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PAPER

Synthesis and fluorescent properties of cationic carbosilane dendrimers containing eugenol linkers for their use in biomedical applications†

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A synthetic strategy has been carried out to prepare a new family of dendrimers containing eugenol linkers between the carbosilane scaffold and the peripheral amine or ammonium groups, of type G_n-[Si(CH$_2$)$_3$C$_6$H$_3$(OMe){O(CH$_2$)$_2$NMe$_2$}]$_m$ or G_n-[Si(CH$_2$)$_3$C$_6$H$_3$(OMe){O(CH$_2$)$_2$NMe$_3^+$I$^-$}]$_m$ respectively. Cationic carbosilane dendrimers have shown fluorescent properties due to the presence of the aromatic rings. A preliminary study of the interaction of dendrimers with two model drugs as examples of Active Pharmaceutical Ingredients (APIs) has been performed by fluorescence and NMR methods. This study determines the ability of these dendrimers to interact with potassium phenoxymethylpenicillin (Penicillin VK) as an example of an anionic low molecular weight drug entity or bovine serum albumin (BSA) as a biopharmaceutical drug protein entity, affording an appropriate dendritic system for its use in certain biological applications like drug delivery.

Introduction

Several dendrimers showing fluorescent properties have been described so far, among them water-soluble fluorescent dendrimers are mainly interesting due to the need for new water-soluble fluorescent labels in biology, thus these dendrimers might afford new tools in this field. A recent review by Majoral and co-workers highlights the most representative examples.[1] Many dendritic compounds containing fluorescent groups have been synthesized so far attaching fluorophore units to dendrimer core, branches or periphery. The solubility in water of these complexes is due to the presence of water solubilising functional groups that can be cationic, anionic or neutral. The number of water-soluble dendrimers that exhibit intrinsically fluorescent properties is much lower and only a few cases have been reported.[2–7] Other types of dendrimers are those which present fluorescent groups as a core of dendrimers. In this case, several examples of ionic water soluble dendrimers containing as core fluorescent phthalocyanines or porphyrins have been described.[8] In these complexes, the presence of the branches prevents aggregation and protects the core against quenching by water. Also, multiply labelled dendritic structures are known. These compounds bear several aromatic groups in their structure which causes a high lipophilicity and a poor solubility in water. To overcome this problem, it is very frequent to design dendrimers that contain simultaneously and specifically both the fluorescent groups and the water-solubilising groups as terminal units.[9]

We have recently reported the synthesis of cationic water-soluble carbosilane dendrimers by functionalization of their periphery with ammonium groups that can be considered as non-viral vectors for gene therapy treatments.[10] These dendrimers are generally non-toxic in a concentration range of 1 to 10 μm, able to bind oligonucleotides and siRNA and transport them to the interior of different types of cells. In addition, these dendrimers protect nucleic material like oligonucleotides or siRNA from sequestration and degradation by serum proteins and nucleases which is of fundamental importance for nucleic material to be able to exert an effect once in the interior of the cell.[11]

In this paper we report the synthesis of a new family of carbosilane dendrimers in which amine terminal functionalities are attached to the carbosilane skeleton through eugenol moieties. The presence of these eugenol moieties in the branches of the dendrimers provides them fluorescent properties. The fluorescence exhibited by these dendrimers has been used

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to complete their characterization and to prove their complexation properties through electrostatic interactions with Active Pharmaceutical Ingredients (APIs) such as potassium phenoxymethylpenicillin (Penicillin VK) or bovine serum albumin (BSA).

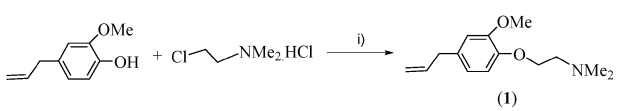
Results and discussion

Amine-terminated dendrimers

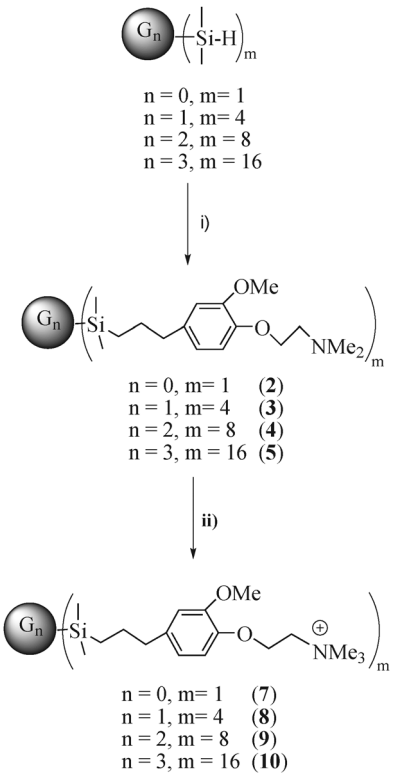
We have designed a new family of amine-terminated carbosilane dendrimers attaching these functionalities to the dendrimer skeleton using eugenol derivative moieties as bridging groups. The synthetic procedure is based on the hydrosilylation with Si–H terminated dendrimers of the allyl function of a eugenol derivative supporting an amine group ([$(CH_2{=}CHCH_2)C_6H_3$-(OMe)$(OCH_2CH_2NMe_2)$] (**1**)). This compound is prepared by reaction of $ClCH_2CH_2NMe_2{\cdot}HCl$ with [$(CH_2{=}CH{-}CH_2)$-C_6H_3(OMe)(OH)] (eugenol) in refluxing acetone in the presence of NaI/K_2CO_3 for 48 hours (Scheme 1). This procedure leads to the allyl amine **1** as a pale yellow oil with moderate yield.

Triethylsilane (n = 0, m = 1; see Scheme 2) and Si–H terminated carbosilane dendrimers of the type G_n-$(SiH)_m$ (n = 1, 2 and 3; m = 4, 8 and 16 respectively)[12] were used in the hydrosilylation of the allyl amine **1**. The reactions were performed using THF as a solvent and the Karstedt's catalyst for 12 hours at 45 °C, to afford the corresponding system [$Et_3Si(CH_2)_3C_6H_3$(OMe){$O(CH_2)_2NMe_2$}] (**2**) and dendrimers G_n-[$Si(CH_2)_3C_6H_3$(OMe){$O(CH_2)_2NMe_2$}]$_m$ (n = 1, m = 4 (**3**); n = 2, m = 8 (**4**); n = 3, m = 16 (**5**)) in high yield as yellow oils (see Scheme 2), in which n means the number of generation G, and x states the number of peripheral units. All of these dendrimers are soluble in chlorinated solvents and aromatic hydrocarbons, whereas solubility in aliphatic hydrocarbons diminishes with increasing generation, and are insoluble in water.

The analytical and NMR spectroscopic data of compounds **1–5** are consistent with their proposed structures (Scheme 2). The ^{1}H-NMR spectra of compounds **1** and **2** show a singlet around 3.81 ppm for the methoxide group and another singlet around 2.31 for the dimethylamino fragment in both cases. The main difference between these two complexes is the presence of three signals due to the allyl fragment for **1** that appear at 5.92, 5.02 and 2.52 ppm whereas the methylenic groups appear at 2.53, 1.57 and 0.68 ppm after the hydrosilylation with triethylsilane in compound **2**. ^{13}C-NMR data of both complexes are also in agreement with the proposed structures and show similar values in both complexes except for the allyl groups that appear as three signals at 133.2, 115.6 and 39.8 in compound **1** and at 39.7, 26.1 and 11.2 for the CH_2 groups in compound **2**. The APCI mass spectrum of compound **1** shows the molecular peak at 236.16 $[M + H]^+$.



Scheme 1 Synthesis of compound **1**. (i) K_2CO_3, Ether Crown 18-C-6, NaI, acetone, reflux, 48 h.



Scheme 2 Synthesis of neutral and cationic dendrimers. (i) **1**, THF, 45 °C, [Pt]; (ii) MeI excess, Et_2O.

The ^{1}H-NMR spectra of carbosilane dendrimers **3–5** show, for the carbosilane skeleton, almost identical chemical shifts for analogous nuclei in different generations, although the signals appear less defined and broader when the generation of the dendrimer is increasing. With respect to the outer group, the signals due to the protons *a*, *b* and *c* (see assignments in Fig. 1) appear in compounds **3–5** in the same zone as that in compound **2**. The rest of the signals of the phenylamine fragment also show similar values to those observed in compounds **1** and **2** for the same groups. ^{13}C-NMR spectra of these dendrimers show also the expected values. Fig. 1 shows an example of the ^{1}H and ^{13}C-NMR spectra for the second generation dendrimer **4**. With regard to ^{29}Si-NMR data, in all cases, only the signals due to the two outer silicon atoms are observed. The gHMBC-{^{1}H–^{29}Si} experiments of these compounds show a signal around 1.7 ppm for the outer Si atom and another signal around 1.0 ppm for the Si atom just before. MALDI-TOF spectra have been registered for the three generation dendrimers but only in the case of the first generation dendrimer the molecular peak was observed. This behaviour has been observed for other carbosilane dendrimers elsewhere.[12e,13]

Ammonium-terminated dendrimers

Ammonium-terminated derivatives were easily prepared by addition of methyl iodide (MeI) over the amine functionalized compounds **1–5** (see Scheme 2). Again, we have prepared initially monofunctional derivatives by addition of MeI over compounds **1** and **2** in diethyl ether, leading to the synthesis of [$(CH_2{=}CHCH_2)C_6H_3$(OMe)$(OCH_2CH_2NMe_3{}^+I^-)$] (**6**) and

