Carbosilane dendrimers peripherally functionalized with dansyl fluorescence tags and their cellular internalization studies†

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This paper describes the synthesis of carbosilane dendrimers containing dansyl groups at their periphery showing two topologies: as per-dansylated systems of type G_n-[$Si(CH_2)_3NH(Dans)]_m$ or as random partially surface-modified carbosilane dendrimers containing both amine and dansyl groups G_2-{[$Si(CH_2)_3NH_2]_x$[$Si(CH_2)_3NH(Dans)]_y$}. The dendritic systems were tested for *in vitro* toxicity on primary cell cultures (dendritic and peripheral blood mononuclear cells) and their cellular uptake was studied using confocal fluorescence microscopy. The results suggest that both types of dendritic topologies are acting as fluorescent labels proving the internalization of the carbosilane skeleton into cells.

Introduction

There is a significant number of recent publications concerning the use of dendrimers in biomedicine.[1–3] Dendrimers are extensively applied as transfection agents for gene therapy (GT), as carriers for delivering selectively active drugs, as contrast agents for magnetic resonance imaging (MRI) or in boron neutron capture therapy (BNCT) for cancer treatment. The most common approach regarding their use in GT as carriers of nucleic acid molecules has been the electrostatic interaction between peripherally ammonium-functionalized cationic dendrimers and nucleic acids (oligonucleotides, siRNA or plasmids) which are negatively charged. In order to find out how these dendrimers distribute within the cell, most studies have been performed using PAMAM dendrimers,[4,5] though other types of dendrimers have also been tested.[6,7] To follow internalization of a dendrimer or a nucleic acid–dendrimer complex (dendriplex), fluorescence tags have been linked to the dendrimer system to allow intracellular localization by confocal fluorescence microscopy. Furthermore, a number of studies have established that lipophilic moieties, included in PAMAM dendrimers when used as synthetic gene delivery systems,[3] or highly flexible structures, which can easily collapse and swell,[8] favor fusion of the complex particle with endosomal membranes. In this sense, it seems worth studying carbosilane dendrimers for transfection purposes since these macromolecules are constituted by a highly lipophilic and flexible skeleton.

Water-soluble carbosilane dendrimers carrying ammonium or amine groups at their periphery have recently been described by our group as biocompatible molecules with good prospects as non-viral carriers for nucleic material, with the transfection assays showing promising results. However, such assays have always been performed with labelled oligonucleotides[9] and no direct internalization studies of the carbosilane dendrimer skeleton have been performed so far. In order to prove the internalization of carbosilane dendrimers, we describe in this paper the coupling of the dansyl chromophoric group (5-(dimethylamino)-1-naphthalenesulfonamido) at the periphery of these dendrimers as a potent fluorescent label, which is widely used for sensing and labelling purposes.[10]

Results and discussion

Dansyl-terminated dendrimers

Dansyl-terminated carbosilane dendrimers were prepared following the synthetic procedures previously reported for other types of dendrimers.[7,11–16] In short, dansyl chloride reacted with the model compound $Et_3Si(CH_2)_3NH_2$[9] or the analogous amine-terminated carbosilane dendrimers of different generations G_n-[$Si(CH_2)_3NH_2]_m$ (where $n = 1$, 2 or 3; $m = 4$, 8 or 16 respectively, in which n means the number of generation G, and m states the number of peripheral units)[9] in dry dichloromethane and in the presence of NEt_3 to provide the corresponding fluorescence systems [$Et_3Si(CH_2)_3NH(Dans)$] (**1**) or G_n-[$Si(CH_2)_3NH(Dans)]_m$ (where $n = 1$ and $m = 4$ (**2**); $n = 2$ and $m = 8$ (**3**); $n = 3$ and $m = 16$ (**4**), and Dans denotes the dansyl group) as yellowish green oils (see Scheme 1 and Fig. 1). The non-dendritic ligand **1** was isolated in a 95% yield, decreasing to a 60–75% yield in the case of dendrimers **2–4**. All of them were air-stable and well soluble in DMSO, toluene or halogenated solvents like chloroform or dichloromethane, but showed decreasing solubility in diethyl ether

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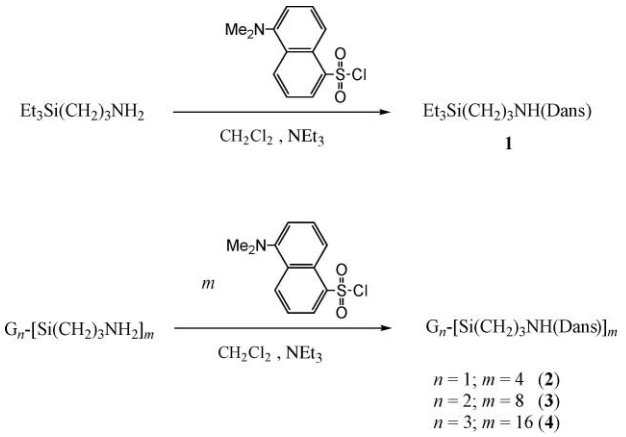


Scheme 1 Synthesis of per-dansylated derivatives.

when increasing the dendrimer generation. Finally, all four systems were insoluble in protic solvents like water or methanol.

