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Synthesis of Cationic Carbosilane Dendrimers via Click Chemistry and Their Use as Effective Carriers for DNA Transfection into Cancerous Cells

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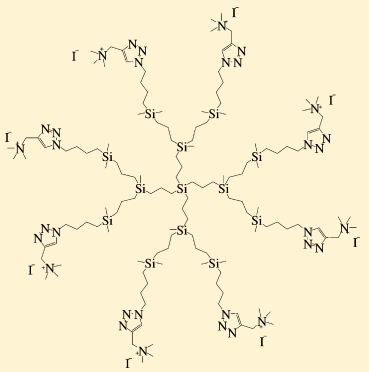
Supporting Information

ABSTRACT: New amine-terminated carbosilane dendrimers have been prepared by a Huisgen cycloaddition ("click chemistry" reaction) of azide-terminated carbosilane dendrimers with two different propargyl amines. The corresponding cationic derivatives with peripheral ammonium groups were obtained by subsequent addition of MeI. Quaternized dendrimers are soluble and stable in water or other protic solvents for long time periods, and have been studied as nonviral vectors for the transfection of DNA to cancer cells. In this study DNA-dendrimeric nanoparticles (dendriplexes) formulated with two different families of cationic carbosilane dendrimers (family 1 (G1, G2 and G3) and family 2 (G1, G2)) were characterized and evaluated for their ability to transfect cells *in vitro* and *in vivo*. Dendriplex derived from second generation dendrimer of family 1 (F1G2 5/1 (+/−)) increased the efficiency of plasmid-mediated gene transfer in HepG2 cells as compared to naked DNA and the commercial control dendrimer. Also, intravenously administered dendriplex F1G3 20/1 (+/−) is superior in terms of gene transfer efficiency *in vivo*.



KEYWORDS: *dendrimer, carbosilane, cationic, click chemistry, gene therapy, cancer*

1. INTRODUCTION

Gene therapy could be an efficient approach for cancer therapy. For this purpose one important goal is to find efficient gene delivery vehicles, because the success of gene therapy in this field is largely dependent on the development of carriers capable of internalizing foreign genes into cancerous cells. For this reason, in recent years, the gene therapy field has expanded its search to find a synthetic gene delivery vector that achieves high-efficiency and nontoxic transgene expression, since nonviral vectors can overcome some of the problems found when viral vectors were used.[1]

Some of these synthetic systems have shown difficulties in reaching high levels of efficiency in some cell lines, mainly in the presence of serum. In this context, dendrimers, due to their unique properties, have been shown as an attractive alternative for the development of effective nonviral vectors for gene therapy purposes.[2] Dendrimers are highly branched macromolecules, with a globular shape. They have a high density of surface groups, are monodisperse and nonimmunogenic and can mediate the delivery of single-stranded and double-stranded, natural or synthetic DNA or RNA of several types and sizes.[3]

Dendrimers form complexes with DNA through sequence-independent electrostatic interactions between negatively charged phosphate groups of the nucleic acid and cationic (positively charged) ammonium groups on the dendrimer surface, giving rise to a particle termed *dendriplex*. These dendriplexes produce very stable and highly soluble RNA/DNA complexes.[4] Another advantage of dendritic polymers is that they have a broader concentration range between transfection and cytotoxicity.[3] It has been also proved that some of these polymers increased the efficiency of plasmid mediated gene transfer *in vivo*.[5]

PAMAM dendrimers are well-known carriers for different drugs including nucleic acids, delivering them efficiently into tumor cells.[6] Their low toxicity *in vitro* and *in vivo* allowed their use as delivery agents for conventional anticancer drugs, but also antibodies and nucleic acids. Szoka et al. showed that dendrimers above generation 4 (G4) can efficiently transfect different tumor cell lines *in vitro*.[7] The ability of PAMAM dendrimers to transfect tumor cells *in vivo* was demonstrated when delivering oligonucleotides to ascitic tumors by intraperitoneal

Special Issue: Biological Applications of Dendrimers

Received: October 24, 2011
Revised: December 9, 2011
Accepted: December 27, 2011
Published: December 27, 2011

injection[8] or plasmid DNA by intratumoral[9] or systemic[10] administration. Nevertheless, pure amino-terminated PAMAM dendrimers are cytotoxic when are used in a higher concentration range, so it is important to find new systems with new properties and lower toxicity to find alternatives to these commercial dendrimers.

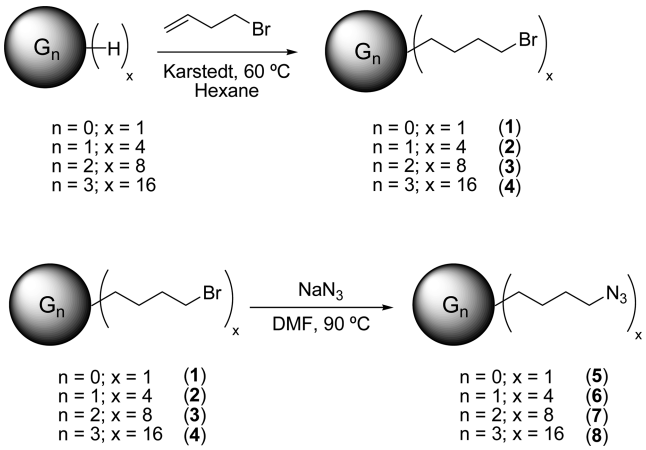
We have recently reported the use of cationic carbosilane dendrimers of second generation as nonviral vehicles for gene therapy against HIV.[11] Dendriplexes formed between carbosilane dendrimers and oligonucleotides or siRNA are able to transfect nucleic acids in several types of cells,[12] protecting these nucleic materials from the attack of serum proteins or nucleases[13] and achieving high levels of efficiency in the reduction of the viral load in HIV infected cells.[11c]

In this work, we describe the use of cationic carbosilane dendrimers up to third generation, prepared by using the synthetic strategy named "click chemistry", as nonviral vectors for transfection of DNA, *in vitro* and *in vivo*. Dendriplexes, prepared with the dendrimers and DNA, proved to be effective for *in vitro* and *in vivo* gene delivery to liver cancer cells (HepG2).

2. RESULTS AND DISCUSSION

2.1. Synthesis and Characterization of Carbosilane Dendrimers. *2.1.1. Carbosilane Dendrimers with Terminal Azide Groups.* First, triethylsilane and Si−H terminated carbosilane dendrimers of type G_n-$(SiH)_x$ (n = 0 (Et_3SiH), 1, 2 and 3; x = 1, 4, 8 and 16)[14] were used in the hydrosilylation of 4-bromobutene to lead to carbosilane dendrimers with $-CH_2Br$ terminal groups. The reactions were performed in ampules with J Young valves, using hexane as solvent and the Karstedt catalyst during 16 h at 60 °C, to afford the corresponding system $[Et_3Si(CH_2)_4Br]$ (**1**) and dendrimers G_n-$(SiCH_2CH_2CH_2CH_2Br)_x$ (n = 1, x = 4 (**2**); n = 2, x = 8 (**3**); n = 3, x = 16 (**4**)) (Scheme 1).

Scheme 1

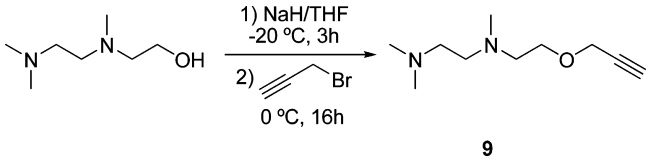


Compounds **1**−**4** were converted to terminal azide carbosilane dendrimers by reaction with sodium azide in DMF at 90 °C giving the monofunctional compound $[Et_3Si(CH_2)_4N_3]$ (**5**) and dendrimers G_n-$(SiCH_2CH_2CH_2CH_2N_3)_x$ (n = 1, x = 4 (**6**); n = 2, x = 8 (**7**); n = 3, x = 16 (**8**)) (Scheme 1). Compounds **1**−**8** were obtained in high yield as colorless oils, in which n means the number of generation G (the generation is stablished on the basis of the silicon concentric shapes present in the structure), and x states the number of peripheral units.

These dendrimers were characterized by elemental analysis and NMR spectroscopy. The most significant signals for compounds **1**−**4** are those due to the methylene groups bonded to bromine that appear about 3.4 and 36.5 ppm in ^1H and ^{13}C NMR respectively, whereas for derivatives **5**−**8**, the methylene groups directly bonded to the azide group appear around 3.24 and 51.1 ppm. A complete description of the NMR data is given in the Experimental Section, and some NMR spectra are shown in the Supporting Information.

2.1.2. Carbosilane Dendrimers with Terminal Amine Groups. New amine-terminated carbosilane dendrimers have been prepared by a Huisgen cycloaddition ("click chemistry" reaction)[15] of azide-terminated carbosilane dendrimers with two different propargyl amines, the commercially available *N*,*N*-dimethyl propargyl amine and a new propargyl diamine (**9**) prepared as described below. This propargyl diamine **9** can be prepared in a two step reaction starting from 2-{[2-(dimethylamine)ethyl]methylamine}ethanol. The addition of NaH to this amino alcohol leads to a sodium derivative that is not isolated and treated *in situ* with propargyl bromide to give $[HCCHCH_2OCH_2CH_2N(Me)CH_2CH_2NMe_2]$ (**9**) as a dark yellow oil in moderate yields (see Scheme 2 and the Experimental Section), that was characterized by elemental analysis and ^1H and ^{13}C NMR spectroscopy.

Scheme 2



Analysis of this propargyl amine by ^1H NMR in $CDCl_3$ revealed the following signals: a doublet at δ 4.07 arising from the methylene group adjacent to the triple bond coupled with the alkyne proton; two triplets due to methylene groups 4 and 5 (see Figure 1) at 3.54 and 2.53 ppm, these signals being shifted downfield due to the proximity of the oxygen atom; protons 7 and 8 giving an A_2B_2 system that appears as two multiplets at 2.4 and 2.3 ppm, the alkyne proton signal being

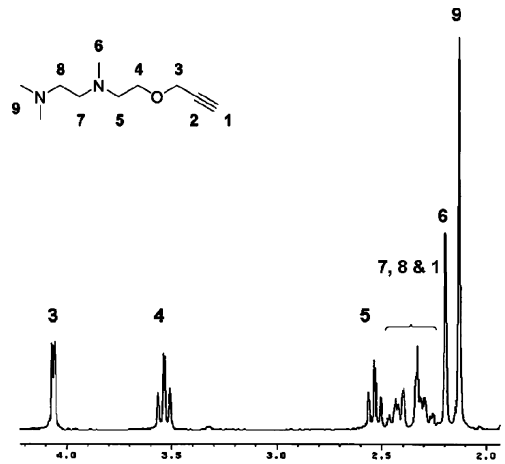


Figure 1. ^1H NMR spectrum of compound **9**.

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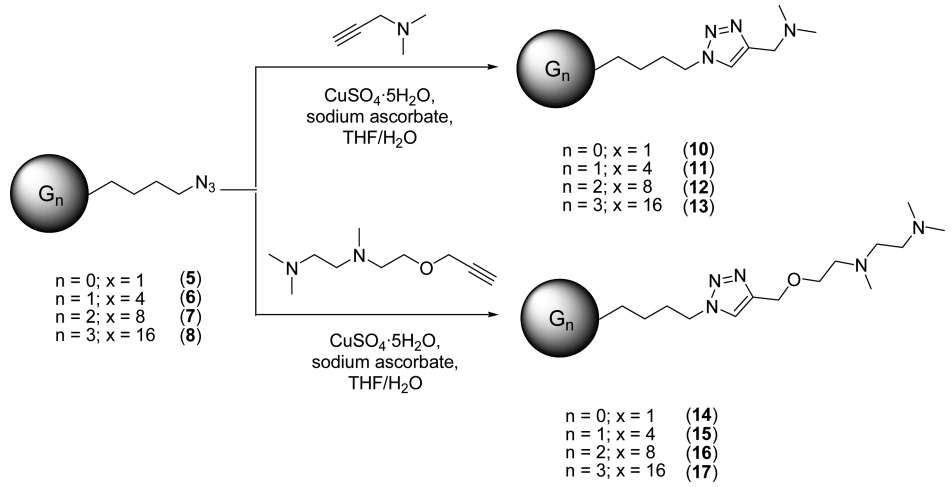
Scheme 3



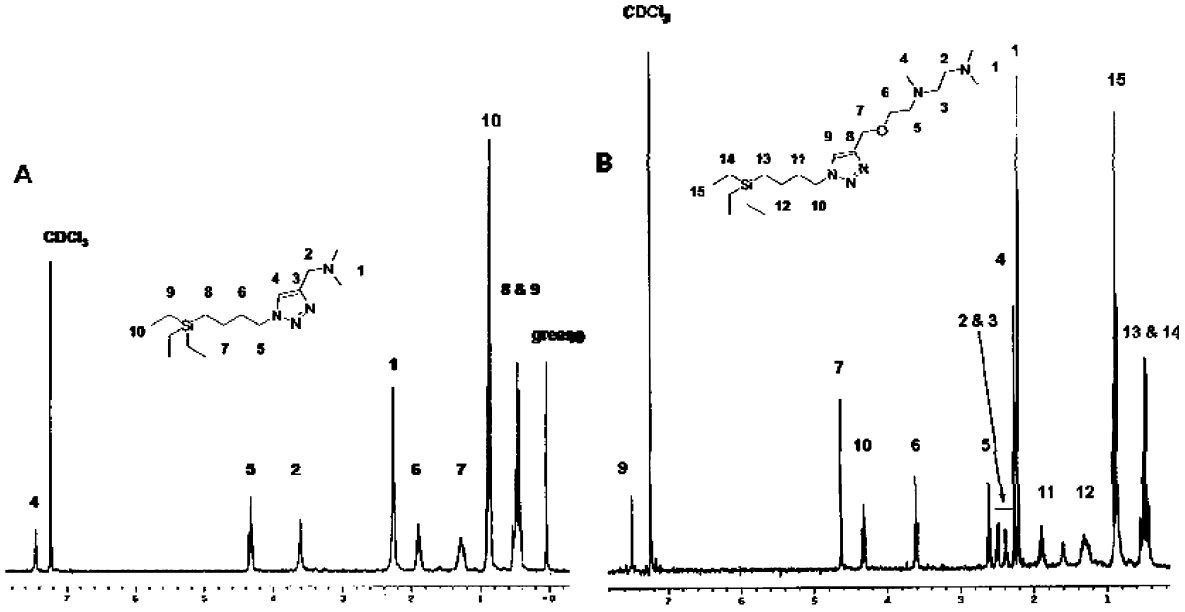


Figure 2. ^{1}H NMR spectra of compounds **10** (A) and **14** (B).

overlapped with this last resonance; finally methyl groups bonded to nitrogen, 6 and 9, appearing as two singlets at 2.20 and 2.13 ppm respectively. Full ^{13}C NMR spectral data are given in the Experimental Section. The most significant signals in this spectrum are those at 79.6 and 74.2 ppm that are assigned to the carbons of the triple bond.