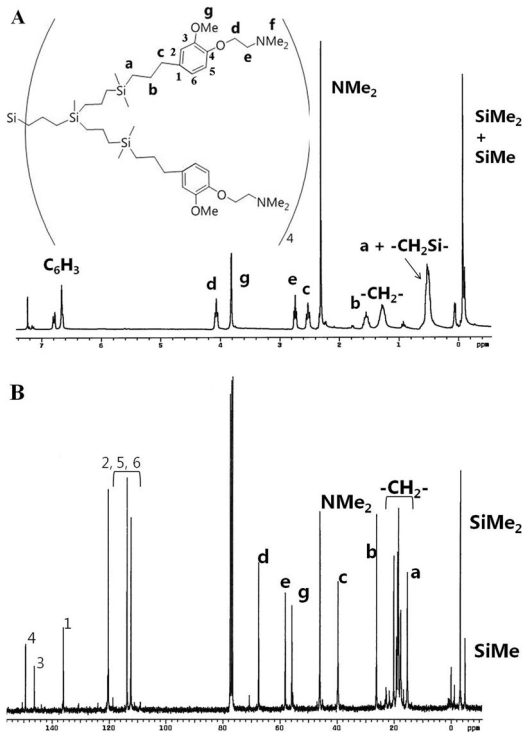


Fig. 1 ^{1}H-NMR (A) and ^{13}C-NMR (B) spectra of second generation dendrimer **4**.

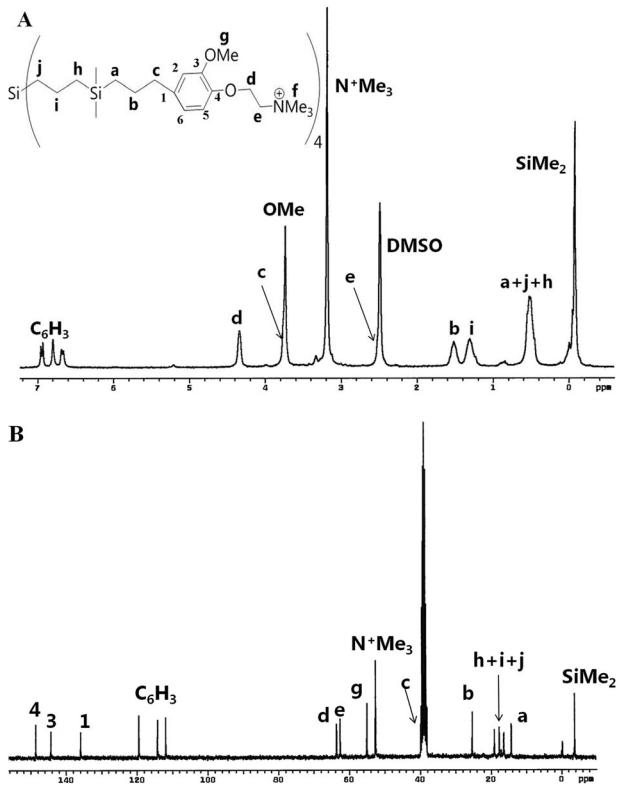


Fig. 2 ^{1}H-NMR (A) and ^{13}C-NMR (B) spectra of cationic first generation dendrimer **8**.

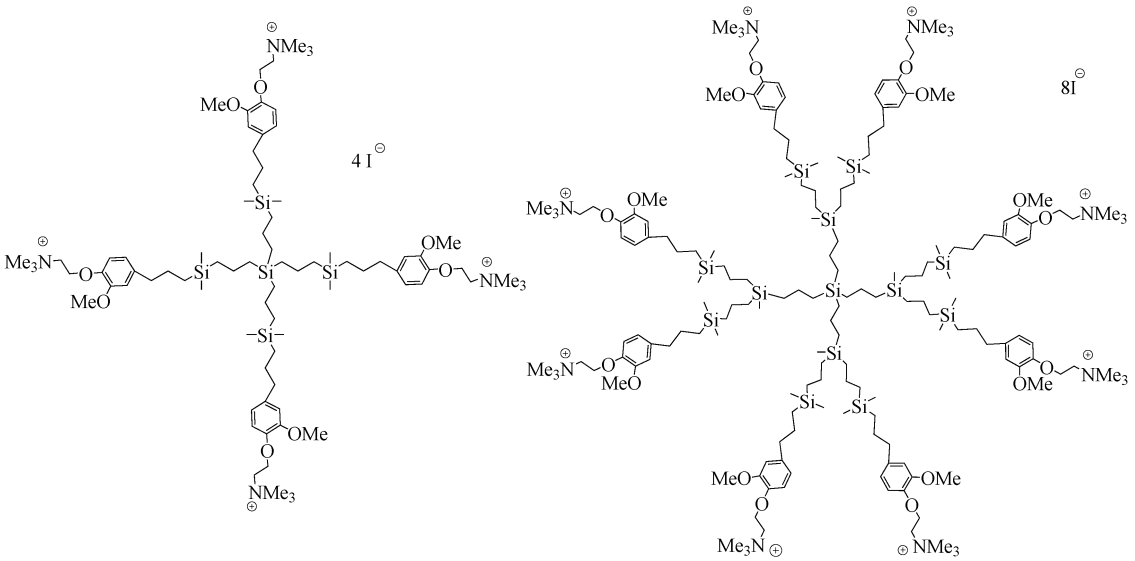
$[Et_3Si(CH_2)_3C_6H_3(OMe)\{O(CH_2)_2NMe_3{}^+I^-\}]$ (**7**). Following a similar procedure we have prepared a new family of cationic dendrimers up to third generation. Therefore, the dendrimers G_n-$[Si(CH_2)_3C_6H_3(OMe)\{O(CH_2)_2NMe_3{}^+I^-\}]_m$ (n = 1, m = 4 (**8**); n = 2, m = 8 (**9**); n = 3, m = 16 (**10**)) are prepared by addition of MeI over dendrimers **3–5** in high yield as white solids. Compounds **6–10** are poorly soluble in water but can be completely dissolved in water by addition of small amounts of DMSO, less than a 5%, which could allow the use of these systems for biomedical studies where the presence of small amounts of DMSO is tolerated. Both cationic non-dendrimer and dendrimer systems are stable in protic solvents and can be stored without decomposition for long time periods.

The cationic compounds **6–10** have been characterized by NMR spectroscopy and elemental analysis. The NMR spectra were recorded in DMSO-d_6 at room temperature. In this solvent the line widths of these spectra tended to be broader than those of the derivatives soluble in common organic solvents. Also broader signals were seen when the dendrimer generation increases.

The ^{1}H-NMR spectra of cationic compounds show that the quaternization of the amine group results in a deshielding of the chemical shifts of the geminal methylene (e) and methyl (f) groups directly bound to the charged nitrogen atom (see Fig. 2) with respect to the same signals in the neutral compounds. Also vicinal methylene groups (d) are affected by the positive charge of the nitrogen, but in this case a small downfield shift is observed. Beyond these positions no significant displacement is observed but aromatic protons appear now as three signals instead of the two signals present in the neutral counterparts. A similar behaviour is observed for the carbon atoms in the ^{13}C-NMR spectra. As an example, Fig. 2 also shows the spectrum of the first generation dendrimer **8** in which the methyl groups bonded to nitrogen give a signal at 52.7 ppm, whereas the geminal and vicinal methylene groups appear at 62.6 and 63.6 ppm respectively. The rest of the signals are in the expected values. The ^{29}Si-NMR spectra also were registered for the cationic derivatives, nevertheless for the silicon atoms the chemical shifts obtained in these cases are very similar to those presented by the amine terminated counterparts as a consequence of the big distance with the positive charge in these derivatives. Mass spectra for dendrimers **8–10** showed again the molecular peak only in the case of first generation dendrimer **8**. Analogously, this behaviour has been also reported by other authors for different types of dendrimers.[12e,13]

Host–guest interactions

Cationic carbosilane dendrimers prepared in this work (Fig. 3) have shown fluorescent properties due to the presence of the aromatic ring of the eugenol moiety used as a bridging ligand between carbosilane skeleton and ammonium functionalities. A spectrofluorimetric study has been carried out in order to obtain the excitation and emission spectra of cationic carbosilane dendrimers **8–10** in aqueous medium. They were dissolved in distilled, deoxygenated water containing a small amount of DMSO (4%) that was added to assure complete solubility of these dendrimers. In these conditions, dendrimers **8–10** show maximum excitation and emission signals at λ_{EX} = 285 and λ_{EM} = 315 nm respectively. We have studied the variation of the intensity of the fluorescence signal of the



