

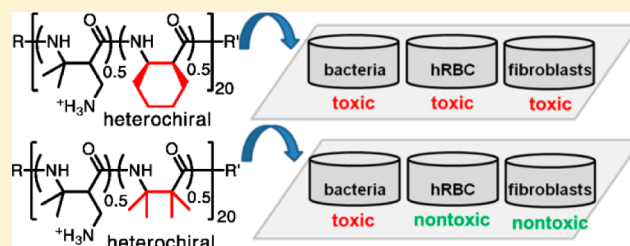
# Tuning the Biological Activity Profile of Antibacterial Polymers via Subunit Substitution Pattern

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**S** Supporting Information

**ABSTRACT:** Binary nylon-3 copolymers containing cationic and hydrophobic subunits can mimic the biological properties of host-defense peptides, but relationships between composition and activity are not yet well understood for these materials. Hydrophobic subunits in previously studied examples have been limited mostly to cycloalkane-derived structures, with cyclohexyl proving to be particularly promising. The present study evaluates alternative hydrophobic subunits that are isomeric or nearly isomeric with the cyclohexyl example; each has four sp<sup>3</sup> carbons in the side chains. The results show that varying the substitution pattern of the hydrophobic subunit leads to relatively small changes in antibacterial activity but causes significant changes in hemolytic activity. We hypothesize that these differences in biological activity profile arise, at least in part, from variations among the conformational propensities of the hydrophobic subunits. The  $\alpha,\alpha,\beta,\beta$ -tetramethyl unit is optimal among the subunits we have examined, providing copolymers with potent antibacterial activity and excellent prokaryote vs eukaryote selectivity. Bacteria do not readily develop resistance to the new antibacterial nylon-3 copolymers. These findings suggest that variation in subunit conformational properties could be generally valuable in the development of synthetic polymers for biological applications.



## INTRODUCTION

Eukaryotes deploy a broad range of “host-defense peptides” (HDPs) to discourage infection by prokaryotes.<sup>1–3</sup> Many of these peptides appear to act by compromising the barrier function of bacterial membranes, although the precise mechanism of disruption remains uncertain, as does the relative importance of membrane-directed vs alternative modes of action.<sup>4,5</sup> HDPs are typically rich in both hydrophobic residues and cationic residues,<sup>6–8</sup> and their positive charge is thought to underlie their preference for attacking bacterial membranes relative to eukaryotic membranes. Numerous prokaryote-selective synthetic oligomers, containing  $\alpha$ -amino acid residues and/or other types of subunits, have been designed based on principles that are believed to underlie the function of natural HDPs.<sup>9–26</sup>

Designed peptides (i.e., oligomers of  $\alpha$ -amino acids) and other discrete oligomers are interesting as tools to establish the features essential for a HDP-like biological activity profile, but synthetic difficulties may hamper practical applications of this type of molecule. Rigorous control of subunit sequence typically requires solid-phase synthesis, which is labor-intensive and expensive.<sup>3</sup> This consideration has prompted recent explorations of sequence-random copolymers as functional mimics of HDPs. Diverse backbones have been evaluated, including polystyrene,<sup>27</sup> poly(norbornene),<sup>28</sup> polymethacrylate,<sup>29–31</sup> poly- $\beta$ -peptide (nylon-3),<sup>32–35</sup> polyacrylamide,<sup>36</sup> polyolefin,<sup>37</sup> polyvinylpyridinium-polymethacrylate,<sup>38,39</sup> pepti-

do-polysaccharide,<sup>40</sup> polycarbonate,<sup>41,42</sup> and poly(vinyl ether).<sup>43</sup> Most studies have focused on identifying a hydrophobic-cationic balance that supports potent antibacterial activity while limiting toxicity toward mammalian cells, which is typically assessed in terms of red blood cell lysis (“hemolytic activity”).<sup>44</sup> Hydrophobic-cationic balance is generally tuned by varying side chain hydrophobicity (carbon atom number) and/or altering the hydrophobic:cationic side chain proportion.

The research described here explores how the biological activity profiles of cationic-hydrophobic copolymers are influenced by changes in the arrangement of side chain carbon atoms within hydrophobic subunits, rather than by changes in the number of side chain carbon atoms, which is related to hydrophobicity. This aspect of molecular design has received little attention in studies of antibacterial polymers, in part because most polymer systems explored to date would not easily support such changes. It is well-known that subtle variations in the structure of  $\alpha$ -amino acid residues exert a significant impact on the folding and function of peptides and proteins. For example, the isomers leucine and isoleucine are comparable in terms of hydrophobicity, but they have divergent conformational preferences, favoring  $\alpha$ -helical and  $\beta$ -sheet secondary structure, respectively. Glycine is more flexible than all other residues because of the lack of a side chain.<sup>45–47</sup>

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Many nonribosomal peptide antibiotics contain aminoisobutyric acid (Aib) residues, the *gem*-dimethyl substitution pattern of which causes a distinctive helix-favoring propensity.<sup>48–50</sup>

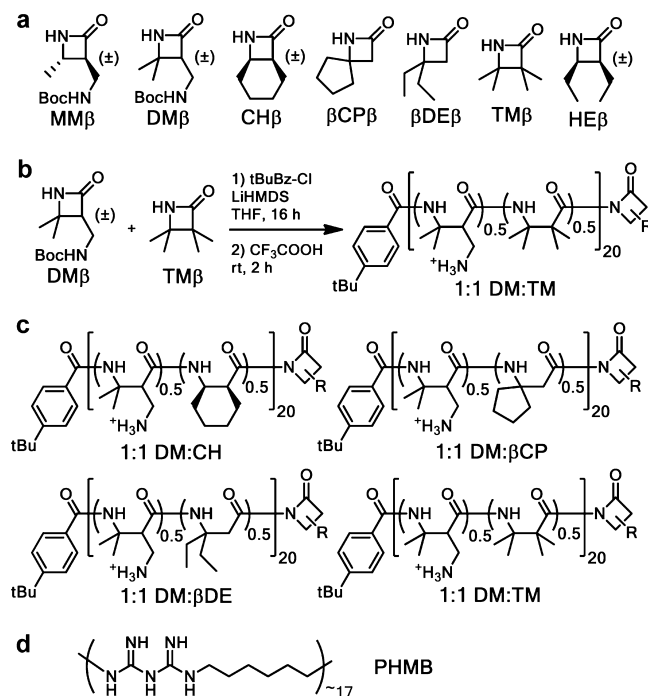
We propose that the broad range of potencies and selectivities manifested among natural antibacterial peptides reflects evolutionary optimization of both hydrophobic-cationic balance and backbone conformational propensity. Based on this hypothesis, we predict that variation of both properties could lead to antibacterial polymers with improved properties. In many systems, the position at which side chains are traditionally modified is too far from the backbone to affect conformational behavior.<sup>27–31,36,38–40</sup> Nylon-3 polymers, on the other hand, offer considerable latitude for modulation of conformational propensity, because each subunit contains a pair of adjacent sp<sup>3</sup> carbon atoms in the backbone, and the substitution pattern at each of these positions can be varied independently. Here we show that evaluation of a small set of related subunits, each containing four side chain sp<sup>3</sup> carbons, leads to new nylon-3 copolymers with diverse biological activity profiles; one of the new polymers appears to match the profile that is characteristic of the very best peptides and polymers previously reported. These findings suggest that the ability to alter the subunit substitution pattern may be an important criterion in selecting polymer systems to be developed for specific biological applications.

