Strategies for Penicillin V dendronization with cationic carbosilane dendrons and study of antibacterial properties

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Strategies for Penicillin V dendronization with cationic carbosilane dendrons and study of antibacterial properties


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Abstract

Strategies to synthesize a cationic carbosilane dendron containing the antibiotic penicillin V potassium salt (PenVK) at the focal point are discussed. The preparation of such compound requires the use of systems with no donor atoms, such as N or S, in their framework because they produced the rupture of the penicillin β-lactam ring. The antibacterial activity of the new dendron containing ammonium groups, at the periphery, and the PenV moiety, at the focal point, against gram-positive *Staphylococcus aureus* strains was evaluated. These results were compared with those obtained for free PenVK, a related cationic dendron without penicillin moiety at the focal point, and also with an equimolar mixture of this last dendron with free PenV. The data obtained indicate that conjugation or interaction of PenV with cationic dendrons reduces its activity in comparison with free PenVK. On the other hand, the penicillin-dendron is able to release the antibiotic in the presence of esterase, due to the breaking of the ester bond in this derivative.

**Keywords**: dendritic molecules, carbosilane dendrons, antibacterial, penicillin, antibiotic.
1. Introduction

The widespread use and misuse of antibiotics have favored the appearance of antibiotic-resistant bacteria, creating a major public health concern. The problem is worsened by the ability of bacteria to create biofilms, which are involved in the majority of infection diseases caused by bacteria. Thereby, the need of finding compounds with non-specific antibacterial activity to fight resistance and/or reduce antibiotic dependence is a priority.

The bacterial cell walls are formed with negative lipoproteins and divalent cations, such as Mg$^{2+}$ and Ca$^{2+}$, acting as glue. Thus, quaternary ammonium salts (QAS) have become popular antimicrobials due to their ability to replace these cations and destroy the cell wall. Another advantage of some QAS is their solubility in water, which allows them to kill bacteria in water solution. The incorporation of QAS to polymers generates polycations that present a high surface charge. The multivalency of these polyammonium macromolecules, such as polymers, hyperbranched polymers and dendrimers, increases the activity with respect to monofunctional molecules.

Dendrimers and dendrons are multibranched macromolecules designed step by step, which leads to well-defined structures. Dendrimers are spherical molecules, whereas dendrons are cone-shaped molecules, presenting an extra active moiety, the focal point, which can be used to attach a second functionality or to dendronize materials. The well-known structures of these systems make easier to establish structure-activity relationships. Several types of ammonium dendrimers, depending on their dendritic structure, have shown antibacterial properties. Their activity is increased by the presence of hydrophobic chains, which enables their penetration into the phospholipid bilayer leading to the disintegration of the bacterial membrane. Regarding dendrons, antimicrobial studies are scarce and usually they are associated to the generation of dendronized materials, since the focal point is an excellent anchorage position for functionalization.

Dendrimers have also been employed as drug carriers by electrostatic interaction between drugs and surface groups, conjugation of drug to dendrimer, or by encapsulation of drug inside hyperbranched...
framework.\textsuperscript{31, 32} For example, it has been reported that some dendrimers enhance solubility and activity of antibiotics.\textsuperscript{33-37} Moreover, synergistic effect has been observed for some combinations of dendrimers with antibacterial properties and antibiotics.\textsuperscript{38-40}

One type of dendrimers is based on carbosilane framework, which contains very low polar C-C and Si-C bonds.\textsuperscript{41, 42} The decoration of dendrimer surface with ammonium functions introduces antimicrobial properties and water solubility to these dendrimers.\textsuperscript{43-46} Recently, we have published an evaluation of antibacterial activity of cationic carbosilane dendrimers and dendrons by comparing generation, core of dendrimers, focal point of dendrons, and type of ammonium groups.\textsuperscript{47} These results highlight the good activity obtained for low generation systems (generation one for dendrimers and two for dendrons with six and four ammonium groups, respectively) against both gram-positive and gram-negative bacteria and also against resistant \textit{S. aureus} strains. Moreover, these systems did not generate resistance after continuous treatments. We have also reported the synergy produced after combination of a cationic dendrimer with an amoebicide compound (chlorhexidine digluconate).\textsuperscript{48}

