

**Functionalization of silica with amine and ammonium alkyl chains, dendrons and dendrimers: synthesis and antibacterial properties**

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## **Abstract**

Materials modified with ammonium groups on the surface have shown antibacterial activity. In this paper, alkyl chains, carbosilane (CBS) dendrimers and dendrons and poly(amidoamine) (PAMAM) dendrimers containing amine and ammonium groups have been grafted to silica surface and the influence of molecule structure on the stability and on antibacterial activity have been evaluated. These materials have been characterized by thermogravimetric analysis (TGA), zeta potential, scanning electron microscopy (SEM), infrared spectroscopy (IR) and nuclear magnetic resonance ( $^{13}\text{C}$  CP MAS NMR). The degree of silica functionalization depends on type of outer groups, amine or ammonium, type and core of dendrimer, and length of chains. The Z potential measurements of these materials were used to test their stability. These measurements showed, for some of the modified silicas, the diminishing of Z potential from positive values toward zero, probably due to interaction of the functional groups over silica surface. This variation was also dependent on ligand structure and peripheral functions. Finally, studies of inhibition of bacteria growth stand out again the relevance of ligand structure and number of functional groups on silica surface. The most active systems were those with more surface covered, those with cationic groups further away from silica surface and higher dendritic generation.

**Keywords:** silica, carbosilane dendrimer, carbosilane dendron, ammonium salts, antibacterial materials.

## 1. Introduction

Resistance of microorganisms to traditional antibiotics<sup>1</sup> and microorganism contamination<sup>2, 3</sup> are major concerns of public health systems.<sup>4, 5</sup> For example, infected material devices or implants are the main responsible of nosocomial infections due to this bacteria resistance. To reduce the use of antibiotics, the development of materials with antibacterial properties is of prime importance.<sup>6-9</sup> With this idea, materials with microbicide properties focused to water storage and disinfection or food protection have been made.<sup>10-13</sup>

Two different types of active surfaces have been designed: microbicide releasing or antimicrobial by contact.<sup>8, 14</sup> For the first type, it can be expected that their activity would be limited to the amount of microbicide charge and delivering time; whereas for the second one, drawbacks as saturation of surface or difficulties to interact with bacteria has to be considered. However, for some applications (water treatment, food storage, health care material) the second one would be desirable since with those type of materials the killing of bacteria or their growing would not imply the release of new chemical compounds, which safety should be evaluated.<sup>15</sup>

The divalent cations  $Mg^{2+}$  and  $Ca^{2+}$  help to keep the structure of bacterial walls acting as glue with negative lipoproteins.<sup>16</sup> The displacement of these cations destabilizes the membrane leading to its lysis and death of bacteria. This substitution can be afforded introducing other type of cations, as for example quaternary ammonium salts (QAS), which have been widely explored as bactericides, the target being the cytoplasmic membrane in bacteria.<sup>16-20</sup> An improvement of QAS activity has been achieved by incorporating QAS moieties to polyfunctional macromolecules. This process generates polycations that present a high surface charge,<sup>21-24</sup> observing and increase of the activity with respect to monofunctional molecules.<sup>21, 25-27</sup>

On the other hand, surfaces of materials such as glass, clays, inorganic oxides (e. g.  $SiO_2$ )<sup>28</sup> or metals<sup>29</sup> coated with QAS have shown bactericide properties.<sup>12</sup> These materials are the main components of different type of membranes for water purification (clays and oxides) or surgery equipment (metals).<sup>15, 30</sup> The presence of biocidal moieties grafted on them creates active system with dual activity.<sup>31, 32</sup> Functionalization of surfaces with QAS has been done by homogenous distribution of monomers or also by grafting polymers containing the QAS moieties. With this

last procedure it is claimed that the density of active groups increases, although polymer structure can be determinant in biocide properties.<sup>13, 33</sup>

Dendritic systems are polymer-like derivatives with a well defined shape and structure, synthesized by an iterative sequence of controlled reactions, which lead to hyperbranched monodisperse macromolecules.<sup>34</sup> Two main topologies can be distinguished for dendritic macromolecules, spherical dendrimers and dendrons or dendritic wedges, with a periphery similar to dendrimers and an additional function known as focal point. As for traditional polymers, the multivalency of dendritic systems makes easier their anchorage to surfaces. In the particular case of dendrons, the presence of the focal point allows the introduction of a linker group while maintaining the functional groups at the periphery, enabling the transference of dendron properties to other materials while keeping control on the molecular structure.<sup>35-37</sup>

Antibacterial cationic dendrimers containing QAS with different frameworks, polyamidoamine (PAMAM),<sup>38</sup> polypropyleneimine (PPI),<sup>39</sup> phosphorus-viologen,<sup>40</sup> carbosilane (CBS),<sup>41</sup> poly(propyleneoxide) amines,<sup>42</sup> have been described. The mode of action of these macromolecules is related with that of QAS, displacing the divalent cations and modifying membrane permeability. The presence of hydrophobic chains in dendrimers enable their penetration into the phospholipid bilayer leading to the disintegration of the bacterial membrane.<sup>17, 20</sup> Regarding this last consideration, our group has developed a library of ammonium CBS systems, observing that an adequate hydrophobic/hydrophilic balance is essential for the antibacterial activity.<sup>43-46</sup> In this case, the hydrophobic moiety belongs to the carbosilane framework, made of apolar C-C and Si-C bonds, whilst the hydrophilic moiety is due to the ammonium groups. Moreover, the more active derivatives were lower generation systems, which simplify the synthesis, showing microbicide properties against Gram-positive and Gram-negative bacteria, methicillin resistant *S. aureus*, did not generate resistance and were active against *Acanthameba* trophozoites.<sup>46, 47</sup> These attractive microbicide properties of ammonium CBS dendrimers and dendrons have also been moved to other materials using dendrons, as for example nanoparticles.<sup>48</sup>

Taking into account these previous results, we have explored the functionalization of silica SiO<sub>2</sub>, as a model of inorganic oxide, with CBS dendrimers and dendrons containing amine and ammonium (QAS) groups with the aim to give silica antibacterial properties. With this in mind, we have studied the influence of topology, generation, and type of functional group (amine -NH<sub>2</sub> or -NMe<sub>2</sub> and ammonium -NH<sub>3</sub><sup>+</sup> or -NMe<sub>3</sub><sup>+</sup>). Moreover, the behavior of these systems has been also compared with ammonium monomers containing both short and long alkyl chains between them and the silica surface.

## 2. Materials and Methods

**2.1. General Considerations.** All reactions were carried out in an inert atmosphere and solvents were purified employing MBraun Solvent Purification System if necessary. Thiol-ene reactions were carried out with an HPK 125W Mercury Lamp from Heraeus Noblelight with maximum energy at 365 nm in normal glassware after deoxygenation bubbling argon. Solution NMR spectra were recorded on a Varian Unity VXR-300 (300.13 MHz (<sup>1</sup>H), 75.47 (<sup>13</sup>C)) or on a Bruker AV400 (400.13 MHz (<sup>1</sup>H), 100.60 MHz (<sup>13</sup>C)). <sup>13</sup>C CP-MAS NMR spectra were recorded on a Bruker AV400 WB with a multinuclear CP MAS probe (100.60 MHz (<sup>13</sup>C)). Chemical shifts (δ) are given in ppm. <sup>1</sup>H and <sup>13</sup>C resonances were measured relative to solvent peaks considering TMS = 0 ppm. Elemental analyses were done on a LECO CHNS-932. Compounds [GnX(S-NH<sub>3</sub>Cl)<sub>m</sub>] (X = Si, n = 0, m = 4;<sup>49</sup> X = Si, n = 1, m = 8;<sup>45</sup> X = O<sub>3</sub>, n = 1, m = 6;<sup>43</sup> X = O<sub>3</sub>, n = 2, m = 12<sup>43</sup>), NH<sub>2</sub>G<sub>n</sub>(S-NMe<sub>2</sub>)<sub>m</sub>,<sup>50</sup> PhtG<sub>3</sub>V<sub>8</sub><sup>50</sup> were synthesized as previously published. All other compounds were obtained from commercial sources and used as received but SiO<sub>2</sub>, which was dried under vacuum at 120°C for 3 weeks and stored under inert atmosphere. Reactions with silica were done using 250 ml DMF per 1 g of SiO<sub>2</sub>.

**Thermogravimetric analysis (TGA):** were performed using a TGA Q500. Dry and pure samples (2-10 mg) were placed into platinum sample holder under nitrogen atmosphere. The measurements were recorded from 25 to 1000 °C, with heating rate of 10 °C/min.

**Scanning electron microscopy (SEM):** were performed using a Hitachi TM-100 in samples previously lyophilized.

**Zeta potential:** was measured in a Zetasizer Nano ZS (Malvern Instruments Ltd., UK) at 25 °C using disposable Malvern plastic cuvettes (1 mL), by suspended the silica (1 mg) in purified water (1 mL), which was previously filtered through 0.22 µm filter. Water stability assays were done with 22.5 mg, keeping the suspension stand for 15 days and then stirring.

## 2.2. Synthesis of compounds.

A description of the synthesis of representative examples follow, being the procedures and data for all compounds collected in the supporting information.