The click chemistry reaction of propargyl amine with azide functionalized compounds **5**−**8** leads to $[Et_3Si(CH_2)_4(N_3C_2H)CH_2NMe_2]$ (**10**) and dendrimers *ck*-G_n-$[Si(CH_2)_4(N_3C_2H)CH_2NMe_2)]_x$ (n = 1, x = 4 (**11**); n = 2, x = 8 (**12**); n = 3, x = 16 (**13**)) (Scheme 3) through a Huisgen cycloaddition using a Cu(I) catalyst, generated from sodium ascorbate and copper sulfate, in a mixture THF/H_2O as solvent.

In a similar way, the Huisgen cycloaddition reaction of dendrimers **5**−**8** with the propargyl diamine **9** leads to $[Et_3Si(CH_2)_4(N_3C_2H)CH_2OCH_2CH_2N(Me)CH_2CH_2NMe_2]$ (**14**) and dendrimers *ck*-G_n-$[Si(CH_2)_4(N_3C_2H)CH_2OCH_2CH_2N(Me)CH_2CH_2NMe_2]_x$ (n = 1, x = 4 (**15**); n = 2, x = 8 (**16**); n = 3, x = 16 (**17**)) (Scheme 3).

Compounds **10**−**17** were characterized by elemental analysis, mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy. In this section, only NMR data of mononuclear compounds **10** and **14** used as models are described. These spectra show the disappearance of the alkyne units and the appearance of the triazole units. The dendrimers show similar chemical shifts for the terminal groups to those of **10** and **14** and broader and less structured signals for the carbosilane skeleton, when the dendrimer generation is increasing (see the Experimental Section). The ^{1}H NMR spectrum of compound **10** shows a singlet at 7.45 ppm corresponding to the proton of the triazole ring. The methylene groups close to nitrogen atoms, 5 and 2, appear as a triplet and a singlet at 4.33 and 3.61 ppm respectively. Terminal methyl amine groups give a singlet at 2.27 ppm. Finally, the other methylene groups and the ethyl groups bonded to silicon give several multiplets in the expected region (see the Experimental Section and Figure 2). ^{13}C NMR data are in agreement with the ^{1}H NMR spectrum, and the most significant signals are those corresponding to the triazole ring that appear at 145.0 and 121.9 ppm for carbons 3 and 4

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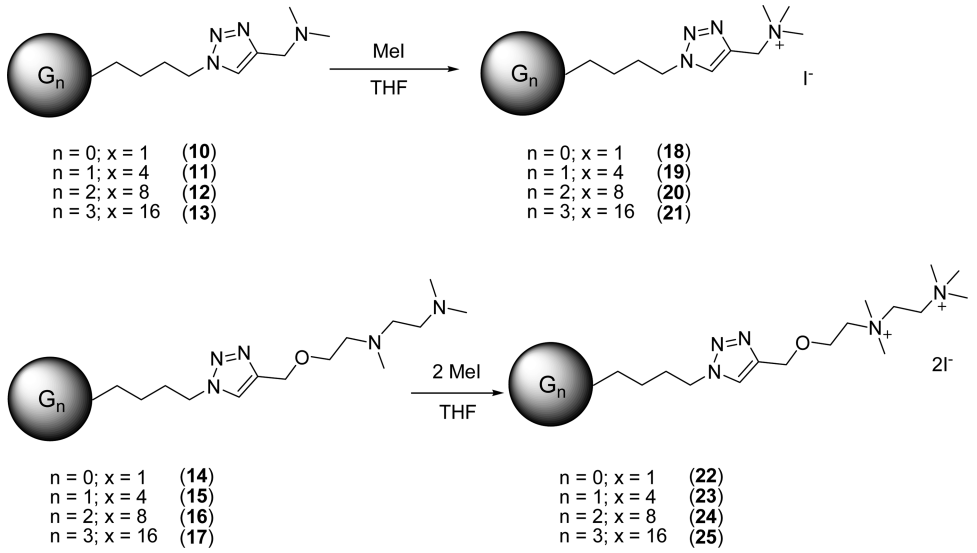
Scheme 4



n = 0; x = 1 (**10**)
n = 1; x = 4 (**11**)
n = 2; x = 8 (**12**)
n = 3; x = 16 (**13**)

n = 0; x = 1 (**18**)
n = 1; x = 4 (**19**)
n = 2; x = 8 (**20**)
n = 3; x = 16 (**21**)

n = 0; x = 1 (**14**)
n = 1; x = 4 (**15**)
n = 2; x = 8 (**16**)
n = 3; x = 16 (**17**)

n = 0; x = 1 (**22**)
n = 1; x = 4 (**23**)
n = 2; x = 8 (**24**)
n = 3; x = 16 (**25**)

respectively. The methyl groups bonded to nitrogen appear at δ 45.0 whereas the methylene groups close to nitrogens (2 and 5) gives signals at δ 54.3 and 49.7. The ^{15}N NMR spectrum of **10** shows four signals attributed to the aliphatic amine (−352 ppm), the pyrrole-like nitrogen (−138 ppm) and the two pyridine-like nitrogens (−32 and −22 ppm).

The ^{1}H NMR spectrum of compound **14** (see Figure 2) shows signals for the triethylsilyl fragment bonded to the triazole ring similar to those describe for compound **10**. The diamine fragment bonded to this ring gives the following signals, two singlets at 7.50 and 4.63 ppm for the proton of the heterocyclic ring, 9, and the methylene group bonded to oxygen, 7, respectively. The methylene groups bonded to nitrogen atoms give three triplets at 4.32, 3.61, and 2.61 that are assigned to the methylene fragments 10, 6 and 5 respectively, whereas the other two methylene groups, 2 and 3, appear as two multiplets at δ 2.50 and 2.37. Finally the methyl groups bonded to the inner and outer nitrogen atoms give two singlets at 2.26 and 2.21 ppm respectively. Again the ^{13}C NMR spectrum confirms this assignment and shows as more significant signals two resonances at 144.7 and 122.1 for carbons 8 and 9 of the heterocyclic ring. Analogously, compound **14** shows in the ^{15}N NMR five signals attributed to the triazole ring, at −131 ppm for the pyrrole-like nitrogen and at −31 and −18 ppm for the pyridine-like nitrogens, and the two aliphatic nitrogens, at −355 ppm for the outer amine group and at −351 pp for the inner one.

2.1.3. Carbosilane Dendrimers with Terminal Ammonium Groups. The ammonium-terminated dendrimers were purely prepared by adding a little excess of MeI to parent derivatives **10**−**17** in THF (see Scheme 4). The addition of MeI over the amine functionalized dendrimers produces also the corresponding cationic ammonium-terminated derivatives of type *ck*-G_n-$[Si(CH_2)_4(N_3C_2H)CH_2N^+Me_3\ I^-)]_x$ (n = 0, Et_3Si, x = 1 (**18**); n = 1, x = 4 (**19**); n = 2, x = 8 (**20**); n = 3, x = 16 (**21**)) in the case of compounds with just one nitrogen atom per branch (family 1), or derivatives of type *ck*-G_n-$[Si(CH_2)_4(N_3C_2H)$-$CH_2OCH_2CH_2N^+(Me_2)CH_2CH_2N^+Me_3,\ 2I^-]_x$ (n = 0, Et_3Si, x = 1 (**22**); n = 1, x = 4 (**23**); n = 2, x = 8 (**24**); n = 3, x = 16 (**25**)) in the case of compounds with two positive charged nitrogen atoms per branch (family 2). When the quaternization reactions were performed in an excess of MeI at room temperature, only the aliphatic groups were quaternized. No evidence of quaternization of the nitrogen atoms of the triazole ring were observed by ^{1}H and ^{15}N NMR spectra (vide infra).

The NMR spectroscopic and analytical data for compounds **18**−**25** are consistent with their proposed structures (Scheme 4 and Supporting Information). Structures of second generation derivatives are depicted in Figure 3. The ^{1}H and ^{13}C NMR spectra of the quaternized dendrimers in DMSO-d_6 exhibit identical resonance patterns to those observed in their neutral counterparts **10**−**17** for the carbosilane framework, although broader signals are seen in these deuterated solvents. In this section, only NMR data of mononuclear compounds **18** and **22** used as models are described. The dendrimers show similar chemical shifts for the terminal groups to those of **18** and **22** and broader and less structured signals for the carbosilane skeleton, when the dendrimer generation is increasing.

In general for the ^{1}H NMR spectrum of **18** (see Figure 4), the quaternization of the amine groups result in a deshielding of the chemical shifts of the geminal methylene (2) and methyl (1) groups directly bound to the charged nitrogen that appears at 4.68 and 3.06 ppm respectively. The proton present in the triazole ring (4) appears as a singlet at 8.44 ppm, and the methylene groups between the triazole ring and the silicon atom (5−8) give four multiplets at 4.43, 1.85, 1.22, and 0.44 respectively. Finally, the ethyl group gives place to a triplet centered at 0.85 ppm for the methyl groups and a multiplet due to the methylene protons that appears overlapped with the signal of 8 at 0.4 ppm. ^{13}C NMR spectrum of **18** (Supporting Information) is very clean and allows the assignment of all signals. The carbon atoms of the triazole ring give two singlets at 134 (3) and 127.3 (4) ppm. The methyl (1) and methylene (2) groups directly bonded to the charged nitrogen show two signals at 51.3 and 58.4 ppm which also represents a downfield shift respecting the analogous neutral compound, **10**, as a result of the positive charge present in this nitrogen. The aliphatic chain between the triazole ring and the silicon atoms shows four signals at 49.7, 32.8, 19.6, and 9.6 ppm assigned to carbons 5, 6, 7 and 8 respectively. Finally, the ethyl groups give the expected signals at 6.7 and 2.2 ppm. In the ^{15}N NMR spectrum of **18**, only the nitrogen on the ammonium groups is altered

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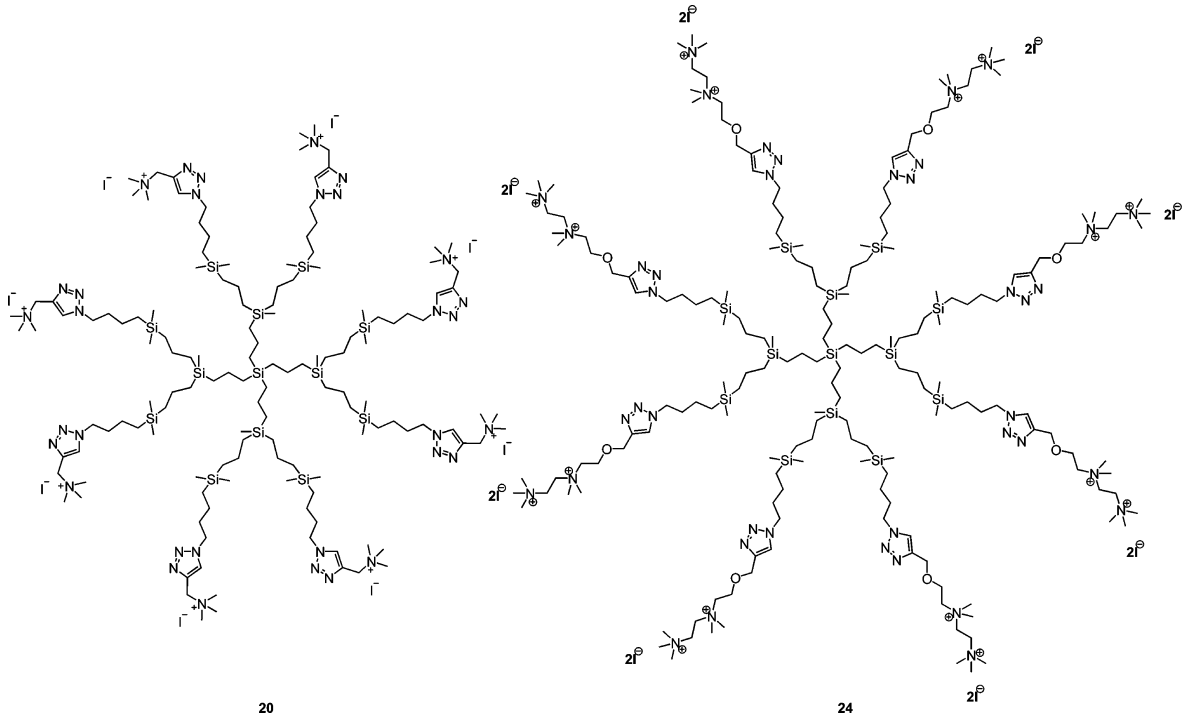

Figure 3. Structures of dendrimers **20** and **24**.

