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Structure-Activity Relationship Study of Cationic Carbosilane Dendritic Systems as Antibacterial Agents

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Abstract

This work focuses on the antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and the hemolytic properties of two types of ammonium cationic carbosilane systems: dendrimers and dendrons. The effects of: i) the generation, ii) the type of peripheral groups near the cationic charges (a $SiMe_2$ moiety or a S atom depending on the synthetic procedure, hydrosilylation or thiol-ene addition, respectively), iii) the core of dendrimers (polyphenoxo *vs*. Si atom) and iv) the focal point of dendrons ($-N_3$, $-NH_2$, -OH) have been assessed. The structure-activity relationship analysis indicates the importance of an adequate balance between the hydrophilic and lipophilic fragments of these molecules to reach the best antibacterial activity. Regarding hemolysis, lowest toxicity values were registered for dendritic systems with a sulfur atom close to the surface and, in the particular case of dendrons, for those with a hydroxyl focal point. One dendrimer and one dendron, both bearing a sulfur atom close to the surface, scored best in the activity-toxicity relationship analysis and were chosen for resistance assays. No changes in the inhibitory and bactericidal capacity in the case of the dendron and only a slight increase of these values for the dendrimer were observed after 15 subculture cycles. Furthermore, these two compounds stayed active towards different strains of resistant bacteria and avoid formation of biofilm at concentrations over the minimum inhibitory concentration (MIC).

Keywords: dendritic molecules, cationic, antibacterial, resistance assay, biofilm.

1. Introduction

Microbial infections are becoming increasingly difficult to fight because the number of antibiotic-resistant microbial strains is growing faster than the number of useable antibiotics.[1-3] Moreover, the residual toxicity of conventional antimicrobial agents is associated with serious problems they may cause to the environment. Furthermore, bacterial contamination is a major concern in biomedical equipment and the creation of materials with non-antibiotic microbicide surfaces would improve their efficiency.[4] The problem is worsened by the ability of bacteria to create biofilms,[5] which are involved in the majority of infection diseases caused by bacteria.[6] Thus, the search for alternative approaches to fight microbial antibiotic resistance has been raised as a priority.[7, 8]

Quaternary ammonium salts (QAS) are popular antimicrobial agents due to their activity against both Gram-positive and Gram-negative bacteria. The majority of bacterial cell walls are negatively charged and ammonium compounds interact with them replacing metal cations and destroying the cell wall.[9-11] Another advantage of some QAS is their solubility in water, which allows them to kill bacteria in this medium.[12] The incorporation of QAS to polymers generates polycations that present a high surface charge. This multivalency of polymeric macromolecules increases the activity with respect to monofunctional molecules. Further advantages of these systems, when compared with small-molecule antimicrobial agents, are their non-volatility, chemical stability, long-term antimicrobial activity and also the broader range of possibilities to introduce modifications for improving their behaviour.[13] The activity and mode of action of antimicrobial polycations may be affected by several factors, such as molecular weight, polydispersity, spacer length between the active site and the main scaffold, hydrophilic/hydrophobic balance, and nature of counterions.[7, 14] Different types of macromolecules containing QAS fragments, like polymers,[13] dendrimers,[15, 16] and hyperbranched polymers,[17] have been studied as antimicrobial agents, exhibiting high activity due to their polyvalency.

Dendrimers are uniformly decorated spherical molecules, whereas dendrons are cone-shaped molecules that present an extra active moiety, the focal point, which can be used to attach a second functionality or to

dendronize materials. Both types of multifunctional molecules are designed step by step leading to well-defined structures.[18-20] Dendrimers and dendrons, though bearing the same peripheral functions may exert different interactions with a target, for example, as a consequence of the molecular flexibility or the influence of the focal point. An advantage of dendritic molecules over traditional polymers is their well-known structures, which ease the establishment of structure-activity relationships. Moreover, the dendron focal point is an excellent anchorage position for additional functionalization. Several types of cationic dendrimers, like PAMAM,[21] PPI,[22] phosphorus-viologen,[23] carbosilane,[24] poly(propyleneoxide) amines,[25] have shown antibacterial properties. The mode of action of these macromolecules is related with that of QAS, displacing the divalent cations and modifying membrane permeability. Furthermore, the presence of hydrophobic chains in dendrimers enable their penetration into the phospholipid bilayer leading to the disintegration of the bacterial membrane.[11, 26] Other types of dendrimers, such as glycodendrimers[27-29] or peptide-dendrimers[30, 31] with microbicidal properties have also been reported. Some of these dendrimers neither induced antibiotic resistance in bacteria[32], nor avoided biofilm formation by themselves or in combination with drugs.[30, 33] On the other hand, to our knowledge, the study of antimicrobial properties of dendrons is scarce[34] and usually associated to generation of dendronized materials.[35-37]

With respect to carbosilane derivatives, the antibacterial properties of ammonium functionalized dendrimers and hyperbranched polymers have been studied by our group previously.[24, 38 39, 40] The better behavior of well-defined dendrimers compared to hyperbranched polymers and the importance of groups proximal to the ammonium functions were observed. The relevance of the structure in carbosilane dendrimers and hyperbranched polymers is highlighted in derivatives decorated by thiol-ene addition. The former compounds showed good solubility in water whilst the latter were poorly water-soluble, hence making the proper assessment of their antibacterial properties difficult in this medium.[41] Chen *et al.* have also reported that hyperbranched PPI polymers were less effective as biocides than PPI dendrimers.[22]

Herein, we have extended our research to explore the influence on the antibacterial activity and toxicity of several structural aspects of cationic carbosilane dendrimers and dendrons: i) the generation; ii) the type

of peripheral groups near the cationic charges (a $SiMe_2$ moiety or a S atom depending on the synthetic procedure, hydrosilylation or thiol-ene addition, respectively); iii) the core in the case of dendrimers (polyphenoxo *vs*. Si atom); iv) the focal point for dendrons ($-N_3$, $-NH_2$, -OH). The comparison of antibacterial activity and toxicity of the systems discussed in this work led to the selection of one dendrimer and one dendron to carry out resistance studies, as one important drawback of traditional antibiotics is the generation of resistant strains. Finally, the effect of the selected dendritic molecules on biofilm formation and stability has also been evaluated.

2. Results and Discussion

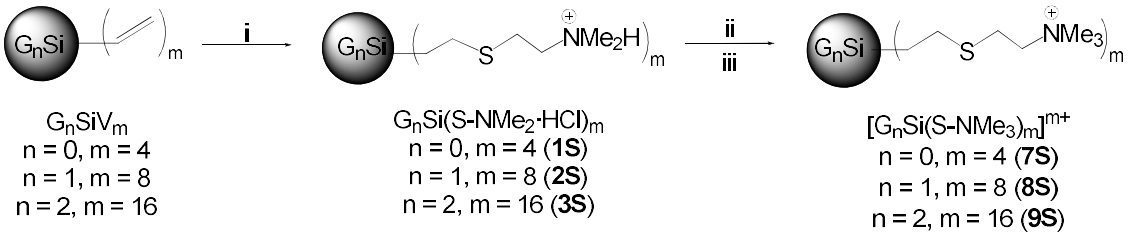
2.1. Synthesis and characterization of dendrimers.

In order to clarify the discussion of the compounds studied herein, the following nomenclature has been used: Dendrimers are named as $[GnX(Y\text{-}Z)_m]$, where n indicates the generation, X refers to the core (Si for a silicon atom and O_3 for the polyphenoxo core), Y indicates functionalization of the periphery by hydrosilylation (Y = Si) or hydrothiolation (Y = S), and Z and m correspond to the peripheral functional groups and their numbers on the surface. Dendrons are named as $[XGn(Y\text{-}Z)_m]$, where X refers to the focal point. Moreover, all compounds will be quoted with a number followed by S or Si meaning that they were obtained by thiol-ene addition or hydrosilylation, respectively, using the same number for compounds of the same core and generation.

