Synthesis and Antifungal Activity of Novel Cationic Pneumocandin B_0 Derivatives

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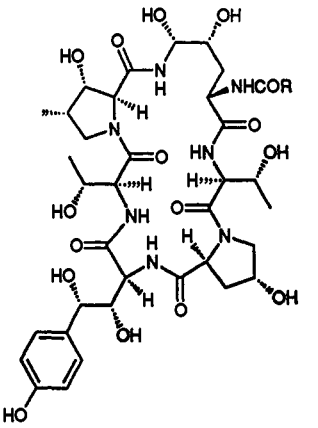
Received November 8, 1993

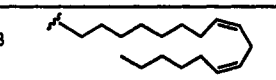
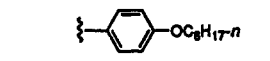
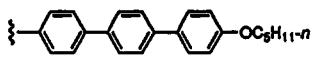
The need for new drugs to treat systemic fungal infections has intensified due to the rapid growth of the immunocompromised patient population.[1] The increase in the frequency of fungal infections has been accompanied by an increase in the variety of opportunistic and pathogenic fungi which now include *Candida*, *Cryptococcus*, *Aspergillus*, *Pneumocystis*, *Histoplasma*, *Coccidiodes*, and *Fusarium*.[2]

Amphotericin B (AmB) remains the most effective drug for the treatment of acute, life-threatening mycoses; however, it has significant drawbacks related to its toxicity.[3] Use of the newer and safer triazoles in the management of disseminated fungal infections is increasing,[4] although prolonged treatment with these fungistatic agents is often necessary to prevent relapse and reports of resistance are appearing.[5]

The echinocandins are a class of fungicidal cell-wall active lipopeptides which are specific inhibitors of β-(1,3)-D-glucan synthesis.[6] β-(1,3)-D-Glucan is a carbohydrate polymer essential for the structural integrity of the cell wall of many of the medically important fungi. Initially thought to be relatively narrow spectrum agents restricted to certain pathogenic strains of *Candida*, the spectrum of the echinocandins has recently been expanded to include *Pneumocystis carinii*[7] and *Aspergillus fumigatus*.[8] *P. carinii* pneumonia (PCP) is a life-threatening opportunistic infection most frequently found in patients with AIDS. Trimethoprim–sulfamethoxazole (TMP/SMZ) and pentamidine isethionate are used to treat and prevent PCP, but both are associated with a high incidence of adverse reactions.[9] Atovaquone has recently been approved for the treatment of mild to moderate PCP infections in patients intolerant of TMP/SMZ; however, an improved oral formulation is needed.[10] While less common than candidiasis or PCP, invasive aspergillosis has a mortality rate surpassing 60%.[11] AmB remains the treatment of choice, although the new triazole drug, itraconazole, is under study as an alternative.[4a]

Echinocandin B (1) is the major component of a family of lipopeptides isolated in 1974 from an *Aspergillus* culture.[12,13] Cilofungin (2), a semisynthetic side-chain derivative of echinocandin B, is the most thoroughly studied of this class of compounds.[6c] Although found effective against disseminated candidiasis in phase II clinical trials, it was withdrawn due to toxicity associated with its solubilizing vehicle.[14] Continuing their study of side-chain structure–activity relationships, Lilly scientists recently described LY303366 (3), a more potent variation of cilofungin currently undergoing preclinical evaluation, in which the (octyloxy)phenyl side chain has been replaced by (pentyloxy)terphenyl.[15]



Compound	R
1 echinocandin B	
2 cilofungin	
3 LY303366	

The pneumocandins are a complex of echinocandin-like lipopeptides produced by the fungus *Zalerion arboricola*. Their isolation, structure elucidation, and biological evaluation by scientists from these laboratories have recently been reviewed.[16,17] Pneumocandin B_0 (4, L-688786) differs from echinocandin B in three ways: (1) a 10,12-dimethylmyristoyl side chain replaces the linoleoyl side chain; (2) a 3-hydroxyglutamine residue replaces the threonine adjacent to the homotyrosine (hTyr); and (3) a 3-hydroxyproline residue substitutes for the 3-hydroxy-4-methylproline present in echinocandin B. Notable among these differences is the additional site for selective chemical modification of the nucleus provided by the primary amide of the Glu residue in addition to the phenol and the S_N1-active hydroxyl groups of the Orn and hTyr residues. The aqueous insolubility of 4 was addressed by selective phosphorylation of the phenol in the presence of the base-sensitive hemiaminal to provide the water-soluble phosphate prodrug 5 (L-693989) which was equipotent to 4 in rodent models of disseminated candidiasis and PCP.[18] In an effort to find an intrinsically water-soluble and less labile derivative of 4, further chemical modification was focused on the hemiaminal and the Glu residue.