**[G<sub>0</sub>Si(S-NH<sub>2</sub>)<sub>3</sub>(Si(OEt)<sub>3</sub>)] (1).** Dendrimer [G<sub>0</sub>Si(SNH<sub>3</sub>Cl)<sub>4</sub>] (0.766 g, 1.30 mmol) was suspended in dry CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and excess NEt<sub>3</sub> (5.20 mmol) was added to neutralize the dendrimer. The suspension was stirred for 10 min and then slightly default of 3-(triethoxysilyl)-propylisocyanate (1.25 mmol) was also added. The reaction was stirred for 16 h, hexane (50 ml) was mixed with the solution and the solution was filtered. Volatiles were removed under vacuum and the crude product was washed first with Et<sub>2</sub>O (25 ml), second with hexane (25 ml) and finally dried under vacuum, leading to compound **1** as yellowish wax (yield 76%). This compound was used without further purification for grafting to silica. Data for **1** (C<sub>26</sub>H<sub>61</sub>N<sub>5</sub>O<sub>4</sub>S<sub>4</sub>Si<sub>2</sub>, Fw = 692.22 g/mol): NMR (DMSO-d<sub>6</sub>): <sup>1</sup>H NMR: 0.58 (m, 2 H, OSiCH<sub>2</sub>), 0.95 (m, 8 H, SiCH<sub>2</sub>), 1.20 (m, 2 H, SiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.38 (m, 9 H, CH<sub>3</sub>CH<sub>2</sub>O), 2.62 (m, 8 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 2.78 (m, 8 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.23 (m, 10 H, CH<sub>2</sub>NH<sub>2</sub>, SCH<sub>2</sub>CH<sub>2</sub>NHC(O), NHC(O)NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 3.83 (m, 6 H, CH<sub>3</sub>CH<sub>2</sub>O). <sup>13</sup>C{<sup>1</sup>H}NMR: 13.5 (SiCH<sub>2</sub>CH<sub>2</sub>S), 13.8 (OSiCH<sub>2</sub>), 18.6 (CH<sub>3</sub>CH<sub>2</sub>OSi), 25.1 (OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.9 (SiCH<sub>2</sub>CH<sub>2</sub>S), 36.2 (SCH<sub>2</sub>CH<sub>2</sub>N), 41.1 (SCH<sub>2</sub>CH<sub>2</sub>N), 45.6 (OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 58.3 (CH<sub>3</sub>CH<sub>2</sub>OSi), 158.3 (C=O). IR (KBr, cm<sup>-1</sup>): 3368 ν(N-H), 1636 ν(C=O).

**[(EtO)<sub>3</sub>SiG<sub>1</sub>(S-NMe<sub>2</sub>)<sub>2</sub>] (7).** A solution of neutral dendron NH<sub>2</sub>G<sub>1</sub>(S-NMe<sub>2</sub>)<sub>2</sub> (0.363g; 1.47 mmol) and NEt<sub>3</sub> (1.50 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (50 ml) was treated with slightly default of 3-(triethoxysilyl)-propylisocyanate (1.39 mmol) and the reaction was stirred for 16 h. Afterwards, hexane was added (50 ml), the solution was filtered and volatiles removed under vacuum. The crude product was washed first with Et<sub>2</sub>O (25 ml), second with hexane (25 ml) and finally dried under vacuum, leading to compound **2** as yellowish wax (yield 83%, 0.50 g). This compound was

used without further purification for grafting to silica. Data for **7**: NMR (CDCl<sub>3</sub>): <sup>1</sup>H NMR: -0.09 (s, 3 H, SiMe), 0.43 (m, 4 H, CH<sub>2</sub>Si), 0.80 (m, 4 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 1.21 (m, 13 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si, C(O)NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si and CH<sub>3</sub>CH<sub>2</sub>O), 1.38 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 2.14 (s, 12 H, NMe<sub>2</sub>), 2.48 (m, 4 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 2.52 (m, 4 H, SCH<sub>2</sub>CH<sub>2</sub>N), 2.57 (m, 4 H, CH<sub>2</sub>N), 3.18 (m, 4 H, CH<sub>2</sub>NH), 3.83 (m, 6 H, CH<sub>3</sub>CH<sub>2</sub>O); <sup>13</sup>C{<sup>1</sup>H} NMR: -5.5 (SiMe), 13.2 (CH<sub>2</sub>Si), 14.3 (SiCH<sub>2</sub>), 18.6 (CH<sub>3</sub>CH<sub>2</sub>OSi), 20.9 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 25.1 (OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 27.4 (SiCH<sub>2</sub>CH<sub>2</sub>S), 29.6 (SCH<sub>2</sub>CH<sub>2</sub>N), 36.8 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 45.2 (NMe<sub>2</sub>), 45.6 (OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 58.3 (CH<sub>3</sub>CH<sub>2</sub>OSi), 59.0 (SCH<sub>2</sub>CH<sub>2</sub>N), 158.3 (C=O); <sup>29</sup>Si NMR: δ 2.5 (G1-SiMe). Anal. Calcd. C<sub>27</sub>H<sub>62</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub>Si<sub>2</sub> (627.11 g/mol): C, 51.71; H, 9.97; N, 8.93; S, 10.23. IR (KBr, cm<sup>-1</sup>): 3374 ν(N-H), 1658 ν(C=O).

**[(EtO)<sub>3</sub>SiG<sub>1</sub>(S-NMe<sub>3</sub>I)<sub>2</sub>] (10)**. To a solution of neutral dendron **7** (0.605 g; 0.97 mmol) in dry Et<sub>2</sub>O (50 ml) excess MeI was added (0.30 mmol). The reaction was stirred for 16 h. Afterwards, volatiles were removed under vacuum and the crude product was washed with Et<sub>2</sub>O (2 x 25 ml) and dried under vacuum, leading to compound **10** as yellowish solid (yield 80%, 0.707 g). Data for **10**: NMR (DMSO-d<sub>6</sub>): <sup>1</sup>H NMR: -0.07 (s, 3 H, SiMe), 0.60 (m, 4 H, CH<sub>2</sub>Si), 0.90 (m, 4 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 1.22 (m, 9 H, CH<sub>3</sub>CH<sub>2</sub>O), 1.33 (m, 4 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si and OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH), 1.56 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 2.67 (m, 4 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 2.92 (m, 4 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.14 (s, 18 H, NMe<sub>3</sub><sup>+</sup>), 3.18 (m, 4 H, CH<sub>2</sub>NHC(O)), 3.58 (t, 4 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.83 (m, 6 H, CH<sub>3</sub>CH<sub>2</sub>O). <sup>13</sup>C{<sup>1</sup>H} NMR: δ -5.7 (SiMe), 12.3 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 13.5 (SiCH<sub>2</sub>CH<sub>2</sub>S), 13.8 (OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 18.6 (CH<sub>3</sub>CH<sub>2</sub>OSi), 19.8 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 23.1 (SCH<sub>2</sub>CH<sub>2</sub>N), 25.1 (OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.3 (SiCH<sub>2</sub>CH<sub>2</sub>S), 30.3 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 38.5 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 45.6 (OSiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 51.7 (NMe<sub>3</sub><sup>+</sup>), 58.3 (CH<sub>3</sub>CH<sub>2</sub>OSi), 63.9 (SCH<sub>2</sub>CH<sub>2</sub>N), 158.3 (C=O). Anal. Calcd. C<sub>29</sub>H<sub>68</sub>I<sub>2</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub>Si<sub>2</sub> (910.98 g/mol): ESI: q = 1 (783.33 [M-I]<sup>+</sup>), q = 2 (328.01 [M-2I]<sup>2+</sup>). IR (KBr, cm<sup>-1</sup>): 3354 ν(N-H), 1632 ν(C=O).

**PhtG<sub>3</sub>(S-NHBoc)<sub>8</sub> (13)**. To a mixture of vinyl dendron PhtG<sub>3</sub>V<sub>8</sub> (0.217 g, 0.22 mmol) and 2-(Boc-amino)ethanethiol (0.358 g, 1.94 mmol) in THF/MeOH (9 ml, 1:2), DMPA (2,2-dimethoxy-2-phenylacetophenone) was added (0.02 g, 0.09 mmol, 2.5% with respect to total number of vinyl groups). The mixture was bubbled with Ar and stirred under UV irradiation for

1.5 h. Then, again the same amount of DMPA was added (0.02 g, 0.09 mmol), the suspension was bubbled with Ar and stirred for other 1.5 h. Afterwards, volatiles were removed under vacuum and crude product was purified by size exclusion chromatography (acetone as eluent). Compound **13** was obtained as white solid (71% yield, 0.378 g). Data for **13**: NMR (CDCl<sub>3</sub>): -0.13 (s, 9 H, SiMe), -0.04 (s, 12 H, SiMe), 0.46 (m, 24 H, SiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 0.62 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 0.83 (m, 16 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 1.23 (m, 14 H, SiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si and NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 1.38 (s, 72 H, CMe<sub>3</sub>), 1.66 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 2.55 (m, 16 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 2.62 (m, 16 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.26 (m, 16 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.63 (t, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 5.17 (s, 8 H, NH), 7.64 (m, 2 H, Pht), 7.82 (m, 2 H, Pht). <sup>13</sup>C{<sup>1</sup>H} NMR: -5.4 and -5.1 (SiMe), 14.5 (SiCH<sub>2</sub>CH<sub>2</sub>S), 18.2–18.8 (CH<sub>2</sub>), 27.0 (SiCH<sub>2</sub>CH<sub>2</sub>S), 28.2 (CMe<sub>3</sub>), 31.9 (SCH<sub>2</sub>CH<sub>2</sub>N), 39.6 (SCH<sub>2</sub>CH<sub>2</sub>N), 78.9 (CMe<sub>3</sub>), 123.0 (C-H. Pht), 132.1 (C<sub>ipso</sub>, Pht), 133.9 (C-H, Pht), 155.4 (C=O, Boc), 167.9 (C=O, Pht). <sup>29</sup>Si NMR: 2.1 (G3–SiMe), 0.9 (G2–SiMe). ESI: q = 1 (2389.22 [M+H]<sup>+</sup>). Anal. Calcd. C<sub>109</sub>H<sub>213</sub>N<sub>9</sub>O<sub>18</sub>S<sub>8</sub>Si<sub>7</sub> (2391.03 g/mol): C, 54.75; H, 8.98; N, 5.27; S, 10.73.

