New Biocide with Both *N*-Chloramine and Quaternary Ammonium Moieties Exerts Enhanced Bactericidal Activity

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Considering the rise of antibiotic resistance, the development of new antibacterial agents with improved biocidal functions is urgently required. In this study, ionic 5,5-dimethylhydantoin (DMH) analogues containing either a quaternary ammonium moiety (2)–4) or a phosphonate functional group (5),–6), were designed and synthesized to investigate the possible enhancing effect of quaternary ammonium moieties on the antibacterial performance of *N*-chloramines. These ionic DMH analogues were converted to their *N*-chloramine counterparts either in free form or after being covalently immobilized on a polymer surface via the "click" chemistry method. In the subsequent antimicrobial assessment against multi-drug-resistant *Escherichia coli* (MDR-*E. coli*) and methicillin-resistant *Staphylococcus aureus* (MRSA), chlorinated 2 and 3, the cyclic *N*-chloramines with a structural cation, exhibited distinctly enhanced biocidal functions in solution and after immobilization on surfaces.

1. Introduction

Healthcare associated infections (HAIs) are defined as infections obtained by patients during their stay in healthcare facilities or as a result of healthcare interventions. Recent decades have witnessed a rapid increase in the incidence of HAIs caused by antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA).[1] A community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) surveillance conducted by the Minnesota Department of Health (MDH) in Minnesota indicated that the number of CA-MRSA infections increased 10-fold during the period 2000 to 2005.[2] For HAIs to occur there must be a source of infectious organisms and a means of transmission. Infectious microorganisms can shed from other patients and then contaminate the environment. Healthcare personnel as well as environmental surfaces including privacy curtains, nursing uniforms, and laboratory bench surfaces serve as major reservoirs for infectious microorganisms, which can be passed onto subsequent patients or staff via airborne and vehicle-borne transmission. Of the 1561 nosocomial outbreaks studied, 40.3% could be attributed to index patients, 21.1% to contaminated equipment or devices, and 19.8% to the environment.[3] Consequently, to promote public health, there is an urgent need for research and development in agents with potent antimicrobial activity which can be grafted or coated onto various surfaces in healthcare facilities to intervene the transmission of infectious microorganisms as a supplemental measure to infection control and prevention measures such as handwashing.

Three major categories of broad-spectrum biocides that can be used in solution as well as coated onto surfaces are: silver, quaternary ammonium compounds (QACs), and *N*-chloramines. Silver-based biocides, including silver nanoparticles (AgNPs), silver nitrate, and silver sulfadiazine, have been widely used in medical settings. It has been proven that silver-coated catheters, compared to conventional catheters, reduce infection rates.[1] Also, AgNPs coated wound-care dressings have achieved success in reducing the bacterial burden in burn wounds and preventing infections. However, several bacterial strains including *E. coli* K-12, O157:H7, MRSA 133/03, 151, and 152 have been found to contain a silver-resistant gene: silE.[4,5] Even though the widespread usage of silver has not been found to induce cross-resistance to antibiotics[6] and increased antibiotic resistance may not be associated with increased biocide resistance,[7] the emergence of silver-resistant bacterial strains entails frequent monitoring of the occurrence of silver-resistant bacteria and necessitates a continuous effort in searching for new powerful biocides.

There are also reports of bacterial resistance against QACs[8,9] but none against *N*-chloramines. Whereas it is difficult to

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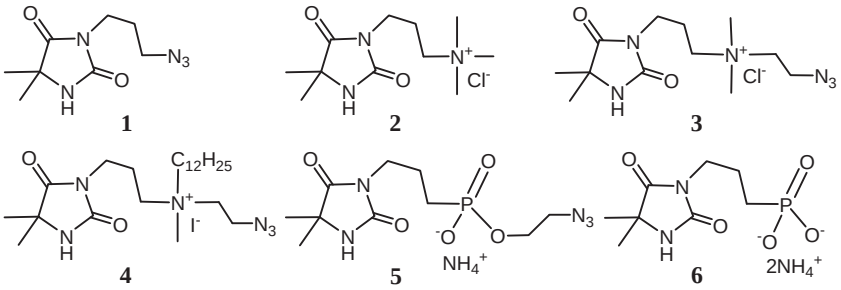
DOI: 10.1002/adhm.201200018



Scheme 1. Structures of *N*-chloramine precursors used in this research.

develop an entirely new category of effective biocides, it is beneficial to study the possible synergistic effects among the existing biocides. Recently we found that poly(2-(*N*-chloroacrylamido)-2-methylpropane-1-sulfonic acid) (poly(CAMPS)) grafted cotton fabrics showed a lower antibacterial efficacy against *E. coli* K-12 than cotton fabrics grafted with poly(*N*-chloro-*N*-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)acrylamide) poly(CTHMA) despite the former sample having a higher active chlorine content (332 ppm vs. 141 ppm).[10] The less effective biocidal property might be caused by negative charges on the poly(CAMPS) grafted cotton, which can repel *E. coli* cells because of the negative charges on their cell walls. It is hypothesized that a molecule with both cationic and *N*-chloramine moieties could demonstrate enhanced antibacterial efficacy and that further combining long-chain antibacterial QAC (QAC becomes antibacterial when its alkyl substitution reaches 6-carbons or longer) with *N*-chloramine may produce a synergistic antibacterial activity. The central point of this hypothesis is that cations might boost the antibacterial efficacy of *N*-chloramine when these two moieties are covalently bonded together. Worley and coworkers prepared a copolymer that consists of a *N*-chloramine siloxane block and a QAC siloxane block, but only improved water solubility was achieved without detectable enhanced disinfection efficacy.[11,12] This may be due to the fact that the highly effective biocidal effect of the *N*-chloramine block overshadowed the possible boosting effect of the QAC moieties.

In this research we designed and prepared nonionic **1**[13] and ionic **2-6** (**Scheme 1**) derivatives of 5,5-dimethyl hydantoin (DMH), one of the most studied precursors of cyclic *N*-chloramine biocides.[11,14–16] All the synthetic small molecules were converted into corresponding *N*-chloramine forms by chlorination in solution or after being covalently bonded onto PET and cotton fabrics using "click" chemistry. Antibacterial assessment was performed and an enhanced antimicrobial performance was observed for chlorinated **2** and **3**, the DMH analogues that possess both the structural cation and *N*-chloramine moieties.

2. Result and Discussion

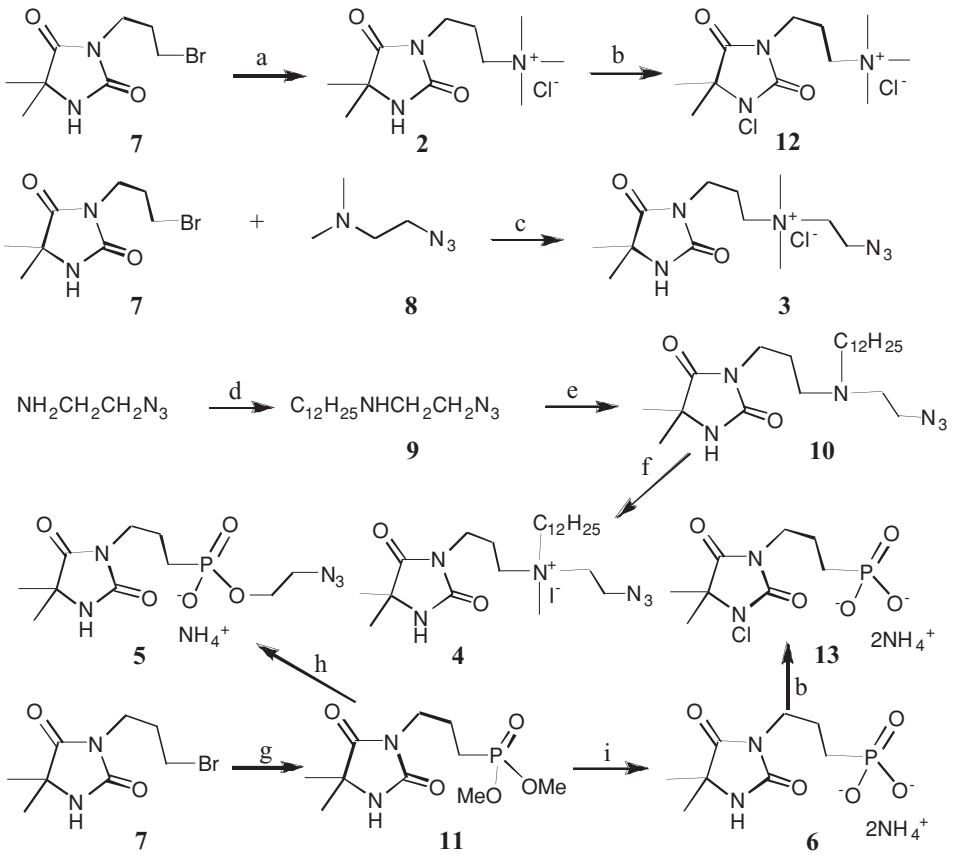
2.1. Model Study–Antibacterial Performance of Ionic DMH Derivatives 12 and 13

To test the hypothesis that introducing a positive charge into the structure of *N*-chloramine may enhance its antibacterial efficacy, compound **2**, a hydantoin derivative with cationic charge, was synthesized and converted to its *N*-chloramine counterpart (compound **12** in **Scheme 2**). Compound **6**, a hydantoin derivative with anionic charge, was also synthesized and converted to *N*-chloramine (compound **13**) for comparison. Both compounds **2** and **6** were used to serve as controls.

