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Total Synthesis and Conformational Analysis of the Antifungal Agent (−)-PF1163B

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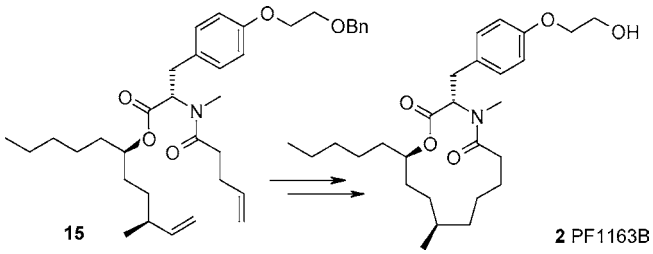
ABSTRACT



(−)-PF1163B, a new macrocyclic antifungal antibiotic isolated from *Streptomyces* sp., has been prepared in eight steps from (*S*)-citronellene. The key step is a ring-closing metathesis reaction of an ester and amide derivative obtained from a substituted *N*-methyl-L-tyrosine.

The ergosterol biosynthesis cascade is a known target of antifungal agents such as allylamines, azoles, and morpholines. Recently, two new compounds, PF1163A **1** and PF1163B **2** (Figure 1), were isolated from a fermentation broth of *Penicillium* sp.[1,2] and subsequently shown to be the first known inhibitors of ERG25p, a C-4 methyl oxidase.[3] ERG25p is the enzyme that converts 4,4-dimethylzymosterol to zymosterol by oxidation of the 4α-methyl group to a carboxylic acid followed by decarboxylation. The antifungal activity of **1** is 4 times higher than that of **2**, the former being a more potent inhibitor than fluconazole for ergosterol synthesis in *Candida albicans*. Thus, these compounds are interesting leads for the development of new antifungal agents.

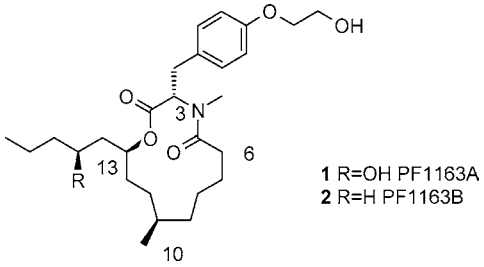


Figure 1.

They are characterized by the presence of a 13-membered macrocycle (incorporating both lactone and lactam moieties) derived from *O*-2-hydroxyethyl-*N*-methyl-L-tyrosine. Although several bioactive cyclic depsipeptides incorporating *N*-methyl tyrosine such as geodiamolides have been reported,[4] the recently isolated cytotoxic spongidepsin is the

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(1) Nose, H.; Seki, A.; Yaguchi, T.; Hosoya, A.; Sasaki, T.; Hoshoko, S.; Shomura, T. *J. Antibiot.* **2000**, *53*, 33.
(2) Sasaki, T.; Nose, H.; Hosoya, A.; Yoshida, S.; Kawaguchi, M.; Watanabe, T.; Usui, T.; Ohtsuka, Y. Shomura, T.; Takano, S.; Tatsuta, K. *J. Antibiot.* **2000**, *53*, 38.
(3) Nose, H.; Fushumi, H.; Seki, A.; Watabe, H.; Hoshiko, S. *J. Antibiot.* **2002**, *55*, 969.

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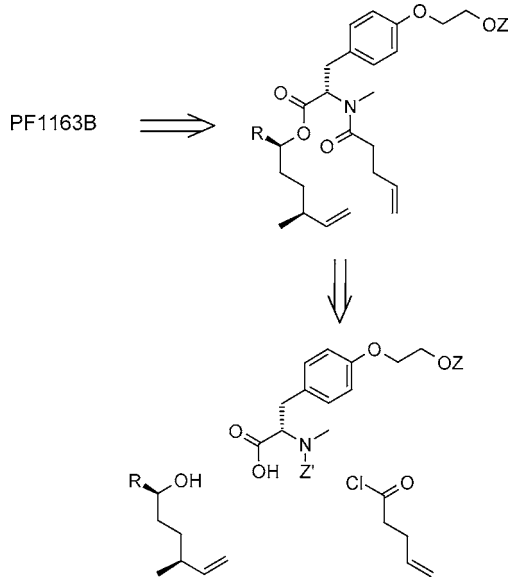
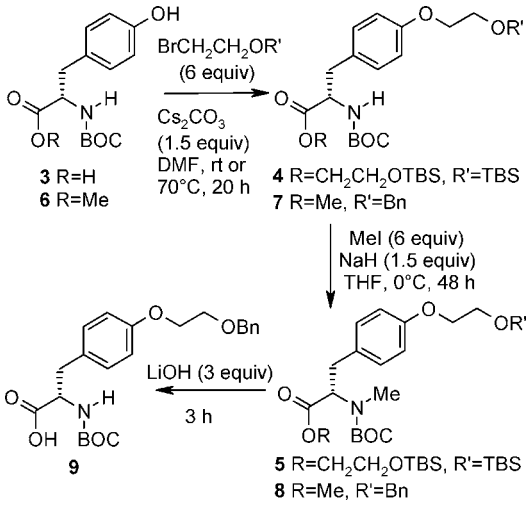
only known 13-membered macrocycle bearing similar lactone and lactam functional groups.[5] PF1163A differs from PF1163B by the presence of an extra hydroxyl group in the side chain. Their structures have been determined by NMR, degradation studies,[2] and total synthesis.[6] The latter has been carried out in 13 steps from (*R*)-citronellol and L-tyrosine. We now report a shorter and more flexible synthesis of (−)-PF1163B from (*S*)-citronellene using ring-closing metathesis (RCM) as a key step (Scheme 1).

