Convergent Total Synthesis of Khafrefungin and Its Inhibitory Activity of Fungal Sphingolipid Syntheses

Shū Kobayashi,*,† Kouhei Mori,† Takeshi Wakabayashi,† Satoshi Yasuda,‡ and Kentaro Hanada‡

Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, and Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases (former National Institute of Health), Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

skobayas@mol.f.u-tokyo.ac.jp

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A convergent total synthesis of khafrefungin, a novel inhibitor of fungal sphingolipid syntheses isolated from the fermentation culture MF6020, has been developed. Alkenylboronic acid **5** and alkenyliodide **6**, key fragments for the total synthesis, were prepared from the corresponding achiral aldehydes using tin(II)-catalyzed and Zr(IV)-catalyzed asymmetric aldol reactions, respectively. The Suzuki coupling reaction of these two fragments was successfully performed to give **17** in good yield. Through the total synthesis, epimerization of the C4 position having a rather highly acidic proton did not occur, indicating that khafrefungin was under strict conformational constraints to prevent the epimerization process. This characteristic stability of khafrefungin has also been discussed using semiempirical calculation and synthesis. Finally, khafrefungin derivatives have also been synthesized, and their antifungal activities have been measured to obtain information on the structure−activity relationships.

Introduction

Sphingolipids are members of cellular membranes and are composed of a ceramide backbone combined with polar ends such as monosaccharides, oligosaccharides, or phosphocoline. Recently, these lipids have been shown to act as a second messenger for regulating signal transduction pathways and directly influence various cellular events such as proliferation, differentiation, and apoptosis; however, little is yet known about the precise functions of sphingolipids.[1]

The proposed pathway for the syntheses of sphingolipids is initiated by the condensation of L-serine with palmitoyl CoA catalyzed by serine palmitoyltransferase (SPT) to provide 3-ketosphinganine, which is converted to sphinganine with a reductase. These steps are observed in the syntheses of sphingolipids of both mammals and fungi. In mammals, the amino group of sphinganine is subsequently acylated by sphinganine *N*-acyltransferase to give dihydroceramide. After dihydroceramide is converted to ceramide, various saccharides or phosphocoline is added to the resulting ceramide to provide the corresponding glycosphingolipids or sphingomyelin, respectively. In fungi, on the other hand, a hydroxyl group is introduced at the C4 position of sphinganine, probably because of its more aerobic circumstance, affording phytosphingosine. *N*-Acylation of phytosphingosine followed by condensation with inositol phosphate provides inositol phosphorylceramide (IPC), which is further modified by an addition of mannose to make mannosyl inositol phosphorylceramide (MIPC) and the addition of a second inositol phosphate group to make mannosyl diinositol diphosphorylceramide ($M(IP)_2C$). It is worth mentioning that such a difference in the biosynthesis of sphingolipids between mammals and fungi is shown, suggesting that specific inhibitors of fungal sphingolipid syntheses are considered to be attractive as targets for antifungal therapy.

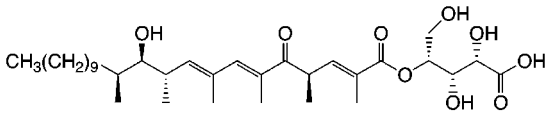

Figure 1. Khafrefungin.

Khafrefungin is a novel antifungal agent isolated from the fermentation culture MF6020 by a Merck group in 1997 (Figure 1).[2] It has been shown to inhibit IPC synthase, which catalyzes the previously mentioned fungal specific step, in *Sacchromyces cerevisiae* and pathogenic fungi such as *Candida albicans* and *Cryptococcus neoformans*. Unlike other inhibitors that inhibit the corresponding enzyme in fungi and mammals to the same extent, khafrefungin does not impair sphingolipid synthesis of mammals.

We already performed the total synthesis of sphingofungins B and F that have been shown to inhibit SPT,[3,4] and we also investigated the antifungal activities through the synthesis of its derivatives.[4] In addition, quite recently, we have achieved the complete stereochemical

† The University of Tokyo.

‡ National Institute of Infectious Diseases.