**NH<sub>2</sub>G<sub>3</sub>(S-NHBoc)<sub>8</sub> (14)**. Compound PthG<sub>3</sub>(S-NHBoc)<sub>8</sub> (**13**) (0.378 g, 0.16 mmol) and excess hydrazine (0.10 ml, 1.58 mmol) were heated in a teflon valved ampoule at 80°C for 4 d, using MeOH as solvent (30 ml). Afterwards volatiles were removed under vacuum and Et<sub>2</sub>O/NH<sub>4</sub>Cl (aq) extraction was done. The organic phase was dried with MgSO<sub>4</sub> and volatiles were removed under vacuum, yielding **14** as yellowish wax (83%, 0.26 g). Data for **14**: NMR (CDCl<sub>3</sub>): <sup>1</sup>H NMR: -0.13 (s, 9 H, SiMe), -0.04 (s, 12 H, SiMe), 0.46 (m, 24 H, SiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si) 0.62 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 0.83 (m, 16 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 1.23 (m, 14 H, SiCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si and NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 1.38 (s, 72 H, CMe<sub>3</sub>), 1.66 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 2.55 (m, 16 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 2.62 (m, 16 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.26 (m, 16 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.43 (t, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si), 5.17 (s, 8 H, NHC(O)O); <sup>13</sup>C{<sup>1</sup>H} NMR: -5.4 and -5.1 (SiMe), 14.5 (SiCH<sub>2</sub>CH<sub>2</sub>S), 18.2–18.8 (CH<sub>2</sub>), 27.0 (SiCH<sub>2</sub>CH<sub>2</sub>S), 28.2 (CMe<sub>3</sub>), 31.9 (SCH<sub>2</sub>CH<sub>2</sub>N), 39.6 (SCH<sub>2</sub>CH<sub>2</sub>N), 79.0 (CMe<sub>3</sub>), 155.5 (C=O); <sup>29</sup>Si NMR: 2.1 (G3–SiMe), 0.9 (G2–SiMe). ESI: q = 1 (2259.21 [M+H]<sup>+</sup>). Anal. Calcd. C<sub>101</sub>H<sub>211</sub>N<sub>9</sub>O<sub>16</sub>S<sub>8</sub>Si<sub>7</sub> (2260.93 g/mol): C, 53.65; H, 9.41; N, 5.58; S, 11.35.



**[(EtO)<sub>3</sub>Si(CH<sub>2</sub>)<sub>2</sub>S(CH<sub>2</sub>)<sub>2</sub>NMe<sub>2</sub>HCl] (17)**. Vinyltriethoxysilane ((EtO)<sub>3</sub>SiV, 3 ml, 13.90 mmol) was dissolved in THF and a EtOH suspension of 2-(dimethylamino)ethilenethiol hydrochloride (2.117 g, 14.9 mmol) and DMPA (0.182 g, 0.70 mmol, 2.5% with respect to total number of vinyl groups) was added. The suspension was bubbled with Ar and stirred under UV irradiation for 2 h. Then, again DMPA (0.182 g, 0.70 mmol, 2.5% with respect to total number of vinyl groups) was added, the suspension bubbled with Ar and stirred under UV irradiation for another 2 h. Afterwards the volatiles were removed under vacuum leading to **17** as yellowish solid. This compound was used for next step without further purification. Data for **17**: NMR (CDCl<sub>3</sub>): <sup>1</sup>H NMR: 0.91 (t, 2 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 1.18 (t, 9 H, CH<sub>3</sub>CH<sub>2</sub>O), 2.69 (t, 2 H, SCH<sub>2</sub>CH<sub>2</sub>N), 2.83 (s, 6 H, NMe<sub>2</sub>H), 2.96 (t, 2 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 3.16 (t, 2 H, SCH<sub>2</sub>CH<sub>2</sub>N), 3.78 (q, 6 H, CH<sub>3</sub>CH<sub>2</sub>O); <sup>13</sup>C{<sup>1</sup>H} NMR: 1.8 (SiCH<sub>2</sub>CH<sub>2</sub>S), 18.5 (CH<sub>3</sub>CH<sub>2</sub>O), 25.3 (SCH<sub>2</sub>CH<sub>2</sub>N), 27.3 (SiCH<sub>2</sub>CH<sub>2</sub>S), 42.4 (NMe<sub>2</sub>H), 57.3 (SCH<sub>2</sub>CH<sub>2</sub>N), 58.6 (CH<sub>3</sub>CH<sub>2</sub>O).

**(EtO)<sub>3</sub>Si(CH<sub>2</sub>)<sub>2</sub>S(CH<sub>2</sub>)<sub>2</sub>NMe<sub>2</sub> (18)**. Compound **17** (0.600 g; 1.81 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, NEt<sub>3</sub> was added (1.01 ml; 7.24 mmol) and the mixture was stirred for 30 min. Afterwards volatiles were removed under vacuum and compound **18** was extracted with Et<sub>2</sub>O, which after evaporation yielded **18** as pale yellow oil. This compound was used for silica grafting without further purification, since any impurity is easily removed from the material. Data for **18**: NMR (CDCl<sub>3</sub>): <sup>1</sup>H NMR: 0.91 (t, 2 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 1.18 (t, 9 H, CH<sub>3</sub>CH<sub>2</sub>O), 2.23 (s, 6 H, NMe<sub>2</sub>), 2.52 (t, 2 H, SiCH<sub>2</sub>CH<sub>2</sub>S), 2.64 (t, 4 H, SCH<sub>2</sub>CH<sub>2</sub>N and SCH<sub>2</sub>CH<sub>2</sub>N), 3.78 (q, 6 H, CH<sub>3</sub>CH<sub>2</sub>O); <sup>13</sup>C{<sup>1</sup>H} NMR: 12.3 (SiCH<sub>2</sub>CH<sub>2</sub>S), 18.5 (CH<sub>3</sub>CH<sub>2</sub>O), 26.5 (SiCH<sub>2</sub>CH<sub>2</sub>S), 29.8 (SCH<sub>2</sub>CH<sub>2</sub>N), 45.3 (NMe<sub>2</sub>), 58.8 (SCH<sub>2</sub>CH<sub>2</sub>N), 59.1 (CH<sub>3</sub>CH<sub>2</sub>O).

**1-SiO<sub>2</sub>**. Compound **1** (1.086 g; 1.30 mmol) was dissolved in DMF (126 ml) and SiO<sub>2</sub> was added (0.480 g, 30 g SiO<sub>2</sub>/0.232 g per amine group). The mixture was stirred at 80°C for 72 h. Next, solution was filtered off and remaining solid was washed with MeCN, Et<sub>2</sub>O and n-hexane (three times with 20 ml for each solvent). The yellowish powder was dried under vacuum to give **1-SiO<sub>2</sub>** (0.58 g). IR (cm<sup>-1</sup>): 3402 (ν SiO-H, br), 3000-2800 (ν C-H), 1668 (ν Si-O), 1575-1384 (δ C-H, δ C-C and δ C-N), 1095 (ν Si-O-Si and ν<sub>as</sub> Si-O-C), 797 (δ Si-O-Si).

**15-SiO<sub>2</sub>**. In a schlenck flask, 7.49 ml of TFA were added over 0.075 g of **15Boc-SiO<sub>2</sub>** and the suspension was stirred for 24 h. The material was washed by centrifugation with water (three times) and MeOH (once) and dry under vacuum (Z potential:  $-23 \pm 1.2$  mV). Over this material a NaCl saturated water solution was added, the suspension was stirred for 10 min and then centrifuged to remove water. This process was repeated three times. Afterwards, the material was washed with distilled water and centrifuged (three times) and then was washed with MeOH. Finally, **15-SiO<sub>2</sub>** was dried under vacuum (yield 76%, 0.06 g). IR (cm<sup>-1</sup>): 3402 ( $\nu$  SiO-H, br), 3000-2800 ( $\nu$  C-H), 1668 ( $\nu$  Si-O), 1575-1384 ( $\delta$  C-H,  $\delta$  C-C and  $\delta$  C-N), 1095 ( $\nu$  Si-O-Si and  $\nu_{as}$  Si-O-C), 797 ( $\delta$  Si-O-Si).

**18-SiO<sub>2</sub>-b**. Silica **18-SiO<sub>2</sub>-a** (0.021 g) were suspended in MeCN (2.5 ml) and MeI was added (7.19  $\mu$ l, 0.12 mmol). The reaction was heated with stirring at 60°C for 3 days. Afterwards, solution was filtered off, the remaining solid was washed with MeCN (three times) and then with n-hexane (once). After drying under vacuum, material **18-SiO<sub>2</sub>-b** was obtained (81%, 0.17 g). IR (cm<sup>-1</sup>): 3402 ( $\nu$  SiO-H, br), 3000-2800 ( $\nu$  C-H), 1668 ( $\nu$   $\delta$ Si-O), 1575-1384 ( $\delta$  C-H,  $\delta$  C-C and  $\delta$  C-N), 1095 ( $\nu$  Si-O-Si and  $\nu_{as}$  Si-O-C), 797 ( $\delta$  Si-O-Si).

### 2.3 Antimicrobial activity

Methods used for microbial susceptibility tests in vitro followed instruction M7-A7 of Clinical and Laboratory Standards Institute (CLSI).<sup>51, 52</sup>

Compounds for analysis were suspended in small amount of demineralized sterile water and then in Mueller-Hinton Broth (BioMaxime) for testing of bacteria, and in RPMI-1640 Medium (Sigma) for yeasts. Serial two-fold dilutions were prepared in a microtiter tray in range 500-3.9 mg/L.

