

Communications

Synthetic Mimic of Antimicrobial Peptide with Nonmembrane-Disrupting Antibacterial Properties

Gregory J. Gabriel,[†] Ahmad E. Madkour,[†] Jeffrey M. Dabkowski,[‡] Christopher F. Nelson,[‡] Klaus Nüsslein,[‡] and Gregory N. Tew^{*,†}

Department of Polymer Science and Engineering and Department of Microbiology, University of Massachusetts-Amherst, Amherst, Massachusetts 01003

Received July 31, 2008; Revised Manuscript Received August 22, 2008

Polyguanidinium oxanorbornene (**PGON**) was synthesized from norbornene monomers via ring-opening metathesis polymerization. This polymer was observed to be strongly antibacterial against Gram-negative and Gram-positive bacteria as well as nonhemolytic against human red blood cells. Time-kill studies indicated that this polymer is lethal and not just bacteriostatic. In sharp contrast to previously reported SMAMPs (synthetic mimics of antimicrobial peptides), **PGON** did not disrupt membranes in vesicle-dye leakage assays and microscopy experiments. The unique biological properties of **PGON**, in same ways similar to cell-penetrating peptides, strongly encourage the examination of other novel guanidino containing macromolecules as powerful and selective antimicrobial agents.

Introduction

We report that poly guanidinium oxanorbornene (**PGON**) possesses a remarkable combination of antimicrobial activity against both Gram-negative and Gram-positive bacteria *as well as* low hemolytic activity against human red blood cells (RBCs). Standard antibacterial, bacteria cell-viability, and hemolysis assays all give data that is encouraging for the development of powerful, yet nontoxic, antibacterial materials.¹ Additionally, the properties of **PGON** surpass that of other cationic polymers, in particular **Poly-1** and **Poly-3** from our previous study.² Vesicle investigations show that **PGON** is not membrane-disruptive, supporting a previous study indicating it also has properties similar to poly arginine and other cell-penetrating peptides (CPPs) like HIV-TAT.³ As a result, **PGON** represents an exciting and fundamentally novel entry in the expanding field of synthetic mimics of antimicrobial peptides (SMAMPs).^{1,2,4,5}

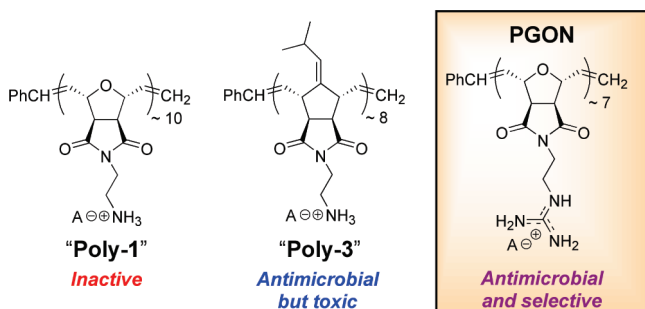
have rarely used the guanidine functionality. Instead, the focus has been almost exclusively on amines.^{2,6} In this work, a new Boc-protected guanidinium oxanorbornene monomer was polymerized via ring-opening metathesis polymerization (ROMP) using the third generation Grubbs' catalyst to afford synthetic poly arginine, **PGON** (PDI = 1.07, MW of the deprotected polymer-TFA salt = 2.5 kDa). This strategy is in contrast to a postpolymerization functionalization technique recently published that afforded polynorbornenes that efficiently promote cellular internalization.⁷ Lastly, polyamine norbornenes, **Poly-1** and **Poly-3**, with similar MWs were prepared for comparison.

Experimental Section

Materials. Solvents and reagents for the monomer synthesis were purchased from Aldrich and used without further purification. For the polymer synthesis the second generation Grubbs' catalyst, tricyclohexylphosphine, (1,3-dimesitylimidazolidine-2-ylidene)benzylideneruthenium dichloride was purchased from Aldrich. This compound was converted to a Grubbs' third generation catalyst by reaction with 3-bromopyridine from Aldrich. Polymerizations were performed in dry CH₂Cl₂ from Acros (sealed under nitrogen with molecular sieves). Chloroform solutions of lipids were obtained from Avanti Polar Lipids. Calcein dye was purchased from Aldrich. The BacLight Staining Kit L-7012 was obtained from Invitrogen Corp.