The degree of dansylation was determined by NMR spectroscopy and found to be virtually quantitative, on the basis of the resonance attributed to the methylene group bonded to nitrogen, $-CH_2NH_2$, that is shifted in the ^{1}H NMR spectra from 2.62 ppm to 2.86 ppm when the dansyl group is present (see ESI for NMR spectra†). In addition, UV–VIS spectroscopy has been used to confirm the per-dansylation of the dendritic branches and satisfactory data were found. The reference compound **1** in THF solution exhibits an intense absorption band in the near UV spectral region ($\lambda_{max} = 334$ nm, $\varepsilon_{max} = 3850$ M^{-1} cm^{-1}). Each dendrimer shows an absorption band almost at the same wavelength as **1**. The molar absorption coefficient increases linearly with an increasing number of dansyl units in the dendrimers which allows the determination of the number of dansyl groups (see Table 1 and ESI†). All compounds were characterized by their fully assigned ^{1}H, ^{13}C and ^{29}Si NMR and UV–VIS spectroscopies and elemental analysis (see Experimental section). In agreement with this, the GPC revealed that the dendrimers prepared were monodisperse as indicated by their polydispersity values that range from 1.02–1.09 (see ESI†). MALDI-TOF mass spectra of the carbosilane dendrimers were obtained for dendrimers **2** and **3** using dithranol as the matrix in which the molecular peaks were identified. No molecular peak was detected for the third generation dendrimer **4** where the ionization becomes more difficult.[9b,17]

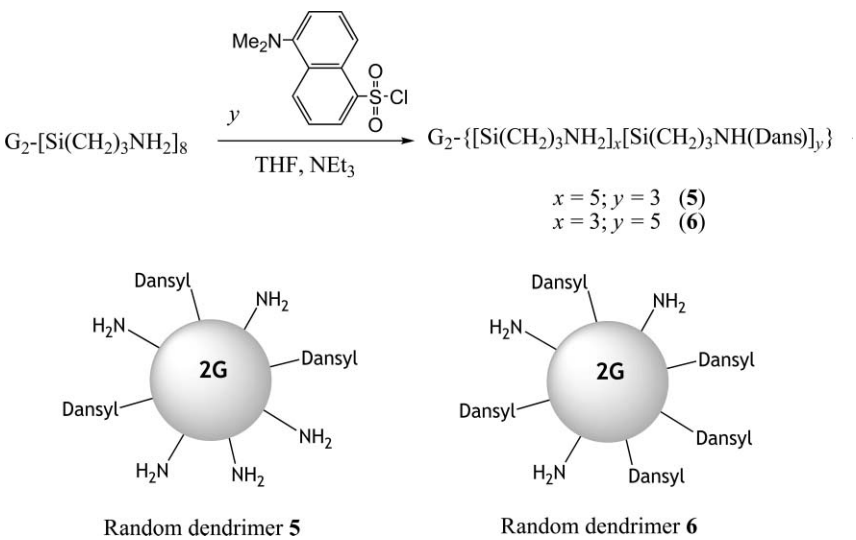
Table 1 Experimental and theoretical values of amino and dansylated branches determined by ^{1}H NMR and UV–VIS spectroscopies of per-dansylated dendrimers and random partially dansyl surface-modified dendrimers of type G_n-{[Si(CH$_2$)$_3$NH$_2$]$_x$[Si(CH$_2$)$_3$NH(Dans)]$_y$}

Dendrimer	Theoretical		Experimental			
			^{1}H-NMR		UV–VIS	
	x	*y*	*x*	*y*	*x*	*y*
2	0	4	0	4.0	0	4.1
3	0	8	0	8.0	0	7.9
4	0	16	0	16.0	0	16.2
5	5	3	5.2	2.8	5.0	3.0
6	3	5	2.6	5.4	3.1	4.9

