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Synthesis of the Antifungal β-1,3-Glucan Synthase Inhibitor CANCIDAS (Caspofungin Acetate) from Pneumocandin B_0

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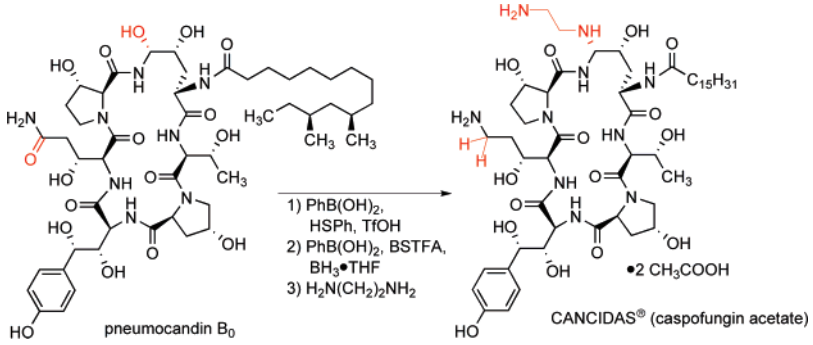

A novel three-step synthesis of the highly functionalized antifungal agent CANCIDAS (caspofungin acetate, **2**) is described, starting from the natural product pneumocandin B_0 (**1**). The highlights of the synthesis include a stereoselective formation of a phenylthioaminal, a remarkable chemoselective, high-yielding, one-step borane reduction of a primary amide, and a stereoselective substitution of the phenylthioaminal with ethylenediamine producing **2** in a 45% overall yield.

Introduction

Serious and life-threatening fungal infections have increased dramatically over the past several decades owing to the expanded use of invasive medical procedures and broad-spectrum antibiotics, as well as a burgeoning immune-compromised patient population resulting from cancer and organ transplantation chemotherapy, hematologic malignancies, and AIDS.[1] The few antifungal agents available are often limited by their toxicity, drug interactions, and growing antifungal resistance.[2]

In 1974, a novel family of lipopeptide antifungal natural products entitled the echinocandins was discovered.[3] These compounds are macrocyclic hexapeptides containing a labile hemiaminal moiety and a fatty acid chain that is N-linked to the peptide core. Their fungal-specific mode of action is the inhibition of the biosynthesis of β-(1,3)-D-glucan, an essential cell wall component of many pathogenic fungi that is absent in the mammalian host.[4] Several semisynthetic drug candidates[5−8] have been advanced from the echinocandin natural products

* Corresponding author.

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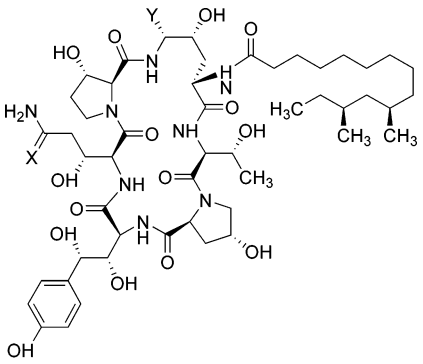

1, pneumocandin B_0 X = O, Y = OH

2, caspofungin (acetate) X = H, H; Y = $NHCH_2CH_2NH_2$ (•2 CH_3CO_2H)

FIGURE 1. Structures of pneumocandin B_0 and caspofungin acetate.

including those derived from the pneumocandin B_0 (**1**) member which was first described in 1992 (Figure 1).[9] It was not until 2001 that this search for a human antifungal drug came to fruition when CANCIDAS (caspofungin acetate, **2**),[10] a semisynthetic compound based on pneumocandin B_0, was approved by the U.S. FDA for invasive aspergillosis in patients who are refractory to or intolerant of standard therapy and subsequently for primary treatment of a variety of *Candida* infections. Most recently, caspofungin was approved as empirical therapy for presumed fungal infections in febrile neutropenic patients.[11] Caspofungin is proving to be a valuable antifungal agent because of its specific mode of action, broad spectrum, and low toxicity.[12]

The synthesis of caspofungin acetate (**2**) from pneumocandin B_0 (**1**) requires chemical modification at two sites of the peptide core—a reduction of a primary amide to an amine, and condensation of the hemiaminal moiety with ethylenediamine. These two transformations present significant synthetic challenges due to the need to control the chemo-, regio-, and stereoselectivity of the reactions during modification of the highly functionalized peptide core. Chemical reactions with **1** and its derivatives also present numerous processing issues as a result of physical characteristics that are inherent to this class of lipopeptides (e.g., poor solubility in organic solvents, micellar and soaplike behavior, hygroscopicity in the solid state, poor crystallinity, and instability[13] to both acid and base because of lability imparted by the hemiaminal). In addition to these challenges, the process had to be amenable to handling changes in the impurity profile of the natural product **1** as the fermentation process evolved.[14] Multiple structurally similar analogues of pneumocandin B_0 are generated as byproducts in this fermentation process, many of which were identified as impurities in **1** following isolation.[15] The first syntheses of **2** and similar primary amines bearing pneumocandin B_0 derivatives employed a five-step process providing less than 10% overall yield.[6,7] The amide to amine conversion was accomplished in two steps via dehydration of the primary amide to the nitrile and subsequent reduction to the amine. Installation of the ethylenediamine unit at the hemiaminal position required a three-step procedure via ethylenediamine displacement of an activated thioaminal derived from cysteamine (Scheme 1). While this sequence was suitable for the preparation of gram quantities of **2**, the low yields, lack of robustness of several reactions, and poor regio- and stereoselectivity required an improved synthetic route as the compound progressed through clinical development and into commercial manufacturing. Herein, we disclose an efficient, high-yielding, three-step route to antifungal agent **2** that is suitable for large-scale production. Highlights of the synthesis include a stereoselective formation of a phenylthioaminal, a remarkable one-step, high-yielding chemoselective borane reduction of a primary amide in the presence of higher order amides, and a stereoselective substitution of ethylenediamine with the unactivated phenylthioaminal producing caspofungin acetate (**2**) in a 45% overall yield.

