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Nylon-3 Polymers with Selective Antifungal Activity

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Abstract

Host-defense peptides inhibit bacterial growth but show little toxicity toward mammalian cells. A variety of synthetic polymers have been reported to mimic this antibacterial selectivity; however, achieving comparable selectivity for fungi is more difficult because these pathogens are eukaryotes. Here, we report nylon-3 polymers based on a novel subunit that display potent antifungal activity (MIC = 3.1 μg/mL for *C. albicans*) and favorable selectivity (IC_{10} > 400 μg/mL for 3T3 fibroblast toxicity; HC_{10} > 400 μg/mL for hemolysis).

Natural strategies to fend off microbial infection include production of relatively small peptides that manifest antimicrobial activity, part of the innate immune response.[1] These "host-defense peptides" have diverse sequences and bioactive conformations, and their biological effects appear to arise from multiple mechanisms.[2] Many host-defense peptides can adopt amphiphilic structures in which lipophilic and hydrophilic (usually cationic) side chains are segregated to distinct regions of the molecular surface.[3] This global amphiphilicity is widely believed to underlie the ability of host-defense peptides to compromise bacterial membrane barrier function and thereby inhibit the growth of or kill prokaryotes.[4] Numerous reports describe synthetic peptides or peptidomimetic oligomers designed to be globally amphiphilic that can serve as tools to elucidate the origins of host-defense peptide function and as candidates for therapeutic application.[5] The evaluation of synthetic systems has recently expanded to include random copolymers that contain both hydrophilic and lipophilic subunits, which are much more readily prepared than are sequence-specific peptides or other oligomers.[6]

Antimicrobial agents have the highest potential for application when their deleterious effects are specific for microbial cells relative to human cells. Such selectivity has been achieved with a variety of compounds for bacterial growth inhibition vs. human cell destruction;[6h,6m,7] the latter property is often assessed as lytic activity toward red blood cells ("hemolysis").[5e,8] Fundamental differences between prokaryotic and eukaryotic cellular membranes, including lipid composition and external surface charge density, seem to

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B.W. and S.H.G. are co-inventors on a patent application that covers the polymers described here.

Supporting Information

Experimental details for synthesis and characterization of nylon-3 polymers, antifungal and antibacterial assays, cytotoxicity on 3T3 fibroblasts and hemolysis on human RBCs. This information is available free of charge via the Internet at http://pubs.acs.org/.

facilitate this selectivity.[2,8b] In contrast, it is difficult to target fungal pathogens selectively relative to human cells, because fungi are eukaryotes.[9] For example, many host-defense peptides are not effective inhibitors of fungal growth at physiological ionic strength,[10] and only modest antifungal vs. hemolytic selectivity has been achieved with sequence-specific oligomers.[11] Here we describe a new family of nylon- 3 polymers (poly-β-peptides) that display significant and selective toxicity toward the most common fungal pathogen among humans, *Candida albicans*.[12]

Nylon-3 materials are readily prepared via ring-opening polymerization of β-lactams,[13] and we have previously reported that sequence-random co-polymers that contain a lipophilic and a cationic subunit can manifest significant antibacterial activity but low hemolytic activity if the subunit identities, lipophilic-cationic subunit proportion and other parameters are optimized.[6h,6m,14] The co-polymer shown in Figure 1, for example, displays a particularly favorable antibacterial activity profile.[6h] However, antifungal activity among previously reported nylon-3 copolymer families proved to be inseparable from hemolytic activity (unpublished). The present studies began with the preparation of a new β-lactam, **NM** ("no methyl"; Figure 2), which provides a cationic subunit at or below neutral pH. We were drawn to this subunit because it contains fewer saturated carbon atoms and therefore should have a lower hydrophobicity than previously examined cationic nylon-3 subunits derived from β-lactams **MM** and **DM** ("monomethyl" and "dimethyl").[6m] The synthesis of **NM** (Figure 3) involves cycloaddition of chlorosulfonylisocyanate to an alkene, as in previous cases, but this route differs from the precedents in that the side chain nitrogen is introduced after β-lactam formation.[6h,13f,15] Although the yield of the iodo-β-lactam is only modest, this potentially versatile molecule can easily be prepared on a multi-gram scale.[15–16] The β-lactam bearing a Boc-protected amino group in the side chain was readily incorporated into nylon-3 co-polymers via the base-catalyzed process we have previously employed, in which the N-terminal group on each polyamide chain is specified by the choice of polymerization co-initiator.[13f] All polymers discussed below were prepared with 20-mer average length because previous work indicated that this size range is generally favorable in terms of maximizing antimicrobial activity and minimizing hemolytic activity.[6m]

