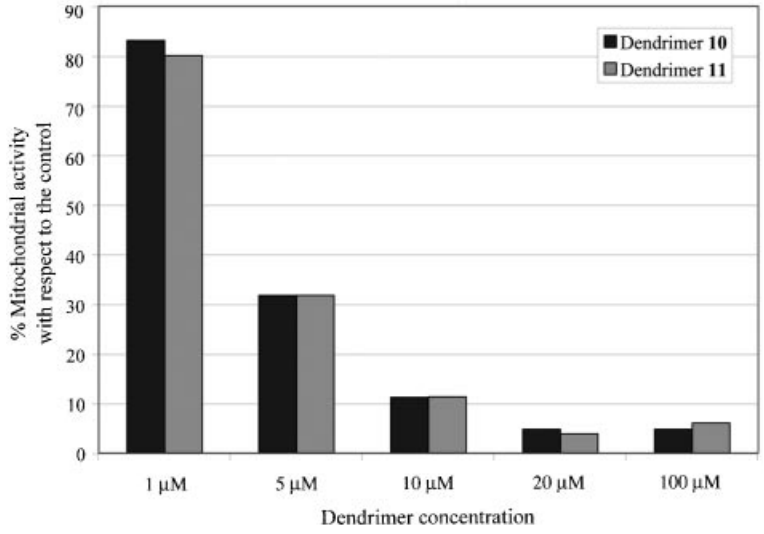


Figure 3. Quantification of mitochondrial activity of cells by MTT test after 48 h of incubation with different concentrations of the carbosilane dendrimers **10** and **11**.

tions lead to the conclusion that the biocompatible concentrations for both dendrimers are between 1 and 5 μM.

A preliminary study of the capacity of these dendrimers in the complexation with the nucleic material to give the so-called dendriplexes was performed by electrophoretic analysis. The technique was carried out in agarose gel using dendrimer **10** and a fluoresceinated phosphorothioate oligodeoxynucleotide (ODN) at different electrostatic charge ratios (+)/(–) (see Figure 4). The retardation of the ODN migration observed in the gel electrophoresis indicates that the ODN is associated with the dendrimer demonstrating a successful complex formation, even at 2:1 charge ratio in which the dendrimer concentration is in the range of biocompatibility mentioned earlier. Because of the hydrolytic problems shown by the carbosilane dendrimers with Si–O bonds, an experiment was performed to ensure that the quaternized dendrimers are responsible for the ODN retardation, rather than charged terminal functional groups detached from the dendritic structure. To this end, mixtures of ODN with the terminal groups [$HOCH_2CH_2NMe_3^+I^-$] alone were subjected to electrophoresis and retardation of the ODN assessed (see also Figure 4). The results clearly show that the terminal branches alone were not able to retard ODN migration, indicating that the whole functionalized dendrimer was necessary to form complexes with ODN. The dendriplexes released the ODN progressively between 6–24 h, which is adequate time for some biomedical applications.

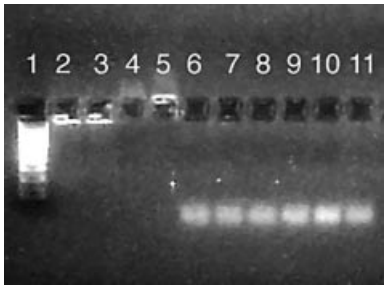


Figure 4. Electrophoresis of dendrimer **10** or [$HOCH_2CH_2NMe_3^+I^-$] and ODN on a 3% agarose gel: (1) a 100 bp DNA ladder as reference; (2) **10**/ODN ratio (+)/(–), 2:1; (3) **10**/ODN ratio (+)/(–), 4:1; (4) **10**/ODN ratio (+)/(–), 20:1; (5) **10**/ODN ratio (+)/(–), 40:1; (6) ODN only; (7) [$HOCH_2CH_2NMe_3^+I^-$]/ODN ratio (+)/(–), 2:1; (8) [$HOCH_2CH_2NMe_3^+I^-$]/ODN ratio (+)/(–), 4:1; (9) [$HOCH_2CH_2NMe_3^+I^-$]/ODN ratio (+)/(–), 20:1; (10) [$HOCH_2CH_2NMe_3^+I^-$]/ODN ratio (+)/(–), 40:1; (11) ODN only.