Results and Discussion

As noted in the Introduction, the required two transformations for the synthesis of **2** from **1** necessitated three overall steps in the synthesis reported herein. The reduction of the amide could be accomplished in a single step, whereas the condensation of the hemiaminal with ethylenediamine required the formation of a phenylthioaminal intermediate followed by displacement with ethylenediamine. During the planning of the synthetic route investigation, the displacement with ethylenediamine to afford the aminal product **2** is, a priori, best reserved for the last step owing to the lability of the aminal group of **2**. The reduction and phenylthioaminal formation steps, in principle, could be carried out in either order as illustrated in Scheme 2. Indeed,

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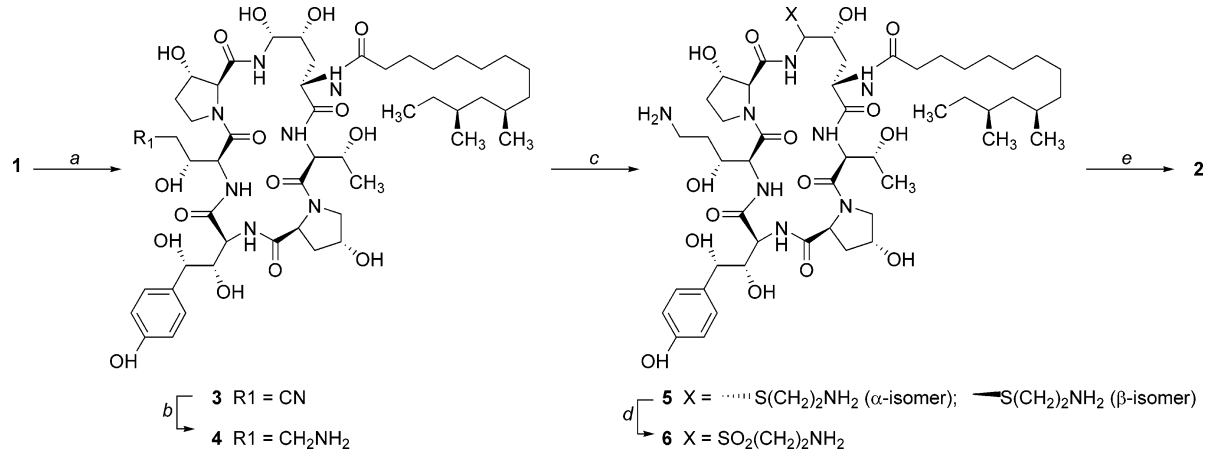
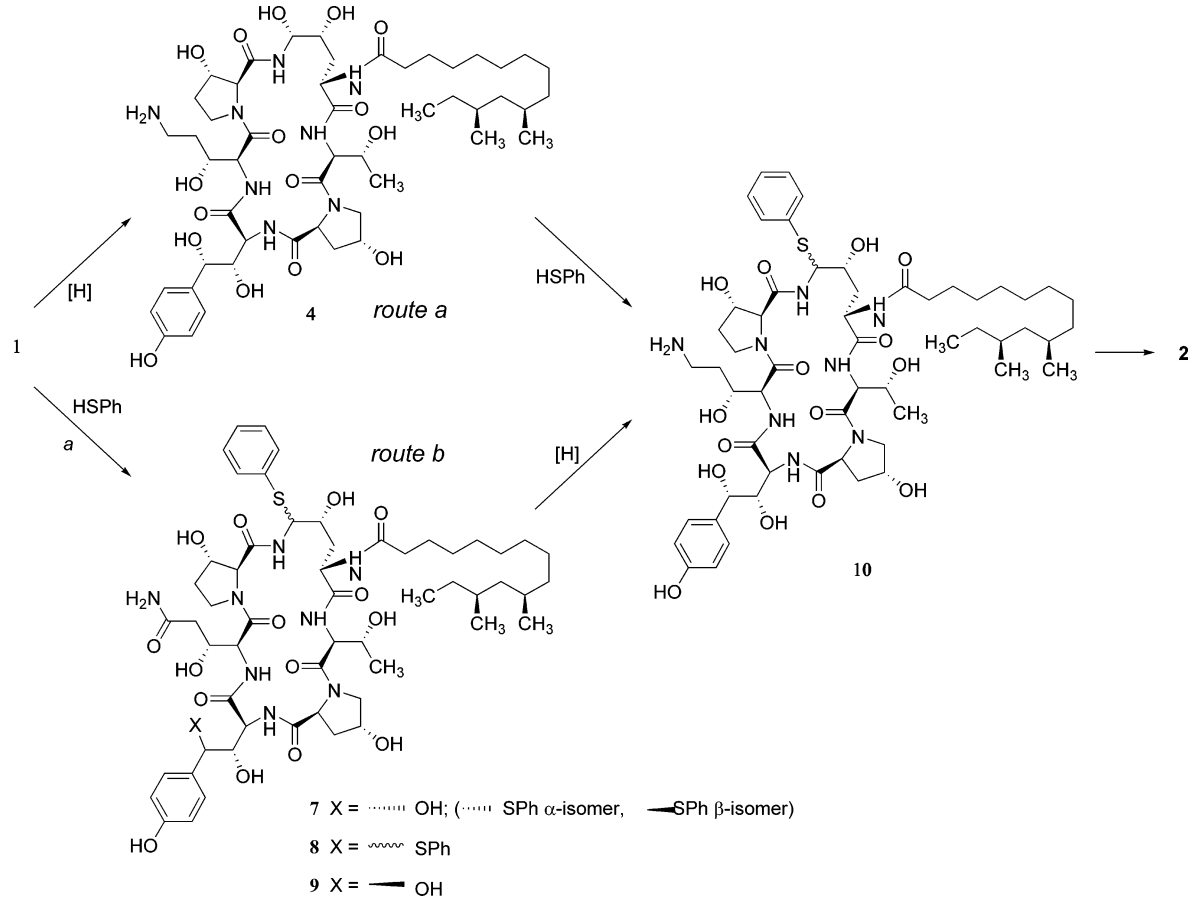
SCHEME 1[a]



[a] Key: (a) cyanuric chloride, DMF, rt; (b) $CoCl_2 \cdot 6H_2O$, $NaBH_4$, CH_3OH, rt; (c) $HS(CH_2)_2NH_3Cl$, CSA, DMF, rt; (d) Oxone, CH_3CN/H_2O, rt; (e) $H_2NCH_2CH_2NH_2$, DMF, rt.

SCHEME 2[a]



[a] Key: (a) 4 equiv of HSPh, 1:9 v/v TFA/CH_3CN, 0 °C, 12 h, yield 83% **7** (95:5 α/β) + 13% **8**.

our earliest preparations of **2** employed reduction of **1** to give amine hemiaminal **4**, followed by phenylthioaminal **10** formation (Scheme 2, route a).

However, as the synthetic process matured and the key discoveries were defined, it was found that formation of phenylthioaminal **7** as the first step provided the most robust process due to the ability of **7** to be isolated as a solid and also because of its increased stability as compared to the hemiaminal **4**. The second step of the synthetic sequence then became the one-step reduction of the primary amide of **7** (route b). The stereoselective displacement of the phenylthioaminal **10** by ethylenediamine to give **2** could be left to the last step as desired.

The details of the design and development of each of the three steps in the synthesis of caspofungin acetate (**2**) from the fermentation product pneumocandin B_0 (**1**) are discussed in this general order (route b) below.

Phenylthioaminal Formation. The most expeditious route to introduction of the ethylenediamine moiety would be the

direct reaction of the hemiaminal (e.g., **1** or **4**) with ethylenediamine. However, all attempts at condensation of **1** with ethylenediamine or its silyl derivatives in the presence of a variety of metal or Brönsted and Lewis acid catalysts failed to give any reaction at the hemiaminal. This lack of reaction is undoubtedly a result of the amines being rendered nonnucleophilic by protonation or coordination under these conditions.

