European Journal of Medicinal Chemistry 44 (2009) 1615–1622

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Enhanced activity of fluorinated quaternary ammonium surfactants against *Pseudomonas aeruginosa*

Lionel Massi [a], Frederic Guittard [a], Richard Levy [b], S. Géribaldi [a,*]

[a] Institut de Chimie de Nice FR 3037 CNRS, Laboratoire de Chimie des Matériaux Organiques et Métalliques, CMOM, UFR Sciences, Université de Nice-Sophia Antipolis, Parc Valrose, 06108 Nice Cedex 2, France
[b] Rohm and Haas France, Laboratoires Européens, Département Process Chemicals and Biocides, 371, rue Beethoven, Sophia Antipolis, 06565 Valbonne, France

ARTICLE INFO

*Article history:*
Received 21 May 2008
Received in revised form 16 July 2008
Accepted 17 July 2008
Available online 5 August 2008

*Keywords:*
Bisammoniums
Fluorinated surfactants
Minimal inhibitory concentration
Preservatives
*Pseudomonas aeruginosa*
Quaternary ammonium salts

ABSTRACT

A novel series of fluorinated quaternary bisammonium surfactants has been synthesized in view to optimize their antimicrobial activities against *Pseudomonas aeruginosa*. As compared with commercial references, most of the new surfactants synthesized exhibit an enhanced activity which is discussed as a function of the nature of the spacer group between the quaternized nitrogen atoms and of the nature of the connector function between the nitrogen atoms and the perfluorinated carbon chains. It appears that the fluorinated "Gemini" surfactants bearing an amide connector can be an interesting alternative to hydrocarbon ammonium salts as preservatives and disinfectants against *P. aeruginosa*.



## 1. Introduction

Quaternary ammonium compounds (QACs) are among the most common products used as preservatives and disinfectants in human health-related domains such as disinfecting in hospital, disinfectants for hard surface cleaning, disinfectants for contact lenses, etc. [1]. However, this type of products suffers from a lack of activity against one of the Gram (−) bacteria that have developed the most important antimicrobial resistance phenomenon today, *Pseudomonas aeruginosa* [2,3]. Indeed this organism possesses the ability to metabolize quaternary ammonium compounds as a source of carbon and nitrogen and it is well known that selective culture media for *Pseudomonas* species contain hexadecyl trimethyl ammonium bromide as only this species of bacteria can live in such medium [4]. This extreme difficulty to prevent contamination from this bacterium with QACs is found even with recent generations of salts exhibiting strong activity and wide antibacterial spectra [5].

However, preservatives and disinfectants based on QACs are widely used in the hospital environment in which *P. aeruginosa* is mostly a nosocomial pathogen [6–13]. So, it would be of the first importance to find new classes of these cheap antibacterial agents that will be also effective against *P. aeruginosa.*

Moreover, among the different classes of QACs used as bactericides, those presenting two quaternised nitrogen atoms, each bearing an hydrophobic tail and called "Gemini" surfactants, seem to exhibit more effective antimicrobial activities than their monoammonium analogues as showed in several recent works [14–16].

To build our strategy for the design of new antimicrobial agents with QAC structure able to possess an enhanced activity against *P. aeruginosa*, we have to consider the specific nature of Gram (−) bacterial membrane. Various mechanisms of antimicrobial resistance can take place in this complex structure and especially for *P. aeruginosa* [17]. On the other hand, the general behavior of biofilms acting as a diffusion barrier and the specific mechanisms employed by these microbial communities to resist the action of classical antibacterial agents such as the hydrocarbon "Gemini" quarternary ammonium salts have to be considered. Taking account of these parameters, we hypothesized that an important change in the structural parameters of this type of surfactants could mislead this Gram (−) bacteria in the expression of its various mechanisms of resistance used as well in its planktonical nature as in its biofilm forms. In this aim, our strategy is to radically change the hydrophilic and lipophilic properties of classical hydrocarbon "Gemini"

*Abbreviations:* cfu, colony forming unit; CMCs, critical micelle concentrations; CPC, cetyl pyridinium chloride; DCC, dicyclohexylcarbodiimide; MIC, minimal inhibitory concentration; QACs, quaternary ammonium compounds; r.t., room temperature.

* Corresponding author. Tel.: +33 (0) 4 92 07 61 12; fax: +33 (0) 4 92 07 61 56.
*E-mail addresses:* Lionel.Massi@unice.fr (L. Massi), Frederic.Guittard@unice.fr (F. Guittard), RLevy@rohmhaas.com (R. Levy), Serge.Geribaldi@unice.fr (S. Géribaldi).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.07.032

surfactants, first by replacement of the lipophilic hydrocarbon tails of these antibacterial agents with semi-fluorinated tails connected to the quaternized nitrogen atoms using various chemical active functions, and secondly by introduction of various spacers, different in nature and in length, between the two quaternized nitrogen atoms.

In the present work, we describe the synthesis of "Gemini" fluorinated surfactants **1**–**16** (Fig. 1) and the evaluation of their antimicrobial activity using measurement of minimal inhibitory concentrations (MICs) in comparison with commercial references, **17**–**20**, and with a hydrocarbon analogue of **3**, noted **21** (Fig. 2).

## 2. Results and discussion

### 2.1. Chemical synthesis

From our previous works concerning the study of antimicrobial properties of heterogeneous series of fluorinated "Gemini" QACs, we observed that the most 'critical' molecular parameter for their biological activity seems to be the nature of connector Q, the fluorinated QACs with an amide connector Q = $NHC(O)CH_2$ exhibiting the best bacteriological activities against various microorganisms (Gram (+) bacteria: *Staphylococcus aureus* ATCC 9144; yeast: *Candida albicans* ATCC 2091 and moulds: *Aspergillus niger* ATCC 6275) [18,19]. In order to confirm this hypothesis on *P. aeruginosa* and to attempt to optimize the other structural parameters of fluorinated "Gemini" surfactants, we decided on the one hand to extend the series with the amide connector by varying the length of spacer $(CH_2)_n$ (Compounds **1**–**7**: Block 1 in Fig. 1) and its nature and rigidity (Compounds **8**–**11**: Block 2 in Fig. 1), and on the other hand to modify largely the nature of the connector Q keeping the spacer R as a constant parameter (Compounds **3** and **12**–**16**: Block 3 in Fig. 1).

Schemes 1 and 2 show that QACs **1**–**12** are synthesized from building blocks **22** and **23**, while **13**–**16** are obtained from the intermediates **24**–**27**, respectively.

