Amine and ammonium functionalization of chloromethylsilane-ended dendrimers. Antimicrobial activity studies

Paula Ortega,[a] Jose Luis Copa-Patiño,[c] Mª Angeles Muñoz-Fernandez,[b] Juan Soliveri,[c] Rafael Gomez*[a] and F. Javier de la Mata*[a]

Received 5th June 2008, Accepted 27th June 2008
First published as an Advance Article on the web 29th July 2008
DOI: 10.1039/b809569h

Novel amine- and ammonium-terminated carbosilane dendrimers of type G_n-[Si{CH_2O-(C_6H_4)-3-NMe_2}]$_x$ or G_n-[Si{CH_2O-(C_6H_4)-3-$NMe_3^+I^-$}]$_x$ have been synthesized and characterized up to second generation by phenolysis of (chloromethyl)silyl-terminated dendrimers with 3-dimethylamine phenol and subsequent quaternization with methyl iodide. Quaternized carbosilane dendrimers are stable in protic solvents and can be solubilised in water after the addition of less than 1% of dimethyl sulfoxide. A study of the antimicrobial activity of these cationic dendrimers of first and second generation against both Gram-positive and Gram-negative bacteria is also described. The results obtained demonstrate that the new ammonium-terminated carbosilane dendrimers can be considered as multivalent biocides.

1. Introduction

Several groups have reported high-yield divergent synthesis of hydrophobic carbosilane dendrimers by using repeating sequences of alternating hydrosilylations with substituted chlorosilanes and alkenylations with Grignard reagents.[1] There are many reports about the employment of these dendrimers as frameworks upon which to attach other functional groups.[2] However, only a few attempts to functionalize these dendrimers with peripheral amphiphilic groups have been published.[3]

Seyferth *et al.*[4] reported the synthesis of stable amphiphilic carbosilane dendrimers through the reaction of (chloromethyl)silyl-terminated dendrimers with several mercapto-substituted amphiphilic compounds. The (chloromethyl)silyl dendrimers are necessary for this purpose since most silicon–heteroatom bonds (*e.g.*, Si–N, Si–OR, Si–S) are moisture sensitive,[5] which means that simple nucleophilic reactions with chlorosilyl-terminated dendrimers are not suitable.

More recently, Astruc *et al.* have reported the nucleophilic substitution by phenol derivatives of (chloromethyl)silyl-terminated groups, helped by the previous exchange of the chloromethyl termini for iodomethyls upon reaction with sodium iodide.[6]

Four years ago, van Leeuwen *et al.* reported the synthesis of (chloromethyl)silyl-terminated dendrimers by hydrosilylation of allylsilane-ended dendrimers with (chloromethyl)dimethyl silane.[7] Here, we show the functionalization of these dendrimers with peripheral amine groups and the subsequent transformation of these amine groups into ammonium units leading to polycationic carbosilane dendrimers with amphiphilic properties.

Cationic dendrimers with ammonium groups in the surface have been described as effective antimicrobial agents.[8] These dendrimers have been shown to be more potent than their small molecule counterparts. The use of these systems as biocides is due to the fact that polyvalent interactions have been frequently used by nature to either induce or combat infections[9] and it has been demonstrated that intermolecular as well as intramolecular interactions are much more enhanced in polymeric systems.[10] In this sense, dendrimers can offer a high local concentration of functional groups and a compact structure with a narrow polydispersity and a well-characterized molecular weight. If these units present biologically active antimicrobial groups, one might expect to see an increased potency associated with the high local concentration. Nevertheless, the dendrimer size is important since it has been shown that quaternary ammonium compounds exert their antimicrobial action by disrupting and disintegrating cell membranes.[11] Thus bulkier dendrimers may not be able to penetrate through the cell membrane barrier and may find difficulties in reaching the target site for the anticipated antimicrobial action.

In this paper, we report the antimicrobial activity of our ammonium terminated carbosilane dendrimers of first and second generation against both Gram-positive and Gram-negative bacteria. We think that the hydrophobic nature of the carbosilane skeleton present in our dendrimers may induce some differences (*e.g.* biopermeability) in the interaction of these systems with membrane cells compared with other kinds of dendrimers described in the literature and may well lead to differences in the antimicrobial action. We have compared the activity of these dendrimers with the activity of the corresponding monofunctional counterpart and have established the range of concentrations in which our dendrimers present bacteriostatic (refers to an agent that halts the growth of bacteria and not necessarily killing them) and bactericidal (refers to an antimicrobial agent or condition that kills a bacterial cell) effects. For the same