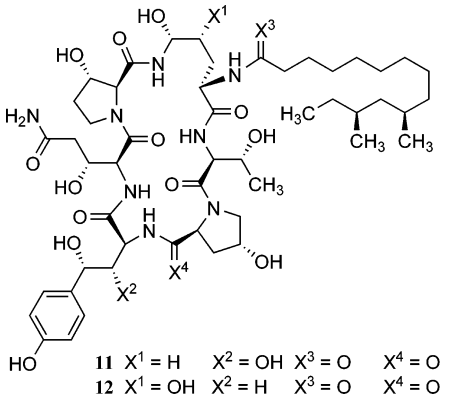
In the initial route to **2**, introduction of the ethylenediamine utilized a three-step procedure wherein the hemiaminal **4** was reacted with cysteamine, the resultant sulfide aminal **5** was activated by oxidation to the sulfone **6**, and that group was displaced with ethylenediamine (Scheme 1). Under the thioaminal formation conditions (CSA/DMF/rt), the thiol substitution was stereoindiscriminate, giving a 65% yield of a 2:3 ratio of the α/*β* isomers of the sulfide aminal **5**. Displacement of the minor α-isomer of the sulfone with ethylenediamine was reasonably stereoselective, giving the aminal **2** in a 9:1 (α/*β*) ratio, whereas the major *β*-isomer gave **2** in a 1:1 (α/*β*) ratio.[6] The lack of stereoselectivity in the substitution with the *β*-sulfone isomer suggested that a stereoselective formation of an α-oriented leaving group for displacement with ethylenediamine would probably be required for a high-yielding synthesis.

It was known that treatment of **1** with thiophenol in neat TFA gave a highly α-stereoselective sulfide substitution at the aminal position; however, concomitant nonstereoselective substitution at the benzylic hydroxyl group giving the bis(phenyl sulfide) **8** as a mixture of epimers also occurred (Scheme 2).[16] We undertook investigations to determine if diluting the TFA reaction solution with a leveling solvent might obviate the undesired substitution at the benzylic center while maintaining the reaction stereoselectivity exhibited by the neat TFA solution. Treatment of **1** with thiophenol in a variety of solvents including DMSO, DMF, or CH_3CN/H_2O containing <40 vol % TFA by volume gave no sulfide formation. Epimerization or reaction at the benzylic center was also not noted. Interestingly, treatment of **1** with thiophenol in the nonleveling control solvent CH_3CN, containing <40 vol % TFA, largely gave the desired phenylthioaminal **7** in a 95:5 (α/*β*) ratio. Optimization of the reaction conditions (4 equiv of HSPh, 1:9 v/v TFA/CH_3CN, 0 °C, 12 h) gave an 83% yield of **7** (95:5 α/*β* ratio) with 13% of the bis(phenyl sulfide) **8** formed (Scheme 2).[17] Extended reaction times led to increased amounts of the bis(phenyl sulfide) **8** and epimerization at the benzylic hydroxyl center to give **9**.

The 10 vol % of TFA was required for a reasonable reaction rate. Acids stronger than TFA predominately gave the bis(phenyl sulfide) diastereomers **8** under similar conditions, as did solutions containing greater than 50 vol % of TFA relative to CH_3CN. Acids weaker than TFA either gave very slow reaction rates or no reaction.

The analogous amine compound **4** also successfully underwent the phenylthioaminal formation under the same conditions; however, the yield was slightly reduced to 75−80% for the resultant amine phenylthioaminal **10**, and the stereoselectivity was reduced to 92:8 (α/*β*). On account of the water solubility of the salts of the amine **10**, the product was isolated after aqueous workup by solid-phase extraction using a hydrophobic adsorbent (e.g., SP-207 or C-18 reversed-phase silica gel). The intermediate **10** was eluted from the adsorbent with methanol in 95% recovery.

In contrast to the water solubility of the amine **4,** the amide **7** could be precipitated as an amorphous solid simply by addition of water. The replacement of the labile hemiaminal with the phenylthioaminal also provided a substantially more stable compound than **4**, and thus, **7** became the preferred process intermediate.



11 X^1 = H X^2 = OH X^3 = O X^4 = O
12 X^1 = OH X^2 = H X^3 = O X^4 = O
13 X^1 = OH X^2 = OH X^3 = H,H X^4 = O
14 X^1 = OH X^2 = OH X^3 = O X^4 = H,H

FIGURE 2. Fermentation analogues and reaction byproducts.

The α/*β* ratio in the formation of phenylthioaminal **7** from **1** appears to be under kinetic control given that the separated diastereomeric products do not interconvert under the same reaction conditions. The diastereomeric ratio also remains largely unchanged with varying conditions. The presence of the (4-ornithine) hydroxyl group vicinal to the reacting hemiaminal center is important in the observed stereoselectivity and rate of reaction. This was illustrated by subjecting the fermentation analogue **11** (Figure 2), which is missing this hydroxyl group, to the same reaction conditions: the resultant phenylthioaminal stereoselectivity was reduced to 2:1 (α/*β*) and the reaction completed 50 times faster. Similarly, the (3-homotyrosine) hydroxyl group vicinal to the benzylic position is important for minimizing the rate of substitution at the benzylic center. Benzylic substitution occurs ca. 75 times faster for the fermentation analog **12** missing the hydroxyl group vicinal to the benzylic position.

These initial reaction conditions giving phenylthioaminal **7** mixed with the bis(phenyl sulfide) compound **8** were used in the initial developmental phases. However, after the subsequent discovery while investigating the reduction chemistry, vide infra, that the vicinal hydroxyl groups of the homotryosine regioselectively form a cyclic boronate ester, we explored the possibility of minimizing the unwanted reactivity of the benzylic hydroxyl group by formation of an in situ cyclic boronate protecting group.

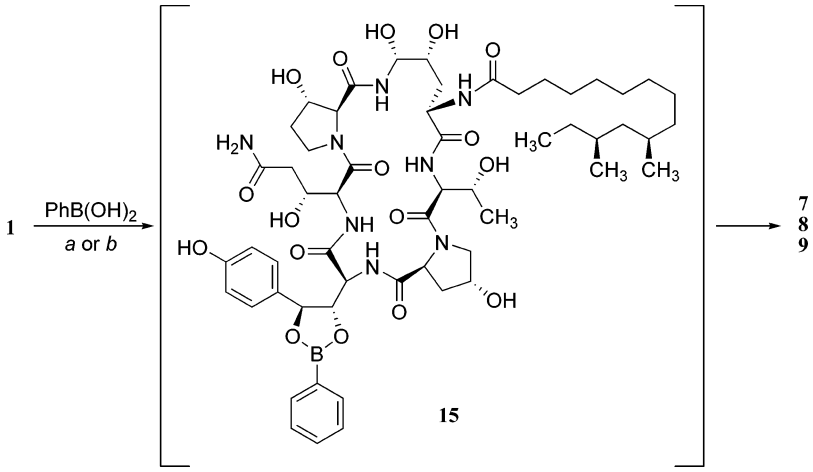
Treatment of **1** with 1 equiv of phenylboronic acid regioselectively formed the desired boronate ester **15** as evidenced by NMR analysis (Scheme 3).[18] Phenylthioaminal formation on this boronate using the same conditions as before (3 equiv HSPh, 1:9 v/v TFA/CH_3CN, 0 °C, 12 h) gave a 94% yield of α- and

(16) Balkovec, J. M.; Black, R. M. Dept. of Medicinal Chemistry, Merck Research Laboratories (personal communication).