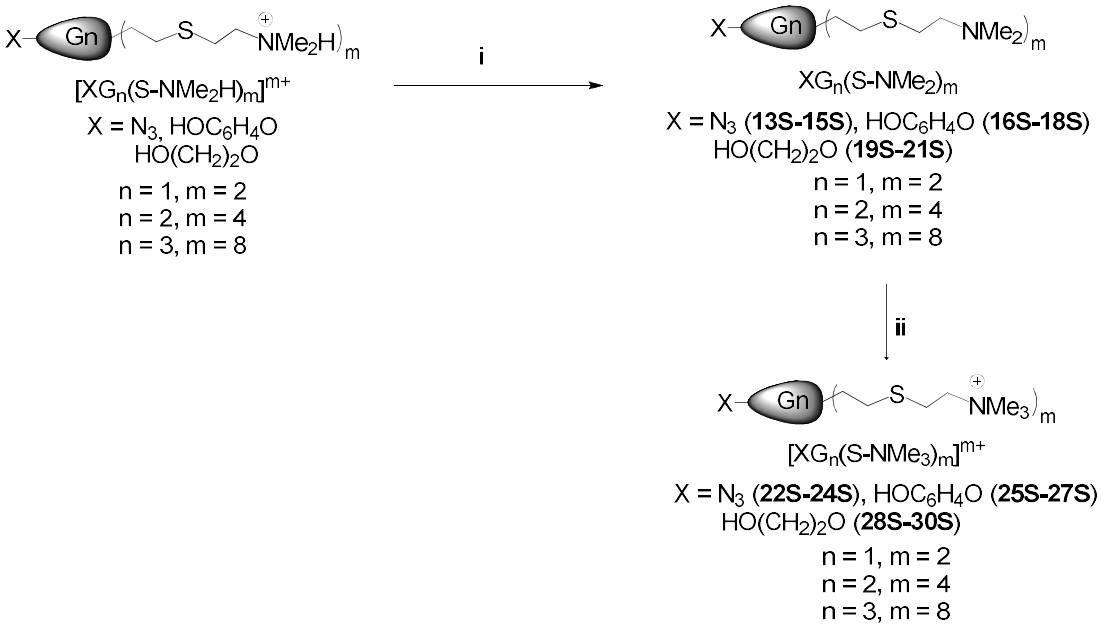
Previously, we have developed cationic dendrimers derived from a polyphenoxo core functionalized by both hydrosilylation, $[G_nO_3(Si\text{-}NMe_3)_m]^{m+}$,[42] and hydrothiolation, $[G_nO_3(S\text{-}NMe_3)_m]^{m+}$ and $[G_nO_3(S\text{-}NH_3)_m]^{m+}$,[39] and also derived from a silicon atom functionalized by hydrosilylation, $[G_nSi(Si\text{-}NMe_3)_m]^{m+}$.[38] To complete the family of spherical compounds for an adequate evaluation of the structure-activity relationship regarding the bactericidal properties of dendritic systems, new cationic dendrimers derived from a Si atom core by thiol-ene addition have been prepared (Scheme 1). The thiol derivative used to yield them contains an ammonium group of the type $–NMe_2H^+$. However, the study of antibacterial

properties of has been carried out after transforming this moiety into $–NMe_3^+$ cationic groups, since it confers two main advantages to carbosilane dendrimers and dendrons with respect to $–NR_2H^+$: i) stability at high pH values, because its deprotonation leading to non-water-soluble compounds is not possible; ii) lower tendency to aggregate, which allows its storage for long periods even in solid state. Furthermore, the $–NMe_3^+$ moieties at the periphery of these new dendrimers allowed us to compare these systems with other cationic dendrimers prepared previously by hydrosilylation, which were decorated with $–NMe_3^+$ functions.

The synthesis of the new compounds was started using carbosilane dendrimers decorated with vinyl moieties G_nSiV_m.[43] In a first step, these compounds were modified by thiol-ene reactions employing 2-dimethylaminoethanethiol hydrochloride, $HS(CH_2)_2NMe_2{\cdot}HCl$, generating $[G_nSi(S\text{-}NMe_2H)_m(Cl)_m]$ (n = 0, m = 4 (**1S**); n = 1, m = 8 (**2S**); n = 2, m = 16 (**3S**)). Subsequently, compounds **1S-3S** were neutralized with base (NaOH) forming $G_nSi(S\text{-}NMe_2)_m$ (n = 0, m = 4 (**4S**); n = 1, m = 8 (**5S**); n = 2, m = 16 (**6S**)). Finally, addition of excess MeI led to $[G_nSi(S\text{-}NMe_3)_m(I)_m]$ (n = 0, m = 4 (**7S**); n = 1, m = 8 (**8S**); n = 2, m = 16 (**9S**)). The cationic derivatives thus obtained were isolated in high yields as pale yellow solids, soluble in water and other polar solvents (DMSO, alcohol). All compounds were characterized by NMR, MS and elemental analysis. The most relevant NMR data for derivatives **1S-3S** were the disappearance of the vinyl resonances of the $Si(CHCH_2)$ fragment and the presence of the resonances associated to the new $Si(CH_2)_2S$ chain formed. Furthermore, ^1H, ^{13}C and ^{15}N NMR spectroscopy of compounds **7S-9S** also revealed the outermost $S(CH_2)_2NMe_3^+$ chain. Since these data are analogous to those found in the related dendrimers derived from a polyphenoxo core[39] and also very similar to the NMR data observed in the related dendrons described below, they will not be further discussed. In a similar way, and following the procedure also used by Rissing and Son,[44] dendrimers of the type $[G_nSi(S\text{-}NH_3)_m(Cl)_m]$ (n = 0, m = 4 (**10S**); n = 1, m = 8 (**11S**); n = 2, m = 16 (**12S**)) were synthesized in high yields and characterized by NMR, MS and elemental analysis. NMR spectra of compounds **11S** and **12S** resembled those reported for **10S** previously.[44]



Scheme 1. Synthesis of cationic dendrimers $[GnSi(S\text{-}NMe_3)_m]^{m+}$ (**7S-9S**). i) $HS(CH_2)_2NMe_2{\cdot}HCl$, DMPA, hυ; ii) NaOH; iii) MeI.



Scheme 2. Synthesis of cationic dendrons $[XG_n(S\text{-}NMe_3)_m]^{m+}$ (n = 1, m = 2; n = 2, m = 4; n = 3, m = 8; X = N_3 (**22S-24S**), HOC_6H_4O (**25S-27S**), $HO(CH_2)_2O$ (**28S-30S**)). i) Na_2CO_3; ii) MeI.

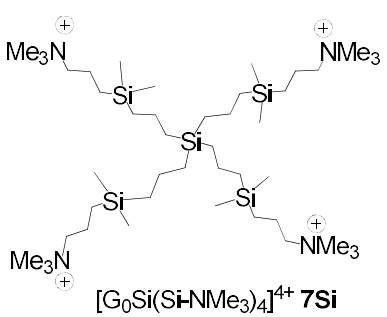
The synthesis of related cationic carbosilane dendrons with different functionalities at the focal point and $-NMe_3^+$ groups on the surface was achieved employing a procedure (Scheme 2) similar to that described above for dendrimers. A neutralization step of dendrons $XGn(S\text{-}NMe_2{\cdot}HCl)_m$ (n = 1, m = 2; n = 2, m = 4; n = 3, m = 8; X = N_3, HOC_6H_4O, $HO(CH_2)_2O$) with Na_2CO_3, and subsequent quaternization with MeI afforded the corresponding dendrons $[XG_n(S\text{-}NMe_3)_m(I)_m]$ (n = 1, m = 2; n = 2, m = 4; n = 3, m = 8; X = N_3 (**22S-24S**), HOC_6H_4O (**25S-27S**), $HO(CH_2)_2O$ (**28S-30S**)). Compounds **22S-30S** were isolated in high

yields as pale yellow solids, soluble in water and other polar solvents, although G1 compounds also showed solubility in chlorinated solvents. Their characterization by NMR, MS and elemental analysis confirmed the formation of the cationic surface bearing -NMe_3^+ moieties, as described for dendrimers, as well as the inalterability of the focal point. This procedure has been used elsewhere to synthesize the related dendron with a primary amine at the focal point $[NH_2G_n(S\text{-}NMe_3)_m]^{m+1}$ (n = 1, m = 2; n = 2, m = 4; n = 3, m = 8 (**31S-33S**)).[45]

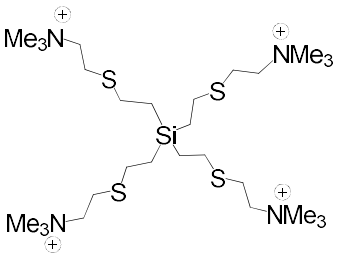
2.2. Antibacterial activity.

