The Echinocandins: Total and Semi-Synthetic Approaches in Antifungal Drug Discovery

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The Echinocandins: Total and Semi-Synthetic Approaches in Antifungal Drug Discovery

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Abstract: The echinocandins are a new class of antifungal lipopeptides for the treatment of serious nosocomial mycoses. The three currently approved drugs, caspofungin, micafungin, and anidulafungin, were each discovered through the synthetic modification of echinocandin natural products obtained from fermentation. This review is intended for the medicinal chemist who is actively pursuing or has a general interest in the synthetic modification of natural products as a means to identify drug candidates. It provides a survey of the synthetic strategies that produced the approved echinocandin therapeutics and a discussion of more recent efforts to identify a new generation of echinocandin drug candidates. Both total synthetic approaches starting from the constituent amino acids and semi-synthetic approaches relying on fermentation-produced lipopeptide are addressed. These various efforts by chemists from industry and academia have not only illuminated the interesting chemistry of these natural products, but have provided the means by which improvements in antifungal potency and spectrum, pharmacokinetic profile, solubility, stability, and safety can be realized. The ultimate success of these efforts can be judged by considering the important role the echinocandins are already playing in the treatment of serious fungal infection.

INTRODUCTION

The increasing prevalence of opportunistic fungal infection in today's immunosuppressed patient population highlights the need for new antifungal therapies that are safe and effective [1]. The increasing prevalence of azole-resistant strains emphasizes the importance of identifying drug leads that act on novel targets. The echinocandins comprise the first new class of antifungal agents to reach the market in more than a decade. Obtained from the fermentation broth of various fungi, these natural products are composed of a highly-oxygenated cyclic hexapeptide linked to a lipophilic side chain. Members of this class include echinocandins B-D as well as the related pneumocandins, mulundocandins, and aculeacin A_γ Fig. (**1**) and (**2**). The echinocandins act through inhibition of β-(1,3)-D-glucan synthase, thereby blocking synthesis of the glucan biopolymers that along with chitin and mannoprotein form the major structural components of the fungal cell wall [2]. They possess potent fungicidal activity against Candida species, including the increasingly prevalent non-albicans strains that have become clinically problematic in recent years. They are also active against Aspergillus species and *Pneumocystis carinii*, but not *Crypotococcus neoformans* or *Fusarium oxysporum*.

With broad spectrum activity against Candida species, minimal toxicity, and efficacy in animal models, the echinocandins quickly attracted the attention of major pharmaceutical companies. Drug discovery efforts during the 1980s and 1990s produced echinocandin analogs with improved potency and spectrum, reduced hemolytic potential, and pharmacokinetic properties that allow once daily dosing. The echinocandins generally exhibit very low oral bioavailability, and early efforts (notably at Eli Lilly) to identify orally available analogs met with little success [3]. However, the need for parenteral administration is not a major drawback, since these drugs are typically used to treat serious mycoses in hospitalized patients. To date, three echinocandin drug candidates have successfully navigated clinical trials and are approved for use in the United States. These include caspofungin (Cancidas) from Merck, micafungin (Mycamine) from Fujisawa, and anidulafungin (Eraxis), which was discovered at Eli Lilly, developed by Vicuron Pharma-ceuticals, and is now marketed by Pfizer Fig. (**1**) and (**2**). Caspofungin and anidulafungin are indicated for the treatment of candidemia and esophageal candidiasis, and caspofungin is additionally approved for invasive asper-gillosis refractory to other fungal therapies. Micafungin has been approved for esophageal candidiasis and for fungal prophylaxis in patients undergoing bone marrow transplant. In the years since its launch in 2002, Cancidas has shown impressive growth in both sales and share of the antifungal market. The rapid acceptance of this drug by physicians is a testament to its safety and efficacy and bodes well for the newer entries from this class.

The intent of this review is to provide practicing medicinal chemists with a survey of the total and semi-synthetic approaches that have been, and are currently being employed in the search for novel antifungal agents of the echinocandin class. In the interest of providing a comprehensive treatment, some overlap with the content of earlier reviews [4-8] of echinocandin structure-activity relationship (SAR) is inevitable. Both total and semi-synthetic approaches have been explored for the synthesis of new echinocandin analogs and the review is organized accordingly. Within each of these

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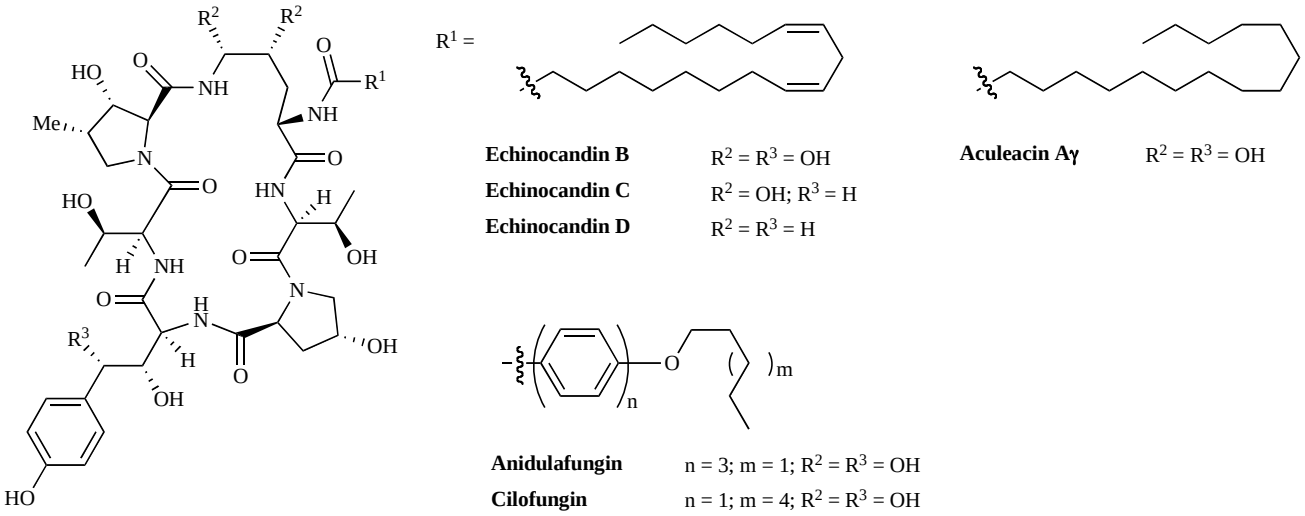


Fig. (1). Chemical structures of echinocandins B-D, aculeacin, and semi-synthetic derivatives anidulafungin and cilofungin.

