ACS Macro Letters

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Functionally Diverse Nylon-3 Copolymers from Readily Accessible β-Lactams

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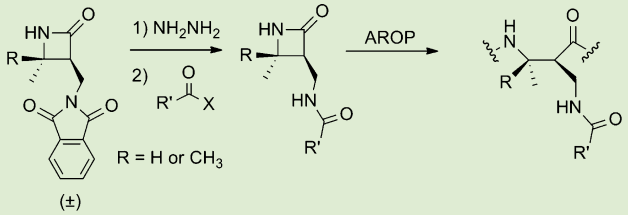
Supporting Information

ABSTRACT: A new family of β-lactams is described that enables anionic ring-opening polymerization (AROP) to prepare nylon-3 materials bearing diverse appended functionality, including carboxylic acid, thiol, hydroxyl, and secondary amine groups. Nylon-3 copolymers generated with the new β-lactams are shown to display distinctive self-assembly behavior and biological properties.



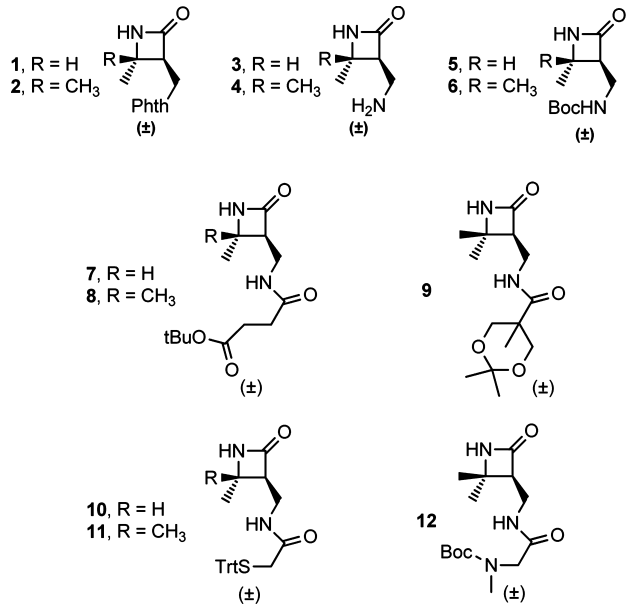
The manifold properties of poly-α-peptides arise from the various functional groups that occur in amino acid side chains.[1] Nylon-3 polymers are poly-β-peptides, and their inherent similarity to proteins at the backbone level raises the possibility that these materials could display interesting biological activities. The exploration of this prospect was initially hindered by limitations in side chain functionality (mostly hydrocarbons in early examples). Nylon-3 chains are generated by anionic ring-opening polymerization (AROP) of β-lactams; however, the most efficient source of β-lactam precursors, cycloaddition of chlorosulfonylisocyanate (CSI) and an alkene, often fails when polar groups are appended to the alkene.[2] Recently we have developed β-lactams (e.g., **1** and **2**) that bear a protected primary amino group in a side chain and enable the preparation of cationic nylon-3 polymers.[3] This synthetic advance allowed us to identify nylon-3 cationic/hydrophobic copolymers that display a variety of interesting biological activities.[4] However, the range of functional groups that can be incorporated into nylon-3 polymers via CSI-derived precursors remains limited. Here we expand the accessible side chain functionality to include carboxylic acids, thiols, hydroxyls, and secondary amines, and we show that the resulting copolymers can display a variety of interesting behaviors.

We sought a concise strategy that would be compatible with diverse side chain groups, so that a variety of β-lactams could be accessed in gram-scale quantities. To this end, we examined the acylation of the primary amino groups of β-lactams **3** and **4**, which are generated via deprotection of **1** and **2** (Chart 1). Previously we had shown that these primary amine groups can be Boc-protected to generate **5** and **6**, which are compatible with the AROP reaction.

In new work, we found that readily available carboxylic acids can be used for the conversion of amines **3** and **4** into β-lactams **7**−**12**, which bear protected carboxylic acid, diol, thiol, or secondary amine groups. These acylations give useful yields of the desired β-lactams in one-pot operations from **1** or **2**.