For the study of the biocidal capacity of cationic dendrimers and dendrons *S. aureus* and *E. coli* were chosen, as a model of Gram-positive and Gram-negative bacteria, respectively. Figure 1 depicts drawings of some representative dendrimers and dendrons and Tables 1-3 summarize the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of dendrimers and dendrons discussed in this section, respectively.

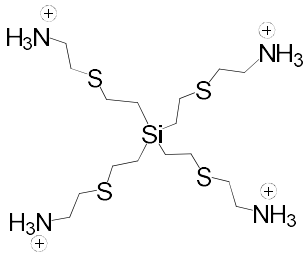
The main goal of this work is the evaluation of the antibacterial behavior of carbosilane dendritic systems as a function of different parameters, such as: i) the dendritic generation, ii) the core of dendrimers or the focal point of dendrons, iii) the nature of the branches, iv) the type of cationic terminal group, v) the topology of the dendritic system or vi) the type of bacteria, in order to find a structure-activity relationship of these compounds that may allow to rationalize the behavior of these dendritic systems as antibacterial agents.



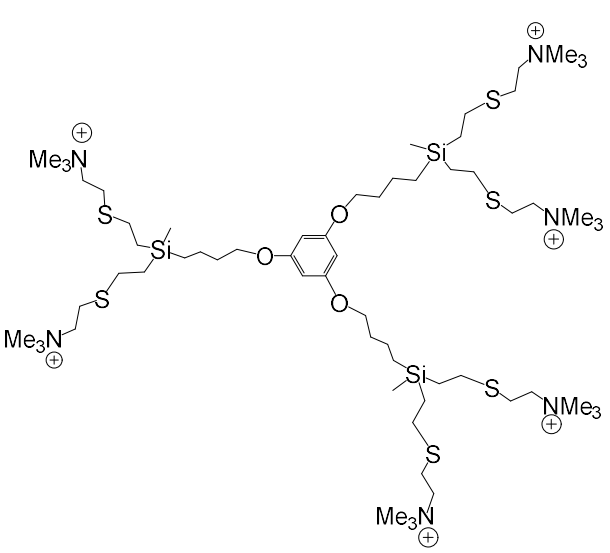
$[G_0Si(Si\text{-}NMe_3)_4]^{4+}$ **7Si**



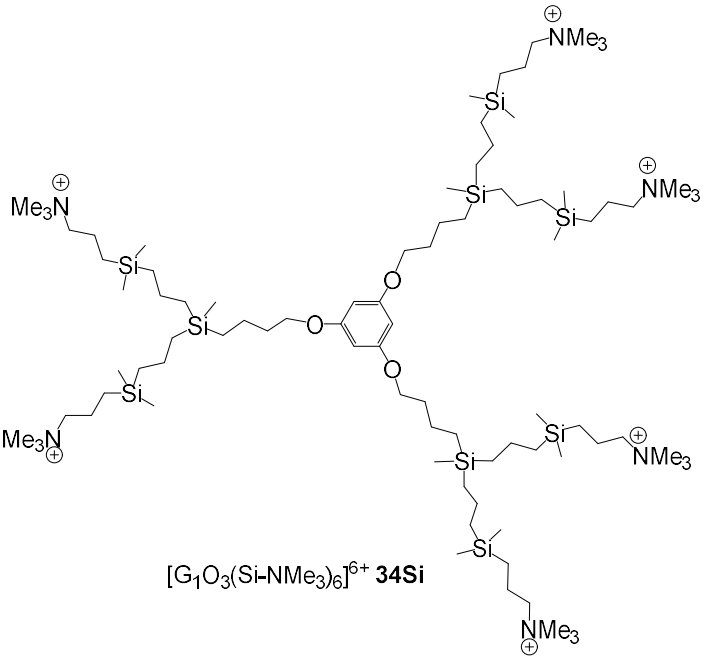
$[G_0Si(S\text{-}NMe_3)_4]^{4+}$ **7S**



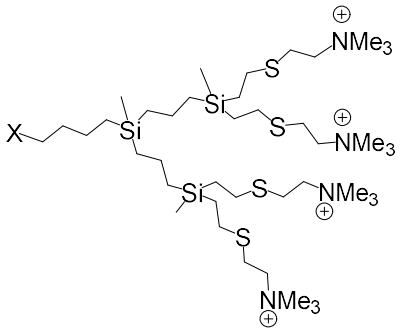
$[G_0Si(S\text{-}NH_3)_4]^{4+}$ **10S**



$[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ **34S**



$[G_1O_3(Si\text{-}NMe_3)_6]^{6+}$ **34Si**



$[XG2(S\text{-}NMe_3)_4]^{4+}$

Figure 1. Drawing of representative cationic dendrimers and dendrons used in this work. G_0 dendrimers derived from Si atom core (**7Si**, **7S**, and **10S**), G_1 dendrimers derived from a polyphenoxo core (**34S** and **34Si**), and G_2 dendrons (X = N_3 (**23S**), HOC_6H_4O (**26S**), $HO(CH_2)_2O$ (**29S**), NH_2 (**32S**)).

	S. aureus		*E. coli*	
	MIC	MBC	MIC	MBC
$[G_0Si(Si\text{-}NMe_3)_4]^{4+}$ (**7Si**)	1	4	4	4
$[G_1Si(Si\text{-}NMe_3)_8]^{8+}$ (**8Si**)	4	8	16	32
$[G_2Si(Si\text{-}NMe_3)_{16}]^{16+}$ (**9Si**)	8	8	64	64

$[G_0Si(S\text{-}NMe_3)_4]^{4+}$ (**7S**)	128	512	256	512
$[G_1Si(S\text{-}NMe_3)_8]^{8+}$ (**8S**)	16	128	16	64
$[G_2Si(S\text{-}NMe_3)_{16}]^{16+}$ (**9S**)	32	32	32	32
$[G_0Si(S\text{-}NH_3)_4]^{4+}$ (**10S**)	128	256	128	256
$[G_1Si(S\text{-}NH_3)_8]^{8+}$ (**11S**)	2	4	4	4
$[G_2Si(S\text{-}NH_3)_{16}]^{16+}$ (**12S**)	512	512	512	512

Table 1. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of dendrimers with a Si atom core obtained by hydrosilylation (**7Si-9Si**) and thiolation (**7S-12S**). Data shown in ppm (mg L^{-1}).

	S. aureus		*E. coli*	
	MIC	MBC	MIC	MBC
$[G_1O_3(Si\text{-}NMe_3)_6]^{6+}$ (**34Si**)	2	2	16	32
$[G_2O_3(Si\text{-}NMe_3)_{12}]^{12+}$ (**35Si**)	4	4	64	64
$[G_3O_3(Si\text{-}NMe_3)_{24}]^{24+}$ (**36Si**)	16	16	64	64
$[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**)	2	2	2	4
$[G_2O_3(S\text{-}NMe_3)_{12}]^{12+}$ (**35S**)	2	2	8	8
$[G_3O_3(S\text{-}NMe_3)_{24}]^{24+}$ (**36S**)	8	8	16	16
$[G_1O_3(S\text{-}NH_3)_6]^{6+}$ (**37S**)	2	2	2	2
$[G_2O_3(S\text{-}NH_3)_{12}]^{12+}$ (**38S**)	4	4	8	8
$[G_3O_3(S\text{-}NH_3)_{24}]^{24+}$ (**39S**)	32	32	128	128

Table 2. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of dendrimers with a polyphenoxo core 1,3,5-$(O)_3C_6H_3$, obtained by hydrosilylation (**34Si-36Si**) and thiolation (**34S-39S**). Data shown in ppm (mg L^{-1}).