8 **9**

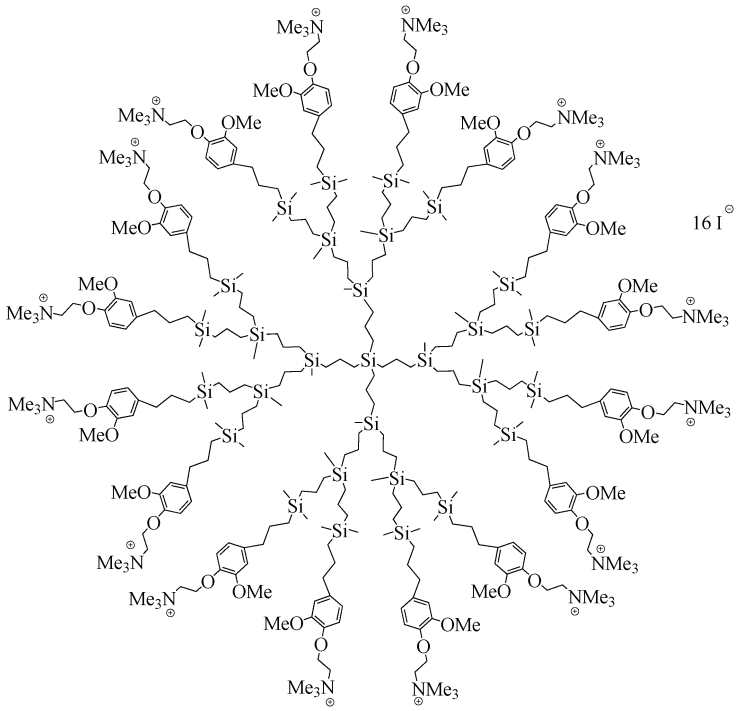


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Fig. 3 Schematic representation of cationic carbosilane dendrimers **8–10**.

second generation dendrimer **9** with its concentration in order to obtain calibration curves that are represented in Fig. 4. The linearity, range, precision and detection limit of the method were investigated essentially following the Validation Pharmaceutical Procedure proposed by U.S.P. 32th[14] and based on International Conference of Harmonization (ICH) guidelines. The correlation of the mean fluorescence intensity (S_F) *versus* variable concentrations of **9** is linear between the range 10–60 μg ml^{-1} (2.5–14 μM). Three standard solutions (4% DMSO in water) of neat **9** dendrimer were prepared and three aliquots were made from them at each level between the range 2.5 to 14 μM. The correlation coefficient of the best fitting straight line of S_F *versus* concentration of pure **9** aqueous solutions was satisfactory (R^2 = 0.9907), being its slope and *y*-intercept (95% confidence interval) of 3.940 ± 0.359 and 1.807 ± 3.007, respectively. Detection limits, based on a signal-to-noise ratio (3 : 1), ranged from 4 to 5 μg ml^{-1} (1–1.25 μM). With regard to the robustness,

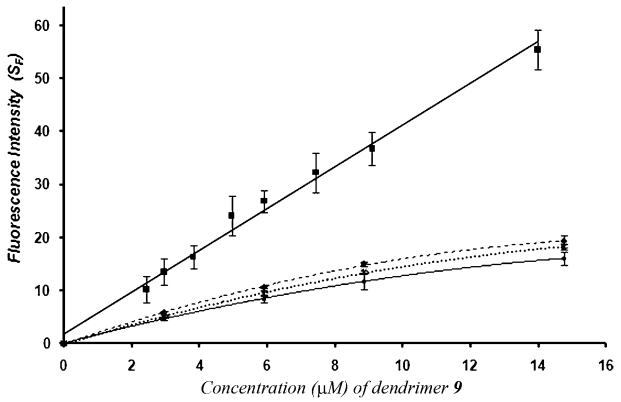

Fig. 4 Relationship of fluorescence intensity (S_F) *versus* dendrimer **9** concentration at 25 °C (■) and the effect of Penicillin VK on fluorescence at different dendrimer/drug ratios: 1 : 2 (◆); 1 : 4 (*) and 1 : 8 (●).

DMSO concentration has influenced the quantum yield of **9**, therefore when DMSO increases from 4% to 5%, the fluorescence signal enlarges linearly ten units at all dendrimer concentrations.

From this curve it is possible to quantify **9** in an unknown solution which can be very useful for the development of dendrimer nanosystems for biomedical applications (drug–carrier interaction, loading drug efficiency, stability studies, *etc.*). Similar calibration curves can be obtained for the first and third generation dendrimers.

Once established the fluorescence properties of our cationic dendritic systems, a preliminary study of the interaction of dendrimers with two model drugs has been carried out by fluorescence and NMR methods. Therefore, Penicillin VK as an anionic low molecular weight drug entity or serum albumin as a biopharmaceutical drug protein entity was used. This study may determine the ability of these dendrimers to form nanoconjugate compounds with these model drugs as a previous step to analyze the use of these cationic systems in drug delivery processes.

Penicillin–dendrimer interactions. We have examined the changes in the fluorescence properties of the second generation dendrimer **9** at 3, 6, 9 and 15 μM concentrations in DMSO 4% aqueous solutions, when variable amounts of an anionic drug such as Penicillin VK were added. Three different ratios of dendrimer/penicillin (1 : 2, 1 : 4 and 1 : 8) have been used to evaluate whether several antibiotic molecules can interact with a dendrimer molecule at the same time. Fig. 4 shows that the addition of penicillin causes a diminishing of the fluorescence signal intensity which slightly decreases on increasing the amount of penicillin with a maximum reduction value of about 70% (70.28% ± 2.77%) when the dendrimer : Penicillin VK ratio was 1 : 8. In addition, the linear relationship between S_F and dendrimer concentration is transformed into a quadratic function, with high level of significance ($p < 0.01$), independently of the ratios used.

Table 1 shows mean score rate ($S_{Fo}/S_{FPeniVK}$) fluorescence intensities of pure **9** dendrimer, in aqueous 4% DMSO solutions, before (S_{Fo}) and after ($S_{FPeniVK}$) addition of Penicillin VK at the three dendrimer/penicillin ratios (1 : 2, 1 : 4 and 1 : 8) and three dendrimer **9** concentrations of 3, 6, 9 and 15 μM studied. The statistical evaluation of these data through the test of two-way analysis of variance (ANOVA) showed significant differences ($p < 0.05$) in $S_{Fo}/S_{FPeniVK}$ due to both the dendrimer concentration and the penicillin proportion.

In order to discriminate where the difference lies, all pairs of mean were examined by the Least Significant Difference test (LSD) (Table 1). It shows that there are not significant differences within samples for 3 μM, 6 μM and, 9 μM dendrimer concentrations at the three levels of penicillin. However, the differences are indeed significant when the concentration of dendrimer is 15 μM at every dendrimer : penicillin proportions (1 : 2, 1 : 4 and 1 : 8). When analyzing the effect of dendrimer : penicillin ratio, keeping constant the concentration of dendrimer, two dissimilar behaviors are seen (column differences between samples), suggesting that Penicillin VK could be arranged in several ways on the dendritic structure according to its concentration.

The interaction between Penicillin VK and dendrimer **9** could be explained considering that at pH 6 (pH of 4% DMSO water solution) the non-ionized drug fraction of Penicillin VK ($pK_a = 2.62$)[15] is very low [$1/(1 + 10^{(pH-pK_a)}) = 3.29\%$]. Thus decay observed on fluorescence intensity can be ascribed to an electrostatic interaction between second generation dendrimer **9**, which contains more than one identical fluorescent unit, and Penicillin VK, that acts as a quencher.

It is feasible to assume that when the ratio of drug increases, more molecules of Penicillin VK may interact with dendrimer **9**, either in the periphery or between the branches. From the statistical results shown in Table 1, as much is this ratio lower is the intensity of fluorescence (S_{FPeni}), because more molecules are interacting, but when maximum interaction was achieved a plateau on the fluorescence rate S_{Fo}/S_{FPeni} should be observed. This happens at ratio of drug twice than that of the dendrimer,

Table 1 Effect of Penicillin VK ratio on fluorescence intensities of dendrimer **9** in aqueous (4% DMSO) solutions, expressed as mean ± s.d. score rate ($S_{Fo}/S_{FPeniVK}$)[a]

Dendrimer **9** conc./μM	Ratio 1 : 2 $S_{Fo}/S_{FPeniVK}$	Ratio 1 : 4 $S_{Fo}/S_{FPeniVK}$	Ratio 1 : 8 $S_{Fo}/S_{FPeniVK}$	($p < 0.05$) differences between samples
3	2.30 ± 0.11	2.72 ± 0.08	2.81 ± 0.21	***1*** : ***2*** ***1*** : ***4*** ***1*** : ***8***
6	2.41 ± 0.15	2.68 ± 0.14	3.01 ± 0.08	***1*** : ***2*** ***1*** : ***4*** ***1*** : *8*
9	2.45 ± 0.23	2.71 ± 0.06	3.15 ± 0.10	***1*** : ***2*** ***1*** : ***4*** ***1*** : *8*
15	3.12 ± 0.20	3.30 ± 0.10	3.75 ± 0.28	***1*** : ***2*** ***1*** : ***4*** ***1*** : *8*
($p < 0.05$) differences within samples	***3***–***6***–***9***–15	***3***–***6***–***9***–15	***3***–***6***–***9***–15	

[a] ANOVA and LSD test ($p < 0.05$). Bold groups show not statistically significant differences.

for the dendrimer concentration ranges from 6 to 9 μM. However, at ratio 1 : 8 a sharp decline in fluorescence (S_{FPeni}) is observed. A similar effect also occurs at 3 μM, but in this case the decrease in fluorescence occurs at a lower ratio (1 : 4). It has been seen (see NMR experiences below) that the radius of the Penicillin VK increases on decreasing the penicillin/dendrimer ratio. This could explain that at lower dendrimer concentration (3 μM) maximum incorporation has been achieved at lower dendrimer : drug ratio.

On the other hand, once it has achieved the highest encapsulation of the drug, more molecules could be incorporated, changing its layout and packaging on the network and therefore the quenching of the fluorophore group. This would explain the statistically significant decrease in the fluorescence and consequently the greatest value.