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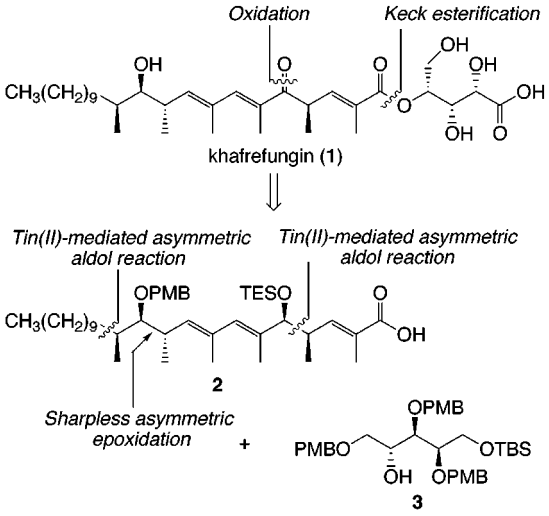
Scheme 1. First-Generation Total Synthesis



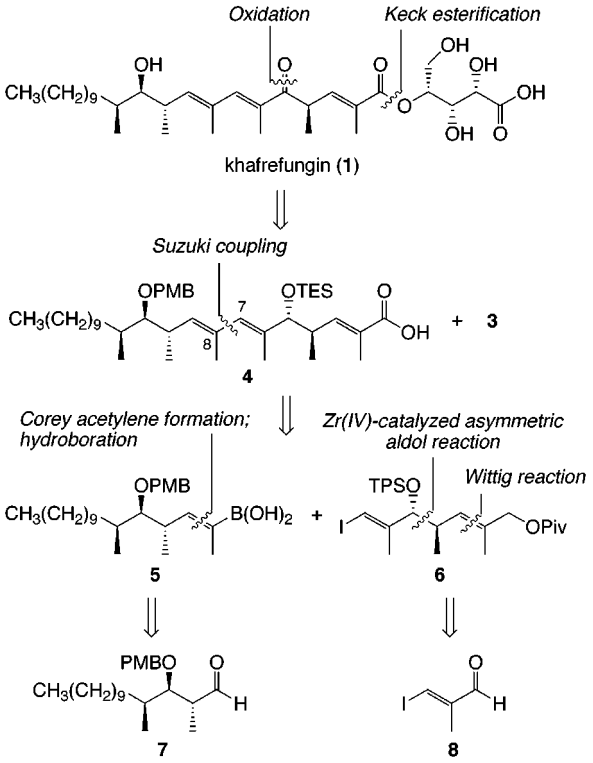
assignment and the first total synthesis of khafrefungin.[5] The strategy of our first-generation total synthesis is summarized in Scheme 1. The polyketide acid part (**2**) was prepared by successive propionate additions from decanal, and one key step was the tin(II)-catalyzed asymmetric aldol reaction.[6] On the other hand, the aldonic acid part (**3**) was synthesized from D-arabinose. In this paper, we report our second-generation, more convergent total synthesis of khafrefungin. The characteristic stability of khafrefungin is discussed using semiempirical calculations and synthesis. In addition, khafrefungin derivatives have also been synthesized, and their antifungal activities have been measured to obtain information on the structure−activity relationships.

Results and Discussion

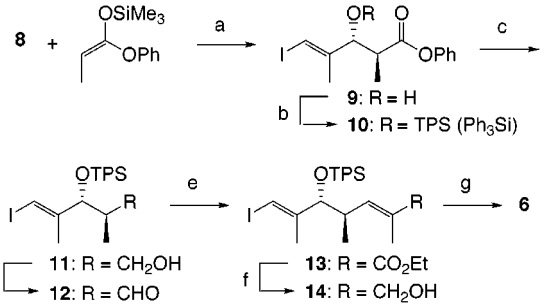
Convergent Total Synthesis. Our synthetic plan of the second-generation total synthesis of khafrefungin is shown in Scheme 2. Khafrefungin is divided into three fragments: the aldonic acid part (**3**), the alkenylboronic acid **5**, and the alkenyliodide **6**. Alcohol **3** was a key intermediate in the first-generation total synthesis and was already prepared from D-arabinose. On the other hand, the C7−C8 bond of **4** would be constructed by the Suzuki coupling reaction of alkenylboronic acid **5** with alkenyliodide **6**. Alkenylboronic acid **5** would be synthesized by the hydroboration of an internal alkyne, which would be prepared from aldehyde **7** according to the Corey protocol. Aldehyde **7** was an intermediate in the first-generation total synthesis and was prepared from decanal via the tin(II)-catalyzed asymmetric aldol reaction as a key step. On the other hand, alkenyliodide **6** would be prepared by the Wittig reaction of the corresponding aldehyde that would be synthesized by the asymmetric aldol reaction of aldehyde **8** with a propionate unit.

