divided by the incorporation measured in parallel control cultures. Generally, enhancements of 2-fold or greater were reproducible and linear with incorporation time.

Acknowledgment. This work was supported by Public Health Service Research Grant CA-11196 from the National Cancer Institute and by grants to the Institute for Cancer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania). We thank Sharon Howard for her assistance with the cell culture studies.

Synthesis and Biological Properties of Chitin Synthetase Inhibitors Resistant to Cellular Peptidases

Ponniah Shenbagamurthi,† Herbert A. Smith,‡ Jeffrey M. Becker,‡ and Fred Naider*,†

Department of Chemistry, College of Staten Island of the 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 July 24, 1985*

The synthesis and biological properties of seven polyoxins (**4–10**) designed to avoid peptidase hydrolysis in *Candida albicans* are presented. Five dipeptidyl and two tripeptidyl polyoxin analogues were synthesized by coupling an amino acid active ester or azlactone to uracil polyoxin C (**2**) or polyoxin D (**1**), subsequent removal of the protecting group, and purification by preparative HPLC. A new and novel route for introducing an *n*-propyl group onto the α-amino group of peptides is reported. With the exception of a carboxamide derivative, **8**, all analogues were resistant to hydrolysis by a cell extract or permeabilized cells of *Candida*. Chitin synthetase inhibition constants were determined for **4–10** and the K_I values ranged from 7.15×10^{-6} M for octanoyl-phenylalanyl-polyoxin D (**10**) to 1.06×10^{-3} M for D-tryptophanyl-uracil polyoxin C (**6**). These novel polyoxins do not compete with the transport of either peptides or uridine into the cell. Millimolar concentrations of compounds **4–10** are required to inhibit growth, cause morphological alterations, or reduce the viability of *C. albicans*.

The polyoxins are a group of closely related peptidyl nucleoside antibiotics produced by species of *Streptomyces*. Previous studies have demonstrated that these compounds are toxic to phytopathogenic fungi.[1,2] The biochemical target for the polyoxins within the cell is chitin synthetase. This membrane-bound enzyme catalyzes the synthesis of chitin, a polysaccharide of *N*-acetylglucosamine (GlcNAc) and an important structural component of yeast and fungal cell walls.[3] Our laboratory has demonstrated that polyoxin D (**1**), one of the most active natural polyoxins, is toxic to the zoopathogenic fungi *Candida albicans* and *Cryptococcus neoformans*. However, this toxicity is manifested only at millimolar concentrations.[4]

The need for development of an antifungal agent for *C. albicans* is clearly indicated by the large number of opportunistic infections caused by *C. albicans* in compromised hosts. Accordingly, we have directed our research efforts toward the synthesis of novel polyoxin compounds that will ultimately find application in the clinical treatment of candidiasis.

In a previous report we showed that a series of dipeptidyl antibiotic analogues were strongly inhibitory to chitin synthetase and that some of these peptidyl antibiotics at millimolar concentrations were effective in killing the yeast *C. albicans*.[5] We also reported that two tripeptidyl polyoxins, leucyl-norleucyl-uracil polyoxin C and leucyl polyoxin D, which were inactive when assayed against chitin synthetase in a membrane preparation, generated toxic compounds within *Candida*.[6] We believe that these tripeptidyl polyoxins represent prodrugs that are activated by intracellular peptidases.

In the above studies we examined the role of cellular peptidases in the intracellular degradation of the polyoxins by *C. albicans*. These hydrolysis studies have shown us that the major difference between polyoxin D and the synthetic di- and tripeptidyl compounds is that polyoxin D is stable to peptidases whereas the synthetic analogues are hydrolyzed inside the yeast.[5,6] Recently polyoxin analogues with a modified peptide bond were found to lose inhibitory activity. However, no attempt to measure intracellular stability was reported in this study.[7]

In order to increase the stability of synthetic polyoxins toward intracellular hydrolysis we modified the backbone and chain ends of the dipeptidyl nucleoside. The chemical modifications we have chosen to circumvent the peptidase hydrolysis problem include the utilization of D-amino acids, N-alkylation of the terminal amine with methyl or propyl groups or replacement of this amine with an aminooxy group (NH_2O), inclusion of a terminal amide in place of the unprotected carboxyl group, utilization of dehydro amino acids in the peptide portion of the polyoxin, and acylation of tripeptidyl polyoxins with a lipophilic group.

Studies have indicated that the above modifications are often tolerated by target molecules yet render the peptide stable to proteolysis.[8,9] In this paper we report on the

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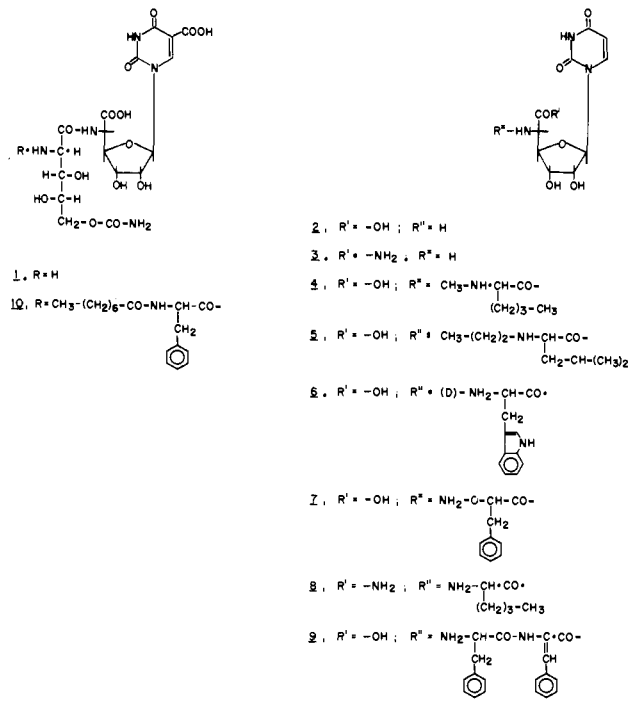

Figure 1. Structural formulae of polyoxins.

synthesis and biological activity of a number of synthetic polyoxins with markedly increased resistance to intracellular degradation. Two of our analogues, **9** and **10**, represent the first report of tripeptidyl polyoxins that are good in vitro inhibitors of chitin synthetase from *C. albicans*.

Chemistry

The vast majority of biological studies on polyoxins have utilized polyoxin D (**1**, Figure 1). Polyoxin L differs from **1**, only in that the 5-carboxyl group (Figure 1) is replaced with a hydrogen atom.[10] The compounds that we synthesized were prepared from uridine and are, thus, analogues of polyoxin L. Most derivatives were prepared from 1-(5′-amino-5′-deoxy-β-D-allofuranosyluronic acid)uracil (**2**), which we have previously designated uracil polyoxin C.[5] The synthesis of most of the final compounds was carried out by condensing the appropriate active ester or azlactone with **2**.[5] The preparation of certain synthetic intermediates required special procedures. Difficulties encountered and routes to circumvent these are described below. The yields of the synthetic polyoxins ranged from 23% to 30% on the basis of **2**. Final products were homogeneous on reversed-phase C_{18} columns and silica thin layers. Their structure was confirmed by using high-resolution nuclear magnetic resonance spectroscopy.