The interaction between cationic dendrimers and Penicillin VK has been also corroborated by NMR spectroscopy through qualitative diffusion experiments (DOSY), NOESY-2D and ^{1}H NMR. For these studies, the cationic second generation dendrimer **9** was chosen and a mixture of D_2O with DMSO-d6 (4%) was used.

For the DOSY experiments, first of all, the diffusion coefficients of each neat reagent were measured at two different concentrations, corresponding to the lower and upper limits that they would be used during the experiences. These measurements showed that in the range studied, the Penicillin VK diffusion value (D_p^0) was kept constant, whereas for dendrimer **9** a concentration dependent behaviour was observed. The latter may be ascribed to dendrimer aggregation, and for that reason only variation of the Penicillin VK diffusion coefficient in the presence of dendrimers was measured in the following experiments. In the first approach, a constant dendrimer concentration and a variable penicillin concentration were used (see Table 2). On decreasing the penicillin/dendrimer **9** ratio, a decrease of the D_p value with respect to neat penicillin (D_p^0) was observed, which corresponds to the increasing radius of the Penicillin VK. In the second approach, the same behaviour was observed when for a constant concentration of Penicillin VK increasing amounts of dendrimer **9** were used (see also Table 2). In both cases, such variation of D_p confirms the existence of interaction between Penicillin VK and dendrimer **9**.

A NOESY experiment for a mixture of Penicillin VK and dendrimer **9**, 4 : 1, was carried out. Besides positive cross peaks

Table 2 Diffusion coefficient of Penicillin VK (D_p) at variable concentration of (a) penicillin or (b) dendrimer **9**

Ratio PenVK/**9**^{a}	D_p (10^{10})	$[D_p^0/D_p]$	Ratio PenVK/**9**^{b}	D_p (10^{10})	$[D_p^0/D_p]$
16/1	1.81	1.60	1/0	2.90	1.00
8/1	1.43	2.03	1/2	2.70	1.07
4/1	1.33	2.18	1/4	2.45	1.18
2/1	0.78	3.68	1/8	1.17	2.47

^{a} Penicillin VK diffusion coefficient data using a constant dendrimer concentration and modifying the amount of Penicillin VK. ^{b} Penicillin VK diffusion coefficient data using a constant Penicillin VK concentration and modifying the amount of dendrimer **9**. D_p^0 diffusion coefficient of Penicillin VK measured in D_2O : DMSO-d6 (4%) at 25 °C.

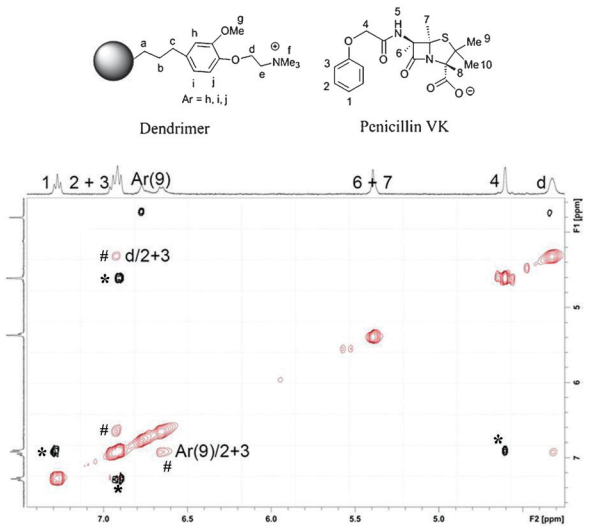

Fig. 5 Fragment of the NOESY-2D spectrum of a mixture of Penicillin VK and dendrimer **9** (ratio 4 : 1). *X* axis, the ^{1}H NMR spectrum of the Penicillin VK and dendrimer **9** mixture. *Y* axis, the ^{1}H NMR spectrum of Penicillin VK. (#) peaks denote cross peaks for Penicillin VK and dendrimer **9** interaction. (*) peaks denote intramolecular NOESY-2D peaks.

corresponding to intramolecular interactions of the protons of each molecule, negative cross peaks corresponding to interactions between protons of dendrimer **9** and Penicillin VK were also observed (see Fig. 5), as a consequence of exchange between free and encapsulated Penicillin VK. These last cross peaks were those belonging to the aromatic rings of both molecules and also to the CH_2O group of dendrimer **9**. Thus, this result points toward an internalization of the phenyl ring of Penicillin VK, with a π–π stacking interaction between the phenyl rings of both molecules. With this situation, the hydrophobic aromatic ring of Penicillin VK would be hidden inside the dendrimer framework with both hydrophilic regions (NMe_3^+ and CO_2^- groups) in the outer sphere.

The ^{1}H NMR spectra of free Penicillin VK and dendrimer **9** were compared with those obtained when different amounts of Penicillin VK were added to a dendrimer **9** solution (see Fig. 6). This comparative study showed that increasing Penicillin VK concentration provokes a clear shifting to higher frequency of the resonances belonging to Penicillin VK and a low frequency shifting of some of the resonances of the cationic dendrimer **9**. These resonances were mainly due to the eugenol aromatic ring and the groups close to it. This modification of chemical shifts should be indicative of host–guest interaction between Penicillin VK and dendrimer **9** through π–π stacking corroborating the results found in the NOESY experiment. Surprisingly, the original resonance attributed to the cationic moiety NMe_3^+ of dendrimer **9** was hardly modified, probably as a consequence that the ionic pair is away from a close contact, although other authors have ascribed such an effect to the steric hindrance around the quaternary nitrogen atom, thus reducing the electrostatic interaction.[16]

Albumin–dendrimer interactions. Taking advantage of the fluorescent properties of dendrimers **8–10**, the possibility that

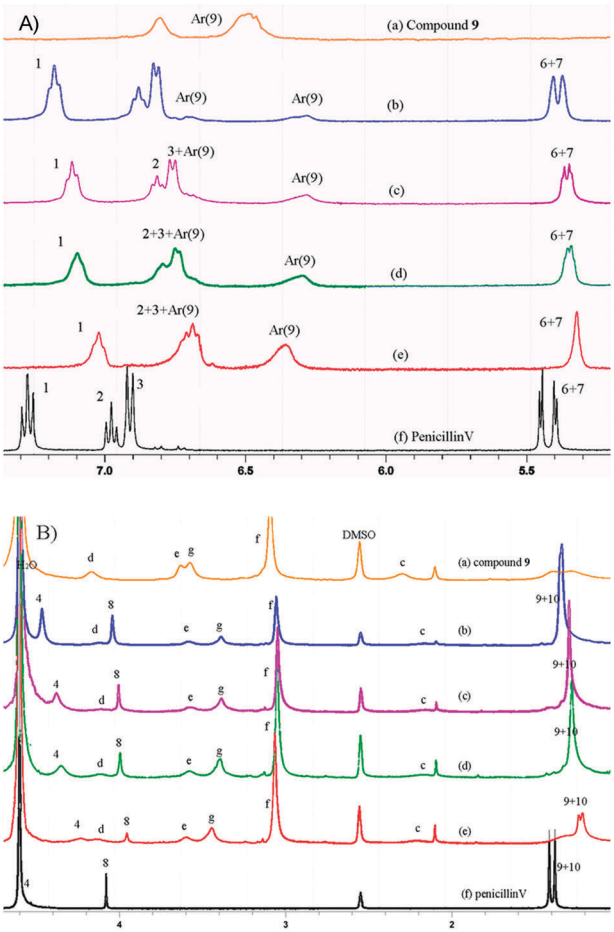

Fig. 6 ^{1}H NMR spectra of Penicillin VK, dendrimer **9** and mixtures in (A) the aromatic and (B) the aliphatic regions. (a) Dendrimer **9**; (b) ratio of Penicillin VK : **9**, 16 : 1; (c) ratio of Penicillin VK : **9**, 8 : 1; (d) ratio of Penicillin VK : **9**, 4 : 1; (e) ratio of Penicillin VK : **9**, 2 : 1; (f) Penicillin VK.

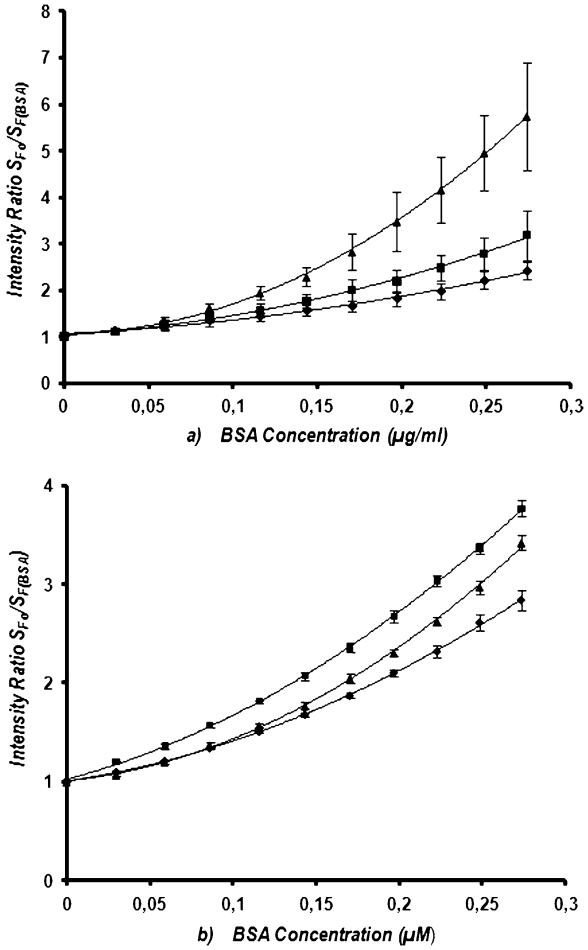

Fig. 7 Stern–Volmer representation of fluorescence quenching of the carbosilane dendrimers (**8–10**) by interaction with BSA: (A) at pH = 7.2; (B) at pH = 9.0. Symbol notation: ◆ dendrimer **8**, ■ dendrimer **9** and ▲ dendrimer **10**.

some kind of interaction could take place between them and protein molecules such as BSA has been analyzed by spectrofluorimetry, at two different pH values. When a molecule is added to a fluorophore solution and, after photon absorption, it does not emit light with the same efficiency than in its basal state, a quenching effect is produced and a complex formation could be considered. Seeing that fluorescence decreases on increasing the BSA concentration, we have used the Stern–Volmer equation [$S_{Fo}/S_{F(Quencher)} = 1 + K_{SV}(C_{Quencher})$] to study this process, considering BSA as a quencher. This equation correlates the ratio of neat dendrimer fluorescence intensities (S_{Fo}), without protein, and in the presence of BSA ($S_{F(BSA)}$) as a function of its concentration (C_{BSA}) (see Fig. 7).

