Total Synthesis and Structural Elucidation of Khafrefungin

Takeshi Wakabayashi, Kouhei Mori, and Shū Kobayashi*

Contribution from the Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Received October 25, 2000

Abstract: Total synthesis and structural elucidation of khafrefungin, a novel antifungal agent isolated from the fermentation culture MF6020, have been achieved. Unlike other inhibitors that inhibit the corresponding enzyme in fungi and mammals to the same extent, khafrefungin does not impair sphingolipid synthesis of mammals. The basic strategy for the structural elucidation is to prepare all stereoisomers of the structurally simplified khafrefungin mimics **1** and **2** that were designed for the elucidation of C10,11,12 and C2′,3′,4′ relative stereochemistry, respectively. The comparison of their spectra with those of natural khafrefungin would result in the identification of eight possible stereoisomers, and the analytical details of these eight stereoisomers have led to the complete stereochemical assignment. On the basis of the structural elucidation, the total synthesis of khafrefungin has been accomplished by using tin(II)-catalyzed asymmetric aldol reactions as key steps.

Khafrefungin is a novel antifungal agent isolated from the fermentation culture MF6020 by a Merck group in 1997.[1] It has been shown to inhibit the biosynthesis of inositol phosphorylceramide (IPC) in *Sacchromyces cerevisiae* and pathogenic fungi such as *Candida albicans* and *Cryptococcus neoformans* in picomolar and nanomolar concentrations and causes ceramide accumulation. Unlike other inhibitors that inhibit the corresponding enzyme in fungi and mammals to the same extent, khafrefungin does not impair sphingolipid synthesis of mammals.[2] Khafrefungin is composed of C5 aldonic acid esterified at the C4 hydroxy group with C22 linear polyketide acid including four chiral centers, and its absolute and relative configuration until now had remained unknown due to both the limited availability from nature and the complexity of the chemical degradation. In this paper, we report the complete stereochemical assignment and the first asymmetric total synthesis of khafrefungin.

Our basic strategy is to prepare all stereoisomers of the structurally simplified khafrefungin mimics **1** and **2** that were designed for the elucidation of C10,11,12 and C2′,3′,4′ relative stereochemistry, respectively (Figure 1). The comparison of their spectra with those of natural khafrefungin would result in the identification of eight possible stereoisomers, so that the analytical details of these eight stereoisomers would lead to the complete stereochemical assignment as well as the total synthesis of khafrefungin.

The synthesis of **1** was performed according to Scheme 1. The catalytic asymmetric aldol reaction of decanal with the silyl enol ether derived from *S*-ethyl propanethioate (**3**) by using 0.2 equiv of a tin(II)−chiral diamine complex afforded the corresponding aldol adduct (**4**) in 83% yield with 94% ee.[3] The deoxygenation of the aldol adduct was performed by thiocarbonylation of the hydroxy group of **4** followed by treatment with tributyltin hydride in the presence of a catalytic amount of AIBN.[4] Reduction of the resulting thioester by lithium aluminum hydride (LAH) followed by Swern oxidation gave the key aldehyde (**5**). The aldol reaction of **5** with silyl enol ether **3** with 0.1 equiv of $Sc(OTf)_3$[5] proceeded smoothly in propionitrile to afford four stereoisomers of the adduct **6** in 92% yield (**6a**:**6b**:**6c**:**6d** = 22:38:9:31).[6] The easily separated **6d** was reduced by using lithium borohydride, and the secondary hydroxy group of the resulting diol **7d** was protected selectively as its *p*-methoxybenzyl (PMB) ether via hydride addition of the corresponding *p*-methoxybenzylidene acetal to give the primary alcohol, which was converted to the aldehyde **8d** by Swern oxidation. The Wittig reaction of **8d** with (carbethoxyethylidene)triphenylphosphorane afforded the corresponding ester stereoselectively (*E*/*Z* = >95/5), which was reduced with