Strains of *Escherichia coli* (*E. coli*) a typical Gram-negative bacterium and *Staphylococcus aureus* a typical Gram-positive bacterium were studied. In the model study we investigated the bactericidal performance of small molecules **12** and **13** against three strains for each bacterium at the concentration of 15 ppm. As shown in **Table 1**, **12** demonstrated a total kill of all six bacterial strains within 5 min whereas no significant reduction was observed for **13** within the same time frame. For **13**, a total kill or >3 log reduction was only achieved for a contact time of 20 min except for MRSA #77090. This indicates that a positive charge of the *N*-chloramine compound contributes to a faster bacterial killing as compared to a negative charge. The fact that >3 log reduction or total kill (except MRSA # 77090) can still be achieved using compound **13** by extending the contact time to 20 min led us to a conclusion that the negative charge just impedes the killing kinetics without compromising the overall antibacterial capacity of **13**.

2.2. Design and Synthesis of "Clickable" Ionic Antibacterial Precursors

Following the encouraging model study in solution, we moved to the next step: grafting DMH derivatives onto poly(ethylene terephthalate)(PET) and study the possible synergistic effect between immobilized QACs and *N*-chloramines. One major advantage of *N*-chloramine biocides is that once immobilized onto a surface, a rechargeable self-disinfecting property can result as the chlorination and de-chlorination (bacterial killing) is reversible. Previously we have successfully developed a PET platform bearing an alkyl handle that allows easy immobilization of azido compounds using "click" chemistry.[13] Accordingly, for the construction of ionic azido precursors of DMH analogues for grafting, at least three segments are to be combined: DMH, a positive or negative charge center, and an azide functional group that can be easily clicked onto the alkyl handle on the PET platform. As chemical modification of the methyl group in DMH involves intense synthetic chemistry usually with low yield,[17] it is better to designate DMH as a terminal moiety. Either a positive or a negative charge center could serve as a bridge linking two terminal functional groups (DMH and azide). Linear azido precursors with cation **3** and **4** were synthesized in a route similar to the synthesis of **1**.[13] As for the negatively charged moiety, it should also act as a shoulder pole to carry the azide handle and the DMH moiety at each end. Therefore phosphoric acid, a commonly used triprotic acid, was herein adopted and the final azido precursor with anion was designed as compound **5**. We introduced a C-P bond into the structure because the resultant phosphonate (C-P) is more

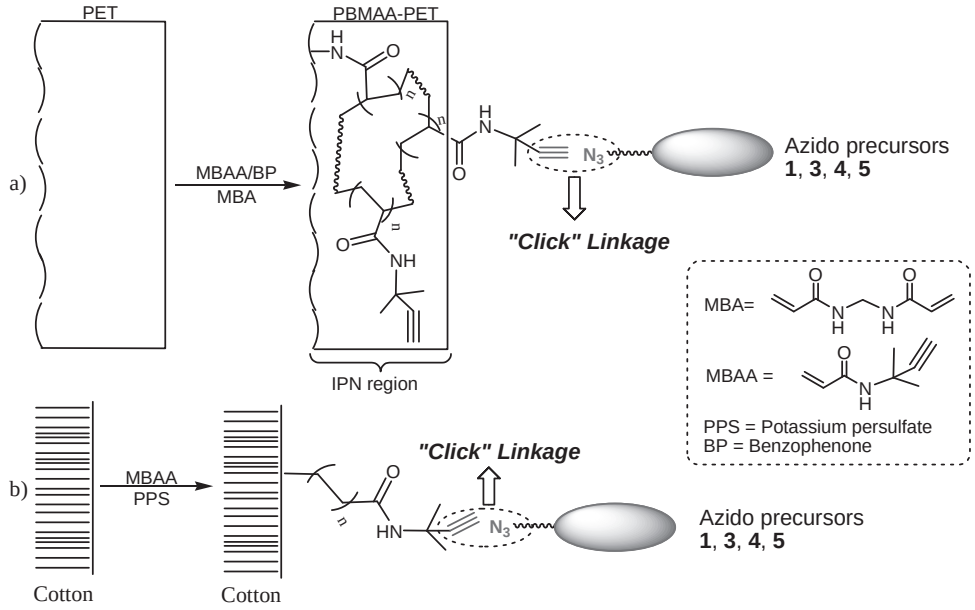


Scheme 2. Chemical synthesis of **2–6**. Reagents and conditions: a) Aqueous dimethylamine, EtOH, reflux, overnight, then Cl^- exchange, 90%; b) *t*-butyl hypochlorite, *t*-BuOH/H_2O (4:1), rt, overnight, quantitive; c) CH_3CN, reflux, 14 h, then Cl^- exchange 99%; d) anhydrous K_2CO_3, DMF, 70 °C, 14 h, 60%; e) K_2CO_3, DMF, 70 °C, 14 h, 72% f) MeI, CH_3CN, rt, 10 h, 88%; g) Dimethylphosphite, NaH/DMF, 24 h, 65%; h) trimethylsilylbromide (TMSBr), CH_2Cl_2, rt, 12 h then 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl), azidoethanol, pyridine, rt, 24 h, and NH_4^+ exchange, 64% in two steps; i) TMSBr, CH_2Cl_2, rt, 12 h, then NH_4^+ exchange, 90%.

Table 1. Antibacterial efficacy of **12** and **13** against 3 *E. coli* and 3 MRSA strains.

Bacteria[a]		Synthetic compounds[b]	Bacteria reduction at various contact times [min]					
			5		10		20	
			%	Log_{10}	%	Log_{10}	%	Log_{10}
Gram-negative	*E. coli* ATCC 25922	**12**	100	6.63	100	6.63	100	6.63
		13	28.5 ± 3.4	0.15	99.96 ± 0.00	3.40	100	6.63
	MDR-*E. coli* (#70094)	**12**	100	6.17	100	6.17	100	6.17
		13	35.6 ± 1.9	0.19	66.8 ± 0.5	0.48	100	6.17
	MDR-*E. coli* (#95882)	**12**	100	6.67	100	6.67	100	6.67
		13	4.6 ± 1.2	0.02	99.75 ± 0.02	2.59	99.94 ± 0.03	3.24
Gram-positive	MRSA ATCC 33592	**12**	100	6.60	100	6.60	100	6.60
		13	6.2 ± 0.9	0.028	98.83 ± 0.12	1.94	99.94 ± 0.01	3.19
	MRSA (#70527)	**12**	100	6.76	100	6.76	100	6.76
		13	32.5 ± 3.5	0.17	99.78 ± 0.00	2.97	100	6.76
	MRSA (#77090)	**12**	100	6.16	100	6.16	100	6.16
		13	37.1 ± 10.6	0.2	52.8 ± 4.5	0.33	74.2 ± 0.5	0.59

[a]Inoculum concentration: 1.46 – 5.87 × 10^6 CFU/mL; [b]Compounds **2** and **6** were used as controls.



Scheme 3. Immobilization of azido-precusors onto a) PET and b) cotton via "click" chemistry.

stable compared to alkyl phosphates (C-O-P) especially when treated with trimethylsilyl bromide (TMSBr) for methyl deprotection.[18] The synthesis of **2–6** is depicted in Scheme 2 and described in the Experimental Section.

2.3. Immobilization of "Clickable" Ionic Antibacterial Precursors on PET

Attachment of **1**, **3**, **4**, and **5** on a PET surface was completed by forming an interpenetrating network (IPN)[19] of poly(*N*-(2-methylbut-3-yn-2-yl)acrylamide) poly(MBAA) (PMBAA, **Scheme 3**a) on the PET surface (named as PMBAA-PET) followed by a "click" linkage reaction.[13] After the covalent immobilization, all PET samples were chlorinated to activate their biocidal function.

We chose one MDR-*E. coli* strain (#70094) to challenge these modified PET samples. **Table 2** outlines the antibacterial results

Table 2. Antibacterial efficacy of modified PET fabrics against MDR-*E.coli*.