Test strains were inoculated into each well of a microtiter plate at 10<sup>6</sup> CFU of bacteria and 10<sup>4</sup> CFU of yeasts per 1 mL. After 24 h incubation at 37° C for bacteria and 48 h at 25° C for yeasts, increase in turbidity at 595 nm was measured with microplate reader (MR 680 Bio-Rad). MIC (minimal inhibitory concentration) values were the lowest concentrations where there was no measurable increase in optical density. MBC (minimal bactericidal concentration) was the

lowest concentration at which the compound killed all cells, there being no growth in subculture on the surface of appropriate rich agar for each organism in Petri dish after 24 or 28 h of incubation at the appropriate temperature.

### 3. Results and Discussion

#### 3.1 Synthesis of dendrimers and dendrons

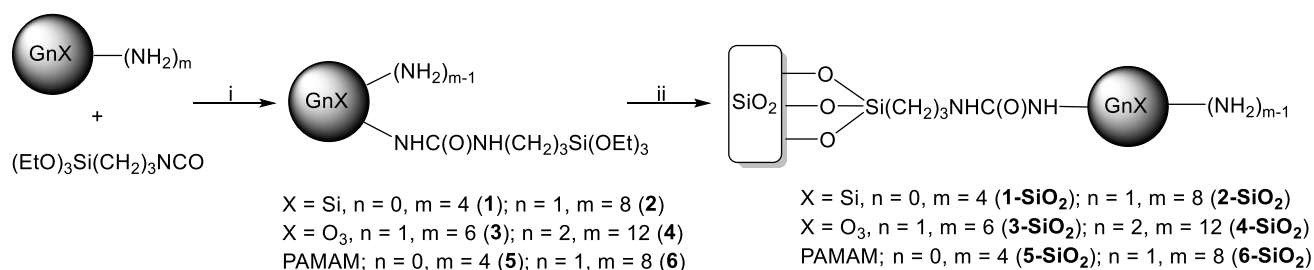
The nomenclature used for dendrimers is of the type  $[G_nX(S-Y)_m]$  (see Figure 1, S1 and S2), where  $G_n$  corresponds with the carbosilane (CBS) or poly(amidoamine) (PAMAM) framework and respective dendrimer generation (n); X refers to the core (Si for a silicon atom and  $O_3$  for the polyphenoxo core), or PAMAM for PAMAM dendrimers; and  $(S-Y)_m$ , indicate the type of peripheral groups (Y), its number (m), and the presence of a sulfur atom (in CBS dendrimers) close to the terminal groups due to the preparative method (thiol-ene addition, S). Regarding dendrons, they are named as  $XG_n(S-Y)_m$  (see Figure 1, S3 and S4), where X refers to the type of focal point;  $G_n$  corresponds with the carbosilane (CBS) framework and respective dendron generation (n); and  $(S-Y)_m$ , indicating the type of peripheral groups (Y), its number (m), and the presence of a sulfur atom close to the terminal groups due to the preparative method (thiol-ene addition, S).

The anchorage of dendrimers and dendrons to the silica surface has been done through the reaction of a  $(EtO)_3Si-$  moiety, introduced in both dendritic systems, with Si-OH groups of silica. Hence, we had first to incorporate this  $(EtO)_3Si-$  fragment to dendrimers and dendrons. For this, we used the reaction between 3-(triethoxysilyl)propyl isocyanate and an amine  $-NH_2$  function, which have to be present at the periphery of dendrimers or at the focal point of dendrons.

In previous works, we found that the type of core and generation of CBS dendrimers influenced notably in their antibacterial activity. For example, lower generation system derived from a polyphenoxo core and  $-NH_3^+$  peripheral groups (six charges) showed one of the best behaviors, whilst lower generation dendrimer derived from a Si atom core (four charges) showed, on the other hand, one of the worst behavior, being for this type of dendrimer the generation two

dendrimer clearly more active.<sup>43</sup> For this reason, we have chosen these two families of dendrimers for their binding to silica, so we can compare the different CBS structures.

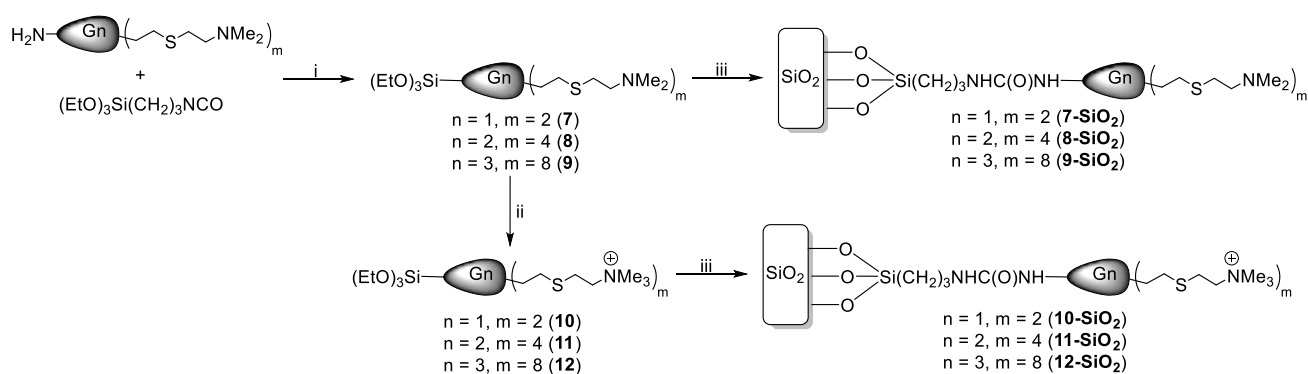
Therefore, the direct reaction of the corresponding dendrimers containing neutral amine  $-NH_2$  groups with an isocyanate derivative containing the  $(EtO)_3Si-$  unit, led to dendrimers containing both amine  $-NH_2$  functions and one  $(EtO)_3Si-$  fragment (reaction ratio 1/1) (Scheme 1). This procedure allowed us the obtaining of dendrimers  $[GnX(S-NH_2)_{m-1}(Si(OEt)_3)]$  ( $X = Si$ ,  $n = 0$ ,  $m = 4$  (**1**);  $X = Si$ ,  $n = 1$ ,  $m = 8$  (**2**);  $X = O_3$ ,  $n = 1$ ,  $m = 6$  (**3**);  $X = O_3$ ,  $n = 2$ ,  $m = 12$  (**4**)) (Figure 1 and S1). The modification of dendrimers was detected by NMR spectroscopy. In the  $^1H$  NMR spectra, the new methylene groups bound to the urea moiety ( $\delta$  ca. 3.3) and the terminal OEt groups were observed ( $\delta$  ca. 3.9 for  $OCH_2$  and  $\delta$  ca. 1.3 for Me groups, respectively); in the  $^{13}C$  NMR spectra the relevant carbon atom belonging to the urea unit was observed at  $\delta$  ca. 160.8. IR spectroscopy was also useful, since it was observed the disappearance of the isocyanate band ( $N=C=O$ ,  $2250\text{ cm}^{-1}$ ) together with the appearance of the band corresponding to carbonyl of the new urea bond ( $1635\text{ cm}^{-1}$ ). The same procedure was successful to prepare amine terminated PAMAM dendrimers of generations zero (**5**) and one (**6**) (Scheme 1, Figure S2).



**Scheme 1.** Synthesis of amine dendrimers containing a  $(EtO)_3Si-$  moiety and silica modified with CBS and PAMAM dendrimers. i)  $NEt_3$ , r. T. 24 h; ii)  $SiO_2$ , DMF,  $80^\circ C$ , 72 h.

Regarding dendrons, we observed that CBS dendrons presenting  $-NMe_3^+$  groups showed the best antibacterial activities.<sup>45</sup> For this reason, dendrons with peripheral functions  $NMe_2$  and  $-NMe_3^+$  have been chosen for silica dendronization, and thus also the effect of neutral or cationic groups on surface functionalization can be evaluated.

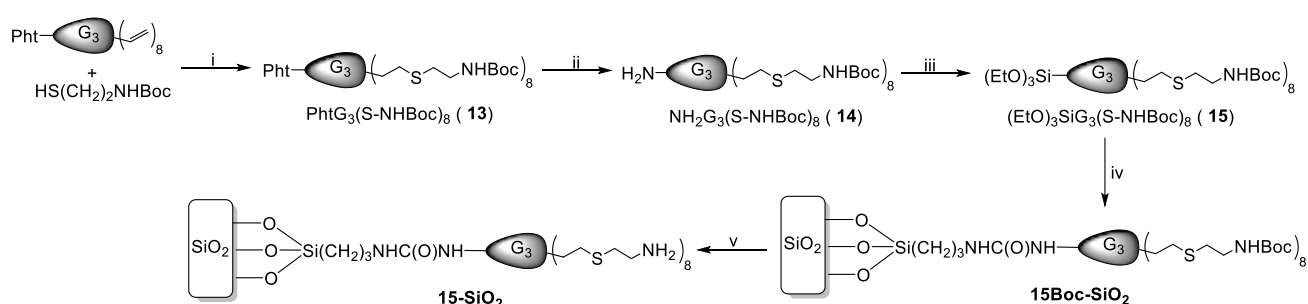
Reactions of neutral dendrons of the type  $\text{NH}_2\text{G}_n(\text{S-NMe}_2)_m$  with 3-(triethoxysilyl)propyl isocyanate led to the corresponding dendrons  $(\text{EtO})_3\text{SiG}_n(\text{S-NMe}_2)_m$  ( $n = 1, m = 2$  (**7**);  $n = 2, m = 4$  (**8**);  $n = 3, m = 8$  (**9**)<sup>53</sup>) (Scheme 2, Figure 1 and S3). These compounds were obtained as pale yellow oils with high yields. NMR and IR spectroscopy were again very useful to confirm the transformation of the focal point with formation of the urea moiety, similar to the data described for dendrimers. These dendrons were transformed into the respective cationic ones by addition of methyl iodide (MeI), thus obtaining the dendrons  $(\text{EtO})_3\text{SiG}_n(\text{S-NMe}_3^+)_m$  ( $n = 1, m = 2$  (**10**);  $n = 2, m = 4$  (**11**);  $n = 3, m = 8$  (**12**)) (Scheme 2, Figure 1 and S4). Dendrons **7-12** were obtained as pale yellow solids in high yield. <sup>1</sup>H NMR spectroscopy clearly showed the shifting to higher frequency of the MeN and CH<sub>2</sub>N resonances after nitrogen methylation.