Synthesis of PGON. The synthesis of **PGON** starts with oxanorbornene **1**,¹ and to this salt (1.4 g, 4.34 mmol) *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamide (4.0 g, 13.02 mmol) and triethylamine (4.1 g, 40 mmol) were added with 60 mL of solvent (90% CH₃CN, 10% H₂O). This solution was stirred at room temperature for 12 h. The solution was then diluted with ethyl acetate and washed twice each with H₂O and brine. The organic layer was evaporated and purified by silica column chromatography using a MeOH in CH₂Cl₂ gradient to afford the Boc-protected guanidinium oxanorbornene monomer, **2**.

This monomer was polymerized by ring-opening metathesis polymerization (ROMP) using a derivative of the second generation Grubbs' catalyst, [(H₂Imes)(3-Br-py)₂(Cl)₂Ru=CHPh].² The polymerization for



While several antimicrobial peptides (AMPs) contain the amino acid arginine along with other basic residues, SMAMPs

* To whom correspondence should be addressed. E-mail: tew@mail.pse.umass.edu.

[†] Department of Polymer Science and Engineering.

[‡] Department of Microbiology.

PGON entailed adding to a test tube monomer (100 mg) plus catalyst (27.4 mg). The test tube was capped with a septum and purged with N_2 for 5 min, then 1 mL dry CH_2Cl_2 was injected. The N_2 line was removed and the clear, brown solution was stirred at 28 °C for 30 min after which 0.4 mL of ethyl vinyl ether was injected to terminate the polymer. After stirring for 15 min, the solution was added dropwise to 300 mL of stirring pentane to precipitate the polymer. The pentane solution was stirred an additional 30 min and left standing undisturbed for an hour. The precipitate was then collected by a fine sinter funnel. The polymer was then redissolved in 1 mL of CH_2Cl_2 , reprecipitated, collected, and then dried by vacuum for 8 h.

The Boc-protected polymers were deprotected by stirring 100 mg in 8 mL of 1:1 TFA/ CH_2Cl_2 for 2 h. The solution was concentrated to an oil by rotary evaporator set at 40 °C and residual TFA was removed by sonicating the oil in more CH_2Cl_2 and evaporating the solvent again by rotary evaporator. The resulting solid was placed under vacuum for 2 h. Finally, the solid was fully dissolved in 4 mL of H_2O and filtered through a polyethersulphone (PES) syringe filter (Whatman, 25 mm diameter, 0.45 μm pore) and freeze-dried for 48 h to give an eggshell colored soft solid. Final deprotected polymers were stored at -20 °C.

Boc-Protected Guanidinium Oxanorbornene Monomer, 2. 1H NMR (300 MHz, DMSO- d_6): δ 11.44 (1H, s), 8.35 (1H, t, $J = 6.0$ Hz), 6.56 (2H, s), 5.10 (2H, s), 3.52 (2H, m), 3.43 (2H, m), 2.85 (2H, s), 1.47 (9H, s), 1.36 (9H, s). ^{13}C NMR (75 MHz, DMSO- d_6): δ 176.3, 156.6, 153.0, 136.5, 83.3, 80.9, 47.6, 39.0, 38.4, 28.3, 28.1. HR-MS (FAB $^+$) calcd, 451.49; found, 451.22.

Boc-Protected PGON. 1H NMR (300 MHz, DMSO- d_6): δ 11.51 (1H, br), 8.47 (1H, br), 5.90 (*trans*) and 5.70 (*cis*; 2H total, br), 4.89 (*cis*) and 4.39 (*trans*; 2H total, br), 1.45 (9H, s), 1.38 (9H, br), *cis/trans* ratio = 46:54.

GPC of Boc-protected **PGON**: $M_n = 3.2$ kDa, PDI = 1.07 (After deprotection the TFA-salt **PGON** has an approximate weight of 2.5 kDa).

PGON. 1H NMR (300 MHz, DMSO- d_6): δ 7.88 (1H, br), 7.36 (4H, br), 5.96 (1H, br), 5.74 (1H, br), 4.92 (1H, br), 4.43 (1H, br) 3.47 (4H, br).