The antifungal activity of new **NM**-containing co-polymers (Figure 4) was evaluated with a clinically isolated strain of *C. albicans* (K1).[17] The minimum inhibitory concentration (MIC) was measured using a protocol suggested by the Clinical and Laboratory Standard Institute (previously known as the National Committee for Clinical Laboratory Standard)[18]. In order to assess the effects of the new polymers on mammalian cells, we determined the concentration necessary for 10% lysis of human red blood cells (HC_{10}), and the concentration necessary to induce 10% cell death in NIH 3T3 fibroblasts (IC_{10}). Previously we have used the minimum hemolytic concentration (MHC) as a metric of red blood cell disruption, but we shifted to HC_{10} for the present studies because it was sometimes difficult to identify the lowest polymer concentration that displayed a non-zero extent of hemolysis.[6h,6m] The fibroblast assays provide an alternative measure, relative to hemolysis, of toxicity toward mammalian cells. Amphotericin B (AmpB), which is used clinically for *C. albicans* infections but associated with high toxicity toward mammalian cells, served as a positive control in these studies.[19] Results are summarized in Table 1.

We began by examining random co-polymers (Figure 4) formed from new β-lactam **NM** and cyclohexyl β-lactam **CH**, because the latter had given rise to selective antibacterial copolymers when paired with the cationic subunit derived from **MM** (Figure 1).6h All of the new polymers bore a p-*t*-butylbenzoyl group at the N-terminus, as in previous antibacterial examples. The maximum proportion of the cyclohexyl subunit that could be used without compromising aqueous solubility, 60:40 **CH:NM**, led to weak antifungal activity and weak hemolytic activity (MIC and HC_{10} ~ 100 μg/mL). Antifungal activity steadily increased

(i.e., MIC decreased) as the proportion of the lipophilic subunit declined, and no co-polymer containing > 50% of the cationic subunit manifested detectable hemolytic activity. Members of this polymer family were generally not toxic toward mouse fibroblasts. The activity levels observed for **CH:NM** co-polymers with ≥ 80% of the cationic subunit, on a μg/mL basis, approached that of AmpB, but were accompanied by substantially less fibroblast cytotoxicity than AmpB. Replacing the p-*t*-butylbenzyol end-group with an acetyl end-group did not alter the biological activity of poly-**NM**. The **NM** homopolymer displayed antifungal activity comparable to that of the most active **CH:NM** copolymers. Follow-up studies showed that poly-**NM** is fungicidal at the MIC, rather than merely inhibitory toward fungal growth.[20]

The excellent activity profile observed for poly-**NM** contrasts with the behavior observed for two other cationic nylon-3 homopolymers, poly-**MM** and poly-**DM** (Table 1). Poly-**MM** shows very little antifungal activity, and this homopolymer is also not hemolytic or toxic toward 3T3 fibroblasts. Poly-**DM**, on the other hand, approximately matches poly-**NM** in activity against *C. albicans*, but poly- **DM** is hemolytic and moderately toxic toward 3T3 fibroblasts.

Poly-**NM** was evaluated for antibacterial activity against a panel of four species that we have previously used to assess poly-**MM** and poly-**DM** as well as cationic-hydrophobic copolymers (Table 2).[6m] The antibacterial effects of poly-**NM** were generally comparable to those of the other two cationic nylon-3 homopolymers: significant activity was observed for *Bacillus subtilis*, which seems to be highly susceptible to a wide array of peptides and peptidomimetic oligomers and polymers, but all three homopolymers were considerably less active against *Escherichia coli*, *Enterococcus faecium* and *Staphylococcus aureus*. The generally low antibacterial activity of poly-**MM** and poly-**DM** has previously been rationalized in terms of their lack of hydrophobic subunits (e.g., the subunit derived from **CH**), which may limit their ability to disrupt bacterial membranes.[6m,14] From this perspective, the relatively low antibacterial activity of poly- **NM** is not surprising. The potent antifungal activity of poly- **NM** is noteworthy in the context of this limited antibacterial activity.