Conclusion

A synthetic strategy has been developed for the preparation of new peripheral amine- or ammonium-terminated carbosilane dendrimers. It consists of the alcoholysis of well-known chlorosilane-terminated dendrimers with *N*,*N*-dimethylethanolamine and the subsequent quaternization reaction with MeI. This procedure was shown to be suitable for the growth of high generations, although partial quaternization was observed in the more crowded third-generation dendrimer **7**. Evaluation of dendrimer toxicity by phase-contrast light microscopy and MTT assay revealed that the second-generation dendrimers **10** and **11** show good toxicity profiles in primary cell culture models over extended periods for concentrations between 1 and 5 μM, making them attractive for potential use as biocompatible drug carriers. All these systems are sensitive towards hydrolysis by breaking of the Si–O bonds with the subsequent liberation of their peripheral units. This feature may have a detrimental effect on the dendrimer properties, however, such a process could be useful in the design of carriers for controlled release of drugs. In addition, the capacity of dendrimer/oligonucleotide complex formation was confirmed by electrophoresis. These experiments open the way to use these dendritic macromolecules as drug delivery systems by an electrostatic approach and their posterior release by means of the hydrolytic process. Further studies are in progress to modify the external ammonium motif of the carbosilane dendrimers in order to control their stability towards hydrolysis.

Experimental Section

General: All manipulations of oxygen- or water-sensitive compounds were carried out under argon using standard Schlenk techniques or an argon-filled glovebox. Solvents were dried and freshly distilled under argon prior to use: hexane from sodium-potassium, toluene from sodium, tetrahydrofuran and diethyl ether from sodium benzophenone ketyl, and dichloromethane from P_4O_{10}. Unless otherwise stated, reagents were obtained from commercial sources and used as received. *n*G-$(SiCl_y)_x$ dendrimers were prepared according to reported methods.[14]

^{1}H-, ^{13}C-, and ^{29}Si NMR spectra were recorded with Varian Unity VXR-300 and Varian 500 Plus Instruments. Chemical shifts (δ, ppm) were measured relative to residual ^{1}H and ^{13}C resonances for [D_1]chloroform and [D_6]dimethyl sulfoxide which were used as solvents, and ^{29}Si chemical shifts were referenced to external $SiMe_4$ (δ = 0.00 ppm). The integral values of the signals in the ^{1}H NMR spectra of dendrimer complexes represent only 25% of the total amount of hydrogen atoms. C, H, and N analyses were carried out with a Perkin–Elmer 240 C microanalyzer. MALDI-TOF MS samples were prepared in a 1,8,9-trihydroxyanthracene (dithranol) matrix, and spectra were recorded with a Bruker Reflex II spectrometer equipped with a nitrogen laser emitting at 337 nm and operated in the reflector mode at an accelerating voltage in the range 23000–25000 V.

Synthesis of 1G-[Si(OCH$_2$CH$_2$NH$_2$)]$_4$ (1): To a diethyl ether solution (50 mL) of the first-generation chloro-terminated dendrimer 1G-$(SiCl)_4$ (0.35 g, 0.61 mmol) were added a slight excess of NEt_3 (0.40 mL, 2.86 mmol) and ethanolamine (0.16 mL, 2.44 mmol). The reaction mixture was stirred for 12 h at room temp. and then evaporated to dryness to remove residual NEt_3. The residue was extracted with Et_2O (50 mL) and filtered through Celite to remove the ammonium salt NEt_3·HCl. The resulting solution was evaporated under reduced pressure to give **1** as a brown oil (0.21 g, 51%). ^{1}H NMR ($CDCl_3$): δ = 3.55 (t, 2 H, CH_2O), 2.74 (t, 2 H, CH_2N), 1.46 (s, 2 H, NH_2), 1.32 (m, 2 H, SiCH$_2$CH_2CH$_2$SiO), 0.63 (m, 2 H, CH_2SiO), 0.53 (m, 2 H, CH_2Si), 0.07 (s, 6 H, OSiMe_2) ppm. ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 64.8 (*C*H$_2$N), 44.3 (*C*H$_2$O), 21.3 (*C*H$_2$SiO), 17.9, 17.2 (Si*C*H$_2$*C*H$_2$CH$_2$SiO), –1.9 (OSiMe_2) ppm. $C_{28}H_{72}N_4O_4Si_5$ (668.4): calcd. C 50.27, H 10.77, N 8.38; found C 51.01, H 11.03, N 8.06.