Taking into account these results, we have considered the evaluation of cationic carbosilane dendrons of second generation as antibiotic carriers, since it could be expected that combination of biocide compounds with different modes of action could act synergistically.\textsuperscript{49} As antibiotic we have chosen a penicillin derivative (penicillin V, PenV) for its simplicity and the presence of one carboxylate moiety. This anionic group will be use to interact electrostatically with the cationic dendrons\textsuperscript{50} or to allow conjugation to the dendron employing an adequate group at the focal point.\textsuperscript{51} The synthetic procedure to obtain the new dendrons and also the difficulties found to do it, their characterization and antibacterial activity against \textit{S. aureus} are here discussed.

### 2. Experimental Section

#### 2.1. 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 (\(^1\)H), 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, meanwhile \(^{29}\)Si resonances were measured relative to external TMS employing \(^1\)H-\(^{29}\)Si HMBC experiments. When necessary, assignment of resonances was done from HSQC, HMBC, COSY, TOCSY and NOESY NMR experiments. 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. Elemental analyses were performed on a LECO CHNS-932. Mass Spectra were obtained from an Agilent 6210. Compounds HS(CH\(_2\)_2)NMe\(_2\)·HCl 2,2′-dimethoxy-2-phenylacetophenone (DMPA), MeI, HSiMe\(_2\)Cl, HSiMeCl\(_2\), LiAlH\(_4\), NaHCO\(_3\), penicillin V potassium salt (PenVK), platinum(0) 1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex solution (Karsted’s catalyst), porcine liver esterase (PLE) were obtained from commercial sources. Compounds NH\(_2\)G\(_n\)(NMe\(_2\))\(_m\), \([\text{NH}_2\text{G}_n(\text{S-NMe}_3)_m]^{m+1}\), ClG\(_1\)V\(_2\), ClG\(_1\)A\(_2\) were synthesized as published.

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

ClG\(_1\)(Si-NMe\(_2\))\(_2\) (11). An excess of allyl-dimethylamine (0.21 mL, 1.76 mmol) and two drops of Karsted’s catalyst were added to a solution of ClG\(_1\)(SiH)\(_2\) (6) (0.145 g, 0.44 mmol) in THF (2 mL). The reaction mixture was heated at 80 °C in a sealed ampoule under inert atmosphere for one night and then evaporated to dryness to remove the solvent and residual allyl-dimethylamine. Afterwards, hexane (10 mL) was added and the solution was filtered through active carbon and dried under vacuum to yield 11 as pale yellow oil (0.194 g, 88%).

\(^1\)H-NMR (CDCl\(_3\)): δ -0.06 (s, 12 H, SiMe\(_2\)), 0.04 (s, 3 H, SiMe), 0.44 (t, \(J_a = 8.4\) Hz, 4 H, SiCH\(_2\)CH\(_2\)CH\(_2\)N), 0.57 (m, 10 H, SiCH\(_2\)CH\(_2\)CH\(_2\)Si and ClCH\(_2\)CH\(_2\)CH\(_2\)Si), 1.29 (m, 4 H, SiCH\(_2\)CH\(_2\)CH\(_2\)Si), 1.42 (m, 4 H, SiCH\(_2\)CH\(_2\)CH\(_2\)N), 1.72 (m, 2 H, ClCH\(_2\)CH\(_2\)), 2.19 (m, 16 H, CH\(_2\)NMe\(_2\)), 3.47 (t, \(J_b = 6.9\) Hz, 2 H, ClCH\(_2\)). \(^{13}\)C\({}^1\)H\}NMR (CDCl\(_3\)): δ -5.2 (SiMe), -3.4 (SiMe\(_2\)), 11.6
NaI. The mixture was heated at 80 °C in a sealed ampoule under argon atmosphere for 40 hours after which, volatiles were removed. After washing the residues with water, this dendron was obtained as a very hygroscopic white solid (0.300 g, 87%).

\[ \text{CIG}_1(\text{Si-NMe}_3)_2 \] (13). A mixture of 11 (0.220 g, 0.44 mmol) and MeI (0.11 mL, 1.76 mmol) in THF (50 mL) were stirred for 16 h. Afterward, volatiles were removed under vacuum and the remaining solid was washed with Et₂O (50 mL), obtaining 13 as a white solid (0.300 g, 87%).