## RESULTS AND DISCUSSION

### Experimental Design.

Our previous exploration of antimicrobial nylon-3 copolymers focused on hydrophobic subunits with *cis*-cycloalkyl frameworks; hydrophobicity was varied by changes in ring size.<sup>32,33</sup> The cyclohexyl-based subunit derived from  $\beta$ -lactam **CH $\beta$**  proved to be optimal in copolymers prepared with  $\beta$ -lactam **MM $\beta$**  or **DM $\beta$** , which provide cationic subunits after side chain deprotection (Figure 1). Some copolymers derived from **CH $\beta$**  manifested low hemolytic activity and moderate antibacterial activity, behavior reminiscent of HDPs.<sup>32</sup> The 1:1 **DM:CH** copolymer displayed the strongest antibacterial activities we observed,<sup>33</sup> with potencies comparable to the best polymers and HDPs reported by others. However, 1:1 **DM:CH** is hemolytic at low concentrations and therefore inadequate in terms of selectivity.<sup>33</sup> The present study takes 1:1 **DM:CH** as the starting point for examining the impact of variations in hydrophobic subunit substitution pattern on biological activity.

New nylon-3 materials were prepared from 1:1  $\beta$ -lactam mixtures containing **DM $\beta$** <sup>51</sup> as the precursor of the cationic subunit and one of three hydrophobic  $\beta$ -lactams: (1)  **$\beta$ CP $\beta$**  (“ $\beta$ -cyclopentyl”), (2)  **$\beta$ DE $\beta$**  (“ $\beta$ -diethyl”), or (3) **TM $\beta$**  (“tetramethyl”) (Figure 1). The nylon-3 subunits **CH**,  **$\beta$ CP**,  **$\beta$ DE**, and **TM** should be comparable in terms of hydrophobicity because each contains four side chain carbon atoms; however, conformational propensity is expected to vary among these four subunits. The backbone  $C\alpha$ – $C\beta$  bond of **CH** is constrained by the six-membered ring. Subunits  **$\beta$ DE** and  **$\beta$ CP** should be relatively flexible because each contains a CH<sub>2</sub> unit in the backbone; this expectation arises because glycine is the most flexible  $\alpha$ -amino acid.<sup>45–47</sup> **TM**, reminiscent of Aib because of the quaternary backbone substitution pattern at  $C\alpha$  and  $C\beta$ , should have a distinctive conformational propensity. Recently, we showed that removing the six-membered ring constraint, by preparing copolymers from **DM $\beta$**  + **HE $\beta$**  (Figure 1) rather than **DM $\beta$**  + **CH $\beta$** , led to a substantial (and unfavorable) increase in hemolytic activity along with a small



**Figure 1.** (a)  $\beta$ -Lactams used in this study, (b) representative copolymer synthesis, (c) nylon-3 copolymers prepared from equimolar binary  $\beta$ -lactam mixtures and containing 50% **DM** and 50% of a hydrophobic subunit, and (d) PHMB. The **DM** and **CH** subunits are racemic. All polymers are heterochiral.

diminution in antibacterial activity.<sup>52</sup> Based on this observation, one might have predicted that all of the new copolymers would have less desirable activity profiles relative to 1:1 **DM:CH**, because none of the new hydrophobic subunits has a cyclic constraint on the backbone  $C\alpha$ – $C\beta$  bond. This prediction turned out to be correct for copolymers containing  **$\beta$ DE** and  **$\beta$ CP**. We were surprised, however, to discover that polymers containing the **TM** subunit display superior properties, as explained below.

**Synthesis and Evaluation of New Copolymers.** All polymers were prepared via base-catalyzed copolymerization reactions with *p*-*t*-butylbenzoyl chloride as co-initiator.<sup>51,53,54</sup> Thus, all polymer chains bear a *p*-*t*-butylbenzoyl group at the N-terminus (Figure 1c). Use of 5 mol % co-initiator relative to total  $\beta$ -lactam should generate 20-mer average chain lengths. The resulting materials had polydispersity indices (PDI) ranging from 1.05 to 1.33.

Functional comparison of 1:1 **DM:CH** with the three new copolymers is summarized in Table 1. Consistent with previous data,<sup>33</sup> 1:1 **DM:CH** is highly active, displaying low minimum inhibitory concentration (MIC) values against a test panel of four bacteria, including laboratory strains of *Escherichia coli* and *Bacillus subtilis* and clinical strains of vancomycin-resistant *Enterococcus faecium* (VREF) and methicillin-resistant *Staphylococcus aureus* (MRSA). However, 1:1 **DM:CH** is quite destructive toward red blood cells, as indicated by the relatively low concentration at which 10% hemolysis is observed (HC<sub>10</sub>).<sup>33</sup> Among the three new nylon-3 copolymers, 1:1 **DM: $\beta$ CP** and 1:1 **DM:TM** are similar to 1:1 **DM:CH** in their antibacterial activities, while 1:1 **DM: $\beta$ DE** is moderately less active. Larger differences, however, are observed in the hemolytic activities. Both copolymers with hydrophobic subunits containing a backbone CH<sub>2</sub> group, 1:1 **DM: $\beta$ CP**

**Table 1. Antibacterial and Hemolytic Activity of Nylon-3 Copolymers**

polymer	MIC, <sup>a</sup> $\mu\text{g/mL}$				HC <sub>10</sub> , <sup>b</sup> $\mu\text{g/mL}$	SI (HC <sub>10</sub> /MIC <sub>MRSA</sub> ) <sup>c</sup>
	<i>B. subtilis</i>	<i>E. coli</i>	VREF	MRSA		
1:1 DM:CH	$\leq 1.6$	6.3	6.3	6.3	19	3
1:1 DM: $\beta$ CP	3.1	6.3	6.3	6.3	<3.1	<0.5
1:1 DM: $\beta$ DE	6.3	25	25	25	<3.1	<0.1
1:1 DM:TM	$\leq 1.6$	13	3.1	6.3	400	63
PHMB	3.1	3.1	3.1	3.1	13	4
1:1 DM:CH ("skewed")	$\leq 1.6$	13	6.3	6.3	4.7	0.7
daptomycin	$\leq 1.6$	>200	6.3	6.3	>400	>63

<sup>a</sup>MIC, which is the lowest polymer concentration that completely inhibits bacterial growth. <sup>b</sup>Polymer concentration necessary for 10% lysis of RBC. <sup>c</sup>Selectivity index (SI) was calculated based on MIC values for MRSA. VREF is vancomycin-resistant *E. faecium*; MRSA is methicillin-resistant *S. aureus*; and PHMB is polyhexamethylene biguanide.

and 1:1 DM: $\beta$ DE, are strongly hemolytic. In contrast, 1:1 DM:TM is only very weakly hemolytic at high concentrations. Overall, the prokaryote-selective biological activity profile of 1:1 DM:TM is the most favorable among the four copolymers compared here and among all nylon-3 polymers we have examined.<sup>32,33</sup>

These nylon-3 polymers were compared with the commercial antimicrobial polymer polyhexamethylene biguanide (PHMB) and with the clinical antibiotic daptomycin; Figure 1d. As expected, PHMB displays potent antibacterial activity against the four bacteria in our panel (Table 1). However, PHMB is highly hemolytic. Thus, the biological activity profiles of 1:1 DM:CH and PHMB are similar. Daptomycin is a lipopeptide antibiotic that targets bacterial membranes. Therefore, this agent is appropriate for comparison with our nylon-3 polymers, which are believed to act on bacterial membranes. Daptomycin displays potent activity against three Gram positive bacteria but no activity toward *E. coli*. The nylon-3 copolymer 1:1 DM:TM compares favorably to both PHMB and daptomycin in terms of antibacterial activity and prokaryote vs eukaryote selectivity.