Synthesis of 1G-[Si(OCH$_2$CH$_2$NMe$_2$)]$_4$ (2): To a diethyl ether (40 mL) solution of the first-generation chloro-terminated dendrimer 1G-$(SiCl)_4$ (0.85 g, 1.49 mmol) were added a slight excess of NEt_3 (0.86 mL, 6.2 mmol) and *N*,*N*-dimethylethanolamine (0.6 mL, 5.97 mmol). The reaction mixture was stirred for 1 h at room temp. and then evaporated to dryness to remove residual NEt_3. The residue was extracted with Et_2O (30 mL) and filtered through Celite to remove the ammonium salt NEt_3·HCl. The resulting solution was evaporated under reduced pressure to give **2** as a pale yellow oil (0.98 g, 84%). ^{1}H NMR ($CDCl_3$): δ = 3.64 (t, 2 H, CH_2O), 2.40 (t, 2 H, CH_2N), 2.22 (s, 6 H, NMe_2), 1.31 (m, 2 H, SiCH$_2$CH_2CH$_2$SiO), 0.60 (m, 2 H, CH_2SiO), 0.53 (m, 2 H, CH_2Si), 0.07 (s, 6 H, OSiMe_2) ppm. ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 61.5 (*C*H$_2$N), 60.8 (*C*H$_2$O), 46.1 (N*Me*$_2$), 21.3 (*C*H$_2$SiO), 18.1, 17.4 (Si*C*H$_2$*C*H$_2$CH$_2$SiO), –1.7 (OSiMe_2) ppm. ^{29}Si{^{1}H} NMR ($CDCl_3$): δ = 0.49 (G_0-Si), 17.62 (G_1-Si) ppm. $C_{36}H_{88}N_4O_4Si_5$ (780.4): calcd. C 55.36, H 11.28, N 7.17; found C 55.16, H 11.22, N 7.06.

Synthesis of 1G-[Si(OCH$_2$CH$_2$NMe$_2$)$_2$]$_4$ (3): This dendrimer was prepared using a similar method to that described for **2**, starting from 1G-$(SiCl_2)_4$ (0.54 g, 0.87 mmol), *N*,*N*-dimethylethanolamine (0.7 mL, 6.94 mmol), and NEt_3 (1.0 mL, 7.2 mmol) to obtain compound **3** as a colorless oil (0.75 g, 80%). ^{1}H NMR ($CDCl_3$): δ = 3.74 (t, 4 H, CH_2O), 2.43 (t, 4 H, CH_2N), 2.23 (s, 12 H, NMe_2), 1.31 (m, 2 H, SiCH$_2$CH_2CH$_2$SiO), 0.63 (m, 2 H, CH_2SiO), 0.52 (m, 2 H, CH_2Si), 0.09 (s, 3 H, OSi*Me*) ppm. ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 61.4 (*C*H$_2$N), 60.7 (*C*H$_2$O), 46.2 (N*Me*$_2$), 18.7 (*C*H$_2$SiO), 17.7, 17.2 (Si*C*H$_2$*C*H$_2$CH$_2$SiO), –4.4 (OSi*Me*) ppm. ^{29}Si{^{1}H} NMR ($CDCl_3$): δ = 0.47 (G_0-Si), –3.65 (G_1-Si) ppm. $C_{48}H_{116}N_8O_8Si_5$ (1072.4): calcd. C 53.71, H 10.82, N 10.44; found C 53.54, H 11.33, N 10.06. MALDI-TOF MS: *m*/*z* 1095.8 [M + H]$^+$ (calcd. 1095.8).

