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Incorporation of dimethyldodecylammonium chloride functionalities onto poly(propylene imine) dendrimers significantly enhances their antibacterial properties

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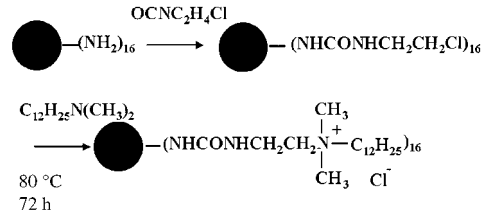
Dimethyldodecylammonium chloride functionalized poly(propylene imine) generation 3 dendrimers have been synthesized and proven to have strong antibacterial properties.

The novel architecture of dendrimers[1–6] provides a very high number of functional groups in a compact space. Thus, it is reasonable to expect that these novel molecules will play a major role in materials whose performance depends on high local concentration, such as drugs or antimicrobial agents. Presently, there are few reports in this field. In this work, DSM Astramol™ poly(propylene imine) (PPI) dendrimers have been successfully used to synthesize dendritic biocides, which are shown to be over two orders of magnitude more potent than their small molecule counterpart, *n*-dodecyltrimethylammonium chloride (DTAC), against *Escherichia coli*.

Quaternary ammonium compounds (QACs) have been widely used as disinfectants. They are effective biocidal agents when they possess an alkyl chain with at least eight carbon atoms. Although the exact mechanism of their antimicrobial action is still unclear, it is mostly attributed to cell membrane disruption, their ability to increase cell permeability, and their possible effects on proteins.[7] Since biocidal action requires interactions with the cell membrane, it will be influenced by both the size of the molecules and the functional group density. Larger molecules tend to have a lower permeation rate through the cell membranes, and thus are less efficient. Therefore, dendritic biocides need to counterbalance the negative side of their bulkier size to be very effective. The antibacterial actions against Gram-positive and Gram-negative bacteria are also different since they have different cell structures. The purpose of this study was to synthesize novel dendritic biocides by converting the surface groups of dendrimers to quaternary ammonium functionalities and to test their biocidal capability. The target biocides not only carry 16 quaternary ammonium groups per molecule but also possess a polycationic structure, which is well-known to increase the permeability of cell membranes and thus aid the killing process.[8]

Commercially available poly(propylene imine) (PPI) dendrimers were used in this study. These dendrimers are well-characterized by various techniques.[9–11] The purity of PPI dendrimers has also been investigated by electrospray mass spectrometry.[12] To synthesize these dendritic biocides, the peripheral primary amine groups of PPI generation 3 dendrimers [DAB-dendr-$(NH_2)_{16}$] were reacted with 2-chloroethyl isocyanate to yield pendent chloroethyl groups.[13,14] Urea protons (CO-NH-CO) were observed at δ 6.72 (^{1}H NMR, $CDCl_3$) and carbonyl carbons (CO-NH-CO) were observed at δ 158.7 (^{13}C NMR, 15% in $CDCl_3$). The modified dendrimers were subsequently quaternized by dimethyldodecylamine to give the dimethyldodecylammonium chloride functionalized dendrimers (Scheme 1).[15] A five-fold excess of dimethyldodecylamine was used to facilitate the reaction and to prevent inter-



Scheme 1 Synthesis of dimethyldodecylammonium chloride functionalized PPI generation 3 dendrimers (the filled circle represents a complete dendrimer molecule except for the end groups).

dendrimer quaternization. Two different carbons in the methyl groups were clearly identified with ^{13}C NMR. The methyl groups connected directly with nitrogen were observed at δ 51.1 while the methyl carbon in the dodecyl groups was observed at δ 13.8. FTIR analysis also showed C–H saturation at 2930.4 cm^{-1}, C=O at 1639.9 cm^{-1}, N–H bend at 1562.3 cm^{-1} and N–H stretch at 3297.9 cm^{-1}. The final product was obtained as a yellow solid in 70% yield. It is very soluble in alcohol and $CHCl_3$, and slightly soluble in water. Although MALDI data for a similar amphiphilic dendrimer structure have been published,[2] our effort to collect MALDI-TOF data was not successful, probably because the sample was extremely hygroscopic and the hydrophobic chains were hard to ionize. The nomenclature used is D3C1NC12, which denotes dimethyldodecyl (C12) ammonium (N) chloride (C1) functionalized PPI generation 3 dendrimers (D3).

