Semisynthetic Chemical Modification of the Antifungal Lipopeptide Echinocandin B (ECB): Structure–Activity Studies of the Lipophilic and Geometric Parameters of Polyarylated Acyl Analogs of ECB

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Echinocandin B (ECB) is a lipopeptide composed of a complex cyclic peptide acylated at the N-terminus by linoleic acid. Enzymatic deacylation of ECB provided the peptide "nucleus" as a biologically inactive substrate from which novel ECB analogs were generated by chemical reacylation at the N-terminus. Varying the acyl group revealed that the structure and physical properties of the side chain, particularly its geometry and lipophilicity, played a pivotal role in determining the antifungal potency properties of the analog. Using CLOGP values to describe and compare the lipophilicities of the side chain fragments, it was shown that values of >3.5 were required for expression of antifungal activity. Secondly, a linearly rigid geometry of the side chain was the most effective shape in enhancing the antifungal potency. Using these parameters as a guide, a variety of novel ECB analogs were synthesized which included arylacyl groups that incorporated biphenyl, terphenyl, tetraphenyl, and arylethynyl groups. Generally the glucan synthase inhibition by these analogs correlated well with *in vitro* and *in vivo* activities and was likewise influenced by the structure of the side chain. These structural variations resulted in enhancement of antifungal activity in both *in vitro* and *in vivo* assays. Some of these analogs, including LY303366 (**14a**), were effective by the oral route of administration.

Introduction

The need for safer and more effective antifungal agents has intensified with the increased number of opportunistic infections.[1,2] This is especially true among patients with immune systems compromised by hematologic malignancy, myelosuppressive therapy, or HIV infection. Currently available antifungal therapy includes amphotericin B (AMB), a polyene, whose effectiveness is often associated with toxicity, and the azoles, such as fluconazole and itraconazole, that are comparatively safe but whose frequent usage makes them a target for resistance development.[3,4] Antifungal agents have been obtained from both natural and synthetic sources. Of these, the natural products provide many structurally diverse classes that are currently under study.[5] One such natural product, echinocandin B (ECB, **1**), a novel antifungal lipopeptide, has potent fungicidal[6] and anti-*Pneumocystis carinii*[7,13a] activities by virtue of its ability to disrupt fungal cell wall development. Several studies have shown that this disruption is the result of noncompetitive inhibition of glucan synthesis, a major component in the architecture of the fungal cell wall.[8] This mode of action, that is selectively targeted to the cell wall of the pathogen, greatly reduces the toxic liability of ECB and related antifungal lipopeptides.[9] Although studies with ECB showed that this agent had mild hemolytic properties, it was found generally free of major organ-related toxicity. Its excellent antifungal potency, unique mode of action against a pathogen-associated target, and low toxicity make ECB an important subject for structure–activity–toxicity studies aimed at development of novel antifungal agents with an improved therapeutic index.

ECB is the archetype of an entire family of lipopeptides[10] that have several structural and biological features in common including excellent antifungal activity.[11] This class also includes the aculeacins, the pneumocandins, mulundocandin, and WF11899.[12] Each of the known members of this family are highly active against several pathogenic fungi including *Candida albicans*.[13] Although initial reports indicated that these antibiotics were primarily active against yeasts, more recent studies show that antimicrobial activity can include *P. carinii* and *Aspergillus* sp.[14]

The structural features common to these lipopeptides include a medium sized cyclic peptide (the "nucleus") composed of six amino acids,[11] whose N-terminus is acylated by a fatty acid group, referred to here as the "side chain" which usually is either unbranched or branched with a chain length of 14–18 carbon atoms. The peptide is cyclized by a novel aminal linkage between the C-terminus and the δ-amino group of the dihydroxyornithine residue. This functional group was readily cleaved at high pH resulting in ring opening and complete loss of antifungal potency.[12e] Full expression of biological activity required that the cyclic peptide and the side chain be covalently linked at the N-terminus, emphasizing the importance of the acyl group to the mode of action of ECB.[15,16]

Enzymatic deacylation of ECB with *Actinoplanes utahensis* provided the corresponding deacylated cyclic peptide (ECB nucleus, **2**) (Scheme 1) which lacked antifungal activity.[17] When the *N*-acyl group was chemically reintroduced, biological activity was restored as long as certain structural requirements were met (see

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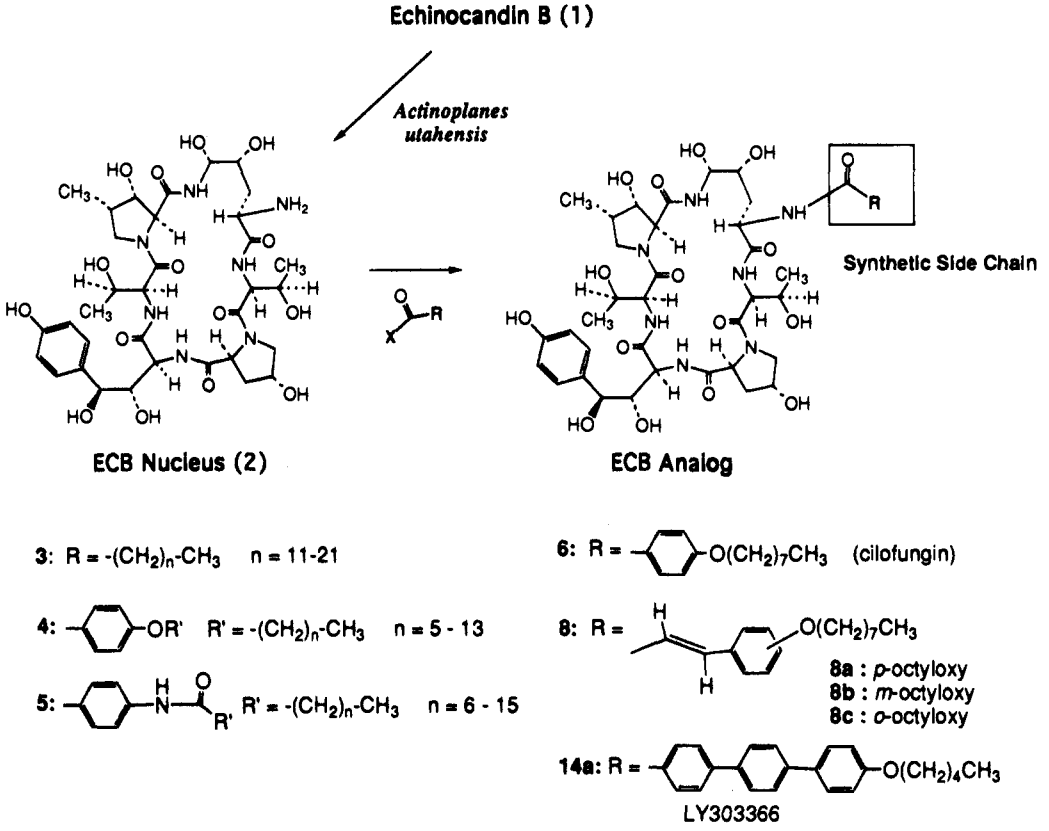
Scheme 1



below).[17] Synthesis of novel ECB analogs by chemical reacylation of **2** with synthetic acyl groups revealed how side chain structure affected antifungal activity and toxicity.[11] Series **3** (see Scheme 1), bearing homologous side chains prepared from alkyl fatty acids, showed that antifungal activity was a function of side chain length. Small acyl groups, such as acetyl, benzoyl, or cyclohexanoyl, were ineffective (data not shown) in restoring activity to **2**. However, increasing the chain length to C_{12} gave the *N*-dodecanoyl ECB analog which had moderate anti-*Candida* activity. This activity was gradually optimized with stepwise extension of the chain length to 17–18 carbons.[11,17] The data reveal that with each series of side chains, there exists a minimal lipophilicity below which antifungal activity is not observed and an optimal lipophilicity at which antifungal effectiveness is maximized.

Two series of active ECB analogs incorporating the 4-alkoxybenzoyl (**4**) and 4-amidobenzoyl (**5**) side chains demonstrated that a diverse range of structures including lipophilic aroyl groups could replace the natural fatty acid moieties. Varying the side chain alkoxy and *N*-amido chain lengths, respectively, modulated the lipophilic character in these series. As with series **3**, antifungal activity was not restored to series **4** and **5** until the hydrophobic alkoxy or amido group was sufficiently lipophilic, this lipophilic "gateway" being a characteristic property of a given side chain type. For series **4**, an alkoxy chain length greater than six was required; longer chain lengths gave ECB analogs that were more potent antifungal agents than ECB. Similarly, optimal activity in the PABA series (**5**) was attained at the *N*-myristoyl-PABA ECB analog. In this case, a larger alkyl component was necessary than that required in series **4** in order to counteract the greater polarity of the PABA acyl group. These series of semisynthetic analogs show that high potency could be achieved by incorporation of substituted aryl groups provided that the overall lipophilicity was adjusted to some optimal level.

The nature of the side chain also influenced toxicity. *In vivo* studies showed that the parent ECB was hemolytic. This toxicity was influenced by the side chain since the deacylated peptide **2** was not hemolytic. Modification within a given series showed that increasing lipophilicity correlated with increasing hemolysis. Fortunately, the structure–activity relationships (SAR) for antifungal activity and hemolysis were not identical, allowing the identification and selection of cilofungin (**6**) as a potent, nonhemolytic ECB analog for clinical study. This demonstrated that modification of the ECB side chain provided a means to control both potency and safety of the ECB series.