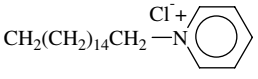
The building block **22** is obtained from the commercially available 2-perfluorohexyethyl iodide. This latter reacts with sodium azide to give 2-perfluorohexylethylazide under phase transfer catalysis conditions. The reduction reaction using hydrazine hydrate catalysed by Raney nickel gives the corresponding amine which reacts with bromoacetic acid using dicyclohexylcarbodiimide (DCC) as coupling reagent to give the expected building block **22**. Then, the reaction of building block **22** with an half-equivalent of *N*,*N*,*N*′,*N*′-tetramethyl-α,ω-alkanediamine leads to QACs **1**–**7**. The same reaction with an half-equivalent of *N*,*N*,*N*′,*N*′-tetrametylcystamine, *trans*-*N*,*N*,*N*′,*N*′-tetramethyl-2-butene-1,4-diamine, 1,3-bis(dimethylamino)-2-propanol, and 1,4-diazabicyclo[2.2.2]octane, gives QACs **8**–**11**, respectively.

The commercially available 2-perfluorohexylethanethiol serves as a starting material for synthesis of building block **23** leading to QAC **12**. First, the thiol is reacted under inert atmosphere to polyoxymethylene and triethylamine in catalytic amounts. Then, phosphorus tribromide is used to convert the intermediate alcohol

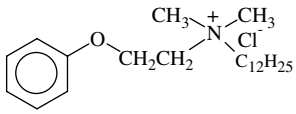
**17**, Empigen bac 50 with n = 14 (50%) n = 12 (40%) and n = 10 (10%)



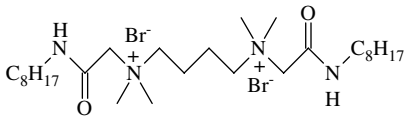
**18**, Catigene 818 with n = 8 and n' = 10 (50%) n= n' = 8 (25%) and n = n' = 10 (25%)



**19**, CPC



**20**, Domiphen bromide

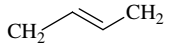
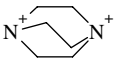


**21**

**Fig. 2.** Structures of the hydrocarbon surfactants **17**–**21** used as standards.

| | Q | R |
|---|---|---|
| **1** | $NHC(O)CH_2$ | $(CH_2)_2$ |
| **2** | $NHC(O)CH_2$ | $(CH_2)_3$ |
| **3** | $NHC(O)CH_2$ | $(CH_2)_4$ |
| **4** | $NHC(O)CH_2$ | $(CH_2)_6$ |
| **5** | $NHC(O)CH_2$ | $(CH_2)_8$ |
| **6** | $NHC(O)CH_2$ | $(CH_2)_{10}$ |
| **7** | $NHC(O)CH_2$ | $(CH_2)_{12}$ |

**BLOCK 1**

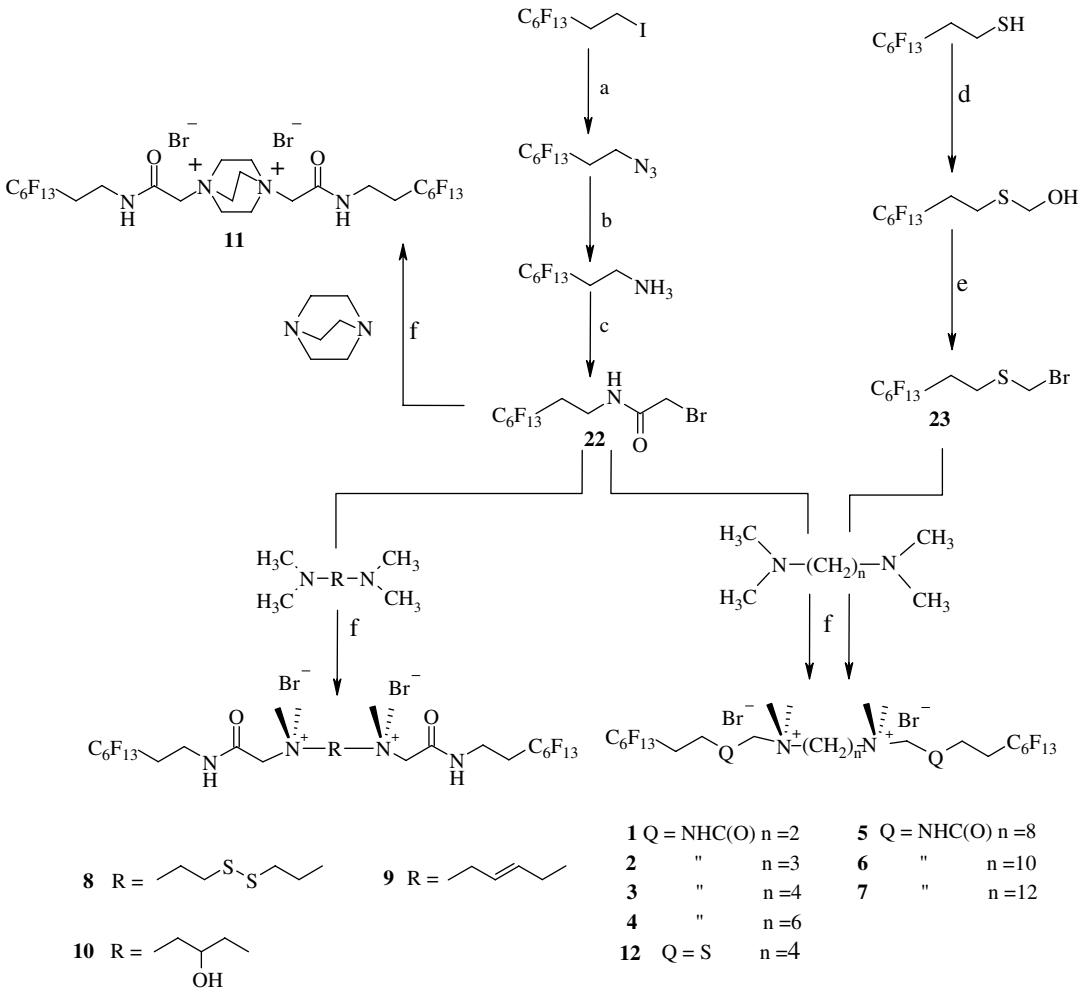
| | Q | R |
|---|---|---|
| **8** | $NHC(O)CH_2$ | $CH_2{-}CH_2{-}S{-}S{-}CH_2{-}CH_2$ |
| **9** | $NHC(O)CH_2$ |  |
| **10** | $NHC(O)CH_2$ | $CH_2{-}CH(OH){-}CH_2$ |
| **11** | $NHC(O)CH_2$ |  |

**BLOCK 2**

| | Q | R |
|---|---|---|
| **12** | $S{-}CH_2$ | $(CH_2)_4$ |
| **13** | No connector | $(CH_2)_4$ |
| **14** | $C(O)NHCH_2CH_2$ | $(CH_2)_4$ |
| **15** | $NHC(O)NHCH_2CH_2$ | $(CH_2)_4$ |
| **16** | $NHC(O)OCH_2CH_2$ | $(CH_2)_4$ |

**BLOCK 3**

**Fig. 1.** Structures of the "Gemini" fluorinated surfactants **1**–**16** synthesized.




Reagents and conditions: (a) $NaN_3$, $H_2O$, ALIQUAT 336 (cat.), (b) 90°C; $CH_3(CH_2)_3OCH_2CH_2OCH_2CH_2OH$, $NH_2NH_2.H_2O$, Raney Ni (cat.), 35°C; (c) DCC, $BrCH_2CO_2H$, $CH_2Cl_2$, 20°C; (d) $N_2$, $Et_3N$, $(CH_2O)_n$, 0 to 20°C, then $H_2O$, 0°C; (e) $PBr_3$, $Et_2O$, 12h, r.t;; (f) corresponding diamine, $Et_2O$, 35°C.