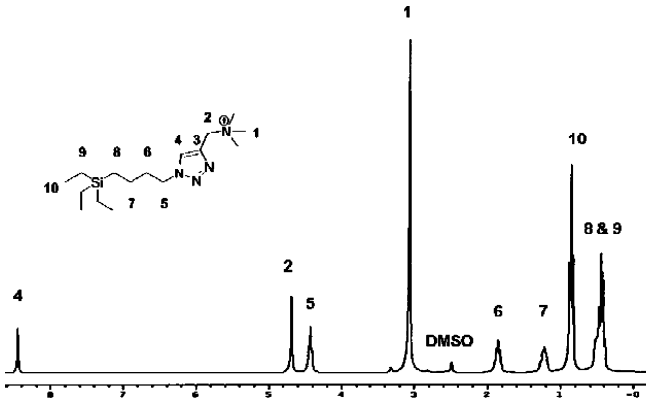

Figure 4. ^{1}H NMR spectrum of compound **18**.

(−310 ppm), appearing downfield of the corresponding nonquaternized amine groups present in compound **10**.

A similar analysis can be made for the ^{1}H and ^{13}C NMR spectra of compound **22** (Figure 5). In this case, the ^{1}H NMR spectrum shows a singlet for the proton of the triazole ring at 8.18 ppm, and other singlet at 4.62 ppm due to the methylene group between the ring and the oxygen atom. The aliphatic chain between the heterocyclic and the silicon atom give four multiplets at 4.35, 1.81, 1.23, and 0.45 corresponding to the methylene groups (10−13). The terminal diammonium chain shows two singlets at 3.19 and 3.16 ppm attributed to the methyl groups (4 and 1) and two multiplets at 3.93 and 3.65 for the methylene groups (2, 3, 6 and 5) directly bonded to the positively charged nitrogen atoms. Finally, the ethyl groups show the expected two signals. The ^{13}C NMR spectrum is also in agreement with the proposed structure, and in this case, a little bit more complex spectrum is obtained due to the similar chemical environments imposed by the oxygen and the positively charged nitrogen atoms. The most significant signals are those of the triazole ring (8 and 9) that appears at 142.4 and 123.4 ppm and those of the methyl groups directly bonded to the positively charged nitrogens that appear at 52.5 and 51.2 ppm. The methylene groups present in this compound give several signals which are described along with their assignment in the Experimental Section. According to the observations in derivative **18**, the ^{15}N NMR spectrum of **22** shows that only the two nitrogens on the ammonium groups are shifted downfield (−331 and −326 ppm) compared with the corresponding amine groups in compound **14**.

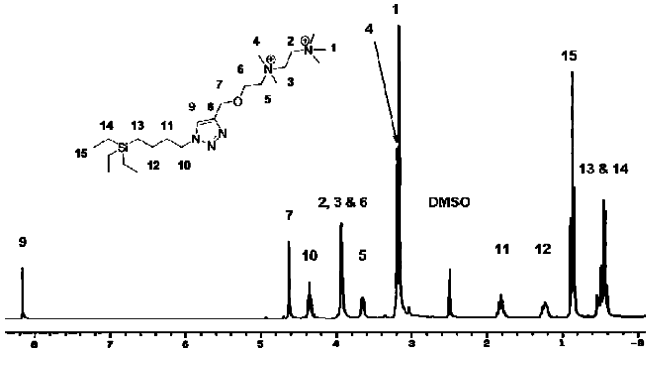

Figure 5. ^{1}H NMR spectrum of compound **22**.

2.2. Physicochemical Characterization of Dendrimers and Dendriplexes Formed by Conjugation of Cationic Dendrimers and DNA. For this study, the cationic dendrimers described previously were divided into two families: family 1 comprises dendrimers from first to third generation **19**−**21** with an ammonium group per branch, and family 2 are dendrimers of first and second generation **23** and **24** that contains two positively charged nitrogen atoms per branch. In all cases a fourth generation PAMAM dendrimer was used as control.

dx.doi.org/10.1021/mp200542j | *Mol. Pharmaceutics* 2012, 9, 433−447

2.2.1. UV–Vis and Fluorimetric Characterization of the Dendrimers. The ammonium-terminated dendrimers were also characterized by means of UV–vis and fluorescence spectrophotometry.

The UV–vis spectrum of these dendrimers (Figure 6) is dominated by an intense band centered at 224 nm (ε = 28400–212400 M^{-1} cm^{-1}), arising from $\pi \rightarrow \pi^*$ transitions of the triazole ring[16] and a low intensity band at 320 nm (ε = 322–1333 M^{-1} cm^{-1}).

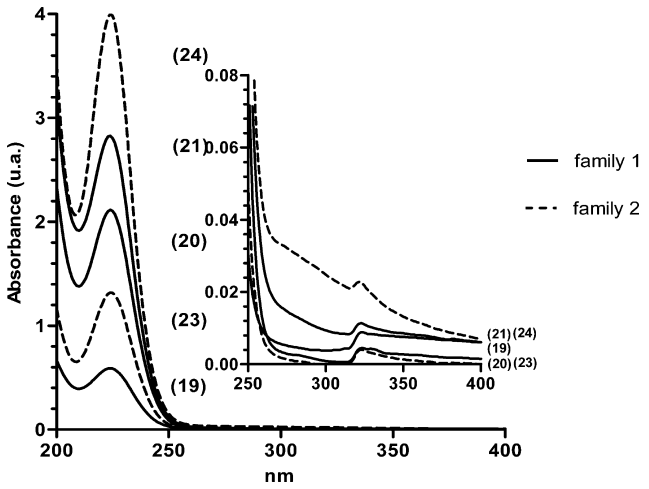

Figure 6. Comparative UV–vis spectra of dendrimers from families 1 and 2.

As expected, the intensity of the band at 224 nm increases as the generation is increased. However, as a proportional connection between the intensity of this band and the number of triazolic groups cannot be found, it can be concluded that some other factors are involved in the absorption of the dendrimers, like the rigidity of the system and the influence of the peripheral groups. On comparing the extinction coefficients, dendrimers from family 2 present a more efficient absorption on both bands, this fact being evidence of the influence of the peripheral ammonium groups on the dendrimer behavior.

Some other dendrimers, like OH-, NH_2- and carboxylate-terminated PAMAM and NH_2-terminated PPI, have shown a pH-dependent fluorescence emission.[17] In particular, for NH_2-terminated dendrimers, the formation of a fluorescence-emitting moiety is in parallel with the protonation and/or oxidization of tertiary amines.[18] Likewise, carbosilane dendrimers in the present work also emitted fluorescence in water, probably arising from the ammonium periphery. With increasing generation, the emission band down-shifted, and fluorescence intensifies proportionally to the number of ammonium groups (Figure 7). A special case is found in **20**, which presents higher fluorescence intensity than expected.

2.2.2. Characterization of Dendriplex Formation. The binding of the dendrimer to DNA and the subsequent dendriplex formation was studied by electrophoresis in agarose gels, and the results are depicted in Figure 8. Dendriplexes were formulated at different charge ratios, which are represented as positive charge equivalents of cationic components (N) to negative charge equivalents of the nucleic acid (P). Gel retardation observed is the result of electrostatic and steric effects due to complex formation between cationic dendrimers

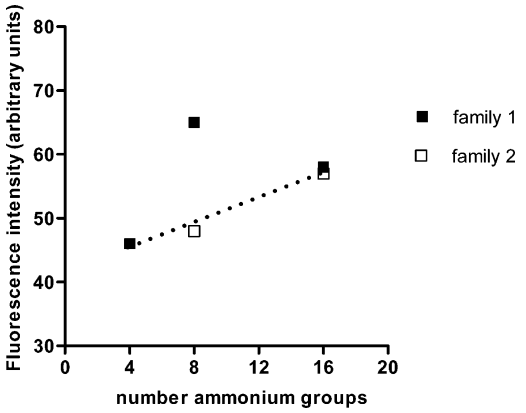

Figure 7. Fluorescence intensity of dendrimers from families 1 and 2, as a function of the number of ammonium groups. Family 1: **19** (Ex 295, Em 400), **20** (Ex 300, Em 350), **21** (Ex 305, Em 380). Family 2: **23** (Ex 300, Em 430), **24** (Ex 350, Em 425).

and DNA. In the first column of the gel (entry 1), DNA bands appear for both the open circular DNA and supercoiled. The second column (entry 2) shows only one band corresponding to DNA complexed with PAMAM dendrimer used as control. The remaining columns show that both families are able to retain and immobilize the pDNA in the gel at all charge ratios, except the 1/1 ratio. It is known that an excess of positive or negative charges leads to obtaining stable complexes in lipid–DNA (lipoplexes) and polymer–DNA (polyplexes). This is usually due to the electrostatic repulsion, which contributes to a better stability. At 1:1 charge ratio (electroneutrality point) a high tendency to get aggregates in these colloidal formulations is observed.

2.2.3. Particle Size of Dendriplexes. Particle size was analyzed by laser diffractometry, and the results obtained for both families at different charge ratios dendrimer/nucleic acid (D/DNA) are described in Table 1. Results show that by increasing the generation there is an increase in the size of the complexes formed. This could be explained because the molecular weights of the two families of dendrimers increase with the generation. In general, for each generation of the two families of dendrimers, it is satisfied that the size of the complexes formed decreases with increasing charge ratio because the condensation capacity increases when the number of positive charges is higher. Similar results were obtained by Ramaswamy et al., in a study with polylysine.[19] This is not observed for the dendrimer **21** at a charge ratio of 20/1. One might think that this size difference could be due to a tendency to aggregation, which is explained because in large generations higher charge ratios result in a decrease in the size of the dendriplexes and a larger area of interaction. Compared with commercial fourth generation PAMAM dendrimer used as control, dendriplexes of family 1 are generally larger than those of the family 2.

2.2.4. Zeta Potential Measurements. Zeta potential measurements were carried out for both families at different charge ratios (D/DNA), and the results are described in Table 2. Dendrimers of family 2 have more positive charges in each generation respecting dendrimers of family 1, which would explain their higher zeta potential values.[20] In all cases, the zeta potential increases when the charge ratio used is higher and reaches a positive value at charge ratios of 5/1, except for the third generation dendrimer, **21**, of family 1, where this value is 10/1. When the charge ratio is 1/1, negative values are founded

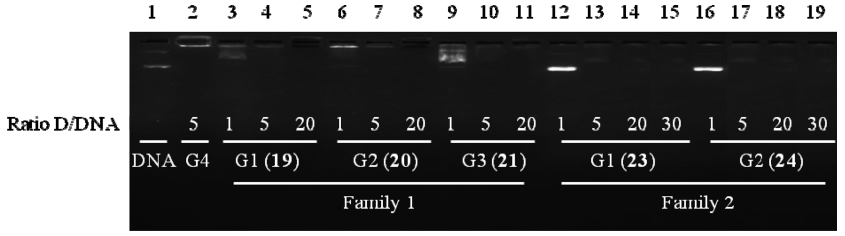

Figure 8. Electrophoresis gel of dendrimers. Line 1: Naked DNA. Line 2: polyplex with commercial G4-PAMAM. Polyplexes of family 1 [lines 3−5 (G1), 6−8 (G2) and 9−11 (G3)] were prepared at charge ratios 1:1, 1:5 and 1:20, and polyplexes of family 2 [lines 12−15 (G1) and 16−19 (G2)] were prepared at charge ratios 1:1, 1:5, 1:20 and 1:30.