Microbial and Hemolysis Assays. Bacteria suspensions of *Ec*, *Sm*, *Sa*, and *Bs*, (*Escherichia coli* D31, *Serratia marcescens* ATCC 43861, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 8037) were grown in Mueller–Hinton Broth (MHB) overnight at 37 °C, diluted with fresh MHB to an optical density of 0.001 at 600 nm (OD_{600}). This OD_{600} gave an initial bacterial concentration of $\sim 10^5$ cells/mL. This suspension was mixed with different concentrations of freshly prepared polymer solutions dissolved in DMSO (40 mg/mL stock solution) by serial dilutions in a 96-well plate, and incubated for 6 h at 37 °C. The OD_{600} was measured for bacteria suspensions that were incubated in the presence of polymer solution or only MHB. Antibacterial activity was expressed as minimal inhibitory concentration (MIC), the concentration at which more than 90% inhibition of growth was observed after 6 h. All experiments were run in quadruplicate.

Hemolytic activity measurements were performed on all polymers. Freshly drawn human red blood cells (RBC, 30 μL), were suspended in 10 mL of Tris saline (10 mM Tris, 150 mM NaCl, pH 7.2, filtered through PES membrane with 0.22 μm pore size) and rinsed three times by centrifugation (5 min at 1500 rpm) and resuspended in Tris saline. Polymer solutions were prepared in DMSO at 40 mg/mL and further diluted as necessary. Freshly prepared polymer solutions with different concentrations were added to 100 μL of the above-prepared RBC suspension to reach a final volume of 200 μL on a 96-well plate. The resulting mixture was kept at 37 °C for 30 min on a stirring plate. Then the plate was centrifuged (IEC Centra-4B, 10 min at 1500 rpm), and the supernatant in each well was transferred to a new plate. Hemolysis was monitored by measuring the absorbance of the released hemoglobin at 414 nm. Hemolysis (100%) was obtained by adding 10 μL of Triton-X (polyoxyethylene isooctylphenyl ether from Sigma-Aldrich) solution (20 vol % in DMSO), a strong surfactant, to the above-prepared HRBC suspension. The upper limit of polymer concentration

that was required to cause 50% hemolysis is reported as HC_{50} , and the absorbance from Tris saline containing no polymer was used as 0% hemolysis. All experiments were run in quadruplicate.

Bactericidal Kinetics Studies. Bactericidal kinetics studies (also known as time-kill assays) were performed on *Sa* to differentiate growth inhibition from cell death. The protocol was adapted from literature.³ Cells were grown in MHB to midexponential phase with an $OD_{600} \sim 0.3$, and then adjusted to an OD_{600} of 0.001. This OD_{600} gave an initial bacterial concentration of $\sim 10^5$ cells/mL, the same as in the MIC studies. Cells were either untreated or treated to **PGON** at $1 \times$ MIC or four times the MIC_{90} concentration and then placed under rotary agitation at 37 °C. Aliquots were removed at different time points (0, 15, 30, 60, 120, and 240 min) and placed back at 37 °C with agitation. Cells were diluted immediately to remove any effect of **PGON**. Serial 10-fold dilutions were spread on MHB agar plates and incubated at 37 °C for 18 h to determine viable colony counts. Viable cell counts were plotted on a log-scale graph (CFU/mL vs time) to determine log-reduction of bacterial growth compared to the untreated control.

Dye-Leakage from Large Unilamellar Vesicles. The following is a general procedure to make large unilamellar vesicles (LUV) having a hydrodynamic radius, $R_h \sim 120$ nm (by dynamic light scattering). Chloroform solutions of POPE (3.23 mg, 0.0045 mmol) and POPG (1.15 mg, 0.0015 mmol; Avanti Polar Lipids, Inc.) were mixed in a 10 mL round-bottom flask, and the chloroform was removed at room temperature by rotary evaporator to form a uniform film. The flask was placed under vacuum for an additional 6 h. The dried film was hydrated with 1 mL of a 40 mM aqueous calcein (Sigma-Aldrich) solution in Tris buffer without added NaCl. The calcein dye solution was adjusted to pH = 7 prior to adding it to the film. The solution was subjected through five freeze/thaw cycles using liquid nitrogen and warm water. The entire volume of 1 mL of solution was subjected to extrusion (a total of 15 passes) through two stacked 400 nm pore PC membranes (Avanti Polar Lipids, Inc.) at room temperature. Finally, the solution was pressurized through a small column packed with Sephadex-25 (Sigma-Aldrich), eluting with Tris-saline buffer to remove nontrapped calcein dye. Fractions (~ 5 drops each) were collected. The vesicle solution can be stored in a vial at 4 °C and diluted as needed for up to 4 days.

