# New cationic amphiphilic compounds as potential antibacterial agents

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## 5.1 | Introduction

Quaternary ammonium compounds (QACs) are readily accessible cationic substances in which the hydrophobicity can be adjusted easily. QACs such as cetylpyridinium chloride or cetyltrimethylammonium bromide (CTAB, Figure 1) are widely used as disinfectives.[1] These compounds exhibit antibacterial activity from their interference with, or destruction of the bacterial cytoplasmic membrane.[2] At low concentrations in water, CTAB forms gels with worm-like micelle structures.[3]

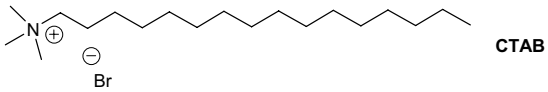


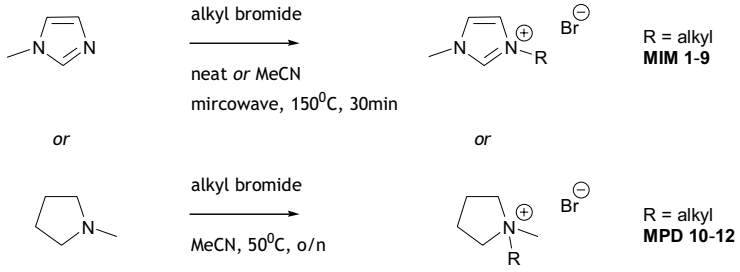

FIGURE 1 | Structure of the QAC cetyltrimethylammonium bromide (CTAB).

Ionic liquids[4] based on quaternary imidazolium salts display amphiphilicity like CTAB. Gelation could be induced in imidazolium-based ionic liquids by addition of organogelators[5] or mesogens (molecules that can exhibit a liquid-crystalline phase).[6] Interestingly, addition of water in low concentrations (~5-40% (wt)) to the ionic liquid *N*-decyl-*N'*-methyl imidazolium bromide was found to induce nearly instantaneous formation of a lyotrophic (*i.e.* concentration-dependent) liquid-crystalline gel phase.[7] The obtained so-called 'ionogels' resisted flow against gravity for an indefinite period of time. As *N*-alkyl-*N'*-methyl imidazolium QACs are expected[8,9] to show antibacterial activity, their encapsulation into gravity-stable gels leads to interesting antibiotic formulations that might be useful for personal (topical) decontamination purposes.[10] This Chapter describes the synthesis and the antibiotic activity of an array of *N*-alkyl-*N'*-methyl imidazolium (MIM) bromides **1-9** and **15-18** (Table 1) as well as *N*-alkyl-*N*-methyl pyrrolidinium (MPD) bromides **10-14**, and of their encapsulation in stable gels obtained by mixing with polar liquids.

## 5.2 | MIM and MPD QACs

### 5.2.1 | Syntheses

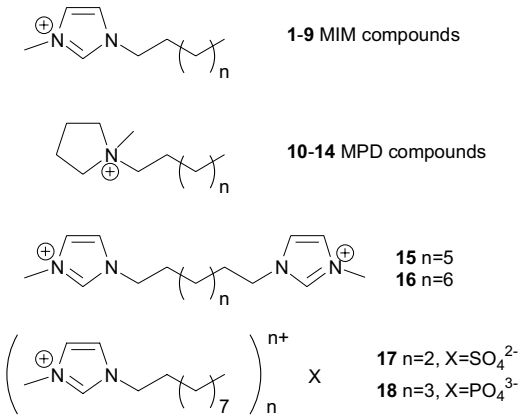
*N*-alkyl-*N'*-methyl imidazolium (MIM) salts were readily obtained through established synthesis procedures, performing the alkylation of *N*-methylimidazole with *n*-alkyl bromides equimolarly, neat or in MeCN,[11] at 150$^0$C for 30min in a microwave oven (Scheme 1). After extraction and vacuum drying at 70$^0$C, compounds **1-9** were obtained in 85-90% yield and were found to be pure apart from some residual $H_2O$. The physical appearances of these MIM salts changed with alkyl chain length. Compounds **1-6** were obtained as ionic liquids whereas **7-9** were solids.




**SCHEME 1** | Preparation of *N*-alkyl-*N'*-methyl imidazolium (MIM) and *N*-alkyl-*N*-methyl pyrrolidinium (MPD) bromides. For the R (alkyl) groups, see Table 1 below.