The data we have presented show that nylon-3 polymers containing subunits derived from the new β-lactam **NM** display potent antifungal activity without a strong tendency to disrupt human red blood cell membranes or strong toxicity toward 3T3 fibroblasts. It is particularly intriguing that poly-**NM** displays such profound differences in biological activity relative to the structurally similar cationic nylon-3 homopolymers poly-**MM** and poly-**DM**. There are several differences among the subunits of these three polymers: (1) the added side-chain carbons in poly-**MM** and poly-**DM** relative to poly-**NM** cause a modest increase in hydrophobicity;[20] (2) the added carbons alter backbone flexibility; (3) the point of attachment of the aminomethyl side chain in **NM** differs from that in **MM** and **DM** (α-carbon vs. β-carbon). Further studies will be necessary to determine the mechanism by which these seemingly subtle molecularlevel changes exert such a substantial influence on biological activity. We have previously proposed that nylon-3 copolymers exert antibacterial effects via disruption of prokaryotic cell membranes, and this hypothesis has been supported by studies of the 40:60 **CH:MM** co-polymer (Figure 1) with synthetic vesicles of varying lipid composition.[14] However, our finding that maximal antifungal activity is manifested by poly-**NM**, the least hydrophobic nylon-3 polymer we have examined to date, raises the possibility that **NM**-containing polymers act via a mechanism that does not involve disturbance of lipid bilayers. The surprising biological activity profile discovered for **NM**-based nylon-3 suggests that antifungal applications of these new materials be pursued.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. (a) Zasloff M. Nature. 2002; 415:389–395. [PubMed: 11807545] (b) Boman HG. J Intern Med. 2003; 254:197–215. [PubMed: 12930229] (c) Hancock REW, Sahl HG. Nat Biotechnol. 2006; 24:1551–1557. [PubMed: 17160061] (d) Steinstraesser L, Kraneburg UM, Hirsch T, Kesting M, Steinau HU, Jacobsen F, Al-Benna S. Int J Mol Sci. 2009; 10:3951–3970. [PubMed: 19865528] (e) Diamond G, Beckloff N, Weinberg A, Kisich KO. Curr Pharm Design. 2009; 15:2377–2392.(f) Yeung ATY, Gellatly SL, Hancock REW. Cell Mol Life Sci. 2011; 68:2161–2176. [PubMed: 21573784]
2. Yeaman MR, Yount NY. Pharmacol Rev. 2003; 55:27–55. [PubMed: 12615953]
3. (a) van't Hof W, Veerman ECI, Helmerhorst EJ, Amerongen AVN. Biol Chem. 2001; 382:597–619. [PubMed: 11405223] (b) Sitaram N, Nagaraj R. Curr Pharm Design. 2002; 8:727–742.
4. Tossi A, Sandri L, Giangaspero A. Biopolymers. 2000; 55:4–30. [PubMed: 10931439]
5. (a) Wade D, Boman A, Wahlin B, Drain CM, Andreu D, Boman HG, Merrifield RB. P Natl Acad Sci USA. 1990; 87:4761–4765.(b) Maloy WL, Kari UP. Biopolymers. 1995; 37:105–122. [PubMed: 7893944] (c) Dathe M, Schumann M, Wieprecht T, Winkler A, Beyermann M, Krause E, Matsuzaki K, Murase O, Bienert M. Biochemistry. 1996; 35:12612–12622. [PubMed: 8823199] (d) Hamuro Y, Schneider JP, DeGrado WF. J Am Chem Soc. 1999; 121:12200–12201.(e) Porter EA, Wang XF, Lee HS, Weisblum B, Gellman SH. Nature. 2000; 404:565–565. [PubMed: 10766230] (f) Liu DH, DeGrado WF. J Am Chem Soc. 2001; 123:7553–7559. [PubMed: 11480975] (g) Tew GN, Liu DH, Chen B, Doerksen RJ, Kaplan J, Carroll PJ, Klein ML, DeGrado WF. P Natl Acad Sci USA. 2002; 99:5110–5114.(h) Porter EA, Weisblum B, Gellman SH. J Am Chem Soc. 2002; 124:7324–7330. [PubMed: 12071741] (i) Raguse TL, Porter EA, Weisblum B, Gellman SH. J Am Chem Soc. 2002; 124:12774–12785. [PubMed: 12392424] (j) Oren Z, Ramesh J, Avrahami D, Suryaprakash N, Shai Y, Jelinek R. Eur J Biochem. 2002; 269:3869–3880. [PubMed: 12180963] (k) Patch JA, Barron AE. J Am Chem Soc. 2003; 125:12092–12093. [PubMed: 14518985] (l) Liu DH, Choi S, Chen B, Doerksen RJ, Clements DJ, Winkler JD, Klein ML, DeGrado WF. Angew Chem Int Edit. 2004; 43:1158–1162.(m) Papo N, Shai Y. Biochemistry. 2004; 43:6393–6403. [PubMed: 15157073] (n) Schmitt MA, Weisblum B, Gellman SH. J Am Chem Soc. 2004; 126:6848–6849. [PubMed: 15174837] (o) Li X, Li YF, Han HY, Miller DW, Wang GS. J Am Chem Soc. 2006; 128:5776–5785. [PubMed: 16637646] (p) Olsen CA, Bonke G, Vedel L, Adsersen A, Witt M, Franzyk H, Jaroszewski JW. Org Lett. 2007; 9:1549–1552. [PubMed: 17352488] (q) Meng H, Kumar K. J Am Chem Soc. 2007; 129:15615–15622. [PubMed: 18041836] (r) Chongsiriwatana NP, Patch JA, Czyzewski AM, Dohm MT, Ivankin A, Gidalevitz D, Zuckermann RN, Barron AE. P Natl Acad Sci USA. 2008; 105:2794–2799.(s) Choi S, Isaacs A, Clements D, Liu DH, Kim H, Scott RW, Winkler JD, DeGrado WF. P Natl Acad Sci USA. 2009; 106:6968–6973.(t) Olsen CA, Ziegler HL, Nielsen HM, Frimodt-Moller N, Jaroszewski JW, Franzyk H. Chembiochem. 2010; 11:1356–1360. [PubMed: 20503219] (u) Hu J, Chen CX, Zhang SZ, Zhao XC, Xu H, Zhao XB, Lu JR. Biomacromolecules. 2011; 12:3839–3843. [PubMed: 21955251]
6. (a) Gelman MA, Weisblum B, Lynn DM, Gellman SH. Org Lett. 2004; 6:557–560. [PubMed: 14961622] (b) Senuma M, Tashiro T, Iwakura M, Kaeriyama K, Shimura Y. J Appl Polym Sci. 1989; 37:2837–2843.(c) Li GJ, Shen JR, Zhu YL. J Appl Polym Sci. 1998; 67:1761–1768.(d) Tiller JC, Liao CJ, Lewis K, Klibanov AM. P Natl Acad Sci USA. 2001; 98:5981–5985.(e) Ilker MF, Nusslein K, Tew GN, Coughlin EB. J Am Chem Soc. 2004; 126:15870–15875. [PubMed: 15571411] (f) Kuroda K, DeGrado WF. J Am Chem Soc. 2005; 127:4128–4129. [PubMed: 15783168] (g) Fuchs AD, Tiller JC. Angew Chem Int Edit. 2006; 45:6759–6762.(h) Mowery BP,