Each of the new β-lactams proved to be compatible with the AROP conditions (treatment with $LiN[Si(CH_3)_3]_2$ and an acylating agent, the latter for in situ co-initiator generation[3b]),

Chart 1. Structures of β-Lactams 1−12



but their reactivities were variable. The side-chain protecting groups could be removed under acidic conditions after polymerization. Access to these unique building blocks enabled us to explore the impact of side chain functionality on nylon-3 copolymer properties.

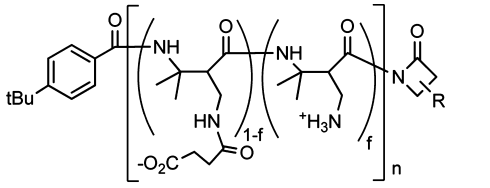
β-Lactam **8** homo- or copolymerizes smoothly in tetrahydrofuran, and the initial products can be converted to water-soluble polyanions after the *tert*-butyl ester groups are removed (see SI, pp S21−23). Previously, nylon-3 polymers with side-chain carboxylic acid groups were generated from the polymerization of glutamate-derived *N*-carboxyanhydrides,[5] but thorough

Received: April 10, 2012
Accepted: May 15, 2012
Published: May 23, 2012

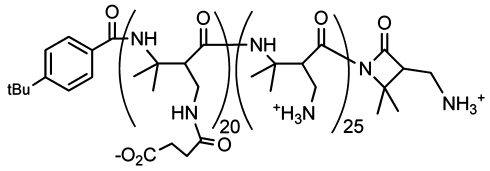
characterization of these materials was impeded by their limited solubility prior to deprotection.

Copolymerization of β-lactam **8** with **5** or **6** leads, after deprotection, to zwitterionic nylon-3 materials (Chart 2).[6]

Chart 2. Structures of P8-*r*-6 and P8-*b*-6



P8-*r*-6 (heterochiral)
Random copolymer



P8-*b*-6 (heterochiral)
Block copolymer

These random copolymers (P8-*r*-6) (M_n = 3000−10 000, M_w/M_n = 1.1−1.2 for the protected polymers) are soluble in aqueous buffers from pH = 1 to pH = 14. Different behavior was observed for a diblock copolymer formed by **8** and **6**. A protected precursor to P8-*b*-6 (M_n = 10 400, M_w/M_n = 1.14), containing a block of ca. 20 units derived from **8** and a block of ca. 25 units derived from **6**, was synthesized through a previously described approach.[3b] Treatment of the protected copolymer with trifluoroacetic acid generated the corresponding zwitterionic diblock copolymer. Polymer P8-*b*-6 is fully soluble in aqueous buffer solution at low pH or at high pH, but solutions near neutral pH are turbid (Figure S1, SI).

We explored the self-assembly behavior of acid−base block copolymer P8-*b*-6. In one experiment, P8-*b*-6 was dissolved in pH 12 buffer, a condition that should cause the side-chain carboxyl groups derived from **8** to be completely ionized and the side-chain amino groups derived from **6** to be uncharged and potentially hydrophobic. This polymer solution was incubated with both a hydrophobic fluorescent dye, Nile red (NR), and a hydrophilic fluorescent dye, 5(6)-carboxyfluorescein (CF), and then dried on a glass slide. Fluorescence microscopy revealed the formation of microspheres (Figure 1). These assemblies could be observed in both the red channel (NR visualized; Figure 1a) and the green channel (CF visualized; Figure 1b). The overlay of the two images (Figure 1c,d) shows that yellow regions (due to combination of red and green colors) are located in the center of each microsphere, while the periphery is green. This observation suggests that the more hydrophobic block (from **6**) may reside closer to the center and the more hydrophilic block (from **8**) may reside closer to the surface. Considering the relatively short chain length of P8-*b*-6 (ca. 45 β-amino acid units), we speculate that these rather large microspheres might result from a hollow vesicle-type structure (see Figure S2, SI). A clearer understanding of the underlying assembly process will require further study. Implementing a comparable procedure with a pH 2 solution polymer P8-*b*-6 produced large irregular structures rather than the regular microspheres seen at pH 12.