Initially, we undertook the synthesis of alkenyliodide **6** (Scheme 3). According to our preliminary results, the

Scheme 2. Synthetic Plan of Convergent Total Synthesis of 1



Scheme 3. Synthesis of 6[a]



[a] Reagents and conditions: (a) 0.1 equiv of $Zr(O^tBu)_4$, 0.12 equiv of (*R*)-3,3′-I_2-BINOL, 0.8 equiv of PrOH, 0.2 equiv of H_2O, toluene, 0 °C, 79%, *syn*/*anti* = 20/80, 96% ee (*anti*); (b) TPSCl, imidazole, DMF, rt; (c) DIBAL, CH_2Cl_2, −78 °C, 76% for two steps; (d) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, followed by Et_3N, rt; (e) $Ph_3P{=}C(Me)CO_2Et$, THF, reflux, 76% for two steps; (f) DIBAL, CH_2Cl_2, −78 °C, 95%; (g) PivCl, pyridine, catalytic amount of DMAP, 90%.

tin(II)-catalyzed asymmetric aldol reaction could not serve as the first step for the preparation of **6**, probably because of the instability of aldehyde **8** toward strong Lewis acids such as metal triflates.[7] On the other hand, our laboratory has recently developed a catalytic asymmetric aldol reaction using a chiral zirconium complex prepared from zirconium tetra-*tert*-butoxide and (*R*)-3,3′-diiodo-1,1′-binaphthalene-2,2′-diol ((*R*)-3,3′-I_2-BINOL), which affords the corresponding *anti* aldol adducts in high enantioselectivities.[8] The catalytic asymmetric aldol reaction of aldehyde **8** with the silyl enol ether derived

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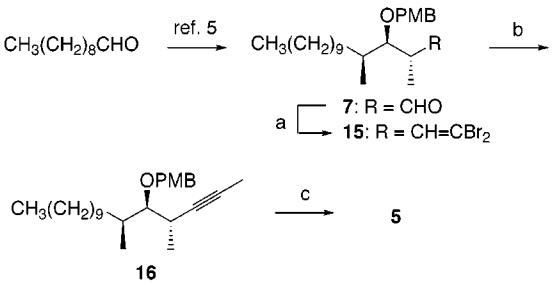
(8) Ishitani, H.; Yamashita, Y.; Shimizu, H.; Kobayashi, S. *J. Am. Chem. Soc.* **2000**, *122*, 5403. For the use of water in preparation of the Zr catalyst, details will be reported in due course.

Scheme 4. Synthesis of 5[a]



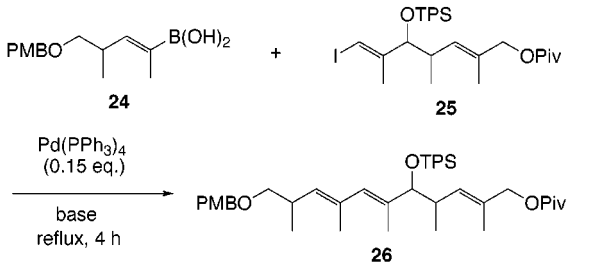
[a] Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2, rt, 95%; (b) BuLi, THF, followed by MeI, −78 °C, 93%; (c) catecholborane, 50 °C, followed by H_2O.

from phenyl propionate using the chiral zirconium catalyst (10 mol %) proceeded smoothly in toluene at 0 °C to give the corresponding aldol adduct **9** in 79% yield with 96% ee (*anti*). Adduct **9** was isolated as white crystals and could be purified by recrystallization (>99% de, >99% ee). The protection of the hydroxyl group of adduct **9** as its triphenylsilyl (TPS) ether followed by reduction using DIBAL gave alcohol **11** in 76% yield for two steps, which was oxidized to provide aldehyde **12**. The Wittig reaction of **12** with (carbethoxyethylidene)triphenylphosphorane gave the ester **13** in 76% yield for two steps with high stereoselectivity (*E*/*Z* = >95/5). The ester **13** was then reduced using DIBAL, and the hydroxyl group of the resulting alcohol (**14**) was subsequently protected as its pivaloyl (Piv) ester to give the key fragment (**6**) in 86% yield for two steps.

