Chemstation was used for all UV spectrophotometric measurements. Complete spectra were quantitatively compared to known standards with use of a large segment of the spectra.

DNA Gyrase Inhibitor Activity. The DNA holoenzyme was prepared according to the procedure described[24] using a heparin-sepharose affinity column. The DNA gyrase supercoiling activity was assayed by a gel electrophoresis technique.[25] A 1% agarose horizontal gel slab was used. The amount of relaxed plasmid (CoE1) band and the supercoil band formed was determined by tracing the photographic negatives of the gel on a LKB Model 2022 Ultroscan densitometer. Because of the noncompetitive nature of the inhibitors, K_i may be determined as the concentration that caused 50% inhibition of the supercoil band formation.

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Synthesis and Biological Evaluation of Dipeptidyl and Tripeptidyl Polyoxin and Nikkomycin Analogues as Anticandidal Prodrugs

Eduardo Krainer,† Jeffrey M. Becker,‡ and Fred Naider*,†

Department of Chemistry, College of Staten Island, City University of New York, Staten Island, New York 10301, and Department of Microbiology and Program in Cellular, Molecular, and Developmental Biology, University of Tennessee, Knoxville, Tennessee 37996. *Received February 8, 1990*

Nine analogues (**1–5**, **9–12**) of the peptidyl nucleoside antibiotics nikkomycin and polyoxin were synthesized and tested for their biological properties against different strains of the pathogenic yeast *Candida albicans*. The tripeptidyl series of analogues (**1–5**) was designed to behave as prodrugs, releasing a toxic moiety upon enzymatic hydrolysis inside the cell. The dipeptidyl series (**9–12**) was designed as double-targeted drugs, being themselves toxic and releasing a toxic amino acid upon hydrolysis. All the analogues were prepared by coupling suitably protected amino acid *p*-nitrophenyl esters to 1-(5′-amino-5′-deoxy-α-D-allofuranuronosyl)uracil (UPOC) or the corresponding polyoxins and nikkomycins, with subsequent removal of the protecting group. Improved coupling yields were observed when DMSO was used as the solvent. Products were purified with use of reversed-phase HPLC and, in one case, diastereomeric products (compound **11**) were resolved by using this procedure. One of the tripeptidyl nikkomycins behaved as a prodrug but none of the compounds, as measured by in vitro testing, proved more effective than nikkomycin as an anticandidal agent.

Introduction

Opportunistic infections by *Candida albicans* are major contributors to morbidity and mortality in immunocompromised hosts.[1,2] Since the drugs currently in use for the treatment of candidiasis suffer from significant clinical limitations, a clear need exists for the development of effective anticandidal drugs.

Polyoxins and nikkomycins, closely related families of peptidyl nucleoside antibiotics, produced by species of *Streptomyces*, are potent competitive inhibitors of *C. albicans* chitin synthetase.[3,4] However, these compounds are not very effective fungicidal agents, when measuring growth of *C. albicans* in culture. These findings could be the result of the failure of these antibiotics to accumulate intracellularly or to their metabolism by the yeast. Degradation inside the cell does not seem to be the problem, since polyoxins have been shown to resist *Candida* peptidases.[5] In contrast, polyoxin and nikkomycin permeation into the cell takes places through peptide permeases; this is the step that appears to be rate limiting in the case of *C. albicans*.[3,6]

To explore increasing the uptake of peptidyl nucleosides by *C. albicans*, we have prepared a number of tripeptidyl nikkomycins and polyoxins using amino acid residues expected to improve recognition by the tripeptide transport system (Table I). These tripeptide prodrugs should not be inhibitors of chitin synthetase but should be hydrolyzed to toxic dipeptides. We have also synthesized a variety of polyoxin analogues containing a known antimetabolite (Table II, **10–12**). These multitargeted drugs have the potential to inhibit chitin synthetase and release the toxic amino acids oxalysine,[7] *m*-fluorophenylalanine[8] and N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP).[9] In this communication we report the synthesis of the above analogues and the evaluation of their stability and anticandidal activity.

Chemistry

1-(5′-amino-5′-deoxy-α-D-allofuranuronosyl)uracil, which we have previously designated UPOC (uracil polyoxin C),[5] is the carboxyterminus amino acid of the synthetic dipeptides **9**, **10**, **11**, **12**, and of tripeptides **1** and **2**. It was synthesized from uridine, according to Damodaran et al.[10]

† College of Staten Island.
‡ University of Tennessee.

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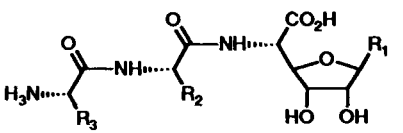
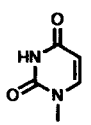
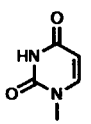
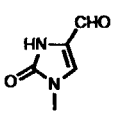
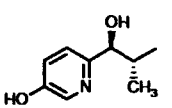
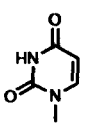
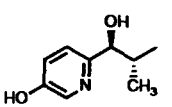
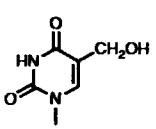
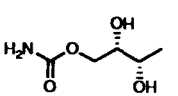
0022-2623/91/1834-0174$02.50/0

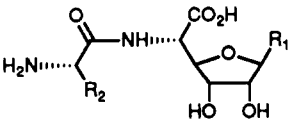
Table I. Synthetic Tripeptide Chitin Synthetase Inhibitors

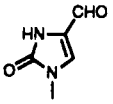
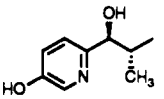
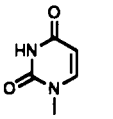
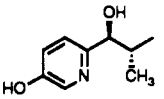
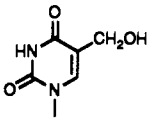
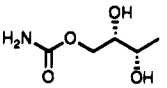
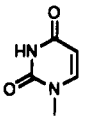
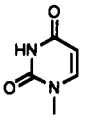
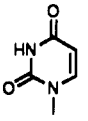
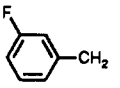
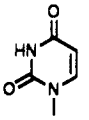
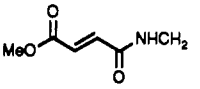


compd	R_1	R_2	R_3	chitin synthetase inhibition: ID_{50},[a] μg/mL	MIC,[b] μg/mL H-317	124	124N5	hydrolysis[c]	% transport inhibition[d] $(Leu)_2$	$(Met)_3$
1 Leu-(oct)Lys-UPOC		$CH_3(CH_2)_6CONH(CH_2)_4$	$(CH_3)_2CHCH_2$	50	>666	>666	>666	0	0	32
2 (oct)Lys-(oct)Lys-UPOC		$CH_3(CH_2)_6CONH(CH_2)_4$	$CH_3(CH_2)_6CONH(CH_2)_4$	80	>666	>666	>666	0	60	56
3 Leu-nikkomycin X			$(CH_3)_2CHCH_2$	30	166	83	333	33	0	22
4 Leu-nikkomycin Z			$(CH_3)_2CHCH_2$	100	>666	666	>666	33	0	28
5 Leu-polyoxin B			$(CH_3)_2CHCH_2$	20	333	333	666	5	0	27