**Scheme 1.** Synthesis of Gemini fluorinated surfactants **1**–**12**.

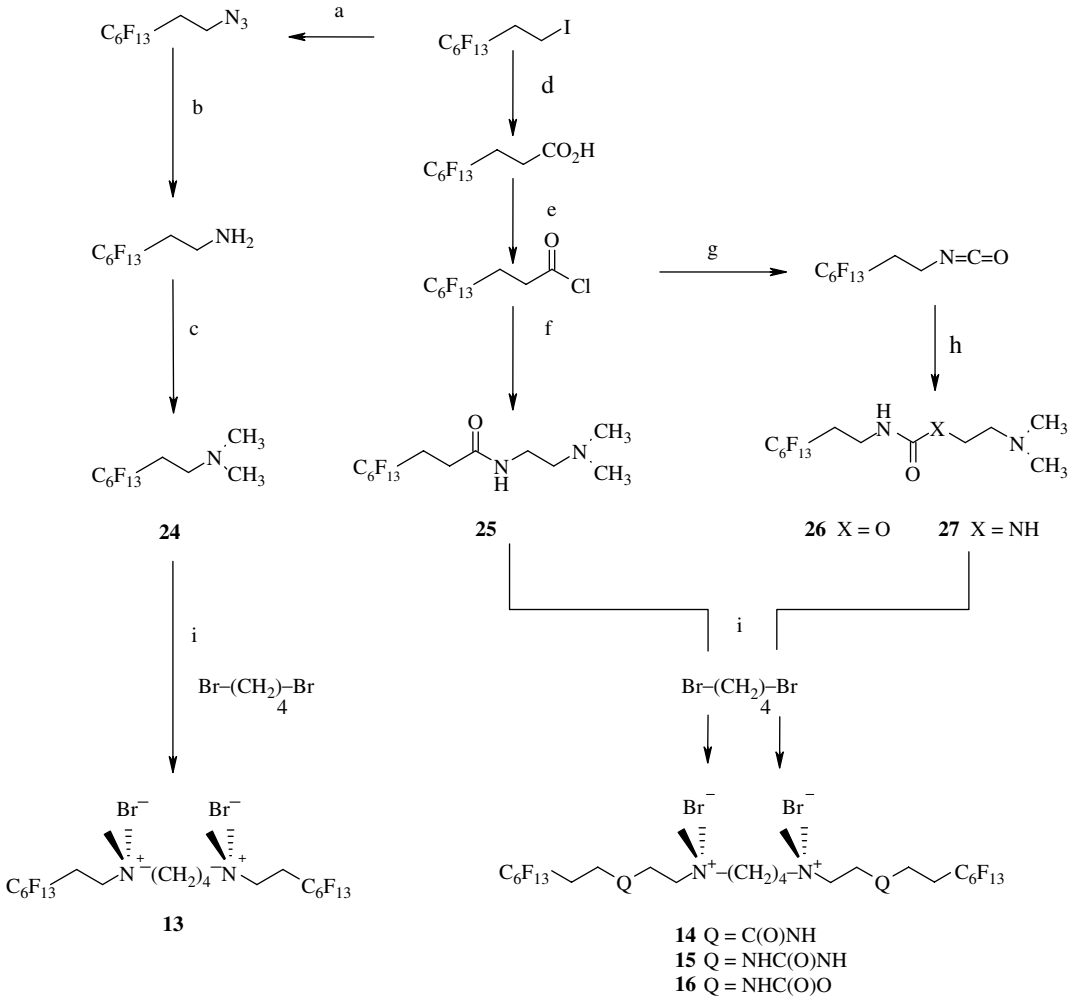
in bromide, affording **23**. The reaction of **23** with an half-equivalent of *N*,*N*,*N*′,*N*′-tetramethyl butanediamine leads to **12**.

As shown in Scheme 2, 2-perfluorohexylethyliodide is the starting material to obtain intermediates **24**–**27**. Then, **24**–**27** are reacted with a half-equivalent of 1,4-dibromobutane to give **13**–**16**, respectively, with good yields. The two first steps for the synthesis of **24** are the same that for **22**. The third step is an Eschweiler and Clarke methylation reaction of primary amines using acetic acid and formaldehyde to afford compound **24** [20]. Concerning **25** and **26**, the two first steps are the same. 3-Perfluorohexylpropanoyl chloride is synthesized by reacting 3-perfluorohexylpropanoic acid, itself obtained from the reaction of carbon dioxide on the organomagnesium derivative of 2-perfluorohexylethyliodide, with phosphorus pentachloride under inert atmosphere. The obtained acyl chloride can then react either with *N*,*N*-dimethylaminoethylamine affording the hydrochloride derivative of compound **25** or with trimethylsilyl azide to give first the perfluorohexylethylisocyanate through a Curtius rearrangement which in turn reacts with dimethylethanolamine or *N*,*N*-dimethylaminoethylamine to afford the carbamate derivative **26** and the urea derivative **27**, respectively.

In order to observe the impact of the fluorinated chain on the antimicrobial activity of such surfactants, we have synthesized **21**, the "Gemini" surfactant homologue of compound **3** according to the synthetic pathway shown in Scheme 3.

### 2.2. Antimicrobial activity and surface activity

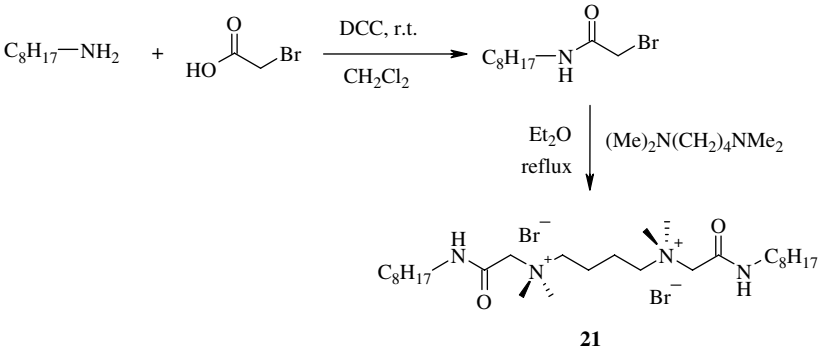
Antibacterial activity of QACs **1**–**21** was evaluated using measurement of minimal inhibitory concentrations (MICs) expressed in μmol/L (see Section 4). These measurement tests were realised on *P. aeruginosa* (CIP A22) Class II type Gram. Benzalkonium chloride (**17**), Catigene 818 (**18**), Cetyl pyridinium chloride (CPC) (**19**), and Domiphen bromide (**20**), were taken as commercial references. Results from the antibacterial evaluation of compounds **1**–**21** are summarized in Table 1 along with the critical micelle concentrations (CMCs) measured using a conductivity technique (see Section 4) and expressed in μmol/L. The MIC values presented are the average of 8–12 measurements for each compound, and the uncertainties are in the order of 10%. Multiple range tests for biological activity by products (Duncan analysis: comparison of averages regarding their standard errors) allowed the ranking of the molecules according to their efficacy for the microorganism tested. Results from the multiple range tests of compounds from Blocks 1, 2 and 3 are summarized in Table 2.