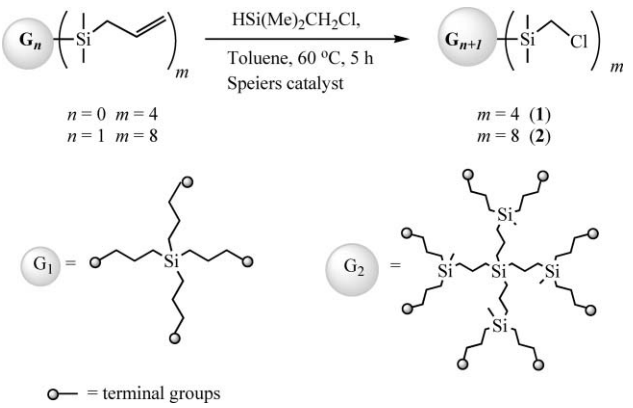
[a]*Departamento de Química Inorgánica, Universidad de Alcalá, Campus Universitario, E-28871 Alcalá de Henares (Spain). Networking Research Center on Bioengineering, biomaterials and nanomedicine (CIBER-BBN), Alcalá de Henares, Spain. E-mail: javier.delamata@uah.es; Fax: +34 91 885 4683; Tel: +34 91 885 4654*
[b]*Laboratorio de Inmunobiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid (Spain). Networking Research Center on Bioengineering, biomaterials and nanomedicine (CIBER-BBN), Spain. E-mail: mmunoz@cbm.uam.es*
[c]*Departamento de Microbiología y Parasitología, Universidad de Alcalá, Campus Universitario, E-28871, Alcalá de Henares, Spain. E-mail: josel.copa@uah.es*

agent, the bacteriostatic concentration may be lower or equal to the bactericidal concentration, but never higher. In other words, higher concentrations are necessary to kill the bacteria.

2. Results and discussion

2.1 Synthesis and structural characterization of carbosilane dendrimers

Preparative details and spectroscopic data for the new compounds are given in the Experimental Section. Only selected data will be presented for this discussion. Dendrimers containing (chloromethyl)dimethylsilane functionalities, G_n-[Si(Me$_2$)CH$_2$Cl]$_m$ (n = 1, m = 4 (**1**); n = 2, m = 8 (**2**), where "n" means the number of generation (G) and "m" denotes the number of branches and hence the number of peripheral groups) were conveniently obtained through the hydrosilylation reaction of allyl-functionalized dendrimers with (chloromethyl)dimethylsilane using Speier's catalyst. The reactions conditions are very similar to those described previously by van Leeuwen[7] for these complexes, but in our case, reaction times are shorter, probably due to the different platinum catalyst used in both cases (see Scheme 1).



Scheme 1

The reaction of the first and second generation chloromethylene-terminated dendrimers, **1–2**, with 3-dimethylaminophenol, 3-(NMe$_2$)-C$_6$H$_4$OH, in the presence of potassium carbonate and potassium iodide, using dimethylformamide as solvent renders high yields of first and second generation amine-terminated dendrimers, G_1-[Si{CH$_2$O-(C$_6$H$_4$)-3-NMe$_2$}]$_4$ (**3**) and G_2-[Si{CH$_2$O-(C$_6$H$_4$)-3-NMe$_2$}]$_8$ (**4**) as yellow oily products. The use of a polar and aprotic solvent clearly helps the nucleophilic substitution reaction due to the presence of naked anions in this kind of solvent, which in this case increases the reactivity of the phenol derivative (see Scheme 2).

Treatment of dendrimers **3** and **4** with methyliodide in acetonitrile during 48 hours causes the quaternization of the peripheral aromatic amines to give the cationic dendrimers G_1-[Si{CH$_2$O-(C$_6$H$_4$)-3-NMe$_3^+$I$^-$}]$_4$ (**5**) and G_2-[Si{CH$_2$O-(C$_6$H$_4$)-3-NMe$_3^+$I$^-$}]$_8$ (**6**) (Scheme 2). The use of acetonitrile is necessary since the quaternization reaction failed in the presence of other organic solvents such as diethyl ether or toluene.

Dendrimers **5** and **6** are obtained as white solids in very high yields and are soluble in acetonitrile, dimethylsulfoxide and methanol. They can be solubilised in water adding small quantities (less than 1%) of dimethylsulfoxide.