Scheme 1. Retrosynthetic Analysis



We first attempted to prepare the known synthon **4** according to the procedure of Tatsuta[6] from *N*-Boc-L-tyrosine **3**. However, N-methylation of **4** to **5** using NaH and MeI

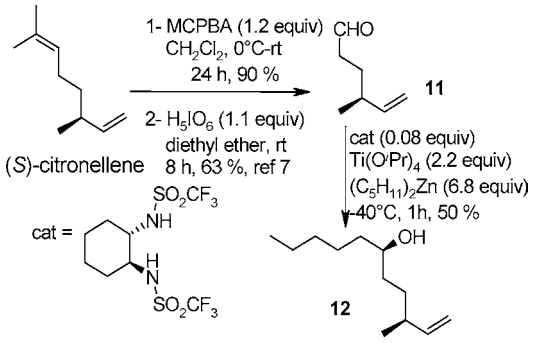
Scheme 2. Preparation of Synthon **9**



was difficult to drive to completion and led to partial cleavage of the silyl protecting group. To avoid the latter difficulty, a benzyl protecting group was used. Treatment of *N*-Boc-L-tyrosine methyl ester **6** with 2-bromo-*O*-benzyl-ethanol and Cs_2CO_3 in DMF at 70 °C[6] thereafter afforded **7** (85%), but chiral-phase HPLC analysis[7] indicated that racemization (60:40 ratio) was significant under these conditions. Indeed, when the same reaction was done at room temperature, **7** was isolated in 70% yield with minimal racemization (97:3 ratio). N-Methylation of **7** cleanly afforded **8** (95%). Hydrolysis of the methyl ester to give **9** was then carried out with LiOH according to the procedure used by Boger.[8]

Alcohol **12** was prepared in three steps from (*S*)-citronellene (Scheme 3).

Scheme 3. Preparation of **12**[a]



[a] Alcohol **12** was prepared in three steps from (*S*)-citronellene (Scheme 3).

The known aldehyde **11** was obtained by cleavage of the trisubstituted epoxide of citronellene with periodic acid in diethyl ether.[9] The dried ethereal solution of **11** was directly treated with dipentylzinc under the conditions of Kobayashi and Knochel[10] to give alcohol **12** (Scheme 4) in 50% yield (ee 98%).[11] Esterification of **9** with alcohol **12** was carried out with DCC in DMF in the presence of DMAP to afford **13** (70%). Removal of the *tert*-butyloxycarbonyl protecting group (TFA, CH_2Cl_2) provided amine **14**, which was then reacted with 4-pentenoyl chloride (THF, Et_3N) to afford amide **15** (94% over two steps). At this stage, chiral-phase HPLC showed a 92:8 ratio of enantiomers, indicating that some epimerization occurred during the deprotection, esterification, and/or amide formation steps.

Then, RCM was attempted using variable amounts of $(PCy_3)_2Cl_2Ru{=}CHPh$, but only limited cyclization was

(4) Chan, W. R., Tinto, W. F.; Manchand, P. S.; Todaro, L. J. *J. Org. Chem.* **1987**, *52*, 3091. Coleman, J. E.; Van Soest, R.; Andersen, R. J. *J. Nat. Prod.* **1999**, *62*, 1137.

(5) Grassia, A.; Bruno, I.; Debitus, C.; Marzocco, S.; Pinto, A.; Gomez-Paloma, L.; Riccio, R. *Tetrahedron* **2001**, *57*, 6257.

(6) Tatsuta, K.; Takano, S.; Ikeda, Y.; Nakano, S.; Miyazaki, S. *J. Antibiot.* **1999**, *52*, 1146.