Lee SE, Kissounko DA, Epand RF, Epand RM, Weisblum B, Stahl SS, Gellman SH. J Am Chem Soc. 2007; 129:15474–15476. [PubMed: 18034491] (i) Sellenet PH, Allison B, Applegate BM, Youngblood JP. Biomacromolecules. 2007; 8:19–23. [PubMed: 17206783] (j) Waschinski CJ, Zimmermann J, Salz U, Hutzler R, Sadowski G, Tiller JC. Adv Mater. 2008; 20:104.(k) Sambhy V, Peterson BR, Sen A. Angew Chem Int Edit. 2008; 47:1250–1254.(l) Palermo EF, Sovadinova I, Kuroda K. Biomacromolecules. 2009; 10:3098–3107. [PubMed: 19803480] (m) Mowery BP, Lindner AH, Weisblum B, Stahl SS, Gellman SH. J Am Chem Soc. 2009; 131:9735–9745. [PubMed: 19601684] (n) Findlay B, Zhanel GG, Schweizer F. Antimicrob Agents Ch. 2010; 54:4049–4058.(o) Stratton TR, Howarter JA, Allison BC, Applegate BM, Youngblood JP. Biomacromolecules. 2010; 11:1286–1290. [PubMed: 20380430] (p) Tew GN, Scott RW, Klein ML, Degrado WF. Accounts Chem Res. 2010; 43:30–39.(q) Nederberg F, Zhang Y, Tan JPK, Xu KJ, Wang HY, Yang C, Gao SJ, Guo XD, Fukushima K, Li LJ, Hedrick JL, Yang YY. Nat Chem. 2011; 3:409–414. [PubMed: 21505501] (r) Li P, Poon YF, Li WF, Zhu HY, Yeap SH, Cao Y, Qi XB, Zhou CC, Lamrani M, Beuerman RW, Kang ET, Mu YG, Li CM, Chang MW, Leong SSJ, Chan-Park MB. Nat Mater. 2011; 10:149–156. [PubMed: 21151166] (s) Wang YQ, Xu JJ, Zhang YH, Yan HS, Liu KL. Macromol Biosci. 2011; 11:1499–1504. [PubMed: 21818858] (t) Timofeeva L, Kleshcheva N. Appl Microbiol Biot. 2011; 89:475–492.