The antibacterial activities of the 1:1 DM:CH and 1:1 DM:TM copolymers were further compared via measurement of minimum bactericidal concentrations (MBC) (Table 2).

**Table 2. Bactericidal Activity of Nylon-3 Copolymers**

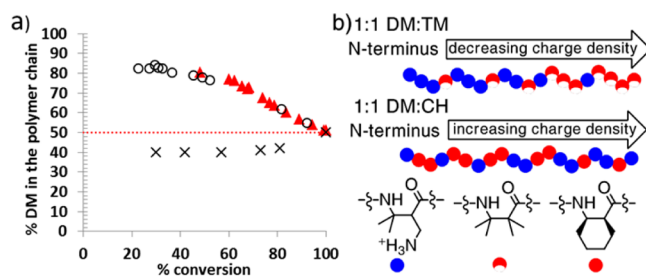
bacterium	1:1 DM:CH		1:1 DM:TM	
	MBC <sup>a</sup>	MBC/MIC <sup>b</sup>	MBC <sup>a</sup>	MBC/MIC <sup>b</sup>
<i>B. subtilis</i>	<3.1	2	<3.1	2
<i>E. coli</i>	13	2	13	1
VREF	>200	>32	200	64
MRSA	25	4	13	2

<sup>a</sup>Minimum bactericidal concentration,  $\mu\text{g/mL}$ . <sup>b</sup>Any polymer with MBC/MIC ratio  $\leq 4$  is considered to be bactericidal for that species.<sup>55</sup>

MIC indicates the lowest polymer concentration at which bacterial growth is inhibited in liquid culture, while MBC indicates the lowest concentration at which all bacterial cells have been killed, as demonstrated by a lack of colony formation on solid medium after the polymer-treated liquid culture is applied to an agar surface. The results for VREF show that inhibition of bacterial growth need not correlate with bacterial killing, because for this organism both copolymers display an

MBC value that is far higher than the MIC. On the other hand, MBC is only slightly higher than MIC for the other three bacteria ( $\leq 4$ -fold), including MRSA, which indicates that the DM:CH and DM:TM copolymers are both potent bactericidal agents for these species.

**Subunit Distribution within Polymer Chains.** Variations in polymer precursor structure can lead to differences in reactivity. For chain-growth copolymerizations, these differences cause deviations from purely random subunit distribution along the backbone. This factor has typically not been considered in previous comparisons among antibacterial copolymers with variable subunits (e.g., different subunit hydrophobicities).<sup>29,32,33,36–39,43,56</sup> For nylon-3 copolymerizations, differences in  $\beta$ -lactamate and/or chain-end reactivity can cause preferential incorporation of one subunit in the early stages of the reaction, which results in compositional drift along the chains.<sup>57,58</sup> Evaluation of the DM $\beta$  + CH $\beta$ , DM $\beta$  +  $\beta$ DE $\beta$ , and DM $\beta$  + TM $\beta$  copolymerizations revealed significant differences in terms of  $\beta$ -lactam incorporation preference, implying differences in subunit distribution within the resulting polymers (Figure 2). For 1:1 DM:CH, there is a small



**Figure 2.** (a) Consumption of  $\beta$ -lactams as a function of reaction progress for the copolymerization of 1:1 DM:CH (x), 1:1 DM:TM (O), or 1:1 DM: $\beta$ DE (red  $\blacktriangle$ ). Reactions were conducted at room temperature with an initial concentration of 50 mM for each  $\beta$ -lactam and 5 mM for the co-initiator tBuBzCl (5 mol % relative to the total amount of  $\beta$ -lactam), to prepare copolymers with an average 20-mer length. Measurement of subunit incorporation is described in the Supporting Information. (b) Cartoons of copolymers showing differences in compositional drift along the polymer chains.

preference for incorporation of CH $\beta$  in the early stages of the reaction, which means that the N-terminal regions of the polymer chains are slightly enriched in CH units, and the C-terminal regions are slightly enriched in DM units. In contrast, for both 1:1 DM:TM and 1:1 DM: $\beta$ DE there is a strong initial preference for DM incorporation; thus, the N-terminal regions of these polymers are highly enriched in cationic DM units, while the C-terminal regions are highly enriched in hydrophobic TM or  $\beta$ DE units. Copolymerization of DM $\beta$  +  $\beta$ CP $\beta$  was so rapid that we could not monitor  $\beta$ -lactam consumption as a function of reaction progress.

The data in Figure 2 indicate that nylon-3 materials generated via DM $\beta$  + TM $\beta$  or DM $\beta$  +  $\beta$ DE $\beta$  copolymerizations have very similar subunit distribution biases along the polymer chains. Therefore, the substantial differences in biological activity profile between these two nylon-3 copolymers, with 1:1 DM:TM displaying higher antibacterial potency and lower eukaryotic cell toxicity relative to 1:1 DM: $\beta$ DE, can be attributed to the different substitution patterns of the isomeric TM and  $\beta$ DE subunits. This result suggests that polymers containing a hydrophobic subunit that is expected to be more flexible ( $\beta$ DE) are more strongly hemolytic than polymers

Table 3. Antibacterial and Hemolytic Activities of Diblock Copolymers

block copolymer	MIC, <sup>a</sup> $\mu\text{g/mL}$				HC <sub>10</sub> , <sup>b</sup> $\mu\text{g/mL}$	SI (HC <sub>10</sub> /MIC <sub>MRSA</sub> ) <sup>c</sup>
	<i>B. subtilis</i>	<i>E. coli</i>	VREF	MRSA		
(DM) <sub>10</sub> (CH) <sub>10</sub>	13	>200	>200	>200	300	<1.5
(DM) <sub>10</sub> ( $\beta$ CP) <sub>10</sub>	13	>200	>200	>200	13	<0.07
(DM) <sub>10</sub> ( $\beta$ DE) <sub>10</sub>	>200	>200	>200	>200	38	<0.2
(DM) <sub>10</sub> (TM) <sub>10</sub>	6.3	>200	200	50	300	6

<sup>a</sup>The lowest polymer concentration that completely inhibits bacterial growth. <sup>b</sup>Polymer concentration required for 10% lysis of RBC. <sup>c</sup>Selectivity index (SI) was calculated based on MIC for MRSA. VREF is vancomycin-resistant *E. faecium*; MRSA is methicillin-resistant *S. aureus*.