(17) Nonisolated reaction yields were determined by quantitative HPLC analysis of reaction mixtures using an analytically pure external standard.

(18) Evidence of monoboronate ester formation was obtained in THF-d_8 and CD_3OD by NMR as discussed below. NMR evidence of boronate formation could not be obtained in CD_3CN as compound **1** was not soluble and the boronate formation did not proceed. Addition of TFA or TfOH to **1** in the presence the phenylboronic acid did give a solution attributed to boronate formation; however, NMR characterization in this mixture was not possible.

SCHEME 3[a]



[a] Key: (a) 1 equiv of $PhB(OH)_2$, 3 equiv of HSPh, 1:9 v/v TFA/CH_3CN, 0 °C, 12 h, 94% yield of **7** (95:5 α/β) + 3% **8** or (b) 2 equiv of $PhB(OH)_2$, 3 equiv of HSPh, 3 equiv of TfOH, CH_3CN, −15 °C, 94% yield of **7** (98:2 α/β) + 2% **8**.

β-**7** (in the same 95:5 ratio) with only 2−3% of the bis(phenyl sulfide) **8** as compared to the previously described reaction without phenylboronic acid that gave an 83% yield of **7** contaminated with 13% of bis(phenyl sulfide) **8**. Greater than 99% of the product **7** could be precipitated by addition of three volumes of water. However, in this strongly acidic aqueous medium, the compound **7** was not stable and extended time cycles led to increasing amounts of the epimer **9** and bis(phenyl sulfide) **8**. This instability could be obviated by neutralization of the TFA with aq NaOAc (2 equiv of NaOAc/TFA, in three volumes water). However, either workup gave a very finely divided precipitate that filtered extremely poorly. Another concern was that NaOAc coprecipitated and could not easily be removed from **7**. Even low levels of residual NaOAc led to a severe decrease in the subsequent borane reduction yield.

In order to reduce the amount of NaOAc needed for neutralization, a reduction in the amount of the acid charge needed to be investigated. We now considered employing the stronger acids that without the use of the boronate protection had previously led to complete bis(phenyl sulfide) **8** formation: methanesulfonic, *p*-toluenesulfonic, and triflic acid all catalyzed the reaction. Triflic acid (TfOH) gave the most rapid rate of reaction with the least amount of acid required, with 3.0 molar equiv of triflic acid proving to be optimum. The use of more TfOH greatly accelerated the reaction leading to uncontrollable amounts of the bis(phenyl sulfide) adducts, and the use of less TfOH resulted in a heterogeneous solution which slowed the reaction.

The rapid reaction also permitted lowering the temperature, which helped decrease the bis(phenyl sulfide) formation and increased the α/β ratio. The optimum conditions (3 equiv of TfOH, 2 equiv of $PhB(OH)_2$, 3 equiv of HSPh, −15 °C) gave a 94% chemical yield of **7** (98:2 α/β) with 2% of the bis(phenyl sulfide) **8**.[19]

The reaction mixture was now quenched by addition of 0.1 volume of water containing 1 equiv of NaOAc (to TfOH) to give a buffered suspension in which **7** is sufficiently resistant to bis(phenyl sulfide) formation and benzylic epimerization. The suspension is subjected to a heat and cool cycle which gives a nicely filtering amorphous solid in 95% recovery that contains <0.5% of the β-isomer and negligible bis(phenyl sulfide) **8** and epimeric **9**. This optimized process provides pure solid phenylthioaminal **7** from pneumocandin **1** in overall 87−91% isolated yield.

Amide Reduction. During our early synthetic studies and preparation of **2**, we followed the original sequence which involved the reduction of **1** as the first step. As mentioned (Scheme 1), the previous synthesis accomplished this in a two-step procedure that employed a dehydration (cyanuric chloride/DMF) of the amide to the nitrile **3**, and a subsequent reduction to the amine **4** with $CoCl_2/NaBH_4$.[6] The reported dehydration conditions were subject to low and very unpredictable yields under conditions that were not amenable to scale up.[20]

In comparison to the two-step dehydration/reduction approach, direct reduction of **1** appears to be a daunting task: it requires a chemoselective reduction of a primary amide in the presence of seven other higher-order amides and nine hydroxyl groups including two that undergo facile acid-catalyzed reduction.[21] The borane complexes, and ionic aluminum and boron hydrides, are the most common reductants for carboxamides; however, tertiary and secondary amides are generally reduced more readily than primary amides with these reagents.[22]

As expected, treatment of **1** with $LiAlH_4$, $NaBH_4$, and related hydrides led either to no reaction or to extensive degradation

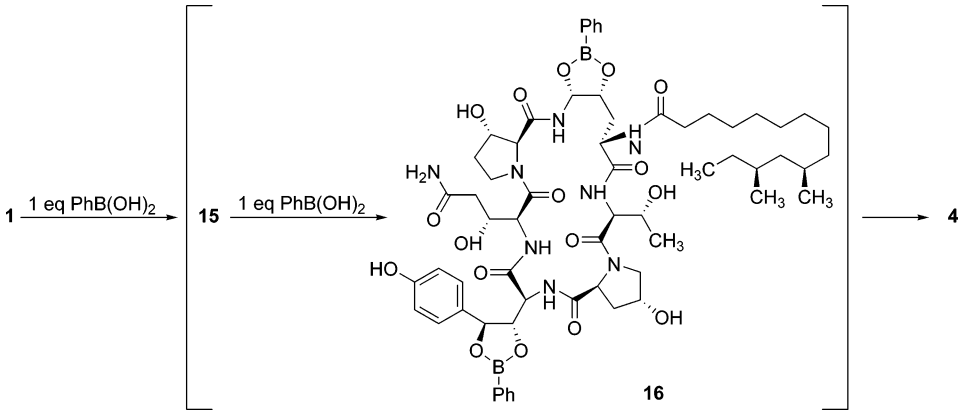
(19) In the presence of 2 equiv of boronic acid, it is unknown whether the bisboronate species is formed under these reaction conditions. Reaction with the monoboronate is presumed; however, an equilibrium of the bisboronate and the monoboronate or reaction of the boronate ester with HSPh may be possible.

(20) The dehydration conditions were optimized by adjusting the charge of cyanuric chloride (100 mol%) and carefully controlling the residual water amount and reaction temperature (<25 mol% of H_2O, −10 °C, 1 h), which gave the desired nitrile in 87% yield. Unfortunately, as a result of these conditions, the nitrile was contaminated with ca. 2% of the benzylic hydroxyl epimer impurity. This epimeric compound or its resultant synthetic impurities could not be removed during the downstream processes and purifications to the required level (<0.2%) in the pharmaceutical product **2**. All attempted variations in dehydration conditions, reagents, solvents, and use of the in situ boronate ester protection did not minimize this benzylic epimer impurity while maintaining desirable yields and this route was abandoned. Dehydration optimization studies for a similar compound can be found in ref. 8b.