Structural characterization of dendrimers **1–6** has been carried out using elemental analysis, ^{1}H, ^{13}C and ^{29}Si- RMN spectroscopy and mass spectrometry. The ^{1}H-NMR spectra of dendrimers **3** and **4** in CDCl$_3$ show a complex group of signals at about 7.10–6.28 ppm corresponding to the aromatic hydrogen atoms, a singlet around 3.53 ppm for the methylene protons of the –SiCH$_2$O– fragment and a singlet at 2.90 ppm due to the methyl groups bonded to nitrogen. The carbosilane framework is insignificantly affected by the addition of the peripheral arylamines, thus the general features of their NMR spectra are almost identical to those described for their precursors.[7]

^{13}C{^{1}H}-NMR spectra show six resonances for the carbon atoms of the aromatic ring due to their unequivalence. The C$_{ipso}$ bonded to the –OCH$_2$Si group appears at 162.7 ppm, while the C$_{ipso}$ bonded to –NMe$_2$ gives a resonance at higher field, 151.8 ppm. The chemical shift of the carbon atom in –OCH$_2$ and the methyl groups of –NMe$_2$ appear at 60.0 and 40.0 ppm respectively. Fig. 1 shows the ^{1}H and ^{13}C{^{1}H}-NMR spectra of the second generation dendrimer **4**. The ^{29}Si chemical shifts of these dendrimers have been determined by a 2D {^{1}H–^{29}Si}-HMBC experiment (see Fig. 2), which shows connectivity of the silicon atoms with the protons

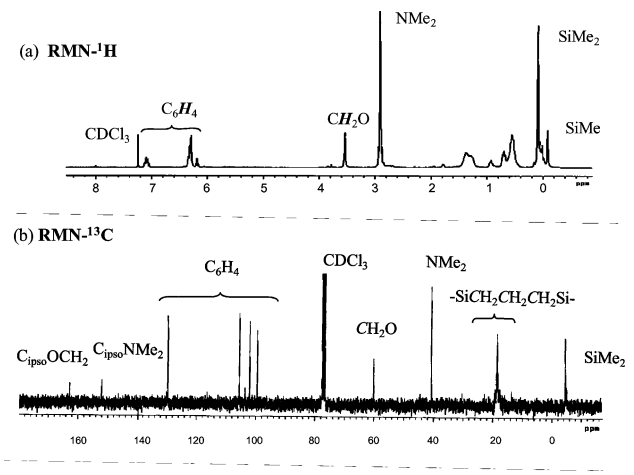
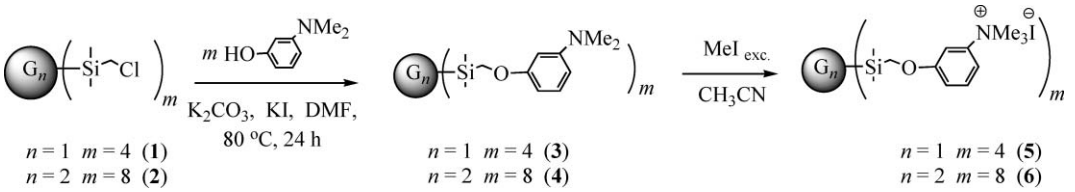
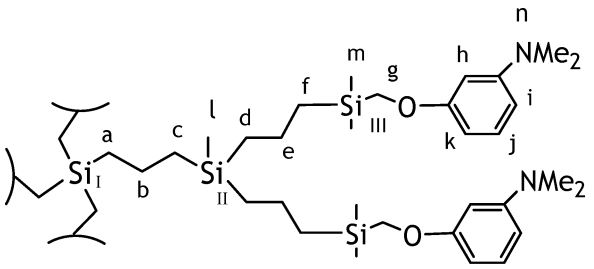


Fig. 1 ^{1}H and ^{13}C-NMR spectra of dendrimer **4**.