Synthesis of 2G-[Si(OCH$_2$CH$_2$NMe$_2$)]$_8$ (4): This dendrimer was prepared using a similar method to that described for **2**, starting from 2G-$(SiCl)_8$ (0.27 g, 0.18 mmol), *N*,*N*-dimethylethanolamine (0.15 mL, 1.47 mmol), and NEt_3 (0.25 mL, 1.79 mmol) to obtain compound **4** as a pale yellow oil (0.31 g, 90%). ^{1}H NMR ($CDCl_3$): δ = 3.64 (t, 4 H, CH_2O), 2.41 (t, 4 H, CH_2N), 2.23 (s, 12 H, NMe_2), 1.30 (m, 6 H, SiCH$_2$CH_2CH$_2$SiO and SiCH$_2$CH_2CH$_2$Si overlapping), 0.65 (m, 4 H, CH_2SiO), 0.53 (m, 8 H, rest of CH_2Si), 0.07 (s, 12 H, OSiMe_2), –0.09 (s, 3 H, Si*Me*) ppm. ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 61.5 (*C*H$_2$N), 60.8 (*C*H$_2$O), 46.1 (N*Me*$_2$), 21.1 (*C*H$_2$SiO), 18.6, 17.9 and overlapped signals (Si*C*H$_2$C*H*$_2$CH$_2$SiO and Si*C*H$_2$*C*H$_2$*C*H$_2$Si overlapping), –1.9 (OSiMe_2), –4.9 (Si*Me*) ppm. ^{29}Si{^{1}H} NMR ($CDCl_3$): δ = 0.93 (G_1-Si), 17.60 (G_2-Si) ppm, G_0-Si was not observed. $C_{88}H_{212}N_8O_8Si_{13}$ (1873.0): calcd. C 56.38, H 11.32, N 5.98; found C 55.98, H 11.20, N 5.78.

Synthesis of 2G-[Si(OCH$_2$CH$_2$NMe$_2$)$_2$]$_8$ (5): This dendrimer was prepared using a similar method to that described for **2**, starting from 2G-$(SiCl_2)_8$ (0.48 g, 0.30 mmol), *N*,*N*-dimethylethanolamine (0.48 mL, 4.77 mmol), and NEt_3 (0.7 mL, 5.02 mmol) to obtain compound **5** as a colorless oil (0.66 g; 90%). ^{1}H NMR ($CDCl_3$): δ = 3.75 (t, 8 H, CH_2O), 2.44 (t, 8 H, CH_2N), 2.24 (s, 24 H, NMe_2), 1.34 (m, 6 H, SiCH$_2$CH_2CH$_2$SiO and SiCH$_2$CH_2CH$_2$Si overlapping), 0.64 (m, 4 H, CH_2SiO), 0.51 (m, 8 H, rest of CH_2Si), 0.09 (s, 6 H, OSi*Me*), –0.10 (s, 3 H, Si*Me*) ppm. ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 61.4 (*C*H$_2$N), 60.7 (*C*H$_2$O), 46.2 (N*Me*$_2$), 18.6 (*C*H$_2$SiO), 17.7, 17.0 and overlapped signals (Si*C*H$_2$C*H*$_2$CH$_2$SiO and Si*C*H$_2$*C*H$_2$*C*H$_2$Si overlapping), –4.4 (OSi*Me*), –4.8 (Si*Me*) ppm. ^{29}Si{^{1}H} NMR ($CDCl_3$): δ = 0.92 (G_1-Si), –3.54 (G_2-Si) ppm, G_0-Si was not observed. $C_{112}H_{268}N_{16}O_{16}Si_{13}$ (2457.0): calcd. C 54.70, H 10.91, N 9.12; found C 54.20, H 10.37, N 9.59.