Modified PET Sample	Active chlorine [ppm]	Reduction of MDR-*E. coli* (#70094)[a)]	Contact angle (After chlorination)
PET	0	0	122 ± 8.1
PMBAA-PET	132 ± 23	0	106.4 ± 7.3
PMBAA-PET-**1**	427 ± 19	46.4 ± 0.5%	90.8 ± 5.6
PMBAA-PET-**3**	433 ± 23	99.8 ± 0.1%	UD[b)]
PMBAA-PET-**4**	434 ± 25	23.2 ± 2.5%	107.1 ± 4.1
PMBAA-PET-**5**	423 ± 31	43.7 ± 2.9%	78.1 ± 10.1

[a)]Inoculum concentration was 1.02×10^7CFU/mL,% reduction after a contact time of 5 min; [b)]Undetectable (too hydrophilic to be detectable).

of the "click"-modified PMBAA-PET samples against MDR-*E. coli* (#70094). The PMBAA-PET samples clicked with various hydantoin derivatives (termed as PMBAA-PET (**1**, **3**, **4**, **5**)) were loaded with a similar amount of active chlorine (around 430 ppm). PMBAA-PET-**3** showed the best antibacterial efficacy which might be because of the cationic charge on **3**. On the other hand, only a 23.2% bacterial reduction, the worst efficacy among all clicked samples, was achieved with PMBAA-PET-**4**, which possesses both *N*-chloramine and long chain QAC moieties. This was unexpected and intrigued us to conduct contact-angle measurements. PMBAA-PET-**4** was still quite hydrophobic with a contact angle of 107.1° ± 4.1°, similar to PMBAA-PET. The surface energy of the PMBAA-PET-**4** sample was not high enough to cause the bacterial suspension to spread on its surface. In the antibacterial test, even if a sterilized beaker was loaded on the top of the fabric assembly between which a bacterial suspension was sandwiched to help create intimate contact, minute beads of the bacterial suspension existed on the hydrophobic surface hindering the contact killing process. For more hydrophilic samples such as PMBAA-PET-**3**, however, the bacterial suspension could spread over the surface immediately after being dispensed so that a sufficient contact with the immobilized biocides was ensured. Therefore, differences in the biocidal efficacies of all the samples were confounded in their differences in hydrophilicity and surface charges (negative, neutral, and positive). The sequence of bactericidal strength was: PMBAA-PET-**3** > PMBAA-PET-**1** > PMBAA-PET-**4** corresponding to their hydrophilicities as denoted by the contact angles (undetectable, 90.8 ± 5.6 and 78.1 ± 10.1). Although we can clearly see that PMBAA-PET-**3** demonstrates the most potent biocidal efficacy among all the samples, no convincing conclusion can be drawn about the effect of the cation center of PMBAA-PET-**3** on its biocidal efficacy. To eliminate the effect of substrate hydrophobicity on the antibacterial efficacy,

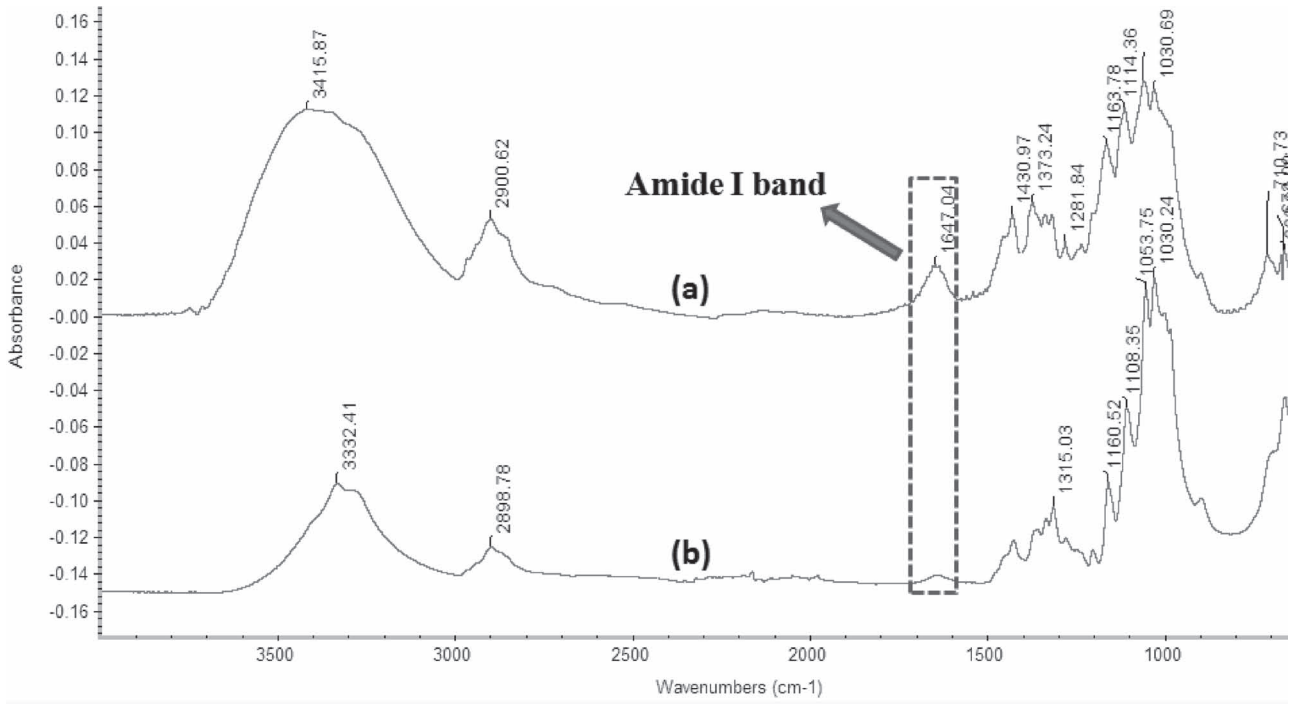


Figure 1. ATR spectra of a) PMBAA-*g*-cotton (graftpercentage = 1.1%); b) untreated cotton.

we decided to graft **1**, **3**, **4**, and **5** onto a hydrophilic cotton substrate.

2.4. Immobilization of "Clickable" Ionic Antibacterial Precursors on Cotton

To bind the synthetic azido precursors on cotton fabrics, PMBAA was first grafted onto cotton (termed as PMBAA-*g*-cotton) via potassium persulfate (PPS) initiated radical grafting polymerization[15] to present surface alkynyl groups (Scheme 3b). In the attenuated total reflectance (ATR) spectrum of PMBAA-*g*-cotton (**Figure 1**a), a new peak appeared at 1647 cm^{-1}, which is characteristic of the carbonyl stretch C=O of amide in PMBAA. N-H stretching gave rise to a broad peak centered at 3421 cm^{-1} in the spectrum of PMBAA-g-cotton (Figure 1a). The ATR results implied successful grafting of PMBAA onto cotton. To visualize the distribution of PMBAA on cotton, we attached 2-azidoethyl 5-(dimethylamino)naphthalene-1-sulfonate (ADNS), an azido fluorescent dye previously synthesized in our research group,[13] onto PMBAA-*g*-cotton using the "click" chemistry method (denoted as PMBAA-*g*-cotton-ADNS, see Scheme S1 in the supporting information). As shown in **Figure 2**, uniform green fluorescence was observed on PMBAA-*g*-cotton-ADNS whereas only blue auto-fluorescence of cotton appeared on the control sample, which indicated the surface PPS-induced grafting polymerization was successful and the alkynyl groups were uniformly distributed on the cotton surface. Next, the synthetic azido-precursors **1**, **3**, **4**, and **5** were attached onto PMBAA-*g*-cotton in the presence of Cu^{2+}/Na ascorbate at room temperature. Afterwards, a chlorination process was carried out converting those clicked

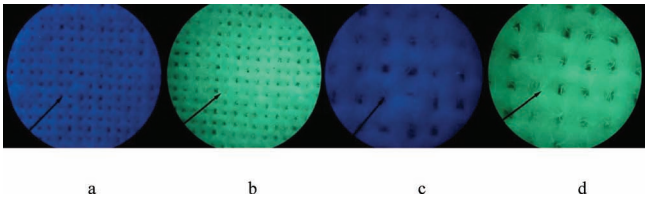


Figure 2. Visualization of PMBAA-*g*-cotton-ADNS under UV light (365 nm). a) and c) Control samples; b) and d) "clicked" samples. Magnification of images: a,b) 40×; c,d) 100×.

cotton samples into their corresponding *N*-chloramines. A similar level of active chlorine on the modified PET was obtained by adjusting the available chlorine of the chlorinating NaClO solution. However, as cotton fabric is hydrophilic, a small change of available chlorine in the chlorination solution can result in a significant variation of the active chlorine on the modified cotton samples. Therefore, we studied the chlorination kinetics of those "click"-modified cotton fabrics.

Based on a previous study,[10] the chlorination reaction could be regarded as being in first-order relationship to the amide concentration according to:

$$v = -\mathrm{d}[\mathrm{amide}]/\mathrm{dt} = k[\mathrm{NaClO}][\mathrm{amide}] \quad (1)$$

where v is the chlorination reaction rate, k is the rate constant and t is the reaction duration.