N-Methylamino acids are constituents of several naturally occurring peptide and depsipeptide antibiotics,[11] and peptides that are N-alkylated are resistant to peptidase hydrolysis.[12,13] Therefore, we decided to synthesize polyoxins containing *N*-methylnorleucine **4** and *N*-*n*-propylleucine **5**. Boc-MeNle (see first paragraph of the Experimental Section for list of abbreviations employed) was prepared from Boc-Nle with use of CH_3I and NaH according to the method of Cheung and Benoiton.[14] The ^1H NMR spectrum showed the expected *N*-methyl singlet at 2.82 ppm and no N–H proton. It was then converted into the *p*-nitrophenyl ester and coupled to **2**, as described in the Experimental Section.

The method of Cheung and Benoiton,[14] however, failed for the attempted introduction of *n*-propyl group using n-C_3H_7I and NaH. Even an improved method employing KH and crown ether did not give alkylation.[15] In a similar manner, the reaction failed when Boc-Leu was treated with n-C_3H_7I and Ag_2O in DMF.[16] Trials with Z-Leu also gave the same results. It was decided, therefore, to use the more reactive allyl iodide in these reactions and reduce the allyl group in a later stage to the *n*-propyl group. Treatment of Z-Leu with allyl iodide and silver oxide gave a mixture of N- and O-allylation. To circumvent this difficulty, Z-Leu-OMe was treated with allyl iodide and Ag_2O in DMF for 18 h. The product was homogeneous as shown by TLC and HPLC. The ^1H NMR spectrum confirmed the structure to be Z-(allyl)Leu-OMe. This ester was saponified with 1 M NaOH and the resulting Z-(allyl)Leu-OH was converted into its *p*-nitrophenyl ester. It was coupled to **2** and the resulting crude Z-(allyl)Leu-uracil polyoxin C was subjected to catalytic transfer hydrogenation and purified on preparative HPLC to give pure **5**.

The introduction of dehydroamino acid is another method for stabilization of bioactive peptides.[17-19] Several methods have been reported in the literature for synthesis of dehydroamino acids.[20-22] Attempts to hydrogenate Z-Δ^Z-Phe resulted in the formation of DL-Phe due to reduction of the double bond. Furthermore, treatment of Z-Δ^Z-Phe with hydrogen bromide/acetic acid resulted in the degradation of the molecule. It was inferred that on treatment with HBr/HOAc, in addition to Z group removal, elimination of ammonia occurred, causing the formation of impurities. This behavior is not surprising in view of the inherent instability of some dehydropeptides.[20] These findings suggested that attempts to prepare Δ^Z-Phe-uracil polyoxin C would not be successful. English and Stammer[17] had reported the coupling of the azlactone of Z-Phe-Δ^Z-Phe with Ala-OMe and subsequent HBr/HOAc treatment to give HBr·Phe-Δ^Z-Phe-Ala-OMe. In this sequence of operations, since the Z group is separated from the dehydro linkage by one Phe residue, no side reactions occur. Following this procedure, the azlactone of Z-Phe-Δ^Z-Phe was coupled with **2** in DMF at room temperature. Subsequent treatment with HBr/HOAc and purification on preparative HPLC yielded pure **9**.

Biological Results and Discussion

Inhibition of Chitin Synthetase by Novel Polyoxins.

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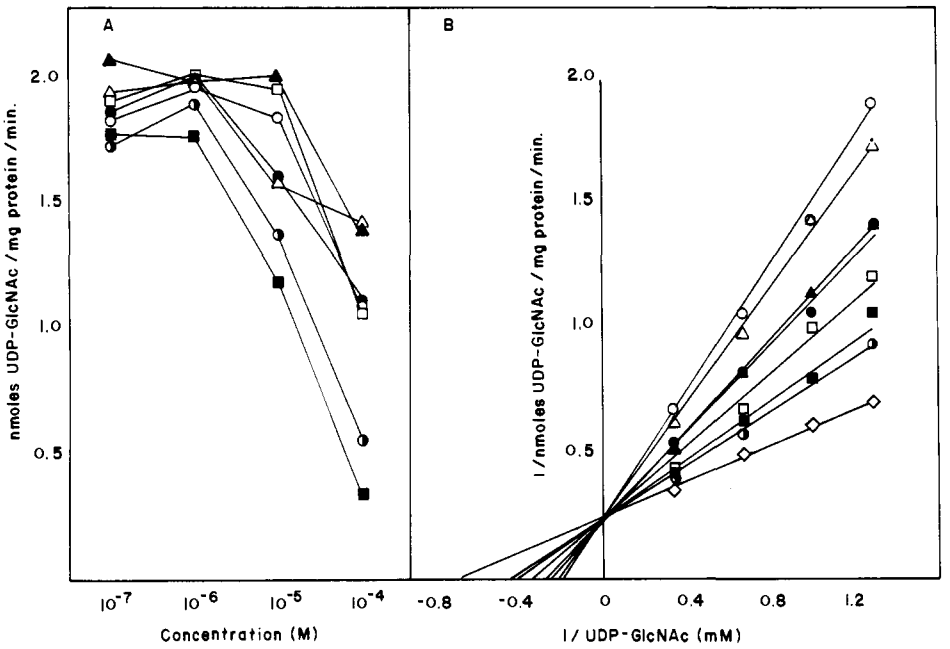

Figure 2. Panel A: chitin synthetase activity in the presence of the polyoxin compounds. The initial rate of enzymatic activity at the corresponding molar concentration of the inhibitor is plotted. Symbols: ○, 4; ●, 5; △, 6; ▲, 7; □, 8; ■, 9; ◑, 10. Panel B: Lineweaver–Burk Plots of chitin synthetase activity in the presence of various polyoxin inhibitors. Symbols and concentration of inhibitors at which the assay was performed: ◇, no inhibitor; ○, 4 (0.1 mM); ●, 5 (0.1 mM); △, 6 (0.5 mM); ▲, 7 (0.5 mM); □, 8 (0.1 mM); ■, 9 (0.01 mM); ◑, 10 (0.01 mM).

Table I. Biological Activities of Polyoxin Analogues

polyoxin analogue	mmf ID_{50},[a] M	mmf K_I,[b] M	perm cell ID_{50},[c] M	hydrolysis[d]	MIC[e]	MEC[f]	viability[g]
1	1.8×10^{-6}	5.9×10^{-7}	1.0×10^{-6}	- (HVPE)	0.06	0.02	1
4	1.2×10^{-4}	2.18×10^{-4}	9.75×10^{-5}	- (HPLC)	>1.0	2.0	>4
5	$>10^{-4}$	1.45×10^{-4}	$>10^{-4}$	- (HPLC)	2.5	>2.5	>1
6	$>10^{-4}$	1.06×10^{-3}	$>10^{-4}$	- (HPLC)	>1.0	4.0	>4
7	$>10^{-4}$	9.78×10^{-4}	$>10^{-4}$	- (HPLC)	3.0	>3.0	>3
8	2.0×10^{-4}	2.46×10^{-4}	$>10^{-4}$	+ (HVPE)	>1.0	>4.0	>4
9	4.0×10^{-5}	1.13×10^{-5}	8.0×10^{-5}	- (HPLC)	>2.0	>2.0	>2
10	4.75×10^{-5}	7.15×10^{-6}	5.0×10^{-5}	- (HPLC)	1.0	1.0	>1

[a] Molar concentration at which the enzyme is inhibited by 50% in the mixed membrane preparation. [b] Inhibition constant (K_I) (in M derived from a Lineweaver–Burk plot) for the mmf chitin synthetase assay performed at constant inhibitor and varying substrate concentrations. [c] Molar concentration at which chitin synthetase is inhibited by 50% in the permeabilized cell assay. [d] (+) Compound was hydrolyzed by *C. albicans* cell extract. (-) No hydrolysis by cell extract. HVPE, hydrolysis monitored by high-voltage paper electrophoresis. HPLC, hydrolysis monitored by high-performance liquid chromatography. [e] Millimolar concentration of the lowest concentration of drug that clearly inhibited growth. [f] Millimolar concentration of the compound at which some morphological effects (about 5% of the cells) was observed microscopically. [g] Millimolar concentration of the drug at which 50% of the cells were not viable.

