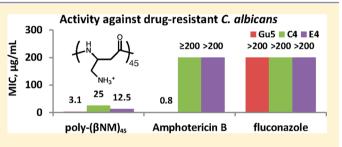# Structure−Activity Relationships among Antifungal Nylon-3 Polymers: Identification of Materials Active against Drug-Resistant Strains of *Candida albicans*

Runhui Liu,[†,‡] Xinyu Chen,[†] Shaun P. Falk,[§] Brendan P. Mowery,[†] Amy J. Karlsson,[∥,⊥] Bernard Weisblum,[§] Sean P. Palecek,[∥] Kristyn S. Masters,*[,‡] and Samuel H. Gellman*[,†]

[†]Department of Chemistry, [‡]Department of Biomedical Engineering, [§]Department of Medicine, and [∥]Department of Chemical and Biological Engineering, University of Wisconsin, Madison, Wisconsin 53706, United States

Ⓢ *Supporting Information*

ABSTRACT: Fungal infections are a major challenge to human health that is heightened by pathogen resistance to current therapeutic agents. Previously, we were inspired by host-defense peptides to develop nylon-3 polymers (poly-β-peptides) that are toxic toward the fungal pathogen *Candida albicans* but exert little effect on mammalian cells. Based on subsequent analysis of structure−activity relationships among antifungal nylon-3 polymers, we have now identified readily prepared cationic homopolymers active against strains of *C. albicans* that are resistant to the antifungal drugs fluconazole and amphotericin B. These nylon-3 polymers are nonhemolytic. In addition, we have identified cationic−hydrophobic copolymers that are highly active against a second fungal pathogen, *Cryptococcus neoformans*, and moderately active against a third pathogen, *Aspergillus fumigatus*.

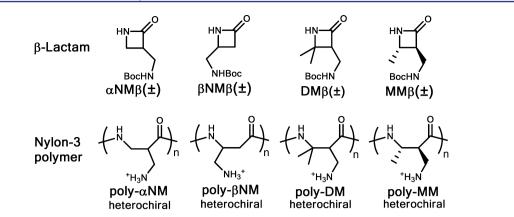Activity against drug-resistant *C. albicans*

INTRODUCTION

Fungal infections are major threats to human health and difficult to treat.[1] Systemic fungal infections are especially dangerous because they are associated with a high mortality rate, which reflects the low efficacy and multiple side effects of current antifungal drugs as well as the emergence of strains resistant to current therapeutic agents.[1] Pathogen resistance to fluconazole is widespread,[2−4] and resistance to amphotericin B (AmpB) is on the rise.[2,3,5] Identifying agents that specifically inhibit fungal growth without deleterious effects on patients is challenging because both fungi and humans are eukaryotes.[1] The discovery of host-defense peptides (HDPs) in the 1980s sparked extensive exploration of these natural antimicrobial agents and synthetic analogues for potential therapeutic applications.[6−9] Many HDP analogues that display antibacterial activity have been documented.[7,10] In contrast, relatively few studies have characterized the effects of HDPs and their analogues on fungi.[11] The antifungal activities reported are very sensitive to conditions, and some assays may not be biologically relevant.[12,13]
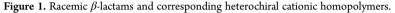
The use of conventional peptides and other sequence-specific oligomers (e.g., D-peptides,[14,15] peptoids,[16,17] β-peptides,[18,19] α/β-peptides[20]) as antimicrobial agents is problematic because production of these types of compounds is extremely costly. This limitation has led many groups to examine synthetic polymers as antimicrobial agents in recent years.[21] Such efforts have been motivated by the observation that diverse natural HDP sequences display similar growth-inhibitory activities toward bacteria, which suggests that the crucial feature may be simply an appropriate balance of hydrophobic and cationic side chains.[22]

Over the past decade, a variety of synthetic polymers have been reported to display prokaryote-specific toxicity.[21,23−35] Selectivity has typically been evaluated by assessing a polymer's ability to lyse human red blood cells ("hemolysis"); antibacterial activity is often thought to depend on disruption of bacterial membranes.[36,37] The ideal profile is low minimum inhibitory concentration (MIC) for bacteria but high minimum hemolytic concentration (MHC) or concentration for 50% hemolysis (HC$_{50}$). In contrast to the numerous examples of antibacterial polymers, there have been few reports of synthetic polymers with antifungal activity. Chan-Park et al. recently described cationic peptidopolysaccharides with good activity against a drug-susceptible strain of *Candida albicans* (MIC = 20 μg/mL) and little or no hemolytic activity.[33] Earlier examples feature nonphysiological assay conditions and/or low activities, and they lack information regarding hemolysis or other measures of toxicity toward mammalian cells.[38,39]

Nylon-3 materials were among the first synthetic polymers reported to display an HDP-mimetic activity profile (substantial growth inhibition for diverse bacterial species and low hemolytic activity).[27] Although many earlier studies had identified synthetic polymers with antibacterial activity,[40−48] such materials were typically not evaluated in terms of toxic effects on mammalian cells (e.g., hemolysis).[21] We were attracted to the nylon-3 polymer class for biological applications because the protein-like backbone, comprised of β-amino acid residues, seemed likely to

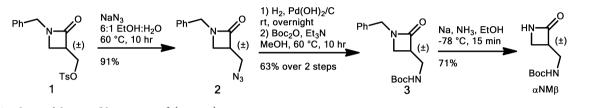**Figure 1.** Racemic β-lactams and corresponding heterochiral cationic homopolymers.

Figure 2. Synthesis of the new β-lactam **αNMβ** (racemic).

confer biocompatibility.[29,49−57] The identity of hydrophobic and cationic subunits can be readily varied among nylon-3 copolymers, which facilitates the optimization of a particular activity profile.[27,28] However, our initial efforts to identify nylon-3 polymers with selective toxicity toward the fungal pathogen *C. albicans* were unsuccessful; antifungal polymers were always found to be quite hemolytic (unpublished).

Recently, we evaluated a new family of nylon-3 polymers that contain the **βNM** subunit (Figure 1).[29] To our surprise, poly-**βNM** with ~20-mer average length displayed strong and selective activity against *C. albicans* strain K1.[58] This homopolymer was fungicidal at the MIC (3.1 μg/mL), but very little hemolysis or toxicity toward 3T3 fibroblasts was detected even at 400 μg/mL. In contrast, the cationic homopolymer poly-**MM** was only weakly active against *C. albicans* K1 (MIC = 200 μg/mL). Cationic poly-**DM** was highly active against *C. albicans* (MIC = 6.3 μg/mL) but also highly hemolytic and moderately toxic to 3T3 fibroblasts.[29]

Favorable preliminary observations regarding the antifungal properties of poly-**βNM** prompted us to undertake the additional studies reported here. We have made comparisons among diverse nylon-3 materials, and the results have elucidated the polymer parameters that are crucial for achieving selective antifungal activity; this information will support future efforts to optimize materials for specific applications. We have identified nylon-3 polymers with substantial activity against pathogenic strains of *C. albicans* that are resistant to AmpB and fluconazole. In addition, we have found nylon-3 polymers that are active against two other pathogenic fungi that commonly cause invasive human infections, *Cryptococcus neoformans* and *Aspergillus fumigatus*.[1]