Table 1. Particle Size (nm) of Dendriplexes (Dendrimer/DNA) Obtained by Laser Diffractometry

		family 1	family 2			
charge ratio (D/DNA)	G4-PAMAM	**19**	**20**	**21**	**23**	**24**
1/1		169 ± 18	182 ± 07	283 ± 15	107 ± 04	226 ± 09
5/1	138 ± 11	116 ± 15	202 ± 15	217 ± 16	88 ± 07	131 ± 02
10/1		108 ± 12	158 ± 05	163 ± 11	86 ± 08	121 ± 07
20/1		77 ± 02	119 ± 20	301 ± 18	84 ± 05	99 ± 11
30/1		93 ± 03	68 ± 01	100 ± 01	57 ± 02	78 ± 01

Table 2. Zeta Potential (mV) of Dendriplexes (Dendrimer/DNA)

		family 1	family 2			
charge ratio (D/DNA)	G4-PAMAM	**19**	**20**	**21**	**23**	**24**
1/1		−28 ± 8	−26 ± 10	−7 ± 4	−34 ± 1	−9 ± 10
5/1	11 ± 1	12 ± 3	14 ± 2	−21 ± 1	36 ± 0	34 ± 1
10/1		22 ± 5	18 ± 3	15 ± 3	33 ± 5	39 ± 3
20/1		29 ± 3	20 ± 6	22 ± 5	31 ± 3	42 ± 8
30/1		41 ± 2	29 ± 10	15 ± 4	30 ± 3	41 ± 0

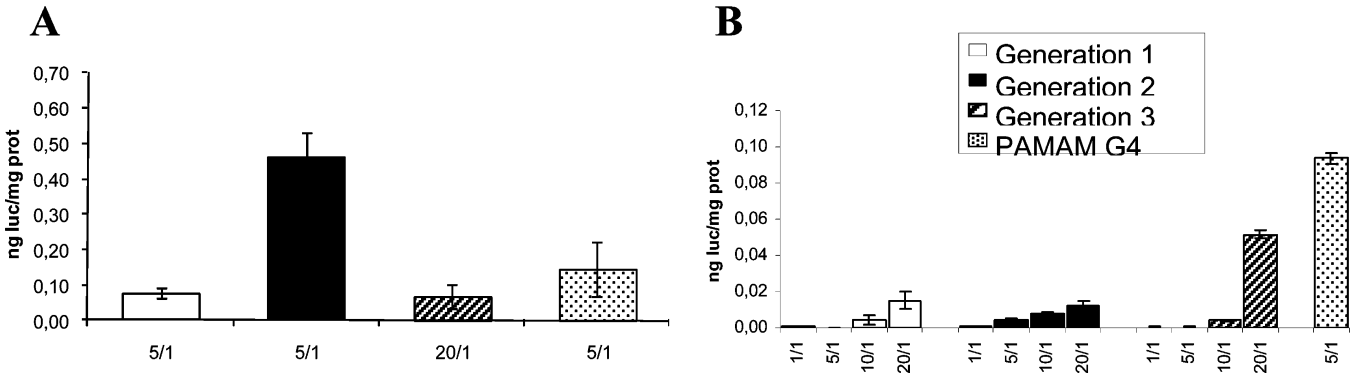

Figure 9. *In vitro* transfection efficiency for family 1 in the cell lines HepG2 (A) and HeLa (B).

probably due to the instability of complexes which are close to electroneutrality. Compared with the control, dendriplexes of family 1 present similar zeta potential values to that of the PAMAM dendriplexes, whereas dendriplexes of family 2 afford significantly higher values, which could be due again to the higher positive charge present in these dendrimers.

2.3. *In Vitro* Transfection Studies. The *in vitro* evaluation of dendriplexes as vehicles for transfection of DNA is presented only for dendrimers of family 1, since these did show the best results for being applied to *in vivo* studies. We carried out studies of toxicity and transfection efficiency in two cell lines: HepG2 (human hepatocellular carcinoma cells) and HeLa (cervix carcinoma cells). The results of transfection efficiency are represented in Figure 9. Cells were transfected with dendriplexes prepared with different generations at different charge ratios. The cells were also transfected with the following formulation used as control: (i) naked DNA to evaluate the differences between free and complexes DNA and (ii) fourth generation commercial PAMAM dendrimer at a charge ratio 5/1 as a control for complex formation.

Dendriplexes obtained with dendrimers of family 1 show the highest transfection efficiency for generation 2 at a charge ratio 5/1 in the case of the HepG2 cell line, and this efficiency value is greater than the value obtained with the commercial PAMAM formulation used as control. In Figure 9A we present the results in HepG2 cells after selecting the best charge ratio for each generation. For the HeLa cell line, Figure 9B shows that the transfection efficiency increases with the charge ratio and with the generation, highest values being found for the third generation dendrimer at a 20/1 charge ratio. Nevertheless,

dx.doi.org/10.1021/mp200542j | *Mol. Pharmaceutics* 2012, 9, 433−447

in this case all carbosilane dendriplexes show lower values than the control.

The two cell lines have very different expression levels, obtaining better results in HepG2 cells. One might think that differences in transfection efficiency of the different dendriplexes and cell lines could be due to differences in their biocompatibility. Some dendrimers could become toxic at certain doses by a strong interaction with the cell membrane and the consequent rupture of the same,[7] but toxicity tests conducted in this study showed that virtually all studied formulations were not toxic, since in all cases cell viability was observed close to 100% and never less than 80% (see the Supporting Information).

2.4. *In Vivo* Studies. Finally, to test whether our dendrimers could be used for *in vivo* gene delivery, the optimal in vitro formulation was the one selected for *in vivo* studies. Dendriplexes obtained for third generation dendrimer of family 1 (**21**) at a charge ratio of 20/1 (+/−) were injected systemically into mice, and their activity was compared to that of the commercial PAMAM G4 5/1 (+/−) formulation.

Figure 10 shows that these dendriplexes led to higher levels of gene expression compared with the PAMAM dendriplexes

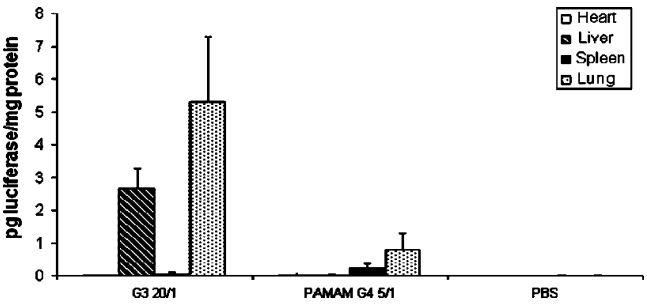

Figure 10. *In vivo* gene expression after intravenous administration of F1G3 20/1 (+/−) dendriplexes and commercial PAMAM G4 5/1 (+/−). The bars represent the mean ± SD (n = 6 animals per group). The data are representative of three independent experiments.

used as control, especially in the liver and the lung. This fact can be due to the formation of a different molecular structure in the carbosilane dendriplexes compared to the commercial PAMAM, which leads to more efficient dendriplexes. No transfection activity was detected by injecting the naked DNA or the control PBS. One possible explanation of these results is that positively charged complexes can be entrapped in the circulatory system of the lung, which represents the first microvascular bed encountered after the tail vein administration. On the other hand, the liver is composed predominately of hepatocytes and Kupffer cells. The Kupffer cells are the primary barrier to hepatocyte access and tend to remove nanoparticles from circulation. We think that, because of the small particle size of our particles, the capture by macrophages and Kupffer cells is avoided and the liver cells which are mainly transfected are the hepatocytes. It is important also to note that no signs of toxicity were noticed when dendriplexes were injected into the mice.

Based on our previous published studies[21] and others from the literature regarding the capacity of lipoplexes and polyplexes to protect DNA against DNase I degradation, we can say that the plasmid contained in the carbosilane dendriplexes is protected against DNase attack. If the plasmid would not be protected from degradation by endonucleases, no *in vivo* gene expression would have been observed.

3. CONCLUSIONS

Water-soluble cationic carbosilane complexes can be prepared using a click chemistry strategy through a Huisgen cycloaddition between azide-terminated carbosilane dendrimers and propargyl amines with subsequent addition of methyl iodide.

The dendrimers developed in this study were shown to generate nanosized particles with plasmid DNA. These dendriplexes had a remarkably low toxicity and good transfection efficiency *in vitro* and *in vivo*. They have the advantages of ease of preparation and economy in comparison with commercial transfection reagents, so they can be proposed as potential nonviral vehicles for transfection of nucleic acids.

4. EXPERIMENTAL SECTION

4.1. General Remarks. All manipulations of oxygen- or water-sensitive compounds were carried out under an atmosphere of argon using standard Schlenk techniques. Solvents were dried and freshly collected from a purification system of MBraun-SPS. Unless otherwise stated, reagents were obtained from commercial sources and used as received. Hydride-terminated carbosilane dendrimers of different generations G_n-$(SiH)_x$ were prepared according to reported methods.[14]

^{1}H, ^{13}C, and ^{29}Si NMR spectra were recorded on 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 chloroform-d_1, DMSO-d_6 used as solvents, and ^{29}Si chemical shifts were referenced to external $SiMe_4$ (0.00 ppm). 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 on a Bruker Reflex II spectrometer equipped with a nitrogen laser emitting at 337 nm and operated in the reflection mode at an accelerating voltage in the range 23−25 kV.

4.2. Fluorescence Experiments. The fluorescence experiments were performed with a Shimadzu RF540 spectrofluorimeter that was calibrated by checking its wavelength accuracy with Raman dispersion line of water and fluorescence intensity by using a quinine solution in sulfuric acid 0.1 N (USP 32nd). All measurements were made at 25 °C, setting the excitation and emission slit widths to 10 and 5 nm, respectively. Excitation and emission spectra of dendrimers were carried out in aqueous solutions (50 μg/mL), previously filtrated and degasified, containing 4% of DMSO, placed on quartz cells with path length of 1.0 cm.

4.2.1. Synthesis of $Et_3SiCH_2CH_2CH_2CH_2Br$ (1). In a glass ampule were dissolved 0.69 mL of triethylsilane (4.3 mmol) in hexane (5 mL) and 0.53 mL of 4-bromobutene (5.16 mmol). Then, 1 drop of a Karstedt catalyst solution was added and the mixture stirred for 16 h at 60 °C. Then, the reaction mixture was concentrated to dryness and redissolved in dichloromethane. The mixture was filtered through charcoal and Celite and taken to dryness to give a yellowish oil that is identified as compound **1** (1.06 g, 99%).

^{1}H NMR ($CDCl_3$): δ 3.41 (2H, t, CH_2Br), 1.85 (2H, m, $SiCH_2CH_2CH_2CH_2Br$), 1.42 (2H, m, $SiCH_2CH_2CH_2CH_2Br$), 0.91 (9H, t, $SiCH_2CH_3$), 0.48 (8H, m, CH_2 bonded to Si). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 36.5 (CH_2Br), 33.6 ($SiCH_2CH_2CH_2CH_2Br$), 22.4 ($SiCH_2CH_2CH_2CH_2Br$), 10.4 ($SiCH_2CH_2CH_2$

dx.doi.org/10.1021/mp200542j | Mol. Pharmaceutics 2012, 9, 433−447

CH_2Br), 7.4 ($SiCH_2CH_3$), 3.2 ($SiCH_2CH_3$). Elemental analysis $C_{36}H_{80}Br_4Si_5$: calcd %, C, 47.80; H, 9.23; found %, C, 47.75; H,9.12.

*4.2.2. Synthesis of G_1-[$SiCH_2CH_2CH_2CH_2Br$]$_4$ (**2**).* In a glass ampule were dissolved 1.52 g of dendrimer G_1-[Si−H] (3.52 mmol) in hexane (5 mL) and 1.61 mL of 4-bromobutene (15.85 mmol). Then, 5 drops of a Karstedt catalyst solution were added and the mixture was stirred vigorously for 5 days at 60 °C. Then, the reaction was concentrated to dryness and redissolved in dichloromethane. The mixture was filtered through charcoal and Celite and taken to dryness to give a yellowish oil that is identified as the first generation dendrimer **2** (2.32 g, 68%).

^{1}H NMR ($CDCl_3$): δ 3.40 (8H, t, CH_2Br), 1.85 (8H, m, $SiCH_2CH_2CH_2CH_2Br$), 1.37 (16H, m, $SiCH_2CH_2CH_2CH_2Br$ and $SiCH_2CH_2CH_2Si$), 0.51 (24H, m, CH_2 bonded to Si), −0.05 (24H, s, $SiCH_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 36.4 (CH_2Br), 33.7 ($SiCH_2CH_2CH_2CH_2Br$), 22.5($SiCH_2CH_2CH_2CH_2Br$), 20.2 ($SiCH_2$), 18.6 ($SiCH_2$), 17.6 ($SiCH_2$), 14.5 ($SiCH_2$), −3.3 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$): 1.9 ($Si(CH_3)_2$). Elemental analysis $C_{36}H_{80}Br_4Si_5$: calcd %, C, 44.44; H, 8.29; found %, C, 44.24; H, 8.18.

*4.2.3. Synthesis of G_2-[$SiCH_2CH_2CH_2CH_2Br$]$_8$ (**3**).* The second generation dendrimer **3** was prepared by a similar synthetic procedure starting from G_2-[Si−H]$_8$ (0.38 g), 4-bromobutene (0.28 mL, 2.7 mmol), hexane (10 mL) and Karstedt catalyst (2 drops). This will get the dendrimer **3** in high yield as a yellowish oil (0.61 g, 83%).

^{1}H NMR ($CDCl_3$): δ 3.40 (16H, t, CH_2Br), 1.85 (16H, m, $SiCH_2CH_2CH_2CH_2Br$), 1.31 (40H, m, $SiCH_2CH_2CH_2CH_2Br$ and $SiCH_2CH_2CH_2Si$), 0.53 (64H, m, CH_2 bonded to Si), −0.05 (48H, s, $Si(CH_3)_2$), −0.09 (12H, s, $SiCH_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 36.8 (CH_2Br), 34.0 ($SiCH_2CH_2CH_2CH_2Br$), 22.9 ($SiCH_2CH_2$), 20.5 ($SiCH_2CH_2$), 19.6 ($SiCH_2CH_2$), 19.3 ($SiCH_2$), 19.1 ($SiCH_2$), 18.9 ($SiCH_2$), 18.2 ($SiCH_2$), 14.9 ($SiCH_2$), −2.8 ($SiCH_3$), −4.5 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$): 1.7 ($Si(CH_3)_2$), 1.0 ($SiCH_3$). Elemental analysis $C_{88}H_{196}Br_8Si_{13}$: calcd %, C, 46.79; H, 8.75; found %, C, 46.51; H, 8.75.