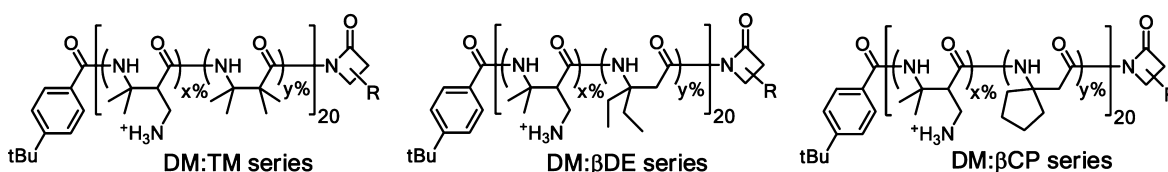


Figure 3. Binary hydrophobic-cationic nylon-3 copolymers containing TM,  $\beta$ DE, or  $\beta$ CP subunits and DM subunits. The DM precursor was racemic, so all copolymers are heterochiral. Polymers within each series have variable subunit proportion;  $x + y = 100$ , with  $x = 40$ –100.

containing isomeric but more conformationally constrained hydrophobic subunits (TM).

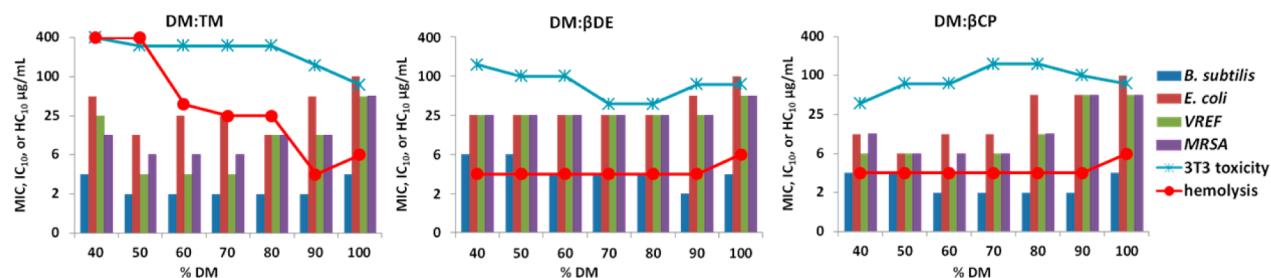
The data in Figure 2 indicate that one must be cautious in drawing conclusions regarding functional differences between the previously studied copolymer 1:1 DM:CH and the copolymer that displays the most favorable biological activity profile, 1:1 DM:TM. This pair differs not only in hydrophobic subunit identity but also in subunit distribution. We therefore undertook a modified approach to  $\text{DM}\beta + \text{CH}\beta$  copolymerization in order to generate a “skewed” version of 1:1 DM:CH with a subunit distribution comparable to that of 1:1 DM:TM.<sup>59</sup> The mechanism of anionic  $\beta$ -lactam ring-opening polymerization features a reactive chain-end (C-terminal imide) and thus constitutes a “living” process.<sup>51,53,54</sup> The skewed version of 1:1 DM:CH was prepared by introduction of the  $\beta$ -lactam precursors in four aliquots, each containing 25% of the total  $\beta$ -lactam but with differing  $\beta$ -lactam proportions. The  $\beta$ -lactam proportions in each aliquot were chosen to match the  $\beta$ -lactam proportions incorporated into growing 1:1 DM:TM chains at 25%, 50%, 75%, and 100% total conversion, according to the data in Figure 2. The resulting “skewed” 1:1 DM:CH copolymer displays antibacterial activities very similar to those of 1:1 DM:TM, and this skewed polymer is much more highly hemolytic than is 1:1 DM:TM (Table 1). Since the biological activity profile of the skewed 1:1 DM:CH copolymer is very similar to that of the original 1:1 DM:CH copolymer (prepared by introducing all of each  $\beta$ -lactam to the reaction vessel at the start of the polymerization), and since both DM:CH samples are much more hemolytic than 1:1 DM:TM, we conclude that the superior prokaryote vs eukaryote selectivity of the DM:TM copolymer arises from the intrinsic properties of the hydrophobic TM subunit, relative to CH, rather than from variations in subunit distribution along the polymer chains.

The subunit distribution bias within the 1:1 DM:TM copolymer (Figure 2) led us to wonder whether complete segregation of the cationic subunits from the hydrophobic subunits might lead to low hemolytic activity for all pairings of the cationic DM subunit with hydrophobic subunits. To address this question, we prepared DM-CH, DM- $\beta$ CP, DM- $\beta$ DE, and DM-TM diblock copolymers using conditions that should provide 20-mer average chain lengths, with a 10-mer average DM block at the N-terminal side and a 10-mer average

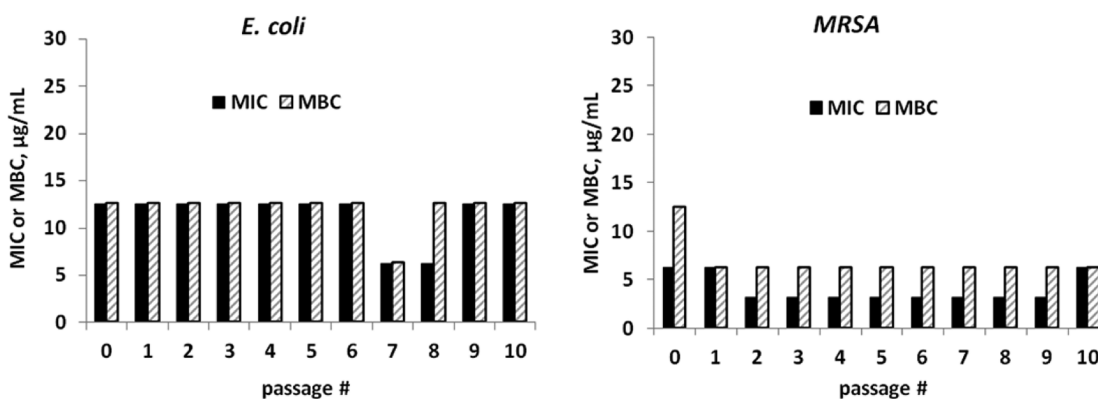
CH, TM,  $\beta$ DE, or  $\beta$ CP block at the C-terminal side.<sup>59</sup> PDI values for these block copolymers were in the range 1.07–1.27. The antibacterial and hemolytic properties of these diblock nylon-3 copolymers are summarized in Table 3. All four diblock copolymers are generally ineffective at inhibiting bacterial growth, other than for *B. subtilis*. There are significant differences among the diblock nylon-3 copolymers in terms of hemolytic activity. DM-CH and DM-TM diblock copolymers are only weakly hemolytic, but DM- $\beta$ DE and DM- $\beta$ CP are strongly hemolytic. Although these latter two block copolymers are somewhat less effective at inducing hemolysis than the corresponding copolymers generated from 1:1  $\beta$ -lactam mixtures (Table 3 vs Table 1), the trend among the four block copolymers mirrors that among the mixed copolymers. Specifically, the presence of hydrophobic subunits that are expected to be relatively flexible, because they contain a backbone CH<sub>2</sub> unit ( $\beta$ DE and  $\beta$ CP), correlates with high hemolytic activity.