The inhibitory activity of seven novel polyoxins, **4–10**, was assayed in two independent chitin synthetase assays. The first assay employed a mixed membrane preparation (mmf) from *C. albicans* H-317, which efficiently catalyzed the formation of chitin from uridine-diphospho-*N*-acetylglucosamine (UDP-GlcNAc). The second assay employed cells that were first permeabilized with digitonin and then assayed for chitin synthetase activity. Using the permeabilized cells, we were able to examine the inhibitory activity of the polyoxin compounds toward chitin synthetase in the presence of cellular peptidases. Dose–response curves were generated for each of the polyoxin compounds in both chitin synthetase assays (Figures 2 and 3). The concentration of inhibitor that resulted in a 50% reduction in chitin synthetase activity (ID_{50}) was interpolated from the data and is presented in Table I. Inhibition constants (K_I values) were also determined for each of the polyoxin compounds by maintaining constant inhibitor concentration and varying substrate concentration in the mmf assay (Figure 2 and Table I).

The ID_{50} values found in the mmf assay were usually quite similar to that determined in the permeabilized cell assay. This result suggests that peptidase degradation is not influencing the activity of most of these compounds (see below). The K_I values presented in Table I allow a quantitative comparison of the inhibitory activities of the synthetic polyoxins. Unfortunately, most analogues are only slightly active. Specifically, incorporation of a D residue near the amine terminus results in an analogue (**6**) with a K_I value of 1.06×10^{-3} M. Thus replacement of L-Trp by D-Trp reduced inhibition by 3 orders of magnitude.[5] Previous studies using chitin synthetase from *Piricularia oryzae* reported that semisynthetic analogues of polyoxin J, containing D residues, were ca. 1 order of magnitude less active than the L isomer.[23] It would appear that the chitin synthetase from *Candida* is more sensitive to this replacement than is the enzyme from *P. oryzae*. The analogues containing aminooxy (**7**), *N*-α-alkyl (**4**, **5**), or a carboxamide (**8**) group had K_i values of $\sim 10^{-4}$ M. Thus modification of the C-terminal carboxyl or alkylation of the α-amine reduce activity by ~100-fold. Clearly, the aminooxy replacement is not tolerated as **7** is 10^3 less active than homophenylalanyl-uracil polyoxin C.[5] Interestingly

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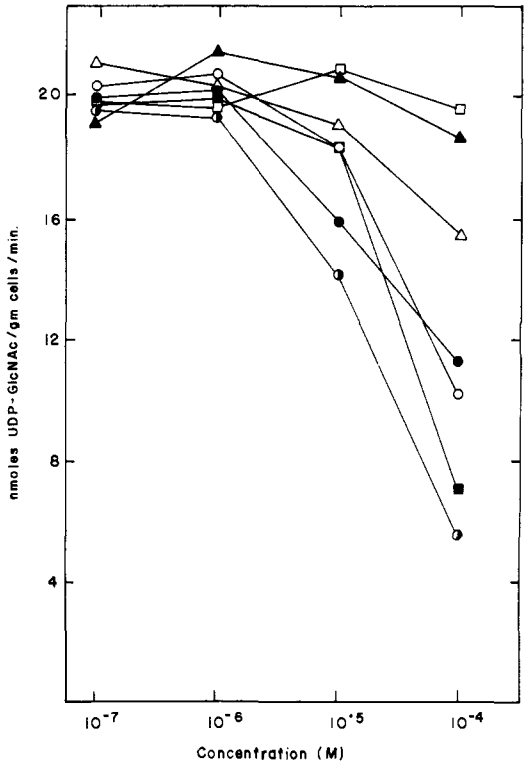

Figure 3. Chitin synthetase activity in digitonin permeabilized cells in the presence of various polyoxin compounds. Symbols: ○, 4; ●, 5; △, 6; ▲, 7; □, 8; ■, 9; ◑, 10.

the two tripeptidyl polyoxins **9** and **10** had the highest inhibitory constants and were effective inhibitors of chitin synthetase. This finding is somewhat surprising in that other tripeptidyl polyoxins we have investigated did not significantly affect chitin synthesis when present at 10^{-4} M.[6] Moreover, it is generally accepted that polyoxins must have a free α-NH_2 to interact with chitin synthetase.[1,24] Yet **10** is acylated on the α-NH_2 with octanoic acid and is only a 5-fold poorer inhibitor than **1**. A number of tripeptidyl polyoxin L analogues were found to be moderately active against five different phytopathogenic fungi.[1] However, no activities against chitin synthetase in vitro were reported. Our results with compound **9** and **10** therefore represent the first example of tripeptidyl polyoxins that are good inhibitors of chitin synthetase from *Candida*.

Degradation of the Polyoxin Analogues by a *C. albicans* Cell Extract. In order to determine the sensitivity of the synthetic polyoxins to peptidases present in *C. albicans*, two peptidase assays employing HVPE or HPLC were developed. To assay for peptidase hydrolysis of the polyoxin compounds by HVPE, cell extract was combined with the polyoxin compound and incubated at 37 °C for 0, 30, and 60 min, at which point portions were withdrawn and spotted onto filter paper prior to electrophoresis. When the peptidase activity of the polyoxin compounds by HPLC was assayed, portions of the reaction mixture were removed at 0, 1, 2.5, 5, 15, and 30 min and chromatographed.

Shown in Figure 4 is the result of an HPLC peptidase assay with the peptidase sensitive Nle-uracil polyoxin C and the peptidase resistant **4**. Nle-uracil polyoxin C is degraded by the cell extract to Nle and **2** with a $t_{1/2}$ equal to 4 min whereas **4** is not detectably degraded within 30 min. Analogue **8** was hydrolyzed by the *C. albicans* cell extract to Nle and **3**. With the exception of **8**, the polyoxin compounds were not detectably degraded by a cell extract of *C. albicans* when the HVPE or HPLC peptidase assays were employed (Table I). These results suggest that the inhibition of chitin synthetase by compounds **9** and **10** is not due to their hydrolysis to active dipeptidyl polyoxins and support our conclusion that **9** and **10** directly interact with chitin synthetase. The octanoyl-phenylalanyl-polyoxin D (**10**) analogue was synthesized because it was expected to have greater membrane partitioning than **1** and might thereby penetrate into the yeast. Once inside the cell we anticipated that the peptide bond between Phe and **1** might be cleaved by a chymotrypsin-like activity. Either such an activity is not present in *Candida* or it is not active under our conditions of assay.

Interaction of the Polyoxin Compounds with Cellular Transport Systems for Peptides and Nucleosides. A goal of our research has been to synthesize polyoxins that will utilize the peptide transport system for cell entry. We were also interested in determining whether the polyoxin compounds were able to utilize the uridine transport system to effect cell entry as the polyoxins we have synthesized are either dipeptidyl or tripeptidyl uridine nucleoside derivatives.[25] To define the ability of the polyoxins to enter the cell, the initial rate of uptake of either radiolabeled trimethionine or uridine was measured in the presence of a 10-fold concentration of the competitor. In control experiments, nonradioactive uridine decreased [^{14}C]uridine uptake by 86% and Gly-Met-Gly decreased [^{14}C]trimethionine uptake by 81% when these compounds were present at a 10-fold M excess. The results of competition experiments indicated that compounds **4–10** do not utilize either transport system to effect their entry into the cell.