As NaClO for chlorination was in excess, k[NaClO] could be regarded as constant k'. Integration of Equation 1 gives:

$$\ln\{[\mathrm{amide}]t/[\mathrm{amide}]0\} = -k't \quad (2)$$

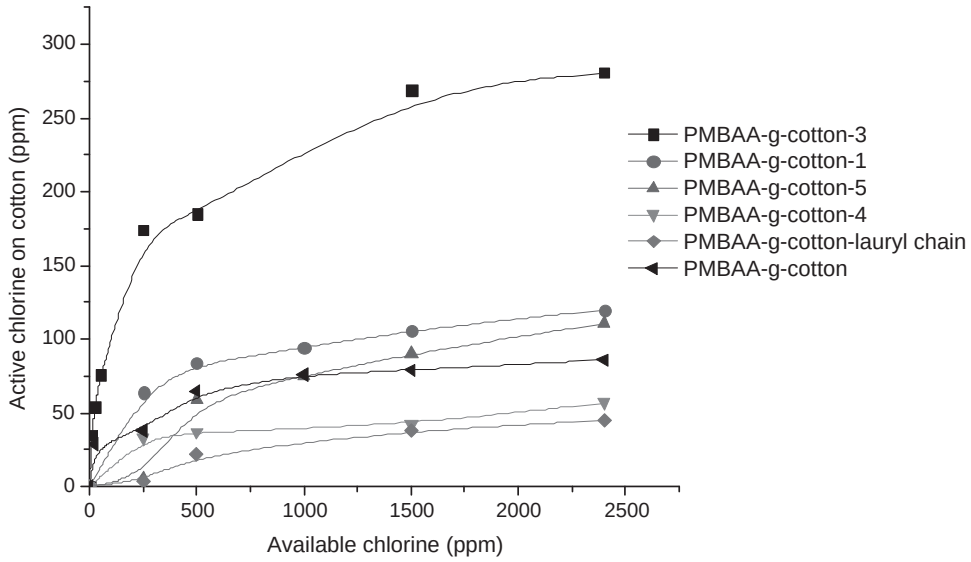


Figure 3. Chlorination progress versus available chlorine in NaClO solution. Reaction duration: 30 min.

where $[amide]_t$ is the amide concentration at the reaction time of t, $[amide]_0$ is the total amide of hydantoin on cotton (which can be calculated from the graft percentage 1.1%), and $k' = k$ [NaClO]. The yield of click-linkage reaction was regarded as 100%, and t was 1800 s. Therefore, based on the obtained active chlorine levels when the available chlorine ([NaClO]) was between 500 ppm and 2400 ppm (**Figure 3**), k' in Equation 2 could be calculated as shown in **Table 3**.

The rate constant k of PMBAA-*g*-cotton-**3** (k(**3**)) was the highest among all the samples. The chlorination of PMBAA-g-cotton-**3** proceeded at a much higher rate probably because of the attraction between the positive charge in **3** and the negatively charged chlorination species ClO^-. However, the similarly positively charged PMBAA-g-cotton-**4** had only a comparable k and even lower active chlorine loadings than the precursor substrate PMBAA-g-cotton, onto which **4** was clicked (as shown in Table 3 and Figure 3) This may be related to the hydrophobicity of PMBAA-g-cotton-**4** and to the fact that steric hindrance of the introduced dodecyl chain impedes the formation of hydrogen bonds between amide hydrogen and hypochlorite oxygen, which has been proposed to be the transition state of the chlorination of amides.[20] To test this hypothesis, we subsequently prepared lauryl azide and attached the long chain azide onto PMBAA-*g*-cotton via the "click" chemistry method. The active chlorine loading on the obtained cotton sample, termed as PMBAA-g-cotton-lauryl chain, was also plotted as a function of the available chlorine of the sodium hypochlorite solution (Figure 3). The active chlorine loadings on PMBAA-g-cotton-lauryl chain were lower than both PMBAA-g-cotton and PMBAA-g-cotton-**4** over the full range of available chlorine (250–2500 ppm). This confirmed that the long alkyl chains retard the chlorination of either the acyclic amide of PMBAA or the cyclic amide of DMH. In addition, it should be noted that the total active chlorine loadings on PMBAA-g-cotton-**3** were more than double that of all other modified cotton fabrics when the available chlorine was greater than 500 ppm, meaning that the positive charge center contributed to not only faster chlorination but also to higher equilibrium active chlorine loading.

Given enough contact time (120–180 min), the cotton fabrics with active chlorine as low as 48 ppm resulted in a 5-log reduction of K-12 *E. coli*.[10] Differences in the antibacterial efficacies of the cotton samples might not be distinguishable after contact with bacterial suspensions for a long time. Also, according to the model study, a cationic charge center majorly contributes to a rapid kill of bacteria. Thus, a short contact time (i.e., 5 min) was adopted in the antibacterial tests. Only a negligible percent reduction of **MDR-***E.coli* (#70094) was observed on the PMBAA-g-cotton sample within 5 min of contact (**Table 4**). This finding is in accordance with previous findings that *t*-butyl acrylamide grafted cotton could neither be easily chlorinated nor demonstrate effective biocidal efficacy.[10] This is because the methyl substitution adjacent to the N-Cl structure impedes the effective chlorine transfer from the N-Cl biocide to biological receptors on bacteria. As shown in Table 4, the biocidal efficacy of

Table 3. Rate constant (k) for the chlorination of modified cotton fabrics.

Modified cotton	PMBAA-g-cotton	PMBAA-g-cotton-**1**	PMBAA-g-cotton-**3**	PMBAA-g-cotton-**4**	PMBAA-g-cotton-**5**
Rate constant k [L mol^{-1} s^{-1}]	7×10^{-5}	1×10^{-4}	4×10^{-4}	7×10^{-5}	2×10^{-4}

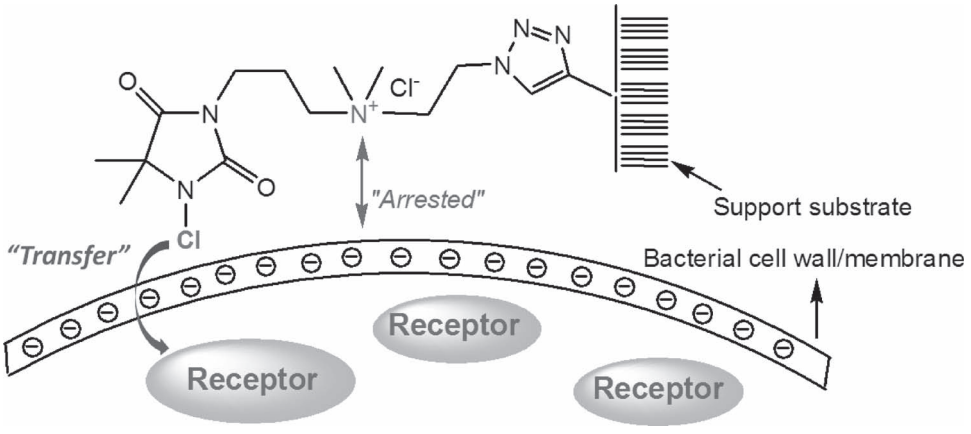
Table 4. Antibacterial efficacy of modified cotton fabrics against MDR-*E. coli* #700094.

Modified cotton sample	Active chlorine [ppm]	Reduction of MDR-*E. coli* (#70094)[a]	
		Percentage reduction	Log_{10} reduction
Cotton	0	0	0
PMBAA-g-cotton	51 ± 5	5.1 ± 0.8%	0.02
PMBAA-g-cotton-**1**	120 ± 8	22.2 ± 3.3%	0.11
PMBAA-g-cotton-**3**	152 ± 12	89.7 ± 3.3%	1
PMBAA-g-cotton-**3**	35 ± 3	37.8 ± 5.3%	0.21
PMBAA-g-cotton-**5**	107 ± 2	18.9 ± 3.3%	0.09
PMBAA-g-cotton-**4**	55 ± 6	28.3 ± 3.4%	0.14

[a]Inoculum concentration was 2.12×10^6 CFU/mL and contact time was 5 min.

PMBAA-g-cotton-**1** was around half that of PMBAA-g-cotton-**3** (with 35 ± 3 ppm active chlorine) even when the latter's active chlorine was much lower (120 vs. 35 ppm). This confirmed the boosting effect of the cationic charge center on the biocidal efficacy of *N*-chloramine. PMBAA-g-cotton-**5** only gave a comparable efficacy to that of PMBAA-g-cotton-**1**, indicating a negligible or no contribution from the negative charge to the biocidal effect. Considering the significantly enhanced bactericidal activity of chlorinated PMBAA-g-cotton-**3**, we propose a possible boosting mechanism as depicted in **Scheme 4**. *E. coli* cells are covered with a lipopolysaccharide layer of 1–3 μm thickness and, hence, become negatively charged. The negatively charged shell can be arrested by the cation in **3** through electrostatic interaction to facilitate the oxidative chlorine transfer from *N*-chloramine to the cell's biological receptors leading to bacterial death. Even with only half of the active chlorine compared to PMBAA-g-cotton-**1** or **5**, PMBAA-g-cotton-**4** showed a comparable biocidal efficacy, if not better, within 5 mins of contact. However, compared to PMBAA-g-cotton-**3**, the boosting effect was less significant. It is deduced that the long alkyl chain shields the electrostatic interaction between the cationic center on the *N*-chloramine structure and the negatively charged *E. coli* cells. The contact time is too short for the QAC moiety to complete the "bubble bursting" action. Under these experimental conditions, no synergist bacterial killing is found between the antibacterial QAC and the *N*-chloramine even when they are covalently bonded with each other.

The finding that a cationic charge center can boost the biocidal efficacy of *N*-chloramine is of great importance for applications. Even though previous research has shown that dimethyloldimethyl hydantoin-treated cotton fabrics with an active chlorine loading of 1100 ppm did not generate any erythema or edema on the bare skin of 8-week-old New Zealand male rabbits after 4-hour skin contact, more evidence is needed about the safety and tolerability of *N*-chloramine modified fabrics before they can be used in close contact to the skin. In this context, it is desirable to present a more potent antibacterial activity with a lower active chlorine loading as in the case of PMBAA-g-cotton-**3**. It is noteworthy that 33 ppm active chlorine on PMBAA-g-cotton-**3** was achieved using a NaClO chlorinating solution with only 10 ppm available chlorine, which is of a similar level as that used in public swimming pools (2–5 ppm). It implies that the biocidal function of PMBAA-g-cotton-**3** can be easily activated to become self-disinfecting and useful in such settings as surgical gowns, nurse uniforms, hospital privacy curtains, etc.