Synthesis of 3G-[Si(OCH$_2$CH$_2$NMe$_2$)]$_{16}$ (6): This dendrimer was prepared using a similar method to that described for **2**, starting from 3G-$(SiCl)_{16}$ (0.20 g, 0.06 mmol), *N*,*N*-dimethylethanolamine (0.10 mL, 0.99 mmol), and NEt_3 (0.16 mL, 1.14 mmol) to obtain compound **6** as a pale yellow oil (0.18 g, 74%). ^{1}H NMR ($CDCl_3$): δ = 3.65 (t, 8 H, CH_2O), 2.42 (t, 8 H, CH_2N), 2.24 (s, 24 H, NMe_2), 1.23 (m, 14 H, SiCH$_2$CH_2CH$_2$SiO and SiCH$_2$CH_2CH$_2$Si overlapping), 0.64 (m, 8 H, CH_2SiO), 0.53 (m, 20 H, rest of CH_2Si), 0.07 (s, 24 H, OSiMe_2), –0.10 (s, 9 H, Si*Me*) ppm. ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 61.5 (*C*H$_2$N), 60.8 (*C*H$_2$O), 46.1 (N*Me*$_2$), 21.1 (*C*H$_2$SiO), 18.6, 17.9 and overlapped signals (Si*C*H$_2$*C*H$_2$CH$_2$SiO and Si*C*H$_2$*C*H$_2$*C*H$_2$Si overlapping), –1.8 (OSiMe_2), –4.8 (Si*Me*) ppm. ^{29}Si{^{1}H} NMR ($CDCl_3$): δ = 0.95 (G_2-Si), 17.95 (G_3-Si) ppm, G_0-Si and G_1-Si were not observed. $C_{192}H_{460}N_{16}O_{16}Si_{29}$ (4058.3): calcd. C 56.77, H 11.33, N 5.52; found C 56.17, H 11.28, N 5.34.

Synthesis of 3G-[Si(OCH$_2$CH$_2$NMe$_2$)$_2$]$_{16}$ (7): This dendrimer was prepared using a similar method to that described for **2**, starting from 3G-$(SiCl_2)_{16}$ (0.19 g, 0.05 mmol), *N*,*N*-dimethylethanolamine (0.17 mL, 1.68 mmol), and NEt_3 (0.24 mL, 1.79 mmol) to obtain compound **7** as a pale yellow oil (0.20 g, 72%). ^{1}H NMR ($CDCl_3$): δ = 3.75 (t, 16 H, CH_2O), 2.44 (t, 16 H, CH_2N), 2.24 (s, 48 H, NMe_2), 1.32 (m, 14 H, SiCH$_2$CH_2CH$_2$SiO), 0.66 (m, 8 H,

$SiCH_2CH_2CH_2SiO$), 0.53 (m, 20 H, rest of $SiCH_2$), 0.07 (s, 12 H, $OSiMe$), –0.10 (s, 9 H, $SiMe$) ppm. $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 61.4 (CH_2N), 60.7 (CH_2O), 46.1 (NMe_2), a range 18.4–17.4 and overlapped signals ($SiCH_2CH_2CH_2SiO$ and $SiCH_2CH_2CH_2Si$ overlapping), –4.7 ($OSiMe_2$), –5.2 ($SiMe$) ppm. $C_{240}H_{572}N_{32}O_{32}Si_{29}$ (5226.3): calcd. C 55.11, H 10.95, N 8.57; found C 56.11, H 11.89, N 8.13.