\[^1^H\text{NMR (DMSO-d}_6\text{): }\delta \text{ -0.07 (s, 3 H, SiMe), 0.00 (s, 12 H, SiMe), 0.38 and 0.56 (m, 14 H, SiCH}_2,\]

1.31 (m, 4 H, SiCH₂CH₂CH₂Si), 1.62 (m, 6 H, CH₂CH₂N⁺ and ClCH₂CH₂), 3.05 (s, 18 H, -NMe₃⁺), 3.25 (t, Jₐ = 7.8 Hz, 4 H, CH₂N⁺), 2.58 (t, Jₖ = 6.5 Hz, ClCH₂). \[ ^{13}\text{C} \{^1^H\}_\text{NMR (DMSO-d}_6\text{): }\delta \text{ -5.6 (SiMe), -3.9(SiMe), 10.6 (SiCH}_2,\]

16.4-17.4 (SiCH₂CH₂Si), 18.6 (CH₂CH₂N⁺), 26.7 (ClCH₂CH₂), 47.8 (ClCH₂), 51.6 (-NMe₃⁺), 67.4 (SiCH₂CH₂CH₂NMe₃⁺). \[ ^{29}\text{Si-NMR (DMSO-d}_6\text{): }\delta \text{ 2.1 (SiMe), 2.4 (SiMe).}\]

ESI: (776.21 g/mol) q=1 (649.31 [M-I⁺]). Anal. Calc. C₂₆H₆₃Cl₂N₃Si₃ (777.31 g/mol): C, 40.17; H, 8.17; N, 3.60; Exp.: C, 40.78; H, 8.39; N, 3.93.

\[(\text{PenV})G_1(\text{Si-NMe}_3)_2\] (15). To a solution of 14 (0.050 g, 0.06 mmol) in dry DMF, PenVK (0.027 g, 0.07 mmol) was added in the presence of ether crown 18C₆ (0.002 g, 0.01 mmol) and a catalytic amount of NaI. The mixture was heated at 80 °C in a sealed ampoule under argon atmosphere for 40 hours after which, volatiles were removed. After washing the residues with water, this dendron was obtained as a very hygroscopic white solid (0.062 g, 89%).

\[^1^H\text{NMR (CDCl}_3\text{): }\delta \text{ 0.00 (s, 12 H, SiMe), 0.04 (s, 3 H, SiMe), 0.57 (m, 14 H, SiCH}_2,\]

1.29 (m, 4 H, SiCH₂CH₂CH₂Si), 1.48 (s, 3 H, CMe₂), 1.58 (s, 3 H, CMe₂), 1.70 (m, 6 H, CH₂CH₂N⁺ and OCH₂CH₂), 3.41 (s, 18 H, -NMe₃⁺), 3.63 (m, 4 H, CH₂N⁺), 4.08 (t, Jₐ = 8.4 Hz, 2 H, (CO)OCH₂), 4.43 (s, 1 H, CH(CMe₂)), 4.53 (s, 2 H, OCH₂CO), 5.56 (d, Jₖ = 4.3 Hz, 1 H, CHS), 5.70 (m, 1 H, NHCH), 6.90 (d, Jₜ = 7.6 Hz, 2 H, CH₃), 7.01 (t, Jₐ = 7.4 Hz, 1 H, CH₃), 7.30 (m, 2 H, CH₃). \[ ^{13}\text{C} \{^1^H\}_\text{NMR (CDCl}_3\text{): }\delta \text{ -5.0 (SiCH}_3,\]

-3.2 (SiMe₂), 9.9 (CH₂Si), 11.4 (SiCH₂CH₂CH₂N⁺), 17.9, 18.0 and 18.3 (SiCH₂CH₂CH₂Si), 19.8 (CH₂CH₂N⁺), 37.1 (CH₂CH₂N⁺), 43.8 (CH₂CH₂N⁺), 47.8 (CH₂CH₂N⁺).
23.2 (OCH₂CH₂), 26.8 and 32.0 (SCMe₂), 53.7 (-NMe₃⁻), 58.0 (NHCH), 64.7 (SCMe₂), 67.1 (CH₂N⁺), 67.7
(OCH₂CO), 68.4 (COOCH₂), 69.5 (CH₇), 70.5 (CHCOO), 114.7 (CH₅), 122.4 (CH₆), 129.8 (CH₇),
156.8 (CO₂), 167.6, 167.8 and 173.0 (C=O). ESI: (1090.32 g/mol) q = 1 (963.42 [M-I]⁻), q = 2 (418.26
7.07; N, 4.43.