Scheme 4. Schematic illustration of boosting microbiocidal function between cation and *N*-chloramine

Table 5. Antibacterial efficacy of modified cotton fabrics against MRSA #77090.

Modified cotton sample	Active chlorine (ppm)	Reduction of MRSA (#77090)[a]	
		Percentage reduction	Log_{10} reduction
cotton	0	0.0%	0
PMBAA-g-cotton	66 ± 3	27.2 ± 2.8%	0.02
PMBAA-g-cotton-**1**	80 ± 14	75.0 ± 1.7%	0.6
PMBAA-g-cotton-**3**	141 ± 8	100.0%	6.3
PMBAA-g-cotton-**3**	33 ± 5	76.5 ± 3.7%	0.63
PMBAA-g-cotton-**5**	84 ± 1	82.3 ± 1.0%	0.75
PMBAA-g-cotton-**4**	59 ± 4	26.0 ± 3.7%	0.11

[a]Inoculum concentration was 2.0×10^6 CFU/mL and contact time was 5 min.

As the dissociation constant of amide *N*-chloramine is less than 10^{-9},[21] its biocidal function is believed to proceed in a direct-contact manner.[22] As for the N-Cl form of **3**, either free or after immobilization, the nature of N-Cl is identical to that of **1**. To further confirm the on-contact killing mechanism as depicted in Scheme 4, we designed a non-contact killing test. Chlorinated cotton and chlorinated PMBAA-g-cotton-**3** were first suspended in phosphate buffered saline (PBS) (0.05 M, pH 7.0) under vortex conditions for 5 and 10 minutes. Then, the extraction buffer was filtered through a syringe filter membrane and added to a bacterial suspension. Viable bacterial colonies were counted to obtain almost constant bacterial concentrations as outlined in the Supporting Information (Figure S1). No bacterial kill was observed when the PMBAA-g-cotton-**3** was not in direct contact with the bacterial suspension. It indicated that contact between *N*-chloramines and bacteria is indispensable for the microorganism inactivation, lending support to the proposed mechanism of enhanced bacterial kill of PMBAA-g-cotton-**3** (Scheme 4).

The modified cotton fabrics were also challenged with a Gram-positive bacterium, healthcare-associated (HA)-MRSA #77090. As shown in **Table 5**, PMBAA-g-cotton-**1** and PMBAA-g-cotton-**5** gave similar reductions of the tested bacterium: 75.0% and 82.3%. Again, it indicated a negligible contribution from the negative charge to *N*-chloramine's biocidal function. A 6.3-log reduction was achieved by PMBAA-g-cotton-**3** with 141 ± 8 ppm active chlorine. The 76.5% bacterial reduction of PMBAA-g-cotton-**3** with an active chlorine loading of 33 ± 5 ppm was comparable to that of PMBAA-g-cotton-**1** and PMBAA-g-cotton-**5**, which possessed a little over twice the active chlorine concentration (80 ± 14 ppm and 84 ± 1 ppm, respectively). Sonohara and coworkers[23] studied the electrophoretic mobility of *E. coli* and *S. aureus* in media with a range of pH and ionic strengths. Based on the mobility formula derived for biological cells by Ohshima and Kondo,[24] Sonohara extracted two parameters from the electrophoretic mobility results: the charge density on the bacterial surface and resistance to liquid flow in the surface layer. Compared to *S. aureus*, the surfaces of *E. coli* cells are more negatively charged and more rigid, thus, they show a higher resistance to liquid flow in the surface layer. As the number density of negative charges on *S. aureus* cells (0.025 m^{-3} at pH 7) is much lower than that of *E. coli* cells (0.145 m^{-3} at pH 7),[23] the contribution of the positive charge in the killing of *S. aureus* was not as obvious as in the case of *E. coli*. The same reason accounts for the less effective antibacterial performance of PMBAA-g-cotton-**4**. If the contribution of the positive charge is diminished, the negative impact of the hydrophobic alkyl chain is magnified. Therefore the PMBAA-g-cotton-**4** was less effective than the PMBAA-g-cotton-**1** and -**5** for the inactivation of MRSA, which is opposite to the case of *E. coli* reduction.

Based on the antibacterial studies against MDR-*E. coli* and HA-MRSA, the same conclusion could be drawn, namely that the cation in **3** contributed greatly to bacterial kill whereas the anion in **5** did not, and no synergistic effect between antibacterial QAC and *N*-chloramine was found. The long alkyl chain in QAC, on the contrary, contributed negatively to the antibacterial efficacy.

The proposed mechanism for the enhanced antibacterial activity is as follows: through an electrostatic attraction of opposite charges, the cation in PMBAA-g-cotton-**3** helps to arrest negatively charged bacterial cells and hence facilitates the oxidative chlorine transfer from *N*-chlorohydantoin to the cell biological receptors causing bacterial death (Scheme 4). Based on this hypothesis, it is possible that the antibacterial activity might be further enhanced if more than one cation is introduced to molecule **3**. We believe that such new products together with **3** would be good candidates to control biofilms, a prominent form of microbial life that causes many chronic infections and environmental contamination.[25] Such designs and synthesis are currently undertaken in our research group.

3. Conclusion

We synthesized a series of ionic DMH analogues (**2–6**) and studied the antibacterial activities of their chlorinated counterparts against MRSA and *E. coli* in solution and on polymer surfaces. It was found that *N*-chlorohydantoin, once covalently linked with a quaternary ammonium cationic charge center, could result in a new antibacterial agent with enhanced antibacterial efficacy both in solution and on surfaces. There was, however, no synergistic effect between antibacterial QAC (with long alkyl chain substitution) and *N*-chloramine. Interestingly, the

cationic charge center was found to have a positive contribution to both the chlorination kinetics and equilibrium active chlorine loading on the modified cotton samples. These findings provide fundamental guidelines for the design and synthesis of novel biocides with more potent broad-spectrum antibacterial activity.

This work also presents clinical application importance as better antibacterial efficacy could result from cotton and PET fabrics with lower active chlorine loadings, minimizing the concern of such adverse effects as skin irritation when the fabrics are used in healthcare settings for decreasing cross-infection. Equally important, the ability of the modified cotton sample (PMBAA-g-cotton-**3**) in picking up positive chlorine atoms from very diluted sodium hypochlorite (10 ppm) will lessen the environmental burden from using chlorine bleach for the activation of the biocidal property, hence allowing the wider use of *N*-chloramine based biocides for battling infectious bacteria.

4. Experimental Section

4.1. Reagents and Materials

All chemicals and solvents were from commercial suppliers such as Fisher Scientific, Aldrich and used as received without further purification. Purification of synthesized compounds was conducted by flash column chromatography on silica gel received from Selecto Scientific Georgia USA. Poly(ethylene terephthalate) (PET) plain woven fabric (#777H) and cotton print cloth (# 400) were purchased from Testfabrics, Inc. (West Pittiston, PA). A clinical isolate of healthcare-associated MRSA (HA-MRSA) isolate #77090, community-associated MRSA (HA-MRSA) #70527, and those of multi-drug-resistant *E. coli* (MDR-*E. coli*) isolate #70094 and #95882 were obtained from the CANWARD (Canadian Ward Surveillance) study assessing antimicrobial resistance in Canadian hospitals, www.canr.ca. *E. coli* ATCC 25922 and MRSA ATCC 33592 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA).

Alkynyl monomer *N*-(2-methylbut-3-yn-2-yl)acrylamide (MBAA), azide-dansyl dye (6-azidohexyl dansyl ester, see Supporting Information), 1-azidoethanol, 2-azidoethylamine, and 2-azido-*N*,*N*-dimethylethanamine were prepared according to known procedures,[26,13,27–29] and the obtained NMR data were identical with those found in the literature.

FTIR spectra were taken on a Nicolet iS10 spectrometer (Thermo Electron Corporation) and NMR spectra were recorded at room temperature in 5 mm NMR tubes on a Bruker Avance 300 MHz NMR spectrometer. Accurate mass measurements were performed using a Perkin Elmer Sciex prOTOF 2000 MALDI-OTOF mass spectrometer.

4.2. Synthesis of Ammonium Salt (2)

To the solution of bromide **7** (1.0 g, 4.0 mmol) in EtOH (5 mL) was added aqueous dimethylamine (2.2 mL, 24 wt%, 8.0 mmol) at room temperature. The resulting solution was heated to reflux overnight under vacuum. Removal of solvent and excess dimethylamine afforded the bromo-quaternary ammonium salt, which was dissolved in a minimum amount of water and slowly passed through an anion-exchange resin (Amberlite R IRA-900, Cl^-) to give **2** as a white solid (Cl^- form, 0.94g, 90%). ^{1}H NMR (D_2O, 300 MHz) δ [ppm]: 3.61 (t, J = 6.9 Hz, 2H;–$CH_2CH_2CH_2N^+$), 3.38 (t, J = 8.4 Hz, 2H; -$CH_2CH_2CH_2N^+$), 3.14 (s, 9H; -$N^+(CH_3)_3$), 2.10–2.20 (m, 2H; -$CH_2CH_2CH_2N^+$), 1.44 (s, 6H; $(CH_3)_2$C-); ^{13}C NMR (D_2O, 75 MHz) δ [ppm]: 185.6 (1′-C = O), 162.1 (3′-C = O), 68.8 (-$CH_2CH_2CH_2N^+$), 64.2 (CH_3 C-), 57.9 (N^+CH_3), 40.4 (-$CH_2CH_2CH_2N^+$), 28.4 (CH_3-C), 26.7 (-$CH_2CH_2CH_2N^+$); HRMS (MALDI-TOF) m/z: $[M\text{-}Cl]^+$ calcd. for $C_{11}H_{22}N_3O_2$, 228.1707; found: 228.1704.