Recent studies of hydrophobic-cationic copolymers in the polymethacrylate and poly(vinyl ether) families indicated that block architecture leads to diminished hemolytic activity relative to random subunit distribution,<sup>30,43</sup> and our results are consistent with this trend. However, diblock and “random” subunit distributions were comparable in terms of antibacterial activity in these two previous studies,<sup>30,43</sup> which contrasts sharply with our observations for four different nylon-3 copolymer compositions, all of which display only weak antibacterial activity in the diblock architecture. Thus, our results suggest that there is no general relationship between relative antibacterial potencies and diblock vs uncontrolled subunit distribution within binary hydrophobic-cationic copolymers.

The similarity in antibacterial profiles among the two forms of 1:1 DM:CH that contain different extents of compositional bias (normal copolymer vs “skewed” in Table 1) and the dramatic decline in antibacterial activities for the DM-CH diblock copolymer relative to mixed copolymers indicate that some degree of subunit intermixing is necessary to maximize inhibition of bacterial growth. This observation may be related to an elegant recent report on materials generated via ring-opening polymerization of cycloalkenes, which indicated that



**Figure 4.** Summary of biological activity profiles (antibacterial activities, 3T3 fibroblast toxicity, and hemolytic activities) as a function of cationic:hydrophobic subunit proportion for the three sets of binary nylon-3 copolymers shown in Figure 3. The lines drawn for 3T3 fibroblast toxicity and hemolysis merely connect data points. MIC is the minimum inhibitory concentration for bacterial growth;  $IC_{10}$  is the polymer concentration required to induce 10% 3T3 fibroblast death; and  $HC_{10}$  is the polymer concentration required to cause 10% lysis of human red blood cells. When the  $IC_{10}$  or  $HC_{10}$  value is  $>400 \mu\text{g/mL}$ , the plot shows a concentration at  $400 \mu\text{g/mL}$ .



**Figure 5.** Antibacterial resistance tests for 1:1 DM:TM with *E. coli* and MRSA. MIC is the minimum inhibitory concentration for bacterial growth; MBC is the minimum bactericidal concentration for 99.9% killing of the bacteria.

$\geq 8$ – $10 \text{ \AA}$  spacing between cationic groups along this backbone is optimal in terms of antibacterial activity.<sup>37</sup>

**Variations in the Proportion of Cationic and Hydrophobic Subunits.** To gain a more complete understanding of composition-activity relationships among copolymers prepared from the hydrophobic  $\beta$ -lactams  $TM\beta$ ,  $\beta DE\beta$ , and  $\beta CP\beta$ , we prepared a series of new binary copolymers via co-reaction of each of these three  $\beta$ -lactams with  $DM\beta$  (Figure 1). Reaction conditions were selected to favor 20-mer average length. Within each copolymer subset, the cationic:hydrophobic subunit proportion was varied (Figure 3). Each polymer was analyzed for antibacterial activity against four species (MIC), hemolysis ( $HC_{10}$ ), and 3T3 fibroblast toxicity ( $IC_{10}$ , the polymer concentration that causes 10% fibroblast toxicity).

Figure 4 summarizes the biological activity profiles of the new copolymer series. For both  $DM:\beta CP$  and  $DM:\beta DE$ , high hemolytic activity and significant 3T3 fibroblast toxicity were observed at all compositions, which indicates that nylon-3 copolymers containing the  $\beta CP$  or  $\beta DE$  subunit are generally not selective for prokaryotic vs eukaryotic cells. In contrast, excellent selectivity can be achieved in the  $DM:TM$  as long as the subunit proportion is properly controlled.

**Pronpensity of Bacteria to Develop Resistance to Nylon-3 Copolymers.** It is difficult for bacteria to develop resistance to HDPs,<sup>60</sup> and we wondered whether the same would be true of nylon-3 copolymers. To evaluate this possibility, we challenged *E. coli* and MRSA with the 1:1 DM:TM copolymer for 10 continuous passages. For each passage, we determined MIC and MBC values for the polymer using a liquid subculture derived from a single colony picked

from an agar plate that had been used for the measurement of MBC in the previous passage. This colony was taken from the plate for the polymer concentration one dilution below the MIC measured for the previous passage, to ensure that the bacteria could grow in the presence of a subinhibitory concentration of 1:1 DM:TM.<sup>61</sup> For both *E. coli* and MRSA, no sign of resistance to 1:1 DM:TM was detected after 10 continuous passages (Figure 5). The variations observed in Figure 5 correspond to a single 2-fold dilution and represent the experimental uncertainty in these measurements. These results suggest that it is difficult for bacteria to develop resistance to an antibacterial nylon-3 polymer, which strengthens the functional analogy between this polymer class and HDPs.

**Further Antibacterial Studies with 1:1 DM:TM.** In addition to the four bacteria used in our standard antimicrobial assessment of nylon-3 polymers (Table 1), we evaluated the activity of the best copolymer, 1:1 DM:TM, against other bacterial pathogens (Figure 6). This polymer displayed only weak activity against *Salmonella enterica* LT2 (MIC =  $200 \mu\text{g/mL}$ ), but the polymer was quite active against *Bacillus cereus* ATCC14579 (MIC =  $25 \mu\text{g/mL}$ ), the uropathogenic *E. coli* CFT073 (MIC =  $50 \mu\text{g/mL}$ ), and *Pseudomonas aeruginosa* PA1066, a strain isolated from a cystic fibrosis patient (MIC =  $12.5 \mu\text{g/mL}$ ).

## CONCLUSIONS

The results reported here show that changes in backbone substitution pattern within the hydrophobic subunit can exert a profound impact on the biological activity profiles of binary

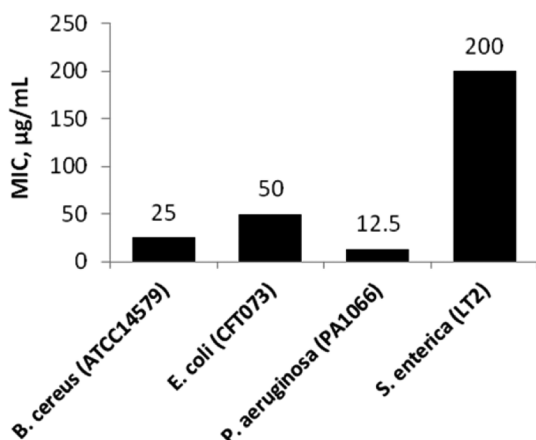


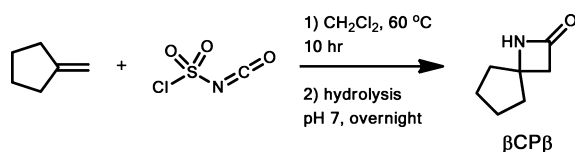
Figure 6. Antibacterial activity of copolymer 1:1 DM:TM against four pathogenic bacteria.

cationic-hydrophobic nylon-3 copolymers. Evolution optimizes polypeptide properties via selection among  $\alpha$ -amino acid residues that vary in both hydrophobicity and conformational propensity, and our findings suggest that exploring comparable variations among synthetic copolymers is useful for tuning functional properties. Implementation of this approach, however, requires a polymer for which alternative backbone substitution patterns can be readily accessed. Nylon-3 polymers are very convenient in this regard, but many common polymer families are not. In addition to their antibacterial properties, nylon-3 polymers have displayed promising behavior in several other areas of biological application,<sup>34,35,62–65</sup> and the approach described here may prove useful in the context of those applications.