Scheme 2



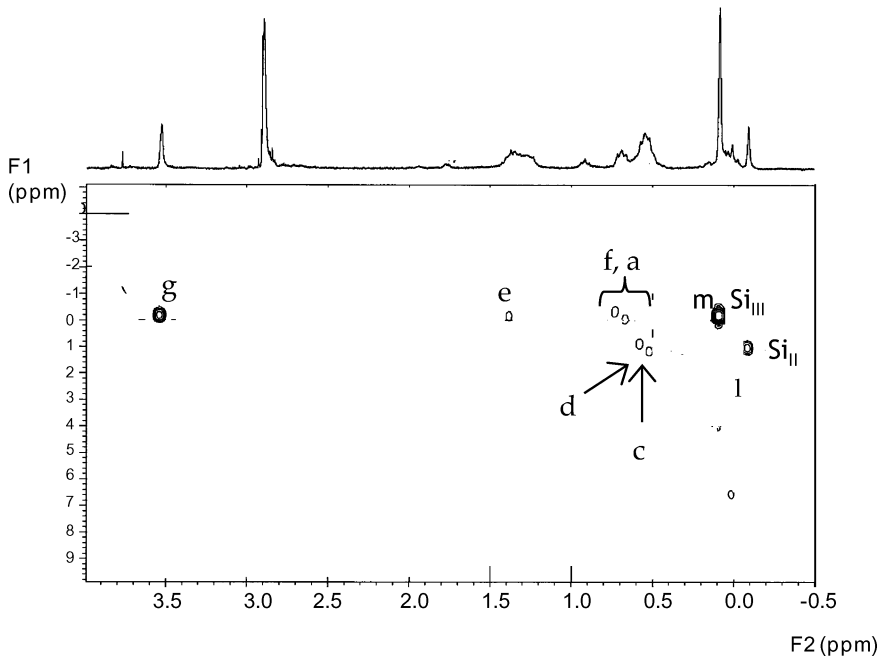


Fig. 2 2D{^{1}H–^{29}Si}-HMBC spectrum of dendrimer **4**.

of the alkyl chains. For example, the spectrum of dendrimer **4** shows for the signal of the $SiMe_2$ fragment (Si_{III}) (−0.22 ppm), two crossed peaks assigned to H_g and H_f and another peak due to H_m. The signal due to SiMe (Si_{II}) (1.05 ppm) gives two crossed peaks corresponding to H_c and H_d, together with a peak produced by H_l. The signal corresponding to Si_I is overlapped by the peak due to Si_{III}.

The ^{1}H, ^{13}C and ^{29}Si NMR spectra of the quaternized dendrimers, **5** and **6**, were recorded in [D_6]DMSO at room temperature. These spectra exhibit resonance patterns identical to those observed in their neutral counterparts, **3** and **4**, for the carbosilane framework, although broader signals are observed with the increasing generation. In general, in the ^{1}H-NMR spectra, the positive charge of the ammonium groups results in the deshielding of about 0.7 ppm, for the methyl group directly bonded to the charged nitrogen atoms whereas small downfield shifts are found for the rest of the protons of the peripheral unit. Analogous behaviour is observed in the ^{13}C NMR spectra for the carbon atoms.

In addition, the proposed structure for dendrimer **3** has been confirmed by mass spectrometry using APCI (*atmospheric pressure chemical ionization*) (see Fig. 3). The molecular peak $[M + H]^+$ appears at 1030 uma (theoretical, 1028.66 uma). Dendrimer **5** was analysed by mass spectroscopy using ESI (electro spray). The spectrum shows a peak to 1470.41 uma corresponding to the molecular peak of the cationic specie after losing an iodide anion. Other important peaks at 1327.48 (corresponding to the loss of one methyl group and two iodide anions) or at 1043.63 (corresponding to the loss of three methyl groups and four iodide anions) are also observed in the spectrum. It has not been possible to analyze dendrimers of second generation **4** and **6** by these techniques since their molecular weight is out of the range in the equipment used.

2.2 Antimicrobial activity

The antibacterial activity of dendrimers **5** and **6** as well as of their monofunctional counterpart, HO-(C_6H_4)-3-$NMe_3^+I^-$ (**7**), has been tested on *Staphylococcus aureus* (Gram+) and *Escherichia coli* (Gram−) and the MIC (minimum inhibitory concentration) and the MBC (minimum bactericidal concentration) have been measured. The MIC indicates the minimal concentration of an agent that can inhibit the growth of a microorganism (bacteriostatic effect) while the MBC means the minimal concentration of an agent that can kill a microorganism (bactericidal effect). For the same agent and microorganism, the value of MIC is lower than or equal to the MBC value (data shown in Table 1).

The results of the antibacterial tests in Table 1 show the high activity of carbosilane dendrimers against both Gram+ and Gram− bacteria. These data also show that the multivalency of