(7) HPLC conditions: Chiralcel OD-H column (100 × 4.6 mm), eluent 2-propanol/hexane (10:90), 0.4 mL/min, UV detection at 276 nm, retention times 7.2 min (D-**7**) and 7.9 min (L-**7**).

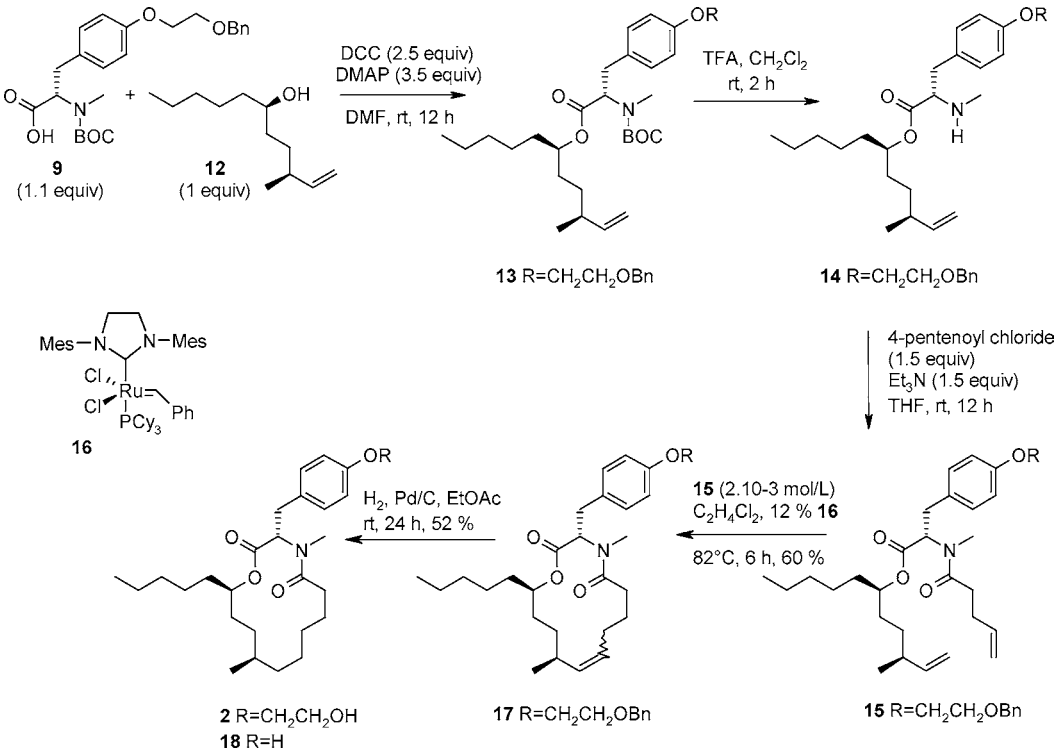
(8) Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1988**, *53*, 487.

(9) Ireland, R. E.; Thaisrivongs, S.; Dussault, P. H. *J. Am. Chem. Soc.* **1988**, *110*, 5768.

(10) (a) Takahashi, H.; Kawakita, T.; Ohno, M.; Yoshioka, M.; Kobayashi, S. *Tetrahedron*, **1992**, *48*, 5691. (b) Knochel, P. *Synlett* **1995**, 393.

(11) For a related example, see: Fürstner, A.; Müller, T. *J. Am. Chem. Soc.* **1999**, *121*, 7814.

Scheme 4. Synthesis of PF1163B



observed.[12] As expected,[13] the use of Grubbs II catalyst **16** led to better results. RCM of **15**, in refluxing $C_2H_4Cl_2$, gave **17** as a mixture of (*E*)- and (*Z*)- diastereoisomers (60%). A minor isomer, which was assumed to result from the aforementioned epimerization of the amino acid moiety, was also isolated (10.5%). Reduction of the double bond and hydrogenolysis of the benzyl group gave PF1163B **2** as a colorless oil in 52% isolated yield ($[\alpha]_D$ −105 (*c* 1.2, MeOH) (lit.[2] $[\alpha]_D$ −111)).