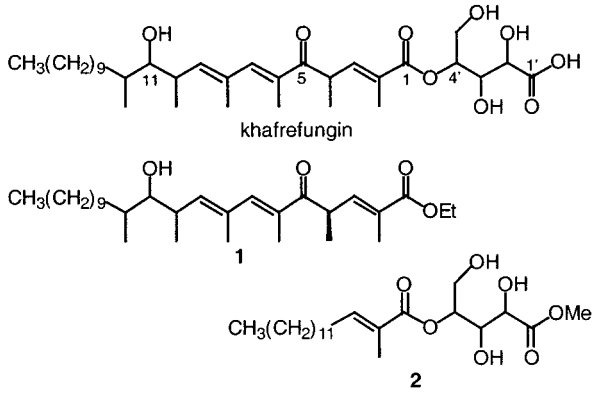


Figure 1. Khafrefungin and its mimics **1** and **2**.

(1) Mandala, S. M.; Thornton, R. A.; Rosenbach, M.; Milligan, J.; Garcia-Calvo, M.; Bull, H. G.; Kurtz, M. B. *J. Biol. Chem.* **1997**, *272*, 32709.

(2) Reviews: (a) Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1532. See also: (b) Dickson, R. C. *Annu. Rev. Biochem.* **1998**, *67*, 27.

(3) (a) Kobayashi, S.; Uchiro, H.; Fujishita, Y.; Shiina, I.; Mukaiyama, T. *J. Am. Chem. Soc.* **1991**, *113*, 4247. (b) Kobayashi, S.; Uchiro, H.; Shiina, I.; Mukaiyama, T. *Tetrahedron* **1993**, *49*, 1761. (c) Kobayashi, S.; Kawasuji, T.; Mori, N. *Chem. Lett.* **1994**, 217. (d) Kobayashi, S.; Horibe, M. *Chem. Eur. J.* **1997**, *3*, 1472. This reaction was already utilized in our total synthesis[20] of sphingofungin B and F that was shown to inhibit serine palmitoyltransferase (SPT) in the biosynthesis of sphingolipid.[21]

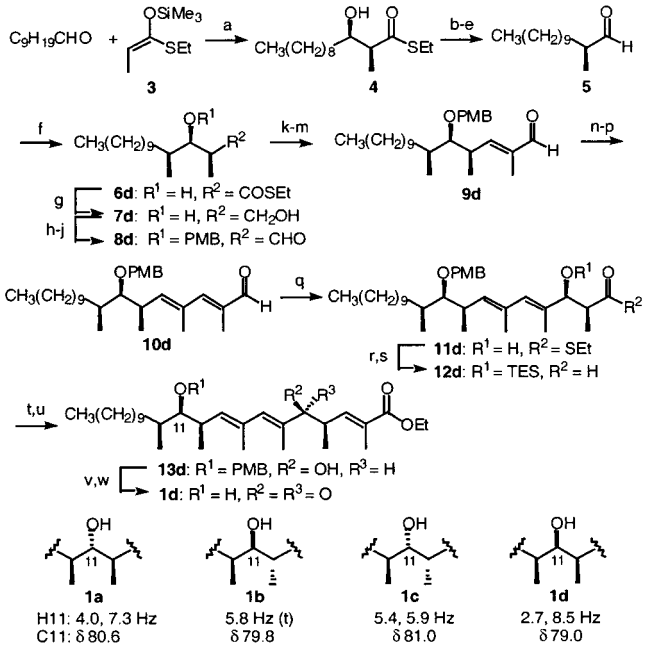
(4) (a) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574. (b) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1983**, *105*, 4059.

(5) (a) Kobayashi, S.; Hachiya, I.; Ishitani, H.; Araki, M. *Synlett* 1993, 472. (b) Kobayashi, S. *Eur. J. Org. Chem.* **1999**, 15.

(6) For stereochemical assignment of **6a**−**d**, see Supporting Information.