■ RESULTS AND DISCUSSION

Location of the Aminomethyl Side Chain Is Unimportant: Poly-αNM vs Poly-βNM. The nylon-3 subunit designated **βNM** in Figure 1 (previously designated "**NM**"[29]) differs in two ways from the **MM** and **DM** subunits. First, **MM** and **DM** both have methyl substituents, while **βNM** does not. Second, the aminomethyl side chain is located on Cα for **MM** and **DM** but

on Cβ for **βNM**. In order to determine whether the position of the aminomethyl side chain is important in terms of biological activity profile, we have now prepared poly-**αNM**. Nylon-3 materials are synthesized via anionic ring-opening polymerization (AROP) of β-lactams;[59,60] synthesis of a precursor for poly-**αNM**, the β-lactam **αNMβ**, is outlined in Figure 2. Racemic β-lactam **1** was prepared according to a reported protocol[61] and then converted to **2** via tosylate displacement. Reduction of the azide to an amino group and Boc protection provided **3**, which was converted to **αNMβ** via reductive debenzylation. AROP of this new β-lactam proceeded smoothly under conditions previously described (Figure 3).[29,60] An acid chloride serves as a co-initiator by forming an *N*-acyl imide in situ; the acyl group remains at the N-terminus of the resulting polymer chains. Two acyl groups were examined in this work, *p-t*-butylbenzoyl (tBuBz) and acetyl (Ac). The side-chain-protected polymer displayed low polydispersity (Table 1). Deprotection under acidic conditions provided poly-**αNM**.

Poly-**αNM** is indistinguishable from previously described poly-**βNM** in terms of biological activity profile, which indicates that the location of the aminomethyl side chain is not functionally significant (Table 1; *p-t*-butylbenzoyl group at the N-termini in both cases). Both nylon-3 polymers are fungicidal at the MIC. Both are substantially more active than α-poly-L-lysine, magainin 2 (an HDP from amphibians), or fluconazole (an antifungal drug). Both nylon-3 polymers are slightly less active than AmpB against *C. albicans* K1; however, both polymers are superior to AmpB in terms of selectivity, based on comparisons of the lowest concentrations required to cause 10% lysis in a sample of human red blood cells (HC_{10}). We define the selectivity index (SI) as the ratio HC_{10}/MIC, with higher values corresponding to greater selectivity for fungal cell destruction. SI > 130 for poly-**αNM** and poly-**βNM**, while SI = 1 for AmpB. Because these two nylon-3 polymers have indistinguishable activities, and because the synthesis of **αNMβ** is considerably more time consuming than the synthesis of **βNMβ**, all subsequent studies focused on poly-**βNM**.
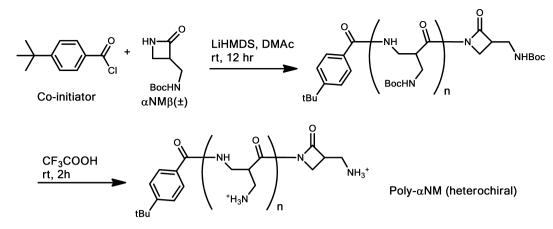
Figure 3. Synthesis of heterochiral poly-**αNM**.

Table 1. Biological Activity Comparison of Poly-αNM, Poly-βNM, and Related Materials[a]

polymer	DP[b]	PDI[c]	MIC[d] (μg/mL)	MFC[e] (μg/mL)	HC$_{10}$[f] (μg/mL)	SI[g]
poly-**αNM**	16	1.4	3.1	3.1	>400	>130
poly-**βNM**[h]	20	1.1	3.1	3.1	>400	>130
α-poly-L-lysine	27−103	ND	>200	ND	ND	ND
magainin 2	NA	NA	>100	ND	ND	ND
fluconazole	NA	NA	>200	ND	ND	ND
AmpB	NA	NA	0.8[h]	0.8[h]	0.8	1

[a]NA means not applicable. ND means not determined. [b]Average degree of polymerization; both nylon-3 homopolymers have a *p-t*-butylbenzoyl at the N-terminus. [c]Polydispersity index. [d]Minimum inhibitory concentration for *C. albicans* (K1 strain) growth in planktonic form. [e]Minimum fungicidal concentration, defined as >99.9% killing of *C. albicans* (K1 strain) cells. [f]Concentration necessary for 10% lysis of human red blood cells. [g]Selectivity index (SI) calculated from HC$_{10}$/MIC. [h]All data for poly-**βNM** and antifungal data for AmpB were reported previously.[29]

Table 2. N-Terminal Group and Polymer Length Effects on the Antifungal and Hemolytic Activities of Poly-βNM[a]

N-terminal group	MIC[b] (μg/mL)							HC$_{10}$ (μg/mL)
	4-mer[c]	6-mer[c]	8-mer[c]	20-mer[c]	45-mer[d]	70-mer[d]	104-mer[d]	
t-BuBz	12.5	6.3	6.3	3.1	3.1	3.1	3.1	>400 for all polymers
Ac	50	12.5	6.3	3.1	3.1	3.1	ND	≥400 for all polymers

[a]The K1 strain of *C. albicans* was tested in planktonic form. [b]Minimum inhibitory concentration for *C. albicans* (K1 strain) growth in planktonic form. The MIC value was also the MFC value. [c]The polymer length was determined by both GPC and proton NMR. [d]The polymer length was determined by proton NMR only because of low solubility in the GPC mobile phase. ND indicates the MIC and HC$_{10}$ values were not determined.

Drug-Susceptible vs Drug-Resistant Strains of *C. albicans*: Importance of Poly-βNM Chain Length. The impact of average chain length on the activity of poly-**βNM** against *C. albicans* K1 was assessed for two polymer series, one bearing *p-t*-butylbenzoyl groups and the other bearing acetyl groups at the N-termini (Table 2). For the *p-t*-butylbenzoyl series, very short chains, with averages in the 4-mer to 8-mer range, are slightly less active than chains with average lengths of 20-mer or higher, up to a 104-mer average. A similar trend is seen in the acetyl series. In all cases, the polymer samples displayed little or no hemolytic activity. The identity of the N-terminal group, *p-t*-butylbenzoyl vs acetyl, influenced antifungal activity only among the very shortest oligomers, for which *p-t*-butylbenzoyl seemed to be slightly more favorable.

The K1 strain of *C. albicans* is highly susceptible to antifungal drug AmpB but less susceptible to fluconazole (Table 1). K1 appears to be resistant to fluconazole when evaluated in terms of the MIC, the lowest concentration that allows no detectable *C. albicans* growth; however, this drug is seen to display modest activity if MIC$_{50}$ (the concentration that inhibits growth by 50%) is considered instead. MIC$_{50}$ is widely used as a measure of

antifungal activity; we find that MIC$_{50}$ = 3.1 μg/mL for fluconazole against the K1 strain (Table 3).