[a] Concentration at which chitin synthetase is inhibited by 50%. [b] Minimum inhibitory concentration; lowest concentration of drug that clearly inhibited growth of strains H-317, 124, and 124N5 of *C. albicans*. [c] Rate of hydrolysis (percent hydrolyzed per 10 min) by cell extract. A value of zero indicates the compound was not hydrolyzed. [d] Percent inhibition of transport of $(Leu)_2$ or $(Met)_3$ into *C. albicans* H-317. A 10-fold molar excess of analogue was used in comparison to the transport substrates $(Leu)_2$ or $(Met)_3$. All measurements were taken at least three times and values obtained were not more than one tube (for MIC) or ten percent greater or lesser than those values reported in all tests.

Table II. Dipeptide Chitin Synthetase Inhibitors



compd	R_1	R_2	chitin synthetase inhibition: ID_{50}, μg/mL	MIC, μg/mL H-317	124	124N5	hydrolysis	% transport inhibition $(Leu)_2$	$(Met)_3$
			Naturally Occurring						
6 nikkomycin X			0.1	5	2.5	333	0	90	30
7 nikkomycin Z			0.3	20	10	666	0	80	20
8 polyoxin B			0.8	40	20	166	nd[a]	0	0
			Synthetic						
9 (oct)Lys-UPOC		$CH_3(CH_2)_6CONH(CH_2)_4$	0.5	>666	666	>666	0	50	10
10 Oly-UPOC		$H_2N(CH_2)_2OCH_2$	100	>1000	>1000	>1000	0	58	0
11 *m*-F-Phe-UPOC			2.0 (>100)[b]	>1000[c]	>1000[c]	>1000[c]	33 (0)[b]	6 (3)[b]	0[c]
12 FMDP-UPOC			100	250	500	1000	0	7	0

[a] nd is not done. [b] Peak I (peak II). [c] Mixture of diastereomers. See footnotes of Table I for explanation of tests and values expressed.

All analogues were prepared by coupling suitably protected amino acid *p*-nitrophenyl esters with UPOC or with polyoxins and nikkomycins (compounds **6–9**) and removing the protecting group. Compound **9** has previously been synthesized in our laboratory.[11] In the former report, Z-(oct)Lys-ONp (*N*-α-(benzoyloxycarbonyl)-*N*-ε-octanoyllysine *p*-nitrophenyl ester) was coupled to UPOC in DMF/H_2O, in the presence of *N*-methylmorpholine (NMM). The overall yield (coupling and deprotection) was only 29%. This was attributed to the low solubility of UPOC in DMF. We achieved a dramatic improvement in yield and product purity in the preparation of **9** by using DMSO as solvent and DIEA as the base. The overall yield of coupling and deprotection was 72%, based on UPOC.

Since the solubility properties of the dipeptides were better, DMF/H_2O was used as the coupling solvent for preparing all tripeptides. The yield of the coupling reaction varied significantly from batch to batch for (oct)-Lys-(oct)Lys-UPOC (**2**). Low yields were consistently accompanied by the appearance of an impurity detected during the monitoring of the reaction by TLC. This impurity had an R_f slightly higher than the starting unprotected dipeptide, but did not give a positive reaction with ninhydrin, indicating that a free amine was not present. The impurity was isolated during the purification of **2**, and NMR analysis showed it to be the N-formylated dipeptide. Formate salts can be formed during precipitation of the products of catalytic transfer hydrogenation where formic acid is the hydrogen donor, and NMR analysis showed that **9** exists at least partially as a formate salt. Formylation must occur by a mechanism that has been described for couplings with active esters of amino components present as acetate salts, and involves the formation of mixed anhydride intermediates.[12,13] The side reaction was not

(10) Damodaran, N. P.; Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1971**, *93*, 3812.

(11) Smith, H. A.; Shenbagamurthi, P.; Naider, F.; Kundu, B.; Becker, J. M. *Antimicrob. Agents Chemother.* **1986**, *29*, 33.

(12) Bodanszky, M. *The Peptides*. E. Gross, J. Meienhoffer, Eds.; Academic Press: New York, 1979; Vol. 1, p 150.

detected during the synthesis of the other tripeptides (1, 3, 4, 5) where the starting dipeptides were previously purified by HPLC and were present as trifluoroacetates.

Polyoxin B was purified according to a previously reported method,[14] and its structure confirmed by ^1H and ^{13}C NMR spectroscopy. The proton NMR of leucyl-polyoxin B (5) in DMSO showed the expected resonances, but the H-1′ doublet as well as one of the CONH doublets were split (see Experimental Section). This was attributed to the coexistence of two stable conformers of roughly the same populations in DMSO.

The two epimers of 11 were separated by HPLC, and the peak with faster mobility (peak I) identified as the L,L diastereomer on the basis of its hydrolysis by *C. albicans* peptidases and inhibition of chitin synthetase. In contrast the slow moving epimer (peak II, D,L diastereomer) was resistant to hydrolysis under the same conditions and did not inhibit chitin synthetase (see Biological Results and Discussion).

Biological Results and Discussion

Inhibition of Chitin Synthetase. The synthetic polyoxins and nikkomycins fall into two distinct categories. As expected, none of the tripeptidyl derivatives (1–5) were efficient inhibitors of chitin synthetase (Table I). The best inhibitor (5) was approximately 25 times less active than the parent compound (8, see Table II). In comparison the nikkomycin derivatives (3 and 4) were a minimum of 270-fold less active as compared to the parent compounds 6 and 7, respectively (Table I and Table II). Of the dipeptidyl polyoxins (9–12) only 9 and 11 (L,L diastereomer) were reasonably good inhibitors (Table II). In the case of the oxalysine containing polyoxin (10) the poor inhibition may be attributed to the high hydrophilicity of the L-4-oxalysine (Oly) residue.[11,15,16] On the basis of their poor inhibition of chitin synthetase, the tripeptidyl antibiotics are expected to be active against *C. albicans* only if they are efficiently transported into this yeast and hydrolyzed to an active drug.