The present report will summarize recent studies of the structure–activity relationships of ECB analogs using novel aryl and polyaryl side chains. The lipophilic and geometric requirements of the side chain structures were clarified using a computer-assisted method for selection of appropriate side chains. The good correlation of glucan synthase inhibition to the antifungal activity by the analogs was helpful in following the emerging series. The biological properties of the analogs were tested by both *in vitro* and *in vivo* methods and revealed significant enhancements of antifungal activity.

Chemistry

Novel ECB analogs were prepared by varying the structure of the *N*-acyl side chain. This side chain was introduced as previously described[11] by N-acylation of the deacylated ECB peptide **2** as shown in Scheme 1.

The preparation of the side chains is summarized in Schemes 2–5. The final analogs are listed in Table 1 which also shows the structure of each respective side chain. In order to simplify tracking, side chains will be referred to by doubling the letter that is used to designate the final ECB analog containing that side chain (e.g., ECB analog **7a** is acylated by side chain **7aa**).

Scheme 2 outlines the synthesis of most of the analogs containing an aryl–alkyl ether linkage **[7(aa–dd), 9(aa–dd), 11aa,cc, 12(aa–dd), 15(aa–ee)]**. The various aryl modules are listed with the normal substitution pattern being the linear, 1,4-substitution pattern. When the aryl group was naphthyl, both the 2,6- and 2,3-substitutions were made. The ethers were formed either by direct alkylation of a phenolic hydroxyl group using base and an alkyl halide or tosylate or by Mitsunobu conditions[18] using the alkyl alcohol, diethyl azodicarboxylate (DEAD), and triphenylphosphine. Some of the biphenyl side chains (**9aa,cc,dd**) used 4-cyano-4′-hydroxybiphenyl as a starting material and necessitated hydrolysis of the nitrile to the acid.

The terphenyl and tetraphenyl side chains **[13ee, 14(aa–dd)]** were prepared using the Suzuki reaction[19] as shown in Scheme 3. When the aryl module was phenyl, the terphenyl was formed, and when the aryl module was biphenyl, a tetraphenyl side chain was the result. Biphenyl boronic acids were coupled to the aryl module iodides or triflates using tetrakis(triphenylphosphine)palladium(0) to give the side chain esters.

Side chains containing an acetylenic linker **[13(bb–dd)]** were synthesized using the stepwise methodology outlined in Scheme 4. The modules $aryl_1$ and $aryl_2$ were either phenyl or biphenyl effectively altering the position of the linker in the phenyl chains. An aryl iodide or triflate segment was coupled to (trimethylsilyl)-acetylene using bis(triphenylphosphine)dichloropalladium(II).[20] After removal of the trimethylsilyl group by methanolysis, a second coupling reaction was performed with the second aryl group to give the final side chain ester. Preparation of **13bb** utilized the commercially available phenylacetylene requiring only a single coupling reaction. Compound **20**, containing a phenolic hydroxyl, was alkylated as shown in Scheme 2 to give the aryl ether side chains.

The synthesis of the straight and branched side chains with a terminal biphenyl group is shown in Scheme 5. These compounds were prepared by condensing 4-hydroxybiphenyl with the appropriate alkyl bromide to give the series **10(aa–dd)**.

The benzylic ether side chain **11bb** was synthesized by coupling (butyloxy)benzene with methyl 4-(chloromethyl)benzoate using ferric chloride as the Lewis acid in a Friedel–Crafts alkylation reaction.[21]

Finally, all of the side chains were hydrolyzed to their carboxylic acid forms. Active esters were synthesized with 2,4,5-trichlorophenol and DCC and then coupled to **2** in DMF giving the final products.

Biological Evaluation

Fungal susceptibility was measured *in vitro* using a microdilution microtiter assay against *C. albicans*, and the results are expressed as the MIC value, the minimum concentration of antifungal agent which completely inhibits visible fungal growth. *In vivo* evaluation of the ECB analogs employed a murine experimental infection model using X-irradiated mice infected with *C. albicans*, and the results are expressed as an ED_{50} value which is the dose (mg/kg) at which 50% of the animals survive the 7 day study. Both antimicrobial evaluation procedures are described in detail in the Experimental Section.

The echinocandin lipopeptides most likely owe their primary mode of antifungal action to their inhibition of the biosynthesis of the fungal cell wall. Exposure of intact yeast cells to cilofungin and other echinocandin-related antibiotics resulted in cell lysis. These antibiotics selectively inhibit glucose incorporation into β-(1,3)-D-glucan, an essential cell wall component, without disruption of synthesis of other biopolymers. This action was shown to be due to inhibition of β-(1,3)-D-glucan synthase from several fungal species.[8d] Echinocandins are noncompetitive inhibitors of this enzyme, but the exact nature of this inhibitory interaction is not well defined. Inhibition is not complete, and at high concentrations is only 75–85%. A purified, solubilized preparation of this enzyme from *C. albicans* was used for the present studies.[22] Table 2 lists the percent glucan synthase inhibition for each analog using this enzyme preparation which generally correlated well with the observed MIC value and provided a valuable guide for selection of analogs for study. However, occasionally this correlation was not followed, and these deviations will be discussed below with the results.

Results and Discussion

Lipophilicity of ECB Side Chains. Side chain lipophilicity was known to play a major role in optimizing the antimicrobial properties of ECB analogs. However, other than building up lipophilic bulk by elongation of alkyl groups, the structural limits of the side chain remained largely unexplored and poorly understood. Using cilofungin (**6**) as the point of departure, its octyloxy group was modified by replacement of its C-3 methylene group by an oxygen atom to give ECB analog series **7a–c** which tested the effect of less lipophilic groups on both antifungal activity and solubility. For **7a** the more polar diether alkoxy group destroyed the antifungal efficacy of **6**, illustrating the sensitivity of the antifungal activity to changes in the lipophilicity of the side chain. Further alkyl extension of this alkoxy group to give compounds **7b,c** restored *in vitro* anti-*Candida* potency to approximately the same level observed with **6**. None of these compounds (**7a–c**) had any greater aqueous solubility than **6**. ECB analog **7d**, incorporating the tricycloalkyl adamantylethoxy group, had greater *in vitro* activity than would be expected on the basis of the activity of the corresponding straight chain C_{12} analog.

Given the major activity changes seen with the addition of a single ether oxygen in series **7**, a reliable method for predicting the suitability of a given acyl group was needed to restrict synthetic efforts to the most effective candidates. Selection of the appropriate chain length in series **3–5** was guided by intuition, which sufficed for these simpler alkyl and arylalkyl side chains. However, it is more difficult to estimate and compare the lipophilic character of structurally complex analogs by inspection. A numerical expression of lipophilicity would be more suitable to predict side chain

Table 1. Synthesis of Echinocandin Analogs

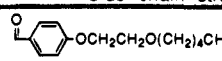
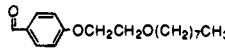
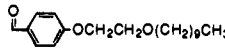
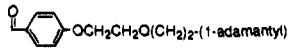
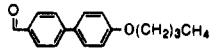
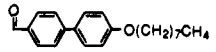
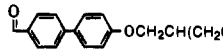
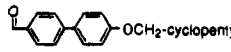
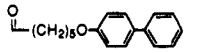
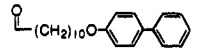
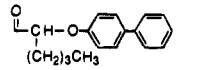
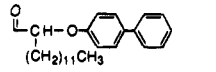
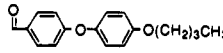
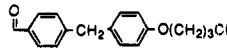
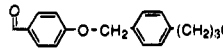
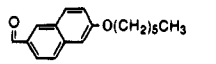
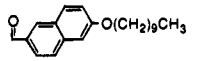
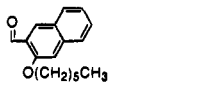
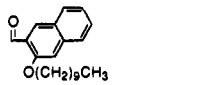
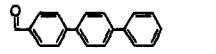
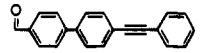
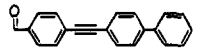
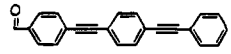
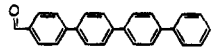
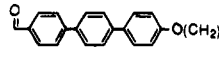
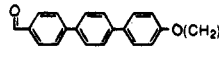
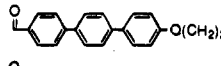
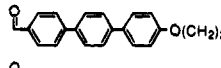
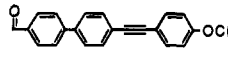
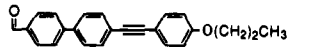
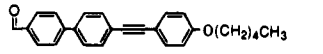
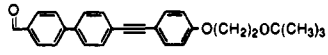
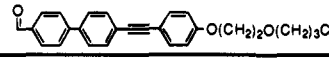
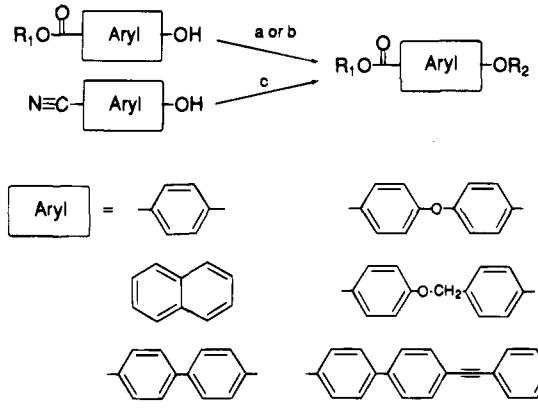
Cmpd	Side chain structure	Purif. Meth.[a]	% Yield	empirical formula[b]	HPLC Ret. Time (min)	HPLC method %ACN[c]
7a		A	18	$C_{48}H_{69}N_7O_{18}$	2.16	40/B
7b		A	23	$C_{51}H_{75}N_7O_{18}$	7.28	40/B
7c		A	15	$C_{53}H_{79}N_7O_{18}$	19.04	40/B
7d		B	91	$C_{53}H_{73}N_7O_{17}$	5.12	45/D
9a		A	18	$C_{51}H_{67}N_7O_{17}$	4.08	40/B
9b		A	39	$C_{55}H_{75}N_7O_{17}$	26.80	40/B
9c		A	27	$C_{53}H_{71}N_7O_{17}$	9.13	40/B
9d		A	22	$C_{53}H_{69}N_7O_{17}$	6.44	40/B
10a		B	85	$C_{52}H_{71}N_7O_{17}$	4.90	80/D
10b		B	94	$C_{57}H_{79}N_7O_{17}$	5.74	50/D
10c		B	89	$C_{52}H_{69}N_7O_{17}$	8.92	40/D
10d		B	81	$C_{60}H_{85}N_7O_{17}$	5.81	70/D
11a		A	15	$C_{51}H_{67}N_7O_{18}$	3.96	40/B
11b		A	10	$C_{52}H_{69}N_7O_{17}$	4.60	40/B
11c		A	22	$C_{52}H_{69}N_7O_{17}$	6.14	40/B
12a		B	99	$C_{51}H_{69}N_7O_{17}$	3.86	45/D
12b		B	94	$C_{55}H_{77}N_7O_{17}$	5.25	45/D
12c		B	99	$C_{51}H_{69}N_7O_{17}$	4.89	60/D
12d		B	91	$C_{55}H_{77}N_7O_{17}$	4.87	40/D
13a		B	13	$C_{53}H_{63}N_7O_{16}$	3.89	40/B
13b		B	14	$C_{55}H_{63}N_7O_{16}$	6.3	40/B
13c		B	30	$C_{55}H_{63}N_7O_{16}$	7.16	40/B
13d		A	58	$C_{57}H_{63}N_7O_{16}$	12.83	40/B
13e		B	93	$C_{59}H_{67}N_7O_{16}$	13.43	40/B
14a		A	46	$C_{58}H_{73}N_7O_{17}$	4.76	50/B
14b		B	19	$C_{59}H_{75}N_7O_{17}$	11.63	50/A
14c		B	72	$C_{59}H_{75}N_7O_{18}$	7.07	45/C
14d		B	36	$C_{59}H_{75}N_7O_{18}$	5.76	45/C
15a		B	36	$C_{56}H_{65}N_7O_{17}$	6.04	40/B