7. Lienkamp K, Madkour AE, Musante A, Nelson CF, Nusslein K, Tew GN. J Am Chem Soc. 2008; 130:9836–9843. [PubMed: 18593128]
8. (a) Haberman E. Science. 1972; 177:314–322. [PubMed: 4113805] (b) Shai Y. Bba- Biomembranes. 1999; 1462:55–70. [PubMed: 10590302]
9. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Sci Transl Med. 2012; 4:165rv113.
10. Helmerhorst EJ, Reijnders IM, van't Hof W, Veerman ECI, Amerongen AVN. Febs Lett. 1999; 449:105–110. [PubMed: 10338113]
11. (a) Karlsson AJ, Pomerantz WC, Weisblum B, Gellman SH, Palecek SP. J Am Chem Soc. 2006; 128:12630–12631. [PubMed: 17002340] (b) Karlsson AJ, Pomerantz WC, Neilsen KJ, Gellman SH, Palecek SP. Acs Chem Biol. 2009; 4:567–579. [PubMed: 19518070] (c) Makovitzki A, Avrahami D, Shai Y. P Natl Acad Sci USA. 2006; 103:15997–16002.(d) Chongsiriwatana NP, Miller TM, Wetzler M, Vakulenko S, Karlsson AJ, Palecek SP, Mobashery S, Barron AE. Antimicrob Agents Ch. 2011; 55:417–420.
12. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Clin Infect Dis. 2004; 39:309–317. [PubMed: 15306996]
13. (a) Králíček J, Šebenda J. Journal of Polymer Science. 1958; 30:493–499.(b) Hall HK. J Am Chem Soc. 1958; 80:6404–6409.(c) Graf R, Lohaus G, Böner K, Schmidt E, Bestian H. Angewandte Chemie International Edition in English. 1962; 1:481–488.(d) de Ilarduya AM, Aleman C, Garcia-Alvarez M, Lopez-Carrasquero F, Munoz-Guerra S. Macromolecules. 1999; 32:3257–3263.(e) Hashimoto K. Prog Polym Sci. 2000; 25:1411–1462.(f) Zhang JH, Kissounko DA, Lee SE, Gellman SH, Stahl SS. J Am Chem Soc. 2009; 131:1589–1597. [PubMed: 19125651] (g) Lee MR, Stahl SS, Gellman SH, Masters KS. J Am Chem Soc. 2009; 131:16779–16789. [PubMed: 19886604] (h) Dane EL, Grinstaff MW. J Am Chem Soc. 2012; 134:16255–16264. [PubMed: 22937875] (i) Liu RH, Masters KS, Gellman SH. Biomacromolecules. 2012; 13:1100–1105. [PubMed: 22455338]
14. Epand RM, Epand RF, Mowery BP, Lee SE, Stahl SS, Lehrer RI, Gellman SH. J Mol Biol. 2008; 379:38–50. [PubMed: 18440552]
15. Tanaka T, Miyadera T. Heterocycles. 1982; 19:1497–1500.
16. (a) Salzmann TN, Ratcliffe RW, Christensen BG, Bouffard FA. J Am Chem Soc. 1980; 102:6161–6163.(b) Brennan J, Richardson G, Stoodley RJJ. Chem Soc-Chem Commun. 1980:49–49.
17. Andes D, Lepak A, Nett J, Lincoln L, Marchillo K. Antimicrob Agents Ch. 2006; 50:2384–2394.
18. NCCLS document M27- A2. 2. National Committee for Clinical Laboratory Standards; Wayne, PA: 2002. Reference method for broth dilution antifungal susceptibility testing of yeast: Approved standard.
19. (a) Chu P, Sadullah S. Curr Med Res Opin. 2009; 25:3011–3020. [PubMed: 19849324] (b) Kagan S, Ickowicz D, Shmuel M, Altschuler Y, Sionov E, Pitusi M, Weiss A, Farber S, Domb AJ,