Analysis of the data obtained for dendrimers (Tables 1 and 2) shows a significant variability of the MIC and MBC values with respect to the core and chain supporting the ammonium functions (with a Me_2Si moiety in hydrosilylation derivatives *vs*. a S atom in hydrothiolation derivatives), which has also been found in the microbicidal capacity studies of all these dendrimers against amoebae.[46] These findings may be explained in terms of the dendrimers' global structure, their antibacterial behavior being controlled by the balance between the lipophilic (the carbosilane framework) and the hydrophilic (external chain) moieties.[22, 47, 48] This balance is mainly affected by two factors: i) the peripheral branches, between the ammonium functions and the outermost dendritic Si atom of the carbosilane scaffold; and ii) the dendrimer core. As for the first factor, thiol-ene derivatives bear five atoms including a polar sulfur atom, whereas hydrosilylation derivatives bear seven atoms including a bulkier non polar $SiMe_2$ group. That is, compounds obtained by hydrosilylation contain a long hydrophobic chain adjacent to the cationic charges. Regarding the second factor, one group of dendrimers present a Si atom core from which four branches grow, whereas the other dendrimer core is derived from $1,3,5\text{-}(HO)_3C_6H_3$, which is bigger and more rigid, and only three branches spread from it. Since all assays were carried out in aqueous media, the carbosilane structure of these dendrimers tends to shrink, minimizing their exposure to this aqueous environment. Therefore, the dendrimer core characteristics make one expect a higher exposure of the inner structure of polyphenoxo derivatives in aqueous solutions, easing the interaction of the hydrophobic region with the bacterial cell walls.[49] These differences determine the hydrophilic/hydrophobic balance given in these derivatives and may well explain the activity data found. Similar considerations concerning the hydrophilic/hydrophobic balance of the dendrimer structure have been made for PPI[22] and PAMAM[50] dendrimers, bearing a hydrophilic core, which showed a better bactericidal response when the periphery was quaternized with long hydrophobic chains.

Regarding the type of cationic ammonium group, either $-NH_3^+$ or $-NMe_3^+$, some differences for dendrimers derived from a Si atom core have been found, achieving best activity values for the first generation compound $[G_1Si(S\text{-}NH_3)_8]^{8+}$ (**11S**), whilst for dendrimers derived from the polyphenoxo core

significant differences were observed between both types of ammonium groups. As for dendrimer generation, results showed that the antibacterial activity of carbosilane dendrimers diminishes with increasing generations, which means that the ammonium groups become less active in larger dendrimers.[38, 39] This might be explained in terms of periphery saturation in higher generation dendrimers with more cationic functions hampering permeation through cell membranes, since the carbosilane framework is less accesible.[22] These findings can also be observed for the new family of derivatives $[G_nSi(Si\text{-}NR_3)_m]^{m+}$ (**7Si-9Si**) obtained by hydrosilylation, although for those derivatives produced by thiol-ene addition $[G_nSi(S\text{-}NR_3)_m]^{m+}$ (**7S-12S**) the best activity was achieved by the first generation and not by generation zero. Nevertheless, in both cases the highest activity was found for low generation derivatives.

With respect to the type of bacteria, each of the compounds behaves better against *S. aureus* than against *E. coli*, or at least equally against both. This is probably due to a more efficient penetration of dendrimer branches through the thick peptidoglycan layer of *S. aureus* than through the outer membrane of *E. coli*, composed by a phospholipid-rich inner leaflet and a thin lipopolysaccharide outer leaflet.[51] Similar results were observed when measuring the concentration of these compounds in ppm or when using concentration values per ammonium group present in the dendritic structure (Tables S1 and S2, supporting information). This latter analysis determines the effectiveness of each ammonium function located on dendrimers, since a lower concentration value means a higher activity of this cationic function, also reflecting the importance of multivalency in the overall activity of the dendrimers. The data found for these cationic carbosilane dendrimers, and in particular for lower generation systems, are in general similar to or slightly better than others reported in the literature. For example, a first generation dendrimer based on poly(propylene oxide) amine (with three $-NH_2$ groups) inhibited *S. aureus* and *E. coli* at a MIC of 1.56 ppm and 3.13 ppm, respectively;[25] whereas PAMAM-NH_2 dendrimers showed best results for the second generation bearing eight $-NH_2$ functions (MIC of 6.25 ppm and MBC of 25.0 ppm for *S. aureus* and *E. coli*).[32] On the other hand, the activity of cationic PPI dendrimers was higher for fourth and fifth generation derivatives than for their lower generation counterparts.[22]

After studying the antibacterial activity of dendrimers a similar analysis with dendrons were carried out to determine the influence of the dendritic topology on the activity of these derivatives. As for the antibacterial behavior of dendrons (Table 3), we have first evaluated the influence of generation within the family of dendrons $[XG_n(S\text{-}NMe_3)_m]^{m+}$ (X = HOC_6H_4O; n = 1, m = 2 (**25S**); n = 2, m = 4 (**26S**); n = 3, m = 8 (**27S**)). In this case, best results were obtained for the second generation dendron **26S**, whose values were comparable to those obtained for the best spherical cationic carbosilane dendrimer. Subsequently, we compared the effect of the different focal points in second generation dendrons $[XG_2(S\text{-}NMe_3)_4]^{4+}$ (X = N_3 (**23S**), HOC_6H_4O (**26S**), $HO(CH_2)_2O$ (**29S**), NH_2 (**32S**)). These moieties lead to minor modifications in dendron features, rendering slightly better results for HO- focal points in **26S** and **29S**. Thus, the antibacterial activity can be ascribed to the terminal ammonium groups. Regarding the type of bacteria, dendrons present also better or equal activity against *S. aureus* in comparison to *E. coli*. When analyzing data according to the concentration of ammonium groups instead of ppm, conclusions drawn for dendrons are similar to those previously commented for dendrimers (Table S3, Supporting Information, molar concentration per ammonium group).

	S. aureus		*E. coli*	
	MIC	MBC	MIC	MBC
$[N_3G_2(S\text{-}NMe_3)_4]^{4+}$ (**23S**)	8	8	8	8
$[(HOC_6H_4O)G_1(S\text{-}NMe_3)_2]^{2+}$ (**25S**)	32	64	32	64
$[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**)	2	4	4	4
$[(HOC_6H_4O)G_3(S\text{-}NMe_3)_8]^{8+}$ (**27S**)	16	16	64	64
$[(HOC_2H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**29S**)	4	4	4	8
$[(NH_2)G_2(S\text{-}NMe_3)_4]^{4+}$ (**32S**)	8	8	8	8

Table 3. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of dendrons (all concentrations data are shown in ppm (mg L^{-1})).

Topology has a marked influence on the antibacterial activity as can be inferred from the comparison of second and third generation dendrons $[HOC_6H_4OG_n(S\text{-}NMe_3)_m]^{m+}$ (**26S-27S**) with dendrimers of the type $[G_nSi(S\text{-}NMe_3)_m]^{m+}$ (**7S-8S**). The pairs of compounds **26S**/**7S** and **27S**/**8S** are covered with the same number of cationic charges and present a sulfur atom close to the ammonium groups, but the better bactericidal features of the second generation dendrons might well be due to their higher flexibility and exposure of the lipophilic skeleton. This reasoning would be consistent with the claim of an adequate balance between hydrophilicity and lipophilicity.

Hence, we believe that the antibacterial activity of these systems is related with the two structural aspects considered above: the cationic charge and the hydrophobic framework. It is very likely that the former causes the exchange with the membrane cations and the latter facilitates the introduction of the dendrimer through the membrane, in accordance with the previous studies commented in the introduction.[9, 11, 26] Some experimental findings that support this idea were provided by the evaluation of cationic carbosilane dendrimers in trophozoites, which showed the formation of holes in the membrane and the release of intracellular material.[52] Furthermore, the capability of this type of dendrimers to internalize into cells has recently been observed by us in eukaryotic cells, despite the more complicated membrane.[53-55]

2.3. Toxicity.

The usefulness of any system as a potential drug should be compatible with living organisms. Therefore, hemolytic studies were carried out to assess specificity of the dendrimers and dendrons discussed in this work towards bacterial or mammalian cells. The toxicity limit used corresponds to the concentration for 20 % hemolysis (H_{20}, Tables S4 and S5, Supporting Information). Figure 2 represents H_{20} *vs* MBC (ppm) only for *E. coli*, since the MBC values for this bacterial strain are equal or higher than those for *S. aureus* (Figures S2-S4, Supporting information). In this figure, the solid line represents equal values

of H_{20} and MBC, compounds with MBC higher than H_{20} is being depicted below this line, whereas compounds with MBC lower than H_{20} above the line.