4.3. Synthesis of Ammonium Salt (3)

To the solution of bromide **7** (1.48 g, 5.9 mmol) in MeCN (15 mL) was added **8** (0.71 g, 6.2 mmol), and the resulting solution was heated to reflux for 14 h. Removal of the solvent and excess **8** under vacuum afforded the crude **3** (Br^- form), which was dissolved in a minimum amount of water and passed through an ion-exchange resin (Amberlite R IRA-900, Cl^-) to give **3** as a white solid (Cl^- form, 1.87g, 99%). ^{1}H NMR (DMSO-d^6, 300 MHz) δ [ppm]: 3.79 (t, J = 4.8 Hz, 2H; -$CH_2CH_2CH_2N^+$), 3.39 (t, J = 5.3 Hz, 2H; -$N^+CH_2CH_2N_3$), 3.27 (t, J = 6.6 Hz, 2H; -N^+CH2CH_2N_3), 3.19 (t, J = 8.1 Hz, 2H; -$CH_2CH_2CH_2N^+$), 2.93 (s, 6H; -$N(CH_3)_2$), 1.77–1.86 (m, 2H; -$CH_2CH_2CH_2N^+$), 1.17 (s, 6H; $C(CH_3)_2$); ^{13}C NMR (DMSO-d^6, 75 MHz) δ [ppm]: 177.4 (1′-C = O), 155.0 (3′-C = O), 61.4 ($N^+CH_2CH_2N_3$), 61.2 (CH_3 C), 57.8 (N^+CH_3), 50.5 (-$CH_2CH_2CH_2N^+$), 44.0 ($N^+CH_2CH_2N_3$), 34.8 (-$CH_2CH_2CH_2N^+$), 24.5 (CH_3C-), 21.3 (-$CH_2CH_2CH_2N^+$); HRMS (MALDI-TOF) m/z: $[M\text{-}Cl]^+$ calcd. for $C_{12}H_{23}N_6O_2$, 283.1877; found: 283.1865.

4.4. Synthesis of Compound (4)

To the lauryl bromide (1.49 g, 6.0 mmol) solution in dimethylformamide (DMF) (15 mL) was added 2-azidoethylamine (0.54 g, 6.27 mmol) and anhydrous K_2CO_3 (2.5 g, 18 mmol) at room temperature. The suspension was maintained at 70 °C under stirring for 14 h before removing the solvent under vacuum. The residue was partitioned between EtOAc and H_2O, and concentration of the organic layer produced the crude compound which was further purified by column chromatography (EtOAc/hexane, 1:1, v/v) to afford **9** as a colorless oil (0.92 g, 60%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ [ppm]: 3.44 (t, J = 6.0 Hz, 2H; -$NHCH_2CH_2N_3$), 2.81 (t, J = 6.0 Hz, 2H; -$NHCH_2CH_2N_3$), 2.63 (t, J = 7.2 Hz, 2H; -$CH_2CH_2NHCH_2CH_2N_3$), 1.52–1.48 (m, 2H;–$CH_2CH_2NHCH_2CH_2N_3$), 1.30–1.27 (m, 18H; lauryl chain), 0.90 (t, J = 6.6 Hz, 3H; $CH_3CH_2CH_2$-); ^{13}C NMR ($CDCl_3$, 75 MHz) δ [ppm]: 51.5 (-$NHCH_2CH_2N_3$), 49.7 (-$NHCH_2CH_2N_3$), 48.6 (-$CH_2CH_2NHCH_2CH_2N_3$) 31.9 (-$CH_2CH_2NHCH_2CH_2N_3$), 30.1, 29.7, 29.6, 29.5, 27.3, 22.7 (30.1 to 22.7 belong to carbon of lauryl chain), 14.1 (-$CH_3CH_2CH_2$-); HRMS (MALDI-TOF) m/z: $[M+H]^+$ calcd. for $C_{14}H_{31}N_4$, 255.2548; found: 255.2540.

To the bromide **7** (0.97 g, 3.9 mmol) solution in DMF (10 mL) was added **2** (1.0 g, 3.9 mmol) and anhydrous K_2CO_3 (1.6 g, 12 mmol) at room temperature. The suspension was maintained at 70 °C under stirring for 14 h before DMF was removed and H_2O (30 mL) and EtOAc (30 mL) were added. The organic layer was concentrated to give the crude compound which was further purified by column chromatography eluting with MeOH/$CHCl_3$ (1:20, v/v) to afford **10** as a slightly yellow oil (1.2 g, 72%). Compound **10** was directly mixed with excess MeI (0.6 mL, 9.6 mmol) in 20 mL CH_3CN at room temperature. The resulting solution was continuously stirred for 10 h before removing the solvent under vacuum to afford the crude compound, which was purified by column chromatography eluting with MeOH/$CHCl_3$ (1:4, v/v) to give the final ammonium salt **4** (1.4 g, 88%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ [ppm]: 7.11 (s, 1H; -NH), 4.13 (t, J = 4.8 Hz, 2H; $N^+CH_2CH_2N_3$), 3.88 (t, J = 4.8 Hz, 2H; $N^+CH_2CH_2N_3$), 3.71–3.67 (m, 4H; $NCH_2CH_2CH_2N^+$ and $CH_2CH_2CH_2N^+$), 3.51–3.46 (m, 2H; -$CH_2CH_2CH_2N^+$), 3.40 (s, 3H; -$N^+(CH_3)_2$), 2.26 (t, J = 7.0 Hz, 2H; $N^+CH_2CH_2CH_2$-), 1.76–1.48 (m, 2H; -$CH_2CH_2CH_2N^+$), 1.30–1.27 (m, 18H; lauryl chain), 0.90 (t, J = 6.6 Hz, 3H; $CH_3CH_2CH_2$-); ^{13}C NMR ($CDCl_3$, 75 MHz) δ [ppm]: 177.2 (1′-C = O), 156.0 (3′-C = O), 61.2 (-$CH_2CH_2CH_2N^+$), 61.0 (CH_3 C), 51.5 ($N^+CH_3C_{11}H_{23}$), 49.7 (-$N^+CH_2CH_2N_3$), 48.6 ($N^+CH_2CH_2N_3$) 34.9 ($NCH_2CH_2CH_2N^+$), 30.1, 29.7, 29.6, 29.5, 27.3, 22.7 (from 30.1 to 22.7, CH_2 of the lauryl chain),

14.1 (CH_3 of the lauryl chain); HRMS (MALDI-TOF) *m/z*: $[M-I]^+$ calcd. for $C_{23}H_{45}N_6O_2$, 437.3600; found 437.3651.

4.5. Synthesis of Compound (5)

Dimethylphosphite (0.93 mL, 10 mmol) was dissolved in DMF (20 mL) and NaH (0.4 g, 10 mmol, 60% dispersion in mineral oil) was dispersed in this resulting solution at 0 °C. The suspension was continuously stirred for 30 min before bromide **7** (2.3 g, 9.2 mmol) was added, and the reaction mixture was then warmed to room temperature. After 24 h the solvent DMF was removed under vacuum and the residue was portioned between H_2O (30 mL) and EtOAc (30 mL). The organic layer was concentrated to yield the crude product which was purified by a short silica gel column to give **11** as a syrup (1.6 g, 65%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ [ppm]: 6.19 (s, 1H; -NH), 3.76 (s, 1H; -OCH_3), 3.72 (s, 1H; -OCH_3), 3.55 (t, J = 6.9 Hz, 2H; N$CH_2CH_2CH_2$P), 1.98–1.86 (m, 2H; $NCH_2CH_2CH_2$P), 1.82–1.71 (m, 2H; -$NCH_2CH_2CH_2$P), 1.44 (s, 6H; C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ [ppm]: 177.3 (1′-C = O), 156.1 (3′-C = O), 58.7 (CH_3 *C*), 52.5 (-OCH_3), 38.6 ($NCH_2CH_2CH_2$P), 24.6 (d, J = 140 Hz; -CH_2P), 21.3 (CCH_3), 21.2 ($NCH_2CH_2CH_2$P); ^{31}P NMR ($CDCl_3$, 120 MHz) δ [ppm]: 33.37; HRMS (MALDI-TOF) *m/z*: $[M+H]^+$ calcd. for $C_{10}H_{20}N_2O_5P$, 279.1110; found 279.1084.