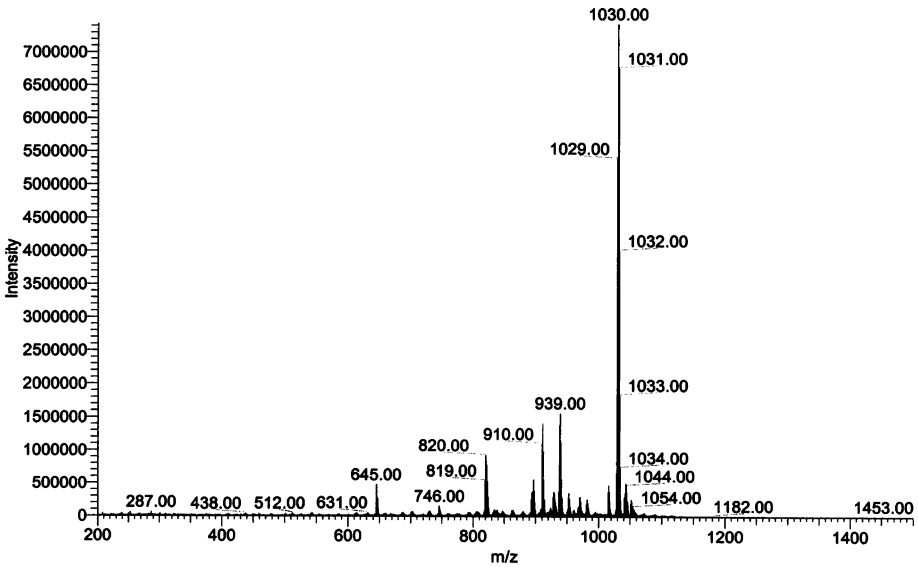


Fig. 3 APCI spectrum of dendrimer **3**.

Table 1 Bactericidal and bactericidal effects of compounds **5**, **6** and **7**

	Products					
	Monofunctional counterpart **7**/mg l^{-1}		Dendrimer **5**/mg l^{-1}		Dendrimer **6**/mg l^{-1}	
Microorganism	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	> 512	> 512	4	4	64	256
Staphylococcus aureus	512	> 512	1	4	8	32

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

dendrimers plays a major role in this antibactericidal activity since the dendrimer biocides are over two orders of magnitude more potent than their monofunctional counterpart. This significant improvement of biocidal action of dendritic biocides can be attributed to the high number of functional groups in a compact space and their polycationic structure. These tests also show a lower MIC for the Gram+ bacteria than for the Gram− bacteria, mainly in the second generation dendrimers. This fact has been observed by other authors[12] in lysine-based dendrimers and is probably due to the structure of the double layer membrane of Gram− bacteria. This underlines the importance of the interaction between dendrimers and membrane in the mode of action of these compounds. In this sense, it has been proposed[8d] that there are electrostatic interactions between the negatively charged bacteria membrane and the dendrimer biocides that present a high positive charge density. These dendrimers displace the divalent surface ions such as calcium and magnesium and may destabilize the membrane structure; this means that dendrimer biocides behave as surface-active organic cations and therefore have a destabilizing effect on phospholipid systems.[13] Nevertheless, the exact mechanism of their antimicrobial action is still unclear and mostly attributed to their ability to increase cell permeability and disrupt cell membranes.

The generation dependence of dendrimer biocides was also evaluated and we observed that the first generation dendrimer is more potent than the second one, primarily in the case of the Gram− bacteria. This result has also been shown by other authors[8d,12] and has been attributed to the size and molecular weight of the dendrimer and its capacity to cross the membrane of a bacteria. The higher decrease of the antimicrobial activity observed for the second generation dendrimer against Gram− bacteria may be due to the size of this dendrimer and its difficulty to cross the first double-layer membrane present in this type of bacteria. Nevertheless, there is always a balance between the disadvantage of the smaller number of functionalites in dendrimers of low generation and the disadvantage of a bulkier size in the dendrimers of high generation. In addition, in our case, we cannot rule out solubility problems in the dendrimer of second generation since this dendrimer is less soluble in water.

3. Conclusions

A new family of amine- and ammonium-terminated carbosilane dendrimers has been synthesized by phenolysis of chloro–carbon bonds in chloromethyl–silicon derivatives and subsequent quaternization with MeI. These dendrimers have been fully characterized using elemental analysis, NMR spectroscopy and mass spectrometry. Quaternized dendrimers are stable in water or other protic solvents for long time periods (NMR solutions of these dendrimers show the same spectra after several months). The antimicrobial

studies showed that the multivalency of dendrimers plays a major role in the antibactericidal activity of these compounds, since the dendrimer biocides are over two orders of magnitude more potent than their monofunctional counterpart. Dendrimers **5** and **6** present antimicrobial activity against both Gram+ and Gram− bacteria. This activity slightly decreases when the generation dendrimer increases, mainly in the case of a Gram− bacteria, which may well be due to the different structure of the cell wall in these systems

4. Experimental details

4.1 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 glove box. Solvents were dried and freshly distilled under argon prior to use: DMF from sodium carbonate, pentane from sodium, diethyl ether from sodium benzophenone ketyl and acetonitrile over calcium hydride.[14] Unless otherwise stated, reagents were obtained from commercial sources and used as received, G_n-[$SiMe_2CH_2CH{=}CH_2$]$_m$ dendrimers were prepared according to reported methods.[2f]

^{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 $CDCl_3$ and [D_6]DMSO 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. APCI samples were prepared in acetonitrile or methanol and spectra were recorded on a Termoquest Finnigan Automass spectrometer.