Recent studies show that Nikkomycin enters *C. albicans* through what appears to be a dipeptide permease.[26,27] However, polyoxin D is a poor substrate for this permease and the entry of polyoxins into *C. albicans* is markedly dependent on the growth conditions.[28] We have found that the uptake of $(Met)_3$ into five different strains of *Candida* is 20–100-fold higher than the entry rate of $(Leu)_2$ (H. Smith, unpublished results). Given the poor uptake of the native polyoxins into *C. albicans*, it is not clear whether the modifications examined in this paper affect the uptake rate of dipeptidyl nucleosides.

Effect of Novel Polyoxins on Cell Morphology and Growth. In a previous study we reported on the morphological alterations and inhibition of growth produced by novel dipeptidyl and tripeptidyl polyoxins synthesized in our laboratory.[6] As noted previously, polyoxin D (**1**) causes severe alterations in the morphology of *C. albicans*.[3] The polyoxin compounds reported in this paper were evaluated to determine the minimum effective concentration (MEC), the lowest concentration at which some (5%) morphologically abnormal cells are observed microscopically (Table I). With the exception of **1**, the polyoxin compounds required high concentrations to observe morphologically altered cells, the major alteration appearing as swollen cells 2–3 times their normal size. Analogues **5** and **7–9** did not produce observable mor-

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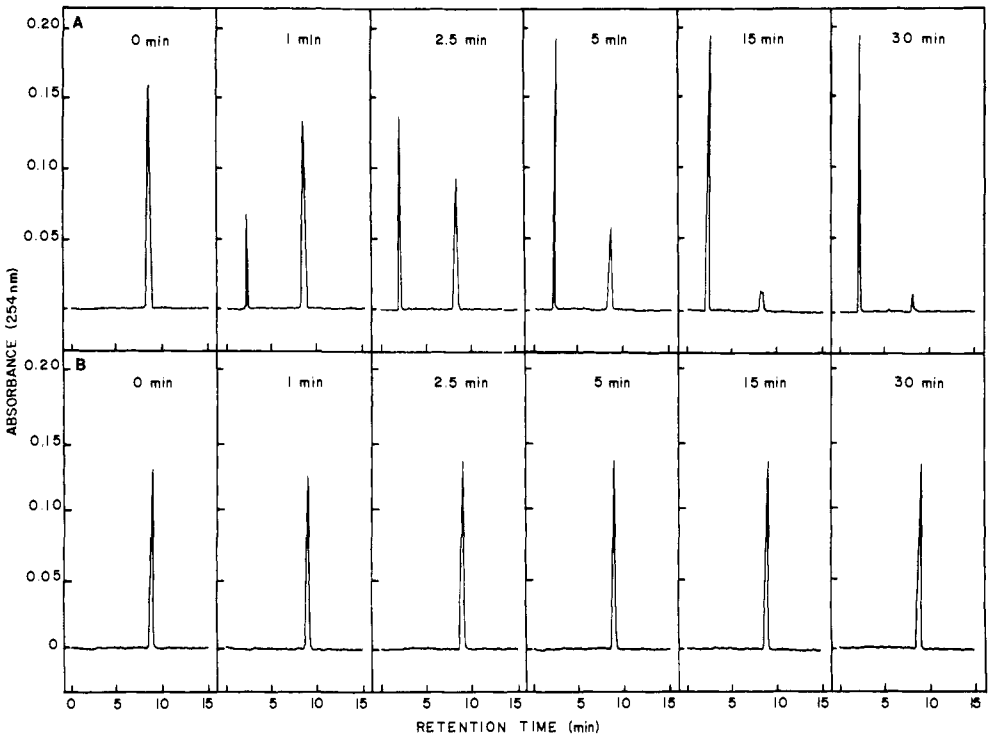

Figure 4. Top panel: high-performance liquid chromatography of Nle-uracil polyoxin C incubated with cell extract for 0, 1, 2.5, 5, 15, and 30 min. Bottom panel: high-performance liquid chromatography of **4** incubated with cell extract for 0, 1, 2.5, 5, 15, and 30 min.

phological alterations. The effect of these polyoxins upon cell viability was determined by the number of colonies formed after culturing a known number of potential viable cells after incubation in the presence of the polyoxin compound for 48 h. With the exception of **1**, these polyoxin compounds did not decrease cell viability. For some of the analogues the minimum inhibitory concentration (MIC) was determined as the lowest concentration of drug that clearly inhibited visible growth. In comparison to **1** with a MIC equal to 0.06 mM, the novel polyoxins had a 20–50-fold lower activity with MIC values in the range of 1–3 mM.

Conclusion

A number of changes in the dipeptidyl moiety of our polyoxin L analogues were found to markedly stabilize these chitin synthetase inhibitors toward degradation by *Candida* peptidases. The synthetic polyoxins also provide new insights into structure–activity relationships for chitin synthetase from *C. albicans*. Specifically, changes in the peptide backbone and the amine or carboxyl terminus alter the compound into a poor enzyme inhibitor. The results suggest that increased stability toward peptidase hydrolysis might best be realized by changing the side chain of the amine terminal residue. Preliminary results toward this end are encouraging, and we are now preparing polyoxin analogues that resist candidal peptidases but remain highly inhibitory toward chitin synthesis in this yeast.

Experimental Section

Standard abbreviations for amino acid derivatives and peptides are according to the IUPAC–IUB Commission on Biochemical Nomenclature (*Biochemistry* **1975**, *14*, 449–462). Additional abbreviations used are as follows: Bis-Tris, bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane; Boc, *tert*-butoxycarbonyl; *n*-BuOH, *n*-butyl alcohol; DMF, *N*,*N*-dimethylformamide; GlcNAc, *N*-acetyl-D-glucosamine; HOAc, acetic acid; HPLC, high-performance liquid chromatography; HVPE, high-voltage paper electrophoresis; ID_{50}, drug concentration resulting in 50% inhibition; MEC, minimum effective concentration; MeNle, *N*-methyl-L-norleucine; MeOH, methanol; MES, 2-morpholinoethanesulfonic acid; MIC, minimum inhibitory concentration; mmf, mixed membrane fraction; NMM, *N*-methylmorpholine; ONp, *p*-nitrophenyl ester; Δ^Z-Phe, α,β-dehydrophenylalanine (*Z* configuration); TFA, trifluoroacetic acid; TLC, thin-layer chromatography; Z, benzyloxycarbonyl.

Chemical Synthesis. The melting points reported are uncorrected. Most of the amino acid derivatives were purchased from Bachem Inc. and were of the L configuration. All the solvents used were of analytical grade and were supplied by Fisher Scientific. Allyl iodide, methyl iodide, silver oxide, and TFA were products from Aldrich.