*4.2.4. Synthesis of G_3-[$SiCH_2CH_2CH_2CH_2Br$]$_{16}$ (**4**).* The third generation dendrimer **4** was prepared by a similar synthetic procedure starting from G_3-[Si−H]$_{16}$ (0.81 g, 0.31 mmol), 4-bromobutene (0.51 mL, 5.04 mmol), hexane (20 mL) and Karstedt catalyst (2 drops). This will get the dendrimer **4** as a yellowish oil (1.27 g, 86%).

^{1}H NMR ($CDCl_3$): δ 3.40 (32H, t, CH_2Br), 1.85 (32H, m, $SiCH_2CH_2CH_2CH_2Br$), 1.30 (88H, m, $SiCH_2CH_2CH_2CH_2Br$ and $SiCH_2CH_2CH_2Si$), 0.53 (144H, m, CH_2 bonded to Si), −0.05 (120H, s, $Si(CH_3)_2$ y $SiCH_3$), −0.09 (12H, s, $SiCH_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 36.4 (CH_2Br), 33.6 ($SiCH_2CH_2CH_2CH_2Br$), 22.5 ($SiCH_2CH_2$), 20.1 ($SiCH_2CH_2$), 19.0−14.5 ($SiCH_2$), −3.3 ($SiCH_3$), −4.8 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$): 2.0 ($Si(CH_3)_2$), 1.1 ($SiCH_3$). Elemental analysis $C_{192}H_{428}Br_{16}Si_{29}$: calcd %, C, 47.74; H, 8.93; found %, C, 48.62; H, 9.00.

*4.2.5. Synthesis of $Et_3SiCH_2CH_2CH_2CH_2N_3$ (**5**).* In a glass ampule were suspended 1.09 g of **1** (4.33 mmol) in DMF (30 mL), to which were then added sodium azide (1.41 g, 21.65 mmol) and a spatula tip of sodium iodide. The mixture was heated to 90 °C for 16 h, then allowed to cool to room temperature and concentrated to dryness. The resulting residue was dissolved in dichloromethane (30 mL) and washed with distilled water (15 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum, yielding the product **5** as a yellowish oil (0.74 g, 80%).

^{1}H NMR ($CDCl_3$): δ 3.24 (2H, t, CH_2N_3), 1.60 (2H, m, $SiCH_2CH_2CH_2CH_2N_3$), 1.35 (2H, m, $SiCH_2CH_2CH_2CH_2N_3$), 0.91 (9H, t, $SiCH_2CH_3$), 0.50 (8H, m, CH_2 bonded to Si). ($^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 51.0 (CH_2N_3), 32.8 ($SiCH_2CH_2CH_2CH_2N_3$), 21.1 ($SiCH_2CH_2$), 10.9 ($SiCH_2CH_2CH_2CH_2N_3$), 7.4 ($SiCH_2CH_3$), 3.2 ($SiCH_2CH_3$).

*4.2.6. Synthesis of G_1-[$SiCH_2CH_2CH_2CH_2N_3$]$_4$ (**6**).* In a glass ampule were suspended 0.5 g of dendrimer **2** (0.52 mmol) in DMF (20 mL), to which were then added sodium azide (0.34 g, 5.16 mmol) and a spatula tip of sodium iodide. The mixture was heated to 90 °C for 16 h, then allowed to cool to room temperature and concentrated to dryness. The resulting residue was dissolved in dichloromethane (20 mL) and washed with distilled water (10 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum, yielding the product **6** as a yellowish oil (0.35 g, 81%).

^{1}H NMR ($CDCl_3$): δ 3.24 (8H, t, CH_2N_3), 1.60 (8H, m, $SiCH_2CH_2CH_2CH_2N_3$), 1.35 (16H, m, $SiCH_2CH_2CH_2CH_2N_3$ and $SiCH_2CH_2CH_2Si$), 0.52 (24H, m, CH_2 bonded to Si), −0.06 (24H, s, $Si(CH_3)_2$). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 51.1 (CH_2N_3), 32.6 ($SiCH_2CH_2CH_2CH_2N_3$), 21.1 ($SiCH_2CH_2$), 20.2 ($SiCH_2CH_2$), 18.6 ($SiCH_2$), 17.5 ($SiCH_2$), 15.0 ($SiCH_2$), −3.3 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$): 1.8 ($Si(CH_3)_2$).

*4.2.7. Synthesis of G_2-[$SiCH_2CH_2CH_2CH_2N_3$]$_8$ (**7**).* This second generation dendrimer was prepared by a similar synthetic procedure to that of **6** starting from **3** (1.00 g, 0.44 mmol), sodium azide (0.43 g, 6.64 mmol) and DMF (30 mL). This leads to dendrimer **7** as a yellowish oil (067 g, 78%).

^{1}H NMR ($CDCl_3$): δ 3.24 (16H, t, CH_2N_3), 1.60 (16H, m, $SiCH_2CH_2CH_2CH_2N_3$), 1.29 (40H, m, $SiCH_2CH_2CH_2CH_2N_3$ and $SiCH_2CH_2CH_2Si$), 0.51 (64H, m, CH_2 bonded to Si), −0.06 (48H, s, $Si(CH_3)_2$), −0.10 (24H, s, $SiCH_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 51.1 (CH_2N_3), 32.6 ($SiCH_2CH_2CH_2CH_2N_3$), 21.2 ($SiCH_2CH_2$), 20.5 ($SiCH_2CH_2$), 19.2 ($SiCH_2CH_2$), 18.8 ($SiCH_2$), 18.6 ($SiCH_2$), 18.5 ($SiCH_2$), 17.7 ($SiCH_2$), 15.0 ($SiCH_2$), −3.3 ($Si(CH_3)_2$), −4.9 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$): 1.7 ($Si(CH_3)_2$), 1.0 ($SiCH_3$). Elemental analysis $C_{88}H_{196}N_{24}Si_{13}$: calcd %, C, 54.04; H, 10.10; N, 17.19; found %, C, 54.81; H, 9.72; N, 12.94.

*4.2.8. Synthesis of G_3-[$SiCH_2CH_2CH_2CH_2N_3$]$_{16}$ (**8**).* This third generation dendrimer was prepared by a similar synthetic procedure to that of **6** starting from **4** (1.82 g, 0.38 mmol), sodium azide (0.50 g, 7.70 mmol) dissolved in a mixture DMF (30 mL) and THF (10 mL). This leads to dendrimer **8** as a yellowish oil (1.33 g, 82%).

^{1}H NMR ($CDCl_3$): δ 3.24 (32H, t, CH_2N_3), 1.60 (32H, m, $SiCH_2CH_2CH_2CH_2N_3$), 1.32 (88H, m, $SiCH_2CH_2CH_2CH_2N_3$ and $SiCH_2CH_2CH_2Si$), 0.54 (144H, m, CH_2 bonded to Si), −0.05 (120H, s, $Si(CH_3)_2$ and $SiCH_3$), −0.09 (12H, s, $SiCH_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 51.1 (CH_2N_3), 32.6 ($SiCH_2CH_2CH_2CH_2N_3$), 21.2 ($SiCH_2CH_2$), 20.0 ($SiCH_2CH_2$), 19.0−17.5 ($SiCH_2$) 15.0 ($SiCH_2$), −3.3 ($Si(CH_3)_2$), −4.9 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$): 1.7 ($Si(CH_3)_2$), 1.0 ($SiCH_3$). Elemental analysis $C_{192}H_{428}N_{48}Si_{29}$: calcd %, C, 54.59; H, 10.21; N, 15.92; found %, C, 56.08; H, 9.58; N, 11.08.

*4.2.9. Synthesis of [$HCC-CH_2OCH_2CH_2N(Me)-CH_2CH_2NMe_2$] (**9**).* This propargyl diamine was prepared following the experimental procedure given below. To 652 mg (16.32 mmol) of sodium hydride 60% was added 10 mL of hexane. The mixture was filtered, and over the solid was added 50 mL of THF. The resulting suspension was cooled to −30 °C,

and 2.21 mL (13.6 mmol) of 2-{[2(dimethylamino)ethyl]-methylamino}ethanol was added dropwise. After 3 h, an addition funnel was attached to the Schlenk flask, and 16 mL of THF containing 1.8 mL (16.32 mmol) of propargyl bromide 80% in toluene was transferred dropwise on the above suspension. The reaction mixture was kept under stirring for 16 h, allowing it to reach room temperature slowly. To stop the reaction, a saturated solution of ammonium chloride was added dropwise, until hydrogen evolution was finished. The mixture was extracted with ethyl acetate (3 × 50 mL), and the organic phases were pooled and washed with 100 mL of a saturated solution of sodium chloride. The organic phase was dried over $MgSO_4$ and filtered, and the solvent was removed under vacuum, obtaining a dark yellow oil (1.27 g, 51%).

^{1}H NMR ($CDCl_3$): 4.14 (2H, d, CCH_2O), 3.62 (2H, t, NCH_2CH_2O), 2.60 (2H, t, CH_2CH_2O), 2.51 and 2.40 (5H, m, CH_2CH_2N, overlapped with CC*H*), 2.28 (3H, s, NCH_3), 2.22 (6H, s, $N(CH_3)_2$). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 79.6 (*C*CH), 74.2 (C*C*H), 67.6 (CH_2CH_2O), 58.0 (CCH_2O), 57.2 (NCH_2CH_2O), 56.9 (NCH_2CH_2N), 55.7 (NCH_2CH_2N), 45.7 ($N(CH_3)_2$), 42.8 (NCH_3). Elemental analysis $C_{10}H_{20}N_2O$: calcd %, C, 65.18; H, 10.94; N, 15.20; found %, C, 64.32; H, 10.56; N, 14.81.

*4.2.10. Synthesis of Et$_3$[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_2$] (**10**).* In a Schlenk flask, 100 mg (0.47 mmol) of the starting azide **5** was dissolved in 5 mL of THF, and 0.06 mL (0.56 mmol) of *N*,*N*-dimethylpropargylamine was added. Then were added freshly prepared solutions of 11 mg (0.06 mmol) of sodium ascorbate in 0.2 mL of distilled water and 6 mg of copper sulfate pentahydrate in 0.2 mL of distilled water. The resulting mixture was stirred at room temperature. After 4 h, the reaction was stopped by adding 0.5 mL of a 23% solution of ammonium hydroxide, and it was stirred for 15 min. The mixture was extracted with ethyl acetate (3 × 5 mL), and the organic phases were combined, washed with a saturated solution of sodium chloride (10 mL) and dried over $MgSO_4$. Then, the material was filtered and concentrated under vacuum to give a yellow oil, characterized as compound **5** (101 mg, 73%).

^{1}H NMR ($CDCl_3$): δ 7.45 (1H, s, NC*H*CN), 4.33 (2H, t, CH_2CH_2N), 3.61 (2H, s, CCH_2N), 2.27 (6H, s, $N(CH_3)_2$), 1.90 (2H, m, $SiCH_2CH_2CH_2CH_2N$), 1.29 (2H, m, $SiCH_2CH_2CH_2CH_2N$), 0.88 (9H, t, $SiCH_2CH_3$), 0.47 (8H, m, $SiCH_2CH_3$ and $SiCH_2CH_2CH_2CH_2N$). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 145.0 (NCHCN), 121.9 (NCHCN), 54.3 (CCH_2N), 49.7 ($SiCH_2CH_2CH_2CH_2N$), 45.0 ($N(CH_3)_2$), 34.0 ($SiCH_2CH_2CH_2CH_2N$), 20.7 ($SiCH_2CH_2CH_2CH_2N$), 10.7 ($SiCH_2CH_2CH_2CH_2N$), 7.2 ($SiCH_2CH_3$), 3.0 ($SiCH_2CH_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −352 ($N_{aliphatic}$), −138 ($N_{pyrrole\text{-}like}$), −32 and −22 ($N_{pyridine\text{-}like}$). Elemental analysis $C_{15}H_{32}N_4Si$: calcd %, C, 60.76; H, 10.88; N, 18.99; found %, C 60.02; H, 10.61; N, 18.96.

*4.2.11. Synthesis of ck-G$_1$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_2$]$_4$ (**11**).* The preparation of the first generation dendrimer **11**, with four terminal amino groups, follows a similar synthetic procedure to that of **10**. Starting from the first generation dendrimer with terminal azide groups **6** (300 mg, 0.37 mmol) dissolved in 20 mL of THF and 0.17 mL (1.61 mmol) of *N*,*N*-dimethylpropargylamine, freshly prepared solutions of 34 mg (0.18 mmol) of sodium ascorbate in 1.7 mL of distilled water and 18 mg (0.07 mmol) of copper sulfate pentahydrate in 0.14 mL of distilled water were added. The reaction was stopped after 15 h, by adding 1.5 mL of a 23% ammonium hydroxide solution, and the workup was done with ethyl acetate (3 × 15 mL) and 20 mL of a saturated sodium chloride solution. This leads to dendrimer **11** as a yellow oil (382 mg, 91%).