(21) Balkovec, J. M.; Black, R. M. *Tetrahedron Lett.* **1992**, *33*, 4529−4532.

(22) For a general review on the reduction of amides to form amines see: Barrett, A. G. M. Reduction of Carboxylic Acid Derivatives to Alcohols, Ethers and Amines. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: Oxford, 1991; pp 248−251.

SCHEME 4



and over-reduction. The use of more reactive hydride systems, such as those generated by addition of thiols, iodine, or acids to $NaBH_4$, led to similar results.[23] The use of transition-metal salts ($CoCl_2$, $ZrCl_4$, and $TiCl_4$) in the presence of $NaBH_4$ also yielded only trace amounts of the desired product **4**.[24]

Much to our delight, treatment of **1** with excess BH_3·THF[25] or BH_3·$S(CH_3)_2$[26] at −10 to 0 °C, cleanly gave reasonable amounts (45−50%) of the desired amine 4. Only small amounts (<2%) of products resulting from the reduction of other amides were produced, the most significant of which were compounds resulting from secondary reduction at the threonine amide to give **13** (Figure 2) or the myristamide side-chain to afford **14**. The remainder of the mixture was recovered starting material.

This result might be considered surprising because primary amides typically require more forcing conditions (refluxing THF) for reduction by borane than do higher-order amides.[27] We attribute this mild and chemoselective reduction to the intramolecular delivery of the coordinated borane reagent by the pendent β-hydroxyl group of the hydroxyglutamide.[28] Indeed, kinetic studies indicated that the reaction is zero-order in borane and the reaction rate is unaffected by concentration, corroborating the supposition of intramolecular hydride delivery. Clearly, other controlling factors such as steric environment or intramolecular hydrogen bonding are at play because three other β-hydroxyamides are also present in the molecule.

All attempts at using more forcing conditions to increase the conversion in the reduction of **1**, such as increased borane equivalents, higher reaction temperature, or extended reaction times, gave higher starting material conversions and increased byproducts, but with no concomitant increase in the yield of **4**. The use of other solvents or borane complexes also gave inferior results.

After an initial reaction period whereupon the conversion is usually 15−30%, the reaction mixture largely gelatinizes, presumably owing to borate oligomer formation from the borane and active hydroxyl groups. The reduction still proceeds in this gelatinous mixture until the maximum 50% conversion is noted. Unfortunately, NMR investigations to understand and optimize the reaction were impossible given this gelatinous mixture. All attempts to dissolve this heterogeneous mixture in nonprotic solvents failed.

As a result, empirical approaches to increasing the reactivity or changing the nature of the product/starting material reaction matrix were attempted. Solvent studies, sonication, and addition of Lewis acids, surfactants, or $NaBH_4$ were investigated without success in raising the reaction yield. Partial silylation of **1** with 5 mol of BSTFA did render the reduction mixture homogeneous for a longer duration; however, the conversion remained at 50%. Persilylation with an excess (>10 molar equiv) of BSTFA completely inhibited the reduction. This result is consistent with intramolecular coordination and delivery of the borane due to the β-hydroxyl group which is blocked under excess silylation conditions.

In an attempt to minimize the formation of the gelatinous mixture (for NMR studies and homogeneous reaction conditions) without masking the β-hydroxyl group, the amide **1** was reacted with 2 equiv of phenylboronic acid in an effort to form cyclic boronates from the vicinal diols (Scheme 4). NMR studies showed that the monoboronate **15** formed within minutes in THF-d_8 or even CD_3OD, whereas it took several hours to form the second boronate, giving the bisboronate **16**.[29]

Unfortunately, after formation of the bisboronate **16**, in situ treatment with BH_3·SMe_2 or BH_3·THF still gave such severely broadened NMR resonances for the hexapeptide core as to be indistinguishable from the baseline despite the observation that gel formation was significantly retarded. This resonance broadening is presumably due to dynamic complexation of the borane with the highly functionalized core. Upon workup, however, an increase in yield from the typical 50% to an improved 62% was noted for the amide reduction of the bisboronate intermediate! A similar yield was obtained with the use of 1 equiv of phenylboronic acid to make the monoboronate, while with 3 equiv of phenylboronic acid the yield fell to 42%.

Derivatization as the boronate is apparently having an effect on the relative rates of the desired reduction versus the reaction

(23) (a) Guida, W. C.; Entreken, E. E.; Guida, A. R. *J. Org. Chem.* **1984**, *49*, 3024−3026. (b) Maki, Y.; Kikuchi, K.; Sugiyama, H.; Seto, S. *Chem. Ind. (London)* **1976**, 322. (c) Bhanu, Prasad, A. S.; Bhaskar, Kanth, J. V.; Periasamy, M. *Tetrahedron* **1992**, *48*, 4623−4628. (d) Wann, W. R.; Thorsen, P. T.; Kreevoy, M. M. *J. Org. Chem.* **1981**, *46*, 2579−2581. (e) Umino, N.; Iwakuma, T.; Itoh, N. *Tetrahedron Lett.* **1973**, 763−766.

(24) Heinzman, S. W.; Ganem, B. *J. Am. Chem. Soc.* **1982**, *104*, 6801−6802 and references therein.

(25) Brown, H. C.; Heim, P. *J. Org. Chem.* **1973**, *38*, 912−916.

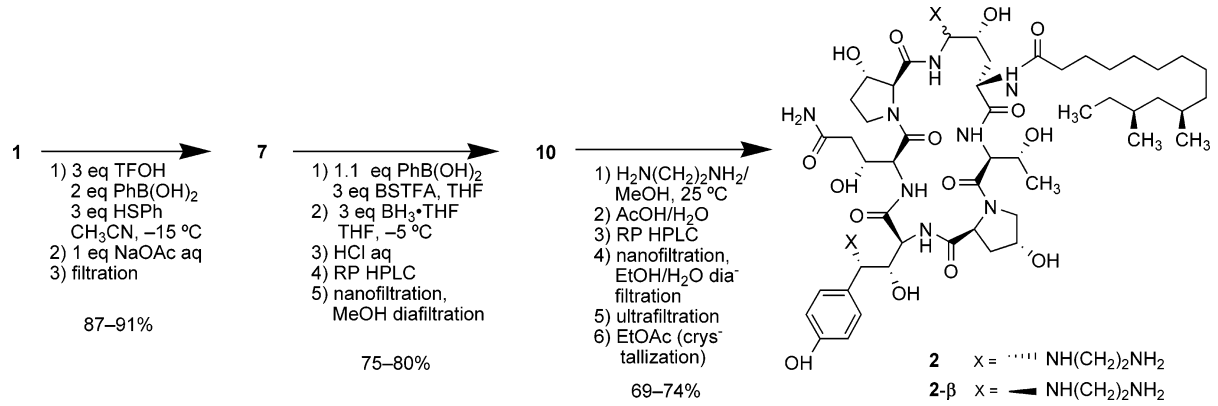
(26) Brown, H. C.; Narasimhan, S.; Choi, Y. M. *J. Org. Chem.* **1982**, *47*, 3153−3163.