Relatively little is known about structure *vs* activity at the hemiaminal. Reductive elimination of the hydroxyl group results in decreased anti-*Candida* activity[7a,19] as does conversion to lower alkyl ethers.[20] However, the aminoethyl ether of tetrahydroechinocandin B is reportedly water soluble in its protonated form.[21] The analogous ether derivative of 4 was prepared and evaluated for antifungal activity. Treatment of 4 with ethanolamine hydrochloride in the presence of HCl directly provides aminal ether 6 (L-705589) as the major product in addition to the β-anomer and bisether.[22,23]

The primary amide of the Glu residue of 4 was selectively converted to an amine in a two-step sequence *via* nitrile 7. Dehydration of the amide with cyanuric chloride[24]

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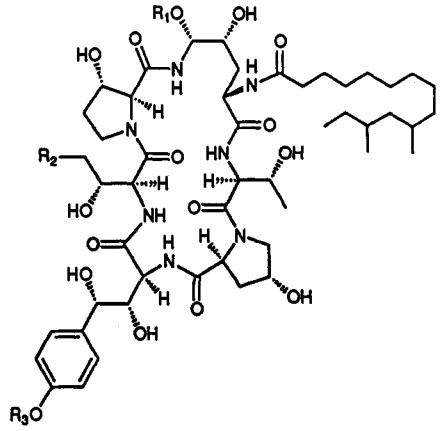

Compound	R_1	R_2	R_3
4	H	$CONH_2$	H
5	H	$CONH_2$	$PO_3H^-Na^+$
6	$CH_2CH_2NH_2$	$CONH_2$	H
7	H	CN	H
8	H	CH_2NH_2	H
9	$CH_2CH_2NH_2$	CH_2NH_2	H

requires carefully controlled conditions in order to avoid degradation of the product by the HCl generated during the reaction. Cyanuric chloride was added in one portion to a solution of 4 in anhydrous DMF at 25 °C and the reaction quenched with 2 M NaOAc after a period of 5.5 min.[25] Increasing the reaction time or lowering the temperature to 0 °C decreased the yield of 7 due to formation of increasing amounts of unwanted byproducts. Other dehydrating reagents which gave less satisfactory results include oxalyl chloride, (trimethylsilyl)polyphosphate, trifluoroacetic anhydride/pyridine, and cyanuric chloride/pyridine. Reduction of the nitrile with $NaBH_4/CoCl_2$[26] provides 8 (L-731373),[27] the first example of an echinocandin with a 3-OH Orn residue adjacent to the hTyr. The aminoethyl ether was prepared as described above by reaction of 8 with ethanolamine hydrochloride in the presence of acid to give the novel bisamine 9 (L-733560) in 38% yield after purification by reverse-phase HPLC.[28,29]

The results of the *in vitro* evaluation of amines 6 and 8 as water-soluble hydrochloride salts and of 9 as a dihydrochloride salt are reported in Table 1. The IC_{50}'s of 6 and 8 *vs* a membrane preparation of the *C. albicans* (MY1208) β-(1,3)-D-glucan synthase are 11 and 10 nM, respectively, a 7-fold improvement relative to 4 (IC_{50} = 70 nM). An overall 70-fold increase in the level of glucan synthesis inhibition compared to 4 was achieved by the combination of the hemiaminal-modified aminoethyl ether with the reduced Glu to give 9 (IC_{50} = 1 nM). The improved IC_{50}'s exhibited by amines 6, 8, and 9 were reflected in their *in vitro* anti-*Candida* activity. The minimum fungicidal concentrations (MFC's) *vs C. pseudotropicalis*, a reference strain, and several clinical isolates were determined in a whole cell broth microdilution assay.[30] The most potent fungicidal activity was found against *C. albicans* and *C. tropicalis* (MFC range = <0.06 μg/mL for 8 and 9 to 0.25 μg/mL for 6) with *C. parapsilosis* (MFC range = 0.125 μg/mL for 8 and 9 to 1 μg/mL for 6) being somewhat less susceptible.