At pH = 7.2, no linear relationship is observed for the ratio $S_{F0}/S_{F(BSA)}$, but a quadratic function is noticed. That is, the dendrimer fluorescence intensity decreases in a non-linear behaviour on increasing the concentration of BSA. It is also worth noting that the quenching effect increases on increasing the generation. At pH = 9.0, analogous effects are shown although the intensity values are greater than those at pH = 7.2 indicating a more effective quenching behaviour. In addition, no correlations between dendrimer generation and quenching effect are observed in this case.

It is known that albumin undergoes conformational changes on α-helices, β-sheets and random coils, depending on the pH.[17] The molecular diameter of BSA increases as a function of the pH, being characterized into five forms, that are known as expanded (E, below pH 2.7), fast (F, below pH 4.3), native (N, pH 7.0), basic (B, above pH 8.0) and age (above pH 10.0). At pHs next to 7, BSA conforms a predominant globular shape and carries a net negative charge due to its isoelectric point in water at 25 °C (pI = 4.7).

In this situation, the larger the dendrimer surface the greater interaction with the protein surface must be observed. However at pH = 9.0 the albumin undergoes a more open structure in which the higher generation dendrimer **10** is more difficult to accommodate and internalize within the open shell compared to the dendrimer of second generation **9**. Nevertheless, both situations are consistent with the existence of interactions between dendrimers and BSA at the two different pHs. Fluorescence studies concerning the interaction of dendrimers with BSA have been reported elsewhere although based on the intrinsic fluorescence of the tryptophan residues of BSA.[18]

Conclusions

A synthetic strategy has been carried out to prepare a new family of dendrimers containing eugenol linkers between the carbosilane scaffold and the peripheral amine or ammonium groups, of type G_n-[Si(CH$_2$)$_3$C$_6$H$_3$(OMe){O(CH$_2$)$_2$NMe$_2$}]$_x$ or G_n-[Si(CH$_2$)$_3$C$_6$H$_3$(OMe){O(CH$_2$)$_2$NMe$_3^+$I$^-$}]$_x$ respectively. It consists in the hydrosilylation reaction of the amine-substituted eugenol ligand with H–Si terminated dendrimers and the subsequent quaternization with MeI to afford the corresponding ammonium dendrimers. Although the presence of an aromatic ring per branch decreases the water solubility, the inclusion of water-solubilizing groups like ammonium terminal units may overcome this problem. This strategy can be envisaged as an example of multiply labelled dendritic systems perfectly defined in an attempt to solve the problems that other alternatives based on statistically labelled fluorescent dendrimers afford, as the uncertainty about the structure, polydispersity or a possible absence of the reproducibility of the experiments, among others. These dendrimers are able to interact with single drugs like penicillin as has been evidenced by fluorescence and NMR experiments, showing that the lipophilic part of the drug is partially inserted within the dendritic structure in the interior pocket through a π–π stacking interaction. This type of host–guest interaction may favour the delivery of the penicillin in contrast to situations in which the anionic guest may only be attached to the cationic dendritic surface by only electrostatic forces, due to the absence of groups that create additional stabilizations, like hydrogen bonding or π–π stacking interaction as in this case. In the same way, the interaction of the dendrimers with BSA has been corroborated by fluorescence experiments without the need to use labelled proteins as usual protocol. All these features indicate that the dendrimers here prepared would work as good delivery systems for anionic drugs while maintaining the fluorescent properties needed for monitoring the corresponding biological processes.

Experimental

General remarks

All manipulations of oxygen- or water-sensitive compounds were carried out under an atmosphere of argon using standard Schlenk techniques or an argon-filled glovebox. Solvents were dried and freshly distilled under argon prior to use: hexane from sodium–potassium, tetrahydrofuran and ethyl ether from sodium benzophenone ketyl.[19] Dry acetone was purchased from Aldrich and used without further purification. Unless otherwise stated, reagents were obtained from commercial sources and used as received. The hydride-terminated carbosilane dendrimers of different generations nG-(SiH)$_x$ were prepared according to reported methods.[12]

^{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$_6$ and water-d$_2$ 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.

DOSY experiments were carried out with a Bruker Advance 400 at 25 °C. The singlet belonging to the protons of the O–CH$_2$–CO group in the Penicillin VK was used to measure the D values (see Fig. 5, position 4 of the Penicillin drawing). The values of mid-point between gradients (Δ) and gradient length (δ) were adjusted for free Penicillin VK (Δ = 0.18 s; δ = 3200 μs) and employed for each set of experiments. The relaxation delay was set to 10 s. However, in the last experience of each set these values were again calculated observing a variation of the diffusion value for Penicillin V lower than 2%. For this reason, all the data are referred to free Penicillin VK.

Fluorescence experiments

The fluorescence experiments were performed with a Shimadzu RF540 spectrofluorimeter that was calibrated by checking its wavelength accuracy with the Raman dispersion line of water and fluorescence intensity by using a quinine solution in sulfuric acid, 0.1 N (USP 32th). 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 **8–10** (50 μg ml^{-1}) were carried out in aqueous solutions, previously filtrated and degasified, containing 4% of DMSO, placed on quartz cells with a path length of 1.0 cm.

For dendrimer/anionic drug interactions, the measurements of the fluorescence signal (S_F) variation were achieved by increasing volumes of aqueous solutions of Penicillin VK (1.33 μg ml^{-1}) or BSA (100 μg ml^{-1}) to water solutions of dendrimers located in the apparatus cell. The experiments were analyzed on the basis of the Stern–Volmer equation where S_{Fo} is the intensity of fluorescence in the absence and S_F in the presence of the quencher (Penicillin VK or BSA).

Statistical evaluation of the results was done by using Analysis of Variance (ANOVA), Least Significant Difference (LSD) and Student–Fisher test (α = 0.05) and fitting of curves by simple or polynomial regression at a confidential level of 95%, through Statgraphics Plus (Statpoint Technologies, Inc., USA).

Bovine serum albumin (BSA) and potassium phenoxymethylpenicillin (Penicillin VK) were obtained from Sigma-Aldrich. Other chemical substances were of analytical grade. Sterile, deionised and degasified water was used to prepare aqueous solutions. Buffered phosphate pH 7.2 and borate pH 9 solutions were prepared according to the procedure described in USP 32th.

For interaction carbosilane dendrimer/Penicillin VK (see Fig. 4): relationship of fluorescence intensity (S_F) *versus* dendrimer **9** concentration at 25 °C (■) $S_F = 3.9384C + 1.8053$ ($R^2 = 0.9907$) (mean ± s.d. n = 9) and the effect of Penicillin VK ratio on fluorescence: **1**:**2** (◆) $S_F = -0.039C^2 + 1.661C^2 + 0.06$ ($R^2 = 0.998$); **1**:**4** (*) $S_F = -0.045C^2 + 1.908C - 0.133$ ($R^2 = 0.993$) and **1**:**8** (●) $S_F = -0.06C^2 + 2.201C - 0.094$ ($R^2 = 0.992$).

For interaction carbosilane dendrimer/BSA (see Fig. 7): Stern–Volmer representation of fluorescence quenching of the carbosilane dendrimers (**8–10**) by interaction with BSA: (A) at pH = 7.2; (B) at pH = 9.0.