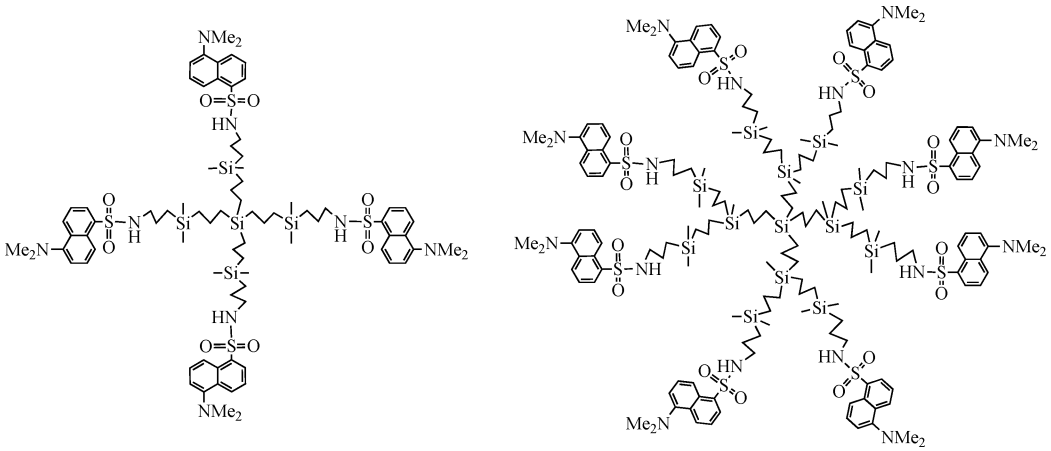
Partially surface-modified dendrimers

The partial assembly of dansyl groups onto the surface of the dendrimers was only carried out using the amino-terminated dendrimer of the second generation G_2-[Si(CH$_2$)$_3$NH$_2$]$_8$. The reaction of G_2-[Si(CH$_2$)$_3$NH$_2$]$_8$ with dansyl chloride in two different proportions per amino group (amino : dansyl = 8 : 3 and 8 : 5 respectively) in THF and in the presence of NEt$_3$, rendered the corresponding materials G_2-{[Si(CH$_2$)$_3$NH$_2$]$_x$[Si(CH$_2$)$_3$NH(Dans)]$_y$} (where theoretically $x = 5$, $y = 3$, (**5**); $x = 3$, $y = 5$, (**6**)) as air stable light-green oils in 70–85% yields (see Scheme 2). The compounds are soluble in chlorinated solvents and THF but insoluble in diethyl ether, DMSO and water.

The chemical structures of the random compounds were established on the basis of ^{1}H NMR and UV–VIS spectroscopies and elemental analysis giving in all cases satisfactory results. The ^{1}H NMR spectra revealed useful information for confirming the real proportions and purity of these materials, due to their high solubility in CDCl$_3$. Evidence of the reactivity was provided by



Scheme 2 Synthesis of random partially surface-modified dendrimers with amino and dansyl units.



2 **3**

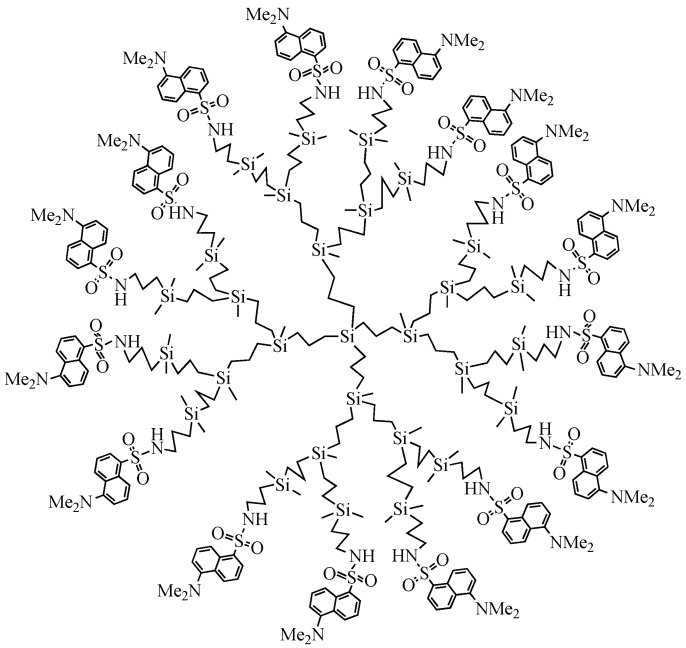


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Fig. 1 Molecular representation of per-dansylated dendrimers **2–4**.

high solubility in $CDCl_3$ and based on the NMR knowledge acquired for the perdansylated dendrimers **2–4** and the model system **1** (see above). The average number of amine or dansyl units present in a given system was determined by ^1H NMR comparing the integration of the proton resonances at 2.63 ppm attributed to the methylene group $–CH_2NH_2$ of the amine-terminated branches and at 7.17 ppm assigned to one of the aromatic protons of the dansyl-ended branches. Unfortunately, the comparison between the respective methylene groups bonded to nitrogen $–CH_2NH_2$ or $–CH_2NH$(Dans) of both types of branches cannot be done, because of the overlapping of the methylene resonance of the dansyl-containing branches with the NMe_2 resonance of the aromatic group, which precluded a better data accuracy. Furthermore, the representation of the full spectrum shows an increase in the dansyl resonances and a decrease in the signals associated with the amine-terminated branches, as expected. The experimental values measured by this method are shown in Table 1 and reasonable accordance between the experimental and theoretical values was obtained. To corroborate the ratio obtained by NMR, UV–VIS spectroscopy has been also used. The average number of dansyl groups measured by this method is shown in Table 1 and correlates well with those found by NMR. The GPC analysis performed for each random partially surface-modified dendrimer revealed the expected statistical distribution by exhibiting peaks of relatively narrow polydispersity, 1.74 for **5** and 1.21 for **6** (see ESI†).