4.2 Synthesis of G_1-[$SiCH_2Cl$]$_4$ (1)

Over a toluene solution of tetraallylsilane, $Si(CH_2CH{=}CH_2)_4$ (0.97 g, 5.09 mmol), $HSi(Me)_2CH_2Cl$ (22.3 mmol, 2.70 ml) and two drops of the Speier catalyst were added. The reaction mixture was stirred at 60 °C during 5 hours. Silane excess and solvent were removed under vacuum and the residue extracted with hexane and then filtered with celite and active carbon to eliminate the catalyst. The resulting solution was evaporated under reduced pressure to give **1** as a colorless oil (2.75 g, 87%). ^{1}H-NMR ($CDCl_3$): δ 2.75 (8H, s, CH_2Cl), 1.30 (8H, m, SiCH$_2$CH_2CH$_2$Si), 0.50 (16H, m SiCH_2CH$_2$CH_2Si), 0.09 (24H, s, $SiMe_2$). ^{13}C-NMR ($CDCl_3$): δ 30.5 (CH_2Cl), 18.6 (SiCH$_2$CH$_2$CH$_2$Si), 18.4 (SiCH$_2$$CH_2CH_2$Si), 17.3 (SiCH$_2CH_2$$CH_2$Si), −4.4 ($SiMe_2$). Elemental analysis calcd. (%) for $C_{24}H_{56}Cl_4Si_5$: C 45.98, H 9.0; found C 45.88, H 8.86.

4.3 Synthesis of G_2-[$SiCH_2Cl$]$_8$ (2)

This dendrimer was prepared using a similar method to that described for **1**, starting from G_1-[$SiCH_2CH{=}CH_2$]$_8$ (1.03 g, 1.47 mmol), $HSi(Me)_2CH_2Cl$ (12.34 mmol, 1.5 ml) and two drops of the Speier catalyst to obtain compound **2** as a colorless oil (1.85 g, 80%). ^{1}H-NMR ($CDCl_3$): δ 2.75 (16H, s, CH_2Cl), 1.33 (24H, m, SiCH$_2$CH_2CH$_2$Si), 0.68 (16H, m SiCH$_2$CH$_2$CH_2SiCH$_2$Cl), 0.54 (32H, m SiCH_2CH$_2$CH_2Si), 0.08 (48H, s, $SiMe_2$), −0.08 (12H, s, SiMe). ^{13}C-NMR ($CDCl_3$): δ 30.5 (CH_2Cl), 18.6–17.6 (SiCH$_2$CH$_2$CH$_2$SiCH$_2$Cl), −4.5 ($SiMe_2$), −5.0 (SiMe). Elemental analysis calcd. (%) for $C_{64}H_{148}Cl_8Si_{13}$: C 49.09, H 9.52; found C 48.56, H 9.43.

4.4 Synthesis of G_1-[$SiCH_2O$-(C_6H_4)-3-NMe_2]$_4$ (3)

Over a solution of **1** (0.27 g, 0.42 mmol) in DMF (30 ml), 3-dimethylaminophenol (0.24 g, 1.69 mmol), 1 g of potassium carbonate and a small amount of KI were added. The reaction mixture was stirred and heated to 80 °C during two days, then evaporated to dryness and the residue extracted with pentane (2 × 20 ml) to give a yellow solution. The solvent was removed under vacuum leading to dendrimer **3** as a yellow oily product (0.36 g, 85%). ^{1}H-NMR ($CDCl_3$): δ 7.10 (4H, s, C_6H_4), 6.35 (12H, m, C_6H_4), 3.54 (8H, s, CH_2O), 2.91 (24H, s, NMe_2), 1.37 (8H, m, SiCH$_2$CH_2CH$_2$Si), 0.68 (8H, m, SiCH$_2$CH$_2$CH_2SiCH$_2$O), 0.59 (8H, m, SiCH_2CH$_2$CH$_2$Si), 0.08 (24H, s, $SiMe_2$). ^{13}C-NMR ($CDCl_3$): δ 162.7 (C_{ipso}OCH$_2$), 151.8 (C_{ipso}NMe$_2$), 129.5, 105.4, 101.9 and 99.3 (C_6H_4), 60.0 (CH_2O), 40.7 (NMe_2), 18.6, 18.4 and 17.3 (SiCH$_2$$CH_2$$CH_2$Si), −4.5 ($SiMe_2$). ^{29}Si-NMR ($CDCl_3$): δ 0.5 (G_0–Si), −0.2 (G_1–Si). Elemental analysis calcd. (%) for $C_{56}H_{96}N_4O_4Si_5$: C 65.31, H 9.40, N 5.44; found C 64.87, H 9.06, N 5.62. APCI: [M + H$^+$] = 1030 uma (calcd. = 1028.66 uma).