Table 1 (Continued)

Cmpd	Side chain structure	Purif. Meth.[a]	% Yield	empirical formula[b]	HPLC Ret. Time (min)	HPLC method %ACN[c]
15b		B	20	$C_{58}H_{69}N_7O_{17}$	3.90	50/B
15c		B	41	$C_{60}H_{73}N_7O_{17}$	8.38	50/B
15d		B	16	$C_{61}H_{75}N_7O_{18}$	2.62	55/B
15e		B	21	$C_{61}H_{75}N_7O_{18}$	2.98	55/B

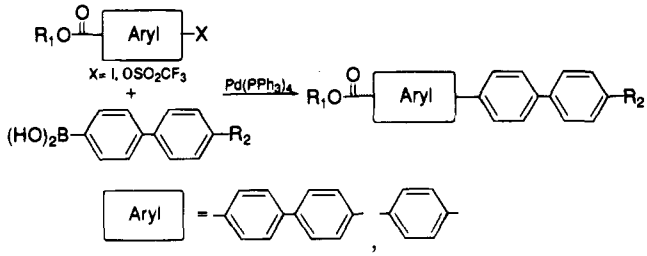
[a] A = HPLC. B = precipitation from methanol/water. [b] All empirical formulas confirmed by high-resolution mass spectra. [c] Percentage of acetonitrile in acetonitrile/water HPLC eluent. Buffer concentrations: A = no buffer, B = 0.5% monobasic ammonium phosphate, C = 0.1% trifluoroacetic acid, and D = 1.0% acetic acid.

Scheme 2[a]

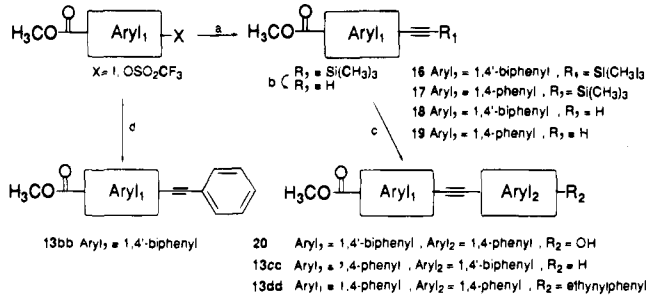


[a] (a) KO*t*-Bu, XR_2; (b) R_2OH, PPh_3, DEAD; (c) i, KO*t*-Bu, XR_2, ii, 50% NaOH (aq), EtOH, iii, HCl, MeOH.

Scheme 3



Scheme 4[a]



[a] (a) (Trimethylsilyl)acetylene, $PdCl_2(PPh_3)_2$; (b) K_2CO_3, MeOH; (c) X-$Aryl_2R_2$, $PdCl_2(PPh_3)_2$; (d) phenylacetylene, $PdCl_2(PPh_3)_2$.

effectiveness in enhancing antifungal activity. Such a method decreases the number of analogs required to develop an efficient, progressively directed SAR while still promoting synthesis of structurally challenging candidates.

The octanol–water partition coefficient, expressed numerically as the log *P* value, has been used in estimating and comparing the lipophilicity of structurally diverse compounds.[23] Although direct determination of the partition coefficient for a large number of compounds is impractical, computer-assisted methods for calculation of log *P* values as the sum of substructural contributions to lipophilicity have been developed for this purpose. One method, the CLOGP algorithm,[24] performs log *P* calculations directly from the structural

Scheme 5

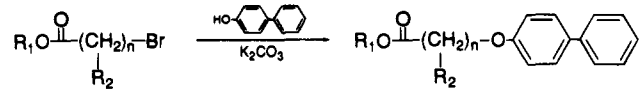


Table 2. Calculated Physical, Enzyme Inhibition, and Antifungal Data of ECB Analogs

compd	CLOGP	MIC[a] (μg/mL)	glucan synthase inhib (%)[b,c]	*in vivo Candida* assay[b,d] ED_{50} (mg/kg) dosing routes: ip	oral
6 (cilofungin)	4.56	0.312	76.0	7.6	>400
7a	2.72	>20	17.1	NT	NT
7b	4.31	2.5	54.8	4.4	NT
7c	5.37	0.625	71.4	9.5	NT
7d	4.66	0.039	72.3	NT	>50
9a	4.11	0.078	71.4	0.8	NT
9b	4.63	0.0025	81.5	0.5	27.3
9c	5.03	0.039	NT	4.6	NT
9d	4.61	0.039	NT	1.2	NT
10a	3.54	1.25	69.1	NT	NT
10b	6.18	0.020	80.2	7.4	NT
10c	4.21	80.0	55.1	NT	NT
10d	8.44	0.312	83.0	NT	NT
11a	4.63	>20	66.7	NT	NT
11b	4.38	>20	43.4	NT	NT
11c	4.86	0.156	68.9	6.7	NT
12a	4.53	0.039	77.7	2.8	NT
12b	6.65	0.005	82.2	1.6	46.1
12c	4.51	20.0	19.4	NT	NT
12d	6.51	20.0	40.6	NT	NT
13a	4.43	0.078	78.8	1.3	>50
13b	4.38	0.039	75.8	0.6	18.8
13c	4.38	0.078	78.2	1.7	NT
13d	4.33	0.010	81.3	4.5	16.0
13e	6.32	<0.010	74.7	0.4	39.7
14a	6.47	0.005	75.0	0.3	7.8
14b	7.00	0.030	NT	0.8	6.8
14c	5.69	0.030	80.1	0.3	13.6
14d	5.34	0.060	76.1	0.4	21.3
15a	4.53	0.020	81.0	NT	19.0
15b	5.36	<0.010	83.5	0.4	7.0
15c	6.42	<0.010	82.3	NT	8.8
15d	5.29	0.020	82.4	0.2	7.6
15e	5.64	0.020	81.9	NT	10.6

[a] Minimum inhibitory concentration. [b] NT = not tested. [c] At 10 μg/mL concentration, single determination. [d] Mouse infection model, mortality end point.

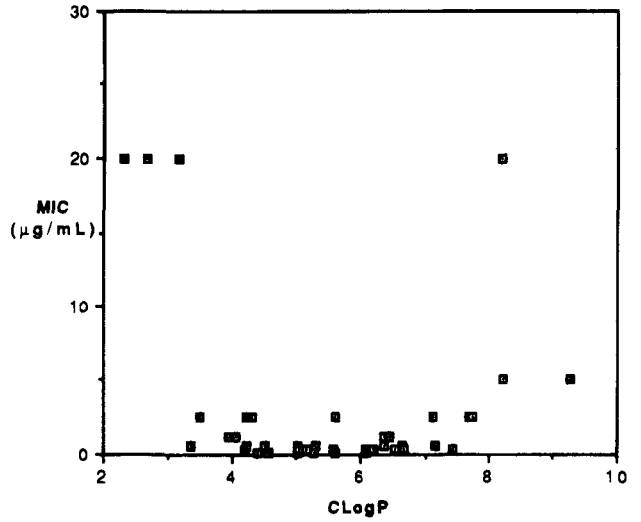


Figure 1. Graph of CLOGP values of compounds from ref 10 versus their MIC values against *C. albicans*.

formula. Rekker[25] showed that the partition coefficient for a whole molecule could be expressed as the sum of partition coefficients of the substructures that compose the molecule. The program determines which substructural features are present directly from the graphic input and completes the summation of their individual contributions to provide a CLOGP value, an estimate of the log *P* of the whole molecule. Since the cyclic peptide portion remained constant for each of the proposed analogs, only the CLOGP values of the side chain structure portion would change, and so only these side chain values need be generated for comparison purposes. For each proposed ECB analog, the structure of the side chain amide was drawn and a calculation performed to provide a CLOGP value.