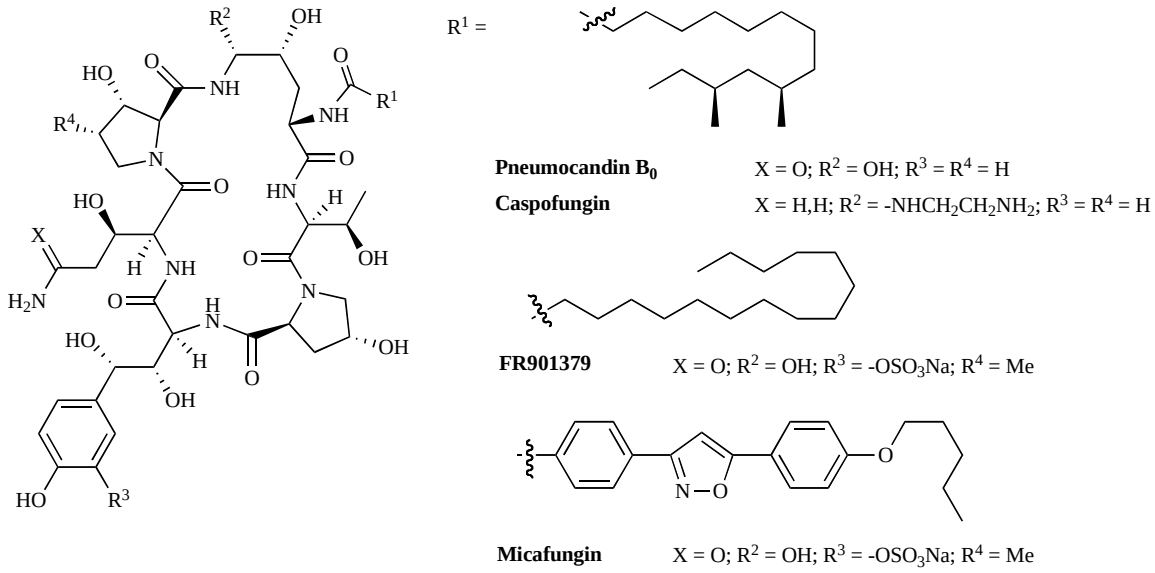


Fig. (2). Chemical structures of pneumocandin B_o, FR901379, and corresponding semi-synthetic derivatives caspofungin and micafungin.

larger sections, the discussion is roughly organized according to the specific location of synthetic modification. Attention will be paid both to the synthetic methodology used to prepare the new analogs as well as the biological activities and SAR that derive from the synthetic modifications. Because standardized methods for antifungal susceptibility testing have been difficult to develop and slow to be widely adopted, we have tried to avoid drawing specific SAR conclusions based on antifungal activities from disparate sources. A discussion of the biological and therapeutic properties of the marketed echinocandins is beyond the scope of this review. The interested reader is instead directed to a number of excellent reviews [9-22].

TOTAL SYNTHETIC APPROACHES TO NATURALLY OCCURRING ECHINOCANDINS AND ANALOGS

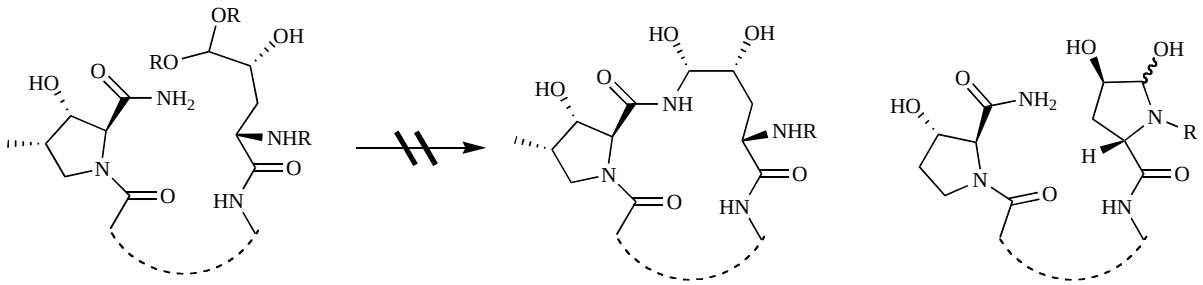
In principle, the total synthesis of echinocandins from their constitutive amino acids should be a relatively straightforward task. The synthetic challenge presented by these molecules is two-fold. First, the highly oxygenated amino acid building blocks must be prepared in an efficient and stereoselective manner. Second, the unusual *N*-acyl hemiaminal connection in the cyclic peptide is a potential site of chemical instability and so a means for its efficient formation must be developed.

In the mid 1980s, Ohfune [23-25] and Evans [26] separately described the total synthesis of echinocandin D, the least complex member of the family in that it lacks both an ornithine hemiaminal function and a benzylic C4 hydroxyl (at homotyrosine). In their total syntheses, the Evans and Ohfune groups developed distinct synthetic routes to the unusual amino acids (2*S*, 3*R*)-3-hydroxyhomotyrosine and (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methyl proline. Ohfune employed the Sharpless asymmetric epoxidation to establish key stereogenic centers in these amino acids, while Evans' route exploits the auxiliary-controlled asymmetric aldol methodology developed in those laboratories. Both syntheses employ the synthetic endgame developed by Ohfune involving diphenyl phosphoryl azide (DPPA) mediated macrocyclization between the ornithine *δ*-amine and the C-terminal carboxylate function of a linear hexapeptide.

For their attempted synthesis of echinocandin C, Ohfune prepared the linear hexapeptide **1** containing a fully oxygenated ornithine residue with a terminal dimethoxy acetal Fig. (**3**). Unmasking of this protected aldehyde was expected to precipitate macrocyclization onto the C-terminal carboxamide, thereby generating the desired hemiaminal function and furnishing echinocandin C. Instead, the uncyclized aldehyde was obtained as its hydrate (**2**) and all further attempts to effect dehydrative macrocyclization of this material failed, yielding instead the pyrrolidine **3**. In fact, pyrrolidines such as **3** are known decomposition products of a number of the echinocandins, as will be discussed later in more detail. While problematic in the context of total synthesis, the reactivity of the hemiaminal can in fact be used to advantage for the preparation of novel echinocandin analogs, as we shall see.

About five years after the appearance of the first total syntheses, a group at Merck reported the preparation of simplified echinocandin analogs using a total synthetic approach [27]. In this work, the 4'-octyloxybenzoyl side chain of cilofungin Fig. (**1**) was combined with cyclic hexapeptides of varying complexity. Potential issues surrounding the hemiaminal linkage were avoided by preparing analogs with a stable amide connection between proline and ornithine. The structurally least complex analogs prepared were found to be completely inactive in both enzymatic and growth inhibition assays. Notably, analogs bearing a tyrosine residue were uniformly inactive while analogs bearing homotyrosine retained significant antifungal activity. Hence, while the one carbon homologation is crucial, the C3 and C4 hydroxyls apparently are not. Replacement of the unusual (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methyl proline residue with simple 4-hydroxy proline precipitated a four-fold loss of antifungal potency and an even greater reduction in the IC_{50} for glucan synthesis. Thus, the most potent of the simplified analogs were those that incorporated both homotyrosine and (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methyl proline residues in the cyclic peptide core. Such analogs were essentially equipotent to the cilofungin comparator, despite the presence of a much more highly oxygenated peptide core in the latter.

