37 °C in an air-tight plexiglass box, which was flushed with 5% oxygen, 5% carbon dioxide, and 90% nitrogen. After 24 h of incubation, cultures were labeled with tritiated hypoxanthine and incubated for an additional 18–20 h prior to harvesting particulate matter on fiber glass strips. Hypoxanthine incorporation in each well was determined by scintillation spectrophotometry and served as an index of specific parasite growth rates. Computer-generated concentration–response curves were analyzed by nonlinear regression, and fifty percent inhibitory concentrations were calculated for each drug (Table III).

Two control *Plasmodium falciparum* clones derived by direct visualization and micromanipulation[33] were utilized.

B. Blood Schizontocidal Activity in Mice. The activity of the α-DQHS ethyl ether was compared with that of arteether and QHS in mice infected with a drug-sensitive strain of *Plasmodium berghei* and a range of drug-resistant lines of this parasite and *P. yoelii*.[27] The compounds were dissolved in water or suspended by sonication with 10 mL of and 0.1% solution of Tween 80. Animals received a range of doses as a single, subcutaneous dose of drug on each of 4 consecutive days, starting 3 h after infection with parasitised donor blood.[34] Parasites were counted in thin blood films made from each animal and from sham-treated controls 1 day after the end of treatment. Activity was assessed by comparing the levels of parasitaemia in treated animals with those in the controls. The 50% and 90% effective levels (ED_{50}, ED_{90}) were estimated from log dose/probit-activity graphs. An index of resistance at the ED_{90} level was also calculated.[34]

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Registry No. 1, 63968-64-9; 2, 112346-66-4; 2-Na, 112419-28-0; 3, 71939-50-9; 4, 71963-77-4; 5, 71939-51-0; 6, 75887-54-6; 7, 82534-75-6; 8, 72826-63-2; 9a, 94668-17-4; 9b, 112419-27-9; 10, 112297-78-6; 11, 112297-79-7; 12, 112346-67-5.

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Synthesis and Anticandidal Properties of Polyoxin L Analogues Containing α-Amino Fatty Acids

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Analogues of polyoxin L containing amino acids with saturated fatty acid like side chains were synthesized from the benzyloxycarbonyl-protected α-amino fatty acid *p*-nitophenyl ester and uracil polyoxin C. Transfer hydrogenolysis using palladium black and formic acid gave diastereomeric, dipeptidyl polyoxin L analogues containing α-aminooctanoic acid (**3**), α-aminododecanoic acid (**4**), or α-aminohexadecanoic acid (**5**) as the amine terminal residue in 40–60% yield. Diastereomers of **3** and **5** were resolved by using high-performance liquid chromatography on a reversed-phase column and designated as **3a**, **3b** and **5a**, **5b**. Analogues **3–5** were excellent inhibitors of chitin synthetase from *Candida albicans*; **4**, the best inhibitor, had an ID_{50} of 0.5 μM. The L,L diastereomers of **3** and **5** were 1–2 orders of magnitude more potent chitin synthetase inhibitors than their D,L homologues. None of the synthetic polyoxin L analogues inhibited transport of trimethionine, but **3a**, **4**, and **5b** caused decreases of 71%, 87%, and 83%, respectively, in the initial rate of uptake of dileucine. Compounds **3–5** were significantly more stable to peptidase degradation than polyoxin L analogues containing naturally occurring α-amino acids. Compound **4** inhibited growth of *C. albicans* in culture at 40–80 μg/mL. All other analogues were less potent antifungals. The results suggest that synthetic polyoxins can be designed to have increased affinity for a peptide transport system and to have increased stability against intracellular degradation in *C. albicans*.

Polyoxins[1] and nikkomycins[2-4] (neopolyoxins[5-7]) are peptidyl nucleoside antibiotics that exhibit marked activity against phytopathogenic fungi. They inhibit the enzyme chitin synthetase,[1,8,9] which catalyzes the biosynthesis of chitin, and bear a structural resemblance to uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), the natural substrate of this enzyme. Our laboratory has demonstrated that polyoxin D, one of the most active natural polyoxins, is toxic to the zoopathogenic fungi *Candida albicans* and *Cryptococcus neoformans* at millimolar concentrations.[10] The large number of infections caused by *C. albicans* in compromised hosts has fueled a major campaign by medicinal chemists to discover a more effective anticandidal drug. One approach to this problem involves the synthesis of novel polyoxin derivatives that will ultimately find application in the clinical treatment of patients with fungal infections.

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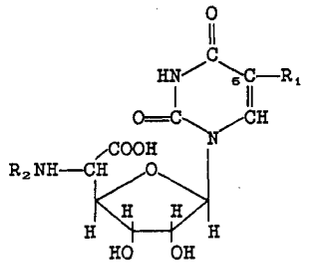
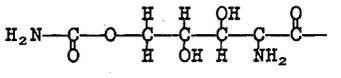
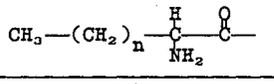


	R_1	R_2
1	COOH	
2	H	H
3 - 5	H	

3: n=5; 4: n=9; 5: n=13

Figure 1. Structural formula of polyoxin antibiotics.

Previous studies have shown that (1) certain polyoxin L analogues retain high activity against chitin synthetase,[11] (2) peptidase hydrolysis drastically reduces the effectiveness of these analogues against *C. albicans* in culture,[11,12] (3) tripeptidyl polyoxins serve as prodrugs,[12] and (4) insertion of chemical linkages known to reduce proteolytic degradation drastically reduces the inhibition of chitin synthetase by the resulting polyoxin analogue.[13,14]

Recently,[15] we have reported synthesis and characterization of two novel polyoxin analogues *N*-ε-octanoyllysyl uracil polyoxin C (Oct-Lys-UPOC) and *N*-γ-octylglutaminyl uracil polyoxin C (Oct-Gln-UPOC). These compounds exhibited high activity against chitin synthetase from *C. albicans* membrane preparations and were resistant to hydrolysis by a cell extract of *C. albicans* H-317.

In an attempt to further increase the stability of our synthetic polyoxins, we decided to incorporate saturated α-amino fatty acids as the amine terminal residue of dipeptidyl polyoxins. Such amino acids might also increase entry of the cell wall inhibitors into the yeast via either the peptide transport system or increased passive diffusion and might inhibit fungal growth due to direct interaction of the hydrocarbon side chain with the *C. albicans* cellular membrane.[16,17] In this paper, we report the synthesis and biological properties of polyoxin analogues containing long alkanelike side chains.