Polacheck I. Antimicrob Agents Ch. 2012; 56:5603–5611.(c) Laniado-Laborin R, Cabrales-Vargas MN. Rev Iberoam Micol. 2009; 26:223–227. [PubMed: 19836985]

20. Please see the supporting information.

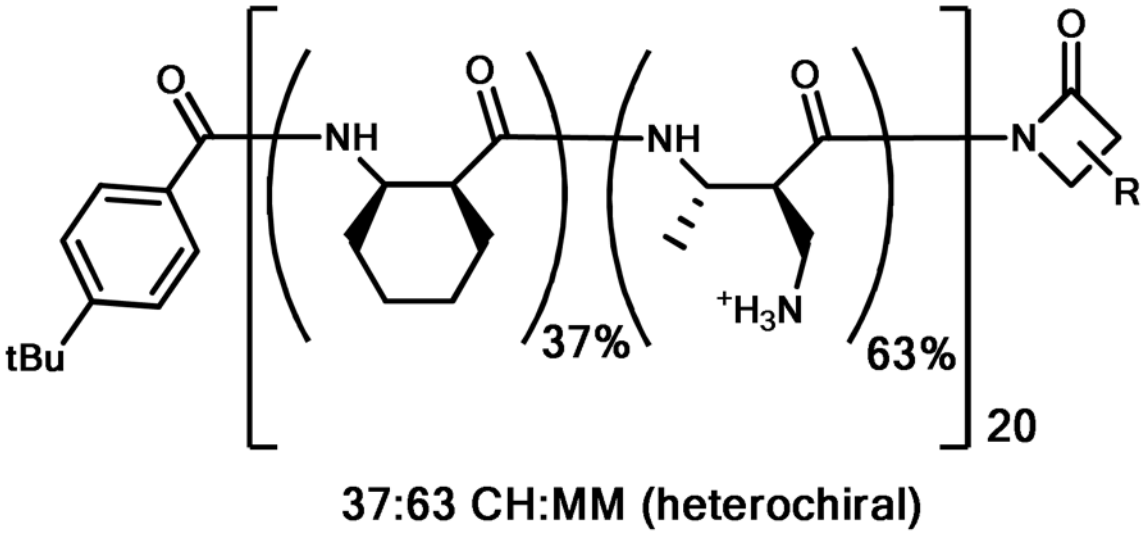

Figure 1.

