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Determination of the Relative and Absolute Configuration of the Dimethylmyristoyl Side Chain of Pneumocandin B_0 by Asymmetric Synthesis

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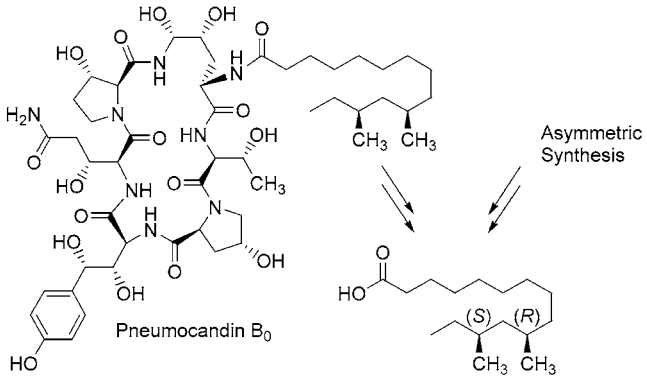
ABSTRACT



The relative and absolute configuration of the pneumocandin B_0 side chain has been established as (10*R*,12*S*)-dimethylmyristoyl by the stereocontrolled synthesis of both antipodes of the side chain acid and their comparison to a sample derived from the natural product.

Serious and life-threatening fungal infections have increased dramatically over the past several decades due to the increased 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, malignancies, and AIDS. The few antifungal agents available are limited by their toxicity, drug interactions, and growing antifungal resistance.[1]

The echinocandin class of natural products has recently emerged as a promising antifungal therapy.[2] Their fungal-specific mode of action is inhibition of the biosynthesis of β-(1,3)-D-glucan, an essential cell wall component of many pathogenic fungi.[3]

One of the echinocandins, pneumocandin B_0 (**1**), was isolated from the fermentation of the fungus *Glarea lozoyensis*.[4] It has been employed in the semisynthesis of several potent antifungal drug candidates,[5] including CANCIDAS

(1) (a) Groll, A. H.; Walsh, T. J. *Curr. Opin. Infect. Dis.* **1997**, *10*, 449. (b) Kauffman, C. A.; Carver, P. L. *Drugs* **1997**, *53*, 539.

(2) For reviews on the antifungal properties and SAR studies of echinocandins, see: (a) Hossain, M. A.; Ghannoum, M. A. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1797−1813. (b) Balkovec, J. M.; Black, R. M.; Bouffard, F. A.; Dropinski, J. F.; Hammond, M. L. *Pharmacochem. Libr. (XIVth International Symposium on Medicinal Chemistry, 1996)* **1997**, *28*, 1−13. (c) Debono, M. *Exp. Opin. Ther. Patents* **1995**, *5*, 771−786. (d) Hammond, M. L. In *Cutaneous Antifungal Agents*; Rippon, J. W., Fromtling, R. A., Eds.; Marcel Dekker: New York, 1993; pp 375−393.

(3) Kurtz, M. B.; Douglas, C. M. *J. Med. Vet. Mycol.* **1997**, *35*, 79−86

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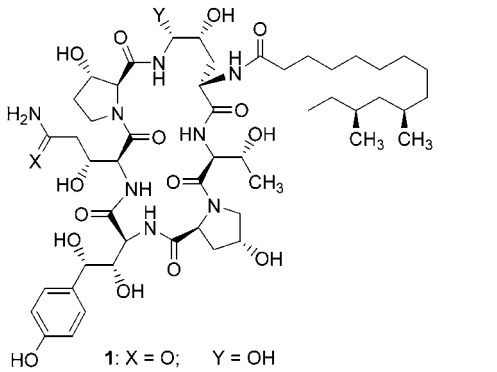
(caspofungin acetate)[6] (**2**), which has recently been approved by the U.S. FDA for treatment of invasive aspergillosis in patients who are refractory to or intolerant of standard therapy. Caspofungin is also in late-stage clinical testing for other indications, including empirical antifungal therapy.[7] Caspofungin is proving to be a valuable antifungal agent due to its specific mode of action, broad spectrum, and low toxicity.