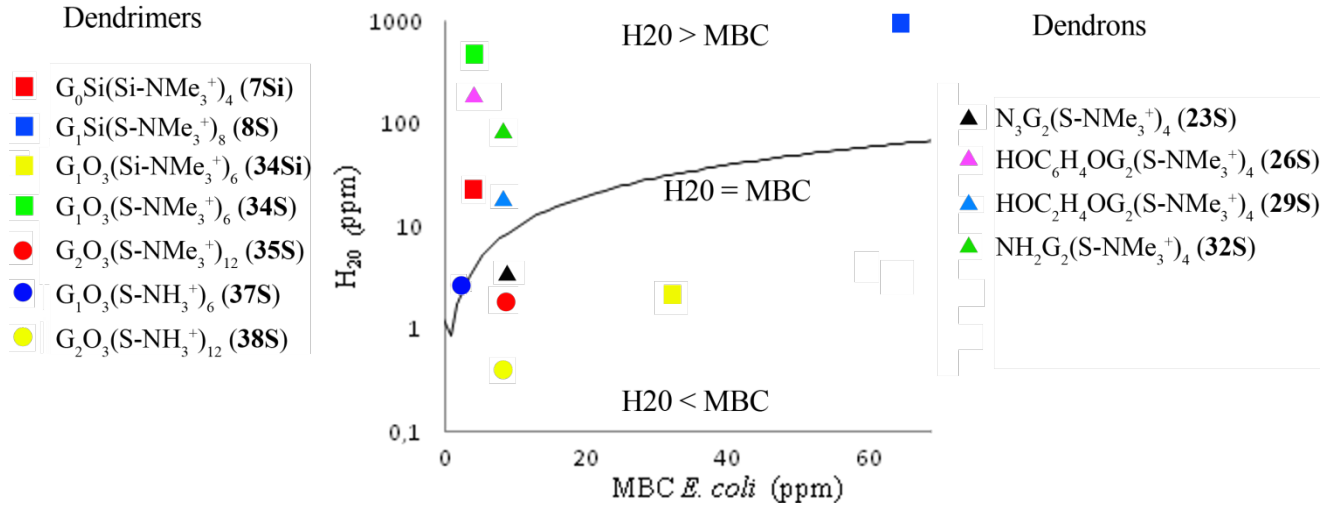


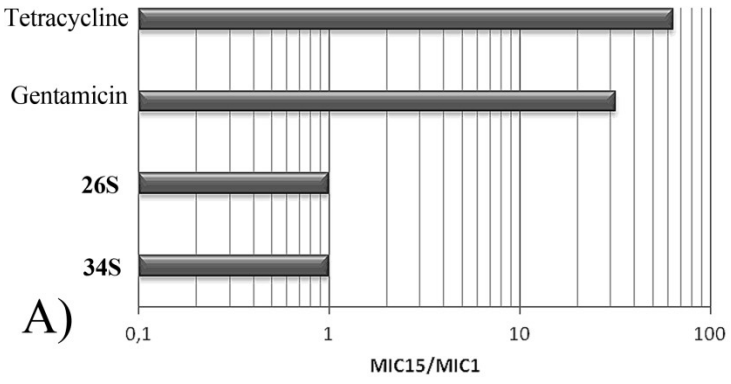
Figure 2. H_{20} *vs* MBC for selected compounds in *E. coli*. H_{20} refers to the concentration corresponding to 20 % hemolysis. Data given in ppm. The line represents equal values of toxicity (H_{20}) and activity (MBC).

The best biocompatibility of these dendritic compounds was observed for those derivatives with a peripheral sulfur atom. Furthermore, a clear dependence of molecular weight and hemolytic concentration was detected, finding the best H_{20}/MBC relation (above the continuous line) for the first generation dendrimer $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**) and the second generation dendron $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**). For dendrimers, the $-NMe_3^+$ ammonium groups significantly reduce the toxicity with respect to $-NH_3^+$ groups.[39] Regarding dendrimer generation, only lower generations of each core were active enough at concentrations above H_{20} values. For dendrons, this limit was exceeded by second generation compounds with –OH and $–NH_2$ focal points, but not with a $-N_3$ moiety. The toxicity of cationic dendrimers in human cell lines, assessed by MTT in HeLa cells and reported by us previously, indicates that the best scoring bactericidal derivatives, low generation dendrimers and dendrons, are non-toxic at concentrations higher than the MBC (Table S6, Supporting Information).[46, 56] Some of these cationic macromolecules behave very well in contrast to quaternary ammonium polymers showing higher toxicity than antibacterial activity as reported previously.[57]

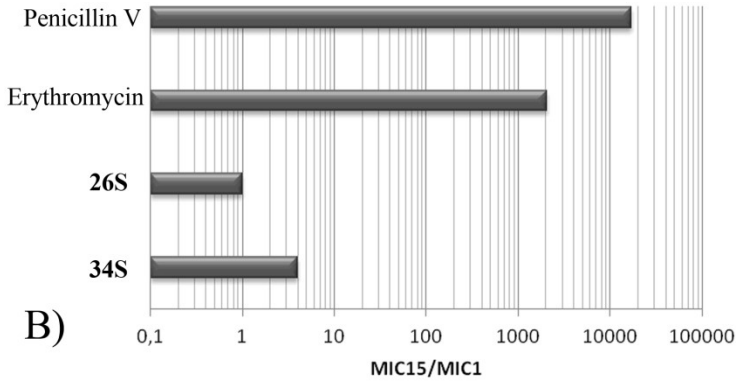
As is described in next sections, we have focused our research in dendron $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**) and dendrimer $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**). For that reason, extra data on toxicity have been collected, observing an important variability depending on the cell line. For example, 80 % cellular viability in peripheral blood mononuclear cells (PBMC) was observed at concentrations of *ca*. 400 ppm for both systems **34S** and **26S**.[54] On the other hand, in more sensible cell lines as prostate epithelial RWPE, IC50 values of 4 ppm and 2 ppm were observed for **34S** and **26S**, respectively.

2.4. Resistance studies.

In the view of the huge problems caused to health and economy arising from antibiotic resistant bacteria,[58] we addressed the ability of dendritic molecules to induce resistance in bacteria. Taking into account the bactericidal and hemolytic behavior of this library of dendrimers and dendrons, we chose dendron $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**) and dendrimer $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**), as models for resistance assays, and compared them with the commercial antibiotics tetracycline or gentamycin, active against *E. coli*, and PenVK or erythromycin, active against *S. aureus* (Figure 3). This assay consists in the daily assessment of the MIC over 15 cycles using a subculture coming from the plate treated the cycle before. The evolution of the inhibitory capacity of the compounds was then determined by comparing the MIC of the last experiment (MIC_{15}) with that obtained in the first one (MIC_1). These data clearly showed that dendron **26S** did not generate resistance in either type of bacteria ($MIC_{15}/MIC_1 = 1$), whilst the MIC of dendrimer **34S** only increased slightly for *S. aureus* and, as expected, commercial antibiotics led to much higher MIC values after the fifteen cycles of treatment.



E. coli	MIC_{15}/MIC_1
Tetracycline	64
Gentamycin	32
26S	1
34S	1



S. aureus	MIC_{15}/MIC_1
PenV	17067
Erythromycin	2048
26S	1
34S	4

Figure 3. Induction of resistance to $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**) and $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**) in *E. coli* (A) and *S. aureus* (B) after 15 survival cycles in the presence of antimicrobials. MIC_{15}/MIC_1 is the ratio obtained from the fifteenth subculture (MIC_{15}) and the first culture (MIC_1).