Competition for Peptide Transport. As shown in Table I, with the exception of 2 none of the tripeptides competed with the uptake of $(Leu)_2$. Unexpectedly compound 2 competed with tripeptide and dipeptide transport. All other tripeptidyl compounds (1, 3–5) competed poorly with the tripeptide substrate $(Met)_3$. The nikkomycins, 6 and 7, have high affinity for the dipeptide transport system (Table II), while polyoxin B (8) does not compete with the uptake of either $(Leu)_2$ nor $(Met)_3$. Among the synthetic dipeptides, only 9 and 10 were reasonably well recognized by the system transporting $(Leu)_2$ (Table II). These competition experiments can not be fully explained on the basis of what is currently known about the structural specificity of peptide transport in *C. albicans*.[3]

Hydrolysis by Cell Extracts. The tripeptidyl polyoxins are ineffective in vitro inhibitors but may be activated by intracellular enzymes to generate polyoxins and nikkomycins which are effective chitin synthetase inhibitors (Tables I and II). We found that cell extracts from *C. albicans* H-317 degraded 3–5 to leucine and the corresponding polyoxin and nikkomycins. Degradation of 3 and 4 was faster than that seen for norleucyl-uracil polyoxin C (Nle-UPOC)[5] whereas degradation of 5 was somewhat slower. 1 and 2 were not degraded at all. These results, as well as the resistance to hydrolysis by 9 and other analogues[11,15] suggest that the very hydrophobic and bulky residue in the second amino acid of 1 and 2 stabilizes both peptide bonds toward peptidase degradation by *Candida* cell extract. The only synthetic dipeptide which was readily hydrolyzed by the cell extract was the L,L diastereomer of 11 (peak I) (see Table II). Peak II was resistant to hydrolysis, indicating that it is the D,L diastereomer.

Table III. Effect of Various Toxic Amino Acids on Growth of *C. albicans* Strains[a]

amino acid	MIC, ug/mL H-317	124	124N5
oxalysine	31	31	31
D,L-*m*-fluorophenylalanine	>1000	>1000	>1000
FMDP[b]	7.8	7.8	15.6

[a] *C. albicans* strains H-317, 124, and 124N5 were grown in the presence of various amounts of toxic amino acid and their minimum inhibitory concentration (MIC) determined. [b] FMDP is N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid.

Inhibition of Growth of *C. albicans*. We studied the effect of peptides (1–12) and the toxic amino acids on the growth of three strains of *C. albicans* (Tables I–III). Two of the strains were wild type (H-317 and 124) whereas strain 124N5 is resistant to nikkomycin. *m*-F-Phe has been found to be inactive against *C. albicans* (strains B311 and 759) unless it is transported inside the cell by peptide carriers.[8,17] Similar results were observed against the strains examined in this investigation (Table III). Oxalysine shows some toxicity against all the strains tested (Table III). In contrast to the reported lack of toxicity of FMDP when tested against *C. albicans* AMB 25 (MIC ≥ 200 μg/mL),[18] this amino acid showed high toxicity toward the three strains tested here, causing growth inhibition at 7.8 μg/mL (Table III).

The dipeptide 12 showed low toxicity (Table II), a fact that can be explained in terms of its failure to inhibit chitin synthetase and its low transportability into the cell. On the other hand, the low toxicity of dipeptide 10, a poor chitin synthetase inhibitor which has a much higher affinity for the $(Leu)_2$ transport system, can be explained in terms of its resistance to hydrolysis to toxic Oly if it reaches the cytoplasm. The nontoxicity of 11 despite its being a good chitin synthetase inhibitor and a good substrate for peptidases, can be explained by its low transportability into the cytoplasm.

None of the tripeptidyl polyoxins were effective anticandidal agents (Table I). Leu-nikkomycin X which was the most potent synthetic analogue had an MIC (minimum inhibitory concentration) = 83 μg/mL against *C. albicans* 124. This MIC is lower than that found for the dipeptide 9 despite the fact that the dipeptide is a 60-fold more efficient inhibitor of chitin synthetase. Thus it appears that 3 behaves as a prodrug. It is transported into the pathogen and hydrolyzed to the toxic nikkomycin X inside the cell. Its low effectiveness is explained by its low affinity for both the di- and oligopeptide transport systems. The low toxicity of 1 and 2 is not surprising because neither of these compounds is hydrolyzed by cell extract peptidases and the intact tripeptides are poor inhibitors of chitin synthetase. In comparison to 3, compound 5 is hydrolyzed at a slower rate inside the cell (see above) and

(13) Bodanszky, M. *Peptides 1968*, Proceedings of the 9th European Peptide Symposium, Orsay, France, 1968; Bricas, E., Ed.; North Holland Publishers: Amsterdam, 1968; p 150.
(14) Shenbagamurthi, P.; Smith, H. A.; Becker, J. M.; Naider, F. *J. Chromatogr.* **1982**, *245*, 133.
(15) Khare, R.; Becker, J. M.; Naider, F. R. *J. Med. Chem.* **1988**, *31*, 650.
(16) Hori, M.; Kakiki, K.; Misato, T. *Agric. Biol. Chem.* **1974**, *38*, 691.
(17) Payne, J. W.; Shallow, A. *FEMS Microb. Lett.* **1985**, *28*, 55.
(18) Andruszkiewicz, R.; Chmara, H.; Milewski, S.; Borowski, E. *J. Med. Chem.* **1987**, *30*, 1715.

releases polyoxin B, which is not as potent a chitin synthetase inhibitor as nikkomycin X (Table II). Those differences explain the limited effectiveness of 5 against the yeast.

The difference in toxicities of 3 and 4 is difficult to explain, since their rate of transport, hydrolysis, and inhibiting power of the released moiety is very similar. One possible explanation is that although their extent of affinity for the oligopeptide transport system is almost the same, leucyl-nikkomycin Z is not translocated to a significant extent to an intracellular site. Supporting this hypothesis are the data of Yadan et al.[6] who showed that in *C. albicans* nikkomycin and dimethionine had similar K_m values for the dipeptide transport system (4 μM and 6 μM, respectively) whereas their respective V_{max} values were 0.15 pmol min^{-1} and 3.5 pmol min^{-1}.