(27) Under these reaction conditions, the nitrile **3** does not appear to be an intermediate: nitrile **3** is not detected by HPLC analysis, and submission of pure **3** to the reduction conditions does not give **4**.

(28) (a) Huang, F. C.; Hsu Lee, L. F.; Mittal, R. S. D.; Ravikumar. P. R.; Chan, J. A.; Shi, C. J.; Caspi, E.; Eck, C. R. *J. Am. Chem. Soc.* **1975**, *97*, 4144−4145, and ref 26.

(29) The monoboronate **12** formed from 1 equiv of phenylboronic acid was stable with extended reaction time in THF-d_8 as evidenced by NMR analysis.

SCHEME 5



pathway that ultimately prevents the reduction.[30] The change in relative rates may be on account of a change in conformation or steric factors. With this hypothesis, the partial silylation that previously proved unsuccessful in increasing the reduction yield was revisited. Treatment of the monoboronate **15** with BSTFA followed by addition of BH_3·SMe_2 gave a further increase in yield for the desired amine **4**. The optimum amount of BSTFA was 3 equiv, which resulted in an 80% yield of **4**. Both the BH_3·SMe_2 and BH_3·THF complexes worked equally well as reductants, and the borane amount could be reduced from 600 to 300 mol %. The reaction now remained homogeneous throughout the process. As was typical, the remainder of the reaction mixture was largely the unconverted substrate, and NMR spectroscopic examination of the reaction mixture still proved uninterpretable.

Since the phenylthioaminal **7** had become our preferred intermediate because of its superior stability and isolation as a solid, the same reduction protocol was attempted on this compound. Treatment with phenylboronic acid followed by BSTFA and reduction with BH_3·THF gave the amine **10** in similar 80% yield (Scheme 5). Once again, the reaction is zero-order with respect to borane, and the reaction rate was unaffected by concentration, again implicating coordination of the borane to the β-hydroxy group of the glutamide.

The reaction is quenched by addition of 2 N HCl to hydrolyze the boronate ester, silyl ethers, and the remaining borane. The separation of the amine product from the neutral starting material could not be accomplished by simple acid extraction. Instead, after dilution of the reaction mixture, solid-phase extraction on a column containing C18 silica gel was employed. Chromatographic elution of the adsorbed reaction products with CH_3CN/H_2O separated the desired amine **10** from the unreacted amide **7** as well as several low-level impurities arising from process byproducts and from fermentation analogues. The chromatographic fractions containing **10** were concentrated by nanofiltration and diafiltered to methanol in preparation for the next step. The optimized reaction conditions provide **10** in 76−81% yield from phenylthioaminal **7.**

Ethylenediamine Aminal Formation via the Phenylthioaminal. Unlike the original cysteamine aminal, the phenylthioaminal **10** does not need to be activated for displacement by ethylenediamine (Scheme 5). Treatment of the pure α-diastereomer of **10** with neat ethylenediamine (20 °C, 2 h) readily gave the desired product aminal **2** along with the β-diastereomer **2**-β in a 95:5 ratio, respectively, whereas the β-diastereomer of **7** is nearly 10-fold more reactive than the α-isomer and afforded only a 2:1 ratio of **2**/**2**-β. The ratio of product diastereomers remained constant throughout the reaction, and subjecting each of the separated diastereomers to the reaction conditions demonstrated that the two products do not interconvert under the reaction conditions. The vicinal α-hydroxyl group again plays a key role in the selectivity of the reaction. In the reaction of the phenylthioaminal produced from the fermentation impurity **11** (Figure 2) in which the vicinal hydroxyl group is absent, the α-isomer afforded a ratio of 77:23 of **2**/**2**-β, while the analogous β-isomer gave nearly the same ratio (70:30, **2**/**2**-β).

In an attempt to optimize the stereoselectivity, the displacement reaction of the typical diastereomeric mixture of **10** was attempted in a variety of solvents including 1:1 to 1:3 v/v mixtures of ethylenediamine with MeOH, *i*-PrOH, ethylene glycol, CH_2Cl_2, THF, water, and CF_3CH_2OH. The reaction was slowed because of dilution, but the α/β ratios remained generally the same as the neat reaction. Of note, however, was that CH_3-CN as a cosolvent gave a 10% lower ratio of **2**/**2**-β.

The reaction was most easily carried out using MeOH as a cosolvent (25 °C, 15−20 h) to aid in agitation and workup. The diastereomerically pure phenylthioaminal **7** resulting from the annealing procedure reduced to give a 93:7 (α/β) mixture of **10**, which was reacted with ethylene diamine resulting in an 85% yield of **2** and a 6% yield of **2**-β for the step. Thus, the product was produced in high stereoselectivity and confirmed our initial judgment that a highly stereoselective formation of an α-oriented leaving group for displacement with ethylenediamine would be required.

Reaction Mixture Workup and HPLC Purification of 2. The ethylenediamine reaction is quenched by addition of acetic acid and water. Partial addition of aqueous acetic acid leads to a high pH solution in which product aminal **2** is unstable. As a result, a constant pH quench procedure was developed in which the solution of **2** and acetic acid is added simultaneously to a vessel containing water which maintains the pH during quench at 5 to 5.5, where **2** has maximum stability. The reaction is then further diluted with water and extracted with heptane to remove thiophenol.

A column containing reversed-phase HPLC adsorbent is used to both extract the desired **2** from the reaction mixture and to

(30) Use of different alkyl- or arylboronate esters gave differing reduction yields, with the maximum yield of 74% for the 1-naphthylboronate. However, following subsequent derivitization of the boronate with BSTFA (vide infra), there was no difference in overall yield (ca. 80%) between the phenyl- or 1-naphthylboronate derivitized reaction. The more stable and inexpensive phenylboronic acid was therefore used.

purify it from echinocandin process impurities including the **2-**β diastereomer, a ring-opened byproduct, and several impurities resulting from fermentation analogues. The same C18 reversed-phase adsorbent and column that was used for the HPLC purification of amine **10** was employed in this final purification. The worked up reaction solution containing 1:5 MeOH/water was loaded on the column to capture the product **2**. The column was eluted with 22:78 v/v acetonitrile/aqueous acetic acid, and the fraction containing **2** of desired purity was collected in 90% recovery.[31]

Final Salt Formation. On small scale, the HPLC fractions of suitable quality for crystallization were lyophilized to a powder. On a larger scale, the HPLC fractions were concentrated from the acetonitrile/aqueous acetic acid eluent using nanofiltration and solvent switched to a mixture of ethanol/water (9:1 v/v) by diafiltration. Acetic acid was added to ensure equivalency, and the solution was subjected to an ultrafiltration to remove endotoxins.[32] Ethyl acetate was added to initiate crystallization of **2** as the diacetate salt. After a seed bed was established, additional ethyl acetate was added to complete the crystallization in 95% recovery. The crystallization was employed to produce a stable crystalline solid and to slightly reduce several echinocandin process impurities. Removal of residual ethanol from the crystalline solid required passing humid nitrogen through the filter cake. This treatment also controlled the residual water level and minimized the formation of product degradates. The final hygroscopic crystalline solid contains 6−8 wt % residual water. The recovery across the column, nanofiltration, and crystallization was typically 86% giving an overall isolated yield of 69−74% for the last step.