We broadened our evaluation of polymer activity to *C. albicans* strains other than K1, focusing on strains that are resistant to standard drugs used clinically for human infections. The Gu5 strain is resistant to fluconazole,[62] and the C4 and E4 strains are resistant to both fluconazole and AmpB.[63] Table 3 compares activities against all four *C. albicans* strains for the poly-**βNM** series with varying average chain length and *p-t*-butylbenzoyl at the N-terminus. The pattern manifested for the Gu5 strain is nearly identical to that for the K1 strain. Poly-**βNM** appears to be less active against the C4 and E4 strains; however, significant activity is observed when the polymer chains are sufficiently long (maximal effect against C4 at 45-mer average length and against E4 at 20-mer average length). Poly-**βNM** is fungicidal at the MIC in all cases. The activity against *C. albicans* C4 and E4 is noteworthy because neither of the clinical drugs, fluconazole or AmpB, displays activity against these strains. The Gu5 strain is resistant to fluconazole because of overexpression of the CDR1 and CDR2 genes, which encode the transmembrane drug transporter proteins Cdr1p and Cdr2p.[62] The C4 and E4 strains are resistant to both fluconazole and AmpB because their

Table 3. Antifungal Activities of Nylon-3 Polymers toward Drug-Resistant Strains of *C. albicans*

compd	MIC^a (μg/mL)			
	K1	Gu5	C4	E4
fluconazole	>200	>200	>200	>200
fluconazole	MIC$_{50}$ = 3.1^b	MIC$_{50}$ > 200^b	MIC$_{50}$ > 200^b	MIC$_{50}$ > 200^b
AmpB	0.8	0.8	≥200	>200
tBuBz-(βNM)$_4$	12.5	12.5	>200	>200
tBuBz-(βNM)$_6$	6.3	6.3	200	100
tBuBz-(βNM)$_8$	6.3	6.3	200	50
tBuBz-(βNM)$_{20}$	3.1	6.3	50	12.5
tBuBz-(βNM)$_{45}$	3.1	3.1	25	12.5
tBuBz-(βNM)$_{70}$	3.1	3.1	25	12.5
tBuBz-(βNM)$_{105}$	3.1	3.1	25	12.5

^{a}Minimum inhibitory concentration for *C. albicans* growth in planktonic form. The MIC values were also the MFC values. ^{b}The concentration to inhibit 50% *C. albicans* growth in planktonic form.
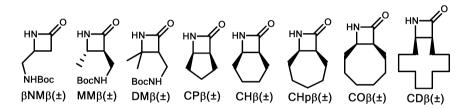

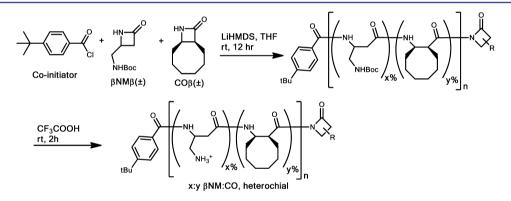**Figure 4.** Racemic β-lactams used to prepare nylon-3 polymers in this study.

Figure 5. Representative synthesis of βNM:CO nylon-3 copolymers. R could be the side chain of either subunit. All other nylon-3 copolymers in this study were prepared in a similar manner using either THF or DMAc as the solvent.

membranes have reduced ergosterol content relative to other *C. albicans* strains.[63] The high activity of poly-βNM against the Gu5 strain suggests that transmembrane drug transporter proteins Cdr1p and Cdr2p cannot effectively counter the antifungal effects of the nylon-3 polymers. The somewhat reduced activity of longer poly-βNM toward the C4 and E4 strains of *C. albicans*, relative to the K1 strain, suggests that ergosterol may play a role in the nylon-3 mode of antifungal activity. However, since the longer forms of poly-βNM manifest very significant activity against the C4 and E4 strains, we conclude that ergosterol is either not the direct target or not the only direct target of these polymers.

Cationic−Hydrophobic Nylon-3 Copolymers. Previously, we conducted an extensive survey of binary nylon-3 copolymers containing both cationic and hydrophobic subunits in an effort to identify the hydrophilic−lipophilic balance that is optimal for inhibition of bacterial growth without harm to eukaryotic cell membranes.[27,28] Two cationic subunits were considered, those derived from **MMβ** and **DMβ** (Figure 4). This study revealed that the identities of the cationic and hydrophobic subunits and their proportion were critical determinants of

antibacterial and hemolytic activities. We have now conducted a comparable study of the antifungal effects of binary nylon-3 copolymers containing βNM and varying hydrophobic subunits, including cyclopentyl (**CP**), cyclohexyl (**CH**), cycloheptyl (**CHp**), cyclooctyl (**CO**), and cyclododecyl (**CD**) (Figures 5 and 6). The impact of copolymer composition and proportion on MIC for *C. albicans* K1 and on hemolysis, as indicated by HC$_{10}$, are summarized in Figure 7A. For comparison, data for two binary copolymer series involving the **MM** cationic subunit are shown in Figure 7B and data for two binary copolymer series involving the **DM** cationic subunit are shown in Figure 7C.

Data for each of the five copolymer series containing the βNM cationic subunit are depicted in separate plots in Figure 7A. The leftmost data points, for the βNM homopolymer, are identical in all five plots. Tracking the hemolytic data across the five plots reveals a trend consistent with previous work: increasing hydrophobic content leads to polymers with greater hemolytic activity (lower HC$_{10}$). For the least hydrophobic subunit, **CP**, none of the binary copolymers display significant hemolytic activity. For the more hydrophobic **CH** subunit, very modest
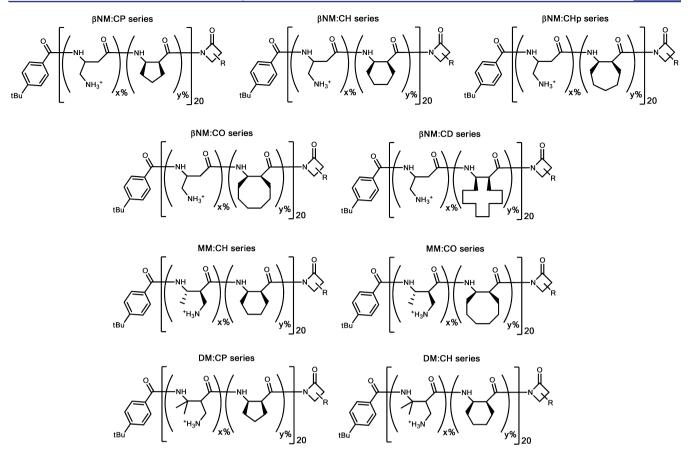
Figure 6. Nylon-3 polymers used for structure−activity relationship studies. All polymers were heterochiral and prepared at an average length of 20-mer. The **MM:CHp** and **MM:CO** copolymers had similar antifungal activity profiles; we provide data for only **MM:CO** copolymers. The **MM:CP** and **MM:CH** copolymers displayed low antifungal activity; we provide data for only **MM:CH** copolymers. Most of the **MM:CD**, **DM:CO**, and **DM:CD** copolymers had limited solubility in water and displayed high hemolytic activity; these polymers were not studied carefully.

hemolytic activity is detected only when the **CH** content reaches 50%. Increasing subunit hydrophobicity, with **CHp** or **CO**, leads to substantial hemolytic activity for ≥20% hydrophobic subunit content. For the most hydrophobic subunit, **CD**, considerable hemolytic activity is evident even at 10% content.