**TABLE 1** | Synthesized QACs.[A]

| Compound | | Compound | |
|---|---|---|---|
| **1** | $C_7$MIM Br | **10** | $C_{10}$MPD Br |
| **2** | $C_8$MIM Br | **11** | $C_{11}$MPD Br |
| **3** | $C_9$MIM Br | **12** | $C_{12}$MPD Br |
| **4** | $C_{10}$MIM Br | **13** | $C_{13}$MPD Br |
| **5** | $C_{11}$MIM Br | **14** | $C_{14}$MPD Br |
| **6** | $C_{12}$MIM Br | **15** | $C_9MIM_2$ 2Br |
| **7** | $C_{13}$MIM Br | **16** | $C_{10}MIM_2$ 2Br |
| **8** | $C_{14}$MIM Br | **17** | $(C_{10}MIM)_2$ $SO_4$ |
| **9** | $C_{16}$MIM Br | **18** | $(C_{10}MIM)_3$ $PO_4$ |




[A] MIM = *N*-alkyl-*N'*-methyl imidazolium, MPD = *N*-alkyl-*N*-methyl pyrrolidinium, where '*N*-alkyl' refers to the linear $C_x$ alkyl group.

Non-aromatic *N*-alkyl-*N*-methyl pyrrolidinium (MPD) bromides were prepared through overnight reaction of *N*-methylpyrrolidine and *n*-alkyl bromide in MeCN at $50^0$C (Scheme 1). [12,13] Pure MPD salts **10-14** (Table 1) were obtained by either spontaneous crystallization or after precipitation upon addition of $Et_2O$. Application of the microwave-based synthesis of these bromides gave rise to the formation of unidentified impurities that could not be removed easily. All MPD bromides were obtained as crystalline white solids in yields of 70-95%. The choice of limiting the array of MPD salts to the ones containing $C_{10}$ to $C_{14}$ alkyl chains was based on the data obtained from MIC determinations of analogous MIM compounds (*vide infra*).

Next, dicationic MIM salts **15** and **16** (structure see Table 1), in which the two cationic sites are connected through a hydrophobic stretch were prepared from *N*-methylimidazole and linear α,ω-dibromoalkanes (0.5eq.) applying the microwave approach. Finally, the 'bis-MIM' sulfate **17** and 'tris-MIM' phosphate **18** were prepared by conversion of compound **4** with either $Ag_2SO_4$ or $Ag_3PO_4$[14] and were included to examine the effect of a multivalent anion.

## 5.2.2 | Biological Evaluation

The minimal inhibitory concentration (MIC) values of all MIM compounds were determined against *Escherichia coli* ATCC 11775. Results are found in Table 2 and show an obvious relationship between length of n-alkyl chain and antimicrobial activity. QACs with alkyl chains < 10 carbon atoms did not display any antimicrobial activity up to 200μM. On the other hand, lengthening the alkyl chain >14 atoms did not increase the MIC value, a finding that agrees with earlier reports.[9a]

**TABLE 2** | MIC[A] and MHC[B] values of MIM and MPD QACs.

| # | MIC (μM) | MHC (μM) | # | MIC (μM) | MHC (μM) |
|---|---|---|---|---|---|
| **1** | >200 | n/d | **10** | >1000 | >1000 |
| **2** | >200 | n/d | **11** | 500 | >1000 |
| **3** | >200 | 1000 | **12** | 250 | 500 |
| **4** | 200 | n/d | **13** | 125 | 250 |
| **5** | 100 | 125 | **14** | 62.5 | 125 |
| **6** | 50 | n/d | **15** | >1000 | n/d |
| **7** | 25 | 125 | **16** | 1000 | n/d |
| **8** | 12.5 | n/d | **17** | 250 | n/d |
| **9** | 12.5 | 31 | **18** | 31 | n/d |

[A] Minimal inhibitory concentration against *E. coli* ATCC 11775; [B] Minimal hemolytic concentration; n/d - not determined. It should be noted that for MIM and MPD QACs different bacterial growth media were used (see Experimental section).

Dicationic MIM species **15** and **16** are virtually devoid of antibacterial activity, which can be explained by the fact that a terminal hydrophobic tail is needed to penetrate the membrane, a feature absent in **15** and **16**. Furthermore, no conclusion can be drawn from the MIC values regarding the effect of multivalent anions; compounds **4** (bromide) and **17** (sulfate) have MIC values in the same range whereas **18** (phosphate) is >6 times as active as **4**.[15] Similarly, the MIC values of the MPD bromides were determined against *E. coli* ATCC 11775. As expected, the same trend was observed as with the MIM salts: a longer alkyl chain constitutes higher antimicrobial activity.[16] Additionally, hemolytic indexes were determined of some of these compounds. MIM compounds **3**, **5**, **7** and **9** and the MPD bromides were tested for their potency to lyse erythrocytes and were found to have MHC (minimal hemolytic concentration, see Table 2) values that were close to the MIC values (1.25-5 fold (MIM) or 2-fold (MPD) the MIC values).