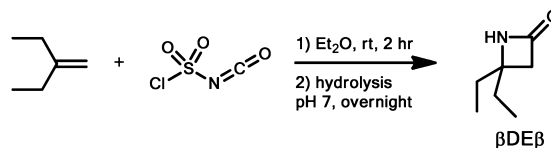
## EXPERIMENTAL METHODS

NIH 3T3 fibroblast cells were obtained from the American Type Tissue Collection (ATCC, Manassas, VA). Dulbecco's modified eagle medium (DMEM) and cell culture supplies were obtained from Invitrogen (Carlsbad, CA). CytoTox-ONE assay kits (G7892) were obtained from Promega (Madison, WI). LB medium (244610) was obtained from BD (Franklin Lakes, NJ). Agar (BP1423500) was obtained from Fisher Scientific (Pittsburgh, PA). PHMB (84428-SMP) was obtained from Lonza (Allendale, NJ) as a 20% aqueous solution ("Vantocil IB"). The solution was lyophilized to give PHMB as a white powder, which was used to generate solutions for biological activity studies. All other chemicals were purchased from Sigma-Aldrich and used without purification. <sup>1</sup>H and <sup>13</sup>C NMR spectra were collected on a Varian MercuryPlus 300 spectrometer at 300 and 75 MHz, respectively, using CDCl<sub>3</sub> or D<sub>2</sub>O as the solvent. <sup>1</sup>H NMR chemical shifts were referenced to the resonance for residual protonated solvent ( $\delta$  7.26 for CDCl<sub>3</sub> and 4.79 for D<sub>2</sub>O). <sup>13</sup>C NMR chemical shifts were referenced to the solvent ( $\delta$  77.16 for CDCl<sub>3</sub>). Mass spectra were acquired using either a Waters (Micromass) LCT mass spectrometer or a Waters (Micromass) AutoSpec mass spectrometer. IR spectra were acquired on a Bruker Tensor 27 instrument with an ATR attachment (Pike Technologies).

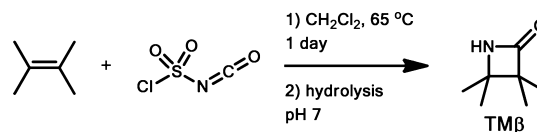
**Synthesis of  $\beta$ -Lactams.** *1-Azaspiro[3.4]octan-2-one*. The product was synthesized by a modification of reported methods.<sup>66</sup> A



solution of methylenecyclopentane (3.9 g, 47.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (24 mL) at rt was treated with chlorosulfonyl isocyanate (4.3 mL, 49.9 mmol), and the mixture was stirred at 60 °C for 10 h. The reaction mixture was poured into an ice-cold buffer solution containing sodium sulfite (12 g, 95.1 mmol) and dibasic sodium phosphate (13.5 g, 95.1 mmol). The mixture was stirred at rt overnight and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  150 mL). The combined organic layers were washed with brine (50 mL), dried over MgSO<sub>4</sub>, and concentrated. The crude product was purified by silica gel chromatography (1:1 hexane:EtOAc) to give  $\beta$ -lactam  $\beta$ CP $\beta$  as a light-yellow viscous oil (1.7 g, 29%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  6.23 (bs, 1H), 2.95 (dd,  $J$  = 8.1, 1.5 Hz, 1H), 1.91 (dd,  $J$  = 15, 4.5 Hz, 1H), 1.71–1.81 (m, 3H), 1.48–1.55 (m, 1H), 1.46 (bs, 3H), 1.24–1.36 (m, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  170.24, 62.86, 60.35, 35.87, 25.85, 24.20, 23.05; EI-HRMS:  $m/z$  calcd for C<sub>7</sub>H<sub>11</sub>NO [M]<sup>+</sup>: 125.0836; found: 125.0832.



*4,4-Diethylazetidone-2-one*. The product was synthesized by a modification of reported methods.<sup>67</sup> A solution of 2-ethyl-1-butene (10 g, 118.8 mmol) in Et<sub>2</sub>O (58 mL) at rt was treated with chlorosulfonyl isocyanate (10.4 mL, 118.2 mmol), and the mixture was stirred at rt for 2 h. The reaction mixture was poured into an ice-cold buffer solution containing sodium sulfite (22.5 g, 178.2 mmol) and dibasic sodium phosphate (25.3 g, 178.2 mmol). The mixture was stirred at rt overnight and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  500 mL). The combined organic layers were washed with brine (100 mL), dried over MgSO<sub>4</sub>, and concentrated to give  $\beta$ -lactam  $\beta$ DE $\beta$  as a colorless oil, which was used without purification (11.8 g, 78%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  6.90 (bs, 1H), 2.51 (d,  $J$  = 1.8 Hz, 2H), 1.60 (q,  $J$  = 7.5 Hz, 4H), 0.82 (t,  $J$  = 7.5 Hz, 6H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  168.49, 57.73, 45.94, 29.46, 8.57; EI-HRMS:  $m/z$  calcd for C<sub>7</sub>H<sub>14</sub>NO [M + H]<sup>+</sup>: 128.1070; found: 128.1068.



*3,3,4,4-Tetramethylazetidone-2-one*. The product was synthesized by a modification of reported methods.<sup>68</sup> A solution of 2,3-dimethylbutene (8.5 g, 101 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6.5 mL) was cooled in an ice-water bath and treated with chlorosulfonyl isocyanate (8.8 mL, 101 mmol). The mixture was removed from the ice-water bath and heated at 65 °C for 1 day. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and poured into an ice-cold buffer solution containing sodium sulfite (19.1 g, 151.4 mmol) and dibasic sodium phosphate (21.5 g, 151.4 mmol). This mixture was stirred at rt overnight and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  250 mL). The combined organic layers were washed with brine (100 mL), dried over MgSO<sub>4</sub>, and concentrated. The crude product was recrystallized from 1:6 EtOAc:Hex to afford  $\beta$ -lactam  $\text{TM}\beta$  as white needle crystals (10.8 g, 84%), mp 101.2–101.9 °C (ref 68, mp 100–101 °C). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  6.68 (bs, 1H), 1.23 (s, 6H), 1.11 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  175.30, 58.04, 54.37, 24.31, 19.01; ESI-HRMS:  $m/z$  calcd for C<sub>7</sub>H<sub>13</sub>NN<sub>2</sub>O [M + N<sub>2</sub>]<sup>+</sup>: 150.0891; found: 150.0886.

**Preparation Nylon-3 Copolymers.** Regular or "random" nylon-3 copolymers were prepared in THF or DMF by adding the entire quantity of each  $\beta$ -lactam to the reaction vessel before polymerization was initiated. These copolymers were purified by precipitation from the polymerization solution with pentane and deprotected using neat trifluoroacetic acid (TFA) by following protocols described previously.<sup>33</sup> The reaction setup and polymerization operations were conducted in a glovebox to maintain the moisture level below 5 ppm. The reaction mixture was removed from the glovebox for purification.