The predictive nature of CLOGP values was tested using published data from series **3**–**5**.[10] The relationship between side chain CLOGP and antifungal potency (MIC values vs *C. albicans*) for these known ECB analogs was determined and depicted in Figure 1. This plot reveals that analogs whose CLOGP values at the more polar end of the scale (3.5 or less) had poor antifungal activity. Conversely, analogs with CLOGP values > 3.5 were more potent antifungal agents. These data reveal the existence of a lipophilic "gateway" near a CLOGP value of 3.5 for each side chain, above which antifungal activity is observed. In the example used above, the calculated value for the CLOGP of **7aa** was 2.72, making its poor *in vitro* activity less surprising, since it was well below the 3.5 threshold. When the terminal alkyl group was elongated as in **7bb**,**cc**, CLOGP values increased to 4.31 and 5.37, respectively, restoring antifungal activity and confirming the applicability of this method of analysis.

The CLOGP method for estimating lipophilicity was applied throughout this study in the design of new side chain structures. CLOGP values are listed in Table 2 along with the evaluation data for the corresponding ECB analog. In most cases only side chains with CLOGP values of 3.5 or greater were coupled to **2** resulting in ECB analogs which had the greatest probability of improved antifungal properties.

Although a CLOGP value of >3.5 was necessary for members of an active series to be in the region of optimum antifungal activity, CLOGP alone was not sufficient to provide potency. Other structural features of the side chain, especially its geometry and conformation, also influenced the potency of the ECB analogs and in some cases overrode the lipophilic requirement.

Geometric Properties of the ECB Side Chains. The greater effectiveness of side chains with a linear shape in enhancing antifungal activity over that of the corresponding angular isomer was established in the earlier studies.[10] Within a set of isomeric aroyl ECB analogs, the antifungal activity of the *para*-isomer was superior to that of the *meta*-isomer and each of these much more active than the *ortho*-isomer. The isomeric (octyloxy)-*trans*-cinnamoyl ECB analogs **8a**–**c** are similarly related (see Scheme 1). Although each member of series **8** had identical CLOGP values, **8a** had *in vitro* activity that was 32 and 500 times greater than that of the *meta*-isomer **8b** and the *ortho*-isomer **8c**. These studies indicate the side chain of a given ECB analog plays a dual role: it imparts the required lipophilic properties and provides the correct geometry and orientation for optimization of antifungal effectiveness.

Several novel series of ECB analogs permitted more detailed study of lipophilic and geometric factors influencing antifungal activity with special focus on the structure of the side chain aryl group. Taking cilofungin (**6**) as reference, its 1,4-phenylene group was replaced with extended and condensed aryls, permitting subtle addition of polarity and rigid linearity simultaneously. Rigidly linear side chains were prepared by linking aryl groups such as biphenyl, terphenyl, and tetraphenyl. Substituted naphthoyl side chains demonstrated that condensed aromatic side chains were useful platforms for varying side chain shape. A rigid, rodlike side chain was attained by spacing 1,4-substituted aryl groups with ethynyl groups which permitted linear extension without increasing the number of aryl groups. "Fine tuning" of side chain lipophilicity of these aryl side chains to the appropriate CLOGP range by incorporation of alkyl or alkoxy groups insured that the lipopeptide analogs would be active antifungal agents.

Inserting an additional phenyl group into the side chain of **6** gave the alkoxybiphenyl ECB series **9**. These analogs had excellent anti-*Candida* activity (MIC range of 0.078–0.0025 μg/mL), a significant enhancement over that of **6** (MIC = 0.312 μg/mL). The interplay of geometry and lipophilicity discussed above is evident in this series. While the unalkylated biphenyl side chain (CLOGP = 2.54) is not sufficiently lipophilic to impart antifungal activity (data not shown), addition of single unbranched 4′-butyloxy and -octyloxy groups (**9a**,**b**, respectively) greatly enhanced anti-*Candida* activity. This *in vitro* activity correlated well with a 16-fold enhancement of *in vivo* anti-*Candida* activity over that of **6** in the murine infection model by the parenteral route. The biphenyl ECB analogs (series **9**) demonstrate that multiple phenyl groups can be introduced into the side chain with advantage resulting in a 100-fold increase in *in vitro* potency relative to **6**. Branched and cyclic hexyloxy-substituted biphenyl ECB analogs (**9c**,**d**) had *in vitro* activity roughly equivalent to that of **9a**,**b** but had lower *in vivo* potency.

A feature of the highly active series **9** is the rigidly linear biphenyl system near the attaching head of the side chain acyl group. When the location of the biphenyl was shifted further out in the side chain by a pentyloxy

spacer, as in **10a**, the CLOGP value was lowered indicating the side chain was more polar, and the MIC increased significantly over those of the **9** series. Lengthening the spacer to a decyloxy unit (**10b**) increased the lipophilicity of the side chain and lowered the MIC closer to the value observed for **9**. Unfortunately this alteration increased the ip ED_{50} value back to the less potent range of **6**. Having the biphenyl unit near the head of the side chain was critical for potent *in vivo* activity. Branching in the chain before the biphenyl group as in **10c,d**, lowers the *in vitro* activity including the glucan synthase values in keeping with the observed preference for linear geometry discussed above. Lengthening the alkyl unit (**10d**) begins to restore activity, though never attaining the higher levels observed for series **9**.

Series **11** further illustrates the sensitivity of side chain effectiveness to structural deviations from linearity. Introduction of a single oxygen or carbon spacer between the biphenyl aryl groups of compound **9a** gave compounds **11a,b**, respectively, which are less active than **9a** even though the CLOGP values for each compound are approximately the same. One major structural consequence of these modifications is conversion of the linear relationship between the **9a** biphenyl aryl groups to an angular one (~120°). However, elongating this bridge by yet another atom (**11c**) relaxes this angular constraint and permits a more linear orientation and restoration of *in vitro* antifungal activity back to the same level observed with **9a**. *In vivo* activity is still much less than that of series **9**. Compound **11a** is one of the few examples in which the enzyme inhibition (66.7%) does not correlate with the MIC (>20). Normally this level of inhibition would imply a much lower MIC, perhaps indicating that this side chain structure was inhibiting transport of the molecule to the site of action in the whole cell. These observations further support the hypothesis that side chains with appropriate lipophilic and geometric character facilitate full expression of antifungal potency.

The naphthoyl group presents another option for studying the linear and angular distribution of groups within the side chain structure. The 2,6-naphthoyl group presents a symmetric linear array of groups but is spatially and electronically more compact than the biphenyls in series **9**. Conversely, the corresponding 2,3-substituted naphthoyl analogs test the effect of a nonlinear substitution pattern. Representative candidates of the 2-carboxy-6-alkoxy- and 2-carboxy-3-alkoxynaphthoyl ECB analogs comprise series **12**. The linear 2-carboxy-6-alkoxynaphthoyl ECB analogs **12a,b** were very potent antifungal agents whose activity increased as the alkoxy group was varied from hexyl to decyl. However, *in vivo* activity was slightly less than the equivalent straight chain analogs in the biphenyl series (**9a,b**). Conversely, the nonlinear 3-alkoxy-2-naphthoyl ECB analogs **12c,d**, which, respectively, have the same alkoxy groups and CLOGP as **12a,b**, each had poor *in vitro* anti-*Candida* activity. Increasing alkoxy chain length in **12c,d** did not improve its inherently poor biological activity. This result is in agreement with the assertion made above with series **8** and **11** that a preferred orientation exists between the side chain and the cyclic peptide which is facilitated by a linearly rigid side chain and the absence of steric interference about the bond by which they are linked.

Continuing this theme, analogs with totally rigid linear side chains were constructed by linking aryl groups or spacing them with ethynyl linkages (see Table 1). Insertion of a single or a pair of *para*-substituted phenyl groups into the biphenoyl side chain gave the corresponding terphenoyl and tetraphenoyl ECB analogs **13a,e**, respectively, which had excellent anti-*Candida* activity. ECB analogs **13b–d** have rigidly linear side chains, synthesized by spacing three phenyl groups with one or more ethynyl groups. These analogs have essentially identical CLOGP values, and each were highly effective antifungal agents, comparable in activity to **13a**. Interchanging the position of the ethynyl group in **13b,c** did not change side chain length or anti-*Candida in vitro* activity. A second ethynyl spacer (**13d**) increased this activity only moderately. The *in vivo* (ip) antifungal activity of **13b–d** ranged from 0.6 to 4.5 mg/kg, slightly favoring **13b**. The biological properties of this series demonstrate that there are remarkably few restrictions on the chain length of side chains extended by the addition of multiple 1,4-aryl or 1,2-ethynyl groups. The decrease of solubility with the larger side chains does affect their ability to be synthesized and provides a practical limit to the length of the chains that can be examined.

The terphenyl and biphenyl 4″-(ethynylphenyl) ECB analogs (**13a,b**) have side chains that are sufficiently lipophilic in their own right to impart potent antifungal activity both *in vitro* and *in vivo* in experimental murine *Candida* infections by the parenteral route. In series **3–5**, *in vivo* activity was limited to the parenteral route of administration reflecting their poor oral absorption. In the course of the present studies, preference for active, orally absorbed agents required testing of analogs for efficacy by this route. Oral administration of analogs **13a,b** to infected mice revealed that, while the oral activity of **13a** was low, **13b** showed a moderate level of oral anti-*Candida* activity (see Table 2). This discovery led to a further investigation of the SAR of oral activity by modification of the **13a,b** side chains to give series **14** and **15**. The results of these studies are summarized in Table 2.