Synthesis of 1G-[Si(OCH$_2$CH$_2$NMe$_3^+$I$^-$)]$_4$ (8): To a diethyl ether (10 mL) solution of **2** (0.12 g, 0.15 mmol) was added a MeI solution (0.4 mL, 0.8 mmol, 2 M in Et_2O). The resulting solution was stirred for 48 h at room temp. and then evaporated under reduced pressure to remove residual MeI. The residue was washed with Et_2O (2 × 5 mL) and dried under vacuum to give **8** as a white solid (0.20 g, 96%). ^1H NMR (DMSO): δ = 3.94 (m, 2 H, CH_2O), 3.43 (m, 2 H, CH_2N), 3.09 (s, 9 H, NMe_3^+), 1.29 (m, 2 H, $SiCH_2CH_2CH_2SiO$), 0.66 (m, 2 H, CH_2SiO), 0.56 (m, 2 H, CH_2Si), 0.11 (s, 6 H, $OSiMe_2$) ppm. $^{13}C\{^1H\}$ NMR (DMSO): δ = 65.8 (CH_2N), 55.8 (CH_2O), 52.6 (NMe_3^+), 19.7 (CH_2SiO), 16.9, 16.1 ($SiCH_2CH_2CH_2SiO$), –2.6 ($OSiMe_2$) ppm. $C_{40}H_{100}I_4N_4O_4Si_5$ (1348.0): calcd. C 35.61, H 7.42, N 4.15; found C 36.67, H 7.65, N 4.42.

Synthesis of 1G-[Si(OCH$_2$CH$_2$NMe$_3^+$I$^-$)$_2$]$_4$ (9): This first-generation dendrimer was prepared using a similar method to that described for **8**, starting from **3** (0.17 g, 0.16 mmol) and a MeI solution (0.80 mL, 1.6 mmol, 2 M in Et_2O). Compound **9** was isolated as a white solid (0.29 g, 86%). ^1H NMR (DMSO): δ = 4.12 (m, 4 H, CH_2O), 3.54 (m, 4 H, CH_2N), 3.17 (s, 18 H, NMe_3^+), 1.29 (m, 2 H, $SiCH_2CH_2CH_2SiO$), 0.75 (m, 2 H, CH_2SiO), 0.54 (m, 2 H, CH_2Si), 0.20 (s, 3 H, $OSiMe$) ppm. $^{13}C\{^1H\}$ NMR (DMSO): δ = 65.8 (CH_2N), 56.0 (CH_2O), 52.7 (NMe_3^+), 17.3 (CH_2SiO), 16.5, 15.9 ($SiCH_2CH_2CH_2SiO$), –5.3 ($OSiMe$) ppm. $C_{56}H_{140}I_8N_8O_8Si_5$ (2207.6): calcd. C 30.44, H 6.34, N, 5.07; found C 31.47, H 6.47, N, 5.19.

Synthesis of 2G-[Si(OCH$_2$CH$_2$NMe$_3^+$I$^-$)]$_8$ (10): This dendrimer was prepared using a similar method to that described for **8**, starting from **4** (0.25 g, 0.13 mmol) and a MeI solution (0.6 mL, 1.2 mmol, 2 M in Et_2O). Compound **10** was isolated as a white solid (0.35 g, 87%). ^1H NMR (DMSO): δ = 3.96 (m, 4 H, CH_2O), 3.45 (m, 4 H, CH_2N), 3.11 (s, 18 H, NMe_3^+), 1.29 (m, 6 H, $SiCH_2CH_2CH_2SiO$ and $SiCH_2CH_2CH_2Si$), 0.65 (m, 4 H, $SiCH_2CH_2CH_2SiO$), 0.52 (m, 8 H, rest of $SiCH_2$), 0.11 (s, 12 H, $OSiMe_2$), –0.10 (s, 3 H, $SiMe$) ppm. $^{13}C\{^1H\}$ NMR (DMSO): δ = 65.7 (CH_2N), 55.9 (CH_2O), 52.6 (NMe_3^+), 19.7, 17.5, 16.9 and overlapped signals ($SiCH_2CH_2CH_2SiO$ and $SiCH_2CH_2CH_2Si$), –2.5 ($OSiMe_2$), –5.3 ($SiMe$) ppm. $C_{96}H_{236}I_8N_8O_8Si_{13}$ (3008.2): calcd. C 38.29, H 7.85, N 3.72; found C 38.87, H 8.32, N, 3.79.