The potent *in vitro* anti-*Candida* activity of amine derivatives 6, 8, and 9 correlated with high *in vivo* efficacy in a mouse model of disseminated candidiasis as shown in Table 2. The target organ kidney assay (TOKA) utilizing congenitally immunodeficient DBA/2 mice dosed ip twice daily (bid) has been described previously.[30] $ED_{99.9}$ values for 6, 8, and 9 against *C. albicans* (MY1055) were 0.78, 0.375, and 0.09 mg/kg, respectively, an 8–70-fold increase in potency compared to 4 ($ED_{99.9}$ = 6.0 mg/kg).[31] In the acute PCP model employing dexamethasone-immunosuppressed rats dosed sc,[7a] the ED_{90} values for 6, 8, and 9 were 0.037, 0.037, and 0.019 mg/kg, respectively, a 2–8-fold improvement over 4 (ED_{90} = 0.15 mg/kg). Evaluation of 6, 8, and 9 in a 28-day survival model of disseminated apsergillosis utilizing DBA/2 mice infected with *A. fumigatus* (MF 5668) and dosed ip bid on days 0–4 found ED_{50}'s of 0.06 and 0.03 mg/kg for 6 and 9, respectively. Surprisingly, 8 and 4 were not effective (ED_{50}'s >20 mg/kg), providing the first evidence that a blocked hemiaminal may be necessary for potent *in vivo Aspergillus* activity. This may be understandable in light of the fact that *Aspergillus* spp. are producers of echinocandins.[32]

The pneumocandin B_0 derivatives described in this communication demonstrate that the introduction of an amine provides water soluble compounds which, in addition, exhibit significantly improved *in vivo* efficacy *vs* disseminated candidiasis, aspergillosis (6 and 9), and PCP. In particular, bisamine 9 which combines an aminal aminoethyl ether with a reduced 3-OH Glu residue represents a novel lipopeptide whose exceptional potency and expanded spectrum warrant its further study as an antifungal drug with a new mode of action *vs* the currently marketed antimycotics.

Table 1. *In Vitro* Comparison of Pneumocandin B_0 (4) and Amine Derivatives 6, 8, and 9

		minimum fungicidal concentration (μg/mL)[b]				
	β-(1,3)-D-glucan	*C. albicans*				
compd	IC_{50} (nM)[a]	MY 1028	MY 1055	*C. tropicalis* MY 1012	*C. parapsilosis* MY 1010	*C. pseudotropicalis* MY 2099
4	70	0.25	0.5	0.125	2	1
6	11	0.125	0.125	0.25	1	1
8	10	<0.06	<0.06	<0.06	0.125	0.125
9	1	<0.06	0.125	<0.06	0.125	<0.06

[a] Concentration of test compound causing 50% inhibition of β-(1,3)-D-glucan synthesis in a *C. albicans* (MY 1208) membrane assay (see ref 7a). [b] Median of a minimum of four duplicate determinations in a broth microdilution assay (see ref 30).

Table 2. *In Vivo* Efficacy of Pneumocandin B_0 (4) and Amine Derivatives 6, 8, and 9 in Rodent Models of Candidiasis, Aspergillosis, and *Pneumocystis carinii* Pneumonia

compd	TOKA[a] $ED_{99.9}$ (mg/kg)	*Aspergillus*[b] ED_{50} (mg/kg)	PCP[c] ED_{90} (mg/kg)
4	6.0	>20	0.15
6	0.78	0.06	0.037
8	0.375	>20	0.037
9	0.09	0.03	0.019

[a] Target organ kidney assay employing DBA/2 mice (see ref 30). $ED_{99.9}$ is the minimum dose administered ip bid which decreases the number of *C. albicans* (MY1055) colony forming units/g kidney by 99.9% *vs* untreated controls. Renal clearance ranged from 60 to 80%. [b] DBA/2 mouse model of disseminated *A. fumigatus* (MF5668) infection. ED_{50} is the required dose administered ip bid on days 0–4 which increases the 28-day survival rate by 50%. [c] Acute PCP model employing dexamethasone-immunosuppressed Sprague–Dawley rats (see ref 7a). ED_{90} is the minimum dose administered sc bid for 4 days which decreases the lung cyst count by 90% over untreated controls.

Acknowledgment. We thank Dr. James Heck and Mr. Helmut Kropp for their support of this work, Ms. Amy Hale for providing mass spectral data, and Mrs. Dorothy Levorse for pK_a data.

Supplementary Material Available: 500-MHz ^{1}H-NMR spectra of compounds 6, 8, and 9 (3 pages). Ordering information is given on any current masthead page.

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(22) To a 0.24 M solution of 4 in DMSO containing 20 molar equiv of ethanolamine hydrochloride is added 1 molar equiv of HCl as a 4 M solution in dioxane. The reaction is monitored by reverse-phase HPLC: Zorbax Rx-C18 (4.6 × 250 mm, column temperature 40 °C); 40% A/B (see ref 23) at a flowrate of 1.5 mL/min; UV detection at 210 and 277 nm; t_R (6) = 6.2 min (60% after 65 h at room temperature). The product is isolated by preparative HPLC of the reaction mixture diluted five times with H_2O: Delta-Pak C_{18} (15 μm, 100 Å); 15–30% C/D (see ref 23) at a flowrate of 50 mL/min in 5% step gradients. The mixed HCl/CH_3COOH salt obtained by lyophilization of the product-containing fractions is converted to

the full HCl form on a Bio-Rad AG2-X8 (Cl^-) column eluting with H_2O. Yield = 36%. HPLC purity > 99%. Anal. ($C_{52}H_{86}ClN_9O_{17}$) C, H, N, Cl. HRMS (FAB) MH^+ calcd for $C_{52}H_{86}N_9O_{17}$ 1108.6141, found 1108.6176.