^{1}H NMR ($CDCl_3$): δ 7.41 (4H, s, NC*H*CN), 4.28 (8H, t, $SiCH_2CH_2CH_2CH_2N$), 3.55 (8H, s, CCH_2N), 2.21 (24H, s, $N(CH_3)_2$), 1.85 (8H, m, $SiCH_2CH_2CH_2CH_2N$), 1.25 (16H, m, $SiCH_2CH_2$), 0.49 (24, m, $SiCH_2$), −0.13 (24H, s, $Si(CH_3)_2$). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 145.0 (NCHCN), 122.0 (NCHCN), 54.4 (CCH_2N), 49.8 ($SiCH_2CH_2CH_2CH_2N$), 45.1 ($N(CH_3)_2$), 33.9 ($SiCH_2CH_2CH_2CH_2N$) 20.8−14.7 ($SiCH_2CH_2$ and $SiCH_2$), −3.5 ($Si(CH_3)_2$). ^{15}N NMR {^{1}H} ($CDCl_3$): −351 ($N_{aliphatic}$), −138 ($N_{pyrrole\text{-}like}$), −32 and −22 ($N_{pyridine\text{-}like}$). ^{29}Si NMR ($CDCl_3$): 1.9 ($Si(CH_3)_2$). Elemental analysis $C_{56}H_{117}N_{16}Si_5$: calcd %, C, 58.28; H, 10.13; N, 19.42; found %, C, 58.07; H, 10.61; N, 18.96.

*4.2.12. Synthesis of ck-G$_2$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_2$]$_8$ (**12**).* This second generation dendrimer **12** is prepared by a similar synthetic procedure to that of **11**, starting from the second generation azide dendrimer **7** (300 mg, 0.15 mmol), 0.14 mL (1.29 mmol) of *N*,*N*-dimethylpropargylamine, 29 mg (0.15 mmol) of sodium ascorbate in 1.5 mL of distilled water, and 15 mg (0.06 mmol) of copper sulfate pentahydrate in 0.13 mL of distilled water. This leads to dendrimer **12** as a yellow oil (345 mg, 86%).

^{1}H NMR ($CDCl_3$): δ 7.39 (8H, s, NC*H*CN), 4.26 (16H, t, $SiCH_2CH_2CH_2CH_2N$), 3.52 (16H, s, CCH_2N), 2.19 (48H, s, $N(CH_3)_2$), 1.83 (16H, m, $SiCH_2CH_2CH_2CH_2N$), 1.21 (40H, m, $SiCH_2CH_2$), 0.46 (64H, m, $SiCH_2$), −0.01 (12H, s $SiCH_3$), −0.15 (24H, s, $Si(CH_3)_2$). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 145.0 (NCHCN), 121.9 (NCHCN), 54.3 (CCH_2N), 49.7 ($SiCH_2CH_2$-CH_2CH_2N), 45.0 ($N(CH_3)_2$), 33.8 ($SiCH_2CH_2CH_2CH_2N$) 20.8−14.7 ($SiCH_2CH_2$ y $SiCH_2$), −3.5 ($Si(CH_3)_2$), −5.2 ($SiCH_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −351 ($N_{aliphatic}$), −139 ($N_{pyrrole\text{-}like}$), −31 and −22 ($N_{pyridine\text{-}like}$). ^{29}Si NMR ($CDCl_3$): 1.8 ($Si(CH_3)_2$), 0.9 ($SiCH_3$). Elemental analysis $C_{128}H_{268}N_{32}Si_{13}$: calcd %, C, 58.66; H, 10.31; N, 17.10; observed. %: C 58.50; H, 10.67; N, 16.32.

*4.2.13. Synthesis of ck-G$_3$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_2$]$_{16}$ (**13**).* This third generation dendrimer **13** is prepared by a similar synthetic procedure to that of **11** and **12**, starting from the third generation azide dendrimer **8** (300 mg, 0.07 mmol) dissolved in 30 mL of THF, 0.13 mL (1.21 mmol) of *N*,*N*-dimethylpropargylamine, 27 mg (0.14 mmol) of sodium ascorbate in 1.4 mL of distilled water, and 14 mg (0.06 mmol) of copper sulfate pentahydrate in 0.12 mL of distilled water. This leads to dendrimer **13** as a yellowish oil (252 mg, 64%).

^{1}H NMR ($CDCl_3$): δ 7.42 (16H, s, NC*H*CN), 4.27 (32H, t, $SiCH_2CH_2CH_2CH_2N$), 3.54 (32H, s, CCH_2N), 2.20 (96H, s, $N(CH_3)_2$), 1.84 (32H, m, $SiCH_2CH_2CH_2CH_2N$), 1.22 (88H, m, $SiCH_2CH_2$), 0.47 (144, m, $SiCH_2$), 0.00 (12H, s, $SiCH_3$), −0.14 (120H, s, $Si(CH_3)_2$ and $SiCH_3$). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 144.9 (NCHCN), 122.0 (NCHCN), 54.3 (CH_2N), 49.8 ($SiCH_2CH_2CH_2CH_2N$), 45.0 ($N(CH_3)_2$), 33.9 ($SiCH_2CH_2CH_2CH_2N$), 20.8−14.7 ($SiCH_2CH_2$ y $SiCH_2$), −3.5 ($Si(CH_3)_2$), −5.1($SiCH_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −351 ($N_{aliphatic}$), −139 ($N_{pyrrole\text{-}like}$), −31 and −21 ($N_{pyridine\text{-}like}$).^{29}Si NMR ($CDCl_3$): 1.7 ($Si(CH_3)_2$), ($SiCH_3$) not observed. Elemental analysis $C_{272}H_{572}N_{64}Si_{29}$: calcd %, C, 58.82; H, 10.38; N, 16.14; found %, C 56.67; H, 10.51; N, 11.56.

*4.2.14. Synthesis of Et$_3$[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$OCH$_2$CH$_2$N-(Me)CH$_2$CH$_2$NMe$_2$] (**14**).* This compound **14** is prepared by a similar procedure to that of **10**, starting from the azide **5** (300 mg, 0.47 mmol) dissolved in 15 mL of THF, 259 mg

dx.doi.org/10.1021/mp200542j | Mol. Pharmaceutics 2012, 9, 433−447

(1.41 mmol) of *N,N,N′*-trimethyl-*N′*-(2-(prop-2-inyloxy)ethyl)-ethano-1,2-diamine ($HCCCH_2OCH_2CH_2N(Me)$-$CH_2CH_2NMe_2$),
34 mg (0.17 mmol) of sodium ascorbate in 0.2 mL of distilled water, and 18 mg (0.07 mmol) of copper sulfate pentahydrate in 0.2 mL of distilled water. The reaction was stopped after 4 h, by adding 1.5 mL of a 23% ammonium hydroxide solution, and the workup was done with ethyl acetate (3 × 15 mL) and 30 mL of a saturated sodium chloride solution. This leads to compound **14** as a yellowish oil (510 mg, 91%).

^{1}H NMR ($CDCl_3$): δ 7.50 (1H, s, NC*H*CN), 4.63 (2H, s, CCH_2O), 4.32 (2H, t, SiCH$_2$CH$_2$CH$_2$CH_2N), 3.61 (2H, t, OCH_2CH$_2$N), 2.61 (2H, t, OCH$_2$CH_2N), 2.50 and 2.37 (4H, m, NCH_2CH_2N), 2.26 (3H, s, NCH_3), 2.21 (6H, s, N(CH_3)$_2$), 1.90 (2H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.32 (2H, m, SiCH$_2$CH_2CH$_2$CH$_2$N), 0.89 (9H, t, SiCH$_2$CH_3), 0.48 (8H, m, SiCH_2CH$_3$ and SiCH_2CH$_2$CH$_2$CH$_2$N). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 144.7 (NCHCN), 122.1 (NCHCN), 68.1 (OCH$_2$-CH$_2$N), 64.3 (CCH$_2$O), 56.9 (OCH$_2$CH$_2$N) 56.5 (NCH$_2$CH$_2$N), 55.0 (NCH$_2$CH$_2$N), 49.6 (SiCH$_2$CH$_2$CH$_2$CH_2N), 45.1 (N-(CH_3)$_2$), 42.7 (NCH_3), 34.0 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 20.7 (SiCH$_2$-CH_2CH$_2$CH$_2$N), 10.6 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 7.2 (SiCH$_2$CH$_3$), 3.0 (SiCH_2CH$_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −355 and −351 ($N_{aliphatic}$), −131 ($N_{pyrrole\text{-}like}$), −31 and −18 ($N_{pyridine\text{-}like}$). Elemental analysis $C_{20}H_{43}N_5OSi$: calcd %, C, 60.40; H, 10.90; N, 17.61; found %, C 60.66; H, 11.17; N, 18.47.

*4.2.15. Synthesis of ck-G$_1$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$OCH$_2$CH$_2$N-(Me)CH$_2$CH$_2$NMe$_2$]$_4$ (**15**).* The preparation of the first generation dendrimer **15** follows a similar synthetic procedure to that of **10**−**14**. Starting from the first generation dendrimer with terminal azide groups **6** (300 mg, 0.47 mmol) dissolved in 12 mL of THF and 269 mg (1.46 mmol) of *N,N,N′*-trimethyl-*N′*-(2-(prop-2-inyloxy)ethyl)ethano-1,2-diamine, freshly prepared solutions of 35 mg (0.17 mmol) of sodium ascorbate in 0.2 mL of distilled water and 18 mg (0.07 mmol) of copper sulfate pentahydrate in 0.2 mL of distilled water were added. This leads to dendrimer **15** as a dark yellowish oil (473 mg, 91%).

^{1}H NMR ($CDCl_3$): δ 7.51 (8H, s, NC*H*CN), 4.63 (8H, s, CCH_2O), 4.31 (8H, t, SiCH$_2$CH$_2$CH$_2$CH_2N), 3.61 (8H, t, OCH_2CH$_2$N), 2.61 (8H, t, OCH$_2$CH_2N), 2.52 and 2.40 (16H, m, NCH_2CH_2N), 2.26 (12H, s, NCH_3), 2.23 (24H, s, N(CH_3)$_2$), 1.90 (8H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.28 (16H, m, SiCH$_2$CH_2), 0.52 (24, m, SiCH_2), 0.08 (24H, s, Si(CH_3)$_2$). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 145.0 (NCHCN), 122.2 (NCHCN), 68.4 (OCH$_2$CH$_2$N), 64.4 (CCH$_2$O), 57.1 (OCH$_2$CH$_2$N), 57.0 and 55.6 (NCH$_2$CH$_2$N) 55.6, 49.9 (SiCH$_2$CH$_2$CH$_2$CH_2N), 45.6 (N(CH_3)$_2$), 42.9 (NCH_3), 33.9 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 20.9−14.8 (SiCH$_2$ and SiCH$_2$CH$_2$), −3.5 (Si(CH_3)$_2$). ^{15}N NMR {^{1}H} ($CDCl_3$): −355 and −351 ($N_{aliphatic}$), −130 ($N_{pyrrole\text{-}like}$), −30 and −18 ($N_{pyridine\text{-}like}$).^{29}Si NMR ($CDCl_3$): 1.7 (Si(CH$_3$)$_2$). Elemental analysis $C_{76}H_{160}N_{20}O_4Si_5$: calcd %, C, 58.56; H, 10.35; N, 17.97; found %, C 58.07; H, 10.61; N, 16.96.

*4.2.16. Syntheis of ck-G$_2$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$OCH$_2$CH$_2$N-(Me)CH$_2$CH$_2$NMe$_2$]$_8$ (**16**).* The preparation of the second generation dendrimer **16** follows a similar synthetic procedure to that of **10**−**15**. Starting from the second generation dendrimer with terminal azide groups **7** (494 mg, 0.25 mmol) dissolved in 20 mL of THF and 373 mg (2.02 mmol) of *N,N,N′*-trimethyl-*N′*-(2-(prop-2-inyloxy)ethyl)ethano-1,2-diamine, freshly prepared solutions of 48 mg (0.24 mmol) of sodium ascorbate in 0.25 mL of distilled water and 25 mg (0.10 mmol) of copper sulfate pentahydrate in 0.3 mL of distilled water were added. This leads to dendrimer **16** as a dark yellowish oil (721 mg, 83%).

^{1}H NMR ($CDCl_3$): δ 7.51 (8H, s, NC*H*CN), 4.62 (16H, s, CCH_2O), 4.31 (16H, t, SiCH$_2$CH$_2$CH$_2$CH_2N), 3.61 (16H, t, OCH_2CH$_2$N), 2.61 (16H, t, OCH$_2$CH_2N), 2.50 and 2.37 (32H, m, NCH_2CH_2N), 2.26 (24H, s, NCH_3), 2.20 (48H, s, N(CH_3)$_2$), 1.90 (16H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.28 (48H, m, SiCH$_2$CH_2), 0.53 (80H, m, SiCH_2), −0.08 (12H, s, SiCH_3), −0.10 (48H, s, Si(CH_3)$_2$). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 144.9 (NCHCN), 122.0 (NCHCN), 68.3 (OCH$_2$CH$_2$N), 64.4 (CCH$_2$O), 57.1 (OCH$_2$CH$_2$N), 57.0 and 56.0 (NCH$_2$CH$_2$N), 49.7 (SiCH$_2$CH$_2$CH$_2$CH_2N), 45.6 (N(CH_3)$_2$), 42.8 (NCH_3), 33.8 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 20.8−14.7 (SiCH$_2$ and SiCH$_2$CH$_2$), −3.3 (Si(CH_3)$_2$), −5.0 (SiCH$_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −354 and −350 ($N_{aliphatic}$), −130 ($N_{pyrrole\text{-}like}$), −31 and −19 ($N_{pyridine\text{-}like}$). ^{29}Si NMR ($CDCl_3$): 1.7 (Si(CH$_3$)$_2$), 0.6 (SiCH$_3$). Elemental analysis $C_{168}H_{356}N_{40}O_8Si_{13}$: calcd %, C, 58.83; H, 10.46; N, 16.33; O, 3.73; Si, 10.64; found %, C, 57.61; H, 10.42; N, 15.29.