Polymers at the protected stage (with Boc protection of side chain amine groups) were subjected to gel-permeation chromatography (GPC) characterization using THF as the mobile phase at a flow rate of 1 mL/min at 40 °C, using two Waters columns (Styragel HR 4E, particle size 5  $\mu\text{m}$ ) linked in series. The Shimadzu GPC instrument was equipped with a multi-angle light scattering detector (Wyatt miniDAWN, 690 nm, 30 mW) and a refractive index detector (Wyatt Optilab-rEX, 690 nm).  $M_n$ ,  $M_w$ , and PDI were obtained with ASTRA 5.3.4.20 software using a  $dn/dc$  value of 0.1 mL/g. DP for a polymer was calculated using the obtained  $M_n$  value and the theoretical subunit composition based on the  $\beta$ -lactam proportion used for the polymerization reaction.

The “skewed” form of 1:1 DM:CH, intended to mimic the subunit distribution in “random” 1:1 DM:TM, as shown in Figure 2, was prepared in THF by adding the  $\beta$ -lactams in four portions to the reaction solution. The total amount of  $\beta$ -lactams DM $\beta$  and CH $\beta$  used for this reaction corresponded to a 1:1 (equimolar) mixture, but the  $\beta$ -lactams were divided unequally within each of the four portions. The  $\beta$ -lactam distributions among the four DM $\beta$  + CH $\beta$  portions were chosen based on the proportions of  $\beta$ -lactams TM $\beta$  and DM $\beta$  that were incorporated into growing 1:1 DM:TM chains after 25%, 50%, 75%, and 100% polymerization, according to copolymerization kinetics data (Figure 2). Thus, the first  $\beta$ -lactam portion used to prepare the “skewed” 1:1 DM:CH copolymer contained 25% of the total  $\beta$ -lactam precursors in an 83:17 DM $\beta$ :CH $\beta$  molar ratio. The second portion contained 25% of the total  $\beta$ -lactam precursors in a 73:27 DM $\beta$ :CH $\beta$  molar ratio. The third portion contained 25% of the total  $\beta$ -lactam precursors, in a 36:64 DM $\beta$ :CH $\beta$  molar ratio, and the fourth portion contained 25% of the total  $\beta$ -lactam precursors, in an 8:92 DM $\beta$ :CH $\beta$  molar ratio. Since our studies of DM $\beta$  + CH $\beta$  copolymerization indicated that these reactions are complete within 5 min (rt), we allowed 20 min after the addition of the first, second, and third  $\beta$ -lactam portions before adding the next  $\beta$ -lactam portion. The “skewed” copolymer of 1:1 DM:CH was characterized at the protected stage and deprotected by following the protocol used for “random” nylon-3 copolymers.

**Preparation of Nylon-3 Diblock Copolymers.** Nylon-3 diblock copolymers were prepared in DMAc in a glovebox to keep the moisture level below 5 ppm. The  $\beta$ -lactam monomer for the first block in DMAc solution was mixed with a solution of co-initiator (*tert*-BuZnCl, 0.1 equiv), and a solution of base catalyst (LiHMDS, 0.25 equiv) was added. The reaction mixture was stirred for 2 h at rt, and then a solution of the second  $\beta$ -lactam monomer (1 equiv relative to the first  $\beta$ -lactam) was added. The reaction mixture was stirred for 36 h at rt and then removed from the glovebox. The reaction (total 4 mL) was quenched with a few drops of MeOH, and the protected diblock copolymer was precipitated by adding pentane (45 mL) to the reaction mixture. The precipitated solid was collected from the bottom of the cell culture tube after centrifugation and removal of the solvent by decantation. The crude polymer was dissolved in THF (2 mL) and subjected to additional precipitation/centrifugation operations. After 4–5 cycles of precipitation/centrifugation, the protected polymer was collected and dried under  $\text{N}_2$  to give a white solid. The diblock copolymer was deprotected by treating with neat TFA (2 mL) for 2 h at rt and precipitated by addition of  $\text{Et}_2\text{O}$  (45 mL). The precipitated polymer was collected from the bottom of the culture tube after centrifugation and removal of the solvent by decantation. The collected solid was dissolved in MeOH (1 mL) and subjected to precipitation/centrifugation operations. After a total of three cycles of precipitation/centrifugation, the deprotected diblock copolymer was collected and dried under  $\text{N}_2$  to give a white solid (TFA salt).

The diblock copolymer was characterized at the side-chain protected stage as previously described.<sup>34</sup> A Waters GPC was used for polymer characterization using DMAc (containing 10  $\mu\text{M}$  LiBr) as the mobile phase at a flow rate of 1 mL/min at 80 °C. The GPC was equipped with a single refractive index detector (Waters 2410) and two Waters Styragel HR 4E columns (particle size 5  $\mu\text{m}$ ) linked in series. The columns were calibrated with nine PMMA standards with peak average molecular weight ( $M_p$ ) ranging from 690 to 1944 000. Number-average molecular weight ( $M_n$ ), weight-average molecular

weight ( $M_w$ ), and polydispersity index (PDI) were calculated using Empower software and calibration curves obtained from PMMA standards. The degree of polymerization (DP) for a polymer was calculated from the obtained  $M_n$  value and the theoretical subunit composition based on the  $\beta$ -lactam proportion used for the polymerization reaction.

**Kinetic Studies of Copolymerization.** Kinetic studies of copolymerization for 1:1 DM:CH, 1:1 DM:TM, 1:1 DM: $\beta$ CP and 1:1 DM: $\beta$ DE were conducted using gas chromatography (GC) to detect unreacted  $\beta$ -lactam starting materials at various time points before each polymerization reaction was complete. The base-catalyzed ring-opening copolymerization reactions were conducted in the same way as preparative copolymerization reactions, but these reaction mixtures for kinetic analysis contained triphenylmethane, which is expected to be inert under the reaction conditions, as an internal standard for GC analysis. At different time points during each copolymerization reaction, an aliquot of the reaction solution was transferred to a separate vial that contained about 10 mg of benzoic acid, which rapidly halts the copolymerization reaction. The quenched reaction samples were analyzed on a Shimadzu GC-17A GC instrument equipped with an RTX-5 or a DB-wax column to obtain the peak areas for unreacted  $\beta$ -lactams and the internal standard. Subsequent calculations were based on  $\beta$ -lactam:internal standard peak area ratios. For each time point, the amount of each  $\beta$ -lactam that remained was determined based on a calibration curve ( $\beta$ -lactam:internal standard peak area ratio plotted as a function of  $\beta$ -lactam:internal standard concentration ratio). The kinetic study revealed that the DM $\beta$  + TM $\beta$  copolymerization is much slower than copolymerization of the other three  $\beta$ -lactams with DM $\beta$ . The DM $\beta$  + TM $\beta$  copolymerization is only 81% complete after 5 h, and 92% complete after 80 h. Samples of 1:1 DM:TM used for biological evaluation were prepared in reactions that were terminated after 16 h, because longer reaction times can lead to byproduct formation.<sup>53</sup>