**Scheme 2.** Synthesis of dendrons containing a  $(\text{EtO})_3\text{Si-}$  moiety and silica modified with these CBS dendrons. i)  $\text{NEt}_3$ , r. T., 16 h; ii) MeI; iii)  $\text{SiO}_2$ , DMF, 80° C, 72 h.

Taking into account the results obtained in the bactericidal studies, we have also designed a third generation dendron with protected primary amino groups to synthesize silica surfaces with a dendron of this generation and  $-\text{NH}_3^+$  ammonium functions and, thus, to compare the activity of dendrons of this generation with  $-\text{NH}_3^+$  and  $-\text{NMe}_3^+$  groups. For this purpose, starting from the vinyl dendron  $\text{PhtG}_3\text{V}_8$  (V corresponds to vinyl functions and Pht to a phthalimide function, which is precursor of primary amine group) the thiol-ene addition reaction with 2-(Boc-amino)ethanethiol was carried out, obtaining  $\text{PhtG}_3(\text{S-NHBoc})_8$  (**13**) in high yields (Scheme 3). Transformation of the vinyl functions was clearly observed in NMR spectroscopy by the disappearance of the resonances of this group in both <sup>1</sup>H and <sup>13</sup>C NMR spectra. These spectra

also showed the formation of the new chain  $\text{Si}(\text{CH}_2)_2\text{S}$ , resonances at  $\delta$  0.84 ( $\text{SiCH}_2$ ) and  $\delta$  2.57 ( $\text{CH}_2\text{S}$ ) in the  $^1\text{H}$  NMR spectra and at  $\delta$  14.5 ( $\text{SiCH}_2$ ) and  $\delta$  27.0 ( $\text{CH}_2\text{S}$ ) in the  $^{13}\text{C}$  NMR spectra. The presence of the new peripheral functions were observed at  $\delta$  3.20 ( $\text{CH}_2\text{NHBoc}$ ) in the  $^1\text{H}$  NMR spectra and at  $\delta$  155.5 in the  $^{13}\text{C}$  NMR spectra for the carbonyl carbon atom. Next, the focal point was unprotected with excess hydrazine affording  $\text{NH}_2\text{G}_3(\text{S-NHBoc})_8$  (**14**) (Scheme 3). This compound was treated as previous dendrimers and dendrons to obtain the derivative with a triethoxysilyl group at the focal point  $(\text{EtO})_3\text{SiG}_3(\text{S-NHBoc})_8$  (**15**) (Scheme 3, Figure 1). The NMR and IR changes observed for **15** with respect to **14** were analogous to those described for dendrons **7-9**.



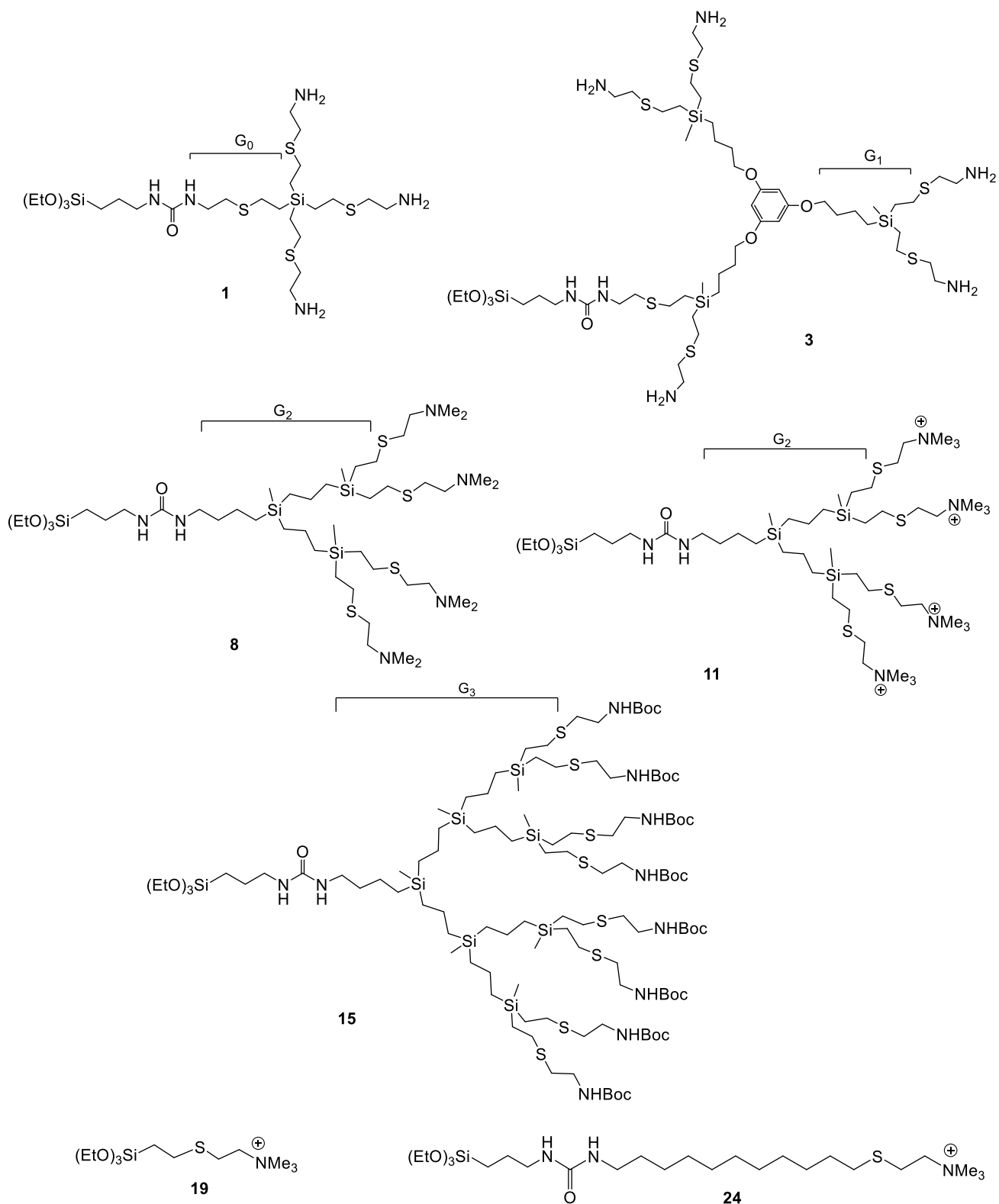
**Scheme 3.** Synthesis of dendron with amine-protected groups and corresponding unprotected silica. i)  $h\nu$ , DMPA (2,2-dimethoxy-2-phenylacetophenone); ii)  $\text{NH}_2\text{-NH}_2$ ,  $80^\circ\text{C}$ ; iii)  $(\text{EtO})_3\text{Si}(\text{CH}_2)_3\text{NCO}$ ; iv)  $\text{SiO}_2$ , DMF,  $80^\circ\text{C}$ ; v) TFA, NaCl.

Dendrimers and dendrons not only present a multivalent periphery, but also their grafting to a surface would move away the active groups from the surface depending on the generation. To inquire in this last effect, amine and ammonium monomers hanging from short and long chains that present in the other end a triethoxysilyl moiety have been prepared.

On one hand, the short length compounds were synthesized (Figure 1, Scheme S1) from triethoxy vinyl silane by thiol-ene addition with  $\text{HS}(\text{CH}_2)_2\text{NR}_2\text{H}^+$  ( $\text{R} = \text{H}, \text{Me}$ ), which led to  $(\text{EtO})_3\text{Si}(\text{CH}_2)_2\text{S}(\text{CH}_2)_2\text{NR}_2\text{H}^+$  ( $\text{R} = \text{H}$  (**16**),  $\text{Me}$  (**17**)). Compound **16** was used as obtained for silica functionalization (see below). Regarding **17**, neutralization led to  $(\text{EtO})_3\text{Si}(\text{CH}_2)_2\text{S}(\text{CH}_2)_2\text{NMe}_2$  (**18**) that was transformed into the cationic derivative

$(\text{EtO})_3\text{Si}(\text{CH}_2)_2\text{S}(\text{CH}_2)_2\text{NMe}_3^+$  (**19**) after addition of MeI. Compounds **16-19** were characterized by NMR spectroscopy in a similar way that analogous dendrons described above.