The trend in antifungal activities among the **βNM**-containing copolymers requires a more complex interpretation than the hydrophobicity-based explanation provided for hemolytic activity trends in the preceding paragraph. Specifically, the data in Figure 7A suggest that the **βNM** subunit manifests intrinsic antifungal activity in nylon-3 chains, and that a sufficiently hydrophobic nylon-3 subunit can compensate, at least partially, for the removal of **βNM** subunits. The data for the **βNM**-CP copolymer series supports the hypothesis that the **βNM** subunit is intrinsically antifungal because introduction of only 10% **CP** causes a substantial decline in antifungal activity (increase in MIC). Further decline is observed as the **CP** proportion grows. A comparable but smaller effect is observed in the **βNM**-CH series, where inclusion of ≥30% **CH** leads to a decline in antifungal activity. Declines in antifungal activity are observed also with the more hydrophobic subunits; however, these decreases in antifungal effect are muted relative to that seen for **CP**, which suggests that the more hydrophobic subunits manifest an antifungal effect that partially compensates for the loss of **βNM** subunits.

The data for the binary nylon-3 copolymers containing **MM** (Figure 7B) suggest that this subunit does not manifest an intrinsic antifungal effect, in contrast to the **βNM** subunit. For the

two series shown, introduction of a hydrophobic subunit leads to a modest increase in antifungal activity (lower MIC), with the more hydrophobic **CO** subunit exerting a stronger antifungal effect than the less hydrophobic **CH** subunit. In contrast, the data for the binary nylon-3 copolymers containing **DM** (Figure 7C) suggest that this cationic subunit behaves more like **βNM** than **MM** in that **DM** units seem to exert an intrinsic antifungal effect. In this set, antifungal activity declines when **DM** is partially replaced with hydrophobic subunits. However, **DM** differs from **βNM** in that the **DM** subunit seems to have an intrinsic hemolytic effect (HC_{10} increases as **DM** subunits are replaced by hydrophobic subunits), while the **βNM** subunit does not. The nature of the apparent intrinsic antifungal effects of the **βNM** and **DM** cationic subunits is unclear at present, as is the origin of the seemingly discontinuous trend among the three cationic subunits, with addition of one methyl group to the backbone (replacement of **αNM** or **βNM** with **MM**) strongly diminishing the antifungal effect but addition of a second methyl group (replacement of **MM** with **DM**) restoring the intrinsic antifungal effect.

The data for the **MM** and **DM** series in Figure 7B and C show that antifungal activity cannot be achieved without promoting hemolytic activity in these binary nylon-3 polymer families. In other words, we were unable to identify a selective antifungal agent within these polymer sets. In this context, the polymers containing **αNM** or **βNM** cationic units stand out for their selective antifungal activity profiles.

Activity against Other Pathogenic Fungi. We evaluated nylon-3 polymers for the ability to inhibit growth of two other
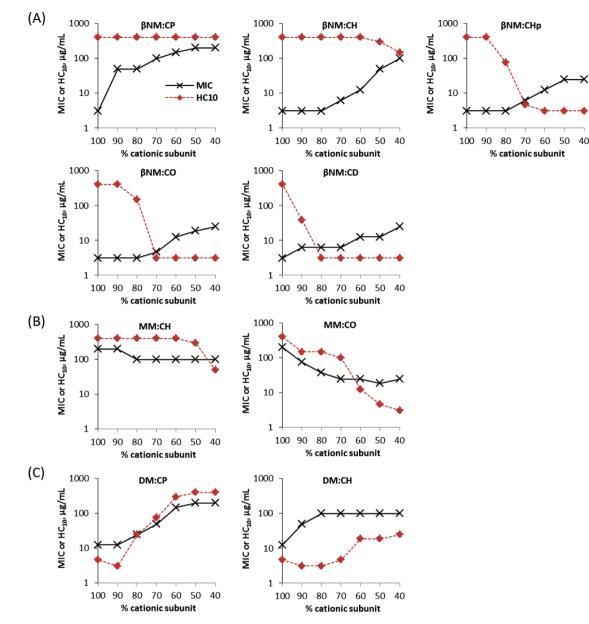
Figure 7. Antifungal activities (MIC) toward *C. albicans* (K1 strain) and hemolytic activities (HC$_{10}$) of nylon-3 copolymers as a function of subunit composition. Black crosses indicate MIC values, and red diamonds indicate HC$_{10}$ values. All polymers had a *p-t*-butylbenzoyl group at the N-terminus. The lines are drawn simply to connect the data points. Structures of the polymers are shown in Figure 6. Results for the *β*NM:CH polymers were reported previously.[29]

fungal species, *C. neoformans* and *A. fumigatus*, both of which cause invasive infections in humans and are associated with high mortality rates.[1] The data in Table 4 show that poly-*β*NM and *β*NM:CH copolymers display potent activity against *C. neoformans* and excellent selectivity relative to mammalian cells, nearly matching the performance of fluconazole. AmpB is slightly more active than the polymers, but as noted above, this drug has poor selectivity. Cationic polycarbonates active against *C. neoformans* have very recently been described.[64] *A. fumigatus* proved to be a more challenging target. Poly-*β*NM is only very weakly active, but moderate activity is observed for *β*NM:CH copolymers with 40−50% CH. *A. fumigatus* is resistant to fluconazole, but AmpB is active against this species.

Table 2 shows that even very short versions of poly-*β*NM display significant activity against the K1 strain of *C. albicans*. All nylon-3 chains bear a C-terminal imide, which contains a *β*-lactam unit. We therefore evaluated *β*-lactam starting materials

*β*NM*β*, MM*β*, and DM*β* for antifungal activity. However, none of theses *β*-lactams displayed any activity against either the K1 or Gu5 strain of *C. albicans*, even at 500 *µ*g/mL (Table S11, Supporting Information).

Conclusions. Data presented here show that nylon-3 polymers containing the cationic *β*NM subunit display favorable antifungal behavior with little or no propensity to induce lysis of human red blood cells. The *β*NM subunit appears to manifest an intrinsic antifungal effect, enabling homopolymers to kill strains of *C. albicans* that are resistant to the widely used drugs AmpB and fluconazole. The isomeric *α*NM and *β*NM subunits are comparable in terms of biological activity profile, but the *β*-lactam precursor for *α*NM is time consuming to prepare, so *β*NM-containing nylon-3 polymers are preferable from a practical perspective. Poly-*β*NM displays potent activity against a second human pathogen, *C. neoformans*, but this homopolymer is not active against *A. fumigatus*. Supplementing *β*NM subunits

**Table 4. Antifungal Activities against *C. neoformans* and
*A. fumigatus***

| compd | MIC[a] (MIC$_{50}$[b]) (μg/mL) | | HC$_{10}$ (μg/mL) | SI[d] |
	C. neoformans	A. fumigatus	red blood cell	
fluconazole	1.6	>200 (>200)	>400	>250
AmpB	0.8	6.3 (4.7)	0.8	1
50:50 βNM:CH	6.3	150 (50)	200	32
60:40 βNM:CH	3.1	150 (50)	>400[c]	>130
70:30 βNM:CH	3.1	200 (75)	>400[c]	>130
80:20 βNM:CH	3.1	200 (100)	>400[c]	>130
90:10 βNM:CH	3.1	>200 (100)	>400[c]	>130
tBuBz-(βNM)$_{20}$	3.1	>200 (200)	>400[c]	>130
tBuBz-(βNM)$_{80}$	3.1	>200 (200)	>400	>130

[a]Minimum inhibitory concentration for fungal cell growth in planktonic form. [b]The concentration to inhibit 50% of *A. fumigatus* growth in planktonic form. [c]These data were reported previously.[29] [d]Selectivity index for *C. neoformans* (HC$_{10}$/MIC). ND means HC$_{10}$ was not determined. The MIC$_{50}$ results for these polymers against *A. fumigatus* are included because MIC$_{50}$ is a recommended activity measurement method by the Clinical and Laboratory Standards Institute (CLSI, previously known as NCCLS).[65]

with hydrophobic **CH** subunits is required to generate nylon-3 copolymers that are moderately inhibitory toward *A. fumigatus*.