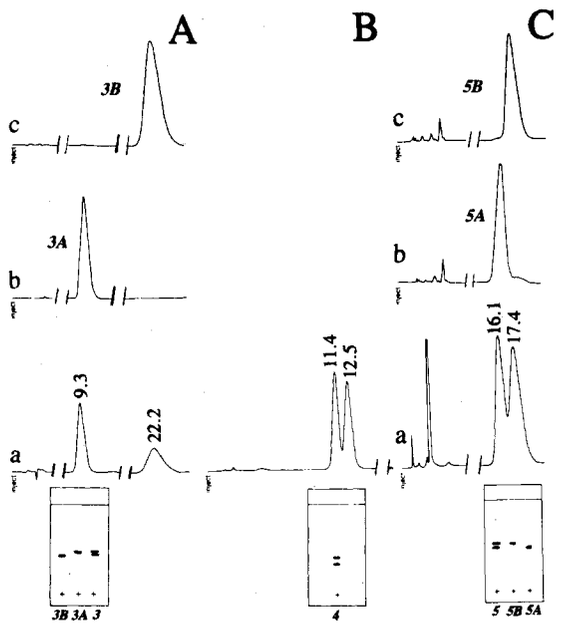


Figure 2. Analytical chromatography of polyoxin L analogues. Panel A, compound **3**. HPLC on a μ-Bondapak C_{18} column, methanol–water (3:97) as the mobile phase. (a) (±)-**3** diastereomers as isolated from hydrogenolysis. (b) **3a**, L,L diastereomer after purification. (c) **3b**, D,L diastereomer after purification. Insert represents silica gel thin layer eluted with 1-butanol–acetic acid–water (4:1:2). Panel B, compound **4**. Conditions as in panel A with the exception that the mobile phase was methanol–water (50:50). Only the diastereomeric mixture of **4** could be isolated. Panel C, compound **5**. Conditions as in panel A with the exception that the mobile phase was methanol–water (50:50). **5a** is the D,L diastereomer; **5b** is the L,L diastereomer. The early peaks in (b) and (c) are related to the solvent used for injection.

Chemistry

Most of the biological investigations on polyoxins have used polyoxin D (1, Figure 1). The polyoxins that we synthesized were prepared from uridine and are, thus, analogues of polyoxin L, which differs from 1 only in that the 5-carboxyl group (Figure 1) is replaced by a hydrogen atom.[18] All derivatives were prepared from 1-(5′-amino-5′-deoxy-β-D-allofuranosyluronic acid)uracil (**2**, Figure 1), which we have previously designated as uracil polyoxin C.[11] This product would be obtained by alkaline hydrolysis of the dipeptidyl bond of polyoxin L. It is synthesized from 2′,3′-*O*-cyclohexylideneuridine following the method of Damodaran et al.[19] The α-amino fatty acids were synthesized from α-bromo carboxylic acids following the method of Horning.[20] These were converted to benzyloxycarbonyl (Z) protected amino acids by use of Z-chloride under Schotten–Baumann conditions.[21] The Z-protected α-amino acids were activated as *p*-nitrophenyl esters according to the method of Shenbagamurthi et al.[22] Uracil

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Table I. Biological Activities of Polyoxin Analogues

analogue	MIC[a]			ID_{50}[b]	transport competition,[c] %		hydrolysis[d]
	H-317	124	124 NIK^R		$(Leu)_2$	$(Met)_3$	
1	640	320	320	2.0	5	10	–
2	>640	>640	>640	>100.0	ND[e]	3	–
3a	160	80	640	2.0	71	6	+
3b	>320	>320	>320	>50.0	14	10	–
4	80	40	80	0.3	87	0	–
5a	>320	>320	>320	>100.0	56	10	–
5b	>320	>320	>320	10.0	83	10	–

[a] Lowest concentration (μg/mL) of analogue that clearly inhibited growth of strains H-317, 124, and 124 NIK^R of *C. albicans*. [b] Concentration (μg/mL) at which chitin synthetase is inhibited by 50%. [c] 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 $(Met)_3$. [d] (+) compound was hydrolyzed by *C. albicans* H-317 extract, (–) no hydrolysis by cell extract. [e] ND is not done.

polyoxin C was then used to prepare a variety of analogues by selectively reacting its 5′-amino group with *p*-nitrophenyl esters of benzyloxycarbonyl (Z) protected amino acids in DMF–water at room temperature.[23] The yield of Z-protected polyoxin analogues ranged from 60% to 80%. They were deprotected by transfer hydrogenation with palladium black–formic acid in methanol[24] in 80–90% yield. All polyoxin analogues prepared by these methods were greater than 98% pure as judged by HPLC.

The hydrophobic polyoxin L analogues were prepared by use of racemic α-amino acid *p*-nitrophenyl esters. The resulting diasteromeric dipeptides had different mobilities on a C_{18} reversed-phase HPLC column with water–methanol as the mobile phase (Figure 2). Although we were able to isolate both isomers from 1-[5′-[(α-aminooctanoyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (**3**) and 1-[5′-[(α-aminohexadecanoyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (**5**), we were less successful with the 1-[5′-[(α-aminododecanoyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (**4**). Although **5a** and **5b** were separated by more than 8 min on a semipreparative C_{18} column, we were unable to find conditions giving base-line separation for the diastereomers from **4**. All attempts to separate these diastereomers resulted in a large sacrifice of material, and we decided to conduct the biological assays on the unresolved mixture (**4**). On the basis of their biological activities (see below), it appears that the faster moving isomer on the C_{18} column from compound **3** (designated **3a**) is the L,L dipeptide. In contrast, the faster moving isomer (**5a**) from compound **5** is D,L. Thus, as the side chain becomes larger, the interaction between the peptide and the C_{18} packing causes a reversal in the order of mobility of the diastereomeric dipeptides. This may explain the difficulty we experienced in isolating pure **4** diastereomers. On silica gel thin layers, the L,L dipeptide was the faster moving isomer for both **3** and **5**. Other workers have reported the reversed-phase separation of diastereomeric peptides.[25,26] To our knowledge, this is the first report of the successful application of this procedure to polyoxins. All compounds were characterized by 200-MHz proton NMR spectroscopy. They exhibit chemical shift values in conformity with those reported in the literature and expected for the peptidyl and nucleoside portions of molecule. Compounds **3–5** gave the expected C, H, N analyses.