On the other hand, the long-chain derivatives were prepared from 11-bromo-1-undecene, which alkyl chain length is equal to that of generation two dendron (Scheme S2, Figure 1). The first transformation step involved the substitution of the bromine atom by a phthalimide group, as precursor of a primary amine function. The  $^1\text{H}$  NMR spectrum of  $\text{Pht}(\text{CH}_2)_9\text{CH}=\text{CH}_2$  (**20**) showed the change from a  $\text{CH}_2\text{Br}$  group ( $\delta$  3.42) to a  $\text{PhtCH}_2$  group ( $\delta$  3.65). Next, following similar protocols than those used for modification of dendrons and monomers of short chain, neutral  $(\text{EtO})_3\text{Si}(\text{CH}_2)_3\text{NHC}(\text{O})\text{NH}(\text{CH}_2)_{11}\text{S}(\text{CH}_2)_2\text{NMe}_2$  (**23**) and cationic  $(\text{EtO})_3\text{Si}(\text{CH}_2)_3\text{NHC}(\text{O})\text{NH}(\text{CH}_2)_{11}\text{S}(\text{CH}_2)_2\text{NMe}_3^+$  (**24**) long chain monomers with a triethoxysilyl unit were obtained. Again, NMR and IR spectroscopy confirmed formation of these compounds by mean of resonances analogous to those observed in dendrimers, dendrons and monomers described above.



**Figure 1.** Examples of G<sub>0</sub> and G<sub>1</sub> dendrimers, G<sub>2</sub> dendrons, G<sub>3</sub> dendron with NHBoc groups, and short- and long-chain models of compounds used in this work with a (EtO)<sub>3</sub>Si-fragment.

### 3.2 Silica modification



Silica	Type	Gn <sup>a</sup>	F <sup>b</sup>	NF <sup>c</sup>	ZP <sup>d</sup>	TGA <sup>e</sup>	NF/100 g <sup>f</sup>
<b>1-SiO<sub>2</sub></b>	CBS	0	-NH <sub>2</sub>	3	28.5±0.7	23.77	0.13
<b>2-SiO<sub>2</sub></b>	CBS	1	-NH <sub>2</sub>	7	24.0±0.8	14.36	0.08
<b>3-SiO<sub>2</sub></b>	CBS	1	-NH <sub>2</sub>	5	42.1±1.8	37.93	0.17
<b>4-SiO<sub>2</sub></b>	CBS	2	-NH <sub>2</sub>	11	31.4±0.8	17.50	0.08
<b>5-SiO<sub>2</sub></b>	PAMAM	0	-NH <sub>2</sub>	3	-20.5±0.9	21.64	0.11
<b>6-SiO<sub>2</sub></b>	PAMAM	1	-NH <sub>2</sub>	7	32.0±2.7	25.62	0.12
<b>7-SiO<sub>2</sub></b>	CBS	1	-NMe <sub>2</sub>	2	X	20.03	0.09
<b>8-SiO<sub>2</sub></b>	CBS	2	-NMe <sub>2</sub>	4	X	26.18	0.12
<b>9-SiO<sub>2</sub></b>	CBS	3	-NMe <sub>2</sub>	8	X	20.61	0.09
<b>10-SiO<sub>2</sub></b>	CBS	1	-NMe <sub>3</sub> <sup>+</sup>	2	35.8±0.5	18.41	0.05
<b>11-SiO<sub>2</sub></b>	CBS	2	-NMe <sub>3</sub> <sup>+</sup>	4	37.5±0.6	19.56	0.05
<b>12-SiO<sub>2</sub></b>	CBS	3	-NMe <sub>3</sub> <sup>+</sup>	8	36.7±0.4	19.81	0.05
<b>15Boc-SiO<sub>2</sub></b>	CBS	3	-NHBoc	8	-2.63±2.0	16.06	0.05
<b>15-SiO<sub>2</sub></b>	CBS	3	-NH <sub>2</sub>	8	33.0± 1.4	11.86	0.05
<b>16-SiO<sub>2</sub>-a</b>	Linear	X	-NH <sub>2</sub>	1	35.5±1.6	23.63	0.15
<b>16-SiO<sub>2</sub>-b</b>	Linear	X	-NH <sub>2</sub>	1	30.0±3.6	30.81	0.19
<b>18-SiO<sub>2</sub>-a</b>	Linear	X	-NMe <sub>2</sub>	1	X	24.49	0.13
<b>18-SiO<sub>2</sub>-b</b>	Linear	X	-NMe <sub>2</sub> -NMe <sub>3</sub> <sup>+</sup>	1	33.9±1.1	42.91	0.08 0.05
<b>19-SiO<sub>2</sub></b>	Linear	X	-NMe <sub>3</sub> <sup>+</sup>	1	34.1±0.8	26.70	0.08
<b>24-SiO<sub>2</sub></b>	Linear	X	-NMe <sub>3</sub> <sup>+</sup>	1	36.0±1.9	21.01	0.06

**Table 1.** Selected data of modified silicas. a) Gn refers to generation; b) F refers to function; c) Number of functions on molecule attached to silica; d) Zeta potential (mV); e) % of organic

mass obtained by thermogravimetric analysis (TGA); f) Number of functions per 100 g of silica (obtained by TGA).

The synthesis of all modified silicas (Table 1) from neutral or cationic compounds described above were done following a similar procedure: predried silica was heated at 80° C with the triethoxysilyl derivatives in dry DMF for 72 h (in the presence of  $\text{NEt}_3$  if precursors with  $-\text{NH}_3^+$  groups were used); the ratio silica/functional group was 30 g  $\text{SiO}_2$  per 0.23 functions, that is, the reactions were done taking into account the number of functional groups at the periphery of the systems. After purification (see experimental section), the corresponding silica materials were obtained (**1-16-SiO<sub>2</sub>**, Schemes 1-3 and S1-S2) as white to yellow powders in high yield. An additional ratio silica/functional group was tested for short chain model (**16**) to compare the relevance of the ratio, and a silica using 30 g  $\text{SiO}_2$  per 0.46 functions was prepared (**16-SiO<sub>2</sub>-b**). On the other hand, the silica covered with protected amino groups **15Boc-SiO<sub>2</sub>** was transformed into the ammonium one by addition of excess TFA, further washing with NaCl to remove trifluoroacetate anions and finally with water, giving **15-SiO<sub>2</sub>**. All these materials were characterized by TGA, IR, SEM, EDX, Z potential.

*-Characterization by thermogravimetric analysis (TGA)*

The degree of silica functionalization was deduced from TGA (Table 1, Figure S5-S25). The data showed that these values depended on the structure of molecules used to grafted silica surface. For silica modified with cationic linear compounds, the number of groups that silica bears when the short chain compound was used (**19-SiO<sub>2</sub>**) was ca. 1.5 fold when compared with silica modified with long linear model (**24-SiO<sub>2</sub>**). This difference can be attributed to higher steric hindrance of longer chains.

On the other hand, neutral short chain model silica **18-SiO<sub>2</sub>-a** contains ca. double number of groups than corresponding silica modified with cationic short chain model **19-SiO<sub>2</sub>**. A similar tendency was observed for each pair of neutral/cationic silicas modified with dendrons (**7/10-SiO<sub>2</sub>**, **8/11-SiO<sub>2</sub>** and **9/12-SiO<sub>2</sub>**). Probably, in this case charge repulsion between ammonium groups conditions silica functionalization. This fact was confirmed when neutral silica **18-SiO<sub>2</sub>-a**

was treated with excess MeI, obtaining a new material **18-SiO<sub>2</sub>-b** with about half of the groups quaternized (58% of -NMe<sub>2</sub>, 42% of -NMe<sub>3</sub><sup>+</sup>).

Dendron generation has no influence in the functionalization degree of silicas. However, the number of groups on dendronized silica were around 66% of functions that cover corresponding silica containing short chain models, but for silica with neutral G2 dendron **8-SiO<sub>2</sub>**, which was close to the short model **18-SiO<sub>2</sub>-a**.

For dendrimers, the type of framework and generation determines the amount of groups on silica surface when compared with silica covered with linear chains. In the case of **1-SiO<sub>2</sub>**, covered with G0 CBS dendrimers with a Si atom core, the number of -NH<sub>2</sub> groups on the surface was close to those on the short linear compound **16-SiO<sub>2</sub>-a**, whilst for **2-SiO<sub>2</sub>** covered with a related G1 dendrimer the functionalization was reduced by half. A similar number of -NH<sub>2</sub> groups was calculated for **4-SiO<sub>2</sub>**, covered with G2 CBS dendrimers with a polyphenoxo core. Surprisingly, the corresponding **3-SiO<sub>2</sub>**, covered with the G1 CBS dendrimers, contains more -NH<sub>2</sub> moieties than the silica covered with the short linear model **16-SiO<sub>2</sub>-a**. Probably, the combination of factors as small branches and a planar core minimize interaction between dendrimers. It is important to remark that this core is clearly bigger than the Si atom core in the other CBS dendrimers, spreading apart the branches from silica surface. Moreover, if the aromatic rings are close enough they could establish  $\pi$ - $\pi$  interactions, which would increase stability. For silica functionalized with PAMAM dendrimers **5-SiO<sub>2</sub>** and **6-SiO<sub>2</sub>**, the number of groups was similar for both silicas and to those of the short model **16-SiO<sub>2</sub>-a**.

The influence of terminal group of short chain compounds, -NH<sub>2</sub> vs -NMe<sub>2</sub>, was little significant for silica functionalization (**16-SiO<sub>2</sub>-a** and **18-SiO<sub>2</sub>-a**). Regarding the reaction ratio silica/number functions, if the amount of the short linear model is increased 100%, the silica functionalization only increases 30% (**16-SiO<sub>2</sub>-b**).

*-Characterization by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX)*

The starting bulk silica was about 500  $\mu\text{m}$  of diameter. The grafting of dendritic molecules and models crushed the silica,<sup>54</sup> showing a diameter of ca. 25  $\mu\text{m}$  (SEM, Figure S26). An EDX

analysis (for Si, S and I atoms, Table S1, Figures S27-S33) showed higher percentage of sulfur atoms for silica covered with the short chain model **18-SiO<sub>2</sub>-a** and also a higher noticeable value for silica covered with G1 CBS dendrimers with a polyphenoxo core **3-SiO<sub>2</sub>**.