*4.2.17. Synthesis of ck-G$_3$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$OCH$_2$CH$_2$N-(Me)CH$_2$CH$_2$NMe$_2$]$_{16}$ (**17**).* The preparation of the third generation dendrimer **17** follows a similar synthetic procedure to that of **10**−**16**. Starting from the third generation dendrimer with terminal azide groups **8** (200 mg, 0.05 mmol) dissolved in 20 mL of THF and 140 mg (0.76 mmol) of *N,N,N′*-trimethyl-*N′*-(2-(prop-2-inyloxy)ethyl)ethano-1,2-diamine, freshly prepared solutions of 18 mg (0.09 mmol) of sodium ascorbate in 0.1 mL of distilled water and 9 mg (0.10 mmol) of copper sulfate pentahydrate in 0.1 mL of distilled water were added. This leads to dendrimer **17** as a dark yellowish oil (155 mg, 46%).

^{1}H NMR ($CDCl_3$): δ 7.51 (16H, s, NC*H*CN), 4.61 (32H, s, CCH_2O), 4.30 (32H, t, CH$_2$CH_2N), 3.60 (32H, t, OCH_2CH$_2$N), 2.62 (32H, t, OCH$_2$CH_2N), 2.50 and 2.37 (64H, m, NCH_2CH_2N), 2.26 (48H, s, NCH_3), 2.21 (96H, s, N(CH_3)$_2$), 1.90 (32H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.27 (96H, m, SiCH$_2$CH_2), 0.56 (0.54 (160H, m, Si CH_2), −0.09 (48H, s, SiCH_3),-0.11 (24H, s, SiCH_3). ^{13}C NMR {^{1}H} ($CDCl_3$): δ 144.9 (NCHCN), 122.0 (NCHCN), 68.3 (OCH$_2$CH$_2$N), 64.4 (CCH$_2$O), 57.1 (OCH$_2$CH$_2$N) 57.0 (NCH$_2$CH$_2$N), 55.7 (NCH$_2$CH$_2$N), 49.8 (SiCH$_2$CH$_2$CH$_2$CH_2N), 45.6 (N(CH_3)$_2$), 42.9 (NCH_3), 33.9 (CH$_2$CH$_2$N), 20.8−14.7 (SiCH$_2$ and SiCH$_2$CH$_2$), −3.5 (Si(CH_3)$_2$), −5.1 (SiCH$_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −353 and −351 ($N_{aliphatic}$), −130 ($N_{pyrrole\text{-}like}$), −31 and −20 ($N_{pyridine\text{-}like}$). ^{29}Si NMR ($CDCl_3$): 1.9 (Si-(CH$_3$)$_2$), 1.0 (SiCH$_3$).

*4.2.18. Synthesis of Et$_3$[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_3^+$, I$^-$] (**18**).* To a solution of the compound with one dimethylamino terminal group **10** (101 mg, 0.34 mmol) in 5 mL of THF was added 0.031 mL (0.51 mmol) of methyl iodide, and it was stirred 13 h at room temperature. The resulting suspension was filtered and dried under vacuum, yielding a white solid identified as **18** (148 mg, 99%).

^{1}H NMR (DMSO-d_6): δ 8.44 (1H, s, NC*H*CN), 4.68 (2H, s, CCH_2N), 4.43 (2H, t, SiCH$_2$CHCH$_2$CH_2N), 3.06 (9H, s, N(CH_3)$_3$), 1.85 (2H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.22 (2H, m, SiCH$_2$CH_2CH$_2$CH$_2$N), 0.85 (9H, t, SiCH$_2$CH_3), 0.46 (8H, m, SiCH_2CH$_3$ and SiCH_2CH$_2$CH$_2$CH$_2$N). ^{13}C NMR {^{1}H} (DMSO-d_6): δ 134.7 (NCHCN), 127.3 (NCHCN), 58.4 (CCH$_2$N), 51.2 (N(CH$_3$)$_2$), 48.7 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 32.8 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 19.6 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 9.6 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 6.7 (SiCH$_2$CH$_3$), 2.2 (SiCH$_2$CH$_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −310 ($N_{aliphatic}$), −138 ($N_{pyrrole\text{-}like}$), −32 and −22 ($N_{pyridine\text{-}like}$).

*4.2.19. Synthesis of ck-G$_1$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_3^+$, I$^-$]$_4$ (**19**).* The preparation of the first generation dendrimer **19** follows a similar synthetic procedure to that of **18**, starting from the first generation dendrimer with terminal amino groups **11** (0.38 g, 0.33 mmol) in 15 mL of THF and 0.13 mL (2.02 mmol) of methyl iodide. Dendrimer **19** is obtained as a yellow solid (478 mg, 84%).

^{1}H NMR (DMSO-d_6): δ 8.39 (4H, s, NC*H*CN), 4.63 (8H, s, CCH_2N), 4.38 (8H, t, SiCH$_2$CHCH$_2$CH_2N), 3.03 (24H, s, N(CH_3)$_3$), 1.82 (8H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.25 (16H, m, SiCH$_2$CH_2), 0.49 (24, m, SiCH_2), −0.12 (24H, s, Si(CH_3)$_2$). ^{13}C NMR {^{1}H} (DMSO-d_6): δ 134.7 (NCHCN), 127.3 (NCHCN), 58.5 (CCH$_2$N), 51.3 (N(CH_3)$_2$), 48.7 (SiCH$_2$-CH$_2$CH$_2$CH$_2$N), 32.8 (SiCH$_2$CH$_2$CH$_2$CH$_2$N) 19.8−13.7 (SiCH$_2$ and SiCH$_2$CH$_2$), −3.9 (Si(CH$_3$)$_2$). ^{15}N NMR {^{1}H} (CDCl$_3$): −310 (N$_{aliphatic}$), −138 (N$_{pyrrole\text{-}like}$), −32 and −22 (N$_{pyridine\text{-}like}$). ^{29}Si NMR (CDCl$_3$): 1.7 (Si(CH$_3$)$_2$). Elemental analysis C$_{60}$H$_{129}$I$_4$N$_{16}$Si$_5$: calcd %, C, 41.85; H, 7.49; N, 13.02; found %, C 41.25; H, 7.60; N, 11.21. UV−vis: λ_{max} = 224 nm (ε = 28401 M^{-1} cm^{-1}) and 320 nm (ε = 333 M^{-1} cm^{-1}).

*4.2.20. Synthesis of ck-G$_2$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_3^+$, I$^-$]$_8$ (**20**).* The preparation of the second generation dendrimer **20** follows a similar synthetic procedure to that of **18** and **19**, starting from the second generation dendrimer with terminal amino groups **12** (0.24 g, 0.08 mmol) in 10 mL of THF and 0.04 mL (0.68 mmol) of methyl iodide. Dendrimer **20** is obtained as a yellowish solid (244 mg, 81%).

^{1}H NMR (DMSO-d_6): δ 8.44 (8H, s, NC*H*CN), 4.64 (16H, s, CCH_2N), 4.40 (16H, s, SiCH$_2$CH$_2$CH$_2$CH_2N), 3.05 (72H, s, N(CH_3)$_3$), 1.81 (16H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.25 (40H, m, SiCH$_2$CH_2), 0.50 (64H, m, SiCH_2),-0.10 (60H, s, SiCH$_3$ and Si(CH_3)$_2$). ^{13}C NMR {^{1}H} (DMSO-d_6): δ 134.8 (NCHCN), 127.3 (NCHCN), 58.5 (CCH$_2$N), 51.3 (SiCH$_2$-CH$_2$CH$_2$CH$_2$N), 48.8 (N(CH$_3$)$_2$), 32.8 (SiCH$_2$CH$_2$CH$_2$-CH$_2$N) 19.8−13.8 (SiCH$_2$CH$_2$ and SiCH$_2$), −3.9 (SiCH$_3$). ^{15}N NMR {^{1}H} (CDCl$_3$): −309 (N$_{aliphatic}$), −138 (N$_{pyrrole\text{-}like}$), −32 and −21 (N$_{pyridine\text{-}like}$). ^{29}Si NMR (CDCl$_3$): 1.7 (Si(CH$_3$)$_2$) and (SiCH$_3$) overlapped. Elemental analysis C$_{136}$H$_{292}$I$_8$N$_{32}$Si$_{13}$: Calcd. C, 43.49; H, 7.84; I, 27.03; N, 11.93; found %, C 43.75; H, 7.63; N, 9.86. UV−vis: λ_{max} = 224 nm (ε = 100000 M^{-1} cm^{-1}) and 320 nm (ε = 340 M^{-1} cm^{-1}).

*4.2.21. Synthesis of ck-G$_3$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)CH$_2$NMe$_3^+$, I$^-$]$_{16}$ (**21**).* The preparation of the third generation dendrimer **21** follows a similar synthetic procedure to that of **18**−**20**, starting from the third generation dendrimer with terminal amino groups **13** (0.32 g, 0.06 mmol) in 15 mL of THF and 0.006 mL (0.09 mmol) of methyl iodide. Dendrimer **21** is obtained as a yellowish solid (302 mg, 68%).

^{1}H NMR (DMSO-d_6): δ 8.38 (16H, s, NC*H*CN), 4.62 (32H, t, SiCH$_2$CH$_2$CH$_2$CH_2N), 4.39 (32H, s, CCH_2N), 3.04 (144H, s, N(CH_3)$_2$), 1.83 (32H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.27 (88H, m, SiCH$_2$CH_2), 0.51 (144, m, SiCH_2), −0.10 (132H, s, Si(CH_3)$_2$ and SiCH_3) ^{13}C NMR {^{1}H} (DMSO-d_6): δ 134.8 (NCHCN), 127.3 (NCHCN), 58.4 (CCH$_2$N), 51.4 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 48.8 (N(CH$_3$)$_2$), 45.0 (CH$_2$N), 32.9 (CH$_2$CH$_2$CH$_2$CH$_2$N) 19.8−13.7 (SiCH$_2$CH$_2$ and SiCH$_2$), −3.9 (SiCH$_3$), −5.1 (SiCH$_3$). ^{15}N NMR {^{1}H} (CDCl$_3$): −310 (N$_{aliphatic}$), −139 (N$_{pyrrole\text{-}like}$), −32 and −21 (N$_{pyridine\text{-}like}$). ^{29}Si NMR (CDCl$_3$): 1.8 (Si(CH$_3$)$_2$) and (SiCH$_3$) overlapped. UV−vis: λ_{max} = 224 nm (ε = 156942 M^{-1} cm^{-1}) and 320 nm (ε =537 M^{-1} cm^{-1}).

*4.2.22. Synthesis of Et$_3$[Si(CH$_2$)$_4$(N$_3$C$_2$H)-CH$_2$OCH$_2$CH$_2$N$^+$(Me$_2$)CH$_2$CH$_2$NMe$_3^+$] 2I$^-$ (**22**).* The preparation of the compound **22** follows a similar synthetic procedure to that of **18**-**21**, starting from the compound **14** (0.50 g, 1.26 mmol) in 20 mL of THF and 0.195 mL (3.14 mmol) of methyl iodide. Compound **22** is obtained as a yellowish solid (751 mg, 88%).

^{1}H NMR (DMSO-d_6): δ 8.17 (1H, s, NC*H*CN), 4.63 (2H, s, CCH_2O), 4.35 (4H, t, SiCH$_2$CH$_2$CH$_2$CH_2N), 3.97 (6H, s, NCH_2CH_2N and OCH_2CH$_2$N), 3.65 (2H, t, OCH$_2$CH_2N), 3.21 (6H, s, N(CH_3)$_2$), 3.18 (9H, s, N(CH_3)$_3$), 1.81 (SiCH$_2$CH$_2$CH_2CH$_2$N), 1.23 (2H, m, SiCH$_2$CH_2CH$_2$CH$_2$N), 0.86 (9H, t, SiCH$_2$CH_3), 0.48 (8H, m, SiCH_2CH$_3$ and SiCH_2CH$_2$CH$_2$CH$_2$N). ^{13}C NMR {^{1}H} (DMSO-d_6): δ 142.4 (NCHCN), 123.5 (NCHCN), 62.7 (CCH$_2$O), 62.6 (OCH$_2$-CH$_2$N), 62.4 (OCH$_2$CH$_2$N) 56.4 and 55.0 (NCH$_2$CH$_2$N), 52.5 (N(CH$_3$)$_3$), 51.2 (N(CH$_3$)$_2$), 48.4 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 33.1 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 19.6 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 9.6 (SiCH$_2$CH$_3$), 6.8 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 2.2 (SiCH$_2$CH$_3$). ^{15}N NMR {^{1}H} (CDCl$_3$): −331 and −326 (N$_{aliphatic}$), −129 (N$_{pyrrole\text{-}like}$), −28 and −17 (N$_{pyridine\text{-}like}$).

*4.2.23. Synthesis of ck-G$_1$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)-CH$_2$OCH$_2$CH$_2$N$^+$(Me$_2$)CH$_2$CH$_2$NMe$_3^+$]$_4$ 8I$^-$ (**23**).* The preparation of the first generation dendrimer **23** follows a similar synthetic procedure to that of **18**−**22**, starting from the first generation dendrimer with terminal amino groups **11** (0.47 g, 0.30 mmol) in 20 mL of THF and 0.16 mL (2.59 mmol) of methyl iodide. Dendrimer **23** is obtained as a yellowish solid (658 mg, 83%).