Representative sequence- and stereo-random nylon- 3 co-polymer containing subunits derived from racemic *cis*-cyclohexyl β-lactam (**CH**) and racemic monomethyl aminomethyl β-lactam (**MM**). R represents the side chain group for either **CH** or **MM**. This co-polymer inhibits the growth of several bacterial species at relatively low concentrations but is only weakly hemolytic.[6h]

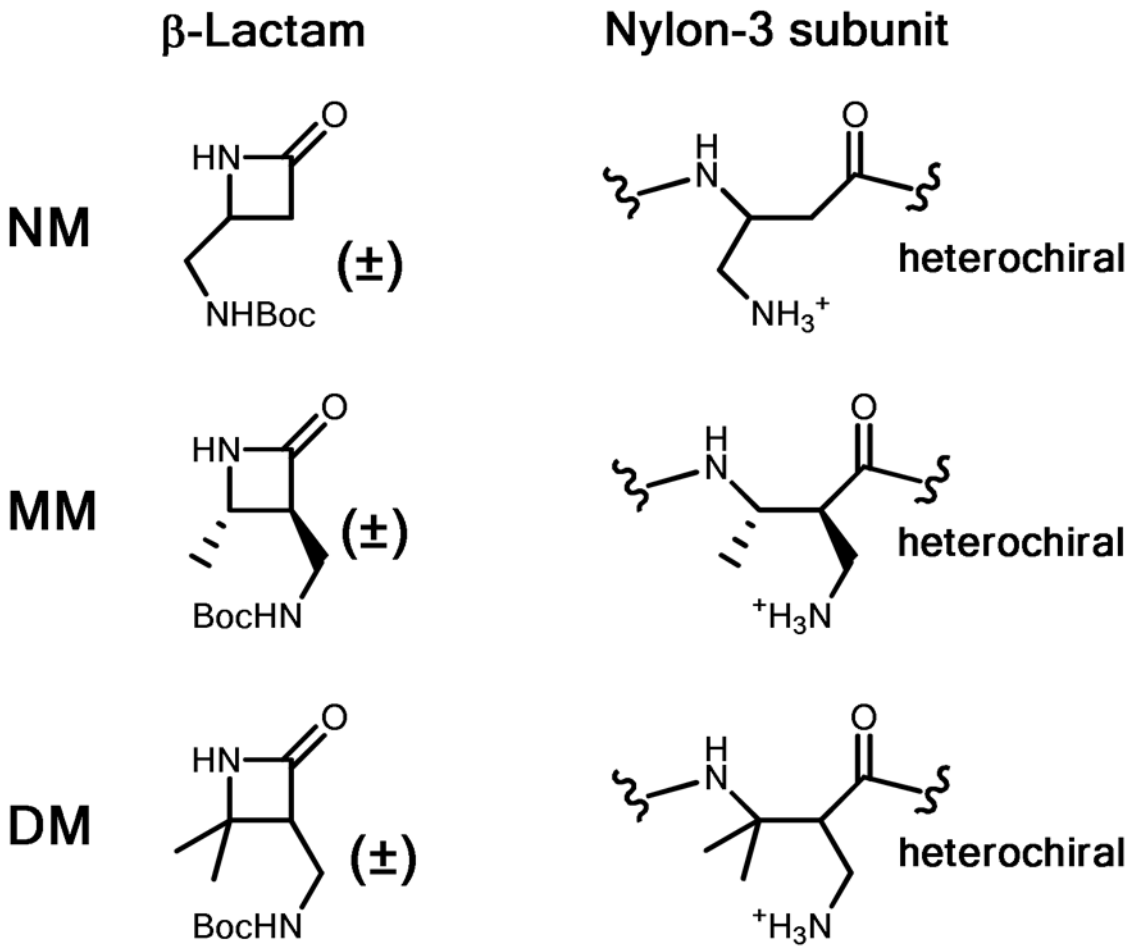

Figure 2.
β-Lactams and corresponding hydrophilic (cationic) subunits within the nylon-3 polymer chain. All β-Lactams are racemic, and the resulting polymers are heterochiral.

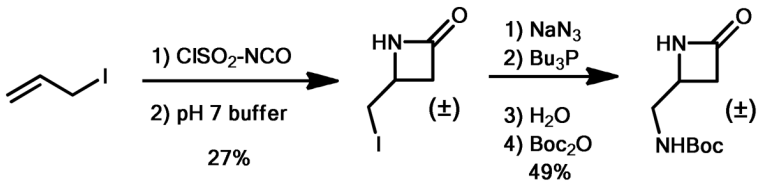

Figure 3.
The synthesis of racemic β-lactam **NM**.