10.1021/ja0057272 CCC: $20.00
Published on Web 01/27/2001

Scheme 1. Synthesis of **1d**



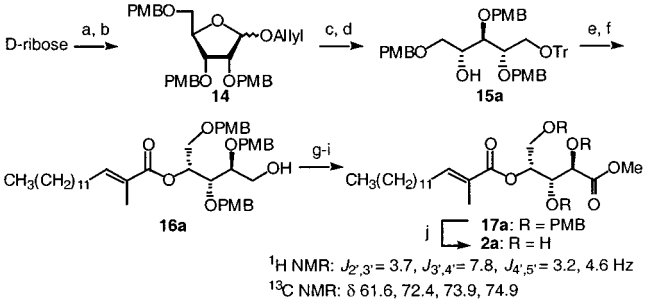
Reagents and conditions: (a) 0.2 equiv. of $Sn(OTf)_2$, 0.2 equiv. of SnO, 0.24 equiv. of (*S*)-1-methyl-2-[(*N*-1-naphthylamino)-methyl]pyrrolidine, CH_2Cl_2, -78 °C, 83%, *syn*/*anti* = 97/3, 94% ee (*syn*); (b) PhOCSCl, pyridine, $ClCH_2CH_2Cl$, reflux; (c) Bu_3SnH, AIBN, toluene, 110 °C; (d) $LiAlH_4$, THF, 0 °C, 86% for 3 steps; (e) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C; then Et_3N, rt, 91%; (f) **3**, 0.1 equiv. of $Sc(OTf)_3$, EtCN, -45 °C, 92%, **6a**:**6b**:**6c**:**6d** = 22:38:9:31; (g) $LiBH_4$, THF, -5 °C, 93%; (h) $PMPCH(OMe)_2$, cat. TsOH, CH_2Cl_2, rt; (i) DIBAL, CH_2Cl_2, rt, 86% for 2 steps, (j) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C; then Et_3N, rt, 92%; (k) $Ph_3P{=}C(Me)CO_2Et$, THF, reflux, 89%, *E*/*Z* = >95/5; (l) DIBAL, CH_2Cl_2, -78 °C, 96%; (m) 0.1 equiv. of TPAP, NMO, CH_2Cl_2, rt, 90%; (n) $Ph_3P{=}C(Me)CO_2Et$, THF, reflux, 83%, *E*/*Z* = >95/5; (o) DIBAL, CH_2Cl_2, -78 °C, 87%; (p) 0.1 equiv. of TPAP, NMO, CH_2Cl_2, rt, 93%; (q) **3**, $Sn(OTf)_2$, $Bu_2Sn(OAc)_2$, (*S*)-1-methyl-2-[(*N*-1-naphthylamino)-methyl]pyrrolidine, CH_2Cl_2, -78 °C, 90%, >98% ds; (r) TESOTf, 2,6-lutidine, CH_2Cl_2, rt, 92%; (s) DIBAL, CH_2Cl_2, -78 °C, 98%; (t) $Ph_3P{=}C(Me)CO_2Et$, THF, reflux, 86%, *E*/*Z* = >95/5; (u) 1 M HCl/EtOH (1/2), rt, 94%; (v) 0.1 equiv. of TPAP, NMO, CH_2Cl_2, rt, 77%; (w) DDQ, H_2O/CH_2Cl_2 (1/20), 0 °C, 88%.

diisobutylaluminum hydride (DIBAL). The resulting alcohol was oxidized with a catalytic amount of TPAP in the presence of NMO[7] to give aldehyde **9d**. A similar transformation from aldehyde **9d** to dienal **10d** was performed. The asymmetric aldol reaction of dienal **10d** with silyl enol ether **3** by using a tin(II)−chiral diamine complex proceeded smoothly to afford the corresponding aldol adduct (**11d**) in 90% yield with >98% ds.[3,8] After the hydroxy group of **11d** was protected as its triethylsilyl (TES) ether, reduction of the thioester group with DIBAL gave aldehyde **12d**. A propionate unit was installed to the aldehyde **12d** by using Wittig reaction to give the corresponding ester stereoslectively (*E*/*Z* = >95/5), which was treated with aqueous HCl in ethanol to afford secondary alcohol **13d**. Finally, TPAP-catalyzed oxidation[7] of **13d** followed by deprotection of the PMB group with DDQ furnished **1d**.