Diluted calcein-loaded vesicle fractions that afforded fluorescence intensities < 80 au without surfactant and > 800 au after addition of surfactant, were used (Ex. = 490 nm used and Em. = 515 nm monitored). A total of 50 μL of 0.2% Triton-X was used as the strong surfactant, which causes complete vesicle disruption and leakage. Typically, chosen vesicle fractions were diluted 6-fold and 25 μL of this stock solution is added to 2 mL of Tris-saline buffer. A fluorescence intensity of < 80 au was verified to be stable over a minute before 25 μL of a polymer stock solution was added (2.5 $\mu g/mL$ final concentration in cuvette), agitated briefly with the pipettor tip, then monitored at 515 nm. After 5 min, Triton-X was added and the corresponding fluorescence was taken as 100% leakage.

Fluorescence Microscopy of Stained Bacteria Cells. An Olympus BX51 Reflected Fluorescence Microscope (Optical Analysis Corp. Nashua, NH) with a 100 W Mercury Lamp (Chin Technical Corp.) was used for fluorescence studies. A BacLight Kit L-7012 (Invitrogen Corp.) was used as the fluorescence dye in a mixture of 1:1 propidium iodide:SYTO9 to examine *Sa* in the presence of **PGON**. It is important to mention that an initial bacterial concentration of $\sim 10^8$ cells/mL was used for microscopy studies (3 orders of magnitude higher than the MIC and bactericidal kinetics studies) for ease of visualization. The dye mixture was incubated with the bacteria at room temperature for 15 min before adding polymer solution (75 $\mu g/mL$ final concentration). Solution of cells, dye, and polymer were allowed to sit for 30 min before 50 μL was placed on a slide, fitted with a coverslip, and visualized. Bacteria were viewed under a green filter (excitation/emission, 420–480 nm/520–800 nm) or a red filter (480–550 nm/590–800 nm). To confirm that these experiments agreed with the time-kill experiments discussed above and to confirm that **Poly-1** is not lethal

Table 1. Biological Activities

polymer (kDa)	MIC ^a ($\mu\text{g/mL}$)					Sel. ^b
	<i>Ec</i>	<i>Sm</i>	<i>Sa</i>	<i>Bs</i>	HC ₅₀	
Poly-1 (3.3)	400 ^d	400	400	200 ^d	2150	5
Poly-3 (2.9)	25 ^d	25	25	12 ^d	1	0.04
PGON (2.5)	6	50	12	12	1500	250
AMP (2.5) ^c	12	>256	12	2	120	10

^a MIC = minimum inhibitory concentration, HC₅₀ = hemolytic concentration, *Ec* = *Escherichia coli*, *Sm* = *Serratia marcescens*, *Sa* = *Staphylococcus aureus*, and *Bs* = *Bacillus subtilis*. ^b Selectivity for *Ec* over RBCs shown (Sel. = HC₅₀/MIC). ^c Previously reported MIC values measured for *Ec*,^{4d} *Sm*,⁹ *Sa*,^{4d} and *Bs*.^{5e} ^d used derivatives of antimicrobial peptide Magainin. ^e Previously reported values.²

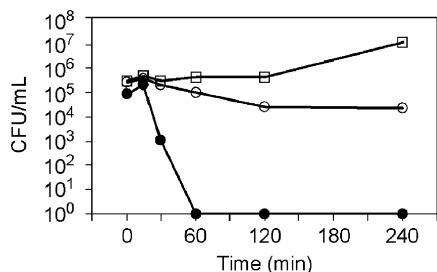


Figure 1. Time kill plots for *S. aureus*. CFU = colony forming unit; squares = untreated cells; white circles = treated with PGON at 1 \times MIC; and black circles = at treated with PGON 4 \times MIC.

or static and that **Poly-3** is lethal, cell counts were taken directly from the microscopy experiments. They confirmed that **Poly-1** is not active with no reduction in cell count compared to the control (at 30 min and two hours), while **Poly-3** and **PGON** showed a reduction of 8 logs in less than two hours. At the 30 min time point, **Poly-3** and **PGON** showed log reductions of 8 and 4, respectively.