At pH = 7.2, no linear relationship is observed for the ratio $S_{F0}/S_{F(BSA)}$, but a quadratic function ($p < 0.05$) is noticed:

Dendrimer **8**: $S_{Fo}/S_{F(BSA)} = 10.26C^2_{(BSA)} + 2.06C_{(BSA)} + 1.04$ ($R^2 = 0.9495$)

Dendrimer **9**: $S_{Fo}/S_{F(BSA)} = 19.42C^2_{(BSA)} + 2.34C_{(BSA)} + 1.03$ ($R^2 = 0.9979$)

Dendrimer **10**: $S_{Fo}/S_{F(BSA)} = 61.74C^2_{(BSA)} + 0.12C_{(BSA)} + 1.07$ ($R^2 = 0.9988$)

At pH = 9.0, analogous effects are shown:

Dendrimer **8**: $S_{Fo}/S_{F(BSA)} = 15.34C^2_{(BSA)} + 2.52C_{(BSA)} + 1.01$ ($R^2 = 0.9997$)

Dendrimer **9**: $S_{Fo}/S_{F(BSA)} = 20.08C^2_{(BSA)} + 4.46C_{(BSA)} + 1.03$ ($R^2 = 0.9997$)

Dendrimer **10**: $S_{Fo}/S_{F(BSA)} = 25.24C^2_{(BSA)} + 1.73C_{(BSA)} + 1.01$ ($R^2 = 0.9992$)

Synthetic procedures

Synthesis of [(CH$_2$=CHCH$_2$)C$_6$H$_3$(OMe)(OCH$_2$CH$_2$NMe$_2$)] (1). To a solution of the phenol derivative (CH$_2$=CHCH$_2$)-C$_6$H$_3$(OMe)(OH) (8.00 g, 48.7 mmol) in dry acetone (50 ml), the ammonium salt ClCH$_2$CH$_2$NMe$_2$·HCl was added (8.02 g, 55.7 mmol) in the presence of K_2CO_3 (26.93 g, 195.0 mmol), crown ether (18-C-6, 2.57 g, 9.74 mmol) and NaI (catalytic amounts). The mixture was refluxed for 48 h, and then a liquid–liquid extraction was performed using a CH_2Cl_2/H_2O mixture. The organic phase contains the desired allyl amine that is obtained unpurified with small amounts of the starting allyl phenol. This suspension is treated with a water solution of HCl (1 M) allowing the separation in the aqueous phase of compound **1** as the corresponding ammonium salt. Then the solution is treated with a 1 M solution of NaOH and extracted with ethyl ether (3 × 20 ml). The organic phase is dried with magnesium sulfate for 2 h, filtered and the solvent removed to vacuum leading to compound **1** as a pale yellow oil (6.04 g, 52%). ^{1}H-NMR ($CDCl_3$): δ 6.79 (d, 1H, C$_6$H$_3$), 6.68 (m, 2H, C$_6$H$_3$), 5.92 (m, 1H, CH$_2$=C*H*CH$_2$C$_6$H$_3$–), 5.02 (m, 2H, C*H*$_2$=CHCH$_2$C$_6$H$_3$–), 4.07 (t, J_{H-H} = 6.2 Hz, 2H, –C$_6$H$_3$OC*H*$_2$CH$_2$NMe$_2$), 3.81 (s, 3H, C*H*$_3$O–), 3.30 (d, J_{H-H} = 6.8 Hz, 2H, CH$_2$=CHC*H*$_2$C$_6$H$_3$–), 2.74 (t, J_{H-H} = 6.2 Hz, 2H, –C$_6$H$_3$OCH$_2$C*H*$_2$NMe$_2$), 2.31 (s, 6H, –NMe$_2$). ^{13}C{^{1}H}-NMR ($CDCl_3$): δ 149.5 (C$_{ipso}$ bonded to –OCH$_2$CH$_2$NMe$_2$), 146.5 (C$_{ipso}$ bonded to –OMe), 137.6 (C$_{ipso}$ bonded to CH$_2$=CHCH$_2$–), 133.2 (*C*H$_2$=CHCH$_2$C$_6$H$_3$–), 120.4, 113.8, 112.3 (C$_6$H$_3$), 115.6 (CH$_2$=*C*HCH$_2$C$_6$H$_3$–), 67.4 (–C$_6$H$_3$O*C*H$_2$CH$_2$NMe$_2$), 58.1 (–C$_6$H$_3$OCH$_2$*C*H$_2$NMe$_2$), 55.8 (CH$_3$O–), 45.9 (–NMe$_2$), 39.8 (CH$_2$=CH*C*H$_2$C$_6$H$_3$–). MS (APCI, CH_3CN/H_2O): *m*/*z* calcd 235.16 [M]; found 236.16 [M + H]$^+$. Elemental analysis of $C_{14}H_{21}NO_2$: calcd%: C, 71.46; H, 8.99; N, 5.95. Found%: C, 71.01; H, 8.63; N, 5.91.

Synthesis of [Et$_3$SiCH$_2$CH$_2$CH$_2$C$_6$H$_3$(OMe)(OCH$_2$CH$_2$NMe$_2$)] (2). Over a solution of the allylamine **1** (0.21 g, 0.87 mmol) in THF (1 ml), $HSiEt_3$ (0.17 ml, 1.04 mmol) and three drops of a solution of the Karstedt's catalyst (3–3.5% Pt in polydimethylsiloxane) were added. The reaction mixture was stirred for 12 h at 45 °C, evaporated to dryness to remove the solvent and then the residue washed with hexane (2 × 5 ml). The resulting product was purified through a size exclusion chromatography column (Bio-Beds S-X1) using toluene as an eluent leading to compound **2** as a yellow oil (0.22 g, 78%). ^{1}H-NMR ($CDCl_3$): δ 6.80 (m, 1H, C$_6$H$_3$), 6.65 (m, 2H, C$_6$H$_3$), 4.07 (t, 2H, –C$_6$H$_3$OC*H*$_2$CH$_2$NMe$_2$), 3.82 (s, 3H, C*H*$_3$O–), 2.74 (t, J_{H-H} = 6.1 Hz, 2H, –C$_6$H$_3$OCH$_2$CH$_2$C*H*$_2$NMe$_2$), 2.53 (m, 2H, –SiCH$_2$CH$_2$C*H*$_2$C$_6$H$_3$–), 2.31 (s, 6H, –NMe$_2$), 1.57 (m broad, 2H, –SiCH$_2$C*H*$_2$CH$_2$C$_6$H$_3$–), 0.88 (m, 9H, C*H*$_3$CH$_2$Si–), 0.68 (m, 2H, –SiC*H*$_2$CH$_2$CH$_2$C$_6$H$_3$–), 0.48 (m, 6H, CH$_3$C*H*$_2$Si–). ^{13}C{^{1}H}-NMR ($CDCl_3$): δ 149.3 (C$_{ipso}$ bonded to –OCH$_2$CH$_2$NMe$_2$), 146.2 (C$_{ipso}$ bonded to –OMe), 136.0 (C$_{ipso}$ bonded to –CH$_2$CH$_2$CH$_2$Si–), 120.2, 113.6, 112.2 (C$_6$H$_3$), 67.4 (–C$_6$H$_3$O*C*H$_2$CH$_2$NMe$_2$), 58.2 (–C$_6$H$_3$OCH$_2$-*C*H$_2$NMe$_2$), 55.8 (CH$_3$O–), 46.0 (–NMe$_2$), 39.7 (–SiCH$_2$-CH$_2$*C*H$_2$C$_6$H$_3$–), 26.1 (–SiCH$_2$*C*H$_2$CH$_2$C$_6$H$_3$–), 11.2 (–Si*C*H$_2$CH$_2$CH$_2$C$_6$H$_3$–), 7.2 (–SiCH$_2$*C*H$_3$), 3.2 (–Si*C*H$_2$CH$_3$).

Synthesis of G$_1$-[SiCH$_2$CH$_2$CH$_2$C$_6$H$_3$(OMe)(OCH$_2$CH$_2$NMe$_2$)]$_4$ (3). Over a THF (3 ml) solution of the first generation Si–H terminated carbosilane dendrimer G$_1$-H$_4$ (0.5 g, 1.16 mmol) were added the allyl amine **1** (1.09 g, 4.64 mmol) and one drop of a solution of the Karstedt's catalyst (3–3.5% Pt in polydimethylsiloxane). The reaction mixture was stirred for 12 h at 45 °C, evaporated to dryness to remove the solvent and the resulting residue washed with hexane (2 × 5 ml). The resulting product was purified through a size exclusion chromatography column (Bio-Beds S-X1) using toluene as an eluent leading to dendrimer **3** as a yellow oil (1.60 g, 99%). ^{1}H-NMR ($CDCl_3$): δ 6.80 (m, 4H, C$_6$H$_3$), 6.65 (m, 8H, C$_6$H$_3$), 4.07 (t, J_{H-H} = 6.2 Hz, 8H, –C$_6$H$_3$OC*H*$_2$CH$_2$NMe$_2$), 3.81 (s, 12H, CH$_3$O–), 2.74 (t, J_{H-H} = 6.2 Hz, 8H, –C$_6$H$_3$OCH$_2$-C*H*$_2$NMe$_2$), 2.52 (t, J_{H-H} = 7.5 Hz, 8H, –SiCH$_2$CH$_2$-C*H*$_2$C$_6$H$_3$–), 2.31 (s, 24H, –NMe$_2$), 1.55 (m broad, 8H, –SiCH$_2$C*H*$_2$CH$_2$C$_6$H$_3$–), 1.28 (m broad, 24H, SiCH$_2$C*H*$_2$CH$_2$-Si–), 0.53 (m, 16H, SiC*H*$_2$CH$_2$C*H*$_2$Si– and –SiC*H*$_2$CH$_2$-CH$_2$C$_6$H$_3$–), −0.07 (s, 24H, –SiMe$_2$). ^{13}C{^{1}H}-NMR ($CDCl_3$): δ 149.4 (C$_{ipso}$ bonded to –OCH$_2$CH$_2$NMe$_2$), 146.3 (C$_{ipso}$ bonded to –OMe), 136.0 (C$_{ipso}$ bonded to –CH$_2$CH$_2$CH$_2$Si–), 120.2, 113.7, 112.3 (C$_6$H$_3$), 67.5 (–C$_6$H$_3$O*C*H$_2$CH$_2$NMe$_2$), 58.2 (–C$_6$H$_3$OCH$_2$*C*H$_2$NMe$_2$), 55.9 (CH$_3$O–), 46.0 (–NMe$_2$), 39.7 (–SiCH$_2$CH$_2$*C*H$_2$C$_6$H$_3$–), 26.3 (–SiCH$_2$*C*H$_2$CH$_2$Ph–), 20.3, 18.6, 17.5 (Si(CH$_2$)$_3$Si–), 15.4 (–Si*C*H$_2$CH$_2$CH$_2$C$_6$H$_3$–), −3.3 (–SiMe$_2$). ^{29}Si{^{1}H}-NMR ($CDCl_3$): δ (G$_0$-Si) is not observed, 1.60 (G$_1$-Si). MS (MALDI/TOF in dithranol dissolved in THF): *m*/*z* calcd 1373.9 [M]; found 1373.9 [M]. Elemental analysis of $C_{76}H_{136}N_4O_8Si_5$: calcd%: C, 66.42; H, 9.97; N, 4.08. Found%: C, 65.11; H, 9.70; N, 3.73.