2.3. DOSY NMR measurements

DOSY experiments were carried out on a Bruker Advance 400 at 25 ºC. The values of mid-point
between gradients (∆) and gradient length (δ) were adjusted for free PenVK and for compound 14 in their
solutions and these values were later used in two different experiments for the mixture PenVK/14, not
observing significant differences in the final result (Figure S6).

2.4. Antibacterial methodology

Bacterial strains. *Staphylococcus aureus* (CECT 240, Gram-positive) were obtained from the Spanish
Type Culture Collection (CECT).

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. 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 10⁷ 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.
3. Results and Discussion

3.1. Synthesis and characterization of dendrons

In order to clarify the discussion of the compounds studied herein, the following nomenclature has been used: dendrons are named as \([XG_n(Y-Z)_m]\), where \(X\) refers to the focal point; \(G_n\) means carbosilane skeleton and generation (Figure 1); \(Y\) indicates functionalization of the periphery by hydrosilylation (\(Y = \text{Si}\)) or hydrothiolation (\(Y = \text{S}\)), and \(Z\) and \(m\) correspond to the peripheral functional groups and their numbers on the surface.

Since the antibacterial results previously obtained by our group for carbosilane dendrons showed the best activity for second generation derivatives containing a sulfur atom close to dendron surface,\(^{47}\) we have focused our efforts on obtaining a second generation dendron with a PenV fragment at the focal point. Nonetheless, the reactions with the first generation dendrons were carried out to find the adequate procedure and characterize properly the compounds.

Initially, we attempted to anchor the penicillin moiety by amide bond involving the carboxylic group of PenV and a primary amine at the focal point of the cationic dendron \(\text{NH}_2G_1(S\text{-NMe}_3)_2\)^{2+}\(^{47}\) or the related neutral derivative \(\text{NH}_2G_1(S\text{-NMe}_2)_2\)\(^{52}\) (Scheme 1). However, the reaction of neutral PenV with these dendrons failed due to instability of the \(\beta\)-lactam ring toward the amino function, observing in the \(^1\text{H NMR}\) spectra the disappearance of the original resonances belonging to this ring (data not shown). This instability of the \(\beta\)-lactam ring in the presence of \(-\text{NH}_2\) functions has been exploited for the preparation of benzylpenicilloyl-dendrimer conjugates to evaluate penicillin allergy.\(^{56}\)

Alternatively, we proceeded to incorporate the PenV moiety to a vinyl dendron by direct reaction of the PenV potassium salt with the dendron containing a Br-C bond at the focal point, \(\text{BrG}_1V_2\), in a similar way to other modifications introduced in these dendrons (Scheme S1).\(^{52}\) Thus, after reaction treatment and purification, the corresponding dendron \((\text{PenV})G_1V_2\) (I) was obtained as a yellowish oil in good yield. The
main NMR data that confirmed this transformation were the resonances corresponding to the CH$_2$ moiety of the new ester bond, observed at δ 4.16 $^1$H NMR spectrum and at δ 65.4 $^{13}$C NMR spectrum respectively.

Scheme 1. Failed attempts to obtained penicillin (PenV) functionalized dendrons containing sulfur atoms.

Unfortunately, subsequent modification of the periphery of 1 via thiol-ene addition failed leading again to the rupture of the β-lactam ring, probably caused by a nucleophilic attack of the thiol function to the carbonyl carbon of this ring (Scheme 1). Nevertheless, the halogen-PenV exchange seemed to be a promising procedure to obtain dendrons with a PenV unit at the focal point. Hence, we designed a cationic dendron with a Cl-C bond at the focal point and cationic peripheral functions at the periphery. The reason for the presence of a Cl-C bond instead of a Br-C bond is the clearly enhanced reactivity of the latter towards amines.