We then synthesized another fragment **5** (Scheme 4). Aldehyde **7**, which was an intermediate in the first-generation total synthesis,[5] was treated with carbon tetrabromide in the presence of triphenylphosphine[9] to give alkene **15**. Elimination of hydrogen bromide of alkene **15** using butyllithium and successive methylation of the resulting lithium acetylide were carried out in the same pot to afford the corresponding alkyne **16**. The regioselective hydroboration of alkyne **16** with catecholborane followed by treatment with water gave alkenylboronic acid **5** as a crude material, which was used in the following transformations without further purification.[10]

With both key fragments in hand, we directed our attention to the following Suzuki coupling reaction for the construction[11] of the highly substituted diene. The model cross-coupling reaction of alkenylboronic acid **24** with alkenyliodide **25** was first planned to be examined. Several reaction conditions were tested, and the results are summarized in Table 1. The use of potassium hydroxide as a base in methanol gave no products. Poor acceleration was also observed using sodium ethoxide and sodium hydroxide as bases in aqueous THF. On the other hand, thallium compounds were reported to be quite efficient as bases used in the Suzuki coupling reaction by Kishi and co-workers.[12] Indeed, the coupling reaction of alkenylboronic acid **24** with alkenyl iodide **25** in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium and 2.5 equiv of thallium ethoxide in aqueous THF proceeded smoothly to afford the desired product **26** in 63% yield. Among the tested cosolvents of water, only THF was efficient for this thallium ethoxide-mediated reaction. It was also noted that the thallium ethoxide was more efficient than thallium hydroxide, freshly prepared from thallium formate and sodium hydroxide according to the reported procedure,[13] for the thallium source used in this reaction. In addition, thallium ethoxide was found to work well in THF−ethanol without any water, affording **26** in 74% yield.

Table 1. Suzuki Coupling of 24 with 26



entry	solvent	base	yield of **26**/%
1	MeOH	K_2CO_3[a]	trace
2	THF−EtOH (4/1)	EtONa[a]	trace
3	THF−H_2O (4/1)	NaOH[a]	20
4	THF−H_2O (4/1)	TlOH[a]	12
5	THF−H_2O (4/1)	TlOEt[b]	63
6	hexane−H_2O (4/1)	TlOEt[b]	trace
7	benzene−H_2O (4/1)	TlOEt[b]	trace
8	DME−H_2O (4/1)	TlOEt[b]	trace
9[c]	THF−EtOH (3/1)	TlOEt[b]	74

[a] 2.0 equiv. [b] 2.5 equiv. [c] 50 °C.

On the basis of these model studies, the key coupling reaction for the total synthesis was next examined (Scheme 5). As expected, the palladium(0)-catalyzed coupling reaction of alkenylboronic acid **5** with alkenyliodide **6** using thallium ethoxide was successfully performed in aqueous THF to give **17** in 63% yield (based on **6**).[14] The undesired deprotection of the *p*-methoxybenzyl (PMB) group at the C12 hydroxyl group of **17** occurred in this coupling reaction (21% yield); however, fortunately, the resulting material was easily converted to **17** by treatment with PMB imidate. After the Piv group of **17** was removed using DIBAL in 81% yield, oxidation of the resulting alcohol (**18**) was conducted using manganese oxide to give aldehyde **19**, which was treated with sodium chlorite to afford carboxylic acid **4** in 73% yield for two steps.[15]

The following steps were similar to those of the first-generation total synthesis. The Keck esterification reaction[16] of carboxylic acid **4** with alcohol **3** followed by treatment with a 1 M aqueous hydrochloric acid solution in THF gave diol **21** in 54% yield for two steps. The Dess−Martin periodinane-mediated oxidation[17] of **21** gave aldehyde **22**, which was treated with sodium chlorite[15] to afford carboxylic acid **23** that had already been converted to khafrefungin (one step) in the first-generation total synthesis.