^{1}H NMR (DMSO-d_6): δ 8.18 (4H, s, NC*H*CN), 4.63 (8H, s, CCH_2O), 4.33 (8H, t, SiCH$_2$CH$_2$CH$_2$CH_2N), 3.94 (24H, s, NCH_2CH_2N and OCH_2CH$_2$N), 3.66 (8H, s, OCH$_2$CH_2N) 3.20 (24H, s, N(CH_3)$_2$), 3.17 (36H, s, N(CH_3)$_3$), 1.79 (8H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.24 (16H, m, SiCH$_2$CH_2CH$_2$CH$_2$N), 0.52 (16, m, SiCH_2), −0.10 (24H, s, Si(CH_3)$_2$). ^{13}C NMR {^{1}H} (DMSO-d_6): δ 142.4 (NCHCN), 123.4 (NCHCN), 62.8 (CCH$_2$O), 62.6 (OCH$_2$CH$_2$N), 62.4 (OCH$_2$CH$_2$N), 56.5 and 55.5 (NCH$_2$CH$_2$N), 52.5 (N(CH$_3$)$_3$), 51.2 (N(CH$_3$)$_2$), 48.5 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 33.0 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 19.8−13.7 (SiCH$_2$ y SiCH$_2$CH$_2$), −3.8 (Si(CH$_3$)$_2$). ^{15}N NMR {^{1}H} (CDCl$_3$): −331 and −326 (N$_{aliphatic}$), −128 (N$_{pyrrole\text{-}like}$), −28 and −18 (N$_{pyridine\text{-}like}$). ^{29}Si NMR (CDCl$_3$): 1.8 (Si(CH$_3$)$_2$). UV−vis: λ_{max} = 224 nm (ε = 176735 M^{-1} cm^{-1}) and 320 nm (ε = 1232 M^{-1} cm^{-1}).

*4.2.24. Synthesis of ck-G$_2$-[Si(CH$_2$)$_4$(N$_3$C$_2$H)-CH$_2$OCH$_2$CH$_2$N$^+$(Me$_2$)CH$_2$CH$_2$NMe$_3^+$]$_8$ 16I$^-$ (**24**).* The preparation of the second generation dendrimer **24** follows a similar synthetic procedure to that of **18**−**23**, starting from the second generation dendrimer with terminal amino groups **12** (0.47 g, 0.30 mmol) in 20 mL of THF and 0.16 mL (2.59 mmol) of methyl iodide. Dendrimer **24** is obtained as a yellowish solid (658 mg, 83%).

^{1}H NMR (DMSO-d_6): δ 8.18 (8H, s, NC*H*CN), 4.62 (16H, s, CCH_2O), 4.33 (16H, t, SiCH$_2$CH$_2$CH$_2$CH_2N), 3.94 (48H, s, NCH_2CH_2N and OCH_2CH$_2$N), 3.66 (16H, s, OCH$_2$CH_2N), 3.20 (120H, s, N(CH_3)$_2$ and N(CH_3)$_3$), 1.79 (16H, m, SiCH$_2$CH$_2$CH_2CH$_2$N), 1.27 (16H, SiCH$_2$CH_2CH$_2$CH$_2$N), 0.51 (56H, m, SiCH_2), −0.10 (24H, s, Si(CH_3)$_2$ y Si(CH_3)). ^{13}C NMR {^{1}H} (DMSO-d_6): δ 142.4 (NCHCN), 123.4 (NCH-CN), 62.8 (CCH$_2$O), 62.7 (OCH$_2$CH$_2$N), 62.4 (OCH$_2$CH$_2$N), 56.4 and 55.6 (NCH$_2$CH$_2$N), 52.5 (N(CH$_3$)$_3$), 51.2 (N(CH$_3$)$_2$), 48.5 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 33.0 (SiCH$_2$CH$_2$CH$_2$CH$_2$N), 19.8−13.7 (SiCH$_2$ and SiCH$_2$CH$_2$), −3.9 (Si(CH$_3$)$_2$). ^{15}N NMR {^{1}H} (CDCl$_3$): −330 and −325 (N$_{aliphatic}$), −129 (N$_{pyrrole\text{-}like}$),

dx.doi.org/10.1021/mp200542j | *Mol. Pharmaceutics* 2012, 9, 433−447

−27 and −17 ($N_{pyridine\text{-}like}$). ^{29}Si NMR ($CDCl_3$): 1.7 ($Si(CH_3)_2$) and ($SiCH_3$) overlapped. UV−vis: λ_{max} = 224 nm (ε = 212383 M^{-1} cm^{-1}) and 320 nm (ε = 1333 M^{-1} cm^{-1}).

4.2.25. Synthesis of ck-G3-[Si(CH2)4(N3C2H)-CH2OCH2CH2N+(Me2)CH2CH2NMe3+]16 32I− (25). The preparation of the third generation dendrimer **25** follows a similar synthetic procedure to that of **18**−**24**, starting from the third generation dendrimer with terminal amino groups **13** (0.16 g, 0.02 mmol) in 5 mL of THF and 0.04 mL (0.70 mmol) of methyl iodide. Dendrimer **25** is obtained as a yellowish solid (140 mg, 60%).

^{1}H NMR (DMSO-d_6): δ 8.20 (16H, s, NC*H*CN), 4.63 (32H, s, CCH_2O), 4.32 (32H, t, $SiCH_2CH_2CH_2CH_2N$), 3.98 (96H, s, NCH_2CH_2N and OCH_2CH_2N), 3.68 (32H, s, OCH_2CH_2N) 3.20 (240H, s, $N(CH_3)_2$ and $N(CH_3)_3$), 1.76 (32H, m, $SiCH_2CH_2CH_2CH_2N$), 1.26 (32H, m, $SiCH_2CH_2CH_2CH_2N$), 0.51 (144, m, $SiCH_2$), −0.10 (132H, s, $Si(CH_3)_2$ and $Si(CH_3)$). ^{13}C NMR {^{1}H} (DMSO-d_6): δ 142.4 (NCHCN), 123.5 (NCHCN), 62.8 (CCH_2O), 62.7 (OCH_2CH_2N), 62.4 (OCH_2CH_2N), 56.4 and 55.6 (NCH_2CH_2N), 52.5 ($N(CH_3)_3$), 51.2 ($N(CH_3)_2$), 48.5 ($SiCH_2CH_2CH_2CH_2N$), 33.0 ($SiCH_2CH_2CH_2CH_2N$), 19.8−13.7 ($SiCH_2$ y $SiCH_2CH_2$), −3.9 ($Si(CH_3)_2$), −5.4 ($SiCH_3$). ^{15}N NMR {^{1}H} ($CDCl_3$): −330 and −326 ($N_{aliphatic}$), −130 ($N_{pyrrole\text{-}like}$), −28 and −18 ($N_{pyridine\text{-}like}$). ^{29}Si NMR ($CDCl_3$): 1.8 ($Si(CH_3)_2$) and ($SiCH_3$) overlapped.

4.3. Biomedical Assays. *4.3.1. Material.* The plasmid pCMVLuc (Bioserve Technologies, Laurel, MD) encoding luciferase was used in the transfection studies. The HEPES glucose buffer was prepared from D-(+)-glucose and *N*-(2-hydroxyethyl)piperazine-*N*′-[2-ethanosulfonic acid] (Sigma-Aldrich). Alamar Blue Dye was purchased from Accumed International Companies (Westlake, OH). For this study, first, second and third generation corresponding to the family 1 and second generation for the family 2 of the synthesized dendrimers were used. Polyamidoamine dendrimer with ethylenediamine core, generation 4, was used as a control.

4.3.2. Cell Culture. HepG2 (human hepatoblastoma) and HeLa (human cervix carcinoma) cells were obtained from the American Type Culture Collection (Rockville, MD). The cells were maintained at 37 °C under 5% CO_2 in Dulbecco's modified Eagle's medium, high glucose (DMEM-HG), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 μg/mL), and L-glutamine (4 mM). All the products were obtained from Gibco BRL Life Technologies (Barcelona, Spain).

4.3.3. Preparation of Dendrimer−DNA Complexes. Complexes were formed by incubating equal volumes of dendrimer and plasmid DNA at room temperature (20−25 °C) for 20 min to obtain different charge ratios (+/−). The final DNA concentration in the dendriplexes was 5 μg/mL. The charge ratios (+/−) are presented as positive equivalents of the cationic component (dendrimer) to negative charge equivalents of the nucleic acid. For each family and generation, complexes with charge ratios (+/−) between 1/1 and 30/1 were obtained. All the complexes were formulated in buffer HEPES glucose (pH 7.4).

4.3.4. Particle Size and Zeta-Potential Measurements. The particle size of complexes was measured by dynamic light scattering and the overall charge by zeta-potential measurements, using a particle analyzer (Zeta Nano series; Malvern Instruments, Barcelona, Spain). Samples of the complexes (200 μL) were diluted in distilled water and measured at least three times.

4.3.5. Gel Retention Studies. Complexes were prepared at different charge ratios (+/−). Particles were incubated for 15 min and then were electrophoresed through a 1.2% agarose gel using 10× TBE buffer (100 mM Tris, 90 mM boric acid, 1 mM EDTA, pH 8.4; everything purchased from Sigma-Aldrich, Spain). One microgram of pDNA was added to each well. After running 2 h at 80 mV, the gel was stained with ethidium bromide and visualized under UV illumination.

4.3.6. In Vitro Gene Transfer. For each transfection, 300,000 cells were seeded in 1 mL of medium in 48-well culture plates (Iwaki, Japan) and incubated for 24 h at 37 °C in 5% CO2. The primary growth medium was removed and replaced with 0.3 mL of serum free media and 0.2 mL of complexes formulated in BHG containing 1 μg of pDNA. After 4 h, complexes were removed and replaced with cell culture medium containing 10% FBS. 48 h later they were washed with phosphate-buffered saline and lysed using 100 μL of Reporter Lysis Buffer (Promega, Madison, WI, USA) at room temperature for10 min, followed by two freeze−thaw cycles. The cell lysate was centrifuged for 2 min at 12000*g* to pellet debris. A 20 μL sample of the supernatant was assayed for total luciferase activity using the luciferase assay reagent (Promega), according to the manufacturer's protocol. A luminometer (Sirius-2; Berthold Detection Systems, Innogenetics, Diagnóstica y Terapéutica, Barcelona, Spain) was used to measure luciferase activity. The Bio-Rad D_c Protein Assay (Bio-Rad Laboratories, USA), using bovine serum albumin as the standard, was used for quantifying protein content. The data were expressed as nanograms of luciferase (based on a standard curve for luciferase activity) per milligram of protein.

4.3.7. Cytotoxicity Assay. Cell viability was quantified by Alamar Blue assay. 300,000 cells per well were seeded and grown overnight in 48-well culture plates. Cells were transfected as described previously. After 4 h, complexes were removed and substituted by new fresh medium. 48 h later, medium was removed and 1 mL of 10% (v/v) Alamar Blue dye in medium supplemented with 10% FBS was added to each well. After 2.5 h of incubation at 37 °C, 200 μL of the supernatant was assayed by measuring the absorbance at 570 and 600 nm. Cell viability was calculated according to the formula

$$\%\text{viability} = 100 \times \frac{(A_{570} - A_{600})_{\text{treated cells}}}{(A_{570} - A_{600})_{\text{control cells}}}$$

4.3.8. In Vivo Studies. Dendriplexes for *in vivo* experiments were prepared with 60 μg of DNA (pCMVLuc) formulated with the dendrimer F1G3 at charge ratio (+/−) 20/1. As controls, PBS and the commercial dendrimer PAMAM G4 were used.

Female Balb-c mice (8−10 weeks of age) were purchased from Harlan Ibérica Laboratories (Barcelona, Spain). All animals were studied in accordance with guidelines established by Directive 86/609/EEC and with the approval of the Committee on Animal Research at the University of Navarra, Pamplona (033/00). Individual mice in groups of 6 were injected via the tail vein with 60 μg of DNA formulated in dendriplexes in a total volume of 200 μL of 5% w/v glucose. Twenty-four hours following iv injection, the mice were sacrificed with a sodium pentobarbital overdose. The heart, lungs, liver and spleen were collected and washed with cold saline twice. The organs were homogenized with lysis buffer

dx.doi.org/10.1021/mp200542j | *Mol. Pharmaceutics* 2012, 9, 433−447

(Promega) using a homogeneizer (Mini-Beadbeater, BioSpec Products, Inc., Bartlesville, OK) and centrifuged at 12000*g* for 3 min at 4 °C. Twenty microliters of the supernatant was analyzed for luciferase activity as described previously.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

This work has been supported by grants from Fondo de Investigación Sanitaria (PI080222) and Proyecto CAM-UAH 2010 (Reference CCG10-UAH/PPQ-5916) to University of Alcalá and by University of Navarra Foundation and the Caja de Ahorros de Navarra (CAN) to University of Navarra. This work was also supported by grants from the Consejería de Educación de la Comunidad de Madrid and Fondo Social Europeo (F.S.E.) for SGG CIBER-BBN is an initiative funded by the VI National R&D&i Plan *2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions* and financed by the Instituto de Salud Carlos III with assistance from the *European Regional Development Fund.*

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