Three other stereoisomers, **1a**, **1b**, and **1c**, were prepared from the corresponding aldol adducts **6a**, **6b**, and **6c**, respectively, in the same way. According to the NMR spectra of the four synthesized compounds and natural khafrefungin (H11: 5.7 Hz (t); C11: δ 79.8), it is reasonable to assume that natural khafrefungin would bear 10,11-anti and 11,12-syn stereochemical relationships.

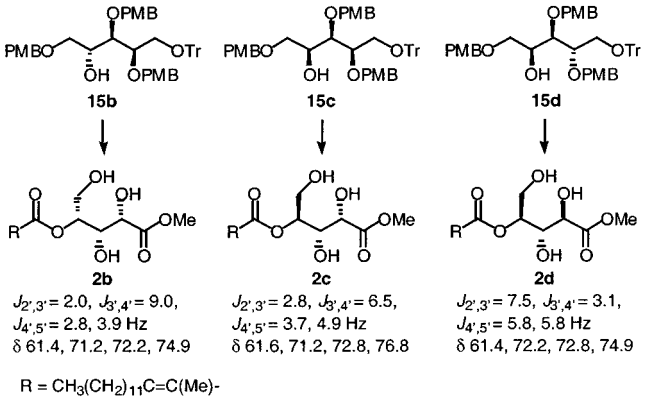
Our attempt to elucidate the C2′−C4′ relative stereochemistry of khafrefungin was initiated by preparation of ribonic acid derivative **2a** (Scheme 2). The anomeric hydroxy group of D-ribose was protected as its allyl ether, and the three remaining hydroxy groups were subsequently protected as their PMB ethers to give compound **14**. Deprotection of the allyl group of **14** and successive reduction of the resulting hemiacetal were carried out in the same pot with DIBAL in the presence of $NiCl_2$(dppp)[9] to afford the corresponding diol, which was protected selectively as its triphenylmethyl (Tr) ether to give secondary alcohol **15a**. DCC-mediated esterification of **15a** with *trans*-2-methyl-2-pentadecenoic acid followed by deprotection of the Tr group afforded alcohol **16a**. TPAP-catalyzed oxidation[7] followed by treatment with sodium chlorite gave the corresponding carboxylic acid. After the carboxylic acid was converted to methyl ester **17a**, deprotection of the three PMB groups of **17a** was successfully performed with BBr_3 to afford **2a**.

Scheme 2. Synthesis of **2a**



Reagents and conditions: (a) allyl alcohol, cat H_2SO_4, 4 °C; (b) PMBCl, Bu_4NBr, 50% aq. KOH/THF (1/1), 90 °C, 82% for 2 steps; (c) DIBAL, 0.01 equiv. of $NiCl_2$(dppp), THF, rt, 87%; (d) TrCl, Et_3N, CH_2Cl_2, rt, 94%; (e) *trans*-2-methyl-2-pentenoic acid, DCC, DMAP, CH_2Cl_2, rt, 84%; (f) HCO_2H, Et_2O, rt, 69%; (g) 0.1 equiv. of TPAP, NMO, CH_2Cl_2, rt,; (h) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, H_2O/*t*-BuOH (1/1), rt; (i) Me_3SiCHN_2, THF/MeOH (1/1), rt, 35% for 3 steps; (j) BBr_3, CH_2Cl_2, -78 °C, 57%.