Homogeneity of all the polyoxins was confirmed by thin-layer chromatography using silica gel plates (Brinkman) with use of the solvent system 1-butanol–acetic acid–water (4:1:2) and the R_f values are reported as R_fA. Detection on TLC plates utilized either ultraviolet light or ninhydrin in butanol. High-performance liquid chromatography (HPLC, analytical) was carried out on a Waters chromatograph (Waters Associates, Milford, MA) equipped with two Model 510 solvent delivery systems, a U6K injector, a Model 481 variable wavelength UV detector, a Model 680 automated gradient controller, and a Model 730 data module. Chromatographic separations were carried out on a Waters μBondapak reversed-phase column (30 cm × 3.9 mm i.d.) employing either MeOH–H_2O–TFA or CH_3CN–H_2O–TFA as the mobile phase at a flow rate of 1.5 mL/min. The absorbance of the column eluants was recorded at 254 nm for all the polyoxin derivatives and at 220 nm for amino acid derivatives. For purification of final polyoxins a Waters Prep LC/system 500 was used with a 1-in. Semiprep column.

NMR spectra were recorded on an IBM 200-MHz instrument, and the chemical shift values are reported in ppm relative to tetramethylsilane as standard. As previously noted synthetic polyoxins are isolated as either formate or trifluoroacetate salts which contain varying amounts of water.[5,6] It is, therefore, often difficult to obtain reliable elemental analyses on synthetic polyoxins.[7] All of the polyoxin analogues are homogeneous in two HPLC systems and on silica thin layers and have the expected NMR resonances with no additional peaks. We believe that the combination of spectroscopic and chromatographic data attests to the purity of the synthetic antibiotics.

Boc-MeNle-ONp. Boc-MeNle-OH was prepared from Boc-Nle with use of methyl iodide and sodium hydride according to the

procedure of Cheung and Benoiton,[14] in 92% yield. This was converted into its *p*-nitrophenyl ester with use of dicyclohexylcarbodiimide in 85% yield: R_f(CH_2Cl_2) 0.47; $[\alpha]^{25}_D$ −44.6° (*c* 0.65, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 8.33 (d, AB quartet, *J* = 7.2 Hz, 2, Ar H), 7.43 (d, AB quartet, *J* = 7.2 Hz, 2, Ar H), 4.69 (m, 1, α-CH), 2.50 (s, 3, NCH_3), 1.93 (m, 2, side-chain CH_2), 1.42 (s, 9, Boc-CH_3), 1.2–1.4 (m, 4, side-chain $(CH_2)_2$), 0.87 (m, 3, side-chain CH_3).

1-[5′-[(*N*-Methylnorleucyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (4). To a solution of **2**[29] (80 mg, 0.24 mmol) and Boc-MeNle-ONp (95.2 mg, 0.26 mmol) in DMF (2 mL) containing water (0.2 mL) was added *N*-methylmorpholine (0.053 mL, 0.48 mmol), and the mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored by TLC (*n*-BuOH–HOAc–H_2O = 4:1:2). When most of the nucleophile had reacted (85%), the reaction mixture was acidified with HOAc (0.1 mL), the solvents were evaporated in vacuo, and the residue was precipitated by adding ether. The crude Boc derivative of **4** (87.4 mg) obtained after filtration (R_fA 0.58) was subjected to acidolysis without purification.

The crude Boc derivative of **4** (87.4 mg) was treated with CH_2Cl_2–TFA: (1:1, v/v, 2 mL) for 30 min at room temperature. The solvents were evaporated in vacuo, and the crude **4** was precipitated by the addition of ether. It was dissolved in 3 mL of CH_3OH–H_2O–TFA (100:900:0.25, v/v/v) and injected onto a μBondapak C_{18} (1 in. Semiprep) column of a Waters Prep LC/system 500, which had been equilibrated with the same solvent system. The column was eluted at a flow rate of 50 mL/min, the fractions were analyzed by analytical HPLC, and those corresponding to the main peak were pooled and evaporated in vacuo at room temperature. The residue was dissolved in water (5 mL) and filtered through a microfilter (0.45 μm), and the filtrate was freeze-dried to give pure **4** (37.5 mg, 29.6% based on **2**). The final product was homogeneous as judged by analytical HPLC on a reversed-phase C_{18} column: *K′* = 2.01 (CH_3OH–H_2O–TFA = 100:900:0.25, v/v/v); *K′* = 5.26 (CH_3CN–H_2O–TFA = 40:960:0.25, v/v/v) and on silica thin layers; R_fA 0.36; $[\alpha]^{25}_D$ +24.50° (*c* 0.2, H_2O); ^{1}H NMR (Me_2SO-d_6) δ 11.44 (m, 1, CONHCO), 9.2 (d, 1, NH), 8.9 (m, 2, NH_2^+), 7.50 (d, *J* = 8.1 Hz, 1, C_6H), 5.79 (d, *J* = 5.3 Hz, 1, C_1'H), 5.68 (d, *J* = 8.1 Hz, 1, C_5H), 4.73 (m, 1, C_5'H), 3.99 (m, 1, C_4'H), 4.15 and 3.82 (m, 3, α-CH, C_2'H, and C_3'H), 2.50 (s, 3, NCH_3), 1.75 (m, 2, side-chain CH_2), 1.26 (m, 4, side-chain $(CH_2)_2$), 0.85 (t, 3, side-chain CH_3).

Z-(allyl)Leu-ONp. Z-(allyl)Leu-OMe was prepared from Z-Leu-OMe with use of allyl iodide and silver oxide following the procedure of Olson,[16] in 94% yield: R_f(CH_2Cl_2) 0.43; $[\alpha]^{25}_D$ −46.3° (*c* 2, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 5, Ar H), 5.82 (m, 1, =CH), 4.9–5.4 (m, 4, Ar CH_2 and CH_2=C), 4.54 (m, 1, α-CH), 3.8–4.14 (m, 2, NCH_2), 3.64 (s, 3, OCH_3), 1.69 (m, 3, side-chain CH_2 and CH), 0.93 (d, 6, $(CH_3)_2$).

Z-(allyl)Leu-OH was obtained from the above by saponification with use of 1 M NaOH for 2 h. Workup gave Z-(allyl)Leu-OH as an oil in 85% yield: R_f(CH_2Cl_2–MeOH–HOAc = 10:1:0.5) 0.71; $[\alpha]^{25}_D$ −50.44° (*c* 0.68, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 5, Ar H), 5.83 (m, 1, =CH), 4.95–5.3 (m, 4, Ar CH_2 and CH_2=C), 4.53 (m, 1, α-CH), 3.5–4.3 (m, 2, NCH_2), 1.70 (m, 3, side-chain CH_2 and CH), 0.93 (m, 6, $(CH_3)_2$).

Z-(allyl)Leu-OH (0.61 g, 2 mmol) was converted into the p-nitrophenyl ester with use of dicyclohexylcarbodiimide, and the product was isolated. Z-(allyl)Leu-ONp was obtained as an oil (0.77 g, 90.4%). The product was homogeneous on silica thin layers: R_f(CH_2Cl_2) 0.64; $[\alpha]^{25}_D$ −61.43° (*c* 0.7, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.20 (d, AB quartet, *J* = 8.7 Hz, 2, NO_2-Ar-H), 7.25–7.32 (m, 7, NO_2-Ar-H and 5 Ar H), 5.89 (m, 1, =CH), 4.9–5.4 (m, 4, Ar CH_2 and CH_2=C), 4.62 (m, 1, α-CH), 3.6–4.4 (m, 2, NCH_2), 1.82 (m, 3, side-chain CH_2 and CH), 0.97 (m, 6, $(CH_3)_2$).