Biological Results and Discussion

Inhibition of Chitin Synthetase. The antibiotics synthesized for this study were designed to be permeable inhibitors of chitin synthetase. Previous investigations showed that polyoxin L analogues containing hydrophobic amino acids such as Oct-Lys-UPOC or Oct-Gln-UPOC were excellent inhibitors of chitin synthetase.[15] For the most part, this property is retained in the compounds of the present investigation (Table I, ID_{50} values). Compounds **3a**, **4**, and **5b** cause 50% inhibition of chitin synthetase at concentrations of 4.4, 0.5, and 17.3 μM, respectively. In contrast, compounds **3b** and **5a** both have ID_{50} values greater than 100 μM. These results suggest that **3a** and **5b** are the L,L diastereomers whereas **3b** and **5a** are the D,L stereoisomers since polyoxin analogues with D-amino acids at the amine terminus are poor inhibitors of the enzyme.[13] The ID_{50} found for **4** is similar to that reported previously for Oct-Lys-UPOC (0.75 μM). Interestingly, compound **4** with a side chain of 10 carbon atoms is a better inhibitor than compound **3a** or compound **5b** with six and 14 carbon atom side chains, respectively. Apparently, the active site of chitin synthetase from *C. albicans* allows or prefers a hydrophobic group at the amino terminus of the inhibitor yet the steric bulk or the overall hydrophobicity that can be tolerated at this position is limited. On the other hand, the chitin synthetase assayed is in a particulate fraction. It is possible, therefore, that **5b** intercalates strongly into the membrane lipid and becomes sequestered from the enzyme. We cannot readily distinguish between these possibilities.

Competition for Peptide Transport. Both polyoxins and nikkomycins may be transported into *C. albicans* via peptide transport systems.[10,27,28] We measured dipeptide and tripeptide transport in the presence and absence of the synthetic analogues to determine if the novel compounds were recognized by peptide transport systems of *C. albicans* H-317 (Table I). None of the compounds were effective competitors for the uptake of the tripeptide $(Met)_3$. In contrast, dipeptide transport as measured by the uptake of $(Leu)_2$ was affected markedly by **4** (87% inhibition), **5a** (56% inhibition), **5b** (83% inhibition) and **3a** (71% inhibition). The result with **5a** is somewhat surprising as D,L peptides normally do not compete with dipeptide uptake into *C. albicans* (Table I, compound **3b** and ref 28–30). Despite this anomaly, it is clear that long

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alkane side chains are well tolerated by the peptide transport system in *C. albicans* H-317 and that the polyoxin L analogues have good affinity for the dipeptide transport system but not for the system transporting $(Met)_3$. Similar conclusions have previously been made concerning uptake of the polyoxin D and nikkomycin Z into *C. albicans*.[28]

Hydrolysis of Analogues by Cell Extracts. Compounds that inhibit essential enzymes in vitro may not be effective against the whole cell because of inactivation by cellular metabolism. Conversely, a compound that is ineffective in vitro may be activated by cell enzymes, leading to growth inhibition. Intracellular hydrolysis of the compounds investigated in this study should render them inactive because the products, fatty acyl amino acids and uracil polyoxin C, are not effective inhibitors of chitin synthetase. We found that cell extracts of *C. albicans* H-317 degraded **3a** to UPOC. Degradation was somewhat slower than that seen for norleucyl uracil polyoxin C (Nle-UPOC).[11] Under the conditions used, this latter analogue was completely hydrolized after a 30-min incubation with cell extract whereas approximately 50% of **3a** remained intact, and **3b** was not degraded at all. This latter result is consistent with our conclusion that **3b** is the D,L isomer. In contrast to **3a**, neither **4**, **5a**, nor **5b** were cleaved by a cell extract of strain H-317. These results suggest that increasing the number of carbons in the alkane side chain of the amine terminal residue results in stabilization of the polyoxin analogues toward peptidase degradation. This finding is consistent with previous results on Oct-Lys-UPOC and Oct-Gln-UPOC, which were highly stable to metabolism by *C. albicans*.[15]

Inhibition of Growth of *C. albicans* by Novel Polyoxins. The effect of **3a**, **3b**, **4**, **5a**, and **5b** on the growth of three strains of *C. albicans* was determined (Table I). Two of the strains were wild type (H-317 and 124) whereas strain 124 Nik^R was isolated on the basis of its resistance to nikkomycin. Under the conditions used, growth on ammonium sulfate as the nitrogen source, polyoxin D (**1**) is only weakly inhibitory toward the cells. Both **3a** and **4** show slightly improved activity compared to **1** against strain H-317 and strain 124. Compounds **3b**, **5a**, and **5b** are inactive. Although some differences were noted when cells were grown on isoleucine (0.5%), as the nitrogen source, **4** remained the most potent inhibitor of growth, and **3b**, **5a**, and **5b** were still inactive. The toxicity results correlate fairly well with the chitin synthetase activity, transport competition, and hydrolysis data. Compound **4**, which is the most potent enzyme inhibitor, seems to have high affinity for the $(Leu)_2$ transport system, is stable to cell extracts, and is the most toxic to the yeast. Lower toxicities observed for **3a** and **5b** can be attributed to cellular metabolism and poor chitin synthetase inhibition, respectively. Despite these findings, the most active analogue (**4**) is still not as good an anticandidal agent as the natural peptidyl nucleoside antibiotic nikkomycin, which is reported to kill *C. albicans* at a concentration of 5–10 μG/mL.[28,30]

Conclusion

The synthetic polyoxin L analogues containing alkane-like side chains near the amine terminus were found to be excellent substrates for the dipeptide transport system in *C. albicans*, efficient chitin synthetase inhibitors, and in some cases stable to a candidal cell extract. A number of the analogues showed activity against *C. albicans* superior to that exhibited by polyoxin D. Unfortunately, the best MIC was 40–80 μg/mL for compound **4**, which is probably not good enough for clinical applications. It is difficult to explain why an efficient, stable, transportable chitin synthetase inhibitor would not be more toxic against the yeast. Perhaps the transport competition reflects affinity but not translocation of the inhibitor into the cell. Studies on nikkomycins suggest that these inhibitors have a high K_m for peptide transport but a V_m that is only 5% of a normal peptide.[28] It is also possible that inhibition of chitin synthesis is not sufficient to kill the cell. Investigations now in progress in our laboratory are attempting to distinguish between these possibilities.