Biological part

The per-dansylated carbosilane dendrimers **2–4** and the random partially surface-modified dendrimers **5–6** along with the precursor G_2-[$Si(CH_2)_3NH_2$]$_8$ as reference, were tested for *in vitro* toxicity by measuring the mitochondrial metabolism (MM) using the standard colorimetric MTT assay on primary cell cultures of peripheral blood mononuclear cells (PBMCs) and immature and mature dendritic cells (from healthy donors) challenging the cells with increased concentrations of the carbosilane dendrimers, ranging from 0.5 μM to 5 μM. The MM only takes place when reductase enzymes are active, and therefore the yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in living cells. Such conversion is often used as a measure of viable (living) cells in which a conventional value lower than 80% of the MM is considered as toxicity. Due to the low solubility of all these systems in water, small quantities of DMSO (less than 5% in water) were added, where no visual aggregation was observed, resulting in clear solutions for the cytotoxicity studies. The exact conditions are given in the Experimental section. The MM values remained above the toxicity limit of 80% against the PBMCs and immature and mature dendritic primary cells for all the molecules tested at concentrations of up to 5 μM (see ESI†). This low toxicity correlates with that observed for the starting material amine-terminated carbosilane dendrimers[9b] or for some neutral dansylated polyamidoamine dendrimers.[6] Surprisingly, no significant generation-dependent toxicity has been observed in all primary cells tested probably due to the particular features of carbosilane dendrimers.

All the per-dansylated dendrimers and partially dansyl surface-modified dendrimers prepared here were clearly observed using confocal fluorescence microscopy within the majority of the cells (see Fig. 2) suggesting cellular internalization of the carbosilane skeleton. The uptake was quantified as explained in the Experimental section. All dendrimers were incubated with dendritic cells over a period of 2 h at a final concentration of 5 μM in cell-culture medium. All per-dansyl generations **2–4** and the partially surface-modified dendrimer **5** were internalized by immature dendritic cells, though the uptake of the third generation dendrimer **4** is significantly increased compared with the smaller generations (dendrimers **2** and **3**). The dendrimer fixation method for confocal microscopy is the same as performed by our group with other analogous carbosilane dendrimers bearing no chromophore in their structure but being complexed to fluoresceinated siRNA.[18] In these cases, *in vivo* experiments based on the filming of a video proved that the dendrimer bioavailability and internalization of the nucleic material depends on the nature of the dendrimer and is not affected by the presence of external stimuli like formaldehyde. Finally, it is worth noting that primary cultures from healthy donors constitute a closer physiological approach compared to the frequently used immortalized cell lines and a more difficult scenario for internalization.

Conclusions

A synthetic approach for carbosilane dendrimers containing dansyl groups at their periphery has been carried out consisting of the preparation of per-dansylated dendrimers of type G_n-[$Si(CH_2)_3NH(Dans)$]$_m$ or random partially surface-modified carbosilane dendrimers of type G_2-{[$Si(CH_2)_3NH_2$]$_x$-[$Si(CH_2)_3NH(Dans)$]$_y$ containing both amine and dansyl groups. Both topologies of dendrimers may act as fluorescent labels with the aim of proving the internalization of the carbosilane skeleton into cells. The evaluation of *in vitro* toxicity showed low values on different primary cell cultures tested with no significant generation-dependent toxicity. The confocal fluorescence microscopy demonstrated that systems based on carbosilane dendrimers can be internalized by cells. The presence of a fluorescent label and a reactive group like the primary amino unit in the random systems opens the way for future studies upon the orthogonal reactivity of both fragments, making the presence of both therapeutic and imaging agents plausible on the same molecule. In addition, the inclusion of polar fragments like oligopeptides as therapeutical units, for example, may increase the water solubility to meet the main condition to perform biological and biomedical experiments.