1: X = O; Y = OH
2: X = H, H; Y = $NH(CH_2)_2NH_2$; $\bullet 2CH_3COOH$

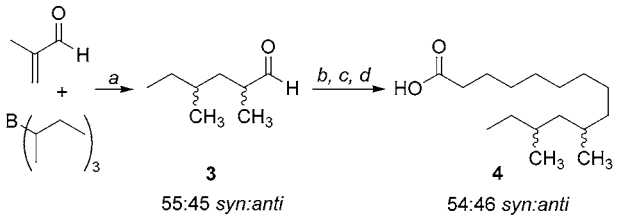
The structures of pneumocandin B_0 (**1**) and caspofungin (**2**) consist of a polar cyclic hexapeptide core with a lipophilic side chain containing two stereogenic methyl groups. The structure and absolute stereochemistry of the peptide core of **1** was determined by NMR, X-ray crystallographic, and chromatographic amino acid analysis; however, the lipophilic side chain was disordered in the crystallographic study, and the stereochemistry of the two methyl groups could not be elucidated.[8] Structure−activity relationship experiments have shown that the structure and stereochemical configuration of the echinocandin side chain is of central importance for both the antifungal potency and the toxicity due to red blood cell hemolysis.[2c,d]

Herein, we report the determination of the relative and absolute stereochemistry of the pneumocandin B_0 dimethylmyristoyl side chain by comparison of the hydrolyzed side chain acid to samples of both antipodes produced by enantioselective synthesis.

A sample of **1** was obtained by fermentation, isolation, and purification as previously described.[9] The sample was 92% pure. The impurities were mostly compounds that contained analogous amino acids. Impurities that differed in the side chain were analyzed at <0.7%. The pneumocandin was methanolized (MeOH, AcCl, reflux, 73 h), and the pentane extract was purified by bulb-to-bulb distillation (95−105 °C oven, 0.35 Torr) to give the methyl ester in 72% yield. The ester was hydrolyzed (THF/H_2O, LiOH, 25 °C, 16 h) to the acid in quantitative yield. The side chain acid was homogeneous by ^1H and ^{13}C NMR analysis, and the specific rotation was determined to be $[\alpha]^{25}_{405}$ +16.6° (*c* 0.010, $CHCl_3$).

To aid in the identification of the relative stereochemistry of the side chain, a short racemic synthesis of a mixture of the syn and anti compounds was conducted (Scheme 1).

Scheme 1[a]



[a] Key: (a) THF, from 0 to 25 °C; (b) 3 equiv of $Br^-Ph_3P^+CH_2(CH_2)_6CO_2H$, THF, 0 °C, LiHMDS, 30 min; (c) Wittig, from 0 °C to rt, 4 h. (d) H_2, Pd/C

Diastereomeric 2,4-dimethylhexanal (**3**) was produced by reaction of methacrolein with *s*-Bu_3B.[10] The product was purified by distillation to provide a 55:45 ratio of aldehyde diastereomers. The major isomer was determined to be syn on the basis of comparison to literature ^{13}C NMR data for the known syn isomer.[11] The mixture was subjected to a Wittig reaction with (7-carboxyheptyl)triphenylphosphonium bromide. The resultant olefin was hydrogenated to provide **4** as a 54:46 ratio of diastereomers. The ratio was determined by quantitative ^{13}C NMR measurements in which 9 of the 16 carbon resonances of the diastereomers are distinguishable. The major isomer (presumed to be syn on the basis of the major aldehyde isomer being carried forward in a similar ratio) matched the ^{13}C NMR spectrum of the side chain acid

(4) (a) Schwartz, R. E.; Sesin, D. F.; Joshua, H.; Wilson, K. E.; Kempf, A. J.; Goklen, K. A.; Kuehner, D.; Gailliot, P.; Gleason, C.; White, R.; Inamine, E.; Bills, G.; Salmon, P.; Zitano, L. *J. Antiobiotics* **1992**, *45*, 1853−1866. (b) Bills, G. F.; Platas, G.; Pelaez, F.; Masurekar, P. *Mycol. Res.* **1999**, *103*, 179−192.