## 5.3 | Gel Formation

Having established the antimicrobial potency of the synthesized MIM and MPD bromides, attention was focussed on the gel formation of selected salts by mixing with water and two other polar liquids (ethylene glycol and glycerol).[17]

### 5.3.1 | Water-based gels (*W*-gels)

Following the procedure in which **4** showed phase transition upon mixing with ~5-40% (wt) $H_2O$, water-based gels (*W*-gels) of MIM bromides **4**-**7** were prepared. In case of MIM bromides **4**-**7** (see Table 3) homogeneous gels were obtained after addition of $H_2O$ at percentages ranging from 10-35% (wt) and homogenization by centrifugation. Obtaining homogeneous gels containing the lowest percentages of additive and longest alkyl chains required additional heating and/or sonication. For the MPD bromides (**10**-**14**, Table 3), gels with water (*W*-gels) could also be prepared with percentage of additive ranging from 10-50% (wt). In contrast, commercially available CTAB (**19**) did not form a *W*-gel. Both MIM and MPD bromides appear to follow a trend in which gel formation of compounds containing longer alkyl chains requires a higher percentage of additive. Some of the prepared *W*-gels ($C_{14}$MIM gels **8**) were found unstable (*i.e.* remained no longer in gel phase) when exposed to the air movements in a fume hood (*vide infra*), very likely due to a decrease in % (wt) of $H_2O$. Gels based on ethylene glycol or glycerol, with

boiling points higher than that of water, were therefore expected to be more stable under such conditions.

TABLE 3 | Gels of selected QACs containing water (*W*).

| | | Additive ($H_2O$ wt%) | | | |
|---|---|---|---|---|---|
| **#** | **Composition** | **a. 10%** | **b. 16%** | **c. 25%** | **d. 35%** |
| **4** | $C_{10}$MIM | Clear gel | Clear gel | Clear gel | Clear gel |
| **5** | $C_{11}$MIM | Clear gel | Clear gel | Clear gel | Clear gel |
| **6** | $C_{12}$MIM | Clear gel | Clear gel | Clear gel | Clear gel |
| **7** | $C_{13}$MIM | Solid | Clear gel | Clear gel | Clear gel |
| **8** | $C_{14}$MIM | Solid | Inh. | Clear gel | Clear gel |
| **9** | $C_{16}$MIM | Solid | Solid | Solid | Solid |
| | | **e. 20%** | **f. 30%** | **g. 40%** | **h. 50%** |
| **10** | $C_{10}$MPD | Clear gel[A] | Clear gel | Fluid | Fluid |
| **11** | $C_{11}$MPD | Inh. | Clear gel | Clear gel | Clear gel |
| **12** | $C_{12}$MPD | Inh. | Clear gel | Clear gel | Fluid |
| **13** | $C_{13}$MPD | Solid | Inh. | Clear gel | Clear gel |
| **14** | $C_{14}$MPD | Solid | Inh. | Clear gel | Clear gel |
| **19** | CTAB | Solid | Solid | Solid | Solid |

[A] A gel with 10% additive could also be constructed from this compound (**10a**); Inh. – inhomogeneous.

## 5.3.2 | Ethylene glycol-based gels (*E*-gels)

Compounds **4**, **5**, **10** and **19** could not be transformed into a gel with ethylene glycol (*E*-gel) in a range of 10-40% (wt). Whereas gelation of **9** was not induced with $H_2O$, 35% (wt) ethylene glycol caused gel formation.

TABLE 4 | Physical appearances of QACs upon addition of ethylene glycol (*E*).

| | Additive (ethylene glycol wt%) | | | |
|---|---|---|---|---|
| **Compound** | **i. 10%** | **j. 20%** | **k. 30%** | **l. 40%** |
| **4** $C_{10}$MIM | Fluid | Fluid | Fluid | Fluid |
| **5** $C_{11}$MIM | Fluid | Fluid | Fluid | Fluid |
| **6** $C_{12}$MIM | Inhomogeneous | Clear gel | Fluid | --- |
| **7** $C_{13}$MIM | Inhomogeneous | Inhomogeneous | Clear gel | Clear gel |
| **8** $C_{14}$MIM | Solid | Inhomogeneous | Inhomogeneous | Clear gel |
| **9** $C_{16}$MIM | Solid | Solid | Inhomogeneous | Clear gel[A] |
| **10** $C_{10}$MPD | Fluid | Fluid | Fluid | Fluid |
| **11** $C_{11}$MPD | Clear gel | Clear gel | --- | --- |
| **12** $C_{12}$MPD | Clear gel | Clear gel | Fluid | --- |
| **13** $C_{13}$MPD | Inhomogeneous | Clear gel | Clear gel | --- |
| **14** $C_{14}$MPD | Solid | Inhomogeneous | Clear gel | Fluid |
| **19** CTAB | Solid | Solid | Solid | Solid |

[A] 35% (wt) instead of 40% (wt) of ethylene glycol was used. --- not prepared.

In contrast to some of the $C_{14}$MIM *W*-gels, the *E*-gel **8l** proved to be stable towards air movements in a fume hood. Although the trend observed for *W*-gels regarding % additive/alkyl chain length also applies to *E*-gels, a higher % (wt) of additive was necessary to form homogeneous *E*-gels with increasing alkyl chain length as compared to *W*-gels.