1-[5′-[(*N*-*n*-Propylleucyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (5). To a solution of Z-(allyl)-Leu-ONp (140.6 mg, 0.33 mmol) and **2** (100 mg, 0.3 mmol) in a mixture of DMF (3 mL) and water (0.3 mL) was added NMM (0.066 mL, 0.6 mmol), and the mixture was stirred at room temperature for 20 h. The reaction mixture was acidified with HOAc (0.2 mL), the solvents were evaporated in vacuo, and the residue was precipitated by adding ether. The crude 1-[5′-[(Z-allylleucyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (120 mg) (R_fA 0.7) obtained was subjected to catalytic transfer hydrogenation without purification.

To a solution of the Z derivative obtained above (120 mg) in CH_3OH (3 mL) was added Pd-black (~30 mg). Formic acid (90%, 0.5 mL) was added with stirring at room temperature. After 30 min, the catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The residue was subjected to purification on a Waters Prep LC/system 500, as described under **4**, using MeOH–H_2O–TFA (100:900:0.25, v/v/v) as eluent. The yield of **5** was 39 mg (23.4%). The final product was >99.8% pure as judged by analytical HPLC on a reversed-phase C_{18} column: *K′* = 4.74 (CH_3OH–H_2O–TFA = 100:900:0.25, v/v/v); *K′* = 0.79 (CH_3CN–H_2O–TFA = 80:920:0.25, v/v/v) and on silica thin layers; R_fA 0.37; $[\alpha]^{25}_D$ +28.33° (*c* 0.18, H_2O); ^{1}H NMR (Me_2SO-d_6) δ 11.44 (m, 1, CONHCO), 9.25 (d, *J* = 8.3 Hz, 1, NH), 8.85 (m, 2, NH_2^+), 7.51 (d, *J* = 8.1 Hz, 1, C_6H), 5.80 (d, *J* = 5.3 Hz, 1, C_1'H), 5.66 (d, *J* = 8.1 Hz, 1, C_5H), 4.72 (m, 1, C_5'H), 3.7–4.25 (m, 4, α-CH, C_2'H, C_3'H, and C_4'H), 2.70 (m, 2, NCH_2), 1.61 (m, 5, side chain), 0.88 (m, 9, Leu side chain $(CH_3)_2$ and CH_3 of *n*-Pr group).

1-[5′-(D-Tryptophanylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (6). Compound **6** was prepared by using the same procedure employed for the synthesis of L-Trp-uracil polyoxin C[5] (40 mg, 22.7%). The final product was >99.8% pure as judged by analytical HPLC on a reversed-phase C_{18} column: *K′* = 0.85 (CH_3OH–H_2O–TFA = 250:750:0.25, v/v/v); *K′* = 1.05 (CH_3CN–H_2O–TFA = 80:920:0.25, v/v/v) and on silica thin layers; R_fA 0.34; $[\alpha]^{25}_D$ −49.2° (*c* 0.12, H_2O); ^{1}H NMR (Me_2SO-d_6) δ 11.4 (m, 1, CONHCO), 11.0 (m, 1, Trp ring NH), 9.28 (d, 1, NH), 7.55 (d, *J* = 8.1 Hz, 1, C_6H), 7.78 (d, *J* = 7.4 Hz, 1, Trp ring H), 7.38 (d, *J* = 7.9 Hz, 1, Trp ring H), 7.0–7.22 (m, 3, Trp ring H), 5.82 (d, *J* = 4.9 Hz, 1, C_1'H), 5.64 (d, *J* = 8.1 Hz, 1, C_5H), 4.68 (m, 1, C_5'H), 3.9–4.2 (m, 4, α-CH, C_2'H, C_3'H, and C_4'H), 3.05 (m, 1, β-CH of Trp), 3.25 (m, 1, β-CH of Trp).

Z-L-2-(Aminooxy)-3-phenylpropionic Acid *p*-Nitrophenyl Ester. Z-L-2-(Aminooxy)-3-phenylpropionic acid (2.1 g, 6.6 mmol) (prepared according to the procedure of Briggs and Morley[30]) was converted into the *p*-nitrophenyl ester (2.0 g, 69%). The product was homogeneous on silica thin layers: R_f(CH_2Cl_2) 0.54; $[\alpha]^{25}$ −53.4° (*c* 0.5, MeOH); mp 98–99 °C; ^{1}H NMR (Me_2SO-d_6) δ 10.85 (s, 1, NH), 8.30 (d, AB quartet, *J* = 9.0 Hz, 2, NO_2-Ar-H), 7.15–7.45 (m, 12, 10 Ar-H and NO_2-Ar-H), 5.12 (s, 2, Ar CH_2), 4.83 (t, *J* = 6.4 Hz, 1, α-CH), 3.2 (m, 2, β-CH_2 of Phe).

1-[5′-[[2-(Aminooxy)-3-phenylpropionyl]amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (7). Compound **7** was prepared from **2** and Z-L-2-(aminooxy)-3-phenylpropionic acid *p*-nitrophenyl ester by using procedures described above (40.8 mg, 28.9%). The product was >99.8% pure as judged by analytical HPLC on a reversed-phase C_{18} column: *K′* = 1.4 (CH_3OH–H_2O–TFA = 260:740:0.25, v/v/v); *K′* = 1.82 (CH_3CN–H_2O–TFA = 120:880:0.25, v/v/v) and on silica thin layers; R_fA 0.41; $[\alpha]^{25}_D$ −18.75° (*c* 0.08, H_2O); ^{1}H NMR (Me_2SO-d_6) δ 11.41 (m, 1, CONHCO), 7.95 (d, *J* = 8.3 Hz, 1, NH), 7.50 (d, *J* = 8.1 Hz, 1, C_6H), 7.25 (m, 5, Ar H), 5.78 (d, *J* = 5.37 Hz, 1, C_1'H), 5.65 (d, *J* = 8.1 Hz, 1, C_5H), 4.6 (m, 1, C_5'H), 4.0–4.18 (m, 4, α-CH, C_2'H, C_3'H, and C_4'H), 3.0 (m, 1, β-CH), 2.69 (m, 1, β-CH).

1-(5′-Amino-5′-deoxy-β-D-allofuranosyluronamide)uracil (3). 1-(5′-Azido-2′,3′-*O*-cyclohexylidene-5′-deoxy-β-D-allofuranosyluronamide)uracil[26] (980 mg, 2.5 mmol) was dissolved in a mixture of dioxane (40 mL) and water (80 mL) and Dowex-50 W resin (H^+ form) (12 g) was added to it. The mixture was stirred and heated to 90 °C. The progress of the removal of cyclohexyl group was monitored by TLC. (eluent CH_2Cl_2–MeOH–HOAc = 10:1:0.5). When the reaction was complete (3 h), the resin was filtered off and the filtrate evaporated in vacuo. 1-(5′-Azido-5′-deoxy-β-D-allofuranosyluronamide)uracil was obtained as a gum (588 mg, 75.4%). The gum was dissolved in MeOH (15 mL) and Pd-black (~200 mg) was added. HCOOH (90%, 1.5 mL) was added to the reaction mixture and stirred at room temperature

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for 30 min. The catalyst was removed by filtration through Celite, and the filtrate was evaporated in vacuo. 1-(5′-Amino-5′-deoxy-β-D-allofuranosyluronamide)uracil–formic acid (**3**) was precipitated by the addition of ether (595 mg, 95%). The product was used for the preparation of compound **8** without further purification.