Another explanation is that if multiple chitin synthetases exist in *C. albicans* as has been shown for *S. cerevisiae*,[19-21] then it is possible that the in vitro activity measured does not accurately reflect in vivo chitin synthesis of the cell. Further studies must be carried out to determine the number of isozymes of chitin synthetase present in *C. albicans*.

Conclusion

This study reports the successful synthesis of novel dipeptides and tripeptides which are analogues of polyoxins and nikkomycins. Dipeptides containing Oly or FMDP were resistant to Candidal peptidases and were consequently ineffective drugs. A polyoxin analogue containing *m*-F-Phe was not recognized by the peptide transport system.

One tripeptidyl nikkomycin (3) was hydrolyzed to an active component after entering the yeast and therefore appears to behave as a prodrug. The results show that the structural specificity for peptide transport in *C. albicans* may be much more complex than previously believed. It is clear that additional knowledge of those molecular interactions which are involved in the actual translocation of a peptide into the yeast must precede the design of chitin synthetase inhibitors which will efficiently kill this pathogen.

Experimental Section

High-performance liquid chromatography (HPLC, analytical, semipreparative) was carried out on a Waters chromatograph (Waters Associates, Milford, MA) equipped with two Model 510 solvent delivery units, a U6K injector and a Model 481 variable wavelength UV detector, a Model 680 automated gradient controller, and a Model 730 data module. Analytical separations were carried out on a Waters micro-Bondapak C18 reversed-phase column (30 cm × 3.9 mm, particle size 10 μm) or an Aquapore spheri-5 RP18 column (22 cm × 4.6 mm). The mobile phase was MeOH–H_2O–TFA at a flow rate of 1.4 mL/min. The absorbance of the column eluants was recorded at 254 nm for all polyoxins. For purification of final products two Waters C18 semipreparative columns (30 cm × 7.8 mm and 15 cm × 19 mm) or an Aquapore RP-18 (25 cm × 10 mm) were used. TLC analysis was carried out on silica gel 60 F_{254} plates, 0.25 mm (Merck, Darmstadt).

The NMR spectra were recorded on a Bruker WP-200SY instrument, and the proton chemical shift values are reported in ppm relative to internal tetramethylsilane. ^{13}C chemical shifts are relative to external C_6D_6 (δ = 128.0). Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. For elemental analyses, freeze-dried samples were further dried in vacuum over P_2O_5 at 65 °C for 48 h. Elemental analyses were performed by Galbraith laboratories, Knoxville, TN. Nikkomycin X was purified by chemochromatography[4] from a crude obtained from Bayer Pflanzenschutz Leverkusen. Nikkomycin Z (Calbiochem, San Diego, CA) was purified before use by HPLC (solvent, TFA 0.10%; flow rate, 4.50 mL/min; 19 cm × 150 mm column; 2-mg shots). Polyoxin B was obtained from an agricultural fungicide preparation supplied by Kaken Chemical (Honkomagome, Tokyo, Japan) as described in ref 14, where it is referred to as peak I. UPOC (uracil polyoxin C) was prepared according to Damodaran et al.[10] and obtained mainly as a zwitterion. *N*-α-(benzyloxycarbonyl)-*N*-ε-octanoyllysine *p*-nitrophenyl ester was prepared as described in ref 11. L-4-Oxalysine was obtained as a gift from Dr. Hong-Long Zhang, Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai. N^2-(*tert*-Butoxycarbonyl)-N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid was prepared according to ref 9. D,L-*m*-Fluorophenylalanine was purchased from Chemical Dynamics Corp. (South Plainfield, NJ).

Polyoxin B (8). Polyoxin B was purified according to ref 14 and was HPLC homogeneous. Its structure was confirmed by ^1H and ^{13}C NMR. The assigned proton resonances at 200 MHz in DMSO-d_6 were δ_H 4.7 (1 H, dd, H-5′), 5.3–5.7 (m, OH), 5.81 (1 H, d, J = 6 Hz, H-1′), 6.53 (2 H, br s, $CONH_2$), 7.39 (1 H, s, H-6), 8.0 (3 H, br, NH_3^+), 9.0 (1 H, d, J = 8.2 Hz, CONH), 11.48 (1 H, br s, NH-3). The ^{13}C resonances at 50 MHz were $\delta_C^{H_2O}$ 55.2, 56.8 (α-CH), 57.0 (CH_2OH), 69.1, 70.2, 70.4 (CH, ribose and polyoxamic acid side chain), 73.0 (CH, ribose), 83.3 (CH, ribose), 91.0 (CH, ribose), 65.8 (CH_2-5″), 114.4 (C-5), 140.4 (C-6), 152.0 (C-2), and 159.5, 165.3, 168.3, and 172.2 (C-4, CONH, $OCONH_2$, and CO_2H, unassigned).

***N*-ε-Octanoyllysyl-uracil Polyoxin C ((Oct)Lys-UPOC) (9).** Z-(oct)Lys-ONp[11] (116 mg, 0.22 mmol) and UPOC (60 mg, 0.2 mmol) were suspended in DMSO (2 mL). Diisopropylethylamine was added (35 μL, 0.2 mmol) (pH ≃ 7) and the mixture stirred at room temperature for 20 h. (The solution cleared up after a few hours.) After acidification with acetic acid (0.1 mL), the solvents were evaporated in vacuo to dryness, and the residue suspended in 1% acetic acid, triturated with a spatula, filtered, washed with water, and dried under vacuum. The solid was resuspended in ether, triturated, and filtered. Yield 0.108 g (80%). The product, Z-(oct)Lys-UPOC, was homogeneous on silica thin layers with *n*-BuOH–AcOH–H_2O (4:1:2, R_f = 0.59) as eluant and was subjected to transfer hydrogenation using Pd black (≃30 mg) and 90% formic acid (0.3 mL) in methanol (3 mL). The initial suspension clears with time and after 3 h the catalyst was removed by filtration through Celite, the filtrate evaporated to dryness, and the residue suspended in ether, triturated, and filtered, yield = 78 mg (72%). (Oct)Lys-UPOC was 97% pure as judged by HPLC, and its mobility identical with a standard,[11] R_f = 0.45 (1-butanol–acetic acid–water, 4:1:2).