Synthesis of G$_2$-[SiCH$_2$CH$_2$CH$_2$C$_6$H$_3$(OMe)(OCH$_2$CH$_2$NMe$_2$)]$_8$ (4). This dendrimer was prepared using a similar method to that described for **3** starting from second generation Si–H terminated carbosilane dendrimer G$_2$-H$_8$ (0.32 g, 0.27 mmol), allyl amine **1** (0.51 g, 2.17 mmol) and one drop of a solution of the Karstedt's catalyst (3–3.5% Pt in polydimethylsiloxane) to obtain compound **4** as a yellow oil (0.38 g, 48%). ^{1}H-NMR ($CDCl_3$): δ 6.80 (m, 8H, C$_6$H$_3$), 6.66 (m, 16H, C$_6$H$_3$), 4.08 (t, J_{H-H} = 6.0 Hz, 16H, –C$_6$H$_3$OC*H*$_2$CH$_2$NMe$_2$), 3.81

(s, 24H, CH_3O–), 2.74 (t, J_{H-H} = 6.2 Hz, 16H, –$C_6H_3OCH_2$-CH_2NMe_2), 2.52 (t, J_{H-H} = 7.9 Hz, 16H, –$SiCH_2CH_2$-$CH_2C_6H_3$–), 2.30 (s, 48H, –NMe_2), 1.55 (m broad, 16H, –$SiCH_2CH_2CH_2C_6H_3$–), 1.28 (m broad, 24H, –$SiCH_2CH_2$-CH_2Si–), 0.53 (m broad, 64H, –$SiCH_2CH_2CH_2Si$– and –$SiCH_2CH_2CH_2C_6H_3$–), −0.07 (s, 48H, –$SiMe_2$), −0.10 (s, 12H, –SiMe). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ 149.4 (C_{ipso} bonded to –$OCH_2CH_2NMe_2$), 146.3 (C_{ipso} bonded to –OMe), 136.0 (C_{ipso} bonded to –$CH_2CH_2CH_2Si$–), 120.2, 113.7, 112.3 (C_6H_3), 67.4 (–$C_6H_3OCH_2CH_2NMe_2$), 58.2 (–$C_6H_3OCH_2CH_2NMe_2$), 55.9 (CH_3O–), 46.0 (–NMe_2), 39.6 (–$SiCH_2CH_2CH_2C_6H_3$–), 26.2 (–$SiCH_2CH_2CH_2C_6H_3$–), 20.1, 18.8, 18.6 (–$Si(CH_2)_3Si$–), 15.4 (–$SiCH_2CH_2CH_2C_6H_3$–), −3.2 (–$SiMe_2$), −4.9 (–SiMe). $^{29}Si\{^1H\}$-NMR ($CDCl_3$): δ (G_0-Si) is not observed, 1.00 (G_1-Si), 1.70 (G_2-Si). Elemental analysis of $C_{168}H_{308}N_8O_{16}Si_{13}$: calcd%: C, 65.91; H, 10.14; N, 3.66. Found%: C, 64.81; H, 9.44; N, 3.24.

Synthesis of G_3-[$SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2NMe_2)]_{16}$ (5). This dendrimer was prepared using a similar method to that described for **3** starting from third generation Si–H terminated carbosilane dendrimer G_3-H_{16} (0.42 g, 0.16 mmol), allyl amine **1** (0.71 g, 2.70 mmol) and one drop of a solution of the Karstedt's catalyst (3–3.5% Pt in polydimethylsiloxane) to obtain compound **5** as a yellow oil (0.78 g, 77%). ^{1}H-NMR ($CDCl_3$): δ 6.76 (m, 16H, C_6H_3), 6.65 (m, 32H, C_6H_3), 4.06 (t, J_{H-H} = 6.0 Hz, 32H, –$C_6H_3OCH_2CH_2NMe_2$), 3.79 (s, 48H, CH_3O–), 2.75 (t, J_{H-H} = 6.0 Hz, 32H, –$C_6H_3OCH_2$-CH_2NMe_2), 2.51 (t, J_{H-H} = 7.3 Hz, 32H, –$SiCH_2CH_2$-$CH_2C_6H_3$–), 2.32 (s, 48H, –NMe_2), 1.55 (m broad, 24H, –$SiCH_2CH_2CH_2C_6H_3$–), 1.27 (m broad, 56H, –$SiCH_2CH_2$-CH_2Si–), 0.53 (m broad, 144H, –$SiCH_2CH_2CH_2Si$– and –$SiCH_2CH_2CH_2C_6H_3$–), −0.07 (s, 96H, –$SiMe_2$), −0.09 (s, 36H, –SiMe). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ 149.7 (C_{ipso} bonded to –$OCH_2CH_2NMe_2$), 146.6 (C_{ipso} bonded to –OMe), 136.4 (C_{ipso} bonded to –$CH_2CH_2CH_2Si$–), 120.6, 113.9, 112.6 (C_6H_3), 67.4 (–$C_6H_3OCH_2CH_2NMe_2$), 58.5 (–$C_6H_3OCH_2$-CH_2NMe_2), 56.2 (CH_3O–), 46.2 (–NMe_2), 40.1 (–$SiCH_2CH_2$-$CH_2C_6H_3$–), 26.7 (–$SiCH_2CH_2CH_2C_6H_3$–), 20.6, 19.2, 18.9 (–$Si(CH_2)_3Si$–), 15.8 (–$SiCH_2CH_2CH_2C_6H_3$–), −2.8 (–$SiMe_2$), −4.5 (–SiMe). $^{29}Si\{^1H\}$-NMR ($CDCl_3$): δ (G_0-Si) and (G_1-Si) not observed, 1.0 (G_2-Si); 1.8 (G_3-Si). Elemental analysis of $C_{352}H_{652}N_{16}O_{32}Si_{29}$: calcd%: C, 65.69; H, 10.21; N, 3.48. Found%: C, 65.26; H, 9.91; N, 3.42.

Synthesis of {[$(CH_2{=}CHCH_2)C_6H_3(OMe)(OCH_2CH_2N^+$-$Me_3)$]$I^-$} (6). To a diethyl ether solution of **1** (0.78 g, 3.31 mmol) was added slight excess of MeI (0.5 ml, 8.01 mmol). The resulting solution was stirred for 12 h at room temperature and then evaporated under reduced pressure to give **6** as a white solid (1.19 g, 95%). ^{1}H-NMR (DMSO): δ 6.99 (m, 1H, C_6H_3), 6.68 (m, 1H, C_6H_3), 6.71 (m, 1H, C_6H_3), 5.92 (m, 1H, $CH_2{=}CHCH_2C_6H_3$–), 5.03 (m, 2H, $CH_2{=}CHCH_2C_6H_3$–), 4.37 (m, 2H, –$C_6H_3OCH_2$-$CH_2N^+Me_3$), 3.75 (s broad, 5H, CH_3O– and –$C_6H_3OCH_2$-$CH_2N^+Me_3$, overlapped), 3.31 (m, 2H, $CH_2{=}CHCH_2Ph$–), 3.19 (s, 9H, –N^+Me_3). $^{13}C\{^1H\}$-NMR (DMSO): δ 148.7 (C_{ipso} bonded to –$OCH_2CH_2N^+Me_3$), 144.5 (C_{ipso} bonded to –OMe), 137.3 ($CH_2{=}CHCH_2C_6H_3$–), 133.4 (C_{ipso} bonded to –CH_2CH_2-CH_2Si–), 119.7, 114.3, 112.1 (C_6H_3), 115.1 ($CH_2{=}CHCH_2C_6H_3$–), 63.6 (–$C_6H_3OCH_2CH_2N^+Me_3$), 62.6 (–$C_6H_3OCH_2CH_2N^+Me_3$), 55.1 (CH_3O–), 52.7 (–N^+Me_3), 39.8 ($CH_2{=}CHCH_2C_6H_3$–). MS (APCI and CH_3CN/H_2O): m/z calcd 379.10 [M]; found 250.18 [M − I]$^+$. Elemental analysis of $C_{15}H_{26}INO_2$: calcd%: C, 47.50; H, 6.91; N, 3.69. Found%: C, 47.17; H, 6.19; N, 3.75.