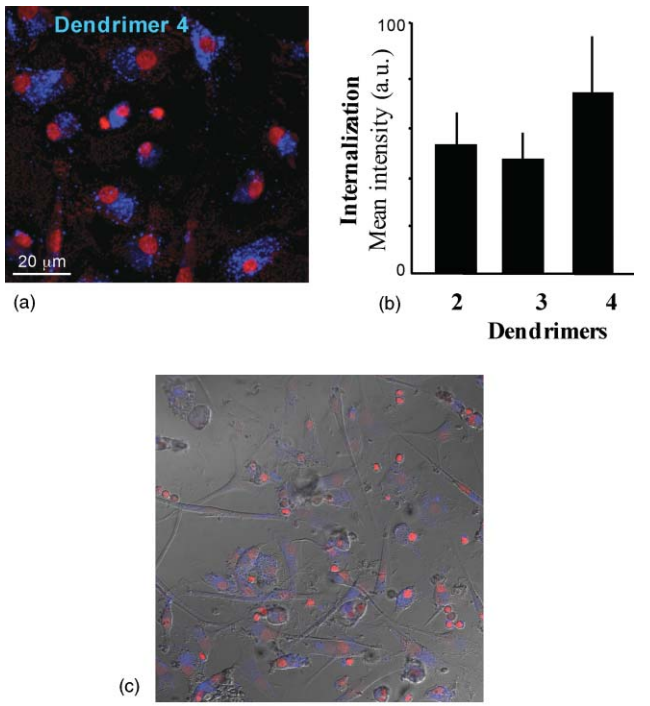

Fig. 2 Merged image of dendritic cells by confocal microscopy incubated with 5 μM of dansyl dendrimer **4** (**A**) or partially surface-modified dendrimer **5** (**C**) (both in blue) for 2 h, and co-stained for nuclei (red). Quantification of dansyl internalization in dendritic cells (**B**). Histograms represent the average mean intensities of internalized dansyl generations ± SD ($n > 60$ cells).

Experimental section

General remarks

All manipulations of oxygen- or water-sensitive compounds were carried out under an argon atmosphere using standard Schlenk techniques or an argon-filled glove box. 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

over P_4O_{10}. Unless otherwise stated, reagents were obtained from commercial sources and used as received. $Et_3Si(CH_2)_3NH_2$ or the analogous amine-terminated carbosilane dendrimers of different generations G_n-$[Si(CH_2)_3NH_2]_m$ were prepared according to reported methods.[9] ^1H, ^{13}C, ^{19}F 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 ^1H and ^{13}C resonances for $CDCl_3$ used as solvent, while ^{19}F was referenced to $CFCl_3$ 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. ESI and 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 of 23–25 kV. Analytical scale GPC was performed on a Varian HPLC with a 2 × Plgel 5 μm MIXED-D 300 × 7.5 mm column, (Polymer Laboratories); GPC, PL-ELS 1000 detector, mobile phase THF, flow rate 1 mL min^{-1}, RT, calibrated with linear polystyrene standards. UV–VIS spectra were taken on a Perkin-Elmer Lambda 18 spectrometer.

Synthesis of $[Et_3Si(CH_2)_3NH(Dans)]$ (1)

A solution of 5-dimethylamino-naphthalene-1-sulfonyl chloride (dansyl chloride) (239 mg, 0.88 mmol) in dry dichloromethane was added to a solution of dry triethylamine (0.14 ml, 1 mmol) and $Et_3Si(CH_2)_3NH_2$ (157 mg, 0.88 mmol) in dry dichloromethane. The reaction mixture was stirred for 12 h at room temperature in the dark 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 bright yellow-greenish oil (252 mg, 70%). ^1H-NMR ($CDCl_3$): δ = 8.54 (d, 1H, H_{arom}), 8.25 (t, 2H, H_{arom}), 7.55 (m, 2H, H_{arom}), 7.20 (d, 1H, H_{arom}), 4.60 (t, 1H, NH), 2.86 (s, 6H, NMe_2), 2.82 (t, 2H, CH_2N), 1.30 (m, 2H, C$H_2$$CH_2$NH), 0.77 (t, 9H, C$H_3$$CH_2$Si), 0.34 (q, 6H, CH_3CH_2Si), 0.26 (m, 2H, SiC$H_2$$CH_2$$CH_2$NH). ^{13}C-NMR ($CDCl_3$): δ = 151.8 (C_{ipso} bonded to SO_2), 134.9 (C_{ipso} bonded to NMe_2), 130.3, 129.6, 128.3, 123.2, 118.6, 115.1 (C_{arom}), 46.6 (CH_2N), 45.4 (NMe_2), 24.2 ($SiCH_2$$CH_2$$CH_2$NH), 8.2 (Si$CH_2$$CH_2$$CH_2$NH), 7.32 ($CH_3$$CH_2$Si), 3.0 ($CH_3$$CH_2$Si). Elemental analysis calcd. (%) for $C_{21}H_{34}N_2O_2SSi$: C 62.01, H 8.73, N 6.90; found: C 62.15, H 8.65, N 6.81. UV–VIS (THF): λ_{max} (ε) = 334 nm (3850 M^{-1} cm^{-1}).