For the synthesis of this new dendron (Scheme S2), we started from ClG$_1$V$_2$ and carried out a thiol-ene addition with cysteamine hydrochloride (HS(CH$_2$)$_2$NMe$_2$·HCl) under UV irradiation, obtaining [ClG$_1$(S-NMe$_2$H)$_2$]$^{2+}$ (2) as a white solid. The formation of this compound was confirmed by NMR spectroscopy: the resonances of the vinyl groups disappeared in both $^1$H NMR and $^{13}$C NMR spectra; the new chain formed Si(CH$_2$)$_2$S presented in the $^1$H NMR two multiplets at δ 0.87 (SiCH$_2$) and at 2.60 (CH$_2$S) and in the $^{13}$C NMR two signals at δ 13.5 (SiCH$_2$) and at 26.0 (CH$_2$S); and also were observed the resonances of the new chain introduced S(CH$_2$)$_2$NMe$_2$. Next, basic treatment of 2 led to the neutral dendron ClG$_1$(S-NMe$_2$) (3), which was obtained as a yellowish oil. The $^1$H NMR spectrum showed a clear
shifting of the NMe$_2$ groups from $\delta$ 2.73 in 2 to $\delta$ 2.24 in 3. Finally, addition of MeI to 3 afforded the cationic dendron [ClG$_1$(S-NMe$_3$)$_2$]$_2$$^{2+}$ (4), which was isolated as a pale yellow solid. In this case, the resonances of the Me$_3$N$^+$ groups were shifted to higher frequency, being observed in the $^1$H NMR spectrum at $\delta$ 3.07.

Again, the reaction of dendron [ClG$_1$(S-NMe$_3$)$_2$]$_2$$^{2+}$ (4) with PenVK (Scheme 1) did not render the desired dendron, once again observing the rupture of the $\beta$-lactam ring by means of modification of their resonances in the $^1$H NMR spectrum. In this case, this $\beta$-lactam ring probably suffered a nucleophilic attack of the thioether function. It is important to note that this reaction requires heating over 80º C.

In order to bypass all these drawbacks in the synthesis of a cationic carbosilane dendron containing a PenV moiety, we moved to modification of the dendron surface by hydrosilylation processes instead of thiol-ene addition, with the aim to avoid the presence of any donor atom in the final dendron. This methodology successfully allowed us to prepare cationic dendrons with PenV at the focal point.

**Scheme 2.** Synthesis of carbosilane dendrons containing peripheral Si-H bonds and a Cl-C bond at the focal point ClG$_n$(SiH)$_m$ (n = 1, m = 2 (6); n = 2, m = 4 (10)). i) HSiMe$_2$Cl, 60º C, [Pt], 4 h; ii) LiAlH$_4$, Et$_2$O, 0º C to r. T., 16 h; iii) HSiMeCl$_2$, 60º C, [Pt], 4 h; iv) BrMg(allyl), Et$_2$O, 0º C to r. T., 16 h.
Scheme 3. Synthesis of dendrons \[\text{(PenV)}\text{G}_n(\text{Si-NMe}_2)_m\]m+ (n = 1, m = 2 (15); n = 2, m = 4 (16)) with PenV at the focal point and cationic peripheral groups derived by hydrosilylation. i) (allyl)NMe2, 60º C, [Pt]; ii) MeI, THF, r. T., 16 h; iii) PenVK, ether crown 18C6, NaI, DMF, 80º C, 40 h.