Conclusion

The antifungal agent caspofungin acetate (**2**) was prepared in three chemical steps from the fermentation product pneumocandin B_0 (**1**) in 45% overall yield. This synthesis demonstrates that subtle features of a complex substrate reacting with appropriate reagents and conditions can provide exquisitely chemo-, regio-, and stereoselective reactions.

Regioselective in situ formation of the cyclic monoboronate derivative of **1** prior to formation of the phenylthioaminal **7** suppressed the formation of undesired the bis(phenyl sulfide) **8**, and the use of TfOH/MeCN led to a highly stereoselective reaction in 87−91% isolated yield.

A chemoselective borane reduction of the primary amide in **7** was demonstrated, and the conversion was increased by in situ derivitization with phenylboronic acid and BSTFA giving an overall yield of 75−80% for amine **10**.

Finally, reaction of the diastereomerically pure **10** with ethylenediamine gave stereoselective substitution on the unactivated phenylthioaminal producing the desired product **2** in 69−74% yield after chromatographic purification and crystallization.

These reactions combine to form a robust synthesis that is currently used as the manufacturing process for caspofungin acetate.

Experimental Section

Phenylthioaminal 7. To CH_3CN (3 L) were added finely divided pneumocandin B_0 (**1**) (100 g, corrected for residual H_2O as assayed by KF titration, 94.0 mmol), phenylboronic acid (22.9 g, 188 mmol), and thiophenol (29.0 mL, 282 mmol). The suspension was cooled and maintained at −15 °C where triflic acid (24.9 mL, 282 mmol) was added and the reaction maintained until HPLC assay indicated reaction completion (2.5 h). The reaction was quenched and the product precipitated by addition of $NaOAc\cdot 3H_2O$ (38.3 g, 282 mmol, 1.0 mol/mol triflic acid) in 333 mL of water. The suspension was warmed 17 °C, maintained for 2 h, and then cooled to 0 °C. The product was isolated by filtration and washed with 1:9 (v/v) H_2O/CH_3CN to give **7** (93.4 g, 94 wt % purity by HPLC vs a pure standard) in 88% yield: ^{1}H NMR (399.87 MHz, CD_3OD) δ 7.57−7.55 (om, 2H), 7.29−7.24 (om, 3H), 7.13 (m, 2H), 6.74 (m, 2H), 5.55 (d, J = 2.0, 1H), 5.05 (d, J = 3.2, 1H)), 4.96 (d, J = 3.2, 1H), 4.57 (dd, J = 11.2, 7.2, 1H), 4.52−4.49 (om, 2H), 4.44 (dd, J = 12.9, 4.8, 1H), 4.38−4.34, om, 2H), 4.29 (dd, J = 8.0, 1.2, 1H), 4.26 (d, J = 8.0, 1H), 4.22 (d, J = 2.8, 1H), 4.18 (ddd, J = 10.0, 5.6, 2.0, 1H), 4.00−3.89 (om, 3H), 3.76−3.66 (om, 2H), 2.76 (dd, J = 15.7, 4.0, 1H), 2.45 (dd, J = 15.7, 9.6, 1H), 2.40 (om, 1H), 2.16−1.95 (om, 6H), 1.83 (m, 1H), 1.54 (m, 2H), 1.49−1.18 (om, 15H), 1.11 (d, J = 6.4, 3H), 1.10−1.00 (om, 2H), 0.91 (t, J = 6.8, 1H), 0.86 (t, J = 7.6, 3H), 0.84, (d, J = 6.8, 3H), 0.83 (d, J = 6.4, 3H); ^{13}C NMR (101 MHz, CD_3OD) 176.9, 175.7, 174.3, 173.4, 172.45, 172.43, 171.68, 168.9, 158.4, 134.83, 134.78 (2C), 133.1, 129.9 (2C), 129.7 (2C), 128.8, 116.2 (2C), 77.0, 75.8, 74.5, 71.3, 70.6, 70.5, 69.8, 68.2, 62.5, 61.6, 58.6, 57.1, 56.1, 55.7, 51.1, 46.8, 45.9, 39.7, 38.5, 38.1, 36.8, 36.0, 34.7, 32.9, 31.24, 31.16, 30.7, 30.6, 30.4, 30.3, 28.0, 27.1, 20.7, 20.2, 19.5, 11.6; IR (KBr) 3800−2300, 3345, 2925, 2853, 1633, 1516, 1439, 1234, 1086 cm^{-1}; HRMS (HPLC/MS ESI-TOF) calcd for $C_{56}H_{84}N_8O_{16}S$ 1157.5799 (M + H$^+$), found 1157.5839 (M + H$^+$).

Amine 10. To THF (3 L) were added the phenylthioaminal **7** (75.7 g, corrected for residual H_2O as assayed by KF titration, 64.8 mmol) and then phenylboronic acid (8.69 g, 71.3 mmol). The suspension was brought to reflux and azeotropically dried (to <35 mol % H_2O) by passing the refluxate through molecular sieves, 3A (250 mL). The solution was cooled and maintained at 20 °C where BSTFA (52 mL, 194 mmol) was added and the mixture stirred for 1 h. The solution was cooled to −5 to 0 °C, and $BH_3\cdot$THF (1 M in THF, 195 mL, 195 mmol) was added. The mixture was maintained at −5 to 0 °C for 2 h. (**Caution:** Hydrogen gas is released during the addition, reaction, and workup of $BH_3\cdot$THF.) The reaction mixture was quenched with 2 N aq HCl (178 mL) and stirred for 2.5 h at −5 to 5 °C. HPLC assay showed 85% conversion and 76% reaction yield of the amine salt (54.7 g).

The reaction mixture was diluted with chilled water (3 L) while maintaining the solution at <10 °C. Chromatographic purification was carried out on Kromasil C-18 adsorbent, eluting sequentially with 10:90 CH_3CN/H_2O, 29:71 CH_3CN/0.014 M aq HCl, and 29:71 CH_3CN/H_2O.

The fractions containing **10** were concentrated by nanofiltration and diafiltered against MeOH. The MeOH solution contained **10** (35.8 g by HPLC assay) in 99% recovery across the column/nanofiltration/diafiltration. The MeOH solution was used as is in the next reaction.