Synthesis of G_1-{$[Si(CH_2)_3NH(Dans)]_4$} (2)

A solution of 5-dimethylamino-naphthalene-1-sulfonyl chloride (dansyl chloride) (250 mg, 0.92 mmol) in dry dichloromethane was added to a solution of dry triethylamine (0.15 ml, 1.07 mmol) and first-generation NH_2-terminated dendrimer G_1-$[Si(CH_2)_3NH_2]_4$ (152 mg, 0.23 mmol) in dry dichloromethane. The reaction mixture was stirred for 12 h at room temperature in the dark 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 **2** as a pale yellow oil. Column chromatography (silica gel, dichloromethane to eliminate the free dansyl chloride and then ethyl acetate as eluent) gave the per-dansylated G_1 dendrimer as a bright yellow-greenish oil (218 mg, 60%). ^1H-NMR ($CDCl_3$): δ = 8.51 (d, 4H, H_{arom}), 8.28 (d, 4H, H_{arom}), 8.21 (d, 4H, H_{arom}), 7.49 (m, 8H, H_{arom}), 7.14 (d, 4H, H_{arom}), 4.82 (t, 4H, NH), 2.86 (s, 24H, NMe_2), 2.82 (t, 8H, CH_2N), 1.31 (m, 8H, C$H_2$$CH_2$NH), 1.17 (m, 8H, $SiCH_2$C$H_2$$CH_2$Si), 0.43 (m, 16H, SiC$H_2$$CH_2CH_2$Si), 0.28 (m, 8H, SiC$H_2$$CH_2$$CH_2$NH), –0.21 (s, 24H, $SiMe_2$). ^{13}C-NMR ($CDCl_3$): δ = 151.8 (C_{ipso} bonded to SO_2), 134.9 (C_{ipso} bonded to NMe_2), 130.2, 129.7, 129.4, 128.2, 123.2, 118.7, 115.1 (C_{arom}), 46.5 (CH_2N), 45.5 (NMe_2), 24.4 ($SiCH_2$$CH_2$$CH_2$NH), 20.0, 18.5, 17.5 (Si$CH_2$$CH_2$$CH_2$Si), 12.2 (Si$CH_2$$CH_2$$CH_2$NH), –3.4 ($SiMe_2$). ^{29}Si-NMR ($CDCl_3$): δ = 0.50 (G_0-Si), 1.7 ppm (G_1-Si). MS (MALDI-TOF-dithranol): m/z monoisotopic mass calcd. for $C_{80}H_{124}N_8O_8S_4Si_5^+$: 1594.7, found 1594.8. Elemental analysis calcd. (%) for $C_{80}H_{124}N_8O_8S_4Si_5$: C 60.26, H 7.84, N 7.03; found: C 60.79, H 7.62, N 6.64. UV–VIS (THF): λ_{max} (ε) = 334 nm (15 990 M^{-1} cm^{-1}). GPC (THF): M_w = 1710, PDI = 1.02.

Synthesis of G_2-{$[Si(CH_2)_3NH(Dans)]_8$} (3)

This dendrimer was prepared using a method similar to that described for **1**, starting from G_2-$[Si(CH_2)_3NH_2]_8$ (232 mg, 0.14 mmol), NEt_3 (0.17 ml, 1.21 mmol) and dansyl chloride (306 mg, 1.13 mmol) to obtain the per-dansylated G_2 dendrimer as a bright yellow-greenish oil (251 mg, 55%). ^1H-NMR ($CDCl_3$): δ = 8.51 (d, 8H, H_{arom}), 8.28 (d, 8H, H_{arom}), 8.20 (d, 8H, H_{arom}), 7.48 (m, 16H, H_{arom}), 7.15 (d, 8H, H_{arom}), 4.82 (m, 8H, NH), 2.85 (s, 48H, NMe_2), 2.83 (t, 16H, CH_2N), 1.25 (m, 40H, C$H_2$$CH_2$NH and $SiCH_2$C$H_2$$CH_2$Si), 0.46 (m, 48H, SiC$H_2$$CH_2CH_2$Si), 0.27 (m, 16H, SiC$H_2$$CH_2$$CH_2$NH), –0.11 (s, 12H, SiMe), –0.21 (s, 48H, $SiMe_2$). ^{13}C-NMR ($CDCl_3$): δ = 151.8 (C_{ipso} bonded to SO_2), 135.1 (C_{ipso} bonded to NMe_2), 130.2, 129.8, 129.6, 128.2, 123.2, 118.8, 115.1 (C_{arom}), 46.4 (CH_2N), 45.3 (NMe_2), 24.4 ($SiCH_2$$CH_2$$CH_2$NH), 20.0–17.5 (Si$CH_2$$CH_2$$CH_2$Si), 12.2 (Si$CH_2$$CH_2$$CH_2$NH), –3.4 ($SiMe_2$), –4.9 (SiMe). ^{29}Si-NMR ($CDCl_3$): δ = 0.50 (G_0-Si), 1.4 (G_1-Si), 2.1 (G_2-Si). MS (MALDI-TOF-dithranol): m/z monoisotopic mass calcd. for $C_{176}H_{284}N_{16}O_{16}S_8Si_{13}^+$: 3501.87, found 3501.5. Elemental analysis calcd. (%) for $C_{176}H_{284}N_{16}O_{16}S_8Si_{13}$: C 60.36, H 8.17, N 6.40; found: C 60.39, H 7.78, N 5.79. UV–VIS (THF): λ_{max} (ε) = 334 nm (30 372 M^{-1} cm^{-1}). GPC (THF) M_w = 3477, PDI = 1.09.