Experimental Section

Abbreviations. Standard abbreviations for amino acid derivatives and peptides are according to the IUPAC–IUB Commission on Biochemical Nomenclature (*Biochemistry* **1974**, *14*, 149–462). Additional abbreviations used are as follows: 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; ID_{50}, compound concentration resulting in 50% inhibition; MIC, minimum inhibitory concentration; NMM, *N*-methylmorpholine; ONp, *p*-nitrophenyl ester; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; UPOC, uracil polyoxin C; Z, benzyloxycarbonyl.

Chemical Synthesis. The melting points reported are uncorrected. All the solvents used were supplied by Fischer Scientific and were of analytical grade. *n*-Octanoic acid, dodecanoic acid, and hexadecanoic acid were purchased from Aldrich, Sigma, and Fischer Scientific, respectively.

All the polyoxins were homogeneous on silica gel thin layers (Brinkmann) with 1-butanol–acetic acid–water (4:1:2) as solvent and R_f values reported as R_fA. TLC plates were detected by utilizing either ultraviolet light or ninhydrin in butanol. 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 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 μ-Bondapak reversed-phase column (30 cm × 3.9 mm i.d.). The mobile phase was either MeOH–H_2O–TFA or MeOH–H_2O at a flow rate of 1.5 mL/min. The absorbance of the column eluants were recorded at 254 nm for all polyoxins. For separation of diastereoisomers, a Waters C_{18} semipreparative column (30 cm × 7.8 mm i.d.) was used.

The NMR spectra were recorded on an IBM 200-MHz instrument, and the chemical shift values are reported in ppm relative to tetramethylsilane. 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. As noted previously, the synthetic polyoxins are isolated as salts, which contain various amounts of acid and water.[11,12] The combined spectroscopic, chromatographic, and analytical data prove the authenticity of the synthetic antibiotics.

Synthesis of α-Bromoalkanoic Acids. The different α-bromoalkanoic acids were prepared from the corresponding alkanoic acids by use of red phosphorus and bromine following the method of Blatt.[31] α-Bromooctanoic acid, α-bromododecanoic acid, and α-bromohexadecanoic acid were all contaminated with unreacted starting material. Attempts to drive the reaction to completion resulted in side reaction. These impure α-bromoalkanoic acids were subjected to ammonolysis without further purification.

(±)-α-Aminooctanoic Acid. To a 100-mL flask were added ammonium hydroxide (21.84 mL, 28–30% NH_3) and α-bromooctanoic acid[31] (6.68 g, 0.029 mol). The flask was sealed and allowed to stand for 1 week at room temperature. The solid product was filtered, washed thoroughly with ethanol, and dried: yield 3.3 g (69%); mp 260–262 °C. This compound was homo-

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geneous on silica gel thin layers with 1-butanol–acetic acid–water (4:2:5, R_f 0.74) as eluant: NMR (0.25 mL of $AcOHd_4$ and 0.25 mL of TFA) δ 7.62 (br, NH_3), 4.33 (m, 1, α-CH), 2.25–1.95 (m, 2, β-CH_2), 1.65–1.20 (complex (c), 8, $(CH_2)_4$), 0.91 (c, 3, CH_3).

(±)-*N*-(Benzyloxycarbonyl)-α-aminooctanoic Acid. To a clear solution of α-aminooctanoic acid (1.5 g, 9.42 mmol) and aqueous NaOH (4.7 mL, 2 N) at 10 °C was added dropwise a suspension of benzyloxycarbonyl chloride (2.89 g, 16.95 mmol) in aqueous NaOH (16.92 mL, 2 N). After complete addition, the reaction mixture was stirred for 3.0 h, neutralized with 6 N HCl, and extracted with ethyl acetate. The ethyl acetate layer was dried with anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue was reprecipitated from ethyl acetate with petroleum ether to give a white crystalline solid, which was homogeneous on silica gel thin layers (1-butanol–acetic acid–water 4:2:5, R_f 0.8): yield 1.88 g (68%); mp 90–91 °C; NMR ($CDCl_3$) δ 7.35 (c, 5, Ar H), 5.22 (d, J = 7.99 Hz, 1, NH), 5.12 (s, 2, OCH_2), 4.39 (m, 1, α-CH), 2.0–1.6 (m, 2, β-CH_2), 1.27 (c, 8, $(CH_2)_4$), 0.87 (t, J = 5.9–6.4 Hz, 3, CH_3).

(±)-*p*-Nitrophenyl *N*-(Benzyloxycarbonyl)-α-aminooctanoate. (Benzyloxycarbonyl)-α-aminooctanoic acid (0.6 g, 2.046 mmol) and *p*-nitrophenol (0.284 g, 2.046 mmol) were dissolved in tetrahydrofuran (5.0 mL). The solution was cooled to 0 °C, and dicyclohexylcarbodiimide (0.421 g, 2.04 mmol) was added. Stirring was continued at 0 °C for 2 h and at room temperature for 17.0 h. The dicyclohexylurea that formed was removed by filtration, and the filtrate was evaporated to dryness under vacuum. The residue was dissolved in absolute ethanol and precipitated with petroleum ether. The crude product was subjected to rapid column chromatography on silica gel with petroleum ether and ether (1:1) as eluant. The white solid product (0.55 g, 65%), mp 80–81 °C, was homogeneous on silica gel thin layers with petroleum ether–ether (1:1) as eluant (R_f 0.57): NMR ($CDCl_3$) δ 8.27 (d, J = 8.8 Hz, 2, Ar H), 7.33 (c, 7, Ar H), 5.24 (d, J = 7.15 Hz, 1, NH), 5.14 (s, 2, OCH_2), 4.59 (m, 1, α-CH), 2.1–1.72 (m, 2, β-CH_2), 1.5–1.15 (c, 8, $(CH_2)_4$), 0.89 (t, J = 6.1–6.7 Hz, 3, CH_3). Anal. Calcd for $C_{22}H_{26}N_2O_6$: C, 63.75; H, 6.32; N, 6.75. Found: C, 63.52; H, 6.48; N, 6.71.