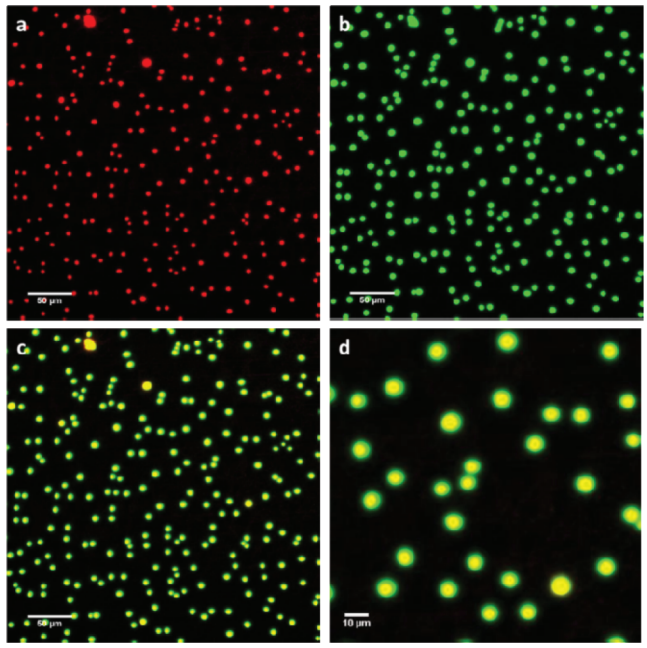
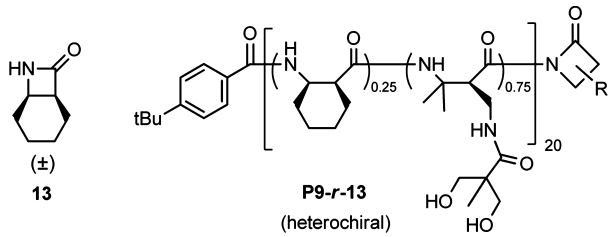


Figure 1. Fluorescent microscopic (nonconfocal) images of assemblies formed by polymer P8-*b*-6 at pH 12 (1 mg/mL) after staining with NR and CF followed by drying on a glass surface (see text for details). (a) Assemblies viewed in the red channel (NR). (b) Assemblies viewed in the green channel (CF). (c) Overlay of images from a and b. (d) Overlay at higher magnification.

The new β-lactams described here allowed us to evaluate the use of polar but uncharged side chains to confer water solubility. Nonionic amphiphilic polymers have recently been shown to display useful solubilizing behavior with intrinsic membrane proteins.[7] β-Lactam **9** leads, after polymerization and deprotection, to a subunit with two hydroxyl groups in each side chain; this building block is considerably easier to prepare than is a β-lactam we have previously described that generates a different type of dihydroxyl subunit.[3a] After removal of the cyclic acetal protecting group, a nylon-3 random copolymer (P9-*r*-13) (Chart 3; M_n = 5800, M_w/M_n = 1.07 for the protected polymer)

Chart 3. Structures of β-Lactam 13 and Polymer P9-*r*-13



P9-*r*-13
(heterochiral)

generated from **9** and cyclohexyl β-lactam **13** was readily soluble in water regardless of the pH.

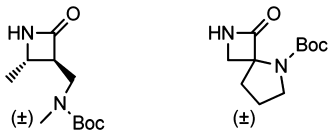
Nylon-3 polymers containing side-chain thiol groups could be used for postpolymerization modification or for polymer chain cross-linking.[8] Previously we have been able to place a single thiol group at one terminus of a nylon-3 chain,[4d,9] and we anticipated that β-lactam **10** or **11** would enable us to incorporate multiple thiol groups along the polymeric backbone. These β-lactams displayed little tendency toward homopolymerization, perhaps because of the bulky trityl protecting group. However, in small proportions each could be copolymerized with a more reactive

dx.doi.org/10.1021/mz300172y | *ACS Macro Lett.* 2012, 1, 714−717

β-lactam (e.g., **5**) to yield nylon-3 random copolymers containing up to 20% of subunits bearing a side-chain thiol (see SI, p S26). This level of incorporation is sufficient for many conjugation or cross-linking applications.