*-Characterization by infrared spectroscopy (IR)*

IR spectra of silicas (Figures S34-S37) showed bands corresponding to both SiO<sub>2</sub> and linear or dendritic systems (C-H and N-H around 2900 cm<sup>-1</sup>, C-C and C-N around 1500 cm<sup>-1</sup>). These last bands were not observed in pristine silica.

*-Characterization by zeta (Z) potential*

Measurement of Z potential of silica suspensions in water gave important information about stability of these materials in these media (Figure S38). These analyses showed that Z potential diminished with time, the rate and final Z potential values depending on type and number of functions and also on distance of functions to surface. Unfortunately, Z potential of some neutral silicas could not be measured because the material was not adequately dispersed in water.

Silicas covered with higher number of functions, were more stable. Silicas covered with neutral groups were also more stable than those covered with cationic systems (for those systems which Z potential could be measured). These results are outstanding for silicas with short chain models, since cationic silica **19-SiO<sub>2</sub>** showed a sharp change in its Z potential, from ca +35 mV to 0 mv in 2 days, whilst Z potential for silica containing neutral and ammonium groups **18-SiO<sub>2</sub>-b** got closer to 0 mv after 30 days. Unfortunately, the Z potential for neutral silica **18-SiO<sub>2</sub>-a** could not be measured. On the other hand, cationic silica covered with long alkyl chains **24-SiO<sub>2</sub>** kept its Z potential value close to +35 mV for months. Since this silica contains less groups than cationic silica **19-SiO<sub>2</sub>**, this datum also reflects the relevance of the shifting apart of groups from surface.

Regarding silica covered with short chain models functionalized with -NH<sub>2</sub> moieties, Z potential of **16-SiO<sub>2</sub>-a** moved from ca. +35 mV to 0 mV in 10 days while Z potential of **16-SiO<sub>2</sub>-b**, containing 30% more groups, moved to +15 mV after 35 days. Hence, more protected are silica surface more stable are the silicas. The behavior observed is probably consequence of the collapse of outer groups over silica surface, which is covered with neutral hydroxyl and anionic

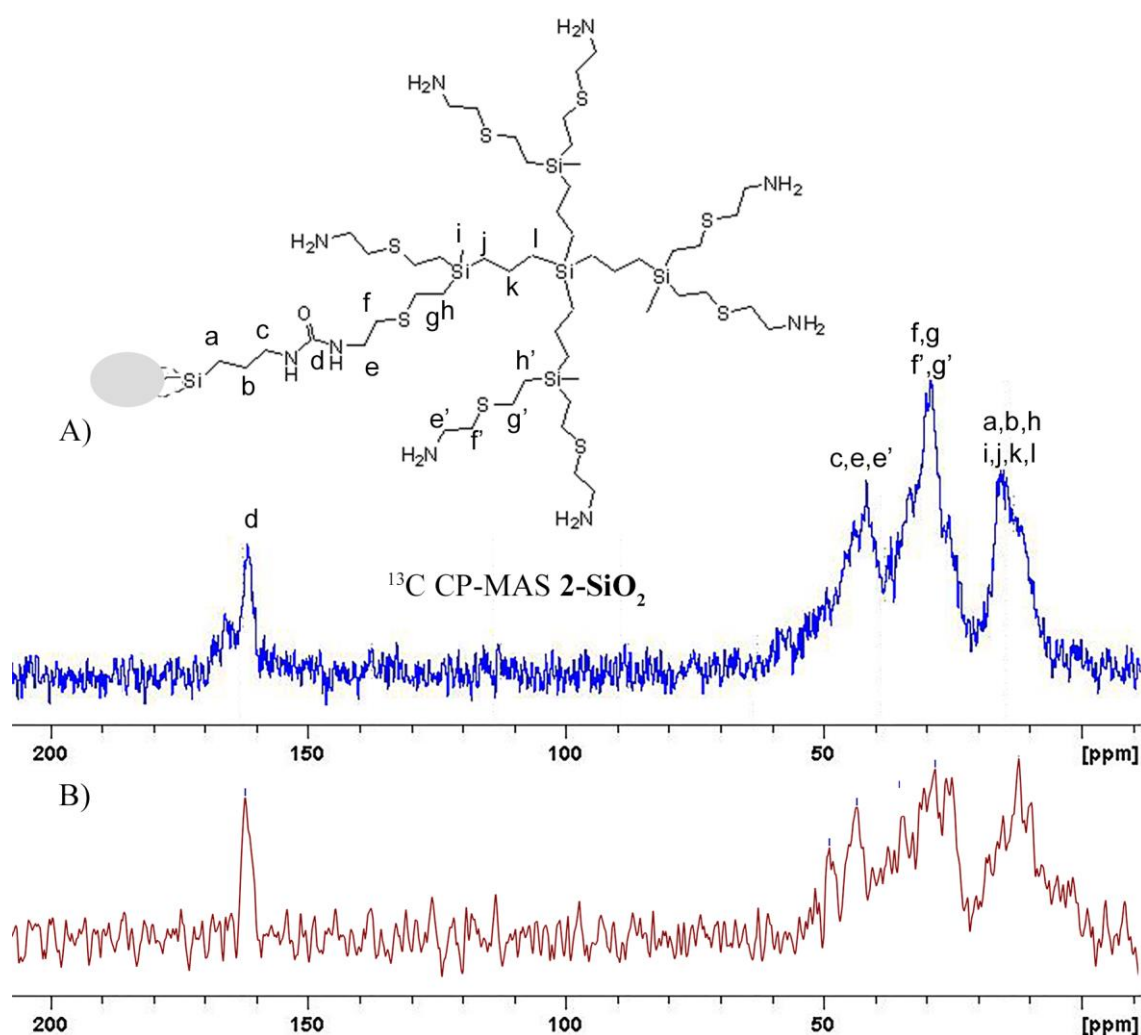
siloxo groups, being this interaction favored for cationic functions. This assessment was confirmed by adding NaCl to cationic silica **19-SiO<sub>2</sub>**, which clearly improved its stability, observing that after 10 days the Z potential was still ca. +15 mV. In this case, Na<sup>+</sup> cations covered the surface hampering the interaction of the cationic moieties of **19-SiO<sub>2</sub>** with the silica surface. In a different experience, a saturated NaCl water solution was added to a silica suspension, **16-SiO<sub>2</sub>-b**, that was kept in water for 35 days. Then, the Z potential changed in the opposite way, from ca. +15 mV to ca. +30 mV, again pointing that outer amine and ammonium groups interact with silica surface.

In general, silica covered with dendrons and dendrimers were clearly more stable than silica covered with with lineal alkyl chains used as models. As for models, the Z potential of silicas covered with neutral dendrons could not be measured. The most stable systems were silica **3-SiO<sub>2</sub>**, functionalized with G1 CBS dendrimer with a polyphenoxo core, and **12-SiO<sub>2</sub>**, functionalized with cationic G3 CBS dendrons. In the first case, the reason is that this silica is the most functionalized whereas the reason for the second case is that the ammonium groups are attached to the longest chains, making more difficult the folding of the chains. Regarding silicas modified with PAMAM dendrimers, **5-SiO<sub>2</sub>** and **6-SiO<sub>2</sub>**, they showed also an initial Z potential value close to +30 mV. Stirring these silicas in water suspensions caused the appearing of several peaks that became closer to 0 mV (Figure S39). The slightly different behavior of these silicas with the analogous silicas covered with CBS dendrimers with a Si atom core could be related with the differences in the framework. PAMAM framework is hydrophilic and contains amine and amide units that can also interact with silica surface, whilst the hydrophobic CBS framework would hardly interact with this surface.

*-Characterization by nuclear magnetic resonance (<sup>13</sup>C CP-MAS NMR)*

The variations of Z potential of the silicas discussed above have been justified by collapse of outer groups on silica surface, supposing that no structural modification of the skeleton was produced. This lack of structural change was confirmed by <sup>13</sup>C CP-MAS NMR spectroscopy of two different types of silicas (Figures 2 and S40). One of them, **19-SiO<sub>2</sub>**, presents a thioether group close to silica surface, is covered with the cationic short chain model and its Z potential

moved down until 0 mV faster than in any other silica. The other one, **2-SiO<sub>2</sub>**, contains a urea group close to the silica surface and is covered with a G1 CBS dendrimer. The spectra of these two silicas were carried out after their synthesis and were compared with the spectra obtained after being suspended and stirred in water until Z potential moved down to 0 mV. The only changed observed in the spectra was broadening of the resonances, probably because the interaction of the external groups on silica surface reduced mobility of branches, but meaning that compounds grafted on the silica remained structurally unaltered.