(±)-1-[5′-[(α-Aminooctanoyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil Formate (3). To a solution of uracil polyoxin C[19] (0.075 g, 0.263 mmol) and *p*-nitrophenyl (benzyloxycarbonyl)-α-aminooctanoate (0.12 g, 0.288 mmol) in dimethylformamide–water (20 mL, 1:1) were added *N*-methylmorpholine (0.049 mL, 0.45 mmol) and 1-hydroxybenzotriazole (0.043 g, 0.288 mmol), and the reaction mixture was stirred at room temperature for 20 h. The progress of the reaction was monitored by high-performance liquid chromatography, and after completion, the solution was acidified with acetic acid and evaporated to dryness under vacuum. The residue on trituration with ether yielded a light brown solid, which was washed with ether, dried, and reprecipitated from dimethylformamide with 2% aqueous acetic acid, yield 0.078 g (52.5%). The product was homogeneous on silica gel thin layers with *n*-BuOH–HOAc–H_2O(4:1:2, R_f 0.61) as eluant and had minor impurity as judged by reversed-phase HPLC [t_R 5.26 min (methanol–water, 50:50)]. It was used for transfer hydrogenation without further purification. To a suspension of the Z derivative obtained above (0.075 g, 0.133 mmol) in methanol (6.0 mL) and dimethylformamide (2.0 mL) was added palladium black (34.0 mg) and formic acid (0.3 mL, 88%). After the mixture was stirred for 1.0 h, TLC indicated no starting material. The catalyst was removed by filtration through Celite. The filtrate was evaporated to dryness under vacuum, and a white solid was recovered after trituration with ether, yield 0.055 g (90%). On silica gel thin layers, the product showed two ninhydrin positive spots corresponding to two diastereoisomers with *n*-BuOH–HOAc–H_2O (4:1:2, R_{f3b} 0.44, R_{f3a} 0.48) as eluant. Two isomers were also evident in NMR as H_6 and H_5 both appeared as two doublets and on reversed-phase HPLC [t_{R3a} 9.3 min, t_{R3b} 22.2 min, (H_2O–MeOH, 97:03)] (panel A of Figure 2).

The two isomers were separated by HPLC using a semipreparative reversed-phase C_{18} column (30 cm × 7.8 mm) and water–methanol (90:10) as the mobile phase.

3a: $[\alpha]^{24}_D$ 40.4° (*c* 0.11, H_2O); NMR (DMSO-d_6) δ 11.30 (br, 1, NH (uracil)), 8.22 (m, 1, CONH), 7.86 (d, J = 8.0 Hz, 1, C_6H), 5.76 (d, J = 6.9 Hz, 1, $C_1'H$), 5.58 (d, J = 8.0 Hz 1, C_5H), 4.34 (c, 1, $C_5'H$), 4.11 (c, 3, $C_2'H$, $C_3'H$, $C_4'H$), 3.73 (c, 1, α-CH), 1.59 (m, 2, β-CH_2), 1.23 (c, 8, $(CH_2)_4$), 0.86 (t, J = 6.0–6.3 Hz, 3, CH_3). Anal. Calcd for $C_{18}H_{28}N_4O_8 \cdot 1.3H_2O$: C, 47.84; H, 6.82; N, 12.39. Found: C, 47.67; H, 6.06; N, 12.73.

3b: $[\alpha]^{24}_D$ 10.4° (*c* 0.11, H_2O); NMR (DMSO-d_6) δ 11.30 (br, 1, NH (uracil)), 8.16 (m, 1, CONH), 7.91 (d, J = 8.0 Hz, 1, C_6H), 5.77 (d, J = 6.9 Hz, 1, $C_1'H$), 5.57 (d, J = 7.9 Hz, 1, C_5H), 4.33 (c, 1, $C_5'H$), 4.1 (c, 3, $C_2'H$, $C_3'H$, $C_4'H$), 3.89 (dd, J = 6.4–6.64 Hz, 1, α-CH) 1.58 (m, 2, β-CH_2), 1.20 (c, 8, $(CH_2)_4$), 0.82 (t, J = 5.8–6.7 Hz, 3, CH_3). Anal. Calcd for $C_{18}H_{28}N_4O_8 \cdot 1.7H_2O$: C, 47.19; H, 6.69; N, 12.23. Found: C, 46.89; H, 6.72; N, 12.58.

(±)-α-Aminododecanoic Acid. To a 100-mL flask were added ammonium hydroxide (40.96 mL, 28–30% NH_3) and α-bromododecanoic acid[31] (11.76 g, 0.042 mmol). The flask was sealed and allowed to stand for 1 week at room temperature. The solid product was filtered, washed with ethanol, and dried: yield 6.01 g (66%); mp 232–234 °C. This compound was homogeneous on silica gel thin layers with 1-butanol–acetic acid–water (4:2:5, R_f 0.74) as eluant. NMR (0.25 mL of $AcOHd_4$ and 0.25 mL of TFA) δ 7.60 (br, NH_3), 4.35 (m, 1, α-CH), 2.13 (m, 2, β-CH_2), 1.48 (c, 16, $(CH_2)_8$), 0.90 (t, 3, J = 5.8–6.6 Hz).

(±)-(Benzyloxycarbonyl)-α-aminododecanoic Acid. To a clear solution of α-aminododecanoic acid (1.0 g, 4.65 mmol) and aqueous NaOH (2.4 mL, 2 N) at 10 °C was added dropwise a suspension of benzyloxycarbonyl chloride (1.42 g, 8.37 mmol) in aqueous NaOH (8.4 mL, 2 N). After addition was complete, the reaction mixture was stirred at 10 °C for 1 h, neutralized with 6 N HCl, and extracted with ethyl acetate. The ethyl acetate layer was dried with anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue was reprecipitated from ethyl acetate with petroleum ether to yield a white crystalline solid, which was homogeneous on silica gel thin layers with *n*-BuOH–HOAc–H_2O(4:1:5, upperphase R_f 0.82): yield 0.75 g (46.2%); mp 84–86 °C; NMR ($CDCl_3$) δ 7.35 (c, 5, Ar H), 5.23 (d, J = 8.14 Hz, 1, NH), 5.12 (s, 2, OCH_2), 4.38 (m, 1, α-CH), 1.74 (c, 2, β-CH_2) 1.25 (c, 16, $(CH_2)_8$), 0.87 (t, J = 5.9–6.5 Hz, 3, CH_3).