### 5.3.3 | Glycerol-based gels (*G*-gels)

Preparation of MIM and MPD gels containing glycerol (*G*-gels) yielded similar trends as did the *E*-gels; all three **4**, **5** and **10** would not form a gel with 10-40% glycerol (Table 5). In general, higher percentages of glycerol were needed to form gels than was necessary with ethylene glycol. $C_{16}$MIM **9** could not be transformed into a *G*-gel, nor could CTAB **19**.

TABLE 5 | Physical appearances of QACs upon addition of glycerol (*G*).

| | Additive (glycerol wt%) | | | |
|---|---|---|---|---|
| **Compound** | **m. 10%** | **n. 20%** | **p. 30%** | **q. 40%** |
| **4** $C_{10}$MIM | Fluid | Fluid | Fluid | Fluid |
| **5** $C_{11}$MIM | Clear gel | Fluid | Fluid | --- |
| **6** $C_{12}$MIM | Inhomogeneous | Clear gel | Clear gel | --- |
| **7** $C_{13}$MIM | Inhomogeneous | Inhomogeneous | Clear gel | Clear gel |
| **8** $C_{14}$MIM | Solid | Inhomogeneous | Inhomogeneous | Clear gel |
| **9** $C_{16}$MIM | Solid | Solid | Solid | Solid |
| **10** $C_{10}$MPD | Fluid | Fluid | Fluid | Fluid |
| **11** $C_{11}$MPD | Clear gel | Clear gel | --- | --- |
| **12** $C_{12}$MPD | Inhomogeneous | Clear gel | Clear gel | Fluid |
| **13** $C_{13}$MPD | Inhomogeneous | Inhomogeneous | Clear gel | Fluid |
| **14** $C_{14}$MPD | Solid | Solid | Inhomogeneous | Clear gel |
| **19** CTAB | Solid | Solid | Solid | Solid |

* Inhomogeneity is due to inability to mix QAC and additive thoroughly ('partly gelated'). --- not prepared.

## 5.4 | Gel Stability

Gel stability against a range of external factors determines their potential applicability. Preliminary results of studies towards the resistance of gels against gravity, temperature and water are discussed in the following paragraphs.

### 5.4.1 | Gravity

The *W*-gels **4b**, **5b** and **6b**, as well as **7c** and **8c** were examined for their ability to resist flow against gravity.[18] They were found to be resistant against gravity for at least 5 consecutive days, whereas the other MIM *W*-gels were stable for at least 6h (the longest period examined for these gels). MPD *W*-gels all were gravity-stable (at least 5 days), as were both all MIM and MPD *E*-gels. In the *G*-gel series, only MIM gel **6p** and MPD gel **12n** were found to be gravity- sensitive.

### 5.4.2 | Temperature

When examined for thermal stability, nearly all gels proved to be stable (*i.e.* did not become fluid, Table 6) up to temperatures of 60-65$^0$C for 30min. Exceptions were *W*-gels **4b** and **4c** (already becoming fluid at slightly elevated temperatures within seconds), and **10a** and **11f** that liquefied after heating to 65$^0$C after a few minutes. Increase in stability is observed in gels of **5** and **6** with increasing % (wt) of $H_2O$; the 30% versions are more stable than are their 16% counterparts. The results obtained with *E*-gels show that none of the MIM gels tested are stable except $C_{16}$MIM **9l**, whereas those composed of the corresponding MPD salts easily seem to handle heating to 60-65$^0$C for 30min. *G*-gels all appeared to be stable under the conditions given.

### 5.4.3 | Water resistance

Water resistance is an important aspect regarding applicability. It is undesirable for antimicrobial gels used as protective coatings outdoors to be easily removed *e.g.* at the event of rain. In this light, preliminary studies on the 'water-stability' of the prepared gels show that $C_{16}$MIM gel **9l** is a promising candidate. This particular gel, when dispensed on a vertical glass plate, showed the ability to withstand a one-minute, continuous drop-wise flow of 20mL/min of tap water. Although **9l** was not completely unaffected by this treatment, the water stability observed was not found in the vast majority of the gels reported in this study.