1-[5′-(Norleucylamino)-5′-deoxy-β-D-allofuranosyluronamide]uracil (8). Compound **8** was prepared from **3** and Z-Nle·ONp as described earlier (28 mg, 27.3%). The product was >99.6% pure as judged by analytical HPLC on a reversed-phase C_{18} column: K' = 0.99 (CH_3OH–H_2O–TFA = 100:900:0.25, v/v/v); K' = 2.76 (CH_3CN–H_2O–TFA = 40:960:0.25, v/v/v) and on silica thin layers; R_fA 0.38; $[\alpha]^{25}_D$ +7.5° (*c* 0.1, H_2O); ^{1}H NMR (Me_2SO-d_6) δ 11.42 (m, 1, CONHCO), 8.77 (d, 1 NH), 8.18 (m, 3, NH_3^+), 7.79 (d, *J* = 8.2 Hz, 1, C_6H), 7.59 (s, 1, $CONH_2$), 7.33 (s, 1, $CONH_2$), 5.85 (d, *J* = 7 Hz, 1, C_1'H), 5.66 (d, *J* = 8.1 Hz, 1, C_5H), 4.61 (m, 1, C_5'H), 3.82–4.09 (m, 4, α-CH, C_2'H, C_3'H, and C_4'H), 1.68 (m, 2, side-chain CH_2), 1.25 (m, 4, side chain $(CH_2)_2$), 0.85 (t, 3, side chain CH_3).

1-[5′-[(Phenylalanyl-Δ^Z-dehydrophenylalanyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (9). The azlactone of Z-Phe·Δ^{Z}Phe·OH (85.2 mg, 0.2 mmol) (prepared according to the procedure of English and Stammer[17]) was treated with **2** (66.6 mg, 0.2 mmol) in dry DMF (3 mL) in the presence of NMM (0.044 mL, 0.4 mmol) for 24 h at room temperature. The solvent was evaporated in vacuo and the crude Z derivative of **9** was precipitated by the addition of ether. It was isolated and dried (140 mg, R_fA 0.66). It was dissolved in acetic acid (0.2 mL), and to the solution was added 32% HBr in acetic acid (0.25 mL) and the mixture was stirred at room temperature for 30 min. The solvent was then removed in vacuo and the crude **9** was precipitated by the addition of ether. The crude final product was purified by preparative HPLC using CH_3OH–H_2O–TFA (300:700:0.25, v/v/v) as the eluent (38 mg, 27.4%). The product was >98% pure as judged by analytical HPLC on a reversed-phase C_{18} column: K' = 2.11 (CH_3OH–H_2O–TFA = 340:660:0.25, v/v/v); K' = 2.71 (CH_3CN–H_2O–TFA = 180:820:0.25, v/v/v) and on silica thin layers; R_fA 0.42; $[\alpha]^{25}_D$ −4.67° (*c* 0.15, HOAc); ^{1}H NMR (Me_2SO-d_6) δ 11.41 (m, 1, CONHCO), 10.23 (m, 1, CONHC=), 8.54 (d, *J* = 7.8 Hz, 1, NH), 8.19 (m, 3, NH_3^+), 7.63 (d, *J* = 8.3 Hz, 1, C_6H), 7.36 (m, 10, Ar H), 7.01 (s, 1, C=CH), 5.84 (d, *J* = 4.9 Hz, 1, C_1'H), 5.70 (d, *J* = 8.3 Hz, 1, C_5H), 5.55 (m, 1, OH), 5.23 (m, 1, OH), 4.70 (m, 1, C_5'H), 4.14 (m, 4 H, α-CH, C_2'H, C_3'H, and C_4'H), 3.25 (m, 1, β-CH of Phe), 2.96 (m, 1, β-CH of Phe).

***N*-*n*-Octanoyl-phenylalanyl-polyoxin D (10).** To a solution of Boc-Phe-ONp (42.5 mg, 0.11 mmol) and **1** (63.5 mg, 0.1 mmol) (isolated according to the procedure of Shenbagamurthi et al.[31]) in a mixture of DMF (2 mL) and water (0.2 mL) was added NMM (0.033 mL, 0.3 mmol), and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with DMF and water (1:1, 7 mL). Dowex-50 W resin (H^+ form) (~3 g) was added, and the reaction mixture was stirred for 30 more min to remove the unreacted **1** and salts. The resin was filtered off and the filtrate evaporated to dryness in vacuo. The crude Boc-Phe-polyoxin D was precipitated by adding ether (65 mg, 84.6%): R_fA 0.32.

The Boc-Phe·polyoxin D (65 mg) obtained above was subjected to acidolysis by treatment with a mixture of CH_2Cl_2–TFA (1:1, 2 mL) for 30 min. TFA·Phe-polyoxin D was isolated after precipitation with ether (64 mg, 96.6%).

This salt (54.7 mg, 0.07 mmol) was coupled with *n*-$C_7H_{15}COONp$ (20.4 mg, 0.077 mmol) (prepared according to the procedure of Shenbagamurthi, et al.[32]) as described above (23 mg, 41.3%). The product was >98% pure as judged by analytical HPLC on a reversed-phase C_{18} column: K' = 1.32 (CH_3OH–H_2O–TFA = 600:400:0.25, v/v/v); K' = 2.57 (CH_3CN–H_2O–TFA = 330:670:0.25, v/v/v) and on silica thin layers; R_fA 0.36; $[\alpha]^{25}_D$ +9.0° (*c* 0.1, HOAc); ^{1}H NMR (Me_2SO-d_6) δ 12.15 (m, 1, CONHCO), 8.38 (s, 1, C_6H), 8.02 (d, *J* = 8.1 Hz, 2, 2 NH), 7.25 (m, 6, Ar H and NH), 6.41 (m, 2, $CONH_2$), 5.83 (m, 1, C_1'H), 5.52 (m, 1, OH), 5.35 (m, 1, OH), 4.95 (m, 1, OH), 4.79 (m, 1, OH), 4.59 (m, 2, 2 α-CH), 4.46 (m, 1, α-CH), 4.16 (m, 2, CH_2O), 3.55–4.08 (m, 5, C_2'H, C_3'H, C_4'H, and 2 CHO), 3.07 (m, 1, β-CH of Phe), 2.74 (m, 1, β-CH of Phe), 2.01 (t, 2, CH_2CO), 1.2 (m, 10, $(CH_2)_5$), 0.87 (t, 3, CH_3).

Organisms and Growth Conditions. In this study we utilize the yeast strain *C. albicans* H317, a clinical isolate from the Centers for Disease Control, Atlanta, GA. The culture medium and growth conditions employed have been previously described.[5,6]

Chitin Synthetase Assay. Total chitin synthetase activity obtained in a mixed membrane fraction (mmf) from *C. albicans* H317 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.[5,6]

For determination of the kinetics of inhibition produced by the polyoxin analogues, the following modification to the referenced protocol was employed. The enzyme substrate 50 mM UDP-[^{14}C]-*N*-acetylglucosamine (200 000 cpm/μmol) was added to achieve substrate concentrations of 0.75, 1.0, 1.5, and 3.0 mM. Constant polyoxin inhibitor concentrations were employed between 0.5 and 0.01 mM.