(23) A = 95:5 CH_3CN/H_2O (0.1% CF_3COOH); B = 95:5 H_2O/CH_3CN (0.1% CF_3COOH); C = 95:5 CH_3CN/H_2O (0.1% CH_3COOH); D = 95:5 H_2O/CH_3CN (0.1% CH_3COOH).

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(25) To a 0.08 M solution of **4** in DMF at room temperature is added 2.3 molar equiv of cyanuric chloride in one portion. After a period of 5.5 min, the reaction is quenched by the addition of 2 M NaOAc. Analytical HPLC conditions are as described in ref 22 except for the following: column Zorbax ODS: mobile phase 55% A/B; t_R (**7**) = 5.4 min (66%). The DMF and H_2O are removed *in vacuo*. The residue is slurried in CH_3OH and poured into stirred CH_3CN (50 mL/g **4**). The resulting mixture is filtered. The filtercake is slurried in a minimum volume of CH_3OH (*ca.* 3.5 mL/g **4**), and the slurry is poured into stirred H_2O (50 mL/g **4**). The mixture is filtered, and the filtercake is air-dried, providing crude **7** which is typically 60% pure by HPLC and used as is in the subsequent reduction.

(26) Satoh, T.; Suzuki, S.; Suzuki, Y.; Miyaji, Y.; Imai, Z. Reduction of organic compounds with sodium borohydride-transition metal salt systems. I. Reduction of organic nitrile, nitro and amide compounds to primary amines. *Tetrahedron Lett.* **1969**, 4555–4558.

(27) To a 0.03 M solution of **7** in CH_3OH containing 6.8 molar equiv of $CoCl_2{\cdot}6H_2O$ is added 34 molar equiv of $NaBH_4$ portionwise (**caution!** exotherm and vigorous hydrogen evolution) over a period of 30 min. After HPLC analysis, the reaction is quenched by the addition of 680 molar equiv of HCl added as a 2 N aqueous solution. The reaction mixture is stirred for a period of 30 min and filtered. H_2O is added to the filtrate to the cloud point. CH_3CN is added to the clear point, and this solution is chromatographed. Analytical and preparative HPLC conditions and conversion to the hydrochloride salt are as described in ref 22: t_R (**8**) = 7.9 min. Yield = 30% (two steps). HPLC purity > 99%. Anal. ($C_{50}H_{83}ClN_8O_{16}$) C, H, N, Cl. HRMS (FAB) MH^+ calcd for $C_{50}H_{83}N_8O_{16}$ 1051.5927, found 1051.5901.

(28) Analytical HPLC conditions are as described in ref 22: t_R (**9**) = 4.2 min. Isolation by preparative HPLC is done as described in ref 22 except for the following: mobile phase 15–20% C/D in 5% step gradient. Conversion to the dihydrochloride salt is done as described in ref 22. Yield = 38%. HPLC purity > 99%. Anal. ($C_{52}H_{89}Cl_2N_9O_{16}$) C, H, N, Cl. HRMS (FAB) MH^+ calcd for $C_{52}H_{86}N_9O_{16}$ 1094.6348, found 1094.6338.

(29) The water solubility of **9**·2HCl, **8**·HCl, and **6**·HCl is >50 mg/mL. The amine pK_a's for **9**, determined in 1:1 MeOH/H_2O, are 8.7 and 9.7. Thus **9**, at physiological pH, is dicationic.

(30) Bartizal, K.; Abruzzo, G.; Trainor, C.; Krupa, D.; Nollstadt, K.; Schmatz, D.; Schwartz, R.; Hammond, M.; Balkovec, J.; VanMiddlesworth, F. *In Vitro* Antifungal Activities and *In Vivo* Efficacies of 1,3-β-D-Glucan Synthesis Inhibitors L-671,329, L-646,991, Tetrahydroechinocandin B, and L-687,781, a Papulacandin. *Antimicrob. Agents Chemother.* **1992**, *36*, 1648–1657.

(31) Bisamine **9**, administered iv, gave a similar result.

(32) Conversion of **8** to its hemiaminal methyl ether restores *Aspergillus* activity. Unpublished results from these laboratories.