To a solution of **11** (0.630 g, 2.3 mmol) in CH_2Cl_2 (10 mL) was added trimethylsilicon bromide (TMSBr, 0.91 mL, 6.9 mmol) at room temperature. After 12 h the solvent was removed under reduced pressure and the residue was dissolved in 95% EtOH (3 mL) for 1 h. Then the solution was concentrated thoroughly and the residue was finally dissolved in anhydrous pyridine (4 mL) under N_2. To this resulting solution 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl, 2.06 g, 6.81 mmol) and 2-azidoethanol (0.39 g, 4.55 mmol) were added sequentially. After being stirred overnight, the majority of solvent was removed under vacuum, and the residue was purified by column chromatography eluting with MeOH/$CHCl_3$ (1:10, v/v, $CHCl_3$ contained 1% Et_3N) to give the product **5** in the Et_3NH^+ form. Then it was dissolved in a minimum amount of water and slowly passed through an ion-exchange resin (Amberlite IR-120, NH_4^+). Fractions containing the target compound were collected and concentrated to give the NH_4^+ form of compound **5** (0.48 g, 64% in two steps).

^{1}H NMR (D_2O, 300 MHz) δ [ppm]: 4.00 (dd, J = 4.5 Hz, 6.0 Hz, 2H; -O$CH_2CH_2N_3$), 3.56 (t, J = 6.7 Hz, 2H; N$CH_2CH_2CH_2$P), 3.49 (t, J = 5.9 Hz, 2H; -$OCH_2CH_2N_3$), 1.90–1.78 (m, 2H; $NCH_2CH_2CH_2$P), 1.66–1.55 (m, 2H; $NCH_2CH_2CH_2$P), 1.44 (s, 6H; -C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ [ppm]: 185.9 (1′-C = O), 162.2 (3′-C = O), 68.1 (-$OCH_2CH_2N_3$), 64.1 (CH_3 *C*), 56.3 (-$OCH_2CH_2N_3$), 44.3 (-$NCH_2CH_2CH_2$P), 29.3 (-CCH_3), 28.4 (d, J = 136 Hz; -CH_2P;), 27.5(-$NCH_2CH_2CH_2$P); ^{31}P NMR (120 MHz) δ [ppm]: 27.03; HRMS (MALDI-TOF) *m/z*: $[M-NH_4+2H]^+$ calcd. for $C_{10}H_{19}N_5O_5P$, 320.1129; found 320.1122.

4.6. Synthesis of Compound (6)

To a solution of **11** (0.67 g, 2.4 mmol) in CH_2Cl_2 (10 mL) was added TMSBr (0.95 mL, 7.2 mmol) at room temperature. After 12 h the solvent was removed under reduced pressure and the residue was dissolved in 95% EtOH (3 mL) for 1 h. Then the solution was concentrated thoroughly followed by addition of 2 mL H_2O. Minimum NH_4HCO_3 was added until pH 7 was reached. Concentration under vacuum gave **6** as a white solid (0.54 g, 90%).

^{1}H NMR (D_2O, 300 MHz) δ [ppm]: 3.53 (t, J = 6.9 Hz, 2H; -$CH_2CH_2CH_2$P), 1.74–1.87 (m, 2H; -$CH_2CH_2CH_2$P), 1.58–1.46 (m, 2H; -$CH_2CH_2CH_2$P), 1.43 (s, 6H; C(CH_3)$_2$); ^{13}C NMR (D_2O, 75 MHz) δ [ppm]: 186.0 (1′-C = O), 162.7 (3′-C = O), 64.1 (CCH_3), 44.5 (-$CH_2CH_2CH_2$P), 30.4 (d, J = 135 Hz; -$CH_2CH_2CH_2$P), 28.4 (CH_3C), 27.3 (-$CH_2CH_2CH_2$P); ^{31}P NMR (120 MHz) δ [ppm]: 23.9; HRMS (MALDI-TOF) *m/z*: $[M-Cl]^+$ calcd. for $C_{11}H_{21}ClN_3O_2$, 262.1317; found: 262.1321.

4.7. Chlorination of (2) and (6)

Compound **2**/**6** was suspended in *t*-BuOH (8 mL) and H_2O (2 mL) was subsequently added to make a clear solution. Afterwards, excess *t*-butyl hypochlorite (3 to 4 equiv.) was added to the solution and the mixture was continuously stirred overnight. Removal of excess *t*-butyl hypochlorite and solvent under vacuum afforded the final chlorinated **12**/**13** as a white solid in quantitative amount.

12: ^{1}H NMR (D_2O, 300 MHz) δ [ppm]: 3.69 (t, J = 6.9 Hz, 2H; -$CH_2CH_2CH_2N^+$), 3.43–3.38 (m, 2H; -$CH_2CH_2CH_2N^+$), 3.15 (s, 9H; -N^+CH_3), 2.22–2.12 (m, 2H; -$CH_2CH_2CH_2N^+$), 1.51 (s, 6H; (CH_3)$_2$C); ^{13}C NMR ($CDCl_3$, 75 MHz) δ [ppm]: 181.8 (1′-C = O), 160.4 (3′-C = O), 71.3 (-CH_2 $CH_2CH_2N^+$), 68.7 (CH_3 *C*), 58.0 (N^+CH_3), 41.6 (-$CH_2CH_2CH_2N^+$), 26.6 (CH_3-C), 25.9 (-$CH_2CH_2CH_2N^+$); HRMS (MALDI-TOF) *m/z*: $[M-2NH_4+H]^+$ calcd. for $C_8H_{16}N_2O_5P$, 251.0791; found: 251.0789.

13: ^{1}H NMR (D_2O, 300 MHz) δ [ppm]: 3.64 (t, J = 6.9 Hz, 2H; C$H_2CH_2CH_2$-P), 1.92–1.82 (m, 2H; -$CH_2CH_2CH_2$P), 1.79–1.68 (m, 2H; -$CH_2CH_2CH_2$P), 1.49 (s, 6H; -C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ [ppm]: 182.1 (1′-C = O), 160.8 (3′-C = O), 71.2 (-CH_3 *C*), 45.1 (-$CH_2CH_2CH_2$P), 29.0 (d, J = 130 Hz; -$CH_2CH_2CH_2$-P), 28.4 (CH_3C), 26.1 ($CH_2CH_2CH_2$P); ^{31}P NMR (120 MHz) δ [ppm]: 24.5; HRMS (MALDI-TOF) *m/z*: $[M-2NH_4+H]^+$ calcd. for $C_8H_{15}ClN_2O_5P$, 285.0402; found: 285.0394.

4.8. Grafting Polymerization of MBAA onto Cotton

To the solution of monomer MBAA (1.92 g, 14 mmol) in mixed solvent (acetone 8 mL + DI water 32 mL) was added the initiator potassium persulfate (PPS, 0.43 g, 1.6 mmol). After the initiator was dissolved completely, a piece of cotton fabric (10 cm × 10 cm) was dipped in the resulting solution and padded twice at a required expression (150% wet pickup). The padded fabric was dried at 60 °C for 10 min, cured at 105 °C for 30 min, and then washed with copious amounts of water. The fabric was then extracted with MeOH in a Soxhlet-extractor for 24 h to remove ungrafted monomer and homopolymer. Afterwards, the fabric was air dried and stored in a desiccator for 24 h to reach a constant weight. The resultant modified fabric was referred to as "PMBAA-*grafted*-cotton" (PMBAA-*g*-cotton). The graft-percentage was calculated according to:

$$\text{Graft Percentage (\%)} = (W_2 - W_1)/W_1 \quad (3)$$

where W_1 and W_2 are the weights of the original and grafted fabrics, respectively.

4.9. "Click" Linkage Between Synthetic Precursors (1, 3, 4, 5) and PMBAA-PET/PMBAA-*g*-Cotton

PMBAA-modified PET (PMBAA-PET) was obtained by forming a surface-interpenetrating network of PMBAA and PET as previously described.[13] The "click" reaction between the synthetic precursors and PMBAA-PET was also performed following a previously reported protocol,[13] and these synthetic azides were covalently bonded onto PMBAA-g-cotton in a similar way. PMBAA-g-cotton fabric (1.2 g, grafting percentage = 1.1%) was first immersed in 20 mL mixed solvent (*t*-BuOH/H_2O = 1:1, v/v) containing equivalent amounts of synthetic azides (calculated based on the total PMBAA on grafted cotton). Then Na ascorbate (40% mol) and Cu^{2+} (10% mol) were added to initiate the "click" reaction. After 1 h of shaking, the cotton fabric was taken out and washed thoroughly with DI water and MeOH. The rinsed cotton was then air dried overnight and stored in desiccators until use. The obtained fabrics that were endowed with specific precursors were named as PMBAA-PET-(**1**,**3**,**4**,**5**) or PMBAA-*g*-cotton-(**1**,**3**,**4**,**5**).

Both "click"-modified PET and cotton fabric were chlorinated with sodium hypochlorite solution in a solid/liquid ratio of 1:50 (w/w).

The concentration of the chlorinating solution varied from 15 ppm to 1500 ppm as needed. After continuous shaking for 30 min, the samples were thoroughly rinsed with DI water and then air dried overnight for titration analyses or antibacterial tests.