The antifungal potency and low mammalian cell toxicity of nylon-3 materials based on **βNM** are significant because fungal infections remain major threats to human health, and new agents that can treat or prevent such infections are therefore valuable. Our interest in antimicrobial properties of nylon-3 polymers was originally inspired by HDPs, but it is noteworthy that the ability of many HDPs to inhibit fungal growth may be limited. Synthetic polymers have a substantial synthetic advantage relative to sequence-specific peptides, because peptides require a laborious step-by-step approach while polymers can be rapidly generated on a large scale. The resulting polymeric materials contain many different chain lengths, but our data show that variations in poly-βNM chain length have little effect on either antifungal activity or hemolytic activity. The ease with which βNM-based nylon-3 polymers can be prepared raises the possibility that they may find use for killing fungi on surfaces or perhaps even as clinical antifungal agents.

■ EXPERIMENTAL METHODS

Materials and Methods. The K1 strain of *C. albicans* was a generous gift from Professor David Andes at University of Wisconsin at Madison; the Gu5 strain (MYA-574), the C4 strain (38245), and the E4 strain of *C. albicans* (38248), *C. neoformans* (ATCC MYA-737), and *A. fumigatus* (ATCC 96918) were obtained from American Type Culture Collection; amphotericin B (46006), fluconazole (PHR1160), α-poly-L-lysine 4−15 KDa (P6516), 3-(N-morpholino) propanesulfonic acid (MOPS, M3183), yeast extract peptone dextrose broth (YPD, Y1375), and Sabouraud dextrose broth (S3306) were obtained from Sigma-Aldrich (St. Louis, MO); magainin 2 (20640) was obtained from AnaSpec; RPMI 1640 (31800-089) was obtained from Life Technologies (Grand Island, NY); LB medium (244610) was obtained from BD (Franklin Lakes, NJ); agar (BP1423500) was obtained from Fisher Scientific (Pittsburgh, PA); and Easivial polymethyl methacrylate (PMMA) standards for GPC column calibration (PL2020-0200) were obtained from Polymer Varian (Palo Alto, CA). All other chemicals were purchased from Sigma-Aldrich and used without purification. ^{1}H and ^{13}C NMR spectra were collected either on a Varian MercuryPlus 300 spectrometer at 300 and 75 MHz, respectively, or on a Bruker Avance III spectrometer at 400 and 100 MHz, respectively, using CDCl$_3$ or D$_2$O as

the solvent. ^{1}H NMR chemical shifts were referenced to residual protonated solvent (δ 7.26 for CDCl$_3$ and δ 4.79 for D$_2$O). ^{13}C NMR chemical shifts were referenced to the solvent (δ 77.16 for CDCl$_3$). Mass spectra were acquired using either a Waters (Micromass) LCT mass spectrometer or a Waters (Micromass) AutoSpec mass spectrometer.

Synthesis of β-Lactams. *1-Benzyl-3-((p-toluenesulfonyloxy)-methyl)-azetidin-2-one (1).* Racemic β-lactam **1** was prepared as a racemic mixture by following the reported method,[61] which provided the compound as a white solid. mp 100.8−101.7 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.75 (d, J = 6.5 Hz, 2H), 7.28−7.35 (m, 5H), 7.21 (dd, J = 7.6, 1.8 Hz, 2H), 4.23 (d, J = 5.1 Hz, 1H), 4.20−4.31 (m, 3H), 3.45 (m, 1H), 3.23 (t, J = 5.6 Hz, 1H), 3.08 (dd, J = 5.9, 2.5 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 165.33, 145.22, 135.02, 132.32, 130.01, 128.90, 128.04, 127.99, 127.85, 66.59, 49.02, 46.07, 42.45, 21.68. HRESI-MS: m/z calcd for C$_{18}$H$_{23}$N$_2$O$_4$S [M+NH$_4$]$^+$, 363.1367; found, 363.1374.

1-Benzyl-3-azidomethyl-azetidin-2-one (2). A mixture of β-lactam **1** (3.7 g) and sodium azide (2.1 g) in 6:1 EtOH:water (42 mL) was heated at 60 °C for 10 h. After cooling, the mixture was concentrated *in vacuo* to give a yellow oil. DI water (80 mL) was added to the reaction mixture with shaking, and the crude product was extracted with CH$_2$Cl$_2$ (3 × 200 mL). The combined organic layers were washed with brine (80 mL), dried over MgSO$_4$, and concentrated *in vacuo*. The crude product was purified by silica gel chromatography using 1:1 hexane:EtOAc as the eluent to give racemic β-lactam **2** as a colorless oil (2.1 g, 91% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.28−7.35 (m, 3H), 7.23 (d, J = 7.3 Hz, 2H), 4.30−4.44 (m, 2H), 3.62−3.65 (m, 2H), 3.38 (m, 1H), 3.24 (t, J = 5.5 Hz, 1H), 3.04 (dd, J = 5.8, 2.4 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 166.94, 135.29, 128.93, 128.19, 127.91, 49.26, 49.08, 46.14, 42.81. HRESI-MS: m/z calcd for C$_{11}$H$_{13}$N$_4$O [M+H]$^+$, 217.1084; found, 217.1083.

1-Benzyl-3-(tert-butyloxycarbonyl aminomethyl)-azetidin-2-one (3). Pd(OH)$_2$ on activated charcoal (1.0 g) was added to a solution of β-lactam **2** (2.0 g) in MeOH (50 mL), and the suspension was stirred under a hydrogen atmosphere (~1 atm) overnight. The reaction mixture was then filtered, and the filtrate was concentrated *in vacuo* to give a colorless oil. The oil was dissolved in MeOH (44 mL); Boc$_2$O (5.3 mL) and Et$_3$N (258 μL) were added to this solution. The mixture was heated to reflux for 6 h. After cooling, the mixture was concentrated *in vacuo* to give an oil. DI water (80 mL) was added to the residue with shaking, and the crude product was extracted with CH$_2$Cl$_2$ (3 × 200 mL). The combined organic layers were washed with brine (80 mL), dried over MgSO$_4$, and concentrated *in vacuo*. The crude product was purified by silica gel chromatography using 1:1 hexane:EtOAc as the eluent to give racemic β-lactam **3** as a colorless viscous oil (1.68 g, 63% overall yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.28−7.36 (m, 3H), 7.23 (dd, J = 6.6, 1.8 Hz, 2H), 4.91 (br, 1H), 4.45 (d, J = 15.1 Hz, 1H), 4.30 (d, J = 15.1 Hz, 1H), 3.47 (m, 2H), 3.32 (m, 1H), 3.21 (t, J = 5.6 Hz, 1H), 3.00 (dd, J = 5.9, 2.4 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 168.62, 156.19, 135.54, 128.95, 128.18, 127.86, 79.63, 50.18, 46.10, 42.77, 38.71, 28.42. HRESI-MS: m/z calcd for C$_{16}$H$_{23}$N$_2$O$_3$ [M+H]$^+$, 291.1704; found, 291.1706.