**TABLE 6** | Visual effects upon heating of selected gels.

| Gel | Composition | T ($^0$C)[A] | t (s) | Observation |
|---|---|---|---|---|
| **4b** | $C_{10}$MIM / 16% *W* | 24 | 5 | - |
| **4c** | $C_{10}$MIM / 30% *W* | 24 | 5 | - |
| **5b** | $C_{11}$MIM / 16% *W* | 42 | 90 | +/- |
| **5c** | $C_{11}$MIM / 30% *W* | 65 | 1800[B] | + |
| **6b** | $C_{12}$MIM / 16% *W* | 65 | 600 | +/- |
| **6c** | $C_{12}$MIM / 30% *W* | 65 | 1800 | + |
| **7c** | $C_{13}$MIM / 30% *W* | 65 | 1800 | + |
| **8c** | $C_{14}$MIM / 30% *W* | 65 | 1800 | + |
| **10a** | $C_{10}$MPD / 10% *W* | 65 | 360 | +/- |
| **11f** | $C_{11}$MPD / 30% *W* | 65 | 510 | +/- |
| **12f** | $C_{12}$MPD / 30% *W* | 65 | 1800 | + |
| **13g** | $C_{13}$MPD / 40% *W* | 65 | 1800 | + |
| **14f** | $C_{14}$MPD / 30% *W* | 60 | 1800 | + |
| **6k** | $C_{12}$MIM / 30% *E* | 65 | 360 | +/- |
| **7k** | $C_{13}$MIM / 30% *E* | 65 | 1200 | +/- |
| **8l** | $C_{14}$MIM / 40% *E* | 40 | 30 | - |
| **9l** | $C_{16}$MIM / 35% *E* | 65 | 1800 | + |
| **11i** | $C_{11}$MPD / 10% *E* | 65 | 360 | +/- |
| **12j** | $C_{12}$MPD / 20% *E* | 60 | 1800 | + |
| **13k** | $C_{13}$MPD / 30% *E* | 60 | 1800 | + |
| **6n** | $C_{12}$MIM / 20% *G* | 65 | 1800 | + |
| **7p** | $C_{13}$MIM / 30% *G* | 65 | 1800 | + |
| **8q** | $C_{14}$MIM / 40% *G* | 65 | 1800 | + |
| **11m** | $C_{11}$MPD / 10% *G* | 60 | 1800 | + |
| **12p** | $C_{12}$MPD / 30% *G* | 60 | 1800 | + |
| **13p** | $C_{13}$MPD / 30% *G* | 60 | 1800 | + |
| **14q** | $C_{14}$MPD / 40% *G* | 65 | 1800 | + |

[A] Temperature reached; [B] No apparent changes after prolonged heating to 3600s; + no apparent changes; - becomes fluid; +/- becomes partially fluid at time indicated.

## 5.5 | Biological Evaluation of Gels

Selected gels were assayed for their antimicrobial potency using an ISO standardized film adherence method.[19] In this method, small glass plates are coated with a thin layer of gel. A bacterial suspension containing Gram-negative *Escherichia coli* ATCC 8739 or Gram-positive *Staphylococcus aureus* ATCC 6538P was then added to the coating, covered, and incubated for 24h at 37$^0$C. Surviving colonies were replated and counted. Results are summarized in Tables 7-10 below (key: see Table 10) and show that all *W*-, *E*- and *G*-gels tested killed >99.9% of *E. coli*; a selection of the gels also eradicated Gram-positive *S. aureus* for >99.9%.

**TABLE 7** | Antibacterial evaluation of selected MIM *W*-gels.

| # | Composition | *E. coli* ATCC 8739 | | *S. aureus* ATCC 6538P | |
|---|---|---|---|---|---|
| | | $t_0$ (log CFU)[A,B] | $t_{24}$ (log CFU)[A,C] | $t_0$ (log CFU)[A,B] | $t_{24}$ (log CFU)[A,C] |
| | Negative control | 5.11 | 6.49 | 4.87 | 5.06 |
| **4b** | $C_{10}$MIM/16% | 5.11 | <1.0[D] | 4.87 | <1.0[D] |
| **5b** | $C_{11}$MIM/16% | 5.11 | <1.0 | 4.87 | <1.0 |
| **6b** | $C_{12}$MIM/16% | 5.11 | <1.0 | 4.87 | <1.0 |
| **7c** | $C_{13}$MIM/30% | 5.11 | <1.0 | 4.87 | <1.0 |
| **8c** | $C_{14}$MIM/30% | 5.11 | <1.0 | 4.87 | <1.0 |

**TABLE 8** | Antibacterial evaluation of selected MPD *W*-gels.

| # | Composition | *E. coli* ATCC 8739 | |
|---|---|---|---|
| | | $t_0$ (log CFU)[A,B] | $t_{24}$ (log CFU)[A,C] |
| | Negative control | 4.54 | 5.91 |
| **10e** | $C_{10}$MPD/20% | 4.54 | <1.0[D] |
| **11e** | $C_{11}$MPD/20% | 4.54 | <1.0 |
| **12g** | $C_{12}$MPD/40% | 4.54 | <1.0 |
| **13h** | $C_{13}$MPD/50% | 4.54 | <1.0 |
| **14h** | $C_{14}$MPD/50% | 4.54 | <1.0 |