***N*-ε-Octanoyllysyl-*N*-ε-octanoyllysyl-UPOC (2).** 9, (40 mg, 74 μmol) from the previous step, and Z(oct)Lys-ONp (46.8 mg, 89 μmol) were dissolved in DMF (0.7 mL). *N*-Methylmorpholine (8.15 μL, 74 μmol) was added and the mixture stirred at room temperature for 15 h. After acidification with acetic acid (0.1 mL), the solvents were evaporated in vacuo to dryness. The residue was treated with ether, filtered, washed with 4% citric acid and water, and dried under vacuum. The product was subjected to transfer hydrogenation as described for 9 for 1.5 h. The catalyst was removed by filtration through Celite, the filtrate evaporated to dryness, and the residue suspended in ether and filtered. A total of 36.2 mg of crude (oct)Lys-(oct)Lys-UPOC was obtained, 24 mg of which were purified by reversed-phase HPLC using a 250 × 10 mm semiprep Aquapore RP-18 column with 0.1% TFA–MeOH (47:53) as eluant. A total of 13.9 mg of pure 2 was obtained after concentration, suspension in ether and filtration: yield = 31%, based on 9; R_f = 0.57 (1-butanol–acetic acid–water, 4:1:2) $[\alpha]^{25}_D$ = +8.8 (c = 0.2, DMF); NMR $\delta_H^{DMSO\text{-}d_6}$ 0.85 (6 H, t, J = 6.2 Hz, CH_3), 1.1–1.8 (32 H, m, $-CH_2-$), 2.03 (4 H, m, α-CH_2 of octanoyl), 3.00 (4 H, m, ε-CH_2 of Lys), 3.76, 4.0–4.2 (4 H, m, H-2′, 3′, 4′, α-CH, unassigned), 4.35 (1 H, m, α-CH), 4.50 (1 H, m, α-CH), 5.26 (1 H, br, OH), 5.44 (1 H, br, OH) 5.68 (1 H, d, J = 8.1 Hz, H-5), 5.79 (1 H, d, J = 6.4 Hz, H-1′), 7.54 (1 H, d, J = 8.1 Hz, H-6), 7.79 (2 H, t, 5.8 Hz, ε-NH of Lys), 7.8–8.5 (4 H, unresolved, two broad peaks at 8.05 and 8.44 ppm when run at 400 MHz, NH_3^+ and CONH, respectively), 8.54 (1 H, d, J =

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8.3 Hz, CONH), 11.38 (1 H, br s, NH-3). Anal. Found: C, 51.47; H, 7.33; N, 10.70. Calcd. for $C_{38}H_{65}O_{11}N_7CF_3CO_2H\cdot H_2O$: C, 51.77; H, 7.39; N, 10.56.

N-α-Formyl-N-ε-octanoyllysyl-UPOC. Small amounts (1 mg) of this formylated dipeptide were isolated as an impurity during the HPLC purification of tripeptide **2**: R_f = 0.47 (1-butanol–acetic acid–water, 4:1:2); NMR $\delta_H^{DMSO\text{-}d_6}$ 0.86 (3 H, t, J = 6.1 Hz, CH_3), 1.15–1.74 (16 H, m, $-CH_2-$), 2.02 (2 H, t, J = 7.3 Hz, α-CH_2 of octanoyl), 2.99 (2 H, m, ε-CH_2 of Lys), 3.95–4.15 (3 H, m, H-2′,3′,4′), 4.43 (1 H, m, α-CH), 4.56 (1 H, m, α-CH), 5.28 (1 H, br, OH), 5.44 (1 H, br, OH), 5.64 (1 H, d, J = 8.3 Hz, H-5), 5.79 (1 H, d, J = 6.4 Hz, H-1′), 7.45 (1 H, d, J = 8.3 Hz, H-6), 7.75 (2 H, br t, ε-NH of Lys), 8.03 (1 H, s, CH of formyl), 8.30 (1 H, d, J = 8.8 Hz, CONH), 8.41 (1 H, br, CONH), 11.37 (1 H, s, NH-3). FAB mass spectrometry indicated a molecular ion [M + H$^+$] at 570.2 da. Calcd MW: 569.6 da.

Leucyl-N-ε-octanoyllysyl-UPOC (1). The procedure was similar to the preparation of **2** and was carried out with use of Z-Leu-ONp (51 mg, 132 μmol) and **9** (72.1 mg, 110 μmol) in 1 mL DMF. **9** had been previously purified, according to ref 11 and was a TFA salt. The coupling time was 42 h and 50 mg of Z-Leu-(oct)Lys-UPOC (yield = 58%) was obtained. It was homogeneous on silica thin layers, with n-BuOH–AcOH–H_2O (4:1:2, R_f = 0.67) as eluant. The deprotection took 1.5 h, and 32.5 mg of the tripeptide **1** was obtained (overall yield = 42%) which was homogeneous on silica thin layers, with the same eluant (R_f = 0.54) and did not need further purification. Reversed-phase HPLC analysis (spheri-5 RP-18 column, eluant 0.1% TFA–MeOH 45:55) showed higher than 99% purity: $[\alpha]^{25}_D$ = +8.9 (c = 0.09, DMF); NMR $\delta_H^{DMSO\text{-}d_6}$ 0.86 (9 H, m, CH_3), 1.15–1.75 (19 H, m, $-CH_2-$ and CH), 2.02 (2 H, t, J = 7.2 Hz, α-CH_2 of octanoyl), 2.98 (2 H, m, ε-CH_2 of Lys), 3.67, 4.0–4.4 (6 H, m, H-2′,3′,4′,3 α-CH), 5.26 (1 H, d, J = 4.4 Hz, OH), 5.60 (1 H, d, J = 8.1 Hz, H-5), 5.75 (1 H, d, J = 6.8 Hz, H-1′), 7.67 (1 H, d, J = 8.1 Hz, H-6), 7.82 (1 H, t, J = 5.1 Hz, ε-NH of Lys), 7.95 (1 H, br d, J = 5.9 Hz, CONH), 8.49 (1 H, d, J = 7.3 Hz, CONH). Anal. Found: C, 51.63; H, 7.50; N, 11.62. Calcd for $C_{30}H_{50}O_{10}N_6\cdot 2.5H_2O$: C, 51.49; H, 7.92; N, 12.01.