Modification of **13a** by introduction of flexible alkoxy groups to the totally rigid terphenoyl 4‴ position (series **14a–d**) resulted in an increase in the oral activity. Addition of a 4‴-pentyloxy and -hexyloxy (**14a,b**) increased *in vitro* antifungal activity relative to **13a** as did the more complex 4‴-alkoxy ethyl ether terphenoyl analogs **14c,d**. Each member of series **14** had good *in vivo* anti-*Candida* activity by the parenteral route (ED_{50} range of 0.32–0.89 mg/kg). Introduction of the additional alkoxy groups to **13a** resulted in improvement of the *in vivo* anti-*Candida* activity by oral administration with **14a,b** showing the best activity (ED_{50} range of 6.8–7.8 mg/kg).

A similar modification of **13b** by incorporation of alkoxy groups into the side chain aryl group gave series **15**. Addition of a 4‴-methoxy group gave analog **15a** which retained the oral activity of **13b**. Increasing the size of the alkoxy group from methoxy to propoxy (**15b**) and pentoxy (**15c**) increased *in vitro* potency relative to that of **13b**. However, enlarging this length to octyloxy (data not shown) decreased *in vitro* antifungal activity

showing that there is a limitation on the effect of increased lipophilicity at some distance from the side chain–peptide linkage. Conversely, at this distance it becomes possible to add hydrophilic groups without jeopardizing antifungal activity, modifications which may influence other features, such as pharmacokinetics and absorption. Introduction of the more polar alkoxyethoxy group in **15d,e** resulted in anti-*Candida* activity of the same magnitude as that of the propyloxy and pentyloxy analogs.

The evaluation of the entire series of novel ECB analogs demonstrated that manipulation of the lipophilicity and shape of the side chain resulted in analogs with superior antifungal potency as well as oral activity in murine infection models. One compound, LY303366 (**14a**; see Scheme 1) was selected on the basis of its antimicrobial potency and oral activity for advanced study as a clinical antifungal candidate.

Summary

Chemical reacylation of the ECB nucleus with synthetic acyl side chains permitted a detailed description of the structural features in that region of the molecule that influence the antifungal efficacy of the echinocandins. The superiority of linearly symmetric groups qualitatively presupposes the existence of a cylindrical domain with which this grouping interacts and "fills". For optimal interaction, steric interference about the peptide–side chain coupling point must be minimal to allow proper side chain orientation. Optimal activity could be achieved with polyarylated side chains with or without ethynyl spacers using smaller alkoxy or alkoxy ether groups at the terminal aryl group. While manipulation of side chain geometry and lipophilicity was beneficial in optimizing anti-*Candida* activity, the details of how ECB or its analogs interact with the binding site are not understood.

Experimental Section

Chemistry. General Comments. ^{1}H-NMR spectra were recorded with either a Bruker WM-270 or GE QE-300 spectrometer. Chemical shifts are reported in parts per million (δ) relative to TMS. Spin multiplicities are reported using the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (b). Field desorption (FD) mass spectra were recorded on a VG Analytical ZAB-3F instrument. Fast atom bombardment (FAB) mass spectra were obtained using a VG Analytical ZAB2-SE mass spectrometer. High-resolution mass spectra (HRMS) were generated for all final products and are consistent with the theoretical empirical formulas. A listing of their values is included as supporting information. Thin layer chromatography (TLC) was carried out using Merck plates of silica gel 60 with a fluorescent indicator (F_{254}). Analytical reverse-phase HPLC work was done using the Waters 600E system with Waters μBondapak (C18, 3.9 × 300 mm) columns with a flow rate of 2 mL/min and using UV detection at 230 nm. Preparative HPLC work was performed with a Waters Prep 2000 system using a Rainin Dynamax-60A (C18, 41.4 mm × 25 cm) column. All final products were >90% pure as determined by analytical HPLC. NMR spectral data supported all final structures, and a detailed spectral analysis of **14a** is included as supporting information. Terphenyl-1-carboxylic acid (**13aa**) was commercially available.

Synthesis of Side Chains. 4-[[4-(Butyloxy)phenyl]methyl]benzoic Acid (11bb). (Butyloxy)benzene (4.1 g, 27.0 mmol), methyl 4-(chloromethyl)benzoate (5.0 g, 27.0 mmol) and anhydrous ferric chloride (4.9 g, 30.0 mmol) were combined in dichloromethane (100 mL) and refluxed for 4 h. After cooling, the reaction solution was extracted with saturated sodium bicarbonate solution, dried over magnesium sulfate, and reduced *in vacuo* to give an oil which was dissolved in ethanol and 2 N sodium hydroxide (10 mL) and refluxed for 1 h. The reaction mixture was diluted with a large volume of water and extracted with ether. The aqueous layer was acidified with hydrochloric acid solution and extracted with ether. The ether extract was dried over magnesium sulfate and reduced *in vacuo* to give a solid which was crystallized from ether/pentane to provide **11bb** (14%): ^{1}H-NMR (DMSO-d_6) δ 7.9 (d, 2H), 7.3 (d, 2H), 7.1 (d, 2H), 6.8 (d, 2H), 3.9 (m, 4H), 1.6 (m, 2H), 1.4 (m, 2H), 0.9 (t, 3H); MS (FD) 298 (M^+).

General Methods of O-Alkylation To Prepare 7(aa–dd), 9(aa–dd), 11aa,cc, 12(aa–dd), and 15(aa–ee). Method A. The 1-carboalkoxy-(4 or 4′)-hydroxyaryl compound (1 equiv) was refluxed in acetonitrile (0.25 M) with potassium *tert*-butoxide (1 equiv) and an alkyl iodide, bromide, or tosylate (1.1 equiv) for 17 h. Evaporation of the solvent under reduced pressure gave a residue which was dissolved in ether and extracted with 2 N NaOH. The ether layer was dried over magnesium sulfate and the solvent removed under reduced pressure to give the aryl ether product.

Method B. Diethyl azodicarboxylate (1 equiv) was added dropwise to a solution of the hydroxyaryl compound, the appropriate alcohol (1 equiv), and triphenylphosphine (1 equiv in anhydrous ether (0.25 M). After stirring for 18 h, the solvents were removed under reduced pressure to give a residual solid which either crystallized or was purified by HPLC.

Method C. 4-Cyano-4′-hydroxybiphenyl was O-alkylated by the procedure outlined in method A. This product was dissolved in ethanol by addition of excess 50% NaOH solution and refluxed for 18 h. The slurry was poured into water and acidified (concentrated HCl) to give a precipitate which was collected by filtration. This solid was refluxed in dioxane (0.25 M) and excess 6 N HCl for 18 h, after which the solvent was removed under reduced pressure. The solids were slurried in ether and collected by filtration to give the 4-alkoxy-4′-carboxybiphenyl.

Ethyl 4[(Pentyloxy)ethoxy]benzoate (7aa). Method A using ethyl 4-hydroxybenzoate (4.7 g, 28.8 mmol) and 2-(pentyloxy)-1-tosylethane (8.6 g, 30 mmol) gave **7aa** (64%): ^{1}H-NMR ($CDCl_3$) δ 7.9 (d, 2H), 6.9 (d, 2H), 4.3 (q, 2H), 4.1 (t, 2H), 3.8 (t, 2H), 3.5 (t, 2H), 1.6 (m, 2H), 1.3 (m, 7H), 0.9 (t, 3H); MS (FD) 342 (M^+).

Ethyl 4-[(Octyloxy)ethoxy]benzoate (7bb). Method A using ethyl 4-hydroxybenzoate (13.0 g, 78.7 mmol) and 2-(octyloxy)-1-tosylethane (25.8 q, 78.7 mmol) gave **7bb** (67%): ^{1}H-NMR ($CDCl_3$) δ 7.9 (d, 2H, aryl 2,6-H), 6.9 (d, 2H, aryl 3,5-H), 4.3 (q, 2H, CH_2-ethyl ester), 4.2 (t, 2H, aryl-O-CH_2-CH_2-O), 3.9 (t, 2H, aryl-O-CH_2-CH_2-O), 3.5 (t, 2H, O-CH_2-CH_2-C_6H_{13}), 1.6 (m, 2H, O-CH_2-CH_2-C_6H_{13}), 1.3 (m, 13H, remaining octyl CH_2, CH_3-ethyl ester), 0.9 (t, 3H, octyl CH_3).

Ethyl 4-[(Decyloxy)ethoxy]benzoate (7cc). Method A using ethyl 4-hydroxybenzoate (12.6 g, 76.0 mmol) and 2-(decyloxy)-1-tosylethane (27.0 g, 76.0 mmol) gave **7cc** (78%): ^{1}H-NMR ($CDCl_3$) δ 8.0 (d, 2H), 6.9 (d, 2H), 4.3 (q, 2H), 4.2 (t, 2H), 3.8 (t, 2H), 3.5 (t, 2H), 1.6 (m, 2H), 1.3 (m, 17H), 0.9 (t, 3H); MS (FD) 336 (M^+).

Methyl 4-[(1′-Adamantyl)ethoxy]benzoate (7dd). Method B using methyl 4-hydroxybenzoate (1.0 g, 6.6 mmol) and 1-adamantaneethanol (1.2 g, 6.6 mmol) gave **7dd** (67%) by HPLC purification (silica gel, hexane/ethyl acetate): ^{1}H-NMR (DMSO-d_6) δ 7.9 (d, 2H), 7.0 (d, 2H), 4.1 (d, 2H), 3.8 (s, 3H), 1.9 (bs, 2H), 1.7–1.4 (m, 15H); MS (FD) 314 (M^+).