Scheme 3. Spectral Data of **2b**, **2c**, and **2d**



Arabinonic acid derivative **2b** was also prepared from alcohol **15b** derived from D-arabinose in the same way. On the other hand, xylonic acid derivative **2c** and lyxonic acid derivative **2d** were prepared from alcohols **15c** and **15d**, respectively, which were derived via Mitsunobu reaction[10] of alcohol **15b** and **15a**, respectively. According to ^1H and ^{13}C NMR analyses of the four synthesized compounds **2a**, **2b**, **2c**, **2d**, and natural khafrefungin (^1H NMR: $J_{2'3'} = 1.9$, $J_{3'4'} = 9.1$, $J_{4'5'} = 2.8$, 4.2 Hz; ^{13}C NMR: δ 61.5, 71.3, 71.8, 75.5), the spectral data of **2b** were the most similar to those of khafrefungin, suggesting

(7) Griffith, W. P.; Ley, S. V. *Aldrichim. Acta* **1990**, *23*, 13.

(8) Stereochemical assignment of newly created asymmetric centers was made based on the chirality of the chiral ligand.

(9) Taniguchi, T.; Ogasawara, T. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1136.

(10) Review: Mitsunobu, O. *Synthesis* **1981**, 1.

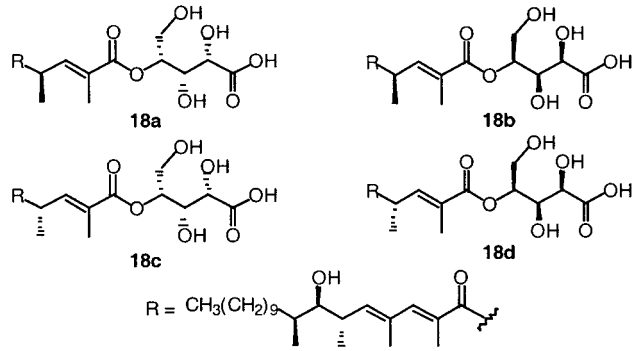
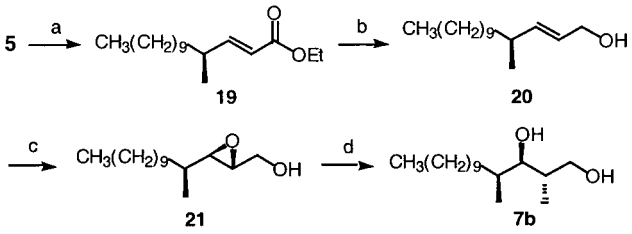


Figure 2. Possible stereoisomers of khafrefungin.

Scheme 4. Stereoselective Synthesis of **7b**



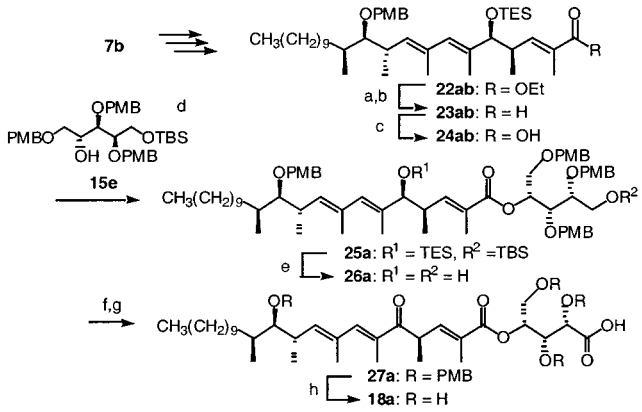
Reagents and conditions: (a) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, rt, 91%, *E*/*Z* = >95/5; (b) DIBAL, CH_2Cl_2, -78 °C, 96%; (c) TBHP, $Ti(OiPr)_4$, D-tartaric acid diethyl ester, CH_2Cl_2, -15 °C; (d) MeLi, CuI, Et_2O, 0 °C, 90% for 2 steps.

that khafrefungin would be an arabinonic acid derivative (Scheme 3).

At this stage, it was assumed that natural khafrefungin would bear 10,11-anti, 11,12-syn, 2′,3′-syn, and 3′,4′-syn stereochemical relationships, which led to the identification of eight possible stereoisomers of natural khafreungin (Figure 2). Our next task is to synthesize four of the eight stereoisomers. Comparison of physical data of these synthesized compounds with those of natural khafrefungin would reveal the real structure of khafrefungin.