**TABLE 9** | Antibacterial evaluation of selected MIM & MPD *E*-gels.

| # | Composition | *E. coli* ATCC 8739 | |
|---|---|---|---|
| | | $t_0$ (log CFU)[A,B] | $t_{24}$ (log CFU)[A,C] |
| | Negative control | 4.54 | 5.91 |
| **7l** | $C_{13}$MIM/40% | 4.54 | <1.0[D] |
| **8l** | $C_{14}$MIM/40% | 4.54 | <1.0 |
| **11j** | $C_{11}$MPD/20% | 4.54 | <1.0 |
| **12i** | $C_{12}$MPD/10% | 4.54 | <1.0 |
| **13j** | $C_{13}$MPD/20% | 4.54 | <1.0 |
| **14k** | $C_{14}$MPD/30% | 4.54 | <1.0 |

**TABLE 10** | Antibacterial evaluation of selected MIM & MPD *G*-gels.

| # | Composition | *E. coli* ATCC 8739 | |
|---|---|---|---|
| | | $t_0$ (log CFU)[A,B] | $t_{24}$ (log CFU)[A,C] |
| | Negative control | 4.54 | 5.91 |
| **5n** | $C_{11}$MIM/20% | 4.54 | <1.0[D] |
| **6n** | $C_{12}$MIM/20% | 4.54 | <1.0 |
| **7q** | $C_{13}$MIM/40% | 4.54 | <1.0 |
| **8q** | $C_{14}$MIM/40% | 4.54 | <1.0 |
| **11n** | $C_{11}$MPD/20% | 4.54 | <1.0 |
| **12p** | $C_{12}$MPD/30% | 4.54 | <1.0 |
| **13p** | $C_{13}$MPD/30% | 4.54 | <1.0 |

Key for Tables 7-10: [A] Average of triplo measurement; [B] At time t=0; [C] At time t=24h; [D] <1.0 equals >99.9% eradication. CFU - colony forming units.

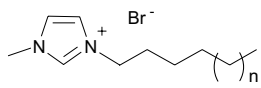
## 5.6 | Conclusion

Alkylated MIM and MPD bromides were synthesized and their antibiotic activity was determined against *E. coli* ATCC 11775. Increased activity was observed along with increasing chain length up to $C_{14}$. The relatively high MIC values, in combination with their MHC values (only a factor ~2 higher than the MIC values) indicate a lack of cell-selectivity and do not allow for systemic use.[2b]

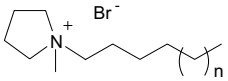
Unlike commercially available CTAB, most (ionic liquid and solid) MIM and MPD bromides could be brought into gel phase by addition of water, ethylene glycol, or glycerol. In general, the length of the alkyl chain appears to impose limitations on gel formation: whereas only an *E*-gel of $C_{16}$MIM bromide **9** could be obtained, CTAB **19** would not form a *W*-, *E*-, or *G*-gel in the range of 10-50% (wt), nor would $C_{18}$MIM (not shown). Preliminary tests to assess the susceptibility of the gels to external factors showed that the majority of the gels resisted flow against gravity and could withstand a temperature of 60-65$^0$C for 30min. Gel **9l** appeared to be also largely stable against running water. A selection of gravity-stable gels were assayed for antibacterial activity against *E. coli* ATCC 8739, and in some cases against *S. aureus* ATCC 6538P. Both these Gram-negative and Gram-positive bacteria were eradicated for >99.9%.

## 5.7 | Experimental Section

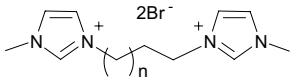
### 5.7.1 | Syntheses




**MIM bromides (1-9).** Typical procedure for the synthesis and analysis of MIM bromides: *n*-alkyl bromide (10mmol) and *N*-methylimidazole (1eq.) were stirred in a microwave oven (Personal Chemistry) at 150$^0$C for 30min with the Absorption leve option set at 'high'. The product was extracted in $Et_2O/H_2O$/ MeOH, removing all unreacted reagents, and the solvents were removed through lyophilization. The bromides were obtained as ionic liquids (1-6) whereas 7-9 were solids. Compounds were analyzed by $^1$H NMR, $^{13}$C NMR and LCMS. All compounds were found to be pure except for residual water (~5-20% (wt) after vacuum drying for 24h at 70$^0$C; percentage increases along with alkyl chain). Yields: $C_{10}$MIM 4: 83%, $C_{11}$MIM 5: 90%, $C_{12}$MIM 6: 87%, $C_{13}$MIM 7: 88%, $C_{14}$MIM 8: 87%. $^1$H NMR data was found to be consistent with the data published.[9a] Representative analysis for $C_{14}$MIM Br 8: $^1$H NMR (MeOD): δ 9.01 (s, 1H, H2), 7.67 (t, 1H, H3), 7.59 (t, 1H, H4), 4.23 (t, 2H, H5), 3.95 (s, 3H, H1), 1.90 (m, 2H, H6), 1.34 (bm, 22H, H7-17), 0.90 (t, 3H, H18). $^{13}$C NMR (MeOD): δ 124.8, 123.5 (C3, C4), 50.6 (C5), 36.3 (C1), 32.8, 30.9, 30.5, 30.2, 29.9, 27.0, 23.5 (C6-17), 14.2 (C18). LC (254nm): Rt 18.2min. ESI-MS: 265.2 $[M–Me+H]^+$, 279.3 $[M]^+$.