Reagents and conditions: (a) $NaN_3$, $H_2O$, ALIQUAT 336 (cat.), 90°C; (b) $CH_3(CH_2)_3OCH_2CH_2OCH_2CH_2OH$, $NH_2NH_2.H_2O$, Raney Ni (cat.), 35°C; (c) $HCO_2H$, HCOH, 0 to 90°C; (d) $N_2$, Mg, $Et_2O$, 35°C; dry $CO_2$, then $H_3O^+$; (e) $N_2$, $PCl_5$, 80°C; (f) $N_2$, $(CH_3)_2NCH_2CH_2NH_2$, $Et_2O$, 20°C; (g) $(CH_3)_3SiN_3$, $N_2$, r.t.; (h) $N_2$, $(CH_3)_2NCH_2CH_2OH$ or $(CH_3)_2NCH_2CH_2NH_2$, $Et_2O$, 20°C; (i) $Br(CH_2)_4Br$, $CHCl_3$, 61°C.

**Scheme 2.** Synthesis of Gemini fluorinated surfactants **13–16**.

In comparison with the reference compounds **17–20** for which the best activity is exhibited by CPC (**19**) with an MIC of 17.60 μmol/L, all the fluorinated "Gemini" surfactants synthesized, **1–16**, are particularly active antibacterial agents against *P. aeruginosa* with MIC ranging from 1.88 to 9.25 μmol/L that is an activity from 2 to 10 times larger than those of references. The comparison of activities of compounds **3** (MIC = 5.94 μmol/L) with **21** (MIC = 51.16 μmol/L) demonstrates that this enhanced activity is due to the presence of




21

**Scheme 3.** Synthesis of the Gemini hydrocarbon surfactant **21**.

**Table 1**
Antibacterial activity of QACs **1**–**21** against *Pseudomonas aeruginosa* according to minimal inhibitory concentrations (MICs) expressed in μmol/L and the corresponding critical micelle concentrations (CMCs) of **1**–**16** expressed in μmol/L

| Biocide no. | MIC (μmol/L) | CMC (μmol/L) | Biocide no. | MIC (μmol/L) | CMC (μmol/L) |
|---|---|---|---|---|---|
| **1** | 5.80 | 0.591 | **12** | 4.59 | Insoluble |
| **2** | 5.19 | 0.546 | **13** | 7.31 | 2.39 |
| **3** | 5.94 | 0.758 | **14** | 1.88 | 0.547 |
| **4** | 5.52 | 0.588 | **15** | 5.38 | 0.532 |
| **5** | 2.89 | 0.361 | **16** | 3.69 | 0.540 |
| **6** | 2.83 | 0.296 | **17** | 47.72 | |
| **7** | 2.76 | 0.269 | **18** | 50.67 | |
| **8** | 4.95 | 0.451 | **19** | 17.60 | |
| **9** | 3.67 | 0.559 | **20** | 40.82 | |
| **10** | 9.25 | 0.541 | **21** | 51.16 | |
| **11** | 5.82 | 0.466 | | | |

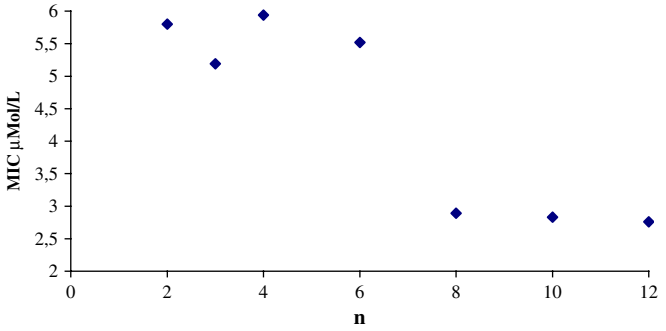



**Fig. 3.** Variations of MIC for the series **1**–**7** as a function of the number *n* of methylene groups in the spacer R.

the perfluorinated chain. This result could be considered as surprising because although the introduction of a perfluorinated chain in the structure of surfactants increases their surface activity, the lipophobicity of surfactants is also increased that could decrease the interaction with the external membrane of the Gram (−) bacteria.

In our previous study, we found the connector Q to have a critical impact on the antimicrobial activity. In view of these previous results, it appears that the most active molecules against various germs have a connector amide, urea or carbamate while connector ester and thioester give QACs with a poor antibacterial activity [18]. The present results are in total agreement with our previous observation since, on one hand compound **3** with an amido connector presented an enhanced activity against *P. aeruginosa* (MIC = 5.94 μmol/L) compared to compound **13** without amido connector (MIC = 7.31 μmol/L) and on the other hand, molecules with amide (**14**), urea (**15**) or carbamate (**16**) connector present very high activities across the series. These results accounts for an important role played by hydrogen bonds in the biological activity against *P. aeruginosa* and are in good agreement with the models established for the prediction of antibacterial activity of various classes of antibacterial agents which state that hydrogen bonding is one of the most important descriptors [21].

However, the "Gemini" surfactant **12** with a sulphide connector shows also a high activity (MIC = 4.59 μmol/L) although it is not a hydrogen bond donor but only a weak hydrogen bond acceptor. What is striking here is that this sulphide derivative shows an important activity against *P. aeruginosa* while the thioester derivative (Q = SC(O)) in which a sulphur lone pair is implied in a conjugation with the carbonyl group and not available to be an hydrogen bond acceptor presents no activity (MIC > 800 μmol/L) [19].

The second structural parameter we studied here is the nature of the spacer R between the two ammonium groups. The comparison of compound **3** with compounds **8**–**11** demonstrates the modulating effect of this spacer on the antibacterial property. At this stage, it is impossible to propose an explanation from the few different spacers we studied in this work.

**Table 2**
Multiple range tests (DUNCAN analysis) for QACs of Blocks 1, 2 and 3 against *Pseudomonas aeruginosa* according to minimal inhibitory concentrations (MICs) expressed in μmol/L

| Block | Ranking (by compounds) |
|---|---|
| Block 1 | **6** ≥ **5** ≥ **7** > **1** ≥ **2** ≥ **4** > **3** |
| | 2.83 ≥ 2.89 ≥ 3.27 > 5.40 ≥ 5.79 ≥ 5.81 > 7.07 |
| Block 2 | **8** > **9** ≥ **11** > **3** > **10** |
| | 3.67 > 5.04 ≥ 5.82 > 7.07 > 9.81 |
| Block 3 | **14** ≥ **16** ≥ **15** ≥ **12** ≥ **3** ≥ **13** |
| | 2.54 ≥ 3.68 ≥ 5.38 ≥ 5.52 ≥ 7.07 ≥ 7.31 |

The third structural parameter studied is the length of the hydrocarbon chain as spacer R between the ammonium groups. Fig. 3 shows the variation of MIC for the series **1**–**7** as a function of the number *n* of methylene groups in the spacer.