Synthesis of 2G-[Si(OCH$_2$CH$_2$NMe$_3^+$I$^-$)$_2$]$_8$ (11): This dendrimer was prepared using a similar method to that described for **8**, starting from **5** (0.10 g, 0.04 mmol) and a MeI solution (0.5 mL, 1.0 mmol, 2 M in Et_2O). Compound **11** was isolated as a white solid (0.17 g, 87%). ^1H NMR (DMSO): δ = 4.13 (m, 8 H, CH_2O), 3.56 (m, 8 H, CH_2N), 3.19 (s, 36 H, NMe_3^+), 1.28 (m, 6 H, $SiCH_2CH_2CH_2SiO$ and $SiCH_2CH_2CH_2Si$), 0.72 (m, 4 H, $SiCH_2CH_2CH_2SiO$), 0.55 (m, 8 H, rest of $SiCH_2$), 0.21 (s, 6 H, $OSiMe$), –0.08 (s, 3 H, $SiMe$) ppm. $^{13}C\{^1H\}$ NMR (DMSO): δ = 65.7 (CH_2N), 56.0 (CH_2O), 52.7 (NMe_3^+); 19.2, 17.2, 16.4 and overlapped signals ($SiCH_2CH_2CH_2SiO$ and $SiCH_2CH_2CH_2Si$), –5.2 ($OSiMe$), –5.6 ($SiMe$) ppm. $C_{128}H_{316}I_{16}N_{16}O_{16}Si_{13}$ (4727.4): calcd. C 32.49, H 6.68, N 4.74; found C 33.32, H 7.03, N, 4.72.

Synthesis of 3G-[Si(OCH$_2$CH$_2$NMe$_3^+$I$^-$)]$_{16}$ (12): This dendrimer was prepared using a similar method to that described for **8**, starting from **6** (0.12 g, 0.03 mmol) and a MeI solution (0.4 mL, 0.8 mmol, 2 M in Et_2O). Compound **12** was isolated as a white solid (0.16 g, 83%). ^1H NMR (DMSO): δ = 3.96 (m, 8 H, CH_2O), 3.47 (m, 8 H, CH_2N), 3.13 (s, 36 H, NMe_3^+), 1.28 (m, 14 H, $SiCH_2CH_2CH_2SiO$ and $SiCH_2CH_2CH_2Si$), 0.65 (m, 8 H, $SiCH_2CH_2CH_2SiO$), 0.52 (m, 20 H, rest of $SiCH_2$), 0.10 (s, 24 H, $OSiMe_2$), –0.08 (s, 9 H, $SiMe$) ppm. $^{13}C\{^1H\}$ NMR (DMSO): δ = 65.8 (CH_2N), 55.9 (CH_2O), 52.6 (NMe_3^+), 19.7, 17.2, 16.0 and overlapped signals ($SiCH_2CH_2CH_2SiO$ and $SiCH_2CH_2CH_2Si$), –2.7 ($OSiMe_2$), –5.5 ($SiMe$) ppm. $C_{208}H_{508}I_{16}N_{16}O_{16}Si_{29}$ (6328.7): calcd. C 39.43, H 8.03, N 3.54; found C 39.19, H 8.19, N, 3.68.

Reaction of 3G-[Si(OCH$_2$CH$_2$NMe$_2$)$_2$]$_{16}$ (7) with MeI: The reaction of **7** with MeI using a similar method to that described for **8** afforded the dendrimer 3G-[Si($OCH_2CH_2NMe_3^+I^-)_2$]$_{16}$ (**13**). However, the NMR analysis revealed that not all the amine groups were quaternized: roughly 29 groups were methylated, based on integration of the corresponding chemical shift of the outer –OCH_2CH_2N– branch for the amine or ammonium groups.