**Antibacterial Assays.** The MIC assay for bacteria was conducted by following a 2-fold broth microdilution protocol previously described.<sup>33</sup> Eight bacteria were tested: *E. coli* JM 109, *B. subtilis* BR151, *E. faecium* A634 (vancomycin-resistant), *S. aureus* 1206 (methicillin-resistant),<sup>33</sup> *Salmonella enterica* LT2, *Bacillus cereus* ATCC14579, the uropathogenic *E. coli* CFT073, and *Pseudomonas aeruginosa* PA1066. Briefly, bacteria were cultured overnight at 37 °C on LB agar plates and then suspended in LB medium at  $2 \times 10^6$  cells/mL. The cell suspension (50  $\mu\text{L}$ ) was mixed with the same volume of polymer solutions in 2-fold serial dilutions (from 400 to 3.13  $\mu\text{g}/\text{mL}$ ) in a 96-well plate, which was incubated for 6 h at 37 °C. Optical density (OD) of each well was measured at 650 nm on a Molecular Devices Emax precision microplate reader. Controls included on the same plate: LB medium only (blank) and cells in LB without polymer (uninhibited growth control). The bacterial cell growth in each well was calculated with the equation (% cell growth =  $(A_{650}^{\text{polymer}} - A_{650}^{\text{blank}}) / (A_{650}^{\text{control}} - A_{650}^{\text{blank}}) \times 100$ ) and plotted against polymer concentration. The MIC value is the minimum concentration of a given polymer necessary to inhibit bacterial growth completely. When repeat measurements of MIC, IC<sub>10</sub>, or HC<sub>10</sub> oscillated between two polymer concentrations, the average of these two is reported (e.g., 75  $\mu\text{g}/\text{mL}$  is reported when the values were 50 and 100  $\mu\text{g}/\text{mL}$ ). In the MIC assay for daptomycin, 0.1 M  $\text{CaCl}_2$  was incorporated for all polymer/bacterial cell mixture at varied polymer concentration.

The MBC for a given polymer was obtained after performing the MIC assay described above. Aliquots of 10  $\mu\text{L}$  of bacterial cell suspension from wells containing the polymer at concentrations ranging from one dilution below the MIC to the highest polymer concentration were plated on LB agar. The plates were incubated overnight at 37 °C, and bacterial colonies were then counted. The MBC is the lowest polymer concentration to result in zero bacterial colonies.

**Antibacterial Resistance Test.** A standard MIC/MBC test of 1:1 DM:TM was conducted with *E. coli* or MRSA using the protocol mentioned above and beginning with the original strain of bacteria (passage 0). The spread plate used for colony forming unit (CFU) counting in the MBC test was used to subculture bacterial cells for this

study. Bacterial colonies were observed on the spread plate that was inoculated with the mixture of bacterial cells and polymer at a concentration one dilution below the MIC, i.e., the polymer concentration is chosen as 3.1  $\mu\text{g}/\text{mL}$  if MIC is identified as 6.2  $\mu\text{g}/\text{mL}$ . One colony, representing a surviving cell from the previous polymer treatment, was carefully picked from this LB-agar plate and designated as passage 1 cells. The colony of passage 1 cells was transferred to a centrifuge tube containing 3 mL of sterile LB medium and dispersed under vortex mixing for 20 s. This cell suspension was subcultured by inoculating on a LB-agar plate and incubating at 37 °C overnight. The cultured cells at passage 1 on a LB-agar plate were suspended in LB medium and used for the next round of standard MIC/MBC test. This operation was repeated to evaluate the impact of 1:1 DM:TM on *E. coli* and MRSA for 10 successive passages.

**Fibroblast Toxicity Assay.** Polymer toxicity was evaluated using NIH 3T3 fibroblasts and the CytoTox-ONE assay kit (Promega), which measures the release of lactate dehydrogenase (LDH) from membrane-damaged cells, as described previously.<sup>34</sup> Briefly,  $1.5 \times 10^4$  cells in DMEM were seeded in each well of a 96-well plate, which was incubated for 24 h at 37 °C. Medium was exchanged for fresh DMEM (phenol red- and pyruvate-free), and cells were incubated for another 2 h at 37 °C. Cells were treated with nylon-3 polymers at varied concentrations in a 2-fold serial dilution series ranging from 400 to 3.13  $\mu\text{g}/\text{mL}$  for 12 h at 37 °C. The cells in each well were then analyzed using the CytoTox-ONE assay kit. On the same plate, wells without polymer and wells treated with lysate solution to cause 100% release of LDH were incorporated as the blank and positive control, respectively. Fluorescence intensity was measured on a Tecan Infinite M1000 microplate reader using ex/em 560/590 nm. Cell death was calculated from  $(\% \text{ death} = (F^{\text{polymer}} - F^{\text{blank}})/(F^{\text{control}} - F^{\text{blank}}) \times 100)$  and plotted against polymer concentration. The  $\text{IC}_{10}$  value is the polymer concentration that causes 10% cell death.

**Hemolysis Assay.** Hemolytic activity was evaluated using human red blood cells (hRBC) following a protocol described previously.<sup>33,34</sup> Briefly, 100  $\mu\text{L}$  of 2% RBC suspension in TRIS-buffered saline (TBS; 10 mM TRIS, 150 mM NaCl, pH 7.2) was treated with an equivalent volume of nylon-3 polymer solution in a 2-fold dilution series in TBS ranging from 800 to 6.25  $\mu\text{g}/\text{mL}$  in a 96-well plate for 1 h at 37 °C. On the same plate, hRBC treated with TBS only (without polymer) and hRBC treated with Triton X-100 (3.2 mg/mL in TBS) were incorporated as the blank and positive control, respectively. After centrifugation, supernatant from each well was transferred to a new 96-well plate, and the optical density (OD) in each well was measured using a Molecular Devices Emax precision microplate reader at 405 nm. The hemolysis for each sample was calculated using the equation  $(\% \text{ hemolysis} = (A_{405}^{\text{polymer}} - A_{405}^{\text{blank}})/(A_{405}^{\text{control}} - A_{405}^{\text{blank}}) \times 100)$  and plotted against polymer concentration. The  $\text{HC}_{10}$  value for a given polymer is the polymer concentration necessary to cause 10% lysis of RBCs. In previous studies,<sup>34</sup> we have used the minimum hemolytic concentration (MHC) to assess polymer impact on red blood cells in a way that is comparable with the index of antibacterial activity, MIC. However, we have switched here to  $\text{HC}_{10}$  because it can be challenging to measure MHC accurately. Other researchers have used  $\text{HC}_{50}$  as a measure of hemolytic activity, but this approach seemed non-ideal because the extent of hemolysis rises slowly as a function of polymer concentration in many cases. For example,  $\text{HC}_{50}$  for 1:1 DM:CH is  $\sim 400 \mu\text{g}/\text{mL}$ , and if we focused on  $\text{HC}_{50}$  we could conclude that this polymer is highly selective for bacteria relative to eukaryotic cells. In contrast,  $\text{HC}_{10}$  for this polymer is 19  $\mu\text{g}/\text{mL}$ , which is not much higher than the MIC values.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Polymer synthesis and compound characterization spectra. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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### Notes

The authors declare the following competing financial interest(s): B.W. and S.H.G. are co-inventors on a patent application that covers the polymers described here.

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