Two plateaus are observed, the first for $n \leq 6$ and the second for $n \geq 8$. This observation can be related to that of Zana and Colleagues for hydrocarbon "Gemini" surfactants $C_mH_{2m+1}N^+(CH_3)_2(CH_2)_nN^+(CH_3)_2C_mH_{2m+1}$, $2Br^-$. These authors found that at constant *m*, the CMC of these surfactants changes with the value of *n*, a small maximum of CMC being observed for $n = 5$–6 that reflects the contribution of a change of conformation of the surfactant at the interface with increasing spacer chain length [22,23]. This implies that a linear relationship must exist between the CMC and the MIC at least for the compounds of the homogeneous series **1**–**7**. Indeed, a linear relationship is found to exist between the CMC of surfactants **1**–**7** and their MIC (Fig. 4) that is in agreement with the results obtained for the first time in 1955 from a homogeneous series of quaternary ammonium chlorides [24] and then observed for various series of antibacterial agents [25–28]. It is noteworthy that no relationship can be established between the MIC and the CMC values neither for the series with variable spacers **8**–**11** nor for the series with variable connectors, **12**–**16**. This can be explained by the complexity and the great number of components of the antibacterial effect of quaternary ammonium salts in which the surfactant effect at the surface of the bacterial cell represents only a small part of the whole effect of the antibacterial agent. In the present case, the variation of the length of alkyl spacers in series **1**–**7** modifies only the interfacial effect of the surfactant reflected by the variation of CMC while the other components of the antibacterial effect are not modified as a whole. On the other hand, the variation of the nature of spacers R in the series of compounds **8**–**11** or of the nature of the connector Q in the series **12**–**16** modifies not only the primary surfactant effect but also the other components of the antibacterial effect that are not reflected by the CMC values.

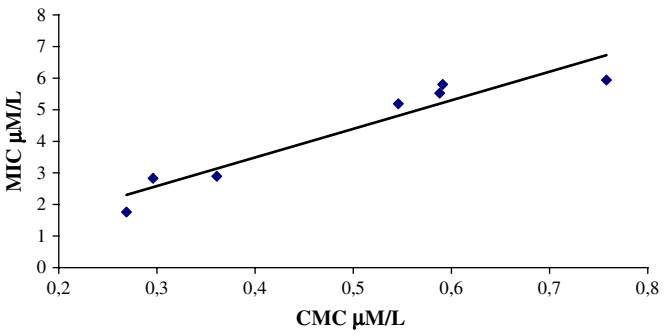



**Fig. 4.** Linear relationships between the CMC and the MIC of surfactants **1**–**7**.

## 3. Conclusion

In this work, we first show the large increase of antibacterial activity of quaternary bisammonium salts against *P. aeruginosa* when a perfluorinated carbon chain is introduced as tails of these "Gemini" surfactants. These fluorosurfactants exhibit an important antibacterial activity as compared to commercial ammonium salt preservatives and to a hydrocarbon homologue. Secondly, we demonstrate that if the length of the alkyl connector between the quaternized nitrogen allows to modulate the antibacterial effect of an homogeneous series of Gemini surfactants by varying their interfacial action with the bacterial cell surface, the variation of the nature of the connector between the charged nitrogen atoms and the fluorinated tails, particularly the presence of hydrogen bond donor group, and of the nature of the spacer between the two quaternized nitrogen atoms modifies more largely the antibacterial effect. These results show the multiplicity of factors occurring in the antibacterial effect of the "Gemini" ammonium surfactants.

## 4. Experimental section

### 4.1. Chemical synthesis

#### 4.1.1. General

NMR data were collected on Brüker Advance 200 spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). $^1$H NMR results are given only for final compounds. The mass spectra of "Gemini" surfactants were recorded on a Finnigan Mat LCQ Classic spectrometer fitted with an Electrospray source API1 ESI-MS mass spectrometer in the positive mode. In this case, only the cationic part of the molecule was detected. As these cations are bischarged (excepted for **21**), the observation of the sole $[M - 2Br]^{++}/2$ peak (where M is the mass of the cationic part of the molecule) is in agreement with the structure. Elemental analysis was performed by analytical laboratory at the Department of Chemistry of University of Nice-Sophia Antipolis on a Thermo-Electron Eager 300 CHNSO apparatus.

As shown in Schemes 1 and 2, the Gemini surfactants **1**–**12** and **13**–**16** were synthesized from building blocks **22** and **23**, and **24**–**27**, respectively. The synthesis of the hydrocarbon surfactant **21** used as reference is resumed in Scheme 3.

#### 4.1.2. Synthesis of building blocks ***22*** *(Scheme 1)*

In the first step (a), 2-perfluorohexylethylazide is obtained by reacting sodium azide on 2-perfluorohexylethyliodide in phase transfer catalysis conditions. (b) Then, the 2-perfluorohexylethylamine is obtained from the reduction of 2-perfluorohexylethylazide by hydrazine hydrate catalysed by Raney nickel (step b). 2-Perfluorohexylethylamine is reacted with DCC and bromoacetic acid to afford compound **22** in 88% yields by coupling reaction (step c).

#### 4.1.3. Synthesis of building block ***23*** *(Scheme 1)*

In the step d, 2-perfluorohexylethanethiol is reacted under inert atmosphere with polyoxymethylene and triethylamine in catalytic amount. Then (step e), phosphorus tribromide is used to convert the alcohol function in bromide, affording the compound **23** in 75% yields.

#### 4.1.4. Synthesis of building block ***24*** *(Scheme 2)*

The steps (a) and (b) are the same that those presented previously. Then (step c), 2-perfluorohexylethylamine is added to a solution of acetic acid and formaldehyde according to the Eschweiler and Clarke methylation reaction of amines to afford compound **24** in 85% yields.

#### 4.1.5. Synthesis of building block ***25*** *(Scheme 2)*

3-Perfluorohexylpropanoic acid is obtained under nitrogen atmosphere from the reaction of dry carbon dioxide on the organomagnesium derivative prepared from 2-perfluorohexylethyliodide and magnesium (step d). (e) 3-Perfluorohexylpropanoyl chloride is synthesized in 90% yields by reacting 3-perfluorohexylpropanoic acid with phosphorus pentachloride under inert atmosphere (step e). Then (step f), *N*,*N*-dimethylaminoethylamine is added at 20 °C and under nitrogen atmosphere to the acyl chloride synthesized previously affording the hydrochloride derivative of compound **25** in 73% yields.