(±)-*p*-Nitrophenyl *N*-(Benzyloxycarbonyl)-α-aminododecanoate. (Benzyloxycarbonyl)-α-aminododecanoic acid (600 mg, 1.72 mmol) and *p*-nitrophenol (0.24 g, 1.72 mmol) were dissolved in tetrahydrofuran (4.0 mL). The solution was cooled to 0 °C, and dicyclohexylcarbodiimide (0.35 g, 1.72 mmol) was added. Stirring was continued at 0 °C for 2 h and then at room temperature for 44 h. The dicyclohexylurea that is formed was removed by filtration, and the filtrate was evaporated to dryness under vacuum. The crude product was dissolved in methylene chloride and subjected to rapid column chromatography using a silica gel column and methylene chloride as eluant. The product (0.51 g, 63%) was a sharp-melting solid, mp 98–100 °C. It was homogeneous on silica gel thin layers with methylene chloride (R_f 0.64) as eluant: NMR ($CDCl_3$) δ 8.27 (d, J = 8.9 Hz, 2, Ar H), 7.34 (c, 7, Ar H), 5.23 (d, J = 7.8 Hz, 1, NH), 5.14 (s, 2, OCH_2), 4.62 (m, 1, α-CH), 1.9 (m, 2, β-CH_2), 1.26 (c, 16, $(CH_2)_8$), 0.88 (t, J = 6.1–6.6 Hz, 3, CH_3). Anal. Calcd for $C_{26}H_{34}O_6N_2$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.25; H, 7.09; N, 5.71.

(±)-1-[5′-[(α-Aminododecanoyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil Formate (4). To a solution of uracil polyoxin C[19] (0.06 g, 0.21 mmol) and *p*-nitrophenyl (benzyloxycarbonyl)-α-aminododecanoate (0.10 g, 0.23 mmol) in dimethylformamide (5.0 mL) and water (3.0 mL) were added *N*-methylmorpholine (0.039 mL) and 1-hydroxybenzotriazole (0.03 g, 0.19 mmol), and the reaction mixture was stirred at room temperature for 44 h. The progress of the reaction was monitored by high-performance liquid chromatography. When no additional reaction could be detected, the solution was acidified with acetic acid and evaporated to dryness. Trituration with ether yielded a light brown solid. The solid was washed with ether, dried, and reprecipitated from dimethylformamide and 2% aqueous acetic acid, yield 0.072 g (55.4%). The product was homogeneous on silica gel thin layers with *n*-BuOH–HOAc–H_2O (4:1:2, R_f 0.71) as eluant and was homogeneous on reversed-phase HPLC [t_R 10.3 min (methanol–water–TFA, 65:35:0.05, v/v)] and was used for transfer hydrogenation without further purification. To a turbid solution of the Z-protected derivative (0.072 g, 0.116 mmol) in methanol (5.0 mL) and dimethylformamide (2.0 mL) were added palladium black (30 mg) and formic acid (0.26 ml, 88%). After the mixture was stirred for 5 h, TLC indicated complete hydrogenolysis, and the catalyst was removed by filtration through

Celite. The filtrate was evaporated to dryness and precipitated with ether to give a light brown solid: yield 0.046 g (75%); $[\alpha]^{24}_D$ 45.5° (*c* 0.11, MeOH). The product exhibited two spots corresponding to two isomers on silica gel thin layers with *n*-BuOH–HOAc–H_2O(4:1:2, R_{f4} 0.35 and 0.43) as eluant. Two isomers are also indicated by NMR and reversed-phase HPLC [t_{R4} 11.36 min and 12.45 min (water–methanol, 50:50, v/v)]. (Figure 2, panel B). NMR (DMSO-d_6) δ 11.25 (br, 1, NH (uracil)), 8.28 (d, J = 3.7 Hz, 1, CONH), 7.82 and 7.87 (2 d, J_1 = 8.2 Hz, J_2 = 8.0 Hz, 1, two isomeric C_6H), 5.77 (d, J = 6.9 Hz, 1, C_1'H), 5.60 and 5.57 (2 d, J_1 = 8.0 Hz, J_2 = 8.0 Hz, 1, two isomeric C_5 H), 3.65–4.40 (c, 5, α-CH, C_5'H, C_2'H, C_3'H, C_4'H), 1.05–1.80 (c, 18, $(CH_2)_9$), 0.82 (t, J = 5.6–6.4 Hz, 3, CH_3). Anal. Calcd for $C_{23}H_{38}O_{10}N_4$ (as formate salt): C, 52.06; H, 7.21; N, 10.56. Found: C, 52.26; H, 7.30; N, 10.75.

(±)-α-Aminohexadecanoic Acid. α-Aminohexadecanoic acid was prepared by ammonolysis of the corresponding α-bromo acid as described for the C_8 and C_{12} carboxylic acids. A white solid product, which was homogeneous on silica gel thin layers with *n*-BuOH–HOAc–H_2O (4:2:5, R_f 0.77) as eluant, was recovered in 30% yield: mp 232–234 °C; NMR (0.25 mL of $AcOHd_4$ and 0.25 mL of TFA) δ 7.64 (br, NH_3), 4.35 (m, unresolved, 1, α-CH), 2.14 (m, 2, β-CH_2), 1.32 (c, 24, $(CH_2)_{12}$), 0.90 (t, unresolved, 3, CH_3).

(±)-*N*-(Benzyloxycarbonyl)-α-aminohexadecanoic Acid. To a suspension of α-aminohexadecanoic acid (0.7 g, 2.58 mmol) and aqueous NaOH (1.28 ml, 2N) at 0 °C was added dropwise a suspension of benzyloxycarbonyl chloride (0.791 g, 4.64 mmol) in aqueous NaOH (4.6 mL, 2 N). After complete addition, the reaction mixture was stirred at 0 °C for 3.5 h, neutralized with 6 N HCl, and extracted with ethyl acetate. The ethyl acetate layer was dried with anhydrous sodium sulphate and evaporated to dryness under vacuum. The crude product was reprecipitated from ethyl acetate with petroleum ether and dried: yield 0.45 g (64.6%); mp 79–81 °C. The product was homogeneous on silica gel thin layers with *n*-BuOH–HOAc–H_2O (4:2:5, R_f 0.94) as eluant: NMR ($CDCl_3$) δ 7.35 (c, 5, Ar H), 5.21 (d, J = 8.3 Hz, 1 NH), 5.12 (s, 2, OCH_2), 4.39 (m, 1, α-CH), 2.0–1.55 (m, 2, β-CH_2), 1.25 (c, 24, $(CH_2)_{12}$), 0.89 (t, J = 6.05–6.66 Hz, 3, CH_3).