Synthesis of G_3-{$[Si(CH_2)_3NH(Dans)]_{16}$} (4)

This dendrimer was prepared using a method similar to that described for **1**, starting from G_3-$[Si(CH_2)_3NH_2]_{16}$ (241 mg, 0.07 mmol), NEt_3 (0.17 ml, 1.21 mmol) and dansyl chloride (290 mg, 1.08 mmol) to obtain the per-dansylated G_3 dendrimer as a bright yellow-greenish oil (304 mg, 62%). ^1H-NMR ($CDCl_3$): δ = 8.50 (d, 16H, H_{arom}), 8.29 (d, 16H, H_{arom}), 8.20 (d, 16H, H_{arom}), 7.48 (m, 32H, H_{arom}), 7.16 (d, 16H, H_{arom}), 4.92 (m, 16H, NH), 2.85 (s, 96H, NMe_2), 2.83 (t, 32H, CH_2N), 1.27 (m, 88H, C$H_2$$CH_2$NH and $SiCH_2$C$H_2$$CH_2$Si), 0.46 (m, 112H, SiC$H_2$$CH_2CH_2$Si), 0.27 (m, 32H, SiC$H_2$$CH_2$$CH_2$NH), –0.11 (s, 36H, SiMe), –0.21 (s, 96H, $SiMe_2$). ^{13}C-NMR ($CDCl_3$): δ = 151.8 (C_{ipso} bonded to SO_2), 135.0 (C_{ipso} bonded to NMe_2), 130.2, 129.6, 129.5, 128.2, 123.2, 118.8, 115.1 (C_{arom}), 46.4 (CH_2N), 45.4 (NMe_2), 24.3 ($SiCH_2$$CH_2$$CH_2$NH), 20.0–17.5 (Si$CH_2$$CH_2$$CH_2$Si),

12.2 (Si*C*H$_2$CH$_2$CH$_2$NH), –3.5 (SiMe$_2$), –4.9 (SiMe). ^{29}Si-NMR (CDCl$_3$): $\delta = 0.90$ (G$_0$-Si), 1.6 (G$_1$-Si and G$_2$-Si). 2.1 ppm (G$_3$-Si). Elemental analysis: calcd. (%) for $C_{376}H_{628}N_{32}O_{32}S_{16}Si_{29}$: C 60.73, H 8.51, N 6.03; found: C 60.36, H 8.39, N 5.04. UV–VIS (THF): λ_{max} (ε) = 335 nm (62 300 M^{-1} cm^{-1}). GPC (THF) $M_w = 7508$, PDI = 1.02.

Synthesis of G$_2$-{[Si(CH$_2$)$_3$NH$_2$]$_5$[Si(CH$_2$)$_3$NH(Dans)]$_3$} (5)