A portion of the solution was evaporated to dryness to obtain the following spectroscopic data: ^{1}H NMR (399.87 MHz, CD_3OD) δ 7.58−7.54 (om, 2H), 7.29−7.26 (om, 3H), 7.11 (m, 2H), 6.74 (m, 2H), 5.36 (d, J = 2.0, 1H), 4.95 (d, J = 3.2, 1H), 4.90 (d, J = 6.0, 1H), 4.61−4.53 (om, 3H), 4.37 (dd, J = 12.9, 4.8, 1H), 4.30−4.28 (om, 2H), 4.23 (dd, J = 8.0, 1.6, 1H), 4.20 (d, J = 4.0, 1H), 4.16 (ddd, J = 10.0, 5.6, 2.0, 1H), 4.05−3.99 (om, 2H), 3.96 (dd, J = 11.2, 3.2, 1H), 3.87−3.73 (om, 3H), 3.04 (t, J = 7.2, 2H), 2.42 (dd, J = 13.3, 7.2, 1H), 2.15−1.99 (om, 7H), 1.93−1.76 (om, 2H), 1.57−1.19 (om, 17H), 1.14 (d, J = 6.4, 3H), 1.18−1.03 (om, 2H), 0.92 (t, J = 7.2, 1H), 0.86 (t, J = 7.2, 3H), 0.84 (d, J = 6.4, 6H); ^{13}C NMR (101 MHz, CD_3OD) 176.3, 174.3, 173.5, 172.6, 172.5, 171.7, 168.8, 158.6, 135.2 (2C), 134.9, 133.0, 130.0 (2C), 129.6 (2C), 129.0, 116.2 (2C), 77.4, 75.5, 74.4, 72.5, 71.4, 70.3, 69.5, 68.3, 62.7, 61.9, 58.3, 57.1, 56.5, 55.9, 51.2, 46.9, 45.9, 39.1,

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(32) Grau, J. P.; Tung, H.-H. PCT Patent, WO 2003028858.

38.5, 38.1, 36.9, 35.6, 34.7, 32.9, 31.3, 31.2, 30.7, 30.61, 30.57, 30.4, 30.3, 28.0, 27.1, 20.7, 20.2, 20.0, 11.5; IR (KBr) 3800−2300, 3344, 2926, 2854, 1633, 1517, 1439, 1235, 1085 cm^{-1}; HRMS (HPLC/MS ESI-TOF) calcd for $C_{56}H_{87}N_8O_{15}S$ 1143.6012 ($M + H^+$), found 1143.5984 ($M + H^+$).

Caspofungin Acetate (2). The solution of **10** as the hydrochloride salt (35.8 g, 30.3 mmol) in methanol (460 mL) was cooled to −10 °C. Ethylenediamine (135 g, 150 mL, 2.25 mol) was added over 15 min while maintaining the temperature at <2 °C. The resulting solution was concentrated by rotary evaporation under vacuum (80−100 mbar, 40 °C bath temperature) to ca. 1:1 (v/v) ethylenediamine/MeOH. This mixture was stirred for 24 h at rt. The reaction was monitored for completion by HPLC assay. The reaction mixture and 280 mL of acetic acid (2.2 mol acetic acid/mol ethylenediamine) were added simultaneously to a receiving vessel containing 1.85 L of chilled water. The addition was carried out such that a constant pH of 5.0−5.5 and a temperature of <20 °C were maintained. The HPLC assay yield of **2** was 31.0 g (85% yield). The solution was stored at 0−5 °C.

The quenched reaction mixture was purified by chromatography in two batches (1050 g of each batch, 13.3 g of assay **2**) on Kromasil C-18 adsorbent, eluting sequentially with 10:90:0.0014 (v/v/v) $CH_3CN/H_2O/CH_3COOH$ and 22:78:0.0012 (v/v/v) $CH_3CN/H_2O/CH_3COOH$. The fractions of desired purity from both batches contained 24.0 g (90% recovery) of **2** by HPLC assay. The fractions were lyophilized.

A portion of the lyophilized solid (20.6 g assay **2**) was dissolved in 274 mL of absolute ethanol, 25.7 mL of water, and 1.3 mL of acetic acid. A seed bed was formed by sequential addition of 108 mL of EtOAc and ca. 10 mg of **2**, and the suspension was maintained with stirring for 1 h. The crystallization was completed by addition of 324 mL of EtOAc and additional aging for 1 h at 20 °C. The solid was isolated by filtration,and washed with ethyl acetate/ethanol/water (17.1:10.7:1.0 v/v/v). Humid nitrogen (30−50% relative humidity) was then passed through the solid at ambient temperature for 6 h to remove the residual organic solvents. An amount of 20.8 g of gross weight crystalline solid (98.6 area % purity by HPLC analysis) was obtained that contained 6.2 wt % of water and 19.5 g of assay **2**, for a 95% crystallization recovery: HPLC area % purity method (Waters Symmetry C18 column, 250 × 4.6 mm, 100 Å, 5 μm, 45 °C, A = CH_3CN, B = 0.1% v/v aq $HClO_4$, linear gradient 67:33 v/v A/B to 66:34 v/v A/B over 10 min, then to 35:65 v/v A/B over 35 min, 1.5 mL/min, 205 nm); ^{1}H NMR (399.87 MHz, CD_3OD) 7.12 (m, 2H), 6.75 (m, 2H), 4.97 (d, J = 3.2, 1H), 4.91 (d, J = 5.8, 1H), 4.66 (d, J = 2.2, 1H), 4.60 (dd, J = 6.2, 3.2, 1H), 4.56−4.51 (om, 2H), 4.48 (dd, J = 12.8, 4.5, 1H), 4.32−4.28 (om, 3H) 4.22 (dd, J = 8.0, 1.7, 1H), 4.18 (d, J = 4.8, 1H), 4.08−3.96 (om, 3H), 3.83 (m, 1H), 3.76 (d, J = 10.4, 1H), 3.05 (t, J = 7.2, 2H), 3.02−2.76 (om, 4H), 2.41 (dd, J = 12.9, 6.8, 1H), 2.29−2.17 (om, 3H) 2.11−1.78 (om, 5H), 1.90 (s, 6H), 1.58 (m, 2H), 1.53−1.19 (om, 15H), 1.16 (d, J = 6.0, 3H), 1.13−1.00 (om, 2H), 0.91 (m, 1H), 0.87 (t, J = 7.6, 3H), 0.85 (degenerate d, J = 6.8, 6H); ^{13}C NMR (101 MHz, CD_3OD) 180.1, 176.3, 174.2, 173.7, 173.5, 172.7, 172.7, 168.9, 158.5, 133.0, 129.6 (2C), 116.2 (2C), 77.3, 75.6, 56.1, 51.2, 47.1, 45.9, 43.9, 40.3, 39.0, 38.5, 38.1, 36.9, 35.8, 34.6, 32.9, 31.2, 31.1, 30.8, 30.8, 30.6, 30.3, 30.3, 28.0, 27.1, 24.1, 20.7, 20.2, 19.9, 11.5;IR (KBr) 3775−2350, 3344, 2925, 1630, 1541, 1456, 1236, 1088 cm^{-1}; $[\alpha]_{405}^{25} = -105$ (c = 1.0, water); HRMS (ESI FTICR) calcd for $C_{52}H_{88}N_{10}O_{15}$ 1093.6514 ($M + H^+$), 547.3294 ($M + 2H^+$), found 1093.6470 ($M + H^+$), 547.3287 ($M + 2H^+$).

Supporting Information Available: General experimental methods and copies of ^{1}H and ^{13}C NMR spectra for compounds **2**, **7**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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