Leucyl-nikkomycin Z (4). Nikkomycin Z (double TFA salt) (38 mg, 50 μmol) and Z-Leu-ONp (23.2 mg, 60 μmol) were dissolved in a mixture of DMF (0.5 mL) and H_2O (0.15 mL). NMM (17 mL, 150 μmol) was added and the mixture allowed to react, with stirring, at room temperature, for 24 h. After acidification with acetic acid (50 μL), the solvents were evaporated in vacuo, and the residue was treated with ether, filtered, and subjected to transfer hydrogenation for 2 h as described above. After filtration through Celite, washing the filter with water, evaporation, and precipitation with ether, 35.4 mg of crude material was obtained, which was purified by HPLC on a semiprep Aquapore RP-18 column with 0.1% TFA–MeOH (95:5) as eluant. Homogeneous **5** (26.1 mg) was obtained, yield = 45%: R_f = 0.27 (1-butanol–acetic acid–water, 4:1:2); $[\alpha]^{25}_D$ = +17.0 (c = 0.1, AcOH); NMR $\delta_H^{DMSO\text{-}d_6}$ 0.66 (3 H, d, J = 6.4 Hz, CH_3-3″), 0.89 (6 H, m, CH_3 of Leu), 1.45–1.70 (3 H, m, β-CH_2 and γ-CH of Leu), 3.8–4.2, 4.5–4.7 (H-2′,3′,4′,5′,2″, α-CH of Leu, unassigned), 4.75 (1 H, br, H-4″), 5.70 (1 H, d, J = 7.8 Hz, H-5) 5.81 (1 H, d, J = 5.4 Hz, H-1′), 7.2–7.4 (2 H, m, H-3‴,4‴), 7.49 (1 H, d, J = 7.8 Hz, H-6), 8.0–8.2 (4 H, br m, NH_3^+ and H-6‴), 8.43 (1 H, d, J = 8.3 Hz, CONH), 8.63 (1 H, d, J = 7.8 Hz, CONH), 9.95 (1 H, br s, ArOH), 11.42 (1 H, s, NH-3). Anal. Found: C, 34.70; H, 4.23; N, 7.45. Calcd for $C_{26}H_{36}O_{11}N_6\cdot 3.5CF_3CO_2H\cdot 8H_2O$: C, 34.41; H, 4.86; N, 7.30.

Leucyl-nikkomycin X (3). Nikkomycin X (double TFA salt) (40 mg, 53 μmol) was processed as described above for nikkomycin Z, with the exception that 1.2 equiv of Boc-Leu-ONp was used as the coupling reagent. Coupling time was 30 h. Deprotection was accomplished with TFA–CH_2Cl_2 (50:50) in 0.5 h. The solvents were evaporated under vacuo, and the residue was precipitated with ether and filtered. Crude product (36.2 mg) was purified by HPLC using a 15 cm × 19 mm C-18 column, with TFA (0.05%)–MeOH (96:4) as the eluant. Homogeneous **3** (20.5 mg) was obtained after concentration and freeze-drying (overall yield = 39%): R_f = 0.29 (1-butanol–acetic acid–water, 4:1:2); $[\alpha]^{25}_D$ = +18.0 (c = 0.1, AcOH). NMR $\delta_H^{DMSO\text{-}d_6}$ 0.66 (3 H, d, J = 6.6 Hz, CH_3-3″), 0.89 (6 H, m, CH_3 of Leu), 1.45–1.70 (3 H, m, β-CH_2 and γ-CH of Leu), 3.75–4.65 (H-2′,3′,4′,5′,2″, α-CH of Leu, unassigned), 4.76 (1 H, broad, H-4″), 5.47 (1 H, d, J = 4.3 Hz, H-1′), 7.2–7.4 (2 H, m, H-3″,4″), 7.72 (1 H, s, H-5), 8.0–8.2 (4 H, br m, NH_3^+ and H-6″), 8.46 (1 H, d, J = 9.0 Hz, CONH), 8.64 (1 H, d, J = 9.5 Hz, CONH), 9.24 (1 H, s, CHO), 11.2 (1 H, s, NH-3). Anal. Found: C, 38.96; H, 4.57; N, 8.84. Calcd for $C_{26}H_{36}O_{11}N_6\cdot 3CF_3CO_2H\cdot 2.5H_2O$: C, 38.60; H, 4.45; N, 8.44%.

Leucyl-polyoxin B (5). Polyoxin B (TFA salt) (62 mg, 0.1 mmol) and Z-Leu-ONp (46 mg, 0.12 mmol) were dissolved in a mixture of DMF (1 mL) and H_2O (0.3 mL). NMM (22.4 μL, 0.2 mmol) was added and the mixture stirred at room temperature for 44 h. After acidification with acetic acid (0.1 mL), the solvents were evaporated in vacuo, and the residue was treated with ether and filtered. A total of 40 mg of Z-Leu-polyoxin B (yield = 53%) was obtained, R_f = 0.72 on silica thin layers with n-BuOH–AcOH–H_2O (1:1:1) as the eluant.

This product was subjected to transfer hydrogenation in methanol (2 mL) with palladium black (~30 mg) and 90% formic acid (0.2 mL) for 24 h. The crude product was purified on a C_{18} reversed-phase column with 0.05% TFA–MeOH (100:1) as eluant. The fractions containing pure **4** were pooled, evaporated under vacuum, and freeze-dried. A total of 7.2 mg of material, showing more than 92% purity by HPLC analysis, was obtained. Other fractions containing **4** were concentrated under vacuum and reinjected onto the 19 cm × 150 mm C-18 column with 0.05% TFA–MeOH (100:2) as the eluant. A total of 4.5 mg of 100% HPLC homogeneous material was obtained after concentration and freeze-drying. (overall yield = 11%): R_f = 0.22 (1-butanol–acetic acid–water, 4:1:2); $[\alpha]^{26}_D$ = +14.0 (c = 0.1, AcOH); NMR $\delta_H^{DMSO\text{-}d_6}$ 0.89 (6 H, d, J = 5.4 Hz, CH_3 of Leu), 1.45–1.80 (3 H, m, β-CH_2 and γ-CH of Leu), 3.6–4.3, 4.4–4.7 (m, unresolved, H-2′,3′,4′,3″,4″, CH_2-5″, CH_2OH, 3 α-CH), 5.81 and 5.82 (1 H, split d, J = 5.9 Hz, H-1′), 6.4–6.8 (2 H, br, $OCONH_2$), 7.42 (1 H, s, H-6), 8.0–8.2 (3 H, m, NH_3^+), 8.65 and 8.90 (1 H, split d, J = 8.1 Hz, CONH), 8.74 (1 H, d, J = 7.8 Hz, CONH), 11.50 (1 H, s, NH-3), 13.15 (1 H, br s, CO_2H). Anal. Found: C, 34.60; H, 3.90; N, 8.46. Calcd for $C_{23}H_{36}O_{14}N_6\cdot 3.5CF_3CO_2H\cdot H_2O$: C, 34.73; H, 4.03; N, 8.10.