A solution of 5-dimethylamino-naphthalene-1-sulfonyl chloride (dansyl chloride) (247 mg, 0.92 mmol) in dry dichloromethane was added drop by drop to a solution of dry triethylamine (0.13 ml, 0.92 mmol) and second-generation NH$_2$-terminated dendrimer G$_2$-[Si(CH$_2$)$_3$NH$_2$]$_8$ (0.50 mg, 0.31 mmol) in dry dichloromethane. The reaction mixture was stirred for 12 h at room temperature in the dark. After complete reaction, the solution was washed once with brine, once with a saturated sodium carbonate solution, and once again with brine. The organic phase was dried over magnesium sulfate and the solvent removed in vacuo, to give **5** as a bright yellow-greenish oil (313.2 mg, 44%). ^{1}H-NMR (CDCl$_3$): *dansylated branch, –CH$_2$NH(Dans)*, δ = 8.51 (d, 1H, H$_{arom}$), 8.28 (d, 1H, H$_{arom}$), 8.20 (d, 1H, H$_{arom}$), 7.48 (m, 2H, H$_{arom}$), 7.15 (d, 1H, H$_{arom}$), 4.82 (m, 1H, NH), 2.85 (s, 6H, NMe$_2$), C*H*$_2$NH(Dans) partially overlapped with NMe$_2$ group; *amine-terminated branch, –CH$_2$NH$_2$*, δ = 2.83 (t, 4H, CH$_2$N); rest of the carbosilane skeleton, 1.25 (m, C*H*$_2$CH$_2$NH and SiCH$_2$C*H*$_2$CH$_2$Si), 0.46 (m, SiC*H*$_2$CH$_2$C*H*$_2$Si), 0.27 (m, SiC*H*$_2$CH$_2$CH$_2$NH), –0.11 (s, SiMe), –0.21 (s, SiMe$_2$). ^{13}C-NMR (CDCl$_3$): *dansylated branch, -CH$_2$NH(Dans)*, δ = 151.8 (C$_{ipso}$ bonded to SO$_2$), 135.1 (C$_{ipso}$ bonded to NMe$_2$), 130.2, 129.8, 129.6, 128.2, 123.2, 118.8, 115.1 (C$_{arom}$), 46.4 (CH$_2$N), 45.3 (NMe$_2$), 24.4 (SiCH$_2$*C*H$_2$CH$_2$NH); *amine-terminated branch, –CH$_2$NH$_2$*, δ = 45.7 (CH$_2$CH$_2$*C*H$_2$NH$_2$), 28.4 (CH$_2$*C*H$_2$CH$_2$-NH$_2$); rest of the carbosilane skeleton, 20.0–17.5 (Si*C*H$_2$*C*H$_2$-*C*H$_2$Si), 12.2 (Si*C*H$_2$CH$_2$CH$_2$NH), –3.4 (SiMe$_2$), –4.9 (SiMe). UV–VIS (THF): λ_{max} (ε) = 334 nm (11 517 M^{-1} cm^{-1}). GPC (THF) $M_w = 1694$, PDI = 1.74.

Synthesis of G$_2$-{[Si(CH$_2$)$_3$NH$_2$]$_3$[Si(CH$_2$)$_3$NH(Dans)]$_5$} (6)

This partially surface-modified dendrimer was prepared using a method similar to that described for **5**, starting from dansyl chloride (411 mg, 1.53 mmol), triethylamine (0.22 ml, 1.53 mmol) and G$_2$-[Si(CH$_2$)$_3$NH$_2$]$_8$ (0.500 mg, 0.305 mmol) to render **6** as a bright yellow-greenish oil (486.6 mg, 57%). The NMR data of both types of branches are identical to those shown by the random system **5** where only the respective signal intensities have changed. λ_{max} (ε) = 334 nm (18 982 M^{-1} cm^{-1}). GPC (THF) $M_w = 2690$, PDI = 1.21.

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. After 24 h of incubation of the cell lines (immature and mature dendritic cells, PBLs) with different concentrations of dendrimers and partially surface-modified dendrimers in a 96-well plate, culture medium containing the dendrimers was replaced with 200 µL of serum-free Optimem. 20 µL of sterile filtered MTT (Sigma) stock solution in PBS pH 7.4 (5 mg mL^{-1}) were added to each well to achieve a final concentration of 0.5 mg MTT per mL. After 4 h, unreacted dye was removed by aspiration and formazan crystals were dissolved in dimethyl sulfoxide (200 µL per well) (Sigma). The concentration of formazan was then determined spectrophotometrically on a plate reader (Biowhittaker microplate reader 2001, Innogenetics®) at a wavelength of 550 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 and partially surface-modified dendrimer concentration was tested in triplicate, according to ATCC directives.

Dansyl uptake quantification

Immature dendritic cells were allowed to adhere to poly-L-lysine (Sigma) coated cover slips in RPMI 1640 medium (Gibco) supplemented with 5% FCS, incubated with 5 µM of three different generations of dansyl dendrimers **2–4** and the partially surface-modified dendrimer **5** for 2 h at 37 °C, fixed with 4% paraformaldehyde, and nuclear stained with Draq5 (Alexis). Internalized dansyls accumulated near the nuclei, as observed in confocal images. For rough evaluation of dansyl uptake, regions of interest (ROIs) were depicted around peri-nuclear internalization compartments. The mean fluorescence intensity detected in each ROI was measured, and the averages ± SD represented in histograms ($n > 60$ cells by dansyl generation, G1, G2, G3). Images were acquired with a Leica confocal microscope (Leica Microsystems, Heidelberg, Germany) using a 63X PL APO NA 1.3 glycerol immersion objective, and exciting with the Diode 405 nm (dansyl group) and the HeNe 633 nm (Draq5) lasers. Measurements and image processing were performed with the Leica software.

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