At least partly inspired by the Merck work, a group that included researchers from Abbott reported recently on a similar study of echinocandin SAR using the total synthetic approach [28]. Of particular note from this work is the finding that the (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methyl proline residue can be replaced with 4β-amino proline and that related analogs, including 4-guanidino proline congeners, possess improved aqueous solubility and good *in vitro* activity. Keeping the 4β-amino proline residue constant, it was determined that replacement of the neighboring threonine residue with other amino acids could be tolerated. For example, the introduction of an ornithine residue at this position produced analogs with improved efficacy in animal models. Exploration of the homotyrosine position confirmed the earlier findings of the Merck team with respect to chain length and also established the importance of the phenolic hydroxyl. Analogs with a 4'-methyl or 4'-amino group on the aromatic ring were less potent *in vitro* and were altogether inactive *in vivo*. Combining the best of the hexapeptide core modifications with an optimized lipophilic side chain produced the analog A-199930 Fig. (**4**), which displayed excellent anti-candida activity *in vitro* and was efficacious in a mouse model of chronic candidiasis. Unfortunately, subsequent pre-clinical studies of A-199930 revealed undesirable cardiovascular (CV) effects in rat and monkey that were linked to a histamine-releasing effect [29]. Subsequent studies showed that this effect was associated with chemotypes containing proximal basic sites (e.g., neighboring ornithine and guanidino proline residues). Further chemical optimization made in light of these findings led to the improved analog A-192411 Fig. (**4**). Here the ornithine is replaced by threonine and an additional basic amine is introduced at the 3' position of the homotyrosine aromatic ring. This compound demonstrated efficacy in a number of murine infection models and displays an improved CV safety profile in both rodents and monkeys [29-31].



1 (R = Me)
2 (R = OH)

Echinocandin C

3

Fig. (3). Attempted ring-closing reaction to form echinocandin C.

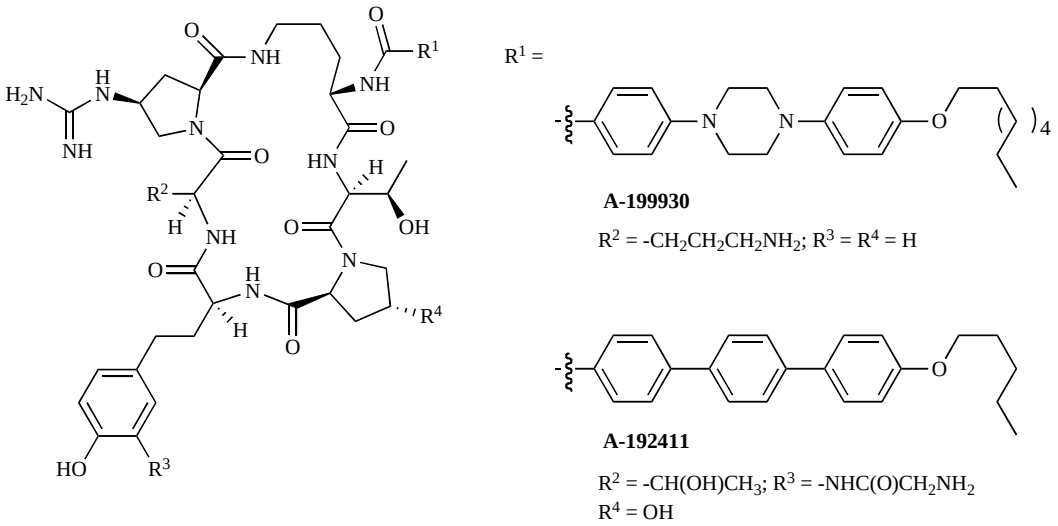


Fig. (4). Lipopeptides A-199930 and A-192411 prepared using a total synthetic approach.

The total synthetic approach as described above has provided valuable echinocandin SAR that would be difficult or impossible to obtain via semi-synthetic modification of the natural products themselves. Important insights gleaned from this work include the highly detrimental effect of homotyrosine truncation to tyrosine and the identification of additional unnatural amino acids (e.g., 4β-amino proline) that can successfully substitute for residues found in the naturally occurring lipopeptides. Although the echinocandins in current clinical use were all discovered via the semi-synthetic approach, the case of A-192411 at least demonstrates that pre-clinical compounds of the echinocandin class are accessible via the total synthetic approach as well.

SEMI-SYNTHETIC APPROACHES TO ECHINOCANDIN ANALOGS

This section describes various semi-synthetic methods that have been developed for the synthesis of novel echinocanidin analogs. It bears noting that this approach relies on the availability of lipopeptide in bulk quantities from fermentation. In examining the structural features of the echinocandins, several salient features will become evident to the synthetic/medicinal chemist. The large number of free hydroxyls in these lipopeptides would appear to limit the scope of chemistries that can be applied without resort to protecting group strategies. The hemiaminal function is a likely site of reactivity, either desired or undesired, and will need to be taken into account when selecting reaction conditions for a given synthetic transformation. The benzylic position of the 3,4-dihydroxy homotyrosine residue is another reactive site that may complicate matters but that might also be exploited in analog synthesis. The phenolic hydroxyl of the homotyrosine residue is the most acidic site in the molecule and might therefore be engaged selectively. If a chemical or enzymatic cleavage of the lipophilic side chain could be achieved, it might be replaced with any number of synthetic variants, thereby producing novel analogs with novel properties. As we shall see in the following discussion, interesting and selective chemistry has been developed at each of these sites, and also at less obvious ones.

Lipophilic Side Chain Replacement

The nature of the lipid side chain in the echinocandins is a determinant for antifungal potency and also plays a role in the hemolytic properties associated with some members of the class. Replacement of the natural side chain with synthetic variants is therefore an obvious strategy for improving antifungal potency and eliminating undesirable properties, such as hemolysis.

Researchers at Eli Lilly conducted extensive studies of echinocandin B analogs bearing various synthetic side chains [32-39]. The natural linoleoyl side chain was removed enzymatically to afford the free cyclic peptide with a single reactive amine substituent [40, 41]. Acylation of this material then provided a wide variety of novel side chain analogs. *In vitro* and *in vivo* testing revealed that side chain length, overall lipophilicity, and geometric factors each contribute to the SAR of these analogs. The most potent analogs tended to have linear side chains of a certain minimal length and fell into a window of lipophilicity (calculated as CLogP) between about 3.5 and 7. Linear aliphatic side chains were effective when at least 11 carbon atoms in length. Also effective were aryl groups substituted with alkoxy substituents of at least six carbons in length, provided that the alkoxy substituent was in the para position. The preeminent example of this type is the 4-(octyloxy)benzoyl analog cilofungin Fig. (**1**), which became an early clinical candidate at Lilly.