(±)-*p*-Nitrophenyl *N*-(Benzyloxycarbonyl)-α-aminohexadecanoate. (Benzyloxycarbonyl)-α-aminohexadecanoic acid (0.25 g, 0.61 mmol) and *p*-nitrophenol (0.086 g, 0.61 mmol) were dissolved in tetrahydrofuran (5.0 mL). The solution was cooled to 0 °C, and dicyclohexylcarbodiimide (0.127 g, 0.61 mmol) was added. Stirring was continued at 0 °C for 2.0 h and then at room temperature for 15.0 h. Dicyclohexylurea was filtered off and washed with tetrahydrofuran. The filtrate was evaporated to dryness under vacuum, and the crude product was subjected to rapid column chromatography on silica gel (35–70 mesh ASTM) with methylene chloride as the eluant. The product (0.2 g, 62%) was a sharp melting white solid, mp 100–102 °C, which was homogeneous on silica gel thin layers with methylene chloride (R_f 0.49) as eluant NMR ($CDCl_3$) δ 8.27 (d, J = 8.9 Hz, 2, Ar H), 7.33 (c, 7, Ar H), 5.23 (d, J = 8.0 Hz, 1, NH), 5.14 (s, 2, OCH_2), 4.58 (m, 1, α-CH), 1.89 (m, 2, β-CH_2), 1.30 (c, 24, $(CH_2)_{12}$), 0.88 (t, J = 6.03–6.77 Hz, 3, CH_3). Anal. Calcd for $C_{30}H_{42}O_6N_2$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.51; H, 7.94; N, 5.18.

(±)-1-[5′-[(α-Aminohexadecanoyl)amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil Formate (5). To a solution of uracil polyoxin C[19] (0.055 g, 0.193 mmol) and *p*-nitrophenyl (benzyloxycarbonyl)-α-aminohexadecanoate (0.111 g, 0.21 mmol) in dimethylformamide (7.0 ml) and water (0.7 mL) were added *N*-methylmorpholine (0.036 mL, 0.33 mmol) and 1-hydroxybenzotriazole (0.028 g, 0.21 mmol), and the reaction mixture was stirred at room temperature for 20.0 h. After the reaction was completed, the solution was acidified with acetic acid and evaporated to dryness. The residue was triturated with petroleum ether, washed with petroleum ether, dried, and reprecipitated from dimethylformamide with 2% aqueous acetic acid to a solid product, yield 0.104 g (80%). The solid was homogeneous on silica gel thin layers with n-BuOH–HOAc–H_2O (4:1:2, R_f 0.73) as eluant and on reversed-phase HPLC [t_R 21.4 min (methanol–water–TFA, 65:35:0.05, v/v)]. This compound was used directly for transfer hydrogenation. To a suspension of the Z-protected product (0.1 g, 0.148 mmol) in methanol (7.0 mL) were added palladium black (37.8 mg) and formic acid (0.33 mL, 88%). After the mixture was stirred for 2.5 h, TLC indicated complete deprotection. The catalyst was removed by filtration through Celite. The filtrate was evaporated to dryness under vacuum, and the residue was triturated with petroleum ether to give a yellowish solid, yield 0.064 g (79%). The product was homogeneous and showed two ninhydrin positive spots corresponding to two diastereoisomers on silica gel thin layers with *n*-BuOH–AcOH–H_2O (4:1:2, R_{f5A} 0.49, R_{f5B} 0.53) as eluant. Two isomers were also evident on reversed-phase HPLC [t_{R5a} 16.1, t_{R5b} 17.4 (H_2O–MeOH, 35:65)] (Figure 2, panel C). Anal. Calcd for $C_{26}H_{44}N_4O_8 \cdot 2H_2O$: C, 54.15; H, 8.39; N, 9.71. Found: C, 54.16; H, 8.33; N, 9.96.

The two diastereoisomers were separated by reversed-phase high-performance liquid chromatography using a reversed phase semipreparative C_{18} column (30 cm × 7.8 mm) and H_2O–MeOH (40:60) as the mobile phase.

5a: $[\alpha]^{25}_D$ 20° (*c* 0.05, MeOH); NMR (DMSO-d_6) δ 11.29 (br, 1, NH (uracil)), 8.19 (m, 1, CONH), 7.89 (d, J = 7.6 Hz, 1, C_6H), 5.76 (d, J = 6.6 Hz, 1, C_1'H), 5.57 (d, J = 7.6 Hz, 1, C_5H), 4.32–3.80 (c, 5, C_2'H, C_3'H, C_4'H, α-CH, C_5'H), 1.57 (m, 2, β-CH_2), 1.23 (c, 24, $(CH_2)_{12}$), 0.85 (t, J = 5.7–6.5 Hz, 3 CH_3).

5b: $[\alpha]^{25}_D$ 18° (*c* 0.05, MeOH); NMR (DMSO-d_6) δ 11.32 (br, 1, NH (uracil)), 8.28 (m, 1, CONH), 7.84 (d, J = 7.6 Hz, 1, C_6H), 5.76 (d, J = 5.92 Hz, 1, C_1'H), 5.59 (d, J = 7.6 Hz, 1, C_5H), 4.3–3.8 (c, 5, C_2'H, C_3'H, C_4'H, α-CH, C_5'H), 1.6 (m, 2, β-CH_2), 1.23 (c, 24 $(CH_2)_{12}$), 0.85 (t, J = 5.8–6.7 Hz, 3, CH_3).

Biological Methods. Organisms. In this study we used the following yeast strains: *C. albicans* H-317, a clinical isolate from the Centers for Disease Control, Altanta, 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.[13,15]

Peptidase Assay. The procedures used to prepare a cell extract of *C. albicans* H-317 in assays for hydrolysis of the polyoxin compounds were previously reported.[13] To assay for hydrolysis, high-performance liquid chromatography (HPLC) was used. Cell extract (100 μL; 200 μg of protein/mL), 100 μL of the polyoxin compounds (1 μg/mL), and 300 μL of water were incubated at 37 °C. At intervals of 0, 1, 5, 15, and 30 min, 100-μL portions were withdrawn, added to 50 μL of water, and frozen in a methanol–dry ice bath. The samples were then chromatographed on a C_{18} μ-Bondapak Waters column (reversed phase, 30 cm × 3.9 mm i.d.) with a 0–90% gradient of methanol in water and 0.025% trifluoroacetic acid.