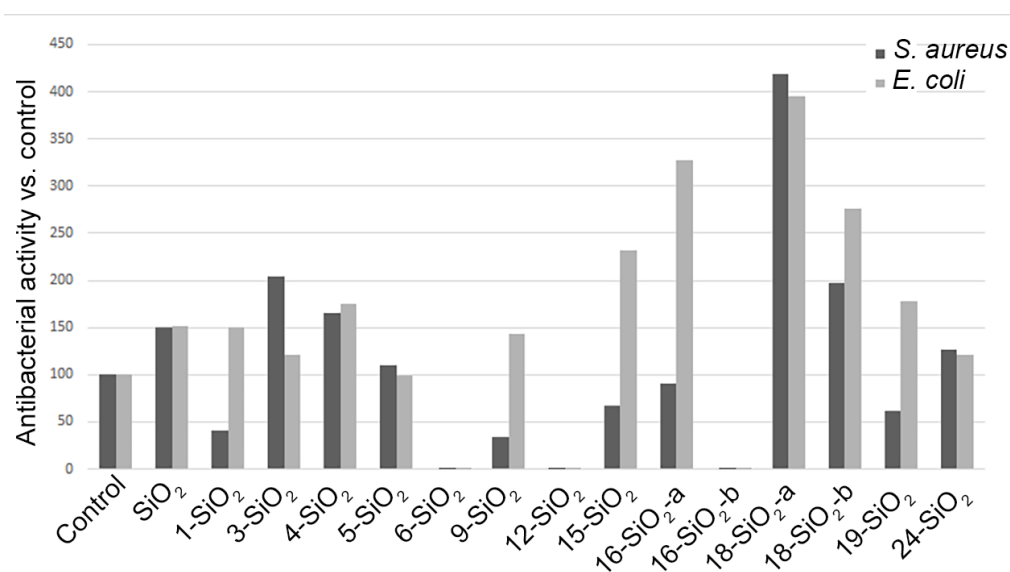


**Figure 2.** <sup>13</sup>C CP-MAS spectra of **2-SiO<sub>2</sub>** before water treatment (A) and after water treatment (B).

### 3.3. Antibacterial activity

A preliminary antibacterial test was carried out for the modified silicas by light scattering analysis. Two type of bacteria were selected for these assays, *S. aureus* as model of Gram-

positive bacteria and *E. coli* as model of Gram-negative bacteria. 96 well plates containing bacteria were treated with two different concentrations of functionalized silicas (20 and 10 mg/ml). The change in light scattering (630 nm) was measured at initial time and after 24 h of treatment (Table S2). The increase of light scattering means that bacteria grow (in red in Table S2). These experiments showed inhibition at 20 ppm for all the systems in both bacteria strains. At 10 ppm, inhibition ability was clearly reduced for almost all the systems in *E. coli*, but for G3 CBS dendrons. On the other hand, all of them were active at this concentration against *S. aureus* but **2-SiO<sub>2</sub>** covered with G1 carbosilane dendrimer with a Si atom core.



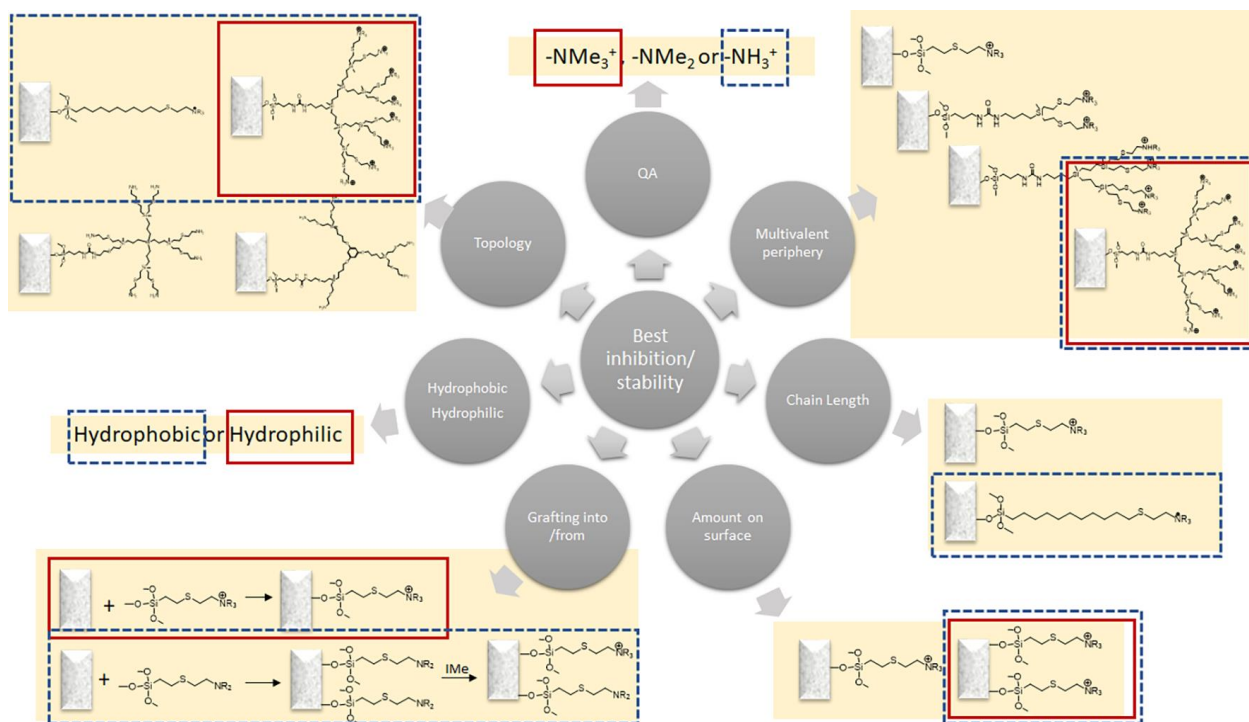
**Figure 3.** Antibacterial activity of selected modified silicas.

A second test was done to analyze antibacterial properties, also in *S. aureus* and *E. coli* bacteria. In this case, bacteria were treated with the materials (10 mg/ml) and then after 24 h the suspensions were centrifuged to remove these materials. The supernatant was used to seed and after 24 h of incubation at 37°C the colony counting units (CFU) were calculated. The results point that antibacterial activity was dependent on functionalization degree, type of peripheral group and dendrimer framework (Figures 3 and 4). Among all the systems studied, only three of them showed remarkable inhibition capacity in both bacteria strains, one dendrimer, one dendron and one linear model. It is noteworthy that some of these combinations, mainly with neutral groups, even make bacteria growing easier, whilst others clearly inhibit it.

For silicas covered with dendrimers, which are functionalized with  $\text{-NH}_2$  groups, the only active one was that covered with G1 PAMAM dendrimer (**6-SiO<sub>2</sub>**). Among other silicas, only **1-SiO<sub>2</sub>** showed some degree of activity in *S. aureus*. We can ascribe this fact to the hydrophilic character of PAMAM dendrimers that favor self-protonation of outer amine functions but also of the internal ones. Moreover, the hydrophilic nature of PAMAM framework can facilitate the exposure of the peripheral groups. CBS dendrimers only contain amine groups at the periphery and their hydrophobic framework can be a hurdle for interaction with surrounding water.

Regarding silicas decorated with 3G CBS dendrons, the most active one was **12-SiO<sub>2</sub>**, which is covered with  $\text{-NMe}_3^+$  moieties. In this case, we believe that the permanent cationic charge on this silica and the size of the dendron, facilitating the exposure of the outermost groups, are responsible of the activity.

For silica covered with models, the highest active system was **16-SiO<sub>2</sub>-b**. This silica is the most functionalized silica, contains 30% more  $\text{-NH}_2$  groups on surface than **16-SiO<sub>2</sub>-a**. In comparison, the other silica with highest functionalization, also containing neutral  $\text{-NH}_2$  groups, **3-SiO<sub>2</sub>**, was not active. We believe that being the silica covered with a small dendrimer, part of the groups cannot be adequately exposed for interaction with bacteria.





**Figure 4.** Summary of compounds with best antibacterial activity (solid red line) and with the best stability in water suspension (discontinuous blue line).

#### 4. Conclusions

The modification of dendrimers, dendrons and alkyl chains with a  $(\text{EtO})_3\text{Si}$ - unit has allowed obtaining a library of silicas with amine and ammonium on the surface. The number of functional groups depends on ligand structure, dendritic generation and type of outermost function. Z potential analysis and  $^{13}\text{C}$  CP MAS NMR spectroscopy have demonstrated that the amine and ammonium groups can interact with silica surface. This interaction is probably due to the backfolding of ligand frameworks, although the structure integrity was kept. This folding is also dependent on ligand structure, dendritic generation and type of outermost function. The most stable materials were those covered with higher number of functions and those keeping further away the active groups from the surface (higher generation and longer chains).

All the above characteristics have also striking influence on the inhibitory activity of these amine and ammonium modified silicas. For example, the folding of the structure would reduce the exposure of the active groups with passing time and thus, the activity would be also reduced. For these reasons, the silica **12-SiO<sub>2</sub>** covered with cationic G3 dendrons was one of the most active and stable systems. Finally, the stability observed will prevent the release to the environment of this ligand from the silica surface. It is also important to note that with this type of system less anchorage points are needed to achieve an active surface, and then G3 dendron should be very suitable to transfer bactericidal properties to other surfaces less prone to suffer modifications. Research to improve water stability of the systems, to study other active antibacterial groups and surfaces are in progress.

#### 5. Acknowledgments.

This work was supported by grants from CTQ2017-86224-P (MINECO), Consortium NANODENDMED II-CM ref B2017/BMD-3703 (CAM), Consortium IMMUNOTHERCAN-CM B2017/BMD-3733 (CAM). CIBER-BBN is an initiative funded by the VI National R&D&i Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by

the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

M. S.-M. wishes to thank UAH for a fellowship (FPI 2015).

## 6. Declaration of Competing Interest

The authors have no competing interests to declare.

**7. Supplementary Data.** Full experimental section, drawing of dendrimers and dendrons, NMR spectra, SEM images, complete antibacterial tables.

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For Graphical Abstract:  
**Functionalization of silica with amine and ammonium alkyl chains, dendrons and  
dendrimers: synthesis and antibacterial properties**

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Antibacterial activity	-	-	+++	+++
Stability in water	-	+++	+++	+

Stability in water and antibacterial activity of silicas covered with carbosilane (CBS) dendrimers and dendrons and PAMAM dendrimers is dependent on ligand structure, generation and peripheral functions.