#### 4.1.6. Synthesis of building blocks ***26*** *and* ***27*** *(Scheme 2)*

In the step g, 2-perfluorohexylethyl isocyanate is prepared under nitrogen atmosphere at 70 °C in 80% yields through a Curtius rearrangement of the derivative obtained at room temperature from reaction of trimethylsilyl azide on the acyl chloride synthesized previously. Then (step h), dimethylethanolamine or dimethylaminoethylamine is added under inert atmosphere to the isocyanate to afford the carbamate derivative **26** in 80% yields and the urea derivative **27** in 90% yields, respectively.

To obtain the "Gemini" surfactants **1**–**12**, the building blocks **22** or **23** synthesized previously were reacted with an half-equivalent of the corresponding diamine (step f in Scheme 1) while "Gemini" surfactants **13**–**16** are obtained from reaction between the corresponding α-ω dibromoalkanes and the building blocks **24**, **25**, **26** or **27**.

#### 4.1.7. General procedure for preparation of Gemini surfactants ***1***–***12*** *(Scheme 1)*

In the step f, the building block **22** or **23** is dissolved in diethyl ether and a half-equivalent of the appropriate diamine is added to the solution. The mixture is stirred at reflux for 12 h. The mixture is filtrated and the residue is washed with diethyl ether. This procedure affords the surfactants **1**–**12** with a yields ranging from 80 to 90%.

#### 4.1.8. General procedure for preparation of Gemini surfactants ***13***–***16*** *(Scheme 2)*

In the step i, the building block **25**, **26** or **27** is dissolved in chloroform (isopropanol for building block **24**), and a half-equivalent of α-ω dibromobutane is added to the solution. The mixture is stirred at reflux for 24 h (72 h for **24**). The solvent is evaporated, and the residue is washed with diethyl ether. This procedure affords the surfactants **13**–**16** with a yields ranging from 60 to 80%.

#### 4.1.9. Synthesis of the Gemini hydrocarbon surfactant ***21*** *(Scheme 3)*

Octylamine dissolved in methylene chloride is added dropwise to a solution of DCC and bromoacetic acid in methylene chloride and stirred 3 h at room temperature. After filtration and purification by column chromatography the octylbromoacetamide is obtained with 91% yields. Then, this precursor is added to *N*,*N*,*N*′,*N*′-tetramethyl-1,4-butanediamine dissolved in refluxing diethyl ether to provide the Gemini hydrocarbon surfactant **21** in 93% yields.

### 4.2. Characterizations of final compounds

Characterization of compounds **1**–**4** and **21** is given elsewhere [18].

#### 4.2.1. Gemini surfactant ***5***

*m*/*z* 504.3 $(M - 2Br)^{++}/2$; $^1$H NMR (methanol-$d_4$) $\delta$ 1.44 (8H, m, $[N–CH_2CH_2CH_2CH_2]_2$), 1.85 (4H, m, $[N–CH_2CH_2]_2$), 2.52 (4H, tt, $^3J_{HH} = 6.9$ Hz, $^3J_{HF} = 19.2$ Hz, $[C_6F_{13}–CH_2]_2$), 3.32 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.61 (8H, m, $[CH_2–NH–C(O)]_2 + [^+N–CH_2CH_2]_2$), 4.17 (4H, s, $[C(O)–CH_2–N^+]_2$). Anal. Calcd for $C_{32}H_{42}Br_2F_{26}N_4O_2$: C 32.89, H 3.62, N 4.79; found: C 32.83, H 3.64, N 4.75.

*4.2.2. Gemini surfactant **6***

*m/z* 518.2 $(M-2Br)^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 1.48 (12H, m, $[N–CH_2CH_2CH_2CH_2\ CH_2]_2$), 1.90 (4H, m, $[N–CH_2CH_2]_2$), 2.57 (4H, tt, $^3J_{HH} = 7$ Hz, $^3J_{HF} = 19.3$ Hz, $[C_6F_{13}–CH_2]_2$), 3.41 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.66 (8H, m, $[CH_2–NH–C(O)]_2 + [^+N–CH_2CH_2]_2$), 4.23 (4H, s, $[C(O)–CH_2–N^+]_2$). Anal. Calcd for $C_{34}H_{46}Br_2F_{26}N_4O_2$: C 34.68, H 3.94, N 4.76; found: C 34.65, H 3.93, N 4.74.

*4.2.3. Gemini surfactant **7***

*m/z* 532.3 $(M-2Br)^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 1.38 (16H, m, $[N^+–CH_2CH_2CH_2CH_2CH_2CH_2]_2$), 1.82 (4H, m, $[N–CH_2CH_2]_2$), 2.50 (4H, tt, $^3J_{HH} = 6.7$ Hz, $^3J_{HF} = 19.3$ Hz, $[C_6F_{13}–CH_2]_2$), 3.32 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.59 (8H, m, $[CH_2–NH–C(O)]_2 + [^+N–CH_2CH_2]_2$), 4.18 (4H, s, $[C(O)–CH_2–N^+]_2$). Anal. Calcd for $C_{36}H_{50}Br_2F_{26}N_4O_2$: C 35.31, H 4.11, N 4.57; found: C 34.51, H 4.11, N 4.56.

*4.2.4. Gemini surfactant **8***

*m/z* 508.2 $(M-2Br)^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 2.52 (4H, tt, $^3J_{HH} = 7.0$ Hz, $^3J_{HF} = 19.6$ Hz, $[C_6F_{13}–CH_2]_2$), 3.39 (16H, m, $[CH_3–N^+–CH_3]_2 + [CH_2–CH_2–S]_2$), 3.61 (4H, t, $^3J_{HH} = 7.0$ Hz, $[CH_2–NH–C(O)]_2$), 4.02 (4H, t, $^3J_{HH} = 7.5$ Hz, $[CH_2–CH_2–S]_2$), 4.30 (4H, s, $[C(O)–CH_2–N^+]_2$). Anal. Calcd for $C_{28}H_{34}Br_2F_{26}N_4S_2O_2$: C 28.59, H 2.91, N 4.76, S 5.45; found: C 28.64, H 2.92, N 4.67, S 5.41.

*4.2.5. Gemini surfactant **9***

*m/z* 475.2 $(M-2Br)^{++}/2$ $^1H$ NMR (methanol-$d_4$) $\delta$ 2.51 (4H, tt, $^3J_{HH} = 7.7$ Hz, $^3J_{HF} = 19.5$ Hz, $[C_6F_{13}–CH_2]_2$), 3.37 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.58 (4H, t, $^3J_{HH} = 7.7$ Hz, $[CH_2–NH–C(O)]_2$), 4.20 (4H, s, $[C(O)–CH_2–N^+]_2$), 4.45 (4H, m, $[CH_2–CH=CH]_2$), 6.51 (2H, m, $[CH_2–CH=CH]_2$). Anal. Calcd for $C_{28}H_{32}Br_2F_{26}N_4O_2$: C 30.29, H 2.90, N 5.05; found: C 30.34, H 2.84, N 5.11.