1-(Butyloxy)-4′-carboxybiphenyl (9aa). Method C using butyliodide (5.8 mL, 51.2 mmol) gave **9aa** (25%): ^{1}H-NMR (DMSO-d_6) δ 12.9 (bs, 1H), 8.0 (d, 2H), 7.7 (d, 2H), 7.6 (d, 2H), 7.0 (d, 2H), 4.0 (t, 2H), 1.7 (m, 2H), 1.5 (m, 2H), 0.9 (t, 3H); MS (FD) 284 (M^+).

1-Carbomethoxy-4′-(octyloxy)biphenyl (9bb). Method A using 1-carbomethoxy-4′-hydroxybiphenyl (5.0 g, 22.0 mmol) and 1-iodooctane (5.4 mL, 30.0 mmol) gave **9bb** (45%): ^{1}H-NMR (DMSO-d_6) δ 8.0 (d, 2H, aryl 2,6-H), 7.8 (d, 2H, aryl 3,5-H), 7.7 (d, 2H, aryl 2′,6′-H), 7.0 (d, 2H, aryl 3′,5′-H), 4.0 (t,

2H, aryl-O-CH_2), 3.9 (s, 3H, ester CH_3), 1.7 (m, 2H, aryl-O-CH_2-CH_2), 1.3 (m, 10H, remaining octyl CH_2's), 0.9 (t, 3H, octyl CH_3).

1-Carboxy-4'-[(1-(2-ethyl)butyl)oxy]biphenyl (9cc). Method C using 1-bromo-2-ethylbutane (7.2 mL, 51.2 mmol) gave **9cc** (21%): ^{1}H-NMR (DMSO-d_6) δ 12.9 (bs, 1H), 8.0 (d, 2H), 7.6 (d, 2H), 7.5 (d, 2H), 7.0 (d, 2H), 3.9 (d, 2H), 1.7 (m, 1H), 1.2 (m, 4H), 0.9 (t, 6H); MS (FD) 298 (M^+).

1-Carboxy-4'-[(cyclopentylmethyl)oxy]biphenyl (9dd). Method C using cyclopentylmethyl tosylate (13.0 g, 51.2 mmol) gave **9dd** (34%): ^{1}H-NMR (DMSO-d_6) δ 12.9 (s, 1H), 8.0 (d, 2H), 7.7 (d, 2H), 7.6 (d, 2H), 7.0 (d, 2H), 3.9 (d, 2H), 2,3 (m, 1H), 1.8 (m, 2H), 1.6 (m, 4H), 1.3 (m, 2H); MS (FD) 296 (M^+).

4-[4-(Butyloxy)phenoxy]benzoic Acid (11aa). Method A was used employing methyl 4-(4-hydroxyphenoxy)benzoate (8.2 g, 33.5 mmol) and butyl iodide (13.8 mL, 33.5 mmol). The resulting alkylated ester was hydrolyzed with aqueous NaOH/EtOH to give 11aa (49%): ^{1}H-NMR (DMSO-d_6) δ 8.1 (d, 2H), 7.1 (d, 2H), 6.9 (m, 4H), 4.0 (t, 2H), 1.8 (m, 2H), 1.5 (m, 2H), 1.0 (t, 3H); MS (FD) 286 (M^+).

Ethyl 4-[(4-Butylphenyl)methoxy]benzoate (11cc). Method B using ethyl hydroxybenzoate (10.1 g, 61.0 mmol) and 4-butylbenzyl alcohol (10.0 mL, 61.0 mmol) gave **11cc** (71%): ^{1}H-NMR ($CDCl_3$) δ 8.0 (d, 2H), 7.3 (d, 2H), 7.2 (d, 2H) 7.0 (d, 2H), 5.1 (s, 2H), 4.4 (q, 2H), 2.6 (t, 2H), 1.6 (m, 2H), 1.4 (m, 5H), 0.9 (t, 3H); MS (FD) 312 (M^+).

Methyl 6-(Hexyloxy)-2-naphthoate (12aa). Method A using methyl 6-hydroxy-2-naphthoate (1.0 g, 4.9 mmol) and hexyl iodide (1.1 mL, 7.4 mmol) gave **12aa** (78%): ^{1}H-NMR (DMSO-d_6) δ 8.5 (s, 1H), 8.0 (d, 1H), 7.9 (d, 1H), 7.8 (d, 1H), 7.4 (s, 1H), 7.2 (d, 1H), 4.1 (t, 2H), 3.9 (s, 3H), 1.7 (m, 2H), 1.4 (m, 2H), 1.3 (m, 4H), 0.9 (t, 3H); MS (FAB) 287 (MH^+).

Methyl 6-(Decyloxy)-2-naphthoate (12bb). Method A using methyl 6-hydroxy-2-naphthoate (1.0 g, 4.9 mmol) and decyl iodide (1.6 mL, 7.4 mmol) gave **12bb** (82%): ^{1}H-NMR ($CDCl_3$) δ 8.5 (s, 1H), 8.0 (d, 1H), 7.9 (d, 1H), 7.7 (d, 1H), 7.2 (d, 1H), 7.1 (s, 1H), 4.1 (t, 2H), 4.0 (s, 3H), 1.9 (m, 2H), 1.5 (m, 2H), 1.3 (m, 12H), 0.9 (t, 3H); MS (FAB) 343 (MH^+).

Methyl 3-(Hexyloxy)-2-naphthoate (12cc). Method A using methyl 3-hydroxy-2-naphthoate (1.0 g, 4.9 mmol) and hexyl iodide (0.8 mL, 5.4 mmol) gave **12cc** (97%): ^{1}H-NMR (DMSO-d_6) δ 8.2 (s, 1H), 7.9 (d, 1H), 7.8 (d, 1H), 7.6 (t, 1H), 7.5 (s, 1H), 7.4 (t, 1H), 4.1 (t, 2H), 3.9 (s, 3H), 1.8 (m, 2H), 1.5 (m, 2H), 1.3 (m, 4H), 0.9 (t, 3H); MS (FAB) 287 (MH^+).

Methyl 3-(Decyloxy)-2-naphthoate (12dd). Method A using methyl 3-hydroxy-2-naphthoate (1.0 g, 4.9 mmol) and decyl iodide (2.1 mL, 9.9 mmol) gave **12dd** (86%): ^{1}H-NMR (DMSO-d_6) δ 8.3 (s, 1H), 7.9 (d, 1H), 7.7 (d, 1H), 7.5 (t, 1H), 7.4 (t, 1H), 7.2 (s, 1H), 4.1 (t, 2H), 4.0 (s, 3H), 1.9 (m, 2H), 1.6 (m, 2H), 1.3 (m, 12H), 0.9 (t, 3H); MS (FAB) 343 (MH^+).

1-Carbomethoxy-4'-[(4-methoxyphenyl)ethynyl]biphenyl (15aa). Method A using **20** (2.0 g, 6.1 mmol) and methyl iodide (0.53 mL, 8.5 mmol) gave **15aa** (95%): ^{1}H-NMR ($CDCl_3$) δ 8.1 (d, 2H, aryl 2,6-H), 7.7 (d, 2H, aryl 3,5-H), 7.6 (s, 4H, aryl 2',3',5',6'-H), 7.5 (d, 2H, aryl 2'',6''-H), 6.7 (d, 2H, aryl 3'',5''-H), 4.0 (s, 3H, aryl-O-CH_3), 3.9 (s, 3H, ester CH_3).

1-Carbomethoxy-4'-[[4-(propyloxy)phenyl]ethynyl]biphenyl (15bb). Method A using **20** (2.0 g, 6.1 mmol) and 1-iodopropane (0.6 mL, 6.1 mmol) gave **15bb** (77%): ^{1}H-NMR (DMSO-d_6) δ 8.0 (d, 2H, aryl 2,6-H), 7.9 (d, 2H, aryl 3,5-H), 7.8 (d, 2H, aryl 2',6'-H), 7.6 (d, 2H, aryl 3',5'-H), 7.5 (d, 2H, aryl 2'',6''-H), 7.0 (d, 2H, aryl 3'',5''-H), 3.9 (t, 2H, aryl-O-CH_2), 3.8 (s, 3H, ester methyl), 1.7 (m, 2H, aryl-O-CH_2-CH_2), 0.9 (t, 3H, propyl CH_3).

1-Carbomethoxy-4'-[[4-(pentyloxy)phenyl]ethynyl]biphenyl (15cc). Method A using **20** (2.0 g, 6.1 mmol) and 1-iodopentane (0.9 mL, 6.7 mmol) gave **15cc** (82%): ^{1}H-NMR ($CDCl_3$) δ 8.1 (d, 2H, aryl 2,6-H), 7.7 (d, 2H, aryl 3,5-H), 7.6 (s, 4H, aryl 2',3',5',6'-H), 7.5 (d, 2H, aryl 2'',6''-H), 6.9 (d, 2H, aryl 3'',5''-H), 4.0 (t, 2H, aryl-O-CH_2), 3.9 (s, 3H, ester methyl), 1.8 (m, 2H, aryl-O-CH_2-CH_2), 1.4 (m, 4H, remaining pentyl CH_2), 0.9 (t, 3H, pentyl CH_3).