The strong preference for para substitution noted above prompted the examination of more rigid, linear side chains. Compared to cilofungin, congeners with a 4'-alkyloxy biphenyl side chain were both more potent *in vitro* and significantly more efficacious in animal models. Homologation to substituted terphenyl side chains produced analogs with improved oral efficacy in animal models (e.g., anidulafungin ED_{50} = 7.8 mg/kg vs. >400 mg/kg for cilofungin). In contrast, analogs with a kinked diphenyl ether side chain were completely inactive, highlighting again the strong preference for linear side-chain geometry. The case of micafungin Fig. (**2**) however demonstrates that more modest departures from linear geometry are tolerated. A recent patent from research-

ers at Aventis describes a series of steroidal side chain analogs [42] and although these are claimed to have glucan synthase activity, no specific data are provided. Although the hemolytic potential of the Lilly analogs tended to track with antifungal activity, it was possible to find specific examples where these two activities are divorced from one another. In this regard, the incorporation of benzoyl (or biphenyl or terphenyl) side chains seems advisable.

In addition to the *N*-acylated analogs discussed above, Lilly scientists also describe *N*-alkylated analogs, which were prepared *via* reductive amination of the deacylated core amine with various long-chain aldehydes [43]. The SAR of the *N*-alkylated side chain congeners parallels that of the *N*-acylated series – increasing lipophilicity generally leads to improved anti-candida activity. However, the *N*-alkylated analogs were found to be completely devoid of activity in a murine cadidiasis model, suggesting that the additional basic amino group in the *N*-alkylated analogs confers unfavorable pharmacokinetic and/or metabolic properties upon these analogs.

The discovery of micafungin at Fujisawa resulted from a similar strategy of side chain replacement, starting with the echinocandin natural product FR901379 Fig. (**2**). This member of the echinocandin family is unusual in that it possesses a sulfated homotyrosine residue and is water soluble [44, 45]. As at Eli Lilly, Fujisawa chemists focused their efforts on side chain modification as the most likely means for improving antifungal potency and reducing the hemolytic properties of FR901379. Replacing the natural side chain of FR901379 with the 4-(octyloxy)benzoyl side chain of cilofungin produced FR131535. This analog exhibited *in vitro* and *in vivo* antifungal activity comparable to the parent but was essentially non-hemolytic [46, 47]. Hence the privileged 4-(octyloxy)benzoyl side chain modification effectively attenuates hemolytic potential when applied to either the echinocandin B or FR901379 core peptide. Further side chain optimization at Fujisawa led to the identification of the pentyloxyl-diphenylisoxazole side chain and the selection of micafungin for clinical development Fig. (**2**). Although no full account of these optimization studies has appeared in the literature, it is striking that the Lilly and Fujisawa teams converged on very similar side chain chemotypes for their respective clinical candidates.

Synthetic Modifications of the Core Cyclic Hexapeptide

As discussed above, medicinal chemistry efforts leading to anidulafungin and micafungin focused primarily on lipid side chain modification, a strategy that proved effective in reducing hemolytic potential as compared to the parent lipopeptides. Pneumocandin B_o in constrast is not hemolytic, and perhaps for this reason chemists at Merck chose to retain the natural 10,12-dimethylmyristoyl side chain and instead explore modifications to the cyclic hexapeptide core. Still other workers have pursued modifications of both the cyclic peptide core and lipophilic side chain, leading for example to analogs such as aminocandin Fig. (**5**). This section surveys the various ways in which chemists have modified the cyclic hexapeptide core of naturally occurring echinocandins so as to impart novel properties to the resulting derivatives. The discussion is organized according to the site of structural modification.

Among sites for chemical alteration, the ornithine hemiaminal function has been the most fertile, yielding to a variety of reduction, substitution, and alkylation chemistries. Cleavage of the cyclic peptide at the hemiaminal has also been used as a means to excise the ornithine residue entirely, allowing for its subsequent replacement with a variety of synthetic surrogates. The homotyrosine residue has likewise served as fertile ground for analog synthesis. The phenolic hydroxyl is a favored site for the incorporation of prodrug moieties while the aromatic ring and benzylic position are additional sites that can be engaged chemically. Notable chemistries at other sites include reduction of the 3-hydroxyglutamine carboxamide to the corresponding amine in the synthesis of caspofungin type analogs.

Ornithine Modifications

A commonly sited motivation for ornithine hemiaminal modification is the chemical instability of echinocandins that contain this function. This instability has been well characterized in the case of pneumocandin B_o [48]. For example, exposure of pneumocandin B_o to mildly basic or acidic conditions produced the inactive degradation products **4** and **5**, respectively Fig. (**6**). Ring-opened degradation products analogous to **4** have also been noted for other members of the echinocandin family [49, 50]. A potential solution to this instability might involve chemical reduction of the hemiaminal to a more stable amide connection. Such "deoxygenated" analogs would be expected to possess improved stability and might therefore be successfully employed in a wider range of subsequent chemistries. This strategy has in fact been widely employed and has proven quite effective, leading to a variety of stable deoxygentated echinocandins with favorable properties.

The first reports of hemiaminal modification date all the way back to the original structural characterization of echinocandins B-D by a group at Sandoz [51-53]. While originally developed for the purpose of structure determination, these chemistries have become widely used by medicinal chemists intent on preparing novel analogs. In the original Sandoz work, it was found that echinocandins (**6**) bearing a hemiaminal function could be converted to the corresponding ether **7a** or thioether **7b** analogs by reaction with excess alcohol or thiol and catalytic acid. While hemiaminal ether formation could be accomplished selectively, thioether formation was accompanied by concomitant addition of thiol at the benzylic position of the homotyrosine residue. Reduction of the carbon sulfur bond(s) in these various intermediates was effected with Raney-Nickel to afford deoxygenated intermediates such as **8**. The proper combination of these substitution and reduction chemistries permitted conversion of the echinocandin B peptide core into that of echinocandin C, aiding in the structural characterization of the latter [53].

Contemporary one-step procedures for hemiaminal reduction employ reducing agents such as triethylsilane or sodium cyanoborohydride in protic solvent, typically acetic acid or trifluoroacetic acid. By controlling the reaction conditions carefully, it is possible to achieve selectivity for reduction at either of the two reactive sites, although yields can