*4.2.6. Gemini surfactant **10***

*m/z* 477.2 $(M-2Br)^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 2.47 (4H, tt, $^3J_{HH} = 7.3$ Hz, $^3J_{HF} = 19.5$ Hz, $[C_6F_{13}–CH_2]_2$), 3.46 and 3.41 (6H + 6H, 2s, $[CH_3–N^+–CH_3]_2$), 3.55 (4H, t, $^3J_{HH} = 7.3$ Hz, $[CH_2–NH–C(O)]_2$), 3.82 (4H, m, $[CH_2–CH(OH)]_2$), 4.36 (2H + 2H, 2d, $^2J_{HH} = 15.2$ Hz, $[C(O)–CH_2–N^+]_2$), 5.05 (1H, m, $[CH_2–CH(OH)]$). Anal. Calcd for $C_{27}H_{32}Br_2F_{26}N_4O_3$: C 29.10, H 2.89, N 5.03; found: C 29.29, H 2.80, N 5.09.

*4.2.7. Gemini surfactant **11***

*m/z* 460.1 $(M-2Br)^{++}/2$. $^1H$ NMR (methanol-$d_4$) $\delta$ 2.61 (4H, tt, $^3J_{HH} = 7.1$ Hz, $^3J_{HF} = 19.1$ Hz, $[C_6F_{13}–CH_2]_2$), 3.71 (4H, t, $^3J_{HH} = 7.1$ Hz, $[CH_2–NH–C(O)]_2$), 4.47 (12H, s, $[N^+(CH_2–CH_2)_3N^+]$), 4.55 (4H, s, $[C(O)–CH_2–N^+]_2$). Anal. Calcd for $C_{26}H_{26}Br_2F_{26}N_4O_3$: C 28.91, H 2.43, N 5.19; found: C 28.95, H 2.45, N 5.16.

*4.2.8. Gemini surfactant **12***

*m/z* 465.1 $(M-2Br)^{++}/2$. $^1H$ NMR (methanol-$d_4$) $\delta$ 1.93 (4H, m, $[N^+–CH_2CH_2]_2$), 2.68 (4H, tt, $^3J_{HH} = 7.3$ Hz, $^3J_{HF} = 19.0$ Hz, $[C_6F_{13}–CH_2]_2$), 3.19 (16H, m, $[CH_3–N^+–CH_3]_2 + [CH_2–S]_2$), 3.61 (4H, m, $[^+N–CH_2CH_2]_2$), 4.88 (4H, s, $[S–CH_2–N^+]_2$). Anal. Calcd for $C_{26}H_{32}Br_2F_{26}N_2S_2$: C 28.64, H 2.96, N 2.57, S 5.88; found: C 28.64, H 2.96, N 2.44, S 5.77.

*4.2.9. Gemini surfactant **13***

*m/z* 419.2: $[M-2Br]^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 2.02 (4H, m, $[N^+–CH_2–CH_2]_2$), 2.99 (4H, tt, $^3J_{HH} = 8.0$ Hz, $^3J_{HF} = 19.0$ Hz, $[C_6F_{13}–CH_2]_2$), 3.31 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.62 (8H, m, $[N^+–CH_2–CH_2]_2 + [C_6F_{13}–CH_2–CH_2–N^+]_2$). Anal. Calcd for $C_{24}H_{28}Br_2F_{26}N_2$: C 28.88, H 2.83, N 2.81; found: C 28.95, H 2.85, N 2.69.

*4.2.10. Gemini surfactant **14***

*m/z* 490.1 $(M-2Br)^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 1.96 (4H, m, $[N^+–CH_2–CH_2]_2$), 2.55 (8H, m, $[CH_2–(CO)]_2 + [C_6F_{13}–CH_2]_2$), 3.21 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.47 (4H, t, $^3J_{HH} = 6.6$ Hz, $[NH–CH_2–CH_2]_2$), 3.58 (4H, m, $[N^+–CH_2–CH_2]_2$), 3.70 (4H, t, $^3J_{HH} = 6.6$ Hz, $[NH–CH_2–CH_2]_2$). Anal. Calcd for $C_{30}H_{38}Br_2F_{26}N_4O_2$: C 31.60, H 3.36, N 4.91; found: C 31.50, H 3.38, N 4.89.

*4.2.11. Gemini surfactant **15***

*m/z* 505.2 $(M-2Br)^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 2.01 (4H, m, $[N^+–CH_2–CH_2]_2$), 2.49 (4H, tt, $^3J_{HH} = 7.3$ Hz, $^3J_{HF} = 19.0$ Hz, $[C_6F_{13}–CH_2]_2$), 3.29 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.57 (16H, m, $[CH_2–NH(CO)NH]_2 + [NH–CH_2–CH_2]_2 + [NH–CH_2–CH_2]_2 + [N^+–CH_2–CH_2]_2$). Anal. Calcd for $C_{30}H_{40}Br_2F_{26}N_6O_2$: C 30.79, H 3.44, N 7.18; found: C 30.96, H 3.33, N 7.22.

*4.2.12. Gemini surfactant **16***

*m/z* 506.1 $(M-2Br)^{++}/2$; $^1H$ NMR (methanol-$d_4$) $\delta$ 1.95 (4H, m, $[N^+–CH_2–CH_2]_2$), 2.47 (4H, tt, $^3J_{HH} = 7.3$ Hz, $^3J_{HF} = 19.0$ Hz, $[C_6F_{13}–CH_2]_2$), 3.22 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.46 (4H, t, $^3J = 7.3$ Hz $[CH_2–NH(CO)O–]_2$), 3.55 (4H, t, $^3J_{HH} = 8.0$ Hz $[N^+–CH_2–CH_2]_2$), 3.78 (4H, m, $[O–CH_2–CH_2]_2$), 4.55 $[O–CH_2–CH_2]_2$. Anal. Calcd for $C_{30}H_{38}Br_2F_{26}N_4O_4$: C 30.73, H 3.27, N 4.78; found: C 31.01, H 3.21, N 4.79.

*4.2.13. Gemini surfactant **21***

*m/z* 563.4, 565.3 $(M-Br)^+$; $^1H$ NMR (methanol-$d_4$) $\delta$ 1.00 (6H, t, $[CH_3–CH_2]_2$), 1.40 (20H, m, $[CH_3(CH_2)_5]_2$), 1.62 (4H, m, $[CH_2CH_2NH]_2$), 201 (4H, m, $[N^+–CH_2CH_2]_2$), 3.31 (4H, t, $[CH_2–NHC(O)]_2$), 3.42 (12H, s, $[CH_3–N^+–CH_3]_2$), 3.89 (4H, m, $[^+N–CH_2–CH_2]_2$), 4;09 (4H, s, $[C(O)–CH_2–N^+]_2$). Anal. Calcd for $C_{28}H_{60}Br_2N_4O_2$: C 52.17, H 9.38, N 8.69; found: C 52.28, H 9.42, N 8.65.