1-Carbomethoxy-4'-[[4[[(*tert*-butyloxy)ethyl]oxy]phenyl]ethynyl]biphenyl (15dd). Method A using **20** (2.0 g, 6.1 mmol) and (*tert*-butyloxy)ethyl tosylate (2.5 g, 9.0 mmol) gave **15dd** (90%): ^{1}H-NMR ($CDCl_3$) δ 8.1 (d, 2H, aryl 2,6-H), 7.8 (d, 2H, aryl 3,5-H), 7.7 (d, 2H, aryl 2',6'-H), 7.5 (d, 2H, aryl 3',5'-H), 7.3 (d, 2H, aryl 2'',6''-H), 6.9 (d, 2H, aryl 3'',5''-H), 4.1 (t, 2H, aryl-O-CH_2), 4.0 (s, 3H, ester CH_3), 3.6 (t, 2H, aryl-O-CH_2-CH_2), 1.2 (s, 9H, *tert*-butyl methyls).

1-Carbomethoxy-4'-[[4-[[(*n*-butyloxy)ethyl]oxy]phenyl]ethynyl]biphenyl (15ee). Method A using **20** (2.0 g, 6.1 mmol) and (*n*-butyloxy)ethyl tosylate (2.5 g, 9.0 mmol) gave **15ee** (95%): ^{1}H-NMR ($CDCl_3$) δ 8.1 (d, 2H, aryl 2,6-H), 7.8 (d, 2H, aryl 3,5-H), 7.7 (d, 2H, aryl 2',6'-H), 7.6 (d, 2H, aryl 3',5'-H), 7.5 (d, 2H, aryl 2'',6''-H), 6.9 (d, 2H, aryl 3'',5''-H), 4.2 (t, 2H, aryl-O-CH_2-CH_2-O), 4.0 (s, 3H, ester methyl), 3.8 (t, 2H, aryl-O-CH_2-CH_2-O), 3.6 (t, 2H, CH_2-O-CH_2-CH_2-CH_2-CH_3), 1.6 (m, 2H, CH_2-O-CH_2-CH_2-CH_2-CH_3), 1.4 (m, 2H, CH_2-O-CH_2-CH_2-CH_2-CH_3), 0.9 (t, 3H, butyl CH_3).

General Procedure for the Preparation and Coupling of Arylboronic Acids To Generate 13ee and 14(aa–dd). A solution of 4-bromo-4'-alkoxybiphenyl (1 equiv) in dry THF (0.25 M) was cooled to −70 °C, and a solution of *sec*-butyllithium (1.3 M in cyclohexane, 1.2 equiv) was added slowly over 90 min to maintain the reaction temperature at −70 °C. After stirring for 2 h, triisopropyl borate (2 equiv) was slowly added, giving initially a blue solution, which eventually became light brown. The reaction mixture was allowed to warm to room temperature over 2 h and then treated with 5 N hydrochloric acid, after which the solvent was removed under reduced pressure. The residue was dissolved in ether, transferred to a separatory funnel, and extracted with 5 N HCl. The organic layer was washed with water and evaporated to give a crystalline residue which was slurried with hexane (1 L) to give a crystalline solid boronic acid.

The boronic acid (1 equiv) was mixed with methyl 4-iodobenzoate (1 equiv) in toluene (0.25 M) containing solid K_2CO_3 (1.5 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.05 equiv), and this mixture was refluxed under an inert atmosphere (N_2) for 7 h. The solution was decanted from solid K_2CO_3, and solvents were removed under reduced pressure. Trituration of the residue with acetonitrile gave a solid product which was collected by filtration.

1-Carbomethoxyquaterphenyl (13ee). The above general procedure was used to couple 4-biphenylboronic acid (5.5 g, 28.0 mmol) with methyl 4-[4-[(trifluoromethyl)sulfonyl]-phenyl]benzoate (5.0 g, 14.0 mmol) to give **13ee**. No NMR data could be obtained due to poor solubility, and the crude product was carried on through the active ester stage without purification: MS (FD) 364 (M^+).

1-Carbomethoxy-4''-(pentyloxy)terphenyl (14aa). 4-Bromo-4'-(pentyloxy)biphenyl (31.0 g, 97.0 mmol) was converted to its boronic acid by the above general procedure and then coupled with methyl 4-iodobenzoate (3.7 g, 14.0 mmol) to give **14aa** (33%): ^{1}H-NMR (DMSO-d_6) δ 8.1 (d, 2H), 7.8 (d, 2H), 7.7 (s, H), 7.6 (d, 2H), 7.0 (d, 2H), 4.1 (t, 2H), 4.0 (s, 3H), 1.8 (m, 2H), 1.4 (m, 4H), 0.9 (t, 3H); MS (FD) 374 (M^+).

1-Carbomethoxy-4''-(hexyloxy)terphenyl (14bb). The boronic acid from 4-bromo-4'-(hexyloxy)biphenyl (10.9 g, 32.8 mmol) was prepared by the above general procedure and then coupled with methyl 4-iodobenzoate (2.8 g, 10.8 mmol) to give **14bb** (30%): ^{1}H-NMR (DMSO-d_6) δ 8.1 (d, 2H), 7.9 (d, 2H), 7.8 (d, 2H), 7.7 (d, 2H), 7.6 (d, 2H), 7.0 (d, 2H), 4.0 (t, 2H), 3.9 (s, 3H), 1.4 (m, 2H), 1.2 (m, 2H), 1.1 (m, 4H), 0.9 (t, 3H); MS (FD) 388 (M^+).

1-Carbomethoxy-4''-[(*n*-butyloxy)ethoxy]terphenyl (14cc). The boronic acid from 4-bromo-4'-[(*n*-butyloxy)ethoxy]-biphenyl (6.2 g, 17.8 mmol) was prepared by the above general procedure and then coupled with methyl 4-iodobenzoate (3.6 g, 13.7 mmol) to give **14cc** (62%): ^{1}H-NMR (DMSO-d_6) δ 8.1 (d, 2H), 7.7 (m, 6H), 7.6 (d, 2H), 7.0 (d, 2H), 4.2 (t, 2H), 4.0 (s, 3H), 3.8 (t, 2H), 3.5 (t, 2H), 1.6 (m, 2H), 1.4 (m, 2H), 0.9 (t, 3H); MS (FD) 404 (M^+).

1-Carbomethoxy-4''-[(*tert*-butyloxy)ethoxy]terphenyl (14dd). The boronic acid from 4-bromo-4'-[(*tert*-butyloxy)-ethoxy]biphenyl (2.5 g, 7.2 mmol) was prepared by the above general procedure and then coupled with methyl 4-iodobenzoate (1.5 g, 5.8 mmol) to give **14dd** (38%): ^{1}H-NMR (DMSO-d_6) δ 8.1 (d, 2H), 7.9 (d, 2H), 7.8 (d, 2H), 7.7 (d, 2H), 7.6 (d, 2H), 7.0 (d, 2H), 4.1 (t, 2H), 3.7 (t, 2H), 1.2 (s, 9H); MS (FD) 404 (M^+).

General Procedure for the Preparation of Arylacetylene Intermediates 16–20 and Side Chains 13(bb–dd). Coupling Procedure. A monosubstituted acetylene (1 equiv), triethylamine (2 equiv), $PdCl_2$ (0.05 equiv), triphenylphosphine (0.1 equiv), and CuI (0.025 equiv) were sequentially added with stirring to a degassed acetonitrile solution (0.2 M) of an aryl triflate or iodide (1 equiv) under N_2, and the solution was refluxed for 2–3 h. The reaction mixture was cooled and the precipitate collected by filtration. The filtrate was concentrated to give a second crop of product. The solids were combined and dried in a vacuum oven at 30 °C to give the arylacetylene.

1-Carbomethoxy-4′-[(trimethylsilyl)ethynyl]biphenyl (16). Using the coupling procedure, (trimethylsilyl)acetylene (40.6 g, 413 mmol) and methyl 4-[4-[(trifluoromethyl)-sulfonyl]phenyl]benzoate (149 g, 413 mmol) were combined to give **16** (85%): MS (FD) 308 (MH^+); ^{1}H-NMR ($CDCl_3$) δ 8.1 (d, 2H, aryl 2,6-H), 7.6 (d, 2H, aryl 3,5-H), 7.5 (s, 4H, aryl 2′,3′,5′,6′-H), 3.9 (s, 3H, ester CH_3), 0.2 (s, 9H, silyl methyls).

Methyl 4-[(Trimethylsilyl)ethynyl]benzoate (17). Using the coupling procedure, (trimethylsilyl)acetylene (4.3 g, 44 mmol) and methyl 4-iodobenzoate (11.5 g, 44 mmol) were combined to give **17** (88%): ^{1}H-NMR ($CDCl_3$) δ 8.0 (d, 2H, aryl 2,6-H), 7.5 (d, 2H, aryl 3,5-H), 3.9 (s, 3H, ester CH_3), 0.2 (s, 9H, silyl methyls).

Trimethylsilyl Solvolysis Procedure. A solution of the aryl(trimethylsilyl)acetylene (1 equiv) in MeOH–CH_2Cl_2 (ratio 1:2, 0.25 M) was treated with anhydrous K_2CO_3 (0.8 equiv) and stirred for 3 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure keeping the temperature under 40 °C to give the solid arylacetylene.

1-Carbomethoxy-4′-ethynylbiphenyl (18). Using the solvolysis procedure, **16** (168 g, 545 mmol) was converted to **18** (81%): ^{1}H-NMR ($CDCl_3$) δ 8.1 (d, 2H), 7.6 (d, 2H), 7.5 (s, 4H), 3.9 (s, 3H), 3.1 (s, 1H); MS (FD) 236 (M^+).

Methyl 4-Ethynylbenzoate (19). Using the solvolysis procedure, **17** (11.2 g, 48 mmol) was converted to **19** (60%): ^{1}H-NMR ($CDCl_3$) δ 8.0 (d, 2H), 7.5 (d, 2H), 3.9 (s, 3H), 3.2 (s, 1H); MS (FD) 160 (M^+).