3-(tert-Butyloxycarbonyl aminomethyl)-azetidin-2-one (αNMβ). To a solution of β-lactam **3** (1.4 g) in EtOH (3 mL) at −78 °C, freshly dried liquid ammonia (100 mL) was added. Sodium metal was added slowly at −78 °C until the reaction solution became dark blue. The reaction mixture was maintained at −78 °C for 15 min and then warmed slowly to rt. After all ammonia had been removed from the reaction flask by a flow of N$_2$, the residue was mixed with aqueous NH$_4$Cl. The crude product was extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with brine (80 mL), dried over MgSO$_4$, and concentrated *in vacuo*. The crude product was purified by silica gel chromatography using 80:1 CH$_2$Cl$_2$:MeOH to give racemic **αNMβ** as a a white solid (0.68 g, 71% yield). mp 132−133 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.75 (br, 1H), 4.91 (br, 1H), 3.45−3.58 (m, 2H), 3.40 (m, 2H), 3.17 (m, 1H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, CDCl$_3$): δ 170.03, 156.24, 79.68, 51.74, 39.63, 38.78, 28.42. HRESI-MS: m/z calcd for C$_9$H$_{17}$N$_2$O$_3$ [M+H]$^+$, 201.1234; found, 201.1241.

Synthesis and Characterization of Nylon-3 Polymers. In a moisture-controlled glovebox under dry N$_2$, β-lactam monomers were

weighed out into a predried glass vial and dissolved in 3 mL of polymerization solvent, dimethylacetamide (DMAc) or THF, depending on the solubility of the resulting polymers. A 0.5 mL solution of co-initiator (t-BuBzCl or AcCl) in DMAc or THF was added to the reaction vessel in a predetermined ratio relative to β-lactams to control the polymer length; e.g., a 1:20 co-initiator:β-lactam molar ratio was used to prepare polymers with 20-mer average length. The monomers and co-initiator were mixed with strong magnetic stirring, and the polymerization reaction was started by adding a 0.1−1 mL solution of lithium bis(trimethylsilyl)amide (LiHMDS) in DMAc or THF to the reaction mixture. The reaction mixture was adjusted to total 4 mL in volume by adding more polymerization solvent and stirred at rt for 12 h. Then, the reaction mixture was removed from the glovebox, and the reaction was quenched by adding a few drops of MeOH. The resulting polymer was isolated by precipitation after a "poor" solvent had been added (see following paragraph).

Polymers containing **αNM** or **βNM** were prepared in DMAc. In these cases, the quenched reaction mixture was combined with pentane (40 mL), which caused a light yellow oil to separate from the solution. The oil was collected after centrifugation and dissolved in THF (2 mL) with the assistance of vortex mixing and ultrasonication. Pentane (40 mL) was then added to induce polymer precipitation. If the oil collected from the first phase separation would not dissolve in THF, MeOH (0.5−1 mL) was used instead, and then Et$_2$O (43 mL) was added to induce polymer precipitation. After four dissolution/precipitation cycles, the side chain amine-protected polymer was collected as a white solid. This material was dried under vacuum overnight and then subjected to gel permeation chromatography (GPC) characterization using DMAc as the mobile phase.

Polymers containing **MM** or **DM** were prepared in THF. In these cases, the quenched reaction mixture was combined with pentane (40 mL), which caused precipitation. The precipitate was collected after centrifugation and dissolved in THF (2 mL), and then, pentane (40 mL) was added to induce polymer precipitation. After three dissolution/precipitation cycles, the side chain amine-protected polymer was collected as a white solid. This material was dried under vacuum overnight and then subjected to GPC characterization using THF as the mobile phase.

To remove Boc protecting groups from side chain amino groups, the protected polymer was treated with neat trifluoroacetic acid (TFA, 2 mL) at rt for 2 h with shaking. The deprotected polymer was precipitated as a white fluffy solid by addition of Et$_2$O (40 mL) into the TFA solution. The solid was collected after centrifugation and dissolved in MeOH (2 mL), and then precipitated again by addition of Et$_2$O (40 mL). After three dissolution/precipitation cycles, the deprotected polymer was collected, dissolved in Milli-Q water, and lyophilized to provide a white powder (TFA salt of the polymer). For the homopolymers poly-**αNM** and poly-**βNM**, the collected solid was sometimes yellowish.

Polymer Characterization by GPC Using DMAc as the Mobile Phase. The side chain amine-protected polymer was dissolved in DMAc (supplemented with 10 μM LiBr) at 2 mg/mL. The polymer was swollen in DMAc for at least 20 min and then passed through a 0.2 μm polytetrafluoroethylene (PTFE) filter before GPC analysis. A Waters GPC instrument was used. The instrument was equipped with a refractive index detector (Waters 2410) and two Waters Styragel HR 4E columns (particle size 5 μm) linked in series. The mobile phase ran at 1 mL/min at a column temperature of 80 °C. The number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (PDI) were calculated using the Empower software provided by Waters and calibration curves obtained from at least nine PMMA standards (peak average molecular weight ranging from 690 to 1 944 000). The degree of polymerization (DP) for a particular polymer was calculated on the basis of the deduced M_n value, the initial ratio of β-lactam monomers in the reaction mixture, and the molecular weight of the β-lactam monomers, as described previously.[28]

Polymer Characterization by GPC Using THF as the Mobile Phase. The side chain amine-protected polymer was dissolved in THF at 10 mg/mL. The polymer was swollen in THF for at least 20 min and then passed through a 0.2 μm PTFE filter before GPC analysis. A Shimadzu GPC instrument was used. The instrument was equipped

with a multiangle light scattering detector (Wyatt miniDAWN, 690 nm, 30 mW), a refractive index detector (Wyatt Optilab-rEX, 690 nm), and two Waters columns (Styragel HR 4E, particle size 5 μm) linked in series. The mobile phase ran at 1 mL/min at a column temperature of 40 °C. M_n, M_w, and PDI were calculated using ASTRA 5.3.4.20 software based on a dn/dc value of 0.1 mL/g. DP for a particular polymer was calculated on the basis of the deduced M_n value, the initial ratio of β-lactam monomers in the reaction mixture, and the molecular weight of the β-lactam monomers, as described previously.[28]

Antifungal Activity Assay (MIC, MIC$_{50}$, and MFC). The MIC and MIC$_{50}$ assays for *C. albicans* and *C. neoformans* were conducted according to a previously described protocol.[66] The MIC and MIC$_{50}$ assays for *A. fumigatus* were conducted using the M38-A2 protocol suggested by the Clinical and Laboratory Standards Institute (CLSI, previously known as NCCLS).[65] *C. albicans* and *C. neoformans* cells were inoculated in YPD broth (for C4 and E4 strains of *C. albicans*) or RPMI medium (for K1 and Gu5 strains of *C. albicans* and *C. neoformans*), and the sample was incubated at 30 °C for 2−3 days. The YPD medium for C4 strain culture was supplemented with 150 units/mL of nystatin.