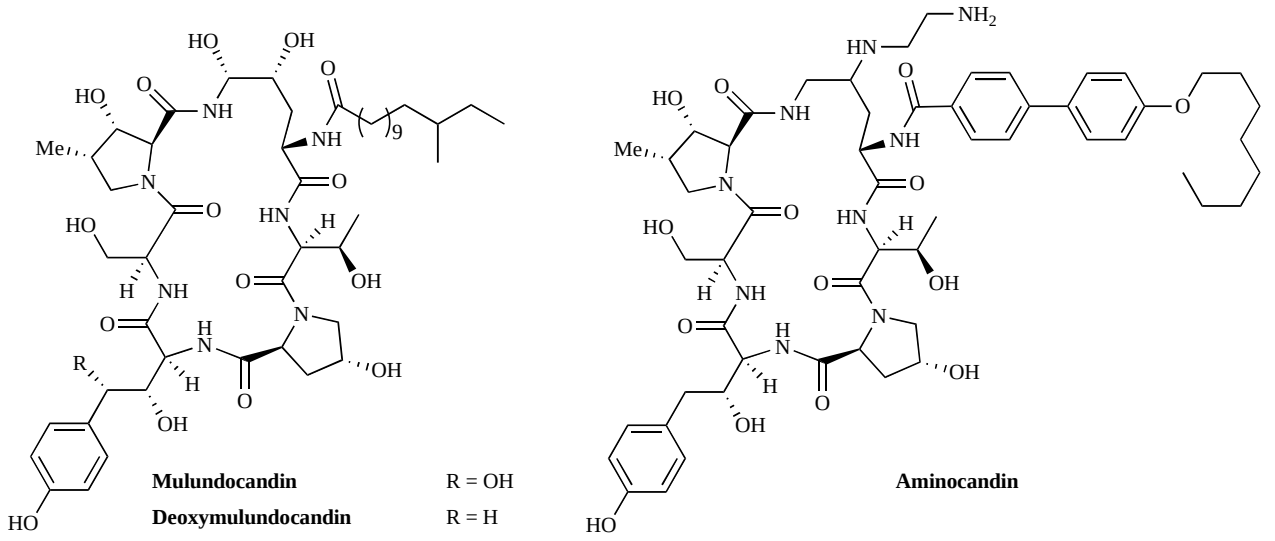


Fig. (5). Chemical structure of mulundocandin and semi-synthetic analog aminocandin.

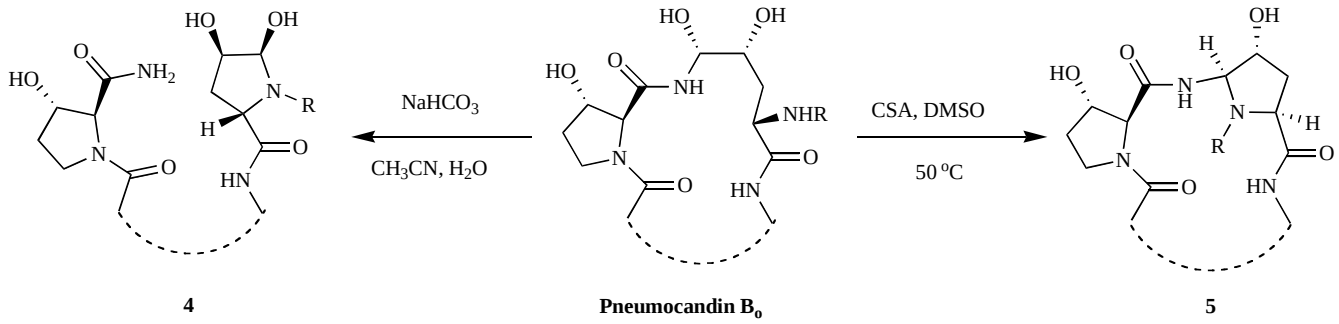


Fig. (6). Degradation products derived from pneumocandin B_o.

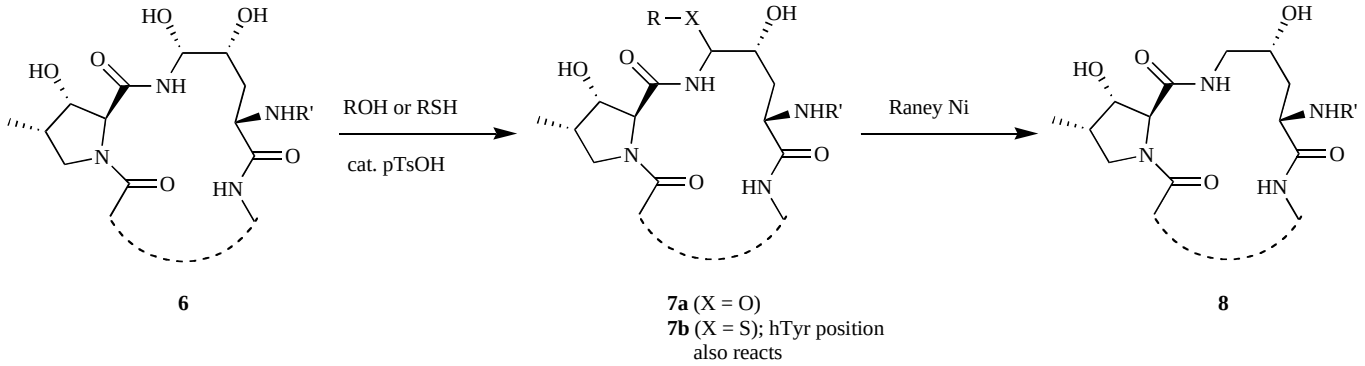


Fig. (7). Formation of hemiaminal ether and thioether derivatives **7a**,**b** and reduction of thioether **7b** to deoxygenated derivative **8**.

be low and over-reduction is often observed [54-58]. More selective, albeit multi-step solutions to this problem involve deactivation of the benzylic position to prevent its reduction, or hemiaminal etherification of unreduced side-product to facilitate its removal from the desired reduced material [59].

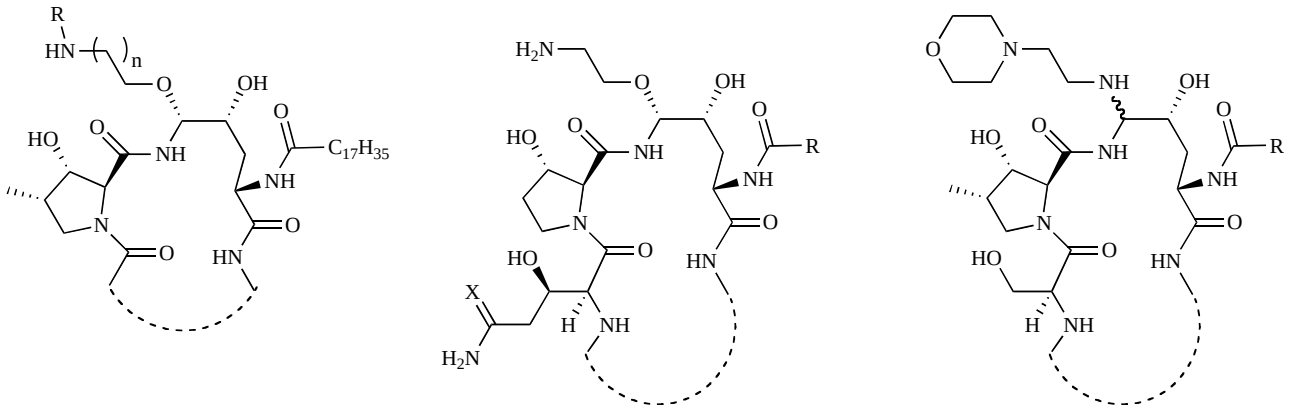
Patent applications from Fujisawa, Eli Lilly, and others indicate that significant time and effort has been invested in the examination of deoxygenated echinocandin analogs [60-67]. In general, these analogs possess antifungal activity comparable to that of their fully oxygenated progenitors. The expected improvement in chemical stability has also been noted. For example, whereas anidulafungin is subject to hemiaminal cleavage at neutral or slightly basic pH, the corresponding dideoxygenated derivative is reported to be stable under even strongly basic conditions [68].