### 4.3. Antimicrobial evaluation

Antibacterial activity of QACs was evaluated using measurement of MIC [29] expressed in μmol/L. These measurement tests were realised on *P. aeruginosa* (CIP A22) Class II type Gram. Bacteria were incubated on TSA (Trypcase Soy Agar) slants for 18 h at 30 °C. MIC tests are run with the third generation of bacteria; samples are taken during the exponential phase of bacterial growth. This third generation of bacterial strains obtained is incubated in an M9 minimal phosphate medium suspension. Benzalkonium chloride (Empigen® Bac 50 purchased from Albright & Wilson®), Catigene 818 (purchased from Stepan®), Domiphen bromide and Cetyl pyridinium Chloride (CPC) (both purchased from ACIMA®) were taken as commercial references. Initial solutions of antimicrobial were prepared at a 500 ppm concentration of active ingredient in distilled water. Bacterial inocula were prepared at $5.10^6$–$5.10^7$ cfu/mL (cfu: colony forming unit). Adjustment was made by optical density measurement (absorbance 0.05 at a wavelength of 660 nm). The Biomek® 1000 (Beckman-Coulter®) apparatus used for our experiments realised automatically dilutions of the tested anti-microbial agent solution in an inoculated 96 wells micro-titration plate. After 24 h of incubation at 30 °C, growth (or its lack) was determined visually. The lowest concentration at which no growth was observed (turbidity) was taken as the MIC. Each measurement is repeated at least 8 times to provide MIC values that are a mean value of a sufficient number of experiments.

### 4.4. Statistics

A full statistic treatment of the antimicrobial activity was carried on. A full analysis of variance (ANOVA) was used to determine the main effects on biological activity. Compounds from Blocks 1, 2 and 3 (Fig. 1) were tested and multiple range tests were done allowing the ranking of the molecules with respect to their activity. Such

tests allow highlighting statistically significant differences in MIC values.

### 4.5. CMC measurements

The CMCs were measured with a Tacussel CDM 210 conductimeter. Samples were thermostated at 25 °C. Conductivity cells were calibrated using 0.1 M potassium chloride solution at 25 °C. All the samples were measured at a constant temperature of 25 °C. The CMCs were determined from the breakpoint of each conductimetry (function of the concentration) curve obtained.

## References

[1] J.M. Ascenzi, Handbook of Disinfectants and Antiseptics, Marcel Dekker Inc., New York, 1996.
[2] L.B. Rice, Clin. Infect. Dis. 43 (Suppl. 2) (2006) S100–S105.
[3] T.F. Mah, B. Pitts, B. Pellock, G.C. Walker, P.S. Stewart, G.A. O'Toole, Nature 246 (2003) 306–310.
[4] V.I. Brown, E.J.L. Lowbury, J. Clin. Pathol. 18 (1965) 752–756.
[5] J. Pernak, J. Rogoza, I. Mirska, Eur. J. Med. Chem. 36 (2001) 313–320.
[6] M. Thuong, K. Arvaniti, R. Ruimy, P. de la Salmonière, A. Scanvic-Hameg, J.C. Lucet, B. Regnier, J. Hosp. Infect. 53 (2003) 274–282.
[7] H. Giamarellou, Int. J. Antimicrob. Agents 16 (2000) 103–106.
[8] P.-R. Hsueh, W.-H. Chen, K.-T. Luh, Int. J. Antimicrob. Agents 26 (2005) 463–472.
[9] E. Kucukates, Jpn. J. Infect. Dis. 58 (2005) 228–231.
[10] M. Arvanitidou, A. Vayona, N. Spanakis, A. Tsakris, J. Appl. Microbiol. 95 (2003) 180–185.
[11] J.A.A. Hoogkamp-Korstanje, J. Roelofs-Willemse, Int. J. Antimicrob. Agents 21 (2003) 547–556.
[12] F. Gunseren, L. Mamikoglu, S. Ozturk, M. Yucesoy, K. Biberoglu, N. Yulug, M. Doganay, B. Sumerkan, S. Kocagoz, S. Unal, S. Cetin, S. Calangu, I. Koksal, H. Leblebicioglu, M. Gunaydin, J. Antimicrob. Chemother. 43 (1999) 373–378.
[13] M.C. Miranda, F. Perez, T. Zuluaga, M.R. Olivera, A. Correa, S.L. Reyes, M.V. Villegas, Biomedica 26 (2006) 424–433.
[14] F.M. Menger, J.S. Keiper, Angew. Chem. Int. Ed. 39 (2000) 1907–1920.
[15] G. Ronsin, A.J. Kirby, S. Rittenhouse, G. Woodnutt, P. Camilleri, J. Chem. Soc., Perkin Trans. 2 (2002) 1302–1306.
[16] F. Zhou, T. Maeda, H. Nagamune, H. Kourai, Biocontrol Sci. 9 (2004) 61–67.
[17] R. Beumer, S. Bloombfield, M. Exner, G.M. Fara, J.K. Nath, E. Scott, International Scientific Forum on Home Hygiene, London, 2000.
[18] L. Massi, F. Guittard, S. Geribaldi, R. Levy, Y. Duccini, Int. J. Antimicrob. Agents 21 (2003) 20–26.
[19] L. Massi, F. Guittard, R. Levy, Y. Duccini, S. Geribaldi, Eur. J. Med. Chem. 38 (2003) 519–523.
[20] E. Farkas, C.J. Sunman, J. Org. Chem. 50 (1985) 1110–1112.
[21] M.T.D. Cronin, A.O. Aptula, J.C. Dearden, J.C. Duffy, T.I. Ntezeva, H. Patel, P.H. Rowe, T. Wayne Schultz, A.P. Worth, K. Voutzoulidis, G. Schürmann, J. Chem. Inf. Comput. Sci. 42 (2002) 869–878.
[22] R. Zana, M. Benrraou, R. Rueff, Langmuir 7 (1991) 1072–1075.
[23] E. Alami, G. Beinert, P. Marie, R. Zana, Langmuir 9 (1993) 1465–1467.
[24] J.A. Cella, L.A. Harriman, D.N. Eggenberger, H.J. Harwood, J. Am. Chem. Soc. 77 (1955) 4264–4266.
[25] T.R.R. Kurup, L.S.C. Wan, L.W. Chan, Pharm. Acta Helv. 66 (1991) 274–280.
[26] F. Devinsky, I. Lacko, D. Mlynarcik, V. Racansky, L. Krasnec, L. Tenside, Detergents 22 (1985) 10–15.
[27] J. Pernak, A. Skrzypczak, M.B. Bogacki, Chem. Pharm. Bull. 43 (1995) 2019–2020.
[28] J. Pernak, A. Skrzypczak, Eur. J. Med. Chem. 31 (1996) 901–903.
[29] M.K. Bruch, in: S.S. Block (Ed.), Disinfection, Sterilization, Preservation, fourth ed. Lea & Febiger, Philadelphia, 1991, pp. 1028–1046.