β-Lactam **12** is a precursor to nylon-3 materials bearing secondary amine groups in side chains. Our previous work has revealed a variety of potentially valuable biological activities for cationic nylon-3 copolymers.[4] To date these efforts have relied exclusively on examples containing primary amino groups (derived from **1** and **2**), but recent reports involving other polymer classes suggest that the degree of nitrogen substitution can influence biological activities of polycations.[10] It is therefore important to compare the properties of nylon-3 copolymers bearing primary versus secondary amino groups. The polymers generated from β-lactams **5** and **12** are not perfectly analogous; therefore, we prepared β-lactam **14** from Boc-protected β-lactam **5** (Chart 4). To broaden the comparison of nylon-3 polyamines,

Chart 4. Structures of β-Lactams 14 and 15



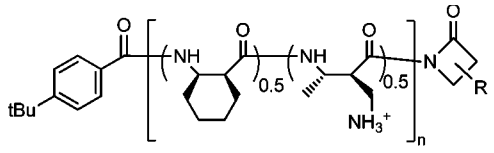
we prepared spiro-β-lactam **15** in two steps from a known precursor.[11] β-Lactams **14** and **15** readily participated in ROP to generate nylon-3 homo- or copolymers (see SI, pp S27−29).

Several groups, including ours, have shown that synthetic cationic polymers can function as antibacterial agents.[4a,c,9,12] We used new β-lactams introduced above to explore the impact of the structure of the cationic subunit on the antibacterial activity of nylon-3 copolymers.

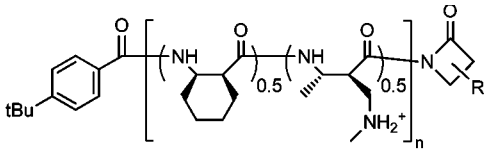
Four hydrophobic−cationic random copolymers were prepared from cyclohexyl β-lactam **13** and one of four amine-bearing β-lactams, **5**, **14**, **12**, or **15**, respectively (Table 1). Each β-lactam pair was copolymerized in 1:1 molar ratio; each resulting copolymer was water-soluble after side-chain deprotection. These polycations were compared in terms of their growth-inhibitory activity toward a panel of bacteria, including species that are Gram negative (*Escherichia coli*[13]) and Gram positive (*Bacillus subtilis*,[14] *Enterococcus faecium*,[15] and *Staphylococcus aureus*[16]). In addition, these copolymers were evaluated for their ability to lyse human red blood cells ("hemolysis"). The antibacterial effects of cationic peptides and polymers are thought to arise from disruption of bacterial membranes, and the most desirable agents are selective toward prokaryotic cells relative to eukaryotic cells (as manifested by potent inhibition of bacterial growth but low hemolytic activity).

Table 1 shows that these nylon-3 copolymers display remarkable differences in biological activity profiles, despite their chemical similarities. As previously reported,[4a] copolymer P13-*r*-5 is very active against all four bacteria (low values for minimum inhibitory concentration (MIC)) but also strongly hemolytic (low value for minimum hemolytic concentration (MHC)). The addition of a methyl group to the side chain amines (P13-*r*-14) leads to a polycation that manifests pronounced selectivity among bacteria (activity retained for *B. subtilis* and *E. faecium* but lost for *E. coli* and *S. aureus*) and that displays diminished hemolytic activity relative to P13-*r*-5. P13-*r*-12, which presents a secondary amine on an extended side chain, displays a different pattern of bacterial selectivity relative to P13-*r*-14, and P13-*r*-12 displays very weak hemolytic activity. Copolymer P13-*r*-15 is completely inactive against the bacteria and very weakly hemolytic. The large difference in activity profile between P13-*r*-5 and P13-*r*-14 demonstrates that switching from primary to secondary ammonium can strongly influence biological effects, and the differences among P13-*r*-14, P13-*r*-12, and P13-*r*-15 reveal unanticipated ways in which altering cationic subunit structure while maintaining a secondary ammonium group can cause large changes in activity.