**MPD bromides (10-14).**Typical procedure for synthesis and analysis of MPD salts: alkyl bromide (15mmol) and *N*-methylpyrrolidine (1eq.) were stirred at 50$^0$C for 16h, yielding a white precipitate in each case. The precipitate was filtered off, washed with $Et_2O$ (3x) removing all unreacted reagents and dried, yielding alkyl MPD bromides as white solids. Yields: $C_{10}$MPD **10**: 85%, $C_{11}$MPD **11**: 92%, $C_{12}$MPD **12**: 92%, $C_{13}$MPD **13**: 96%, $C_{14}$MPD **14**: 70%. All MPD salts were obtained as white solids containing some residual water (~8-20% (wt) after vacuum drying for 24h at 70$^0$C). $C_{13}$MPD contains ~25% (wt) $H_2O$ as exception. Representative analysis for $C_{10}$MPD Br **10**: $^1$H NMR (DMSO-$d_6$): δ 3.52 (m, 4H, H2), 3.38 (m, 2H, H4), 3.02 (s, 3H, H1), 2.09 (bm, 4H, H3), 1.69 (bm, 2H, H5), 1.26 (bm, 14H, H6-12), 0.87 (t, 3H, H13). $^{13}$C NMR (DMSO-$d_6$): δ 63.4 (C2), 63.0 (C4), 47.4 (C1), 31.4, 29.0, 28.8, 28.6, 26.0, 23.0, 22.2, 21.1 (C3, C5-12), 14.0 (C13). ESI-MS: 212.0 $[M\text{-}Me+H^+]^+$, 225.8 $[M^+]^+$, 565.5 $[2M^+ + TFA^-]^+$.



**$MIM_2$ bromides (15, 16).** Typical synthetic procedure: *n*-alkyl-α,ω-di-bromides (3mmol) were treated according to the described microwave procedure using 10mmol of *N*-methylimidazole; MeCN was added to a final volume of 3mL. After repeated of the extraction, residual *N*-methyl imidazole was found to be present by NMR, and compounds were purified by gel filtration using an LH20 column (88x2.8cm) and MeOH as eluent. Yields: $C_9MIM_2$ **15**: 64%, $C_{10}MIM_2$ **16**: 60%. Representative analysis for $C_9MIM_2$·2Br **15**: $^1$H NMR (MeOD): δ 9.23 (s, 2H, 2xH2), 7.82 (t, 2H, 2xH3), 7.74 (t, 2H, 2xH4), 4.35 (t, 4H, 2xH5), 4.05 (s, 6H, 2xH1), 1.95 (m, 4H, 2xH6), 1.37 (bm, 10H, 2xH7, 2xH8, H9). $^{13}$C NMR (MeOD): δ 124.9, 123.6 (C3, C4), 50.7 (C5), 37.0 (C1), 31.0, 29.7, 29.5, 27.0 (C6-C9).

**Metathesis of $C_{10}$MIM (17, 18)**

Bromide **4** (0.5mmol) was dissolved in 1mL MeOH/$H_2O$ 1/1 (v/v), and $Ag_2SO_4$ (**17**) or $Ag_3PO_4$ (**18**), both 1.0eq (taking into account the multivalent anions), were added under the exclusion of light. After stirring for 72h, samples were filtered and solvents were evaporated. After standing for 14d in daylight, newly formed precipitate was filtered off. $^{31}$P NMR ($D_2O$) of **18** showed a single peak. Materials were then subjected to antibacterial assays.

## 5.7.2 | Gel formation

***W*-gels (a-h)**

Gels of MIM compounds **4-8** were prepared by determination of the amount of residual $H_2O$ in a sample by $^1$H NMR (acetone-$d_6$) and then addition of $H_2O$ to obtain a gel with defined weight percentage of $H_2O$ (final concentrations of 10, 16, 25 and 35% (wt)). Gels containing MPD compounds **10-14** were obtained by determination of the $H_2O$ content through $^1$H NMR (DMSO-$d_6$) and subsequent addition of $H_2O$ to obtain the gels (final concentrations of 10, 20, 30 and 40% (wt)).

***E*-gels (i-l) and *G*-gels (m-p)**

Compounds were dried for 16h under vacuum at 70$^0$C. Ethylene glycol (*E*-gels) or glycerol (*G*-gels) was added to samples starting at 10% (wt), and increasing stepwise with 10% (wt) until a homogeneous gel would form (after gently heating/sonication and centrifugation if necessary) to a maximum of 50% (wt).