The first medicinal chemistry efforts involving hemiaminal ether and thioether modifications were carried out by the same Sandoz group that first described the structures of

echinocandins B-D. A series of patent applications from this group [69-72] describe 2-aminoethyl and 3-aminopropyl hemiaminal ether analogs **9** Fig. (**8**). The free amino substituents in these analogs were further modified *via* reductive amination to provide substituted analogs (i.e., **9** where R = alkyl, benzyl, heteroaryl, etc.). Although limited information about these early analogs is available, similar modifications of pneumocandin B_o were subsequently reported by scientists at Merck [73-75]. Reaction of pneumocandin B_o with ethanolamine in the presence of HCl afforded the desired analog **10** along with its β-anomer and double addition side products. The introduction of a second basic amine in the cyclic peptide was accomplished by selective reduction of the terminal carboxamide of the 3-hydroxyglutamine residue. This conversion was accomplished in two steps, involving initial dehydration of the carboxamide to a nitrile followed by reduction of the nitrile to amine [76]. Subsequent introduction of the hemiaminal ether then afforded analogs such as **11** with two basic amine functions. Mono and dibasic analogs such as **10** and **11** possess both improved aqueous solubility and enhanced antifungal properties *in vitro* and *in vivo* [73]. For example, the *in vitro* activity of **10** against β-(1,3)-D-glucan synthase from *C. albicans* improved seven-fold over pneumocandin B_o, while diamine analog **11** afforded a further ten-fold improvement (IC_{50} = 1 nM vs. 70 nM for pneumocandin B_o). Compound **11** likewise exhibited between four and sixteen-fold lower minimum fungicidal concentration (MFC) values than pneumocandin B_o against *C. albicans*, *C. tropicalis*, and *C. parapsilosis* isolates. Compounds **10** and **11** were also shown to be superior to pneumodcandin B_o in three separate animal models of fungal infection. The efficacy of these analogs ($ED_{50} \leq 0.06$ mg/kg i.p.) in a murine model of disseminated aspergillosis is particularly striking considering that pneumocandin B_o was ineffective (ED_{50}> 20 mg/kg) in this same model. This observation led the authors to speculate that a blocked hemiaminal may be a requirement for *in vivo* activity against aspergillus species. Further optimization of the dibasic pneumocandin pharmacophore in **10** and **11**, led to the closely related *N*-alyklamino aminal series [77-79] from which caspofungin was selected for clinical development.

Hemiaminal ethers derived from anidulafungin or mulundocandin have been described more recently by workers from Eli Lilly and Aventis, respectively. The anidulafungin analogs included alkylamino derivatives analogous to **10** and were reported to possess antifungal activities comparable to the parent drug [80]. The mulundocandin analogs included hemiaminal ether and thioethers as well as analogs with an *N*-alkyl aminal function as in caspofungin [81]. Both the thioether and *N*-alkyl aminal analogs exhibited improved chemical stability. For example, while mulundocandin has a half-life of less than an hour at pH 8.5 in acetonitrile-water, the morpholine analog **12** showed less than 10% degradation after 5 days under similar conditions. Unlike with pneumocandin analogs however, the mulundocandin analogs were generally less potent than the parent itself. In a *C. albicans* kidney burden model, ether and thioether analogs exhibited only a modest reduction in fungal burden (typically 1 log) as compared to the fluconazole comparator (> 3 logs).

As we have seen, the introduction of ether, thioether, or amino functions at the hemiaminal has been explored by several groups, producing analogs that in many cases exhibit improved stability and antifungal potency. Somewhat less explored have been analogs with a new carbon-carbon bond at this position. Analogs of this type were first described in a Merck patent application [82] and were prepared from oxidized thioether hemiaminal derivatives such as **13** Fig. (**9**). The thioether sulfone moiety of **13** is displaced by cyanide to afford the nitrile **14** in which the desired carbon-carbon bond has been formed. Reduction of the nitrile then affords the corresponding aminomethyl derivative which was used to prepare additional *N*-alkyl and *N*-acyl analogs. Unfortunately, antifungal activities are reported for only a single analog, the unsubstituted aminomethyl analog **15**. Compound **15** exhibited excellent efficacy (comparable if not superior to that of **11**) in murine models of *C. albicans*, *A. fumigatus* and *P. carinii* infection. Unfortunately, little additional information about these interesting analogs is available, as no account of this work has appeared in the general literature. Similar analogs derived from mulundocandin were described more recently [83] and are reported to possess improved chemical stability, as might be expected.



9

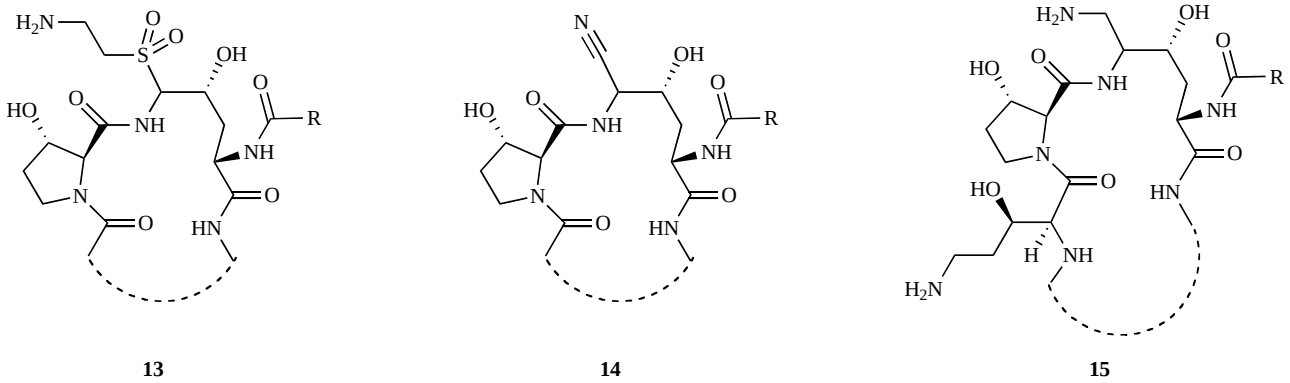
10 (X = O)
11 (X = H, H)

R = 10,12-dimethylmyristoyl

12

R = 12-methylmyristoyl

Fig. (8). Hemiaminal ether analogs **9**-**12**.