Initially, we undertook the stereoselective synthesis of diol **7b** including three contiguous chiral centers. The $Sc(OTf)_3$-catalyzed aldol reaction shown in Scheme 1 proceeded in high yield,[5] albeit the desired stereochemical outcome that yielded the α,β-anti, β,γ-syn aldol adduct was low (38% ds). At this stage, it was necessary to develop a new method for highly stereoselective synthesis of diol **7b**. We attempted several types of aldol reactions. The $TiCl_4$-promoted system gave the desired aldol adduct in moderate stereoselectivity (56% ds). The selectivity was slightly improved by the reaction of the lithium enolate generated from *S*-ethyl propanethioate with aldehyde **5** (66% ds). These results encouraged us to examine some chiral ligands;[11] however, all chiral catalyst-controlled aldol reactions, including chiral tin(II)-catalyzed reactions,[12] failed for the preparation of the desired α,β-anti, β,γ-syn adduct. Thus, the Kishi protocol,[13] including Sharpless asymmetric epoxidation of a chiral allylic alcohol, was then employed instead of the aldol process for stereoselective synthesis of diol **7b** (Scheme 4). Wittig reaction of aldehyde **5** with (carbethoxymethylidene)-triphenylphosphorane gave the corresponding ester (**19**) stereoselectively (*E*/*Z* = >95/5), which was reduced by diisobutylaluminum hydride (DIBAL) to afford allylic alcohol **20** in high yield. Sharpless epoxidation[14] of **20** with D-tartaric acid diethyl ester followed by treatment of the resulting epoxide **21** with Me_2CuLi gave the desired diol (**7b**) in good yield with high stereoselectivity (>90% ds).

Scheme 5. Synthesis of **18a**



Reagents and conditions: (a) DIBAL, CH_2Cl_2, -78 °C, 96%; (b) 0.1 equiv. of TPAP, NMO, CH_2Cl_2, rt, 90%; (c) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, H_2O/*t*BuOH (1/1), rt, 83%; (d) DCC, DMAP, DMAP-HCl, CH_2Cl_2, reflux; (e) 1M HCl/THF (1/3.5), rt, 71% for 2 steps; (f) Dess-Martin Periodinane, pyridine, CH_2Cl_2, rt; (g) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, H_2O/*t*-BuOH (1/1), rt, 77% for 2 steps; (h) BCl_3, CH_2Cl_2, -78 °C, 57%.

We next undertook the synthesis of compound **18a** having a fully functionalized khafrefungin structure (Scheme 5). Diol **7b** was converted to ester **22ab** according to similar procedures in Scheme 1. Reduction of the ester **22ab** was conducted with DIBAL, and successive TPAP-catalyzed oxidation[7] gave aldehyde **23ab**, which was treated with sodium chlorite to provide carboxylic acid **24ab** in high yield. The DCC-mediated esterification reaction of 1 equiv of carboxylic acid **24ab** with a slight excess amount of alcohol **15e**, which was prepared from D-arabinose by a similar procedure shown in Scheme 2, in the presence of (dimethylamino)pyridine (DMAP) and DMAP·HCl (Keck's conditions)[15] proceeded smoothly to afford the corresponding ester **25a**. It was found that the addition of DMAP·HCl was essential to obtain high yield. Both TBS and TES groups of ester **25a** were removed by exposure to aqueous HCl in THF to give diol **26a** in 71% yield for two steps. Oxidation of diol **26a** was successfully performed by Dess−Martin periodinane[16] to afford the keto aldehyde without any epimerized product at the C4 position, which was treated with sodium chlorite to furnish keto carboxylic acid **27a** in 77% yield for two steps. Finally, four PMB groups were removed with BCl_3 to afford **18a** in 57% yield.