L-4-Oxalysyl-UPOC (Oly-UPOC) (10). N^2,N^6-Bis(benzoyloxycarbonyl)-L-4-oxalysine was prepared by standard procedures[22] from L-4-oxalysine and crystallized from ether (R_f = 0.52, CH_2Cl_2–MeOH, 10:3.2). It was converted into the p-nitrophenyl ester by coupling to p-nitrophenol with dicyclohexylcarbodiimide in THF and crystallizing from 2-propanol (yield = 50%). This product was homogeneous on silica thin layers (R_f = 0.76, CH_2Cl_2–MeOH 95:5): NMR $\delta_H^{CDCl_3}$ 3.32–3.47 (2 H, m, ε-CH_2), 3.53–3.66 (2 H, m, δ-CH_2), 3.84 (1 H, dd, J = 9.3 Hz, 3.0 Hz, β-CH), 4.08 (1 H, dd, J = 9.3, 3.0 Hz, β-CH), 4.77 (1 H, m, α-CH), 4.99 (1 H, br, ε-NH), 5.08 (2 H, s, benzylic CH_2), 5.15 (2 H, s, benzylic CH_2), 5.79 (1 H, d, J = 7.2 Hz, α-NH), 7.2–7.4 (12 H, m, phenyl), 8.17 (2 H, d, J = 8.5 Hz, meta of p-nitrophenyl).

α,ε-Bis(benzyloxycarbonyl)-Oly-ONp (85.7 mg, 160 μmol) was coupled to UPOC·HCl (46.0 mg, 145 μmol) in DMSO (1.45 mL) in the presence of DIEA (50.5 μL, 290 μmol) at room temperature for 25 h. The reaction mixture was acidified with acetic acid (300 μL), and the solvents were evaporated in vacuo to dryness. The residue was suspended and triturated in AcOH 1%, filtered, suspended, and triturated in ether, and filtered. This diprotected dipeptide was homogeneous on silica gel thin layers (R_f = 0.29, 1-butanol–acetic acid–water, 4:1:2). It was suspended in MeOH (4 mL) and subjected to transfer hydrogenation with palladium black (~40 mg) and 90% formic acid (0.4 mL) with stirring at room temperature overnight. The catalyst was removed by filtration through Celite, the filtrate evaporated under vacuo to dryness, and the residue suspended in Et_2O, triturated and filtered, yield = 33.4 mg (45%): R_f = 0.09 (1-butanol–acetic acid–water, 4:1:2).

Due to the extremely high hydrophilicity of this material, no satisfactory reversed-phase HPLC analysis could be performed, but its homogeneity was supported by TLC, NMR, and elemental analysis. $[\alpha]^{25}_D$ = +25.8 (c = 0.12, H_2O); NMR $\delta_H^{DMSO\text{-}d_6}$ 2.95 (2 H, m, ε-CH_2), 3.57 (m, β-CH_2, γ-CH_2), 4.02–4.35 (m, H-5′, α-CH, H-2′-3′-4′), 5.62 (1 H, d, J = 8.0 Hz, H-5), 5.78 (1 H, d, J = 6.6

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Hz, H-1'), 7.76 (1 H, d, J = 8.0 Hz, H-6), 8.01 (1 H, d, J = 7.7 Hz, CONH), 8.24 (2 H, s, formate counterion). Anal. Found: C, 38.64; H, 5.92; N, 13.25. Calcd for $C_{15}H_{23}O_9N_5 \cdot 2CH_2O_2 \cdot H_2O$: C, 38.71; H, 5.54; N, 13.28.

***m*-Fluorophenylalanyl-UPOC (11).** (Benzoyloxycarbonyl)-D,L-*m*-fluorophenylalanine was prepared by standard procedures[22] from D,L-*m*-fluorophenylalanine and crystallized from ether, yield = 81% (R_f = 0.56, 1-butanol–acetic acid–water, 4:1:2). It was converted into the *p*-nitrophenyl ester by coupling to *p*-nitrophenol with DCC in THF and crystallizing from 2-propanol, yield = 52%: NMR $\delta_H^{DMSO\text{-}d_6}$ 3.0–3.3 (2 H, m, β-CH_2), 4.61 (1 H, m, α-CH), 5.05 (2 H, s, benzylic CH_2), 7.03–7.43 (11 H, m, phenyl), 8.14 (1 H, d, J = 7.7 Hz, OCONH), 8.32 (2 H, d, J = 9.0 Hz, meta of *p*-nitrophenyl). D,L-Z-*m*-fluorophenylalanine *p*-nitrophenyl ester was coupled to UPOC·HCl as described for compound **10**. The protected dipeptide was homogeneous on silica gel thin layers (R_f = 0.46, 1-butanol–acetic acid–water, 4:1:2). It was subjected to transfer hydrogenation as described above, yield = 59 mg (55%). The two diastereomers were observed on TLC (R_f = 0.17 and 0.21, 1-butanol–acetic acid–water, 4:1:2) and were separated by semipreparative HPLC. A 37-mg sample of the product was injected onto the Waters C_{18} column and eluted with 0.025% TFA in H_2O–acetonitrile (linear gradient of 5%–10% acetonitrile in 1 h). In this way, 18.5 mg of one diastereomer (peak I) and 11.5 mg of the other (peak II) were obtained.

Peak I: $[\alpha]^{25}_D$ = +44.1 (c = 0.17, H_2O); NMR $\delta_H^{DMSO\text{-}d_6}$ 2.8–3.2 (m, β-CH_2), 3.99 (1 H, t, J = 3.8 Hz, H-4'), 4.0–4.3 (3 H, m, α-CH, H-2', H-3'), 4.62 (1 H, m, H-5'), 5.35 (br, OH), 5.51 (1 H, d, J = 5.4 Hz, OH), 5.67 (1 H, d, J = 8.1 Hz, H-5), 5.80 (1 H, d, J = 5.8 Hz, H-1'), 7.02–7.45 (4 H, m, phenyl), 7.53 (1 H, d, J = 8.1 Hz, H-6), 8.2 (very br s, NH_3^+), 8.96 (1 H, d, J = 8.1 Hz, CONH), 11.4 (1 H, br s, NH-3). Anal. Found: C, 43.00; H, 4.08; N, 9.53. Calcd for $C_{19}H_{21}O_8N_4F \cdot C_2F_3O_2H \cdot H_2O$: C, 43.16; H, 4.14; N, 9.59.