Biocompatibility Studies

Cells: PBMC cells were derived from healthy voluntary donors, and obtained from leukophoresed blood by Ficoll™ gradient and elutriation centrifugation. Prior to challenge with dendrimers, PBMCs were stimulated for 48 h with phytohemagglutinin (2 μg/mL) and Interleukin 2 (IL-2, 100 IU/mL). On the day of the challenge, PBMCs were recovered and washed with Phosphate Buffered Saline (PBS), and then seeded in RPMI medium supplemented with 10% of fetal calf serum, 1% L-glutamine, antibiotics, and IL-2 (50 IU/mL), in a 5% CO_2 environment (100 000 cells per well in 96-well plates for the toxicity curve of dendrimer concentrations). The final volume in each well was 200 μL for the 96-well plates and 500 μL for the 24-well plates. For the toxicity curve of dendrimer concentrations, wells were coated with 20 μL of fibronectin/well at a concentration of 5 μg/mL. The intention was not to lose cells through pipetting during the MTT assay. In the case of the 24-well plates, stimulated cells were seeded in 340 μL of complete medium.

Phase-Contrast Light Microscopy: After incubation with dendrimers, changes in morphology and characteristics of PBMCs, such as cell membrane birefringence, were observed using a phase-contrast inverted microscope (Nikon TMS, Nikon, Japan) equipped with a 100X objective (Plan 10/0,30DL/Ph1, Nikon, Japan). Live PBMCs are bright, have a defined spherical shape, and float in the culture medium. Dead cells have a darker appearance, and are mostly present in the bottom of the well. In addition, we assessed the presence or absence of cell aggregation.

MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide] Assay: This method was selected to analyze detrimental intracellular effects on mitochondria and metabolic activity. The colorimetric MTT test, based on the selective ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to purple formazan, relies on intact metabolic activity, and is frequently used for cytotoxicity screening. After 48 h of incubation of PBMCs with different concentrations of dendrimers in a 96-well plate, culture medium containing the dendrimers was replaced with serum-free Optimem (200 μL). Sterile filtered MTT (Sigma) stock solution (20 μL) in PBS pH = 7.4 (5 mg/mL) was added to each well to achieve a final concentration of 0.5 mg MTT/mL. After 4 h, unreacted dye was removed by aspiration, and the formazan crystals were dissolved in dimethyl sulfoxide (200 μL per well) (Merck, Darmstadt, Germany). The concentration of formazan was then determined spectrophotometrically in a plate reader at a wavelength of 570 nm (test) and 690 nm (reference). The spectrophotometer was calibrated to zero absorbance using Optimem medium without cells. The relative cell viability (%) related to control wells

(cells with no dendrimer) was calculated by [A] test/[A] control × 100. Each dendrimer concentration was tested in triplicate, according to ATCC directives.

Formation of Dendrimer/Oligonucleotide Complexes: The fluoresceinated phosphorothioate oligodeoxynucleotide (ODN) sequence was 25 bases long and corresponded to an antisense (complementary) sequence of the HIV Anti-Gag. Complex formation between dendrimer **10** or [$HOCH_2CH_2NMe_3^+I^-$] and ODN was performed using an electrostatic approach. Ratios of ODN to dendrimer were based on the calculation of the electrostatic charge present on each component, for example the number of phosphate groups in the ODN versus the number of terminal ammonium groups on the dendrimer. The dendrimer or [$HOCH_2CH_2NMe_3^+I^-$] was diluted in sterile distilled water at 2 mg/mL, and the ODN concentration for complexation with the carbosilane dendrimer was 0.88 μM (2.57 μg). All complexes or mixtures at different charge ratios were formed in serum-free RPMI medium (60 μL), with an incubation time of 20 min at room temp. Complex formation was assessed by evaluation of migration retardation of fluoresceinated ODN during electrophoresis on 3% agarose gel. A 100 bp DNA ladder was used as reference (Gibco BRL™).

Supporting Information (for details see the footnote on the first page of this article): Selected data as ^{1}H-, ^{13}C-, and ^{29}Si NMR spectra of **1**, **3**–**6**, **9**–**11** (Figure S1–S3 and S6–S10), 2D HMQC and HMBC spectra of **4** (Figure S4–S5), and plots of the hydrolysis processes of **10** and **12** at different concentrations (Figure S11).

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