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(10) Review: Lane, C. F.; Kabalka, G. W. *Tetrahedron* **1976**, *32*, 981.
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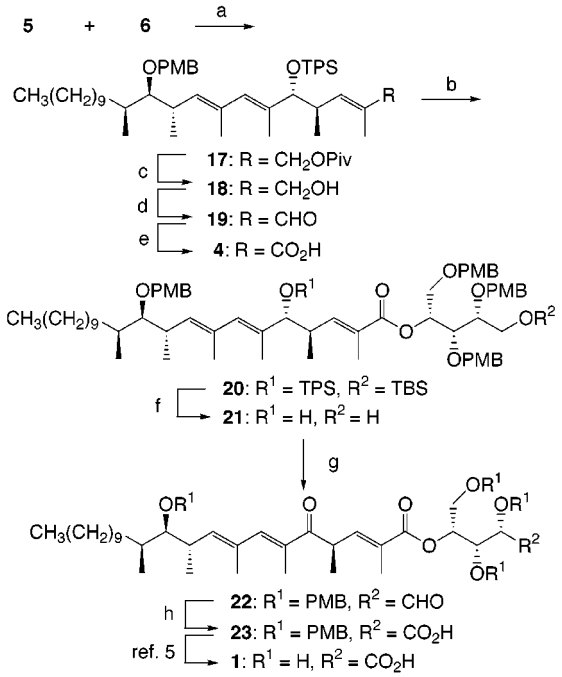
(12) Uenishi, J.; Beau, J. M.; Armstrong, R. W.; Kishi, Y. *J. Am. Chem. Soc.* **1987**, *109*, 4756.
(13) Tyree, S. Y., Jr. *Inorg. Synth.* **1967**, *9*, 52.
(14) The use of THF and ethanol as a mixed solvent did not work well, giving **17** in only 21% yield, probably because of the low solubility of the substrates used.
(15) Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.
(16) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.
(17) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.

Scheme 5. Synthesis of 1[a]



[a] Reagents and conditions: (a) 0.15 equiv of $Pd(PPh_3)_4$, TlOEt, THF, 50 °C, 63% + 21%, see text; (b) DIBAL, CH_2Cl_2, −78 °C, 81%; (c) MnO_2, CH_2Cl_2, rt; (d) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, $^tBuOH/H_2O$ (1/1), 73% for two steps; (e) **3**, DCC, DMAP, DMAP·HCl, CH_2Cl_2, reflux; (f) 1 M HCl/THF (1/3.5), rt, 54% for two steps; (g) Dess−Martin periodinane, pyridine, CH_2Cl_2, rt; (h) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, $^tBuOH/H_2O$ (1/1), 55% for two steps.

Characteristic Stability. The proton at the C4 position of khafrefungin is considered to be rather highly acidic; however, the chirality at the C4 position of khafrefungin was demonstrated to remain intact in the synthesis of khafrefungin and its stereoisomer (*vide infra*).[5] These results seemed to suggest that khafrefungin was under strict conformational constraints to prevent the enolization process. To confirm this, conformation analysis of the model compound (**27**) was performed using MM2 calculations.[18] The results indicated that the dihedral angle between the C5 carbonyl group and the C4−H4 bond in the lowest energy conformer was 164° (Figure 2) and none of the several possible conformers could meet the dihedral demand for enolization.

To assess the influence of the steric environment of this type of compound on enolization, the synthesis and stability of the related compounds (**29**, **32**, and **34**) were next investigated. As was expected, alcohol **28** was oxidized using Dess−Martin periodinane to give ketone **29** having the same type of substitution as khafrefungin in high yield.[17] On the other hand, the oxidation of alcohol **30** lacking the methyl group at the C2 position under the same conditions gave ketone **31** as a single product, which presumably formed via the rapid keto−enol tautomerization of the initially oxidized product (**32**). In addition, the oxidation of alcohol **33** lacking the methyl group at the C6 position was conducted under the same conditions to give a mixture of ketones **34** and **35** (**34**:**35** >14:1). These results clearly explain that the characteristic stability of khafrefungin is due to its steric hindrance, especially due to the methyl group at the C2 position (Scheme 6).[19]