4.10. Antibacterial Assessment of Compounds (12) and (13)

Tryptone Soya Agar (TSA) was used for bacterial culture. After being sub-cultured from stocks, bacteria were allowed to grow at 37 °C for 18–20 hours to obtain logarithmic-phase cultures. Biocidal activity of **12** and **13** were completed as followed. To 20 mL of bacterial suspension (10^6–10^7 colony forming units (CFU)/mL) in a centrifuge tube was added 30 µL of **12** or **13** solutions (0.28 M stock solution) to achieve a final [Cl^+] of 15 ppm. Timing of the exposure to the disinfectant was started immediately with the addition of the synthetic compound **12** or **13**. After contact for 5 min, 10 min, and 20 min, respectively, 1.0 mL aliquots were withdrawn and added to an equal volume of 0.02 N sodium thiosulfate in PBS (0.05 M, pH 7.0). The quenched suspension was serially diluted and 100 µL of each resulting dilution was placed onto nutrient agar plates. The same procedure was also applied to compounds **2** and **6** as controls. After being incubated at 37 °C for 24 hours, the viable bacterial colonies on the plates were counted. Bacterial reduction was reported according to:

$$\text{Percentage reduction of bacteria (\%)} = (A - B)/A \times 100$$

$$\text{Log reduction} = \text{Log (A/B) if B} > 0; = \text{Log(A) if B} = 0 \quad (4)$$

where *A* is the number of bacteria retrieved from controls (CFU/mL), and *B* is the number of bacteria retrieved from **12** or **13** (CFU/mL).

4.11. Antibacterial Assessment of Modified PET and Cotton Samples

The antibacterial properties of chlorinated PMBAA-*g*-cotton-(**1**,**3**,**4**,**5**) were examined against clinical isolates of MDR-*E. coli* (#70094) and HA-MRSA (#77090, healthcare-associated). The click-modified fabrics PMBAA-*g*-cotton-(**1**,**3**,**4**,**5**) were first cut into four small pieces (diameter = 4.8 cm), two of which were put together in a sterilized container. Then 0.5 mL bacterial suspension (10^6–10^7 CFU/mL) was placed onto these two fabric surfaces, and sandwiched by the remaining two portions of the identical fabrics. Immediately another 0.5 mL of bacterial suspension was dispensed on the entire fabric set. After 5 min of contact, 100 mL of 0.03% sodium thiosulfate aqueous solution was added to the container to neutralize any active chlorine. The mixture was then vigorously shaken for 2 min followed by ultrasonic treatment for 5 min. An aliquot of the solution was removed from the mixture and then serially diluted and 100 µL of each dilution was placed onto a nutrient agar plate. The same procedure was also applied to the bleached untreated cotton and bleached PMBAA-g-cotton. The viable bacterial colonies on the agar plates were counted after incubation at 37°C for 24 h. Bacterial reduction was reported according to:

$$\text{Percentage reduction of bacteria (\%)} = (C - D)/C \times 100$$

$$\text{Log reduction} = \text{Log (C/D) if D} > 0; = \text{Log(C) if D} = 0 \quad (5)$$

where *A* is the number of bacteria counted from bleached untreated cotton, and *B* is the number of bacteria counted from modified cotton fabrics.

The antibacterial test for chlorinated PMBAA-PET-(**1**,**3**,**4**,**5**) was carried out against a clinical isolate of MDR-E. coli (#70094) according to our previous report.[13] Briefly, the fabric was cut into two smaller pieces (diameter = 2.4 cm). One of the pieces was put in a sterilized container and 60 µL of an aqueous suspension containing 10^7 CFU/mL of MDR-*E. coli* was placed onto the surfaces of the fabric. The fabric was then "sandwiched" using the other piece of identical fabric. A sterilized 50 mL beaker was placed onto the top of these two fabrics to ensure sufficient contact. After contact for 5 min, the entire "sandwich" was placed into 10 mL of 1.0% sodium thiosulfate aqueous solution to quench the active chlorine on the fabric. The resultant mixture was then vigorously shaken for 2 min before an aliquot (100 µL) of the solution was removed and then serially diluted. 100 µL of each dilution was placed onto a nutrient agar plate. The same procedure was also applied to chlorinated untreated PET as a control. The viable bacterial colonies on the agar plates were counted after incubation at 37 °C for 24 h. Bacterial reduction was reported according to the above equation.

The non-contact killing test was carried out using the following protocol. Chlorinated cotton and chlorinated PMBAA-*g*-cotton-**3** were cut into small pieces and sealed in a nylon bag. The bags containing cotton fabrics were immersed in 10 mL PBS (0.05 M, pH 7.0) and continuously shaken using vortex stirring. At the predetermined time of 5 min and 10 min, 2.0 mL aliquots were taken out by a syringe equipped with a nylon filter membrane (0.45 µm, Fisher) and mixed with 0.5 mL bacterial suspension (10^5–10^6 CFU/mL). The mixture was left to stand for 5 min before 12.5 mL 0.03% sodium thiosulfate aqueous solution was added to quench the "released" active chlorine. Afterwards, the bacterial suspension was serially diluted and 100 µL of each resulting dilution was placed onto nutrient agar plates. After being incubated at 37 °C for 24 hours, the viable bacterial colonies on the plates were counted.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors are grateful for the financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant (Grant No. RGPIN/372048-2009), and the Manitoba Health Research Council (MHRC) Establishment grant.

Received: January 20, 2012
Revised: February 16, 2012
Published online: July 3, 2012

[1] B. Starling, *The Parliamentary Office of Science& Technology* **2005**, *247*, 1.
[2] Minnesota Department of Health, *MDH Disease Control Newsletter* **2007**, *35*, 1.
[3] P. Gastmeier, S. Stamm-Balderjahn, S. Hansen, I. Zuschneid, D. Sohr, M. Behnke, R. Vonberg, H. Ruden, *Am. J. Infect. Control* **2006**, *34*, 603.
[4] S. Silver, *FEMS Microbiology Reviews* **2003**, *27*, 341.
[5] J. V. Loh, S. L. Percival, E. J. Woods, N. J. Williams, C. A. Cochrane, *Int. Wound J.* **2009**, *6*, 32.
[6] S. L. Percivala, P. G. Bowlera, D. Russell, *J. Hosp. Infect.* **2005**, *60*, 1.
[7] A. Fernández-Astorga, M. J. Hijarrubia, M. Hernández, I. Arana, E. Suñen, *Lett. Appl. Microbiol.* **1995**, *20*, 308.
[8] D. E. Townsend, I. Greed, N. Ashdown, W. B. Grubb, *Med. J. Australia* **1983**, *2*, 310
[9] J. M. Tennent, B. R. Lyon, M. T. Gillespie, *Antimicrob. Agents Chemother.* **1985**, *27*, 79.
[10] S. Liu, G. Sun, *Ind. Eng. Chem. Res.* **2009**, *48*, 613.

[11] J. Liang, Y. Chen, K. Barnes, R. Wu, S. D. Worley, T. S. Huang, *Biomaterials* **2006**, *27*, 2495.
[12] L. Kou, J. Liang, X. H. Ren, B. Kocer, S. D. Worley, Y. M. Tzou, T. S. Huang, *Ind. Eng. Chem. Res.* **2009**, *48*, 6521.
[13] L. Li, N. Zhao, S. Liu, *Polymer* **2012**, *53*, 67.
[14] M. Meusel, M. Gutschow, *Org. Prep. Proced. Int.* **2004**, *36*, 391.
[15] S. Liu, G. Sun, *Carbohydr. Polym.* **2008**, *71*, 614.
[16] S. Liu, G. Sun, *Polymer* **2008**, *49*, 5225.
[17] R. G. Murray, D. M. Whitehead, F. Le Strat, S. Conway, *Org. Biomol. Chem.* **2008**, *6*, 988.
[18] L. Li, W. Tang, Z. Zhao, *Chin. J. Chem.* **2009**, *27*, 1391.
[19] S. Liu, N. Zhao, S. Rudenja, *Macromol. Chem. Phys.* **2010**, *211*, 286.
[20] R. P. Mauger, F. G. Soper, *J. Chem. Soc.* **1946**, 71.
[21] L. Qian, G. Sun, *J. Appl. Polym. Sci.* **2003**, *89*, 2418.
[22] D. E. Williams, E. D. Elder, S. D Worley, *Appl. Environ. Microbiol.* **1988**, *54*, 2583.
[23] R. Sonohara, N. Muramatsu, H. Ohshima, T. Kondo, *Biophys. Chem.* **1995**, *55*, 273
[24] H. Ohshima, T. Kondo, *J Colloid Interface Sci.* **1989**, *130*, 281.
[25] L. Hall-Stoodley, J. W. Costerton, P. Stoodley, *Nat. Rev. Microbiol.* **2004**, *2*, 95.
[26] A. Bacchi, M. Costa, B. Gabriele, G. Pelizzi, G. Salerno, *J. Org. Chem.* **2002**, *67*, 4450.
[27] X. Lu, R. Bittman, *J. Org. Chem.* **2005**, *70*, 4746.
[28] S. Angelos, Y. W. Yang, K. Patel, J. F. Stoddart, J. I. Zink, *Angew. Chem. Int. Ed.* **2008**, *47*, 2222.
[29] B. Carboni, A. Benalil, M. Vaultier, *J. Org. Chem.* **1993**, *58*, 3736.
[30] J. Lin, C. Winkelmann, S. D. Worley, J. Kim, C.-I. Wei, U. Cho, R. M. Broughton, J. I. Santiago, J. F. Williams, *J. Appl. Polym. Sci.* **2002**, *85*, 177.