The cultured *C. albicans* or *C. neoformans* cells were collected by centrifugation and suspended in 0.145 M NaCl at 2.5×10^6 cells/mL to generate the stock suspension. *A. fumigatus* cells were inoculated on a Sabouraud dextrose agar plate and incubated at 37 °C for 4−5 days. The plate surface was flooded with 15 mL of RPMI and gently rubbed with a sterile cotton swab. The RPMI suspension was transferred to a 50 mL centrifuge tube and vortexed vigorously for 15 s. After the mixture stood for 15 min, the supernatant was transferred to a new centrifuge tube, and cells were collected after centrifugation.[67] The collected pellet was dispersed in RPMI and subjected to two precipitation/dispersion cycles. The cells were then suspended in RPMI with the density adjusted to $(0.5−1) \times 10^7$ cells/mL to generate the stock suspension. The working suspension for each type of fungal cell was prepared as a 1:1000 dilution of the stock suspension using adjusted RPMI 1640 medium (containing L-glutamine but not sodium bicarbonate) buffered with 0.145 M MOPS. Two-fold serial dilution of nylon-3 polymers, fluconazole, and AmpB was conducted in a 96-well plate using adjusted RPMI to obtain concentrations from 400 to 0.4 μg/mL. Each sample well had a 100 μL compound solution after the 2-fold serial dilution. Then, 100 μL of the cell working suspension was added to each well (except the cell-free blank control), followed by gentle shaking of the plate for 10 s. The plate was then incubated at 37 °C for 2−3 days (E4 strains of *C. albicans* cells were incubated for 4 days), and fungal cell growth was evaluated visually. On the same plate, wells containing RPMI medium only were used as the negative control (cell-free blank); wells containing cells in RPMI without any polymer or antifungal drug were used as the positive control. The antifungal MIC was the lowest concentration of a polymer or antifungal drug that completely inhibited fungal cell growth; that is, no cell colony was visible in the well. The antifungal MIC$_{50}$ was the concentration of a polymer or antifungal drug that inhibited 50% of fungal cell growth. Each experiment was performed in duplicate on a given day, and experiments were repeated on two different days.

The minimum fungicidal concentration (MFC) assay for *C. albicans* was conducted after the MIC test. Aliquots of 10 μL of well-mixed suspension were transferred to YPD agar plates from cell−polymer mixtures containing polymer concentrations from one dilution below the MIC to the highest polymer concentration. The YPD agar plates were incubated at 37 °C for 48 h and then inspected visually for *C. albicans* colony formation. The MFC was defined as the lowest polymer concentration to kill all (>99.9%) the cells, which means no *C. albicans* colony was observed.

Hemolysis Assay. Hemolysis assays were conducted as previously described using human red blood cells (RBCs).[66,68] Human whole blood (5 mL) was washed three times with Tris-buffered saline (TBS, pH 7.2) that was composed of 10 mM Tris and 150 mM NaCl. The collected RBCs were suspended in TBS (250 mL) to obtain a working suspension of 2% RBC relative to total RBCs in the whole blood. Two-fold serial dilution of nylon-3 polymers was conducted in a 96-well plate in TBS to obtain concentrations ranging from 800 to 6.25 μg/mL. Each sample well had 100 μL of compound solution after the 2-fold serial

4340

dx.doi.org/10.1021/ja500036r | J. Am. Chem. Soc. 2014, 136, 4333−4342

dilution. Then, 100 μL of the RBC working suspension was added to each well, followed by gentle shaking of the plate for 10 s. On the same plate, wells containing TBS without polymer were used as the blank; wells containing Triton X-100 (3.2 mg/mL in TBS) were used as the positive control. The plate was incubated at 37 °C for 1 h, and then centrifuged at 3700 rpm for 5 min to precipitate the RBCs. An aliquot of 80 μL of the supernatant from each well was transferred to the corresponding well in a new 96-well plate, and the optical density (OD) at 405 nm was measured using a Molecular Devices Emax precision microplate reader. Measurements were performed in duplicate, and each measurement was repeated on two different days. The percentage of hemolysis at each polymer concentration was calculated from "% hemolysis = $100 \times (A^{polymer} - A^{blank})/(A^{control} - A^{blank})$" and plotted against polymer concentration to give the dose–response curves for hemolysis for each polymer. The HC_{10} value for each polymer was defined as the polymer concentration to cause 10% lysis of RBCs.

■ ASSOCIATED CONTENT

⑤ Supporting Information

Bioassay results and compound characterization spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors
kmasters@wisc.edu
gellman@chem.wisc.edu