Thus, starting from ClG1A254 and using typical reactions for the preparation of carbosilane dendritic systems (hydrosilylation, alkenylation and Cl-H substitution, Scheme 2),58 we obtained dendrons with a Cl-C bond at the focal point and Si-H peripheral groups ClGn(SiH)m (n = 1, m = 2 (6); n = 2, m = 4 (10)). Treatment of these derivatives with allyldimethylamine in the presence of Karstedt’s catalyst59 afforded the neutral dendrons ClGn(Si-NMe2)m (n = 1, m = 2 (11); n = 2, m = 4 (12) (Scheme 3). NMR spectroscopy clearly showed the incorporation of the new propylene chain (Si(CH2)3N) to the dendrimer structure, by means of the resonances in the 1H NMR spectra at δ ca. 0.44 (SiCH2), 1.40 (CH2) and 2.20 (CH3N); and in the 13C NMR spectra at δ ca. 12.8 (SiCH2), 22.1 (CH2) and 63.4 (CH3N). These compounds were easily transformed into the cationic ones [ClGn(Si-NMe3)m]m+ (n = 1, m = 2 (13); n = 2, m = 4 (14) by addition of MeI (Scheme 3, Figure 1). The typical shifting to higher frequency of the methyl (MeN) resonances was observed in the 1H NMR spectra (Figure S1 and S2). Subsequent heating of 13 and 14 with PenVK yielded the goal dendrons \[\text{(PenV)}\text{G}_n(\text{Si-NMe}_3)_m\]m+ (n = 1, m = 2 (15); n = 2, m = 4 (16) (Scheme 3, Figure 1), which were isolated in good yields as highly hygroscopic pale yellow solids. The solubility was generation dependent, being G1 dendron soluble in chlorinated solvents, alcohols and DMSO but not in water,
whereas G2 dendron was soluble in water and other highly polar but not in chlorinated solvents. NMR (Figure 2 and 3), MS (Figure S3) and elemental analysis were in accordance with this formulation. The main NMR data confirming formation of these dendrons were the resonances associated to the ester fragment. The \( \text{CH}_2\text{O} \) group was observed at about \( \delta \) 4.1 and \( \delta \) 67 in the \( ^1\text{H} \) and \( ^{13}\text{C} \) spectra, respectively, and the corresponding carbon atom of the carbonyl group at about \( \delta \) 173 in the \( ^{13}\text{C} \) spectra. Furthermore, the binding of PenV to the dendron was confirmed by DOSY NMR, showing the joint diffusion of both moieties (Figures S3), in comparison with the mixture of PenV and dendron 14 (Figures S6). The formation and stability of the dendrons containing the PenV moiety 14 and 16 following this route confirms that the presence of donor atoms, such as S or N, in the dendron structure was responsible of \( \beta \)-lactam ring degradation.

**Figure 1.** Drawing of cationic dendrons of second generation \([\text{XG}_2(\text{SiNMe}_3)_4]^{4+}\) (\( \text{X} = \text{Cl}, \ (14); \ \text{X} = \) PenV (16)).
Figure 2. $^1$H NMR of $[(\text{PenV})G_2(Si-NMe_3)_4]^{4+}$ (16) in DMSO-D$_6$.

Figure 3. $^{13}$C NMR of $[(\text{PenV})G_2(Si-NMe_3)_4]^{4+}$ (16) in DMSO-D$_6$.

3.2. Antibacterial activity

For the study of the biocidal capacity of dendrons, *S. aureus* was chosen as a model of Gram-positive bacteria, since penicillin is a specific antibiotic for this type of bacteria. Figure 1 depicts drawings of
cationic dendrons employed for the assays. To ascertain the effect of the PenV moiety in dendron


[(PenV)G_2(Si-NMe_3)_4]^{4+} (16), a comparative study of the antibacterial activity of this dendron with its

parent compound [ClG_2(Si-NMe_3)_4]^{4+} (14) and an equimolecular mixture of PenVK salt plus dendron 14

was carried out. Table 1 summarizes the minimum inhibitory concentration (MIC) and minimum

bactericidal concentration (MBC) of these dendrons.

The data obtained point out the following facts: i) slight differences in the antibacterial response of
dendrons with or without PenV at the focal point [ClG_2(Si-NMe_3)_4]^{4+} (14) and [(PenV)G_2(Si-NMe_3)_4]^{4+}
(16); ii) much lower activity of the PenV fragment in 16 than that of the free PenV; and iii) a MIC for the
stoichiometric mixture of PenVK and 14 was similar to that obtained for free PenVK, whereas the MBC
raised to 0.78 ppm (with respect to PenV), much higher than that of free PenVK.