Additionally, bacterial strains treated with dendrimer **34S** during the respective resistant induction assays were treated afterwards with dendron **26S**. The MIC and MBC obtained were the same as those previously observed for non-treated strains. A similar result was produced when the bacteria coming from the resistance assays with dendron **26S** were treated with dendrimer **34S**. These data indicate that dendrimer **44S** and dendron **26S** keep an almost invariable activity against *S. aureus* and *E. coli*.

Finally, the activity of dendron $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**) and dendrimer $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**) was tested against antibiotic resistant bacteria. For these experiments a penicillin-resistant *S. aureus* strain and the induced resistant *E. coli* strain generated in the previous resistance assays were used. Whilst both dendritic compounds maintained their activity towards these strains (Table 4.A and 4.B), the corresponding antibiotics showed much higher MIC and MBC values in these microorganisms than in non-resistant bacteria, as expected (data not showed). A very similar behavior was obtained when a methicillin-resistant *S. aureus* (MRSA) strain was employed (Table 4.A). The respective MIC and MBC values were 1 and 2 ppm for dendron **26S** and 2 and 4 ppm for dendrimer **34S**. These results would be in accordance with their non specific mode of action.

Table 4.A	**34S**	**26S**

	MIC	MBC	MIC	MBC
S. aureus resistant to **PenV**	2	4	2	2
MRSA	2	2	2	4

Table 6.B	**34S**		**26S**	
	MIC	MBC	MIC	MBC
E. coli resistant to **Tetracycline**	2	4	4	4
E. coli resistant to **Gentamycin**	2	2	2	2

Table 4. MIC and MBC of compounds $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**) and $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**) for resistant *S. aureus* and *E. coli* produced in resistance assays and also for methicillin-resistant *S. aureus* (MRSA). Data are given in ppm.

2.6. Biofilm assays.

Two different assays have been carried out to measure the activity of the cations $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**) and $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**) against biofilms: pre-treatment and post-treatment. In these experiments, the presence of biofilm is identified by comparison of turbidity (absorbance 630 nm) of wells containing planktonic cells and wells containing planktonic cells plus biofilm. Moreover, posterior quantification of biofilm was done with methyl violet.

The pre-treatment experiments showed that formation of biofilms was avoided in the presence of compounds **26S** or **34S** at concentrations over MIC values (Figure 4). Below these concentrations, the growing of the biofilm was not inhibited. However, dendrimer **34S** was able to reduce its formation, as can be seen when compared with control, whereas dendron **26S** barely modifies this process. This result is better observed after treatment of biofilms with methyl violet (Figure S4).

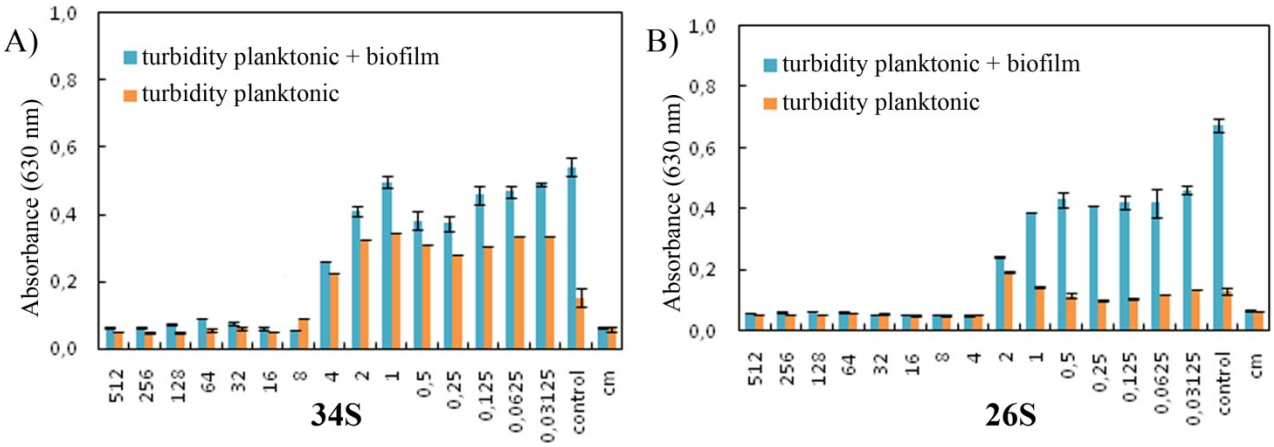


Figure 4. Pre-treatment effect of dendrimer **34S** (A) and dendron **26S** (B) in *S. aureus* CECT 240 biofilm growing. Control: wells without dendritic molecules; cm: wells only with culture medium.

With respect to post-treatment assays, both dendritic cations were unable to reduce significantly the biofilm (Figure S5 and S6) at concentrations close to the MIC, neither were able to release bacteria from it. However, at higher concentrations it is observed some amount of bacteria release, higher for dendrimer than for dendron. The viability of these bacteria was also analyzed (Figure S7) and it was observed higher mortality in the case of dendrimer **34S**, probably because dendrimer **34S** is more active against biofilm than dendron **26S** and slightly more active against the planktonic cells of *S. aureus* CECT 240.

3. Conclusions

The study of the toxicity and the antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* of a library of cationic carbosilane dendrimers and dendrons discussed in this paper show the influence of structural parameters that should be taken into account in the design of antibacterial polycationic compounds, highlighting the relevance of an adequate equilibrium between lipophilicity and hydrophilicity. In this sense, the control of the hydrophilic/hydrophobic balance would allow to design dendritic systems of low generation with very high antibacterial activities (first generation for dendrimers and second generation for dendrons). These are substantial findings for several reasons. First of all, because dendritic systems of low generation are much less toxic than dendrimers of higher generations thus implying a higher therapeutic index for the low generation carbosilane dendritic systems

described in this work. In addition, the preparation of low generation systems is much easier and cheaper, hence opening the door to putative applications of these systems with commercial purposes.

We have considered different parameters that may affect the antibacterial activity of dendritic systems, such as the dendritic generation, the core of dendrimers or focal point of dendrons, the nature of the branches, the type of cationic terminal groups and the topology of the dendritic system. Results have shown that all these parameters can affect individually the antibacterial activity of dendritic systems. But in order to understand the results reported above, the global structure of the dendritic systems must be taken into account, since this global structure is precisely what establishes the adequate hydrophilic/hydrophobic balance that determines the activity of these compounds.

In the particular case of dendrons, we have shown the activity of several compounds presenting different functional groups at their focal point, which allow the binding of other molecules of biological interest providing an added value to these systems in comparison with spherical dendrimers. The effect of the focal point in dendrons on their antibacterial activity is negligible.

The biocompatibility of the systems studied improves with lesser cationic charges and with the presence of sulfur atoms close to the surface. In the case of dendrons, the focal point affects the hemolytic activity, clearly achieving better results when hydroxyl groups are present at this position. Furthermore, the best two compounds, regarding antibacterial and toxicity data, dendrimer $[G_1O_3(S\text{-}NMe_3)_6]^{6+}$ (**34S**) and dendron $[(HOC_6H_4O)G_2(S\text{-}NMe_3)_4]^{4+}$ (**26S**), were tested for resistance development. Dendron **26S** did not generate any resistance and dendrimer **34S** required only a slightly higher amount of compound. Moreover, these cationic compounds kept their activity against *S. aureus* and *E. coli* resistant bacteria, including methicillin-resistant *S. aureus* (MRSA). Furthermore, these two compounds avoided formation of biofilm at concentrations over MIC, although at these concentrations were unable to reduce amount of biofilm once formed.

The fact that best bactericidal activities and least toxicity values were achieved with low generation systems makes them very attractive for future studies for the simplicity of their synthesis. In this sense, the

variety of dendron focal points is of great interest allowing the introduction of modifications capable of improving their behavior or broadening their potential scope of applications. They may well be used as building blocks for new macromolecules, where the focal point can be modified by introducing a second microbicide function, or in the preparation of materials with antimicrobial properties. Research in this sense is currently in progress.