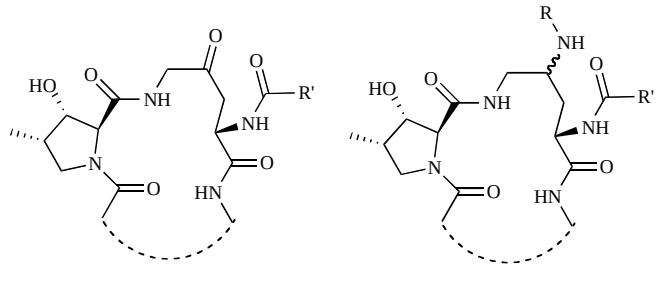


13 **14** **15**

R = 10,12-dimethylmyristoyl R = 10,12-dimethylmyristoyl

Fig. (9). Synthetic intermediates leading to ornithine C5-alkylated analog **15**.

The clinical compound aminocandin Fig. (**5**) is representative of a new generation of echinocandin analogs entering clinical trials [84, 85]. Discovered by Aventis scientists the compound is currently being developed by Indevus Pharmaceuticals. Phase I studies of the compound have revealed a long *in vivo* half life, suggesting the potential for less frequent dosing [86]. Aminocandin is derived from mulundocandin [87-89] and incorporates a synthetic 4'-(octyloxy)-biphenyl side chain and modified ornithine and homotyrosine residues. The ethylenediamine substituent is identical to that in caspofungin, but in aminocandin it is one carbon removed from the hemiaminal position with the latter reduced to an amide linkage. This unique alteration of the ornithine residue derives from an interesting pinacol-like dehydration reaction [49, 90, 91] that in a single step affords ketone intermediates such as **16** Fig. (**10**). Structure-activity studies leading to aminocandin involved the synthesis of various amino (e.g., **17**) and oximino analogs derived from ketone **16**. Unfortunately, the details of these studies have not yet been reported in the general literature. From patent applications [91] however, it is clear that C4 stereochemistry is important in this series. Only one of the two possible epimers exhibits antifungal activity, although the biologically active configuration is not disclosed. With respect to the synthetic side chain, it can be surmised that the 4-(octyloxy) biphenyl group affords a good combination of antifungal potency and low hemolytic potential.



16 **17**

R' = 12-methylmyristoyl

Fig. (10). Novel keto intermediate **16** and corresponding C4 amine analogs **17**.

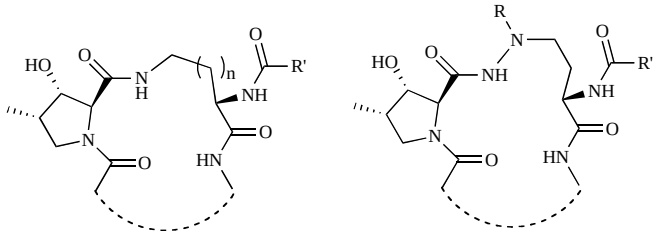
An altogether different strategy for ornithine modification is described in a recent patent application from Eli Lilly [92, 93]. In this approach, a semi-synthetic echinocandin (cilofungin) is converted into the corresponding acyclic hexapeptide by ring opening at the hemiaminal function. The ornithine residue and lipophilic side chain are then excised chemically and the resulting pentapeptide is orthogonally protected with silyl and carbamate protecting groups. This material is then used to regenerate new cyclic hexapeptides bearing ornithine surrogates of various chemotype. Each of the new analogs also incorporates the terphenyl side chain of anidulafungin.

The first set of analogs **18**-**21** are non-hydroxylated ornithine mimics of varying chain length Fig. (**11**). The analog **20** (n = 2) with a "natural" 21-membered ring is as potent as its fully hydroxylated congeners. An epimeric analog prepared with D-ornithine is more than twenty-fold less potent. Analogs such as **18** and **19** of smaller ring size or analog **21** with a 22-membered ring are essentially inactive, indicating that the 21-membered ring present in compound **20** and the natural echinocandins is optimal. Smaller or larger ring sizes may disrupt the transannular hydrogen bonds that are thought to be important for adoption of a biologically active conformation. Another series of analogs prepared by this approach were the acyl hydrazides **22**-**24**. Here the optimal 21-membered ring size is maintained but an additional heteroatom is introduced at the location of the hemiaminal in the natural lipopetides. This new position can be substituted with methyl (**22**) or glycyl susbstituents (**24**) without significant loss of activity. Larger hydrophobic groups (e.g. n-Pr in **23**) are poorly tolerated however.

Homotyrosine Modifications

The total synthetic work discussed in the previous section helped establish the critical importance of the homotyrosine residue for antifungal activity. Using a semi-synthetic approach, Balkovec and co-workers report a systematic exploration of this residue in pneumocandin B_o [94]. Removal of the phenolic hydroxyl led to a complete loss of glucan synthesis inhibition and a significant (>16 fold) reduction in antifungal activity. As expected, elimination the homotyrosine C4 (benzylic) hydroxyl produced no significant loss of activity. Quite surprisingly then, inversion of the homoty-

rosine C4 stereocenter produced a dramatic loss of both enzymatic and antifungal activity. Hence, while the C4 hydroxyl is not essential for binding, incorrect placement of this moiety (as in the C4 epimer) apparently disrupts other important interactions, possibly a crucial interaction with the phenolic hydroxyl.



18-21 (n = 0-3)

22 (R = Me)
23 (R = n-Pr)
24 (R = $COCH_2NH_2$)

R' = pentyloxyterphenyl

Fig. (11). Analogs of anidulafungin prepared using an ornithine excision/replacement strategy.

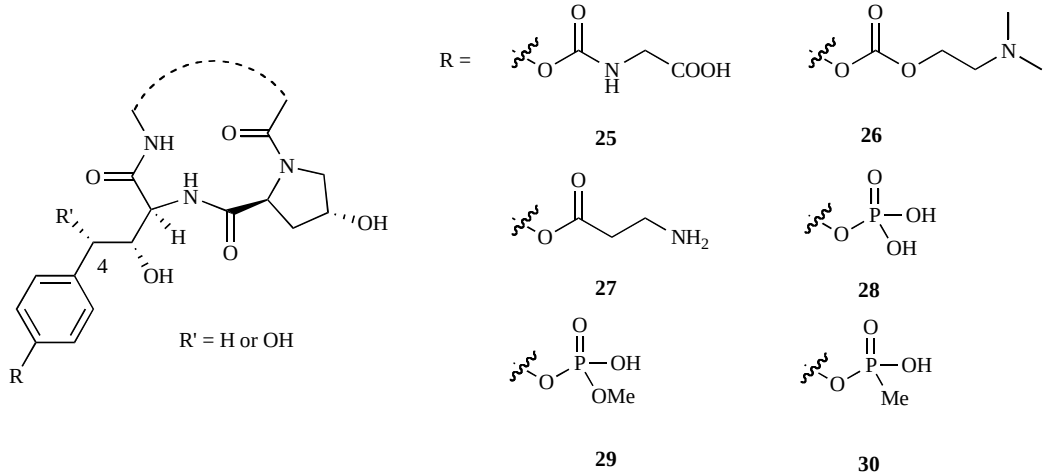
A potential drawback of the echinocandins as drug molecules stems from their generally poor aqueous solubility (typically <0.1 mg/mL). The need to administer these drugs intravenously however necessitates good aqueous solubility so that infusion volumes are not excessive. A number of methods have been explored for improving the solubility of the echinocandins. As we have seen already, the introduction of basic side chains improved the solubility of pneumocandin analogs such as **10** and **11**. Increasing solubility through formulation is another approach, although this has been nontrivial for the echinocandins. Indeed, the clinical development of cilofungin had to be terminated due to toxicity associated with the polyethylene glycol vehicle in which the drug was administered [95]. A third approach is to prepare a water soluble prodrug that is rapidly converted back to parent drug *in vivo*.