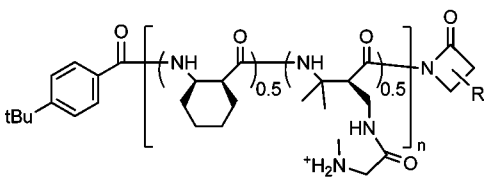
Table 1. Comparison of Biological Activities of Nylon-3 Polymers Containing Charged Primary Amine and Secondary Amine Groups[a]



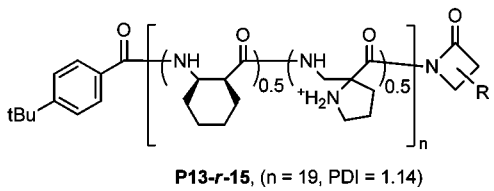
P13-*r*-5, (n = 18, PDI = 1.10)



P13-*r*-14, (n = 20, PDI = 1.06)



P13-*r*-12, (n = 25, PDI = 1.06)



P13-*r*-15, (n = 19, PDI = 1.14)

	MIC[b] (μg/mL)				
polymer	*E. coli*	*B. subtilis*	*S. aureus*	*E. faecium*	MHC[c] (μg/mL)
P13-*r*-5	6.25[d]	1.56[d]	12.5[d]	6.25[d]	12.5[d]
P13-*r*-14	100	3.13	400	6.25	100
P13-*r*-12	25	6.25	>400	200	1600
P13-*r*-15	>400	>400	>400	>400	800

[a]All polymers heterochiral, R = appropriate side chain from either monomer. [b]MIC = minimum inhibitory concentration. [c]MHC = minimum hemolytic concentration. [d]Data from ref 4a.

dx.doi.org/10.1021/mz300172y | *ACS Macro Lett.* 2012, 1, 714−717

Overall, we have shown that a class of easily prepared β-lactams expands the set of peripheral functional groups available for incorporation into nylon-3 materials. The preliminary assessments reported here reveal that newly available nylon-3 copolymers can display distinctive physical or biological properties. Previous work[4] has demonstrated several biological functions for nylon-3 copolymers generated from a relatively small set of β-lactams, and the new β-lactams reported here should prove useful for optimizing established activities and achieving new behaviors.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Nanoscale Science and Engineering Center (NSEC) at UW Madison (DMR-0832760). S.H.G. acknowledges partial support from NIH Grant GM-093265. We thank Saswata Chakraborty for assistance with NMR analysis.

■ REFERENCES

(1) (a) Deming, T. J. *Adv. Mater.* **1997**, *9*, 299−311. (b) Kricheldorf, H. R. *Angew. Chem., Int. Ed.* **2006**, *45*, 5752−5784. (c) Ulijn, R. V.; Smith, A. M. *Chem. Soc. Rev.* **2008**, *37*, 664−675.

(2) (a) Graf, R.; Lohaus, G.; Börner, K.; Schmidt, E.; Bestian, H. *Angew. Chem., Int. Ed.* **1962**, *1*, 481−488. (b) Bestian, H. *Angew. Chem., Int. Ed.* **1968**, *7*, 278−285. (c) Hashimoto, K. *Prog. Polym. Sci.* **2000**, *25*, 1411−1462.

(3) (a) Lee, M. R.; Stahl, S. S.; Gellman, S. H. *Org. Lett.* **2008**, *10*, 5317−5319. (b) Zhang, J.; Kissounko, D. A.; Lee, S. E.; Gellman, S. H.; Stahl, S. S. *J. Am. Chem. Soc.* **2009**, *131*, 1589−1597.

(4) (a) Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Epand, R. F.; Epand, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 15474−15475. (b) Epand, R. F.; Mowery, B. P.; Lee, S. E.; Stahl, S. S.; Lehrer, R. I.; Gellman, S. H.; Epand, R. M. *J. Mol. Biol.* **2008**, *379*, 38−50. (c) Mowery, B. P.; Lindner, A. H.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2009**, *131*, 9735−9745. (d) Lee, M. R.; Stahl, S. S.; Gellman, S. H.; Masters, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 16779−16789. (e) Dohm, M. T.; Mowery, B. P.; Czyzewski, A. M.; Stahl, S. S.; Gellman, S. H.; Barron, A. E. *J. Am. Chem. Soc.* **2010**, *132*, 7957−7967.

(5) Cheng, J. J.; Deming, T. J. *Macromolecules* **2001**, *34*, 5169−5174.