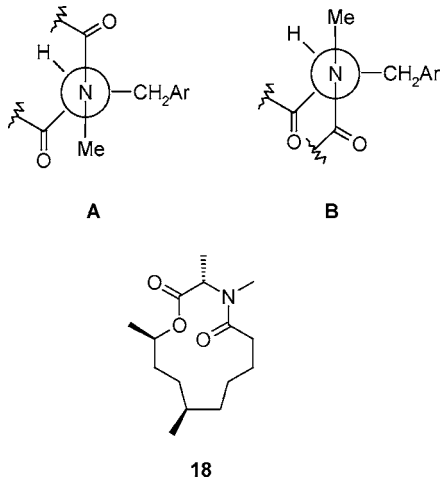
The ^{1}H NMR spectrum of synthetic **2** in DMSO-d_6 is superimposable to the reported one. However, the H-3 signal at 5.62 ppm accounts for only 0.5 H.[14] Furthermore, as reported,[2] broad signals were observed, and this was assumed by the authors to result from *s*-cis and *s*-trans configurations of the amide bond. Indeed, careful examination of ^{1}H NMR spectra in $CDCl_3$ revealed that three isomers are present in solution: H-3 appeared as broad singlets at δ 5.74 (0.5 H) and 3.38 ppm (0.4 H) and as a triplet (J = 7 Hz) at δ 4.51 ppm (0.1 H) (Table 1). In DMSO-d_6, only the lower-field signal (δ 5.62 ppm) could be identified. It was thus obvious that *s*-cis/*s*-trans isomerization of the amide bond was not sufficient to explain such data.

Table 1. Energy and ^{1}H NMR Calculations

conformer	E_{rel} kcal/mol	δ_{calcd} ppm	δ_{obs} ppm	percentage
A_{trans}	0	5.47	5.74	50%
B_{trans}	3.6	2.72	3.38	40%
A_{cis}	4.2	4.14	4.51	10%
B_{cis}	6.2	3.17		0%

Assuming that conformers A and B may be sufficiently stable to be observed on the NMR time scale (Scheme 5),

Scheme 5



energy minima were searched for the four possible structures of **18** (bearing two methyl groups instead of the pentyl and benzyl side chains), at the density functional B3-LYP/6-31G/

(12) For related examples of RCM of peptide derivatives, see: Boruah, A.; Rao, I. N.; Nandy, J. P.; Kumar, S. K.; Kunwar, A. C.; Iqbal, J. *J. Org. Chem.* **2003**, *68*, 5006 and references therein.

(13) (a) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413. (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, 34.

(14) This was not pointed out (see refs 2 and 6).

d3 level using the Gaussian 98 package.[15] It was found that the more stable conformer (A_{trans}) is similar to the reported X-ray structure for **17**, a degradation product of PF1163B.[2] Similar values were obtained for the B_{trans} and A_{cis} conformers, the B_{cis} isomer being slightly less stable.

^{1}H NMR chemical shift calculations[16] revealed significant variations for H-3 (Table 1). On the basis of these figures, it is now proposed that the conformational population of PF1163B in $CDCl_3$ is 50% A_{trans}, 40% B_{trans}, and 10% A_{cis}. To estimate the possibilities of interconversion between these conformers, transition structures were searched, and two of them were found. The first one corresponds to an $A_{trans}-A_{cis}$ isomerization and the second to a $B_{trans}-A_{cis}$ isomerization. These two structures are, respectively, 20.7 and 23.3 kcal/mol above the lowest A_{trans} conformer. Thus, these interconversions are likely to be slow processes at room temperature. Finally, variable-temperature experiments were carried out. The NMR spectrum remained unchanged in $CDCl_3$ up to 50 °C, while the 5.62 ppm signal disappeared at 70−80 °C in DMSO-d_6 in favor of a new signal at ca. 4.6 ppm (in agreement with an averaged signal for the three conformers). This result is in agreement with signal coalescence reported for lauryllactam at 60 °C.[17]

In conclusion, a short synthesis of PF1163B has been completed using RCM as the key step. Such a strategy should be extendable to the preparation of a large set of analogues, including PF1163A, bearing different side chains and ring size. Furthermore, the interesting conformational bias pointed out for such macrocycles should be further studied to pinpoint the geometrical requirements for the biological activity of such compounds.

Acknowledgment. We thank MRT, CNRS, and Région Poitou-Charentes for financial support and F. Seguin (ERM 324) for 500 MHz spectra.

Supporting Information Available: Copies of proton spectra of compounds **7**−**9**, **11**−**15**, and synthetic PF1163B and HPLC of compounds **6**, **12**, **15**, and PF1163B. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

(16) (a) To obtain reliable proton chemical shifts, we have used the GIAO method available in *Gaussian 98*. These chemical shifts were calculated at the Hartree−Fock level using the 6-311+G(2d,p) extended basis set on the B3LYP/6-31G(d) optimized structures, as recommended by Cheeseman et al.[16b] (b) Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, M. J., *J. Chem. Phys.* **1996**, *104*, 5497.

(17) Moriarty, R. M. *J. Org. Chem.* **1964**, *29*, 2748.