Synthesis of {[$Et_3SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2N^+$-$Me_3)$]$I^-$} (7). This compound was prepared using a similar method to that described for **6**, starting from [$Et_3SiCH_2CH_2CH_2$-$C_6H_3(OMe)(OCH_2CH_2NMe_2)$] (**2**) (0.07 g, 0.22 mmol) and MeI (0.05 ml, 0.8 mmol) to obtain **7** as a white solid (0.1 g, 93%). ^{1}H-NMR (DMSO): δ 6.96 (m, 1H, C_6H_3), 6.80 (m, 1H, C_6H_3), 6.65 (m, 1H, C_6H_3), 4.35 (t, 2H, –$C_6H_3OCH_2$-$CH_2N^+Me_3$), 3.75 (s, 5H, CH_3O– and, –$C_6H_3OCH_2$-$CH_2N^+Me_3$ overlapped), 3.18 (s, 9H, –N^+Me_3), 2.49 (m, 2H, –$SiCH_2CH_2CH_2C_6H_3$–, overlapped with the signal of DMSO), 1.54 (m broad, 2H, –$SiCH_2CH_2CH_2C_6H_3$–), 0.87 (m, 9H, CH_3CH_2–), 0.48 (m, 8H, CH_3CH_2– and –$SiCH_2CH_2CH_2C_6H_3$– overlapped).

Synthesis of G_1-{[$SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2N^+$-$Me_3)$]$_4$$4I^-$} (8). To an ethyl ether solution (3 ml) of G_1-[$SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2NMe_2)]_4$ (**3**) (0.61 g, 0.44 mmol) was added an excess of MeI (8.01 mmol). The resulting solution was stirred for 48 h at room temperature and then evaporated under reduced pressure to give **8** as a white solid (0.79 g, 92%). ^{1}H-NMR (DMSO): δ 6.96 (m, 4H, C_6H_3), 6.79 (m, 4H, C_6H_3), 6.68 (m, 4H, –C_6H_3), 4.34 (m, 8H, –$C_6H_3OCH_2CH_2N^+Me_3$), 3.74 (s, 12H, CH_3O– and m, 8H, –$C_6H_3OCH_2CH_2N^+Me_3$ overlapped), 3.18 (s, 36H, –N^+Me_3), 2.49 (m, 8H, –Si–$CH_2CH_2CH_2C_6H_3$–, overlapped with the signal of DMSO), 1.51 (m broad, 8H, –$SiCH_2CH_2CH_2C_6H_3$–), 1.28 (m broad, 8H, –$SiCH_2CH_2CH_2Si$–), 0.51 (m, 24H, –$SiCH_2CH_2$-$CH_2C_6H_3$ and –$SiCH_2CH_2CH_2Si$–), –0.07 (s, 24H, –$SiMe_2$). $^{13}C\{^1H\}$-NMR (DMSO): δ 148.6 (C_{ipso} bonded to –OCH_2-$CH_2N^+Me_3$), 144.3 (C_{ipso} bonded to –OMe), 135.9 (C_{ipso} bonded to –$CH_2CH_2CH_2Si$–), 119.5, 114.2, 111.9 (C_6H_3), 63.6 (–$PhOCH_2CH_2N^+Me_3$), 62.6 (–$C_6H_3OCH_2CH_2N^+Me_3$), 55.1 (CH_3O–), 52.7 (–N^+Me_3), 39.0 (–$SiCH_2CH_2CH_2C_6H_3$–, overlapped with the signal of DMSO), 25.3 (–$SiCH_2CH_2$-$CH_2C_6H_3$–), 19.1, 17.6, 16.4 (–$Si(CH_2)_3Si$–), 14.3 (–$SiCH_2CH_2$-$CH_2C_6H_3$–), −3.7 (–$SiMe_2$). $^{29}Si\{^1H\}$-NMR (DMSO): δ (G_0-Si) was not observed, 1.7 (G_1-Si). Elemental analysis of $C_{80}H_{148}I_4$-$N_4O_8Si_5$: calcd%: C, 49.48; H, 7.68; N, 2.88. Found%: C, 50.97; H, 7.91; N 2.58.

Synthesis of G_2-{[$SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2N^+$-$Me_3)$]$_8$$8I^-$} (9). This dendrimer was prepared using a similar method to that described for **8**, starting from an ethyl ether solution of G_2-$[SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2NMe_2)]_8$ (**4**) (0.38 g, 0.13 mmol) and an excess of MeI (1.12 mmol) to give compound **9** as a white solid (0.45 g, 85%). ^{1}H-NMR (DMSO): δ 6.95 (m, 8H, C_6H_3), 6.78 (m, 8H, C_6H_3), 6.65 (m, 8H, C_6H_3), 4.33 (m, 16H, –$C_6H_3OCH_2CH_2N^+Me_3$), 3.74 (s, 24H, CH_3O– and m, 16H, –$C_6H_3OCH_2CH_2NMe_3^+$ overlapped), 3.18 (s, 72H, –N^+Me_3), 2.49 (m, 16H, –$SiCH_2CH_2CH_2C_6H_3$–, overlapped with the signal of DMSO), 1.50 (m broad, 16H, –$SiCH_2$-$CH_2CH_2C_6H_3$–), 1.29 (m broad, 24H, –$SiCH_2CH_2CH_2Si$–), 0.52 (m, 64H, –$SiCH_2CH_2CH_2C_6H_3$– and –$SiCH_2CH_2CH_2Si$–), −0.09 (s, 48H, –$SiMe_2$), −0.11 (s, 12H, –SiMe). $^{13}C\{^1H\}$-NMR

(DMSO): δ 148.6 (C_{ipso} bonded to $-OCH_2CH_2N^+Me_3$), 144.3 (C_{ipso} bonded to –OMe), 135.9 (C_{ipso} bonded to $-CH_2CH_2CH_2Si-$), 119.5, 114.3, 111.9 (C_6H_3), 63.6 ($-C_6H_3OCH_2CH_2N^+Me_3$), 62.6 ($-C_6H_3OCH_2CH_2N^+Me_3$), 55.1 (CH_3O-), 52.7 ($-N^+Me_3$), 39.0 ($-SiCH_2CH_2CH_2C_6H_3-$, overlapped with the signal of DMSO), 25.3 ($-SiCH_2CH_2CH_2C_6H_3-$), 19.0, 17.7, 17.6 ($Si(CH_2)_3Si$), 14.3 ($-SiCH_2CH_2CH_2C_6H_3-$), −3.8 ($-SiMe_2$), −5.4 (–SiMe). $^{29}Si\{^1H\}$-NMR (DMSO): δ (G_0-Si) was not observed, 1.3 (G_1-Si) and 1.6 (G_2-Si). Elemental analysis of $C_{176}H_{332}I_8N_8O_{16}Si_{13}$: calcd%: C, 50.37; H, 7.97; N, 2.88. Found%: C, 50.08; H, 7.67; N, 2.66.

Synthesis of G_3-{[$SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2N^+Me_3)]_{16}16I^-$} (10). This dendrimer was prepared using a similar method to that described for **8**, starting from an ethyl ether solution of G_3-[$SiCH_2CH_2CH_2C_6H_3(OMe)(OCH_2CH_2NMe_2)]_{16}$ (**5**) (0.22 g, 0.03 mmol) and an excess of MeI (0.04 ml, 0.70 mmol) to give compound **10** as a white solid (0.19 g, 78%). ^{1}H-NMR (DMSO): δ 6.91 (m, 16H, C_6H_3), 6.71 (m, 16H, C_6H_3), 6.61 (m, 16H, C_6H_3), 4.31 (m, 32H, $-C_6H_3OCH_2CH_2N^+Me_3$), 3.69 (s, 48H, CH_3O- and m, 32H, $-C_6H_3OCH_2CH_2N^+Me_3$ overlapped), 3.19 (s, 144H, $-N^+Me_3$), 2.49 (m, 32H, $-SiCH_2CH_2CH_2C_6H_3-$, overlapped with the signal of DMSO), 1.47 (m broad, 32H, $-SiCH_2CH_2CH_2C_6H_3-$) 1.28 (m broad, 56H, $-SiCH_2CH_2CH_2Si-$), 0.49 (m, 144H, $-SiCH_2CH_2CH_2C_6H_3-$ and $-SiCH_2CH_2CH_2Si-$), −0.11 (s broad, 132H, $-SiMe_2$ and –SiMe). $^{13}C\{^1H\}$-NMR (DMSO): δ 148.6 (C_{ipso} bonded to $-OCH_2CH_2N^+Me_3$), 144.3 (C_{ipso} bonded to –OMe), 135.8 (C_{ipso} bonded to $-CH_2CH_2CH_2Si-$), 119.4, 114.4, 111.8 (C_6H_3), 63.6 ($-C_6H_3OCH_2CH_2N^+Me_3$), 62.8 ($-C_6H_3OCH_2CH_2N^+Me_3$), 55.0 (CH_3O-), 52.7 ($-N^+Me_3$), 39.0 ($-SiCH_2CH_2CH_2C_6H_3-$, overlapped with the signal of DMSO), 25.2 ($-SiCH_2CH_2CH_2C_6H_3-$), 19.0, 17.7, 17.5 ($-Si(CH_2)_3Si-$), 14.3 ($-SiCH_2CH_2CH_2C_6H_3-$), −3.9 ($-SiMe_2$), −5.4 (–SiMe). $^{29}Si\{^1H\}$-NMR (DMSO): δ (G_0-Si) and (G_1-Si) were not observed, 1.4 (G_2-Si) and 1.6 (G_3-Si). Elemental analysis of $C_{368}H_{700}I_{16}N_{16}O_{32}Si_{29}$: calcd%: C, 61.02; H, 9.50; N, 2.22. Found%: C, 60.42; H, 8.44; N 2.64.

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