(6) For examples of zwitterionic copolymers, see: (a) Butun, V.; Liu, S.; Weaver, J. V. M.; Bories-Azeau, X.; Cai, Y.; Armes, S. P. *React. Funct. Polym.* **2006**, *66*, 157−165. (b) Rodriguez-Hernandez, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026−202. (c) Li, J. G.; Wang, T.; Wu, D. L.; Zhang, X. Q.; Yan, J. T.; Du, S.; Guo, Y. F.; Wang, J. T.; Zhang, A. *Biomacromolecules* **2008**, *9*, 2670−2676.

(7) (a) Prata, C.; Giusti, F.; Gohon, Y.; Pucci, B.; Popot, J. L.; Tribet, C. *Biopolymers* **2001**, *56*, 77−84. (b) Sharma, K. S.; Durand, G.; Giusti, F.; Olivier, B.; Fabiano, A. S.; Bazzacco, P.; Dahmane, T.; Ebel, C.; Popot, J. L.; Pucci, B. *Langmuir* **2008**, *24*, 13581−13590. (c) Bazzacco, P.; Sharma, K. S.; Durand, G.; Giusti, F.; Ebel, C.; Popot, J. L.; Pucci, B. *Biomacromolecules* **2009**, *10*, 3317−3326.

(8) (a) Gauthier, M. A.; Klok, H. A. *Chem. Commun.* **2008**, 2591−2611. (b) Hoyle, C. E.; Lowe, A. B.; Bowman, C. N. *Chem. Soc. Rev.* **2010**, *39*, 1355−1387.

(9) Zhang, J.; Markiewicz, M. J.; Mowery, B. P.; Weisblurn, B.; Stahl, S. S.; Gellman, S. H. *Biomacromolecules* **2012**, *13*, 323−331.

(10) (a) Palermo, E. F.; Lee, D. K.; Ramamoorthy, A.; Kuroda, K. *J. Phys. Chem. B* **2011**, *115*, 366−375. (b) Palermo, E. F.; Kuroda, K. *Biomacromolecules* **2009**, *10*, 1416−1428. (c) Timofeeva, L. M.; Kleshcheva, N. A.; Moroz, A. F.; Didenko, L. V. *Biomacromolecules* **2009**, *10*, 2976−2986.

(11) (a) Alonso, E.; Lopez-Ortiz, F.; del Pozo, C.; Peralta, E.; Macias, A.; Gonzalez, J. *J. Org. Chem.* **2001**, *66*, 6333−6338. (b) Macias, A.; Ramallal, A. M.; Alonso, E.; del Pozo, C.; Gonzalez, J. *J. Org. Chem.* **2006**, *71*, 7721−7730.

(12) For leading references on other antibacterial polymers, see: (a) Ikeda, T.; Tazuke, S.; Suzuki, Y. *Makromol. Chem.* **1984**, *185*, 869−876. (b) Kawabata, N.; Nishiguchi, M. *Appl. Environ. Microbiol.* **1988**, *54*, 2532−2535. (c) Tiller, J. C.; Liao, C.-J.; Lewis, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5981−5985. (d) 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. (e) Gelman, M. A.; Weisblum, B.; Lynn, D. M.; Gellman, S. H. *Org. Lett.* **2004**, *6*, 557−560. (f) Ilker, M. F.; Nusslein, K.; Tew, G. N.; Coughlin, E. B. *J. Am. Chem. Soc.* **2004**, *126*, 15870−15875. (g) Kuroda, K.; DeGrado, W. F. *J. Am. Chem. Soc.* **2005**, *127*, 4128−4129. (h) Palermo, E. F.; Sovadinova, E.; Kuroda, K. *Biomacromolecules* **2009**, *10*, 3098−3107. (i) Tew, G. N.; Scott, R. W.; Klein, M. L.; DeGrado, W. F. *Acc. Chem. Res.* **2010**, *43*, 30−39.

(13) Yanisch-Perron, C.; Vieira, J.; Messing, J. *Gene* **1985**, *33*, 103−119.

(14) Young, F. E.; Smith, C.; Reilly, B. E. *J. Bacteriol.* **1969**, *98*, 1087−1097.

(15) Weisblum, B.; Demohn, V. *J. Bacteriol.* **1969**, *98*, 447−452.

(16) Nicas, T. I.; Wu, C. Y. E.; Hobbs, J. N.; Preston, D. A.; Allen, N. E. *Antimicrob. Agents Chemother.* **1989**, *33*, 1121−1124.