(5) (a) Bouffard, F. A.; Zambias, R. A.; Dropinski, J. F.; Balkovec, J. M.; Hammond, M. L.; Abruzzo, G. K.; Bartizal, K. F.; Marrinan, J. A.; Kurtz, M. B.; McFadden, D. C.; Nollstadt, K. H.; Powles, M. A.; Schmatz, D. M. *J. Med. Chem.* **1994**, *37*, 222−225. (b) Abruzzo, G. K.; Flattery, A. M.; Gill, C. J.; Kong, L.; Smith, J. G.; Krupa, D.; Pikounis, V. B.; Kropp, H.; Bartizal, K. *Antimicrob. Agents Chemother.* **1995**, *39*, 1077−1081. (c) Bouffard, F. A.; Dropinski, J. F.; Balkovec, J. M.; Black, R. M.; Hammond, M. L.; Nollstadt, K. H.; Dreikorn, S. L-743,872, A Novel Antifungal Lipopeptide: Synthesis and Structure−Activity Relationships of New Aza-Substituted Pneumocandins. *Abstracts*, 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, 1996; American Society for Microbiology: Washington, DC, 1997; F27.

(6) Formerly known as MK-0991 and L-743,872.

(7) (a) Villanueva, A.; Arathoon, E. G.; Gotuzzo, E.; Berman, R. S.; DiNubile, M. J.; Sable, C. A. *Clin. Infect. Dis.* **2001**, *33*, 1529−1535. (b) Arathoon, E. G.; Gotuzzo, E.; Noriega, L. M.; Berman, R. S.; DiNubile, M. J.; Sable, C. A. *Antimicrob. Agents Chemother.* **2002**, *46*, 451−457. (c) Maertens, J.; Raad, I.; Sable, C. A.; Ngai, A.; Berman, R.; Patterson, T. F.; Denning, D.; Walsh, T. Multicenter, Noncomparative Study to Evaluate Safety and Efficacy of Caspofungin in Adults with Invasive Aspergillosis Refractory or Intolerant to Amphotericin B (AMB), AMB Lipid Formulations, or Azoles. *Abstracts*, 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, 2000; American Society for Microbiology: Washington, DC, 2000; J1103.

(8) (a) Hensens, O. D.; Liesch, J. M.; Zink, D. L.; Smith, J. L.; Wichmann, C. F.; Schwartz, R. E. *J. Antibiotics* **1992**, *45*, 1875−1885. (b) Schwartz, R. E., Masurekar, P. S.; White, R. F. In *Cutaneous Antifungal Agents*; Rippon, J. W., Fromtling, R. A., Eds.; Marcel Dekker: New York, 1993; pp 375−393.

(9) (a) Roush, D. J.; Antia, F. D.; Goklen, K. E. *J. Chromatogr. A* **1998**, *827*, 373−389. (b) Conners, N.; Petersen, L.; Hughes, R.; Saini, K.; Olewinski, R.; Salmon, P. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 814−818 and references therein.

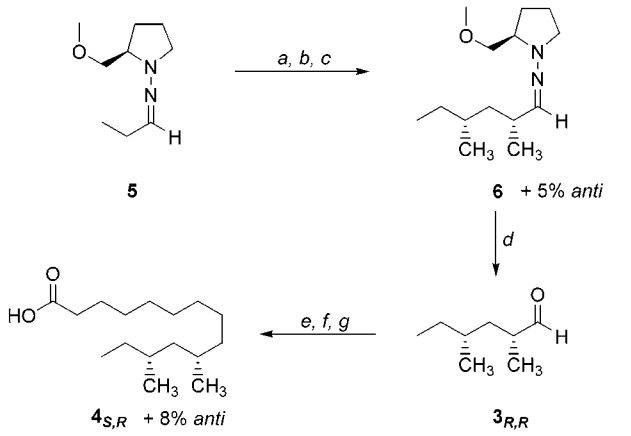
(10) Brown, H. C.; Kabalka, G. W.; Rathke, M. W.; Rogic, M. M. *J. Am. Chem. Soc.* **1968**, *90*, 4165−4166.