In an study by Merck scientists, the properties of various prodrugs of pneumocandin B_o were examined [96]. Selective acylation or phosphorylation of the homotyrosine phenolic hydroxyl was employed to form carbamate, carbonate, ester, and phosphate prodrugs **25**-**28** Fig. (**12**). Each of these was soluble at >20 mg/mL in pH 7 phosphate buffer, as compared to <0.1 mg/mL for the parent drug pneumocandin B_o. Carbamate analogs such as **25** demonstrated excellent hydrolytic stability but were not active in the animal infection models, presumably because they failed to convert to active drug *in vivo*. Although active *in vivo*, carbonate and ester prodrugs such as **26** and **27** were overly prone to hydrolysis ($T_{1/2}$ < 24 h at pH 7). Overall, the phosphate prodrug **28** exhibited the best combination of stability, solubility, and *in vivo* efficacy. Subsequent studies in primates confirmed that **28** is rapidly converted to parent drug *in vivo* [97].

In an effort to improve the aqueous solubility of anidulafungin, Lilly scientists reported [50] the synthesis of a phosphate prodrug analogous to those described above for pneumocandin B_o. Ring opening at the hemiaminal complicated the synthesis of these analogs and necessitated the use of low temperatures (-30 ^oC) and slow addition techniques for optimal results. In subsequent studies, a dideoxygenated analog of anidulafungin was employed that facilitated the preparation of both phosphate (**29**) and phosphonate (**30**) prodrugs [68, 98]. These analogs were reported to possess good aqueous solubility (>20 mg/mL) and *in vivo* activity.

The aromatic ring of homotyrosine can participate in electrophilic aromatic substitution reactions to introduce substituents at the 3' and/or 5'-positions. Starting with either pneumocandin B_o or its dideoxygenated derivative, a Merck group prepared 3'-iodo and 3'-amino derivatives (i.e., **31**, R = I or NH_2) that were used in the preparation of various 3'-alkyl, amino, or amido analogs [99]. The introduction of lipophilic (e.g. allyl, iodo) or hydrophilic (amino, hydroxyethyl) substituents had little effect on either glucan synthase inhibition or antifungal activity. However, analogs bearing 3'-substituents capable of hydrogen bonding to the phenolic hydroxyl (e.g., NO_2, formyl, keto) were poor inhibitors of glucan synthesis. This observation supports the notion that the phenolic hydroxyl is involved in a crucial hydrogen bonding interaction, the disruption of which greatly impacts binding affinity.

In more recent work reported by scientists from Aventis, a Mannich reaction is used to prepare mulundocandin ana-



R' = H or OH

R =

25 **26**

27 **28**

29 **30**

Fig. (12). Water soluble prodrugs of the echinocandin class.

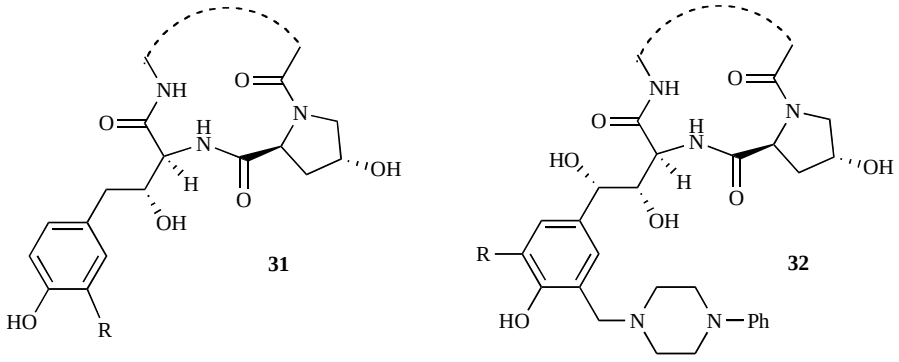


Fig. (13). Echinocandin analogs substituted at the 3' position of the homotyrosine aromatic ring.

logs with heterocyclic rings (e.g., piperidine or piperazine) at the 3' and/or 5' positions of the homotyrosine residue [100, 101]. The best of these analogs (e.g. **32**, R = H) were superior to mulundocandin in a murine *C. albicans* infection model, having activity comparable to fluconazole.

In summary, whereas lipophilic side chain replacement has been employed primarily to improve potency and reduce hemolytic potential, alternation of the cyclic peptide core influences chemical stability, aqueous solubility, and pharmacokinetic profile, while also impacting potency and *in vivo* efficacy. While the first generation of clinical echinocandins resulted from either side chain (anidulafungin, micafungin) or peptide core (caspofungin) modification, a newer generation of clinical echinocandins (e.g. aminocandin) bearing modifications of both core and side chain is now progressing into clinical trials.

CONCLUDING REMARKS

The preceding discussion attests to the skill and creativity of the medicinal chemists who have contributed to the field of echinocandin antifungal drug discovery. The early efforts involving side chain or hemiaminal modification quickly evolved and expanded to include chemical explorations of nearly every nook and cranny of these complex lipopeptides. These manifold efforts have yielded practical solutions to problematic issues such as chemical instability, hemolytic potential, and poor aqueous solubility. These advances, along with the gains in potency, efficacy, and safety that have been realized highlight the power of organic synthesis to alter the properties of natural products for new uses.

The burgeoning clinical and market success of the echinocandins is somewhat ironic given that they represent a dying breed in modern drug discovery − the semi-synthetic natural product therapeutic. The shifting focus of the industry away from infectious disease and towards the treatment of chronic diseases has dovetailed with the current emphasis on genomics and target-based discovery to significantly curtail (or eliminate altogether) the screening of natural product libraries for lead discovery. The logic of this shift has been questioned [102] in the case of antibacterial discovery, given an increasing threat from multi-drug resistant pathogens and the hard realization that genomics approaches in this area are neither as straightforward as expected, nor as likely to succeed as time proven approaches based on antibiotics of natural origin.

Regardless of one's position on these questions, it is certainly the case that very few medicinal chemists in industry today are engaged in natural product based drug discovery and that much valuable experience and chemical know-how has been lost as a result. Despite this, natural products chemistry and total synthesis in particular remain staples of graduate education in synthetic organic chemistry and so the expertise and desire to work with complex molecules is very much alive in the prospective labor pool. Whether this talent will be tapped in a future renaissance of natural products based drug discovery remains to be seen.

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