Results and Discussion

Results from microbiological assays⁸ showed that **PGON** had an MIC of 6 $\mu\text{g/mL}$ against *E. coli* (see Table 1 for abbreviations). Activity against an additional Gram-negative bacteria, *S. marcescens*, which is not typically susceptible to AMPs, and two Gram-positive bacteria, *S. aureus* and *B. subtilis*, were also examined attesting to the broad activity of **PGON**. For comparison, the Magainin derivative, MSI-78, had an MIC of 12 $\mu\text{g/mL}$ against *E. coli*. **PGON**'s close structural analogue, **Poly-1**, having a primary amino group instead of a guanidino group, had MICs of 200–400 $\mu\text{g/mL}$ and was thus considered to be relatively inactive. In contrast, the MICs of the more hydrophobic, isobutylidene-derivatized **Poly-3** were found to be between 12–25 $\mu\text{g/mL}$ for all bacteria, representing good activity. However, and in sharp contrast to **Poly-3**, broadly active **PGON** possessed a lack of toxicity toward RBCs (HC₅₀ = 1500 $\mu\text{g/mL}$). As a result of **PGON**'s high HC₅₀ value, its selectivity of 250 is significantly better than other polymeric SMAMPs.^{1,2,4} Again, as a comparison, MSI-78 had a selectivity of 10 for *E. coli* over RBCs using the HC₅₀/MIC ratio.

Bactericidal kinetics studies (also known as time-kill assays) were performed on *S. aureus* to differentiate growth inhibition from cell death and showed that **PGON** was lethal at 4 \times the MIC with a 5-log reduction in less than 60 min (Figure 1). Starting with $\sim 10^5$ cells/mL, aliquots were removed periodically and viable cells counted via plating techniques. The results clearly showed **PGON**'s ability to kill bacteria (bactericidal activity) not just inhibit growth (bacteriostatic activity).

Many AMPs and their structural mimics have been shown to be membrane-active with the balance of their hydrophobic/

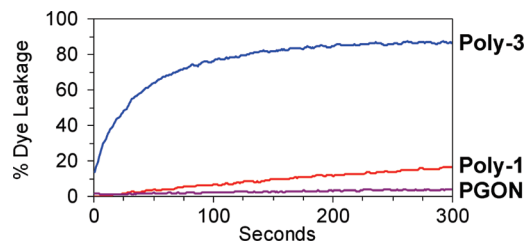


Figure 2. Vesicle dye leakage after polymer addition.

hydrophilic properties critical to their mode of action.¹⁰ Similarly, the biological properties of **Poly-1** and **Poly-3** were previously explained based on their amphiphilicity, in agreement with the concept that the proper balance of hydrophobic and hydrophilic groups is often essential for the activity of AMPs.² **Poly-1** was the most hydrophilic and consequently not membrane-active, presumably because it can not sufficiently disrupt the lipid bilayer. In contrast, **Poly-3**, being significantly more hydrophobic, exhibited good antibacterial activity but unfortunately showed high hemolysis. With its outstanding biological properties, it appeared that **PGON** had attained a favorable amphiphilicity.

High performance liquid chromatography (HPLC) retention times are routinely exploited in the evaluation of a peptide's, or peptidomimetic's relative amphiphilicity.^{5b} Retention times for **Poly-1**, **Poly-3**, and **PGON**, were 19.9, 25.5, and 22.4 min, respectively, indicating that the guanidinium groups of **PGON** resulted in a polymer with intermediate hydrophobic character. Therefore, from the microbiology and HPLC data, it was expected that **PGON** would exhibit strong membrane-lysis in dye-leakage assays using vesicles composed of phosphatidyl ethanolamine (PE) and phosphatidyl glycerol (PG) phospholipids, which are typical of Gram-negative bacteria.¹¹

However, these assays showed that membrane-disruption induced by **Poly-1** and **Poly-3** did reflect their MICs, while **PGON** was completely inactive (Figure 2). In other words, **Poly-1**, which displayed negligible antibacterial activity, caused little dye leakage (16%), while **Poly-3**, with good antibacterial activity, had significant dye release (87%). On the other hand, vesicles exposed to **PGON** were seemingly undisturbed (4% leakage). This lack of membrane disruption activity for **PGON** was unexpected given its high antibacterial efficiency and the fact that its hydrophobicity fell in between the two amine containing polymers.

While dye leakage assays are simple models and quite useful in many studies,^{1b,11} caution should be taken when correlating these results to biological activity. Thus, the effects of these polymers on *S. aureus* were directly observed using a two-component fluorescence stain (Figure 3). This stain uses a green-emitting SYTO9 dye that aids visualization of all cells and red-emitting propidium iodide, which only enters cells with compromised membranes.