(11) White, J. D.; Johnson, A. T. *J. Org. Chem.* **1994**, *59*, 3347−3358.

produced by hydrolysis of **1**. Thus, this relative stereochemical assignment, while equivocal, assisted in the selection of the initial asymmetric route and was corroborated during the enantioselective synthesis of the syn isomer.

With the tentative syn assignment, an enantioselective synthesis of the (10*S*,12*R*)-acid enantiomer ($\mathbf{4}_{S,R}$) was conducted using Enders' chiral hydrazone chemistry[12] to determine the absolute stereochemistry (Scheme 2). Condensation

Scheme 2[a]

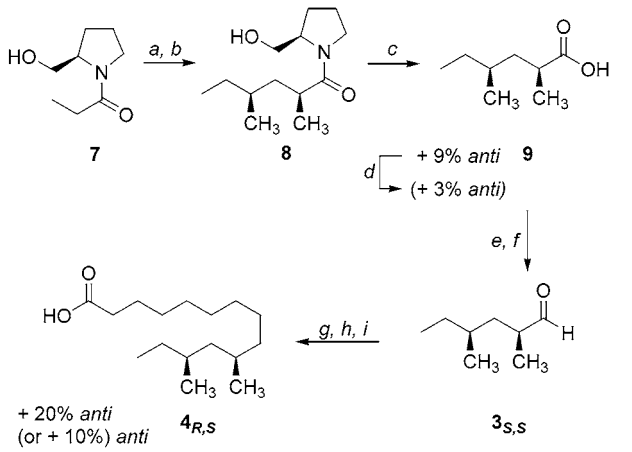


[a] Key: (a) LDA, THF, 0 °C, 7 h; (b) *n*-BuLi, −25 °C, 1 h; (c) 1-iodo-(2*R*)-methylbutane, from −95 to 0 °C, 10 h; (d) O_3, MTBE, −78 °C, 10 min, then 3 Å mol sieves, rt; (e) 5 equiv of $Br^-Ph_3P^+CH_2(CH_2)_6CO_2H$, THF, 0 °C, LiHMDS, 30 min; (f) Wittig, from 0 °C to rt, 5 h; (g) H_2, Pt/C, *i*-PrOAc, rt.

of RAMP with propionaldehyde gave the hydrazone **5** in 96% purified yield.[13] The hydrazone was alkylated with optically pure 1-iodo-(2*R*)-methylbutane[11] to give **6** in 46% yield as a 95:5 (syn/anti) diastereomeric ratio.[14] The alkylated hydrazone was ozonolyzed, and the resultant aldehyde $\mathbf{3}_{R,R}$ was immediately subjected to the Wittig reaction to provide the olefin in 32% purified yield.[15] Hydrogenation of the olefin gave the saturated acid $\mathbf{4}_{S,R}$ in 93% yield as a 92:8 (syn:anti) mixture of diastereomers. The ^{13}C NMR analysis of the major syn (10*S*,12*R*)-isomer matched that of the natural hydrolyzed acid. The specific rotation of the synthetic side chain sample, $[\alpha]^{25}_{405}$ −18.0° (*c* 0.010, $CHCl_3$), was comparable in magnitude to the natural side chain but opposite in sign, indicating the synthesis of the antipode.