1-Carbomethoxy-4′-[(4-hydroxyphenyl)ethynyl]biphenyl (20). Using the coupling procedure, 1-carbomethoxy-4′-ethynylbiphenyl (41.8 g, 177 mmol) and 4-iodophenol (38.9 g, 177 mmol) were combined to give **20** (81%): ^{1}H-NMR (DMSO-d_6) δ 10.0 (s, 1 H, aryl-O*H*), 8.1 (d, 2H, aryl 2,6-H), 7.9 (d, 2H, aryl 3,5-H), 7.8 (d, 2H, aryl 2′,6′-H), 3.9 (s, 3H, ester CH_3).

1-Carbomethoxy-4′-(ethynylphenyl)biphenyl (13bb). Using the coupling procedure, 1-carbomethoxy-4′-iodobiphenyl (6 g, 18 mmol) and phenylacetylene (1.95 mL, 17.75 mmol) were combined to give **13bb** (47%): ^{1}H-NMR ($CDCl_3$) δ 8.1 (d, 2H, aryl 2,6-H), 7.7 (d, 2H, aryl 3,5-H), 7.6 (s, 4H, aryl 2′,3′,5′,6′-H), 7.5 (m, 2H, aryl 2″,6″-H), 7.3 (m, 3H, aryl 3″,5″,5″-H), 3.9 (s, 3H, ester CH_3).

Methyl 4-(4′-Ethynylbiphenyl)benzoate (13cc). Using the coupling procedure, methyl 4-ethynylbenzoate (2 g, 12.5 mmol) and 4-bromobiphenyl (2.9 g, 12.5 mmol) were combined to give **13cc** (24%): ^{1}H-NMR (DMSO-d_6) δ 8.0 (d, 2H, aryl 2,6-H), 7.6 (s, 8H, aryl 3,5,2′,3′,5′,6′,2″,6″-H), 7.5 (t, 2H, aryl 3″,5″-H), 7.4 (t, 1H, aryl 4″-H), 3.9 (s, 3H, ester CH_3).

Methyl 4-[[4′-(Phenylethynyl)phenyl]ethynyl]benzoate (13dd). Using the coupling procedure, 4-(*tert*-butyldimethylsiloxy)iodobenzene (15 g, 44.9 mmol) and phenylacetylene (4.9 mL, 45.0 mmol) were combined to give 4-[[(*tert*-butyldimethylsilyl)phenyl]ethynyl]benzene. The silyl protecting group was cleaved using excess tetrabutylammonium fluoride (1.0 M in THF) at 0 °C and the phenol converted to a triflate using trifluoromethanesulfonic anhydride in pyridine (72%). The 4-(phenylethynyl)phenyl triflate (7.0 g, 21.5 mmol) and **19** (3.5 g, 22.0 mmol) were coupled using the general procedure to give **13dd** (74%): ^{1}H-NMR ($CDCl_3$) δ 8.0 (d, 2H), 7.6 (d, 2H), 7.5 (s, 6H), 7.4 (m, 3H), 3.9 (s, 3H); MS (FD) 336 (M^+).

General Procedure To Prepare Side Chains 10(aa–dd). A solution of the appropriate bromoalkanoate (1.2 equiv), 4-hydroxybiphenyl (1 equiv), and K_2CO_3 (1.2 equiv) in DMF (0.25 M) was stirred for 18 h at room temperature. After removal of the solvent, the residue was taken up in ethyl acetate and washed with 5% HOAc, water, and brine. The organic layer was dried over sodium sulfate and evaporated to give a crystalline product.

***tert*-Butyl 6-(4′-Biphenoxy)hexanoate (10aa).** *tert*-Butyl 6-bromohexanoate (4.4 g, 17.6 mmol) was used to produce **10aa** (73%): ^{1}H-NMR (DMSO-d_6) δ 7.6 (m, 4H), 7.4 (t, 2H), 7.3 (t, 1H), 7.0 (d, 2H), 4.0 (t, 2H), 2.2 (t, 2H), 1.7 (m, 2H), 1.6 (m, 2H), 1.4 (m, 2H), 1.4 (s, 9H); MS (FAB) 340 (M^+).

Methyl 11-(4′-Biphenoxy)undecanoate (10bb). Methyl 11-bromoundecanoate (3.9 g, 14.1 mmol) was used to produce **10bb** (91%): ^{1}H-NMR (DMSO-d_6) δ 7.6 (m, 4H), 7.4 (t, 2H), 7.3 (t, 1H), 7.0 (d, 2H), 4.0 (t, 2H), 3.6 (s, 3H), 2.3 (t, 2H), 1.7 (m, 2H), 1.5 (m, 2H), 1.3 (m, 12H); MS (FAB) 368 (M^+).

Ethyl 2-(4′-Biphenoxy)hexanoate (10cc). Ethyl 2-bromohexanoate (3.9 g, 17.6 mmol) was used to produce **10cc** (95%): ^{1}H-NMR (DMSO-d_6) δ 7.6 (m, 4H), 7.4 (t, 2H), 7.3 (t, 1H), 7.0 (d, 2H), 4.8 (t, 1H), 4.2 (q, 2H), 1.9 (m, 2H), 1.4 (m, 2H), 1.3 (m, 2H), 1.2 (t, 3H), 0.9 (t, 3H); MS (FAB) 312 (M^+).

Ethyl 2-(4′-Biphenoxy)myristate (10dd). Ethyl 2-bromomyristate (5.9 g, 17.6 mmol) was used to produce **10dd** (87%): ^{1}H-NMR (DMSO-d_6) δ 7.6 (m, 4H), 7.4 (t, 2H), 7.3 (t, 1H), 7.0 (d, 2H), 4.8 (t, 1H), 4.2 (q, 2H), 1.9 (m, 2H), 1.4 (m, 2H), 1.3 (m, 18H), 1.2 (t, 3H), 0.9 (t, 3H); MS (FAB) 424 (M^+).

Procedure for Active Ester Formation and Side Chain Attachment to ECBnuc (2) Used for All Final Products. Side chains containing carboxylic esters were converted to their corresponding carboxylic acids by refluxing with dioxane (0.25 M) containing 2 N sodium hydroxide (5 equiv) for 17 h. The solution was neutralized (1 N hydrochloric acid, 10 equiv), poured into a large volume of water to precipitate the carboxylic acid which was collected by filtration, and dried under reduced pressure. A solution of the carboxylic acid (1 equiv), dicyclohexylcarbodiimide (DCC) (1.1 equiv), and 2,4,5-trichlorophenol (1.1 equiv) in dichloromethane (0.25 M) was stirred at room temperature for 5–18 h. After filtration, the solvent was evaporated under reduced pressure. The residue was heated in ether to dissolve the active ester, and the residual dicyclohexylurea was removed by filtration. The solution was dried over magnesium sulfate and reduced *in vacuo* to give the 2,4,5-trichlorophenol active ester.

A solution of **2** (1 equiv) and the 2,4,5-trichlorophenol active ester (1.0–1.5 equiv) in dimethylformamide (0.2 M) was stirred at ambient temperature for 17–65 h while reaction progress was monitored by TLC or HPLC. The solvent was removed under reduced pressure and the residue slurried in ether, collected by filtration, and washed with dichloromethane. The product was purified either by method A–reverse-phase HPLC or method B–dissolution of the product in MeOH and precipitation of the pure product by addition to a large volume of H_2O. Final products are listed in Table 1.

Biology. MIC Determinations. A microdilution microtiter testing procedure was used to determine MICs. Fungal isolates were grown on Sabouraud dextrose agar slants at 35 °C. *C. albicans* A26 (ATCC 62342) conidia were suspended in saline and adjusted to 1×10^5 conidia/mL in antibiotic 3 broth. Cell suspensions were counted using a hemacytometer under phase contrast microscopy. Aliquots containing 100 μL of the above were added to each well of a 96-well microtiter plate. Test compounds were solubilized at 1.0 mg/mL in methanol. Compound solutions were diluted to yield 80 μg/mL in the first well when added in a 100 μL volume. Serial 2-fold dilutions were made to the 11th well. The 12th well served as a positive growth control, and each compound was tested in duplicate. Plates were incubated 48 h at 35 °C. If an end point was not determined, then additional 2-fold dilutions were made until an MIC was obtained. The MICs were defined as the lowest concentrations of drugs which completely inhibited visible growth compared to untreated controls.

ED_{50} Determinations. ICR (Harlan, Sprague–Dawley), 18–20 g, male mice were x-irradiated with 400 r 24 h prior to infection. *C. albicans* A26 (ATCC 62342) was grown overnight at 35 °C on a Sabourauds and dextrose agar slant. Mice were infected iv through the lateral tail veins with a saline suspension containing 2×10^6 cells in 0.1 mL. Parenterally admin-

istered test compounds were suspended in β-cyclodextrin, and mice were treated with a 0.2 mL volume at 0, 4, 24, and 48 h postinfection. For the oral route, test compounds were formulated in a vehicle containing cremophor (5%), linoleic acid (8%), and water, and 0.05 mL was administered using a gavage needle. Mice were dosed b.i.d × 4 beginning immediately postinfection. There were 10 animals per drug level with an upper dose of 10 mg/kg and lower doses determined by 2-fold serial dilutions down to 0.039 mg/kg. The test was concluded after 7 days. Day of death for each treated and each untreated control was compared using the Student's *t*-test. ED_{50} values, the doses in mg/kg at which 50% of the animals survive, were determined according to the method of Reed and Muench.[26]

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Supporting Information Available: Table of HRMS data for all final compounds and an NMR structural assignment of LY303366 (**14a**) (5 pages). Ordering information is given on any current masthead page.

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