From these microscopy studies, **Poly-1** appeared to be inactive (also confirmed by cell viability assays under the same microscopy conditions),⁸ and showed green intact individual *S. aureus* cells (Figure 3A). In contrast, **Poly-3** led to gross aggregation and clear membrane damage inferred from the dramatic increase in red fluorescence intensity (Figure 3D), while under the green filter the cells now appeared more yellow compared to the **Poly-1** or **PGON** trials (compare Figure 3C to 3A and 3E). Cell viability assays confirmed **Poly-3** is lethal under these conditions.⁸ Interestingly, **PGON**, which is significantly bactericidal, as demonstrated by cell viability assays, did not aggregate bacteria nor allow propidium iodide to enter the

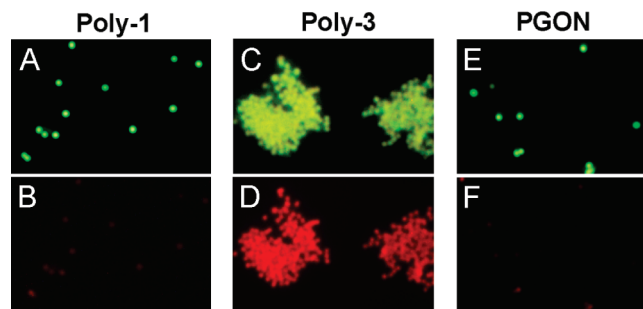


Figure 3. Fluorescence microscopy of *S. aureus* (cells $\sim 1 \mu\text{m}$ in diameter) incubated with a stain to visualize membrane-disruption and polymer for 30 min. Top row shows emission from *S. aureus* using a green filter setting, while the bottom row shows the identical field using a red filter.

cells. These results agreed completely with the dye leakage experiments in which **Poly-1** and **PGON** caused little to no membrane damage relative to **Poly-3**. This apparent ability to transverse the membrane without disruption is also consistent with CPP-like activity.¹⁴ At the same time, studies with a dye-labeled version of **PGON** did support interactions between **PGON** and bacterial cells under the same conditions but due to the small size of bacteria (nominally $1 \mu\text{m}$), it was not possible to see if **PGON** was inside.⁸

Accordingly, these results suggest that **PGON**'s ability to effectively kill bacteria likely occurs via a different mechanism than gross membrane trauma. Likely targets would then be anionic macromolecules such as essential membrane proteins or RNA/DNA. Although no data is yet available on these other targets, CPPs and polyarginine, are well-known to efficiently cross membranes and bind DNA.^{3a,d,12,13} Recently, we demonstrated the ability of these **PGON**'s, with various chain lengths, to traverse membranes as well as other properties reminiscent of CPPs.¹⁴ Therefore, the proposal of intercellular targets is within reason.

Conclusions

There is no doubt that **PGON** is potently antimicrobial without gross membrane disruption. This leads to a lack of RBC lysis and thus improved selectivity compared to AMPs and previous primary amine containing SMAMPs. The recent demonstration that **PGON**-like polymers are effective transduction domains implies intercellular targets are responsible for the antimicrobial properties and lack of mammalian toxicity.^{7,14} Although no data is yet available on these targets, it is clear that these **PGON**s represent an interesting and potentially extremely important new class of molecules. At the same time, highly selective antimicrobial polymers, such as **PGON**, can be extremely valuable in materials designed to prevent Biofilm formation. Targets of opportunity include cardiovascular, orthopedic, and other implants that account for 1 million implant-associated infections and 3 billion U.S. dollars of healthcare costs annually.^{1d}

Acknowledgment. The National Institutes of Health and the Office of Naval Research are acknowledged for their generous support.