A stereoselective synthesis of the other enantiomer was also conducted using a different and complementary asymmetric route to provide additional support for the absolute configuration (Scheme 3). The (2*S*,4*S*)-dimethylhexanal intermediate was prepared according to the method of White and Johnson as follows.[11] (*R*)-Propanoyl-2-pyrrolidinemetha-

Scheme 3[a]



[a] Key: (a) LDA, THF, 5 °C; (b) HMPA, −50 °C, 1-iodo-(2*S*)-methyl-butane; (c) 1 M HCl, reflux; (d) 1 equiv of cinchonidine, 1:1 v/v H_2O/acetone, from 70 °C to rt, two times; (e) Et_2O/THF, $LiAlH_4$, 5 °C; (f) CH_2Cl_2, Swern, from −70 to 0 °C; (g) 3 equiv $Br^-Ph_3P^+CH_2(CH_2)_6CO_2H$, THF, 0 °C, LiHMDS, 30 min; (h) Wittig, from 0 °C to rt, 4 h; (i) H_2, Pd/C.

nol (**7**) was alkylated with commercially available, optically pure 1-iodo-(2*S*)-methylbutane[1616] and hydrolyzed to yield the acid **9**. The acid was reduced to the alcohol and oxidized to give the aldehyde $\mathbf{3}_{S,S}$ as an 83:17 (syn/anti) mixture. Wittig reaction, followed by hydrogenation, gave the side chain $\mathbf{4}_{R,S}$ in 61% yield with an 80:20 (syn/anti) ratio. The specific rotation was determined to be $[\alpha]^{25}_{405}$ +17.1° (*c* 0.010, $CHCl_3$).

A sample of the $\mathbf{4}_{R,S}$ side chain with an improved diastereomeric ratio was produced by purifying a sample of acid **9** having a 91:9 (syn/anti) ratio to >97:3 by twice crystallizing its cinchonidine salt from acetone/water. The resulting acid was carried through the remainder of the synthesis to provide $\mathbf{4}_{R,S}$ as a 90:10 (syn/anti) ratio of diastereomers having a specific rotation of $[\alpha]^{25}_{405}$ +17.0° (*c* 0.010, $CHCl_3$). The small difference in the specific rotations of the samples containing 90:10 vs 80:20 (syn/anti) ratios of the diastereomers indicates that the anti impurity does not significantly contribute to the measured specific rotation.[17]

In summary, the pneumocandin B_0 side chain has been determined to have the syn methyl relative configuration and the absolute stereochemistry of (10*R*,12*S*)-dimethylmyristoyl. This conclusion was based on enantioselective syntheses of the pneumocandin B_0 side chain acid and its antipode and the comparison of the synthetic products' NMR spectra and optical rotation with that of the acid derived from the natural product.

(12) Enders, D. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: Orlando, 1984; Vol. 3, pp 275−339.

(13) Enders, D.; Rendenbach, B. E. M. *Tetrahedron* **1986**, *42*, 2235−2242.

(14) Nicolaou, K. C.; Nadin, A.; Leresche, J. E.; La Greca, S.; Tsuri, T.; Yue, E. W.; Yang, Z. *Angew. Chem., Int Ed. Engl.* **1994**, *33*, 2184−2187.

(15) Yields of reactions were not optimized.

(16) Aldrich Chemical Co., Milwaukee, WI.

(17) As a further indication that the anti diastereomer impurity does not confound the absolute stereochemical assignment, the specific rotation (*c* 0.010, $CHCl_3$) was measured at a variety of wavelengths for the natural side chain acid and the 80:20 syn/anti mixture: (wavelength, $[\alpha]^{23}$ natural side chain, $[\alpha]^{23}$ 80:20 syn/anti) 365, 21.3°, 22.9°; 436, 13.9°, 14.7°; 546, 8.1°, 8.7°; 578, 7.1°, 7.5°; 589, 6.9°, 7.4°.

Supporting Information Available: Experimental procedures and spectral data for $\mathbf{4}_{R,S}$, $\mathbf{4}_{S,R}$, and **6**, the degradation of **1**, and isolation of the natural side chain ester and acid, as well as a table of ^{13}C NMR assignments for the natural side chain acid and synthetic $\mathbf{4}_{R,S}$ (syn) and $\mathbf{4}_{S,S}$ (anti) side chain acids. This material is available free of charge via the Internet at http://pubs.acs.org.

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