Peak II: $[\alpha]^{25}_D$ = −28.6 (c = 0.07, H_2O); NMR (sample slowly frozen, thawed, and spectrum run immediately at 25 °C) $\delta_H^{DMSO\text{-}d_6}$ 2.8–3.2 (m, β-CH_2), 3.9–4.0 (1 H, m, H-4'), 4.0–4.3 (3 H, m, α-CH, H-2', H-3'), 4.53 (1 H, m, H-5'), 5.63 (1 H, d, J = 8.3 Hz, H-5), 5.76 (1 H, d, J = 5.8 Hz, H-1'), 6.97–7.44 (4 H, m, phenyl), 7.61 (1 H, d, J = 8.3 Hz, H-6), 8.83 (1 H, br d, J = 7.0 Hz, CONH), 11.38 (1 H, br s, NH-3). Anal. Found C, 40.44; H, 3.94; N, 9.10. Calcd for $C_{19}H_{21}O_8N_4F \cdot 1.3C_2F_3O_2H \cdot 2.2H_2O$: C, 40.52; H, 4.20; N, 8.75.

***N*³-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoyl-UPOC (FMDP-UPOC) (12).** N^2-(*tert*-Butoxycarbonyl)-N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid was converted into the *p*-nitrophenyl ester by using dicyclohexylcarbodiimide in THF and crystallizing from ethyl acetate–ether, yield = 75%. This product was homogeneous on silica thin layers with CH_2Cl_2/MeOH (8:2, R_f = 0.80) as eluant; NMR $\delta_H^{DMSO\text{-}d_6}$ 1.41 (9 H, s, *tert*-butyl), 3.68 (2 H, m, β-CH_2), 3.73 (3 H, s, OCH_3), 4.40 (1 H, m, α-CH), 6.63 (1 H, d, J = 15.4 Hz, C=CH), 7.02 (1 H, d, J = 15.4 Hz, C=CH), 7.41 (2 H, d, J = 8.9 Hz, *o*-phenyl), 7.58 (1 H, d, J = 7.5 Hz, OCONH), 8.34 (2 H, d, J = 8.9 Hz, *m*-phenyl), 8.82 (1 H, t, J = 5.7 Hz, β-NH).

Boc-FMDP-ONp (104 mg, 238 μmol) was coupled to UPOC·HCl (70 mg, 216 μmol) in DMSO (2.16 mL) with added DIEA (75.3 μL, 432 μmol) by stirring at room temperature for 17 h. The solvent was evaporated in vacuo to dryness, and the residue was suspended and triturated in ether and filtered (R_f = 0.32, 1-butanol–acetic acid–water, 4:1:2). The protecting group was removed by treatment with 2 mL of 2 N HCl/dioxane, and 93.1 mg of product were obtained (R_f = 0.21, 1-butanol–acetic acid–water, 4:1:2, yellow color with ninhydrin spray). A 60-mg sample of this crude product was purified by injection onto the Aquapore RP-18 column in 10-mg shots (solvent, TFA 0.025%; flow rate, 4.50 mL/min). A total of 12 mg of a ≥98% pure fraction was obtained, as well as 26.5 mg of a ~95% pure fraction: overall yield (coupling–deprotection–purification) = 41%; $[\alpha]^{25}_D$ = +20.0 (c = 0.11, H_2O); NMR $\delta_H^{DMSO\text{-}d_6}$ 3.55 (2 H, m, β-CH_2), 3.73 (3 H, s, OCH_3), 3.94–4.26 (4 H, m, H-2',3',4' and α-CH), 4.68 (1 H, dd, H-5'), 5.35 (1 H, d, J = 5.0 Hz, OH), 5.54 (1 H, d, J = 5.7 Hz, OH), 5.67 (1 H, d, J = 8.3 Hz, H-5), 5.78 (1 H, d, J = 6.0 Hz, H-1'), 6.61 (1 H, d, J = 15.6 Hz, C=CH), 6.98 (1 H, d, J = 15.6 Hz, C=CH), 7.45 (1 H, d, J = 8.3 Hz, H-6), 8.24 (br s, NH_3^+), 8.69 (1 H, m, β-NH), 9.09 (1 H, d, J = 8.2 Hz, CONH), 11.44 (1 H, s, NH-3). Anal. Found: C, 35.76; H, 4.00; N, 10.16. Calcd for $C_{18}H_{23}O_{11}N_5 \cdot 1.5C_2F_3O_2H \cdot 2.6H_2O$: C, 35.86; H, 4.26; N, 9.96.

Biological Methods. Organisms. The following yeast strains were studied *C. albicans* H-317, a clinical isolate from the Center for Disease Control, Atlanta, GA, *C. albicans* 124 and *C. albicans* 124 Nik^R, nikkomycin sensitive and resistant strains, respectively, from Dr. William Kingsbury, Smith, Kline and French Laboratories, Philadelphia, PA.

Chitin Synthetase Assay. Total chitin synthetase activity obtained in a mixed membrane fraction from *C. albicans* H-317 was assayed in the presence and absence of polyoxin compounds by measuring the incorporation of *N*-acetylglucosamine into chitin. The detailed procedures have been previously reported.[23,24]

Peptidase Assay. The procedures used to prepare a cell extract of *C. albicans* H-317 in assays for hydrolysis of the antibiotics were previously reported.[5] To assay for hydrolysis, TLC as well as HPLC, were used. A 0.5-mg sample of the antibiotic was dissolved in 50 μL of H_2O–DMSO (50:50) and mixed at 36 °C with 200 μL of cell extract (3.75 mg of protein/mL). The mixture was incubated at 36 °C, and at 20-min intervals small portions were withdrawn and directly chromatographed. Nle-UPOC[5] was used as a control, and was completely hydrolyzed after 1 h under these conditions.

Transport Studies. The procedures and transport conditions to assay for the uptake of labeled trimethionine and dileucine in the presence of polyoxin compounds have been previously described.[23,11] Analogues were added at 10-fold the molar concentration of the transport substrates for competition. At intervals of 0, 1, 2, 3, and 4 min, 180 μL of the reaction mixtures were withdrawn and applied to prewet filters (pore size 0.45 μm) and washed twice with 2 mL of cold distilled water. The rate of uptake was calculated as nanomoles of peptide taken up/milligram of dry weight cells per minute.

Determination of MIC. The methods and procedures employed for the determination of the MIC values in the presence of the polyoxin analogues have been described previously except that ammonium sulfate (0.5%) was used for most experiments as the nitrogen source.[23,11] The MIC was recorded as the lowest concentration of drug that resulted in 5% morphologically aberrant cells as visualized by microscopy after a 24–48-h incubation at 37 °C in yeast nitrogen base (Difco Labs, Detroit, MI).

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