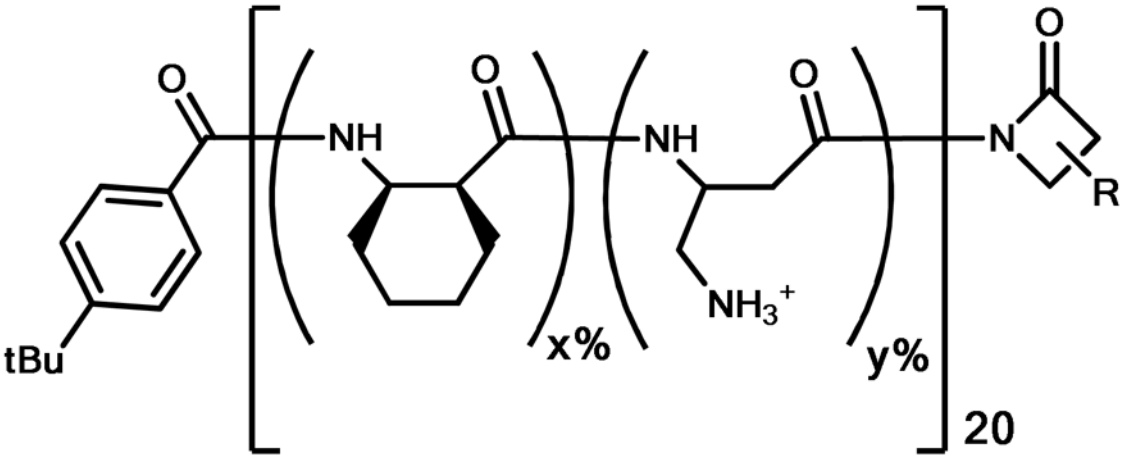

Figure 4.

The structure of **CH:NM** co-polymers. All copolymers are heterochiral and sequence-random. x + y = 100, y = 40, 50, 60, 70, 80, or 90. R represents the side chain group of either **CH** or **NM**.

Table 1

Physical and biological properties of nylon-3 polymers

polymer composition	DP[a]	PDI[b]	MIC, μg/mL[c]	IC_{10}, μg/mL[d]	HC_{10}, μg/mL[e]
60:40 **CH:NM**	23	1.29	100	> 400	100–200
50:50 **CH:NM**	23	1.29	50	> 400	200
40:60 **CH:NM**	21	1.29	13	> 400	> 400
30:70 **CH:NM**	20	1.26	6.3	> 400	> 400
20:80 **CH:NM**	22	1.33	3.1	100–200	> 400
10:90 **CH:NM**	17	1.24	3.1	> 400	> 400
NM	20	1.13	3.1	> 400	> 400
MM	22	1.03	200	> 400	> 400
DM	18	1.13	6.3	50	3.1
Ampho. B[f]	N/A	N/A	0.78	< 1.5	ND

[a] DP (degree of polymerization) indicates average polymer length (number of subunits).

[b] PDI is polydispersity index.

[c] MIC indicates the minimum inhibitory concentration for fungal growth as determined for *C. albicans* in the planktonic form.

[d] IC_{10} indicates the concentration necessary to induce 10% cell death in NIH 3T3 fibroblasts.

[e] HC_{10} indicates the concentration necessary for 10% lysis of human red blood cell.

[f] Amphotericin B was dissolved in 1:1 DMSO:water as the stock solution for bioassay. ND indicates hemolysis data were not obtained. All polymers have an N-terminal p-*t*butylbenzoyl group.

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Table 2

Antibacterial activities of cationic nylon-3 homopolymers

	MIC,[a] μg/mL			
polymer	**E. coli**	**B. subtilis**	**E. faecium**	**S. aureus**
NM	50	6.3	> 200	100
MM	> 200	6.3	> 200	100
DM	100	3.1	100	50

[a]MIC is the minimum inhibitory concentration for bacterial growth.