4.5 Synthesis of G_2-[$SiCH_2O$-(C_6H_4)-3-NMe_2]$_8$ (4)

This dendrimer was prepared using a similar method to that described for **3**, starting from G_2-[$SiCH_2Cl$]$_8$ (0.09 g, 0.06 mmol), 3-dimethylaminophenol (0.07 g, 0.48 mmol), K_2CO_3 (1 g, 7.23 mmol) and a small amount of KI. Second generation dendrimer **4**, is obtained as a yellow-orange oily product (0.11 g, 82%). ^{1}H-NMR ($CDCl_3$): δ 7.09 (8H, s, C_6H_4), 6.29 (24H, m, C_6H_4), 3.53 (16H, s, CH_2O), 2.90 (48H, s, NMe_2), 1.37 (24H, m, SiCH$_2$CH_2CH$_2$Si), 0.69 (16H, m, SiCH$_2$CH$_2$CH_2SiCH$_2$O), 0.55 (32H, m, SiCH_2CH$_2$CH_2Si), 0.08 (48H, s, $SiMe_2$), −0.09 (12H, s, SiMe). ^{13}C-NMR ($CDCl_3$): δ 162.7 (C_{ipso}OCH$_2$), 151.9 (C_{ipso}NMe$_2$), 129.5, 105.3, 101.9 and 99.3 (C_6H_4), 60.0 (CH_2O), 40.7 (NMe_2), 18.6, 18.5 and 17.4 (SiCH$_2$$CH_2$$CH_2$Si, outer fragment), 19.0–18.0 (rest of the CH_2 signals), −4.5 ($SiMe_2$), −5.0 (SiMe). ^{29}Si-NMR ($CDCl_3$): δ 0.6 (G_0–Si), 1.0 (G_1–Si), −0.2 (G_2–Si). Elemental analysis calcd. (%) for $C_{128}H_{228}N_8O_8Si_{13}$: C 64.80, H 9.69, N 4.72; found C 63.85, H 9.51, N 4.55.

4.6 Synthesis of G_1-[$SiCH_2O$-(C_6H_4)-3-NMe_3^+ I^-]$_4$ (5)

A solution of MeI in Et_2O (2 M, 0.10 ml, 1.68 mmol) was added to an acetonitrile (30 ml) solution of **3** (0.29 g, 0.28 mmol). The resulting solution was stirred for 24 hours at room temperature and then evaporated under reduced pressure to remove residual MeI. The residue was washed with Et_2O (2 × 5 ml) and dried under vacuum to yield dendrimer **5** as a white solid compound (0.43 g, 94%). ^{1}H-NMR ([D_6]DMSO): δ 7.48 (12H, m, C_6H_4), 7.15 (4H, s, C_6H_4), 3.71 (8H, s, CH_2O), 3.57 (36H, s, NMe_3^+), 1.39 (8H, m, SiCH$_2$CH_2CH$_2$Si), 0.70 (8H, m, SiCH$_2$CH$_2$CH_2SiCH$_2$O), 0.58 (8H, m, SiCH_2CH$_2$CH$_2$Si), 0.08 (24H, s, $SiMe_2$). ^{13}C-NMR ([D_6]DMSO): δ 161.3 (C_{ipso}OCH$_2$), 147.7 (C_{ipso}NMe$_3^+$), 130.2, 114.8, 111.3 and 106.5 (C_6H_4), 60.5 (CH_2O), 55.9 (NMe_3^+), 17.4, 17.3 and 16.3 (SiCH$_2$$CH_2$$CH_2$Si), −5.18 ($SiMe_2$).^{29}Si-NMR ($CDCl_3$): δ 1.3 (G_0–Si), 0.3 (G_1–Si). Elemental analysis calcd. (%) for $C_{60}H_{108}I_4N_4O_4Si_5$: C 45.11, H 6.81, N 3.51; found C 44.62, H 6.54, N 3.44. ESI: [M − I]$^+$ = 1470.4 uma (calcd. = 1470.6 uma),

$[M - Me - 2I]^+ = 1328.5$ uma (calcd = 1328.7 uma), $[M - 3Me - 4I]^+ = 1044.6$ uma (calcd 1044.8 uma).