The other three stereoisomers **18b**, **18c**, and **18d** were also prepared via esterification reactions of the corresponding carboxylic acids with the corresponding alcohols in the same way. Among the four stereoisomers, the spectra of ^1H and ^{13}C NMR, Rf value of TLC, the retention time of RP HPLC, and the optical rotation of **18a** were completely consistent with those of natural khafrefungin (**18a**: $[\alpha]_D$ −27 (*c* 0.095, MeOH); natural: $[\alpha]_D$ −27 (*c* 0.29, MeOH).[17,18] In addition, among the

(11) Reviews: (a) Carreira, E. M. In *Comprehensive Asymmetric Catalysis*; Jacobsen, E. N., Pfaltz, A., Yamamoto, H., Eds.; Springer-Verlag: Berlin, 1999; p 997. (b) Mahrwald, R. *Chem. Rev.* **1999**, *99*, 1095.

(12) Kobayashi, S.; Ohtsubo, A.; Mukaiyama, T. *Chem. Lett.* **1991**, 831.

(13) Nagaoka, H.; Kishi, Y. *Tetrahedron* **1981**, *37*, 3873.

(14) Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974.

(15) Boden, E.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.

(16) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.

(17) While the optical rotation of natural khafrefungin is $[\alpha]_D$ −27 (*c* 0.29 MeOH), the optical rotations of all four synthesized compounds (**18a**−**d**) show negative values indicating that *ent* **18a**−**d** are not natural khafrefungin. **18b**: $[\alpha]_D$ −3.6; **18c**: $[\alpha]_D$ −6.1; **18d**: $[\alpha]_D$ −34 (MeOH).

(18) The concentration for the measurement of optical rotations of **18a**−**d** was determined by using the extinction coefficient at 286 nm (14300) based on the original procedure. See Supporting Information.

four stereoisomers, only **18a** has been shown to inhibit IPC synthase to almost the same extent as natural khafrefungin.[19] These analyses have revealed the stereochemistry, including the absolute configuration, of khafrefungin to be **18a**, and at the same time, total synthesis of khafrefungin has been completed.

In summary, complete stereochemical assignment and total synthesis of khafrefungin have been achieved by using highly stereocontrolled reactions. Several possible stereoisomers have been synthesized, and comparison of their physical data as well as biological activity with those of an authentic sample have revealed the true structure of khafrefungin. For the total synthesis, the tin(II)-catalyzed asymmetric aldol reaction has been shown to be a powerful synthetic tool for the construction of acyclic compounds containing several chiral centers. After completion of the total synthesis, we also found that khafrefungin exhibits a strict stereochemical requirement for antifungal activity, and biological details, including structure−activity relationships and structural modification, are now under investigation.

Acknowledgment. The authors are grateful to Dr. Guy Harris (Merck) for providing us with an authentic sample of khafrefungin, spectral data, and other valuable information. Drs. Kentaro Hanada and Satoshi Yasuda (National Institute of Infectious Diseases) are also acknowledged for measurement of the biological activity of khafrefungin. The authors also thank Mr. Hiroki Kobayashi for his technical support. This work was partially supported by CREST, Japan Science and Technology Corporation (JST), and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sport, and Culture, Japane. T.W. thanks the JSPS fellowship for Japanese Junior Scientists.

Supporting Information Available: Experimental details and ^{1}H and ^{13}C NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0057272

(19) Yasuda, S.; Hanada, K. Unpublished. Details will be reported in due course.

(20) (a) Kobayashi, S.; Furuta, T.; Hayashi, T.; Nishijima, M.; Hanada, K. *J. Am. Chem. Soc.* **1998**, *120*, 908. (b) Kobayashi, S.; Furuta, T. *Tetrahedron* **1998**, *54*, 10275.

(21) (a) VanMiddlesworth, F.; Gracobbe, R. A.; Lopez, M.; Garrity, G.; Bland, J. A.; Bartizal, K.; Fromtling, R. A.; Polishook, J.; Zweerink, M.; Edison, A. M.; Rozdilsky, W.; Wilson, K. E.; Monaghan, R. L. *J. Antibiot.* **1992**, *45*, 861. (b) Zweerink, M. M.; Edison, A. M.; Well, G. B.; Pinto, W.; Lester, R. L. *J. Biol. Chem.* **1992**, *267*, 25032.