Present Address
[⊥]Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD. 20742

Notes

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Brown, G. D.; Denning, D. W.; Gow, N. A.; Levitz, S. M.; Netea, M. G.; White, T. C. *Sci. Transl. Med.* **2012**, *4*, 165rv13.
(2) Kelly, S. L.; Lamb, D. C.; Kelly, D. E.; Manning, N. J.; Loeffler, J.; Hebart, H.; Schumacher, U.; Einsele, H. *FEBS Lett.* **1997**, *400*, 80.
(3) White, T. C.; Marr, K. A.; Bowden, R. A. *Clin. Microbiol. Rev.* **1998**, *11*, 382.
(4) Lopez-Ribot, J. L.; McAtee, R. K.; Lee, L. N.; Kirkpatrick, W. R.; White, T. C.; Sanglard, D.; Patterson, T. F. *Antimicrob. Agents Chemother.* **1998**, *42*, 2932.
(5) Fanhavard, P.; Capano, D.; Smith, S. M.; Mangia, A.; Eng, R. H. K. *Antimicrob. Agents Chemother.* **1991**, *35*, 2302.
(6) Zasloff, M. *Nature* **2002**, *415*, 389.
(7) Hancock, R. E. W.; Sahl, H. G. *Nat. Biotechnol.* **2006**, *24*, 1551.
(8) Steinstraesser, L.; Kraneburg, U. M.; Hirsch, T.; Kesting, M.; Steinau, H. U.; Jacobsen, F.; Al-Benna, S. *Int. J. Mol. Sci.* **2009**, *10*, 3951.
(9) Yeaman, M. R.; Yount, N. Y. *Pharmacol. Rev.* **2003**, *55*, 27.
(10) Yount, N. Y.; Yeaman, M. R. *Annu. Rev. Pharmacol. Toxicol.* **2012**, *52*, 337.
(11) van der Weerden, N. L.; Bleackley, M. R.; Anderson, M. A. *Cell. Mol. Life Sci.* **2013**, *70*, 3545.
(12) Lee, I. H.; Cho, Y.; Lehrer, R. I. *Infect. Immun.* **1997**, *65*, 2898.
(13) Helmerhorst, E. J.; Reijnders, I. M.; van't Hof, W.; Veerman, E. C. I.; Amerongen, A. V. N. *FEBS Lett.* **1999**, *449*, 105.
(14) Wade, D.; Boman, A.; Wahlin, B.; Drain, C. M.; Andreu, D.; Boman, H. G.; Merrifield, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4761.
(15) Papo, N.; Shai, Y. *Biochemistry* **2004**, *43*, 6393.
(16) Patch, J. A.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 12092.
(17) Olsen, C. A.; Bonke, G.; Vedel, L.; Adsersen, A.; Witt, M.; Franzyk, H.; Jaroszewski, J. W. *Org. Lett.* **2007**, *9*, 1549.
(18) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1999**, *121*, 12200.
(19) Porter, E. A.; Wang, X. F.; Lee, H. S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565.
(20) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2004**, *126*, 6848.
(21) Gelman, M. A.; Weisblum, B.; Lynn, D. M.; Gellman, S. H. *Org. Lett.* **2004**, *6*, 557.
(22) Tossi, A.; Sandri, L.; Giangaspero, A. *Biopolymers* **2000**, *55*, 4.
(23) Lienkamp, K.; Madkour, A. E.; Musante, A.; Nelson, C. F.; Nusslein, K.; Tew, G. N. *J. Am. Chem. Soc.* **2008**, *130*, 9836.
(24) Jiang, Y. J.; Yang, X.; Zhu, R.; Hu, K.; Lan, W. W.; Wu, F.; Yang, L. H. *Macromolecules* **2013**, *46*, 3959.
(25) Kuroda, K.; DeGrado, W. F. *J. Am. Chem. Soc.* **2005**, *127*, 4128.
(26) Palermo, E. F.; Sovadinova, I.; Kuroda, K. *Biomacromolecules* **2009**, *10*, 3098.
(27) 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.
(28) Mowery, B. P.; Lindner, A. H.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2009**, *131*, 9735.
(29) Liu, R. H.; Chen, X. Y.; Hayouka, Z.; Chakraborty, S.; Falk, S. P.; Weisblum, B.; Masters, K. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2013**, *135*, 5270.
(30) Song, A. R.; Walker, S. G.; Parker, K. A.; Sampson, N. S. *ACS Chem. Biol.* **2011**, *6*, 590.
(31) Sambhy, V.; Peterson, B. R.; Sen, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 1250.
(32) Sellenet, P. H.; Allison, B.; Applegate, B. M.; Youngblood, J. P. *Biomacromolecules* **2007**, *8*, 19.
(33) Li, P.; Zhou, C.; Rayatpisheh, S.; Ye, K.; Poon, Y. F.; Hammond, P. T.; Duan, H. W.; Chan-Park, M. B. *Adv. Mater.* **2012**, *24*, 4130.
(34) Nederberg, F.; Zhang, Y.; Tan, J. P. K.; Xu, K. J.; Wang, H. Y.; Yang, C.; Gao, S. J.; Guo, X. D.; Fukushima, K.; Li, L. J.; Hedrick, J. L.; Yang, Y. Y. *Nat. Chem.* **2011**, *3*, 409.
(35) Wang, Y. Q.; Xu, J. J.; Zhang, Y. H.; Yan, H. S.; Liu, K. L. *Macromol. Biosci.* **2011**, *11*, 1499.
(36) Haberman., E. *Science* **1972**, *177*, 314.
(37) Shai, Y. *Biochim. Biophys. Acta* **1999**, *1462*, 55.
(38) Timofeeva, L. M.; Kleshcheva, N. A.; Moroz, A. F.; Didenko, L. V. *Biomacromolecules* **2009**, *10*, 2976.
(39) Cakmak, I.; Ulukanli, Z.; Tuzcu, M.; Karabuga, S.; Genctav, K. *Eur. Polym. J.* **2004**, *40*, 2373.
(40) Vucetic, J. J.; Vandjel, V. H.; Janic, M. D. *Glas. Hem. Drus. Beograd* **1977**, *42*, 389.
(41) Ikeda, T.; Tazuke, S. *Makromol. Chem., Rapid Commun.* **1983**, *4*, 459.
(42) Ikeda, T.; Tazuke, S.; Suzuki, Y. *Makromol. Chem.* **1984**, *185*, 869.
(43) Kawabata, N.; Nishiguchi, M. *Appl. Environ. Microb.* **1988**, *54*, 2532.
(44) Senuma, M.; Tashiro, T.; Iwakura, M.; Kaeriyama, K.; Shimura, Y. *J. Appl. Polym. Sci.* **1989**, *37*, 2837.
(45) Li, G. J.; Shen, J. R.; Zhu, Y. L. *J. Appl. Polym. Sci.* **1998**, *67*, 1761.
(46) Chen, C. Z. S.; Beck-Tan, N. C.; Dhurjati, P.; van Dyk, T. K.; LaRossa, R. A.; Cooper, S. L. *Biomacromolecules* **2000**, *1*, 473.
(47) Tiller, J. C.; Liao, C. J.; Lewis, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5981.
(48) Ohta, S.; Misawa, Y.; Miyamoto, H.; Makino, M.; Nagai, K.; Shiraishi, T.; Nakagawa, Y.; Yamato, S.; Tachikawa, E.; Zenda, H. *Biol. Pharm. Bull.* **2001**, *24*, 1093.
(49) Lee, M. R.; Stahl, S. S.; Gellman, S. H.; Masters, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 16779.

(50) Liu, R.; Vang, K. Z.; Kreeger, P. K.; Gellman, S. H.; Masters, K. S. *J. Biomed. Mater. Res., Part A* **2012**, *100*, 2750.

(51) Liu, R. H.; Masters, K. S.; Gellman, S. H. *Biomacromolecules* **2012**, *13*, 1100.

(52) Liu, R.; Chen, X.; Gellman, S. H.; Masters, K. S. *J. Am. Chem. Soc.* **2013**, *135*, 16296.

(53) 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.

(54) Dane, E. L.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2012**, *134*, 16255.

(55) Dane, E.; Ballok, A.; O'Toole, G. A.; Grinstaff, M. W. *Chem. Sci.* **2014**, *5*, 551.

(56) Chen, L.; Lei, Y.; Shilabin, A. G.; Delaney, J. D.; Baran, G. R.; Sieburth, S. M. *Chem. Commun.* **2012**, *48*, 9604.

(57) Dane, E. L.; Chin, S. L.; Grinstaff, M. W. *ACS Macro Lett.* **2013**, *2*, 887.

(58) Andes, D.; Lepak, A.; Nett, J.; Lincoln, L.; Marchillo, K. *Antimicrob. Agents Chemother.* **2006**, *50*, 2384.

(59) Hashimoto, K. *Prog. Polym. Sci.* **2000**, *25*, 1411.

(60) Zhang, J. H.; Kissounko, D. A.; Lee, S. E.; Gellman, S. H.; Stahl, S. S. *J. Am. Chem. Soc.* **2009**, *131*, 1589.

(61) Bartrum, H. E.; Adams, H.; Caggiano, L.; Jackson, R. F. W. *Tetrahedron* **2008**, *64*, 3701.

(62) Franz, R.; Ruhnke, M.; Morschhauser, J. *Mycoses* **1999**, *42*, 453.

(63) Pierce, A. M.; Pierce, H. D.; Unrau, A. M.; Oehlschlager, A. C. *Can. J. Biochem. Cell Biol.* **1978**, *56*, 135.

(64) Chin, W.; Yang, C. A.; Ng, V. W. L.; Huang, Y.; Cheng, J. C.; Tong, Y. W.; Coady, D. J.; Fan, W. M.; Hedrick, J. L.; Yang, Y. Y. *Macromolecules* **2013**, *46*, 8797.

(65) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard, 2nd ed., CLSI document M38-A2; Clinical and Laboratory Standards Institute: Wayne, PA, 2008.

(66) Karlsson, A. J.; Pomerantz, W. C.; Weisblum, B.; Gellman, S. H.; Palecek, S. P. *J. Am. Chem. Soc.* **2006**, *128*, 12630.

(67) Wetter, T. J.; Hazen, K. C.; Cutler, J. E. *J. Clin. Microbiol.* **2003**, *41*, 4252.

(68) Raguse, T. L.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12774.