Permeabilized Cell Chitin Synthetase Assay. To assay chitin synthetase activity in whole cells, similar procedures to those reported for *Saccharomyces cerevisiae* were employed.[33] Cells obtained from an overnight culture at 37 °C were added to 300 mL of fresh medium and grown for 6–8 h or until the cells were at mid-logarithmic stage. This procedure generally resulted in 1.0 g of cells wet weight. The cells were washed once in cold distilled water and once in 25 mM 2-morpholinoethanesulfonic acid (MES, pH 6.5). Following this the cells were resuspended in 3 times their wet weight in 25 mM MES containing 1% digitonin and incubated for 30 min at 30 °C. The permeabilized cells (as monitored microscopically by the inability to exclude trypan blue) were then washed twice in 25 mM MES (pH 6.5) and resuspended to 3 times their wet weight in 25 mM MES (pH 6.5) and used as such for the chitin synthetase assay. Prior to assay for chitin synthetase activity, the cells were incubated with 100 μg/mL trypsin for 10 min at 37 °C. Trypsin digestion was terminated by the addition of 150 μg/mL soybean trypsin inhibitor. For assay of inhibitor activity, 12.5 μL of each polyoxin analogue of 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6} M was added for each assay. The final reaction mixture contained 0.4 mM ATP, 180 μg/mL phosphatidyl serine, 2.0 mM $MgSO_4$, 14 mM MES, 0.25% digitonin, 30 mM *N*-acetylglucosamine, and 1.0 mM UDP-[^{14}C]-*N*-acetylglucosamine (0.451 μCi/μmol) in a total volume of 125 μL. Aliquots of 25 μL were removed at 5, 20, 40, and 60 min and added to 50 μL of glacial acetic acid; 2 mL of cold distilled water was then added, and the labeled cells were collected by filtration through a Whatman GF/C fiberglass filter prewashed with 20 mM sodium pyrophosphate and then washed twice with a glacial acetic acid–ethanol–water (60:200:740, by volume) solution. The filters were placed in scintillation cocktail, and the label incorporated into chitin was counted by liquid scintillation.

Peptidase Assay. The procedures used to prepare a cell extract of *C. albicans* H317 employed in assays for peptidase hydrolysis of the polyoxin compounds were previously reported.[5] When the peptidase hydrolysis of the polyoxin compds was assayed with high-voltage paper electrophoresis (HVPE), the procedures employed were the same as those previously described.[6] To assay for peptidase hydrolysis employing high-performance liquid chromatography (HPLC), the following protocol was utilized. In a total volume of 700 μL water (sterile, double distilled, deionized water), 52.5 μL of cell extract (200 μg/mL), and 52.5 μL (1 mg/mL) of the polyoxin compounds were incubated at 37 °C. At intervals of 0, 1, 2.5, 5, 15, and 30 min, 100-μL aliquots were withdrawn and added to 50 μL of water and frozen in a methanol/dry ice bath. The samples were then chromatographed on the HPLC. To assay the peptidase hydrolysis products, 100 μL of each time point was injected, resulting in 1 μg of cell extract and 5 μg of the polyoxin compound passing over the column per run.

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Transport Studies. The procedures and transport conditions to assay for the uptake of labeled trimethionine in the presence of polyoxin compounds have been previously described.[34] When assayed for the uptake of uridine, the cells were incubated in 2% dextrose at 37 °C for 10 min and added to an equal volume (0.5 mL) of reaction mixture containing 30 mM Bis-Tris buffer (pH 6.5) and 0.2 mM [^{14}C]uridine (10 mCi/mmol) with or without polyoxin analogues at a 10-fold concentration of uridine.

At intervals of 0, 1, 3, 5, and 7 min, aliquots 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 filters were placed in Bray's scintillation cocktail and were counted. The uptake results were expressed as nanomoles of trimethionine (1.0 mCi/mmol) and picomoles of uridine (10 mCi/mmol) taken up per milligram of dry weight cells.

Determination of MIC, MEC, Growth Inhibition, and Viability. The methods and procedures employed for the determinations of the MIC, MEC, growth inhibition, and viability of *C. albicans* H317 in the presence of the polyoxin analogues has been previously described.[5] For the microtiter assay, the MIC was recorded as the lowest concentration of drug that inhibited clearly visible growth; the MEC was defined as the lowest concentration of drug that results in (5%) morphologically abnormal cells at 48 h; growth inhibition was calculated by comparison of the number of cells at 48 h in the control well (no treatment) with the number of cells in drug-treated wells; the percentage of viability was calculated by comparing the number of viable colonies with the number of potential viable cells by direct counting.

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Synthesis and Antiviral Properties of 5-(2-Substituted vinyl)-6-aza-2′-deoxyuridines†

William L. Mitchell, Paul Ravenscroft, Malcolm L. Hill, Lars J. S. Knutsen, Brian D. Judkins, Roger F. Newton, and David I. C. Scopes*

Chemical Research Department, Glaxo Group Research Ltd., Ware, Hertfordshire SG12 ODJ, England. *Received July 15, 1985*

The following 5-(2-substituted vinyl)-6-aza-2′-deoxyuridines were synthesized: (*E*)-5-(2-bromovinyl) (**2**) (6-aza-BVDU), 5-(2-bromo-2-fluorovinyl) (a mixture of *E* and *Z* isomers) (**3**), (*E*)-5-(2-chlorovinyl) (**4**), (*E*)-5-[2-(methylthio)vinyl] (**5**), 5-(2,2-dibromovinyl) (**6**), and 5-(3-furyl) (**7**). The synthesis of **2–6** utilized Wittig-type reactions on 5-formyl-1-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-6-azauracil (**16**). 6-Aza-BVDU (and its α-anomer) was also synthesized from (*E*)-5-(2-bromovinyl)-6-azauracil (**12**) by using standard deoxyribosidation methodology. Compound **7** was prepared from 5-(3-furyl)-6-azauracil (**33**) via a ribosidation/deoxygenation sequence. An attempt to prepare the corresponding 5-(2,2-difluorovinyl) analogue afforded instead a mixture of the 5-[(2,2-difluoro-2-methoxy)ethyl] and 5-(2,2,2-trifluoroethyl) derivatives **29** and **30**. Compounds **2–7**, **29**, and **30** were tested for in vitro activity against herpes simplex virus types 1 and 2 (HSV-1, HSV-2). 6-Aza-BVDU (**2**) exhibited ID_{50}s of 8 μg/mL vs. HSV-1 and 190 μg/mL vs. HSV-2. BVDU (1) had ID_{50}s of 0.015 and 1.6 μg/mL against HSV-1 and HSV-2, respectively. Compound **4** showed a similar profile of activity, but the other analogues were either weakly active or inactive.

5-(2-Halovinyl)-2′-deoxyuridines are among the most active and selective inhibitors of herpes simplex virus type 1 (HSV-1) and varicella zoster virus (VZV) in cell culture.[1,2] Within this series of compounds (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU; 1) has emerged as the most potent analogue, and its efficacy against HSV-1 and VZV infections has been demonstrated in animal models and in phase I clinical trials.[2,3] However, limitations to the use of BVDU include its poor activity vs. HSV-2 and its rapid degradation by pyrimidine nucleoside phosphorylases.[4] A number of research groups have therefore conducted extensive analogue programs[1] with a view to improving the antiviral profile of BVDU. However, much of this work has been devoted to modification of the vinylic C-5 substituent and the carbohydrate moiety, and little attention has been given to changes in the heterocyclic nucleus.

During the 1960s several 5-substituted 6-aza-2′-deoxyuridines were synthesized as potential antiviral and antitumor agents. However, the nature of the 5-substitution was limited to halogen,[5] methyl[6], hydroxymethyl,[7] and trifluoromethyl.[5,8] Although these earlier studies did not

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(1) For an excellent review on the synthesis and antiviral properties of 5-vinylpyrimidine nucleoside analogues, see: De Clercq, E.; Walker, R. T. *Pharmacol. Ther.* **1984**, *26*, 1.

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