3. Experimental Section

3.1. General Considerations. All reactions were carried out under inert atmosphere and solvents were purified from appropriate drying agents when necessary. NMR spectra were recorded on a Varian Unity VXR-300 (300.13 (^{1}H), 75.47 (^{13}C) MHz) or on a Bruker AV400 (400.13 (1H), 100.60 (^{13}C), 40.56 (^{15}N), 79.49 (^{29}Si) MHz). Chemical shifts (δ) are given in ppm. ^{1}H and ^{13}C resonances were measured relative to internal deuterated solvent peaks considering TMS = 0 ppm, whereas ^{15}N and ^{29}Si resonances were measured relative to external MeNO and TMS, respectively. When necessary, assignment of resonances was done from HSQC, HMBC, COSY, TOCSY and NOESY NMR experiments. Elemental analyses were performed on a LECO CHNS-932. Mass spectra were obtained from a Bruker Ultraflex III and an Agilent 6210. Thiol-ene reactions were carried out employing a HPK 125 W mercury lamp from Heraeus Noblelight with maximum energy at 365 nm, in normal glassware under an inert atmosphere. Compounds, $HS(CH_2)_2NH_2{\cdot}HCl$ (Acros), $HS(CH_2)_2NMe_2{\cdot}HCl$ (Acros), 2,2'-dimethoxy-2-phenylacetophenone (DMPA) (Aldrich), MeI (Aldrich), $HSiMeCl_2$ (Aldrich), K_2CO_3 (Panreac) were obtained from commercial sources. Compounds G_nSiV_m,[43] $[G_nSi(Si\text{-}NMe_3)_m]^{m+}$ (**7Si-9Si**),[38] $[G_nO_3(Si\text{-}NMe_3)_m]^{m+}$ (**34Si-36Si**),[42] $[G_nO_3(S\text{-}NMe_3)_m]^{m+}$ (**34S-36S**) and $[G_nO_3(S\text{-}NH_3)_m]^{m+}$ (**37S-39S**),[39] BrG_nV_m,[59] $XGn(NMe_2{\cdot}HCl)_m$ (X = N_3, HOC_6H_4O, Pht),[59] $[NH_2G_n(S\text{-}NMe_3)_m]^{m+1}$ (**31S-33S**),[45] and $[G_0Si(S\text{-}NH_3)_4]^{2+}$ (**10S**)[44] were synthesized as published.

3.2. Synthesis of selected compounds.

The synthesis of all compounds is described in Supporting Information and just a selection mentioned herein.

$G_0Si(S\text{-}NMe_2\cdot HCl)_4$ (1S). This dendrimer was prepared from G_0SiV_4 (0.250 g, 1.84 mmol), 2-(dimethylamino)ethanethiol hydrochloride (1.042 g, 7.36 mmol), 2,2-dimethoxy-2-phenylacetophenone, DMPA (0.188 g, 0.74 mmol), and a 1:2 THF/methanol solution (3 mL). The reaction mixture was deoxygenated using an argon flow and then irradiated for 1.5 h with UV light at 365 nm. Next, DMPA was again added (5 % mol per vinyl group) and the reaction mixture irradiated for another 1.5 h. ^{1}H-NMR monitoring allowed checking the disappearance of vinyl groups. Afterwards, the initial reaction mixture was concentrated by rotator evaporation and solved in MeOH. Subsequently, the product was precipitated with Et_2O under continuous stirring, eliminating the DMPA remains. After filtering the solution, the precipitate was again solved in water and nanofiltration with membranes of MW = 500 performed in order to eliminate the excess of disulfide. The pure product was vacuum dried to afford **1S** as a white solid (0.400 g, 31 %). The low yield is consequence of the last purification step.

^{1}H-NMR (DMSO-d_6): δ 0.98 (t, J = 8.5 Hz, 8 H, SiC*H*$_2$CH$_2$S), 2.61 (t, J = 8.5 Hz, 8 H, SiCH$_2$C*H*$_2$S), 2.70 (s, 24 H, -N*Me*$_2$HCl), 2.86 (t, J = 7.8 Hz, 8 H, SC*H*$_2$CH$_2$N), 3.16 (t, J = 7.9 Hz, 8 H, SCH$_2$C*H*$_2$N), 10.68 (sa, 4 H, -NMe$_2$*H*$^+$). ^{13}C{^{1}H}-NMR (DMSO-d_6): δ 11.9 (Si*C*H$_2$CH$_2$S), 24.3 (S*C*H$_2$CH$_2$N), 25.6 (SiCH$_2$*C*H$_2$S) 41.6 (-N*Me*$_2$), 55.4 (SCH$_2$*C*H$_2$N). ^{15}N-NMR (DMSO-d_6): -338.2 (-*N*Me$_2$H$^+$). ^{29}Si-NMR (DMSO-d_6): 2.6 (G_0–SiMe). MS: $[M\text{-}3HCl\text{-}Cl^-]^+$ = 557.32 uma (calcd. = 557.32 uma); $[M\text{-}1HCl\text{-}2Cl^-]^{2+}$ = 297.10 uma (calcd. = 297.16 uma). Anal. Calcd. $C_{24}H_{60}Cl_4N_4S_4Si$ (702.92 g/mol): C, 41.01; H, 8.60; N, 7.97; S, 18.25; Exp.: C, 40.48; H, 8.38; N, 7.38; S, 18.88.

$G_0Si(S\text{-}NMe_2)_4$ (4S). To a $H_2O/CHCl_3$ (1:1, 20 mL) solution of **1S** (0.124 g, 0.18 mmol), a NaOH aqueous solution was added drop by drop (0.028 g, 0.70 mmol). The reaction mixture was stirred for 15 minutes at room temperature and finally the aqueous phase removed. The organic phase was dried using Na_2SO_4, filtered and evaporated to obtain **4S** as a pale yellow oil (0.078 g, 80 %).

^{1}H-NMR ($CDCl_3$): δ 0.80 (t, J = 8.6 Hz, 8 H, SiC*H*$_2$CH$_2$S), 2.21 (s, 24 H, -N*Me*$_2$), 2.40 (m, 8 H, SCH$_2$C*H*$_2$N), 2.43 (m, 8 H, SiCH$_2$C*H*$_2$S, overlapped), 2.45 (m, 8 H, SC*H*$_2$CH$_2$N, overlapped). ^{13}C{^{1}H}-NMR ($CDCl_3$): δ 13.0 (Si*C*H$_2$CH$_2$S), 27.3 (SiCH$_2$*C*H$_2$S), 29.9 (S*C*H$_2$CH$_2$N), 45.3 (-N*Me*$_2$), 59.1 (SCH$_2$*C*H$_2$N). ^{15}N-NMR ($CDCl_3$): δ -352.1 (-*N*Me$_2$). ^{29}Si-NMR ($CDCl_3$): δ 2.2 (G$_0$–*Si*Me). MS: $[M+H]^+$ = 557.32 uma (calcd. = 557.40 uma). Anal. Calcd. $C_{24}H_{56}N_4S_4Si$ (557.07 g/mol): C, 51.74; H, 10.13; N, 10.06; S, 23.02; Exp.: C, 52.65; H, 9.46; N, 9.46; S, 22.07.

G$_0$Si(S-NMe$_3$I)$_4$ (7S). To a diethyl ether (20 mL) solution of **4S** (0.078 g, 0.14 mmol) a MeI solution was added (0.05 mL, 0.80 mmol). The resulting solution was stirred for 16 h at room temperature and then evaporated under reduced pressure and washed twice with hexane (20 mL). After drying, **7S** was yielded as a white solid (0.115 g, 70 %).