Supporting Information Available. Synthetic schemes, instrumentation, and dye-labeled polymer synthesis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) (a) Madkour, A. E.; Tew, G. N. *Polym. Int.* **2008**, *57*, 6–10. (b) Gabriel, G. J.; Som, A.; Madkour, A. E.; Eren, T.; Tew, G. N. *Mater. Sci. Eng., R* **2007**, *57*, 28–64. (c) Klibanov, A. M. *J. Mater. Chem.* **2007**, *17*, 2479–2482. (d) Hetrick, E. M.; Schoenfish, M. H. *Chem. Soc. Rev.* **2006**, *35*, 780–789.
- (2) Ilker, M. F.; Nüsslein, K.; Tew, G. N.; Coughlin, E. B. *J. Am. Chem. Soc.* **2004**, *126*, 15870–15875.
- (3) (a) Miyatake, T.; Nishihara, M.; Matile, S. *J. Am. Chem. Soc.* **2006**, *128*, 12420–12421. (b) Henriques, S. T.; Melo, M. N.; Castanho, M. A. R. B. *Biochem. J.* **2006**, *399*, 1–7. (c) Rothbard, J. B.; Kreider, E.; VanDeusen, C. L.; Wright, L.; Wylie, B. L.; Wender, P. A. *J. Med. Chem.* **2002**, *45*, 3612–3618. (d) Futaki, S.; Suzuki, T.; Ohashi, W.; Yagami, T.; Tanaka, S.; Ueda, K.; Sugiura, Y. *J. Biol. Chem.* **2001**, *276*, 5836–5840. (e) Mitchell, D. J.; Kim, D. T.; Steinman, L.; Fathman, C. G.; Rothbard, J. B. *J. Pept. Res.* **2000**, *56*, 318–325.
- (4) For polymeric examples, see: (a) Sambhy, V.; Peterson, B. R.; Sen, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 1250–1254. (b) Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Eppard, R. F.; Eppard, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 15474–15476. (c) Kuroda, K.; DeGrado, W. F. *J. Am. Chem. Soc.* **2005**, *127*, 4128–4129. (d) Arnt, L.; Nüsslein, K.; Tew, G. N. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 3860–3864. (e) Tew, G. N.; Liu, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P. J.; Klein, M. L.; DeGrado, W. F. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5110–5114.
- (5) For peptidomimetic examples, see (a) Gabriel, G. J.; Tew, G. N. *Org. Biomol. Chem.* **2008**, *6*, 417–423. (b) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 417–428. (c) Tew, G. N.; Clements, D.; Tang, H.; Arnt, L.; Scott, R. W. *Biochim. Biophys. Acta* **2006**, *1758*, 1387–1392. (d) Patch, J. A.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 12092–12093. (e) Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565–565.
- (6) Radziszhevsky, I. S.; Rotem, S.; Bourdetsky, D.; Navon-Venezia, S.; Carmeli, Y.; Mor, A. *Nat. Biotechnol.* **2007**, *25*, 657–659.
- (7) Kolonko, E. M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2008**, *130*, 5626–5627.
- (8) See Supporting Information for details.
- (9) Ge, Y.; MacDonald, D. L.; Holroyd, K. J.; Thornsberry, C.; Wexler, H.; Zasloff, M. *Antimicrob. Agents Chemother.* **1999**, *43*, 782–788.
- (10) Brogden, K. A. *Nat. Rev. Microbiol.* **2005**, *3*, 238–250.
- (11) Som, A.; Tew, G. N. *J. Phys. Chem. B* **2008**, *112*, 3495–3502.
- (12) Sakai, N.; Futaki, S.; Matile, S. *Soft Matter* **2006**, *2*, 636–641.
- (13) (a) Pantos, A.; Tsogas, I.; Paleos, C. M. *Biochim. Biophys. Acta* **2008**, *1778*, 811–823. (b) Schroeder, T.; Niemeier, N.; Afonin, S.; Ulrich, A. S.; Krug, H. F.; Braese, S. *J. Med. Chem.* **2008**, *51*, 376–379. (c) Deglane, G.; Abes, S.; Michel, T.; Prevot, P.; Vives, E.; Debart, F.; Barvik, I.; Lebleu, B.; Vasseur, J.-J. *ChemBioChem* **2006**, *7*, 684–692. (d) Fillon, Y. A.; erson, J. P.; Chmielewski, J. *J. Am. Chem. Soc.* **2005**, *127*, 11798–11803. (e) Funhoff, A. M.; Van Nostrum, C. F.; Lok, M. C.; Fretz, M. M.; Crommelin, D. J. A.; Hennink, W. E. *Bioconjugate Chem.* **2004**, *15*, 1212–1220.
- (14) Hennig, A.; Gabriel, G. J.; Tew, G. N.; Matile, S. *J. Am. Chem. Soc.* **2008**, *130*, 10338–10344.

BM800855T