## 5.7.3 | Antibacterial assay in solution

*E. coli* ATCC 11775 were grown on nutrient agar plates and kept at 4$^0$C. Imidazolium salts were dissolved in Luria-Bertani (LB) and MPD salts in Brain-Heart Infusion (BHI) to give a concentration of 200μM and filtered using 0.22μm filter discs. An overnight culture in LB broth was adjusted to 5x10$^6$ CFU/mL and inoculated into the micro titre plate wells containing each 100μL of a serial two-fold dilution (200μM-down) of the tested compound in LB/BHI broth. After incubation for 24h at 37$^0$C, absorbance was measured at 600nm using a μQuant micro plate spectrophotometer. MIC values of compounds were measured in 3-fold and averaged.

### 5.7.4 | ISO Film adherence assay

Antimicrobial activities of the gels were quantitatively established using the film adherence method using Gram-negative *E. coli* ATCC 8739 and Gram-positive *S. aureus* ATCC 6538P. At time $t_0$, in 3-fold, object glasses were thinly coated in an area of $3cm^2$ with 2–5mg of the antimicrobial gel. Subsequently, bacterial suspension (50µL) containing approx. $1x10^5$ CFU was applied onto the coating and covered with a plastic film. Test samples were incubated for 24h at $37^0C$, after which the number of surviving bacteria was determined (*i.e.* $t_{24}$): bacteria were removed with swabs from the glasses, suspended and plated in tryptic soy agar (TSA). The TSA plates were incubated for 3 days at $37^0C$, after which the number of developing colonies was counted. The number of surviving bacteria were calculated in CFU and the antibacterial activity was calculated using log CFU @ $t_0$ (negative control) – log CFU @ $t_{24}$ (test sample).

### 5.7.5 | Hemolysis Assay

Minimal hemolytic concentration (MHC) values were determined by averaging the results of three measurements, according to the method described in see Chapter 1.

## 5.8 | Notes & References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9. During this research, a number of *n*-alkylated imidazolium salts (*N*-alkyl-*N'*-methyl imidazolium (MIM) salts) were indeed found (a) to exhibit antibiotic activity against a variety of bacteria, fungi and the model nematode *C. elegans* (b): (a) Demberelnyamba, D.; Kim, K.-S.; Choi, S.; Park, S.-Y.; Lee, H.; Kim, C.-J.; Yoo, I.-D. *Bioorg. Med. Chem.* **2004**, *12*, 853; (b) Swatloski, R.P.; Holbrey, J.D.; Memon, S.B.; Caldwell, G.A.; Caldwell, K.A.; Rogers, R.D. *Chem. Commun.* **2004**, 668
10. As are for example, the well-known Betadine-gel (also known as povidone-iodine) as in (a) O'Connor Jr, L.T.; Goldstein, M. *J. Am. Coll. Surg.* **2002**, *194*, 407; (b) Eason, E.; Wells, G.; Gerber, G.; Hemmings, R.; Luskey, G.; Gillett, P.; Martin, M. *BJOG* **2004**, *111*, 695; (c) Ostrander, R.V.; Brage, M.E.; Botte, M.J. *Clin. Orthop. Relat. Res.* **2003**, 246; (d) http://www.vidal.fr/Medicament/betadine-2054.htm or a gel containing polymyxin B and a QAC: Langford, J.H.; Artemi, P.; Benrimoj, S.I. *Ann. Pharmacother.* **1997**, *31*, 559
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12. Attempts to alkylate the corresponding *aromatic* heterocyclic compound *N*-methylpyrrole failed using any of the procedures described here; this is likely due to the fact that the nitrogen's lone pair is part of the aromatic system.

13. During this research, the following publication appeared on MPD salts: Baker, G.A.; Pandey, S.; Pandey, S.; Baker, S.N. *Analyst* **2004**, *129*, 890
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15. As a part of the $PO_4^{3-}$ anions will be protonated due to their basicity ($pK_a$ of $HPO_4^{2-}$ 12.32) in solution, compound **18** will not be encountered in this composition. This makes a mono/ multivalent anion comparison rather complicated. Besides, it cannot be excluded that trace amounts of $Ag^+$ (bactericidal in the low µM range) are present in **17** and **18** as the metathesis method used relies on precipitation of AgBr. The minimum achievable level of $Ag^+$ contamination is dictated by the solubility product constant of AgBr in water, $5.2x10^{-13}$ (ref. 14).
16. It should be noted that for MIC determinations for MIM and MPD bromides, different growth media were applied (see Experimental section).
17. Based on their structural similarity: Ivanova, R.; Lindmann, B.; Alexandridis, P. *J. Colloid Interface Sci.* **2002**, *252*, 226
18. A 'gravity-resistant'or 'gravity-stable' gel refers here to the ability of a gel to remain at the same location at the bottom of a test tube if the tube is turned upside down for a defined period of time.
19. Japanese Industrial Standard JIS Z 2801: 2000 (E) Antimicrobial products - Test for antimicrobial activity and efficacy.

# CHAPTER 6 | Fluorous Techniques in Solid-Phase Peptide Synthesis

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