The antibacterial properties of D3C1NC12 against Gram-negative *E. coli* were determined by a bioluminescence method.[16–18] Bioluminescence is observed under normal growth conditions for the recombinant *E. coli* strain TV 1048. Whenever the bacteria are in a biocidal environment, the light-off response corresponds to the toxic effect of the biocide.[19] Fig. 1 shows typical data for the bioluminescence experiment. The result is expressed as the sample bioluminescence normalized to a control (without biocide) *vs.* time. The reduction of luminescence quantitatively shows the antibacterial properties of the

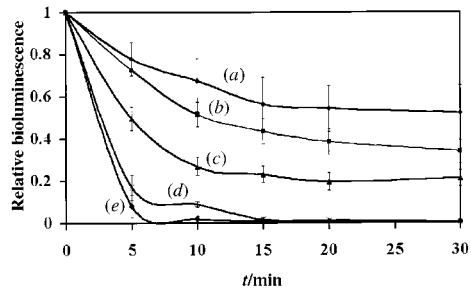


Fig. 1 Time course of the relative bioluminescence of *E. coli* in contact with D3C1NC12: (*a*) 4, (*b*) 8, (*c*) 12, (*d*) 16 and (*e*) 20 μg ml^{-1}. The temperature of experiments was maintained at 30 °C, the optimal temperature for the growth of strain TV 1048. The dendrimer biocides were added to bacterial suspensions with an optical density at 600 nm at 0.2. The data shown are the average of three experiments.

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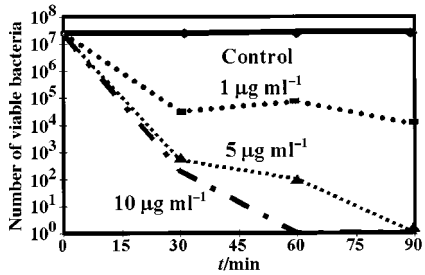

Fig. 2 Biocidal effect of D3C1NC12 on *S. aureus* in suspension tests (10% Tween 80 was used as a neutralizer).

sample. At 4 μg ml^{-1}, the dendrimer inhibited the growth of *E. coli*, but the bacteria could adjust to the environmental stress and survive. At higher concentrations (20 μg ml^{-1}), the bioluminescence decreased very rapidly and was reduced to an undetectable level in 15 min, indicating a strong biocidal effect. In the control experiment, the bioluminescence of the bacteria did not change significantly (5%) if the same concentration of pure PPI generation 3 dendrimers was added (data not shown).

To quantify the level of the biocidal effect of the cationic dendrimer and to compare it to its small molecule counterpart, the *n*-dodecyltrimethylammonium chloride (DTAC) EC_{50} value was determined. EC_{50} is defined as the concentration of the compound which causes a 50% reduction of the bioluminescence in a certain time period. EC_{50} concentrations were determined by interpolation of the bioluminescence against concentration curves at a specific time. A lower EC_{50} indicates a more toxic compound. The EC_{50} of D3C1NC12 is about 12 μg ml^{-1} at 5 min while the EC_{50} of DTAC is about 2000 μg ml^{-1}. The dendrimer architecture increases the potency of DTAC against *E. coli* over 100 times.

Conventional linear or branched polymeric QACs have been investigated for many potential applications.[20–23] Interestingly, it is not enhanced efficiency but low toxicity to humans and the absence of foaming that are often cited as their special features.[7] Compared to conventional linear or branched polymers, dendrimers offer a large number of functional groups in a compact space. These chemical functionalities of the dendrimer biocides are presumably exposed on the surface, while a significant portion of them will be trapped on the interior of random-coil linear polymers. Therefore, the increased potency of dendritic biocides results from their novel dendritic architecture. These biocides could possibly achieve enhanced antimicrobial properties along with low toxicity and the absence of foaming. QACs are not very effective on Gram-negative bacteria such as *E. coli* because they have a complex outer membrane structure that effectively keeps out the antibacterial agents. The combination of their high functional group density and increasing permeability due to their polycationic structure allow dendritic biocides to reach and disrupt cell membranes and eventually lead to cell death. This explanation is supported by the analysis of the bioluminescence data, which give a dilution coefficient of 1.8. The dilution coefficient is similar to the reaction order in chemical kinetics. It represents the change in activity brought about by different concentrations. The higher the dilution coefficient, the more rapidly the antimicrobial agent loses its activity. The dilution coefficient of the novel dendrimer biocides falls into the range of conventional QACs (1–2.5), implying a similar mode of action.[24]

To verify that the biocidal effects of D3C1NC12 were not bacteria dependent, its antibacterial properties against Gram-positive *Staphylococcus aureus* were investigated by suspension tests.[25] Fig. 2 shows that D3C1NC12 inhibited the growth of *S. aureus* at levels as low as 1 μg ml^{-1} and effectively killed them at 10 μg ml^{-1} in 60 min. Similar results have been reported for DTAC.[7] This demonstrates the strong potency of dendritic biocides on typical Gram-positive *S. aureus*.

We have demonstrated that it is feasible to synthesize highly potent dendrimer-based antibacterial agents. The novel dendritic biocides are over two orders of magnitude more potent than their small molecule counterparts against Gram-negative bacteria. They are also very effective against Gram-positive bacteria. The dendritic architecture greatly enhances the effectiveness of common quaternary ammonium disinfectants.

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