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.[13,15] Cells of strain H-317 were harvested at 8×10^6 cells/mL by centrifugation after growth in yeast nitrogen base with isoleucine (0.5%) as nitrogen source. After two washes with sterile distilled water, the cells were resuspended in 2% glucose and warmed at 37 °C for 10 min. An equal volume of cells was added to citric acid (10 mM)–KH_2PO_4 (10 mM) buffer at pH 3.5 with radioactive trimethionine (2.9×10^{-5} M, 1 μCi/μmol)[32] or pH 5.5 buffer with radioactive dileucine (5.6×10^{-5} M, 44 μCi/μmol)[33] to a total volume of 1 mL. 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 filters were placed in 5 mL of scintillation cocktail and counted. 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

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as the nitrogen source.[13,15] The MIC was recorded as the lowest concentration of drug that resulted in 5% morphologically aberant 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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Registry No. 2, 24695-48-5; **3A**, 112139-13-6; Cbz-**3A**, 112139-12-5; **3B**, 112139-14-7; Cbz-**3B**, 112139-26-1; 4 (diastereomer 1), 112139-17-0; 4 (diastereomer 2), 112139-18-1; 4·HCOOH (diastereomer 1), 112139-19-2; 4·HCOOH (diastereomer 2), 112139-20-5; Cbz-4 (diastereomer 1), 112139-16-9; Cbz-4 (diastereomer 2), 112139-27-2; **5A**, 112139-24-9; Cbz-**5A**, 112139-23-8; **5B**, 112139-25-0; Cbz-**5B**, 112139-28-3; (±)-α-bromooctanoic acid, 70610-87-6; (±)-α-aminooctanoic acid, 644-90-6; (±)-*N*-(benzyloxycarbonyl)-α-aminooctanoic acid, 76313-08-1; (±)-*p*-nitrophenyl *N*-(benzyloxycarbonyl)-α-aminooctanoate, 112139-11-4; (±)-α-bromododecanoic acid, 112139-15-8; (±)-α-aminododecanoic acid, 35237-37-7; (±)-*N*-(benzyloxycarbonyl)-α-aminododecanoic acid, 66398-12-7; (±)-*p*-nitrophenyl *N*-(benzyloxycarbonyl)-α-aminododecanoate, 66398-13-8; (±)-α-aminohexadecanoic acid, 98320-69-5; (±)-α-bromohexadecanoic acid, 40305-76-8; (±)-*N*-(benzyloxycarbonyl)-α-aminohexadecanoic acid, 112139-21-6; (±)-*p*-nitrophenyl *N*-(benzyloxycarbonyl)-α-aminohexadecanoate, 112139-22-7; chitin synthetase, 9030-18-6.

Development of a New Physicochemical Model for Brain Penetration and Its Application to the Design of Centrally Acting H_2 Receptor Histamine Antagonists

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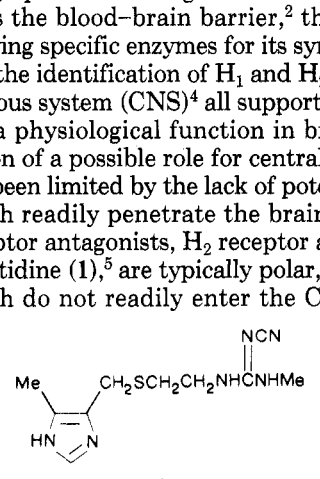
A rational approach to the design of centrally acting agents is presented, based initially upon a comparison of the physicochemical properties of three typical histamine H_2 receptor antagonists which do not readily cross the blood–brain barrier with those of the three brain-penetrating drugs clonidine (**6**), mepyramine (**7**), and imipramine (**8**). A good correlation was found between the logarithms of the equilibrium brain/blood concentration ratios in the rat and the partition parameter, Δ log *P*, defined as log *P* (1-octanol/water) − log *P* (cyclohexane/water), which suggests that brain penetration might be improved by reducing overall hydrogen-bonding ability. This model has been employed as a guide in the design of novel brain-penetrating H_2 antagonists by the systematic structural modification of representatives of different structural types of H_2 antagonists. Although marked increases in brain penetration amongst congeners of cimetidine (**1**), ranitidine (**9**), and tiotidine (**10**) were achieved, no compound was found with an acceptable combination of H_2 antagonist activity ($-\log K_B$ in the guinea pig atrium > 7.0) and brain penetration (steady-state brain/blood concentration ratio > 1.0). Conversely, structural modification of *N*-[[(piperidinylmethyl)phenoxy]propyl]acetamide (**30**) led to several potent, novel compounds which readily cross the blood–brain barrier. One of these, zolantidine (SK&F 95282, **41**), whose $-\log K_B$ is 7.46 and steady-state brain/blood ratio is 1.4, has been identified for use in studying histaminergic H_2 receptor mechanisms in brain. Comparison of Δ log *P* values with the logarithms of the brain/blood ratios for 20 structurally diverse compounds for which data became available confirms a highly significant correlation and supports the general validity of this model.

Histamine has been shown to be present in the brain of many species including humans.[1] While it does not readily cross the blood–brain barrier,[2] the occurrence of accompanying specific enzymes for its synthesis and degradation[3] and the identification of H_1 and H_2 receptors in the central nervous system (CNS)[4] all support the view that histamine has a physiological function in brain. Thus far, investigation of a possible role for central histamine H_2 receptors has been limited by the lack of potent, selective antagonists which readily penetrate the brain. Unlike histamine H_1 receptor antagonists, H_2 receptor antagonists, for example cimetidine (**1**),[5] are typically polar, hydrophilic compounds which do not readily enter the CNS.[6-8]



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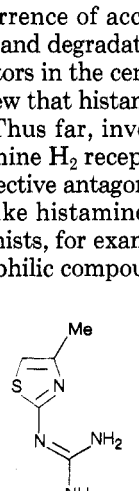

2

A compound which might be considered as a brain-penetrating H_2 antagonist from a report that it caused "CNS stimulation" in animals is ICI 17148 (**2**),[9] and we have since shown that it crosses the blood–brain barrier moderately well in a rat model (see below). The usefulness of **2** as a potential tool for studying histaminergic mechanisms in brain is, however, limited by its low H_2 antagonist activity. In the isolated guinea pig right atrium it

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