4.7 Synthesis of G_2-[$SiCH_2O$-(C_6H_4)-3-NMe_3^+ I^-]$_8$ (6)

This dendrimer was prepared using a similar method to that described for **5**, starting from G_2-[$SiCH_2O$-(C_6H_4)-3-NMe_2]$_8$ (0.18 g, 0.75 mmol) and MeI (0.6 ml, 9.6 mmol). Second generation dendrimer, **6**, is obtained as a white solid compound (0.21 g, 80%). ^{1}H-NMR ([D_6]DMSO): δ 7.49 (24H, m, C_6H_4), 7.26 (8H, s, C_6H_4), 3.71 (16H, s, CH_2O), 3.58 (72H, s, NMe_3^+), 1.36 (24H, m, $SiCH_2CH_2CH_2Si$), 0.69 (16H, m, $SiCH_2CH_2CH_2SiCH_2O$), 0.54 (32H, m, $SiCH_2CH_2CH_2Si$), 0.08 (48H, s, $SiMe_2$), −0.09 (12H, s, SiMe). ^{13}C-NMR ([D_6]DMSO): δ 161.3 ($C_{ipso}OCH_2$), 147.7 ($C_{ipso}NMe_3^+$), 130.2, 114.8, 111.3 and 106.5 (C_6H_4), 60.5 (CH_2O), 55.9 (NMe_3^+), 17.8–17.1 ($SiCH_2CH_2CH_2Si$), −5.1 ($SiMe_2$), −5.2 (SiMe). ^{29}Si-NMR ($CDCl_3$): δ −0.3 (G_0–Si), 1.2 (G_1–Si), 0.0 (G_2-Si). Elemental analysis calcd. (%) for $C_{136}H_{252}I_8N_8O_8Si_{13}$: C 46.57, H 7.24, N 3.19; found C 45.23, H 6.98, N 2.99.

4.8 Synthesis of HO(C_6H_4)-3-NMe_3^+ I^- (7)

A solution of MeI in Et_2O (2 M, 0.10 ml, 1.68 mmol) was added to an acetonitrile (30 ml) solution of 3-dimethylaminophenol (0.21 g, 1.50 mmol). The resulting solution was stirred for 6 hours at room temperature and then evaporated under reduced pressure to remove residual MeI. The residue was washed with Et_2O (2 × 5 ml) and dried under vacuum to give **7** as a purple solid compound (0.35 g, 84%). ^{1}H-NMR ([D_6]DMSO): δ 7.40 (3H, m, C_6H_4), 6.96 (1H, d, C_6H_4), 3.53 (9H, s, NMe_3^+). ^{13}C-NMR ([D_6]DMSO): δ 157.8 ($C_{ipso}OH$), 147.8 ($C_{ipso}NMe_3^+$), 130.2, 116.0, 109.9 and 107.1 (C_6H_4), 55.7 (NMe_3^+). Elemental analysis calcd. (%) for $C_9H_{14}INO$: C 38.73, H 5.06, N 5.02; found C 38.52, H 4.99, N 4.92.

4.9 Antimicrobial activity assay

The minimal inhibitory concentration (MIC) of the products was determined in 96-well tray microplates using the international standard methods ISO 20776–1 by microdilution tray preparation.[15] The assay was carried out in duplicate microplates with three different wells for each analysed concentration. The bacteria used in the analysis were *Escherichia coli* (Gram−) and *Staphylococcus aureus* (Gram+). Both strains were obtained from the "Colección Española de cultivos tipo" (CECT). A stock solution of the products was prepared by dissolving 0.01024 g of the compound with 60 µl of dimethyl sulfoxide (DMSO) and 10 ml of distilled water. After that, the desired concentration was achieved by dilution with distilled water. The microplates were incubated at 37 °C using an ultra microplate reader ELX808iu (Bio-Tek Instruments). The minimal bactericidal concentration (MBC) was calculated inoculating 100 µl of the samples used to calculate the MIC in a Petri dish with Mueller–Hinton agar (ref. 02–136, Scharlau). After 48 h of incubation at 37 °C, the presence of colonies was tested. The MBC was the minimal concentration where no growth was detected.

Acknowledgements

We thank the Ministerio de Ciencia y Tecnología (project CTQ2005-00795/BQU), Fondo de Investigación Sanitaria (PI040993) and Fundación Caja Navarra for financial support.

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