^{1}H-NMR (DMSO-d$_6$): δ 1.00 (t, J = 8.3 Hz, 8 H, SiC*H*$_2$CH$_2$S), 2.68 (t, J= 8.4 Hz, 8 H, SiCH$_2$C*H*$_2$S), 2.93 (t, J = 8.1 Hz, 8 H, SC*H*$_2$CH$_2$N$^+$), 3.25 (s, 36 H, -N*Me*$_3^+$), 3.57 (t, J = 8.2 Hz, 8 H, SCH$_2$C*H*$_2$N$^+$). ^{13}C{^{1}H}-NMR (DMSO-d$_6$): δ 12.3 (Si*C*H$_2$CH$_2$S), 23.2 (S*C*H$_2$CH$_2$N$^+$), 26.2 (SiCH$_2$*C*H$_2$S), 51.8 (-N*Me*$_3^+$), 64.0 (SCH$_2$*C*H$_2$N$^+$). ^{15}N-NMR (DMSO-d$_6$): δ -330.0 (-*N*Me$_3$I). ^{29}Si-NMR (DMSO-d$_6$): δ 2.7 (G$_0$–*Si*Me$_2$). MS: $[M-3I]^{3+}$ = 248.00 uma (calcd. = 247.77 uma). Anal. Calcd. $C_{28}H_{68}I_4N_4S_4Si$ (1124.83 g/mol): C, 29.90; H, 6.09; N, 4.98; S, 11.40; Exp.: C, 29.80; H, 6.31; N, 4.29; S, 10.40.

3.3. Antibacterial methodology.

Bacterial strains. *Escherichia coli* (CECT 515, Gram-negative), *Staphylococcus aureus* (CECT 240, Gram-positive) and S*taphylococcus aureus* with resistances to penicillin (CECT 4004, Gram-positive) were obtained from the Spanish Type Culture Collection (CECT). The *S. aureus* MRSA was obtained from the Hospital Principe de Asturias (Alcalá de Henares, Madrid, Spain) and was resitant to: (penicillin, amoxicillin, ampicillin, clavulanic acid, oxacillin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, azitromycin). The rest of resistant strains were generated in the resistance induction assays carried out in this work.

MIC and MBC. The minimal inhibitory concentration (MIC) of the products was measured in 96-well tray microplates by microdilution tray preparations following the international standard methods ISO 20776-1.[60] Assays were run in duplicate microplates and three different wells for each concentration analyzed in the microplate. Solutions of the products were prepared in the range of 0.25 to 1024 ppm adding in each well 100 µL of one of these solutions, 100 µL of double concentration Mueller Hinton (Scharlau, ref. 02-136) and 5 µL of a bacteria suspension of 2 x 10^7 CFU/mL. Microplates were incubated at 37 ºC for 19 h using an ultra microplate reader ELX808iu (Bio-Tek Instruments), considering the MIC the minimal concentration for which no turbidity was observed. The minimal bactericidal concentration (MBC) was calculated by inoculating Petri dishes containing Mueller-Hinton agar with 3 µl of the samples used for MIC assessment. Samples were tested as droplets on the plates. Microbial growth on plates was monitored after 24 h of incubation at 37 ºC. The MBC was determined as the minimal concentration at which no growth was detected.

Induction of resistance. Initial MIC values (MIC_1) were determined for *Escherichia coli* CECT 515 and *Staphylococcus aureus* CECT 240 as described above. MIC values were monitored daily over the fifteen days as follows: for each compound tested, a bacterial suspension was obtained from the well corresponding to one half of the MIC_n determined in the previous MIC_n assay, and the concentration of this suspension adjusted to 2 x 10^7 CFU/mL in order to measure the MIC_{n+1}. The relative MIC value (MIC_{15}/MIC_1) was determined by calculating the ratio of the MIC obtained for the 15^{th} subculture (MIC_{15}) to the MIC obtained for the 1^{st} culture (MIC_1).

Hemolytic studies. 1.5 mL of a suspension of sheep erythrocytes (RBC, Oxoid sheep erythrocytes in Alsever solution) were diluted in 5 mL of phosphate buffer saline (PBS, pH 7.4; NaCl 137 mM, KCl 2.7 mM, Na_2HPO_4 10 mM and KH_2PO_4 1.8 mM), washed three times by centrifugation (15 min at 3000 rpm) and resuspended in 20 mL of PBS.

Dendrimer were dissolved in PBS or 0.9 % NaCl saline solution, for compounds with $-NH_3^+$ groups in their periphery, at a concentration of 1024 ppm. After preparing stock solutions, PBS solution was added to obtain the desired concentration.

0.5 mL of freshly prepared dendrimer solutions at different concentrations were added to 0.5 mL of RBC suspension in Eppendorf tubes and the resulting mixtures kept at 37 ºC for 30 min under rotatory agitation. Subsequently, tubes were centrifuged (5 min at 1500 rpm) and the supernatant of each tube was transferred to a new tube. Hemolysis was monitored by measuring the absorbance of the released hemoglobin at 412 nm. 100 % hemolysis was achieved by adding 0.5 mL of distilled water to 0.5 mL of RBC solution. The absorbance of PBS containing no dendrimer was used as 0 % hemolysis control value.

The percentage of hemolysis was calculated as $(H_x\text{-}H_0/H_{100}\text{-}H_0)x100$. The dendrimer concentration required to cause 20 % hemolysis rendered the HC_{20}, which was calculated by interpolation between the closest points or by extrapolation when it was above 1024 ppm. All experiments were run in triplicate.

Biofilm formation and quantification assay. The biofilm formation of *S. aureus* CECT 240 was stablished in microtiter plates (96 wells, NUNC). From Petri dish where *S. aureus* was growing, 10 CFU (Colony forming unit) was used to inoculate 4 ml of commercial Trypticase soy broth (TSB) supplemented with 0.4 % glucose and 0.3 % yeast extract for 3 h at 37 ºC in a bath with shaking (110 rpm) for obtaining a pre-inoculum with a value of absorbance at 625 nm of 0.6-0.9 units. Culture were then diluted 1:100 in fresh TSB medium supplemented, and 200 µL was dispensed into each well of the NUNC microtiter plates. After 20 h of incubation at 37 ºC in static the absorbance at 630 nm was measured in an ultra-microplate reader (Biotek, ELx 808). This value was due to planktonic and sessile cells in the well. Next the solution of the well was added to another well in a new microtiter plate and again the absorbance at 630 nm was measured. This value was due to planktonic cells only. The difference between the first and the second measured give information about the amount of sessile cells. Moreover, in the microtiter plates where the solution of the wells were retired, the biofilm formed was stained with 200 µL of 0.1 % crystal violet, rinsed three times with 200 µL of commercial phosphate buffered saline (PBS) and air dried. The crystal

violet-stained biofilm formation was quantified by solubilizing the crystal violet with 200 µL of a solution of 33 % acetic acid. This solution was added to wells in a new microtiter plate and the absorbance at 630 nm was measured in the microplate reader.

Biofilm treatment with dendritic molecules. Two different treatments were made with dendrimer **34S** and dendron **26S**: pre-biofilm and post-biofilm.

Pre-biofilm. It was analyzed the ability of both compounds to inhibit the biofilm formation of *S. aureus*. Solutions of the products were prepared in the range of 0.0625 to 1024 ppm adding in each well 100 µL of one of these solutions, 100 µL of *S. aureus* inoculum done in double concentration commercial TSB. Microplates were incubated at 37 ºC for 20 h in static. The planktonic and sessile cells, and the amount of biofilm formed were measured as was explained above.

Post-biofilm. It was analyzed the ability of both compounds to eliminate a biofilm already formed of *S. aureus*. Once a biofilm has been formed following the method explained above, the liquid of the wells was discarded and 200 µL of the products prepared in the range of 0.0625 to 1024 ppm was adding in each well. Microplates were incubated at 37 ºC for 20 h in static. The planktonic and sessile cells, and the amount of biofilm in the well were measured as was explained above.

Count of viable cells of *S. aureus*. In the post-biofilm assay, after the treatment with de dendrimer **34S** or dendron **26S**, 100 µL of the solution in the wells with the products (range of 0.0625 to 1024 ppm) was collected. Serial dilutions were made and 100 µL of each solution was plated in Petri dish with commercial Plate count agar (PCA). The Petri dish were incubated at 37 ºC 24 h and the CFU per well was calculated by counting the colonies on each Petri dish.

5. Supporting Information

Complete experimental procedures, antibacterial and hemolytic data in ammonium molar concentration and selected NMR spectra.

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Structure-Activity Relationship Study of Cationic Carbosilane Dendritic Systems as Antibacterial Agents

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The bactericidal activity of a library of cationic dendritic systems depends on the hydrophilic/hydrophobic structural balance, being the presence of a sulfur atom proximal to the peripheral ammonium functions of relevance.