The decrease of the bactericide activity (MBC) of the mixture PenVK/14 compare to free PenVK could
be explained in terms of availability of the antibiotic, since PenV is an anionic compound capable of
interacting with the cationic dendron and, thus, likely to diminish its availability. Comparison of the
diffusion coefficients of free PenVK and PenVK in an equimolecular mixture with dendron [ClG_2(Si-
NMe_3)_4]^{4+} (14) by DOSY 2D NMR experiments showed that this coefficient is clearly smaller when the
dendron is present. This means that diffusion of PenVK is affected by the dendron, supporting the
assessment of interaction between both systems (Figure S6). Although in our case the dendron/PenV
interaction seems to difficult the action of the antibiotic, it has been reported that electrostatic interaction
between β-lactam antibiotics and a polymer containing cationic cobaltocenium units exhibit synergistic
effects against methicillin resistant S. aureus by efficiently inhibiting activity of β-lactamase and
effectively lysing bacterial cells.60

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Table 1. Antibacterial activity of PenV, dendrons \([XG_2(Si-NMe_3)_4]^{4+}\) (X = Cl, 14; PenV, 16), and equimolecular mixture of PenVK and 14 against \(S.\ aureus\). \(^a\) \([NR_3^+]\) refers to the \(\mu\)M concentration of ammonium groups. \(^b\) refers to ppm concentration of PenV.

In the case of dendron \([(PenV)G_2(Si-NMe_3)_4]^{4+}\) (16) with a covalently bonded PenV, the higher inhibitory and bactericidal concentration values with respect to free PenVK are probably due to the fact that the ester bond is not adequately split in solution. The activity of PenV requires the availability of the carboxylate moiety, which also favours its stability, since formation of the ester bond to conjugate penicillin to the dendron alter the crucial equilibrium between stability and activity of the \(\beta\)-lactam ring.\(^{61}\) Thus, the presence of this ester bond triggers the reactivity of the ring and, thereby, affecting their activity.

With the aim of facilitating the release of the penicillin fragment by rupture of the ester bond, we tested the antibacterial activity of dendron \([(PenV)G_2(Si-NMe_3)_4]^{4+}\) (16) in the presence of porcine liver esterase (PLE). For this experiment, we initially added one unit of esterase per ester bond. From the first moment the activity increased (MIC = 0.95 ppm and MBC = 0.95 ppm, with respect to the concentration of PenV), confirming the splitting of the ester bond. However, the obtained values were different from those for free PenV in the absence of dendron. This may be due to the electrostatic interactions of free PenV with the dendron or also to partial degradation of penicillin previous to its release from dendron. Addition of excess esterase did not affect the behavior observed in this experiment.

4. Conclusions

Carbosilane cationic dendrons with a penicillin moiety at the focal point can be obtained employing the carboxylate group of penicillin (PenV) to be attached to the dendron. However, the synthetic protocol

<table>
<thead>
<tr>
<th>PenVK/14</th>
<th>0.012(^b)</th>
<th>0.14</th>
<th>0.78(^b)</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>2.91(^b)</td>
<td>33</td>
<td>2.91(^b)</td>
<td>33</td>
</tr>
<tr>
<td>16/esterease</td>
<td>0.95(^b)</td>
<td>3.70</td>
<td>0.95(^b)</td>
<td>3.70</td>
</tr>
</tbody>
</table>
requires avoiding the presence of donor atoms or groups to avoid degradation of the \( \beta \)-lactam ring, which is
the responsible of the penicillin activity.

Evaluation of the activity of this dendron (16) and of a mixture of cationic dendron (14) and free PenV
revealed that both covalent conjugation of PenV to the dendron or electrostatic interaction between PenV
and the cationic dendron reduced penicillin activity. These phenomena can be ascribed to the blocking of
the carboxylate group of penicillin, which also favours the hydrolysis of the \( \beta \)-lactam ring. Hydrolysis of
the ester bond in dendron 16 with an esterase increases the activity, without reaching the values of free
penicillin. However, the release of penicillin in this system could be useful to apply similar compounds in
drug release of drugs where the carboxylate unit don’t play a key role in its therapeutic action.

5. Supporting Information

Complete experimental procedures and selected NMR spectra.

6. Acknowledgments

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7. References

   25-42.
Strategies for Penicillin V dendronization with cationic carbosilane dendrons and study of antibacterial properties


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A cationic carbosilane dendron with penicillin V at the focal point has been prepared. The antibacterial properties of this system have been studied and compared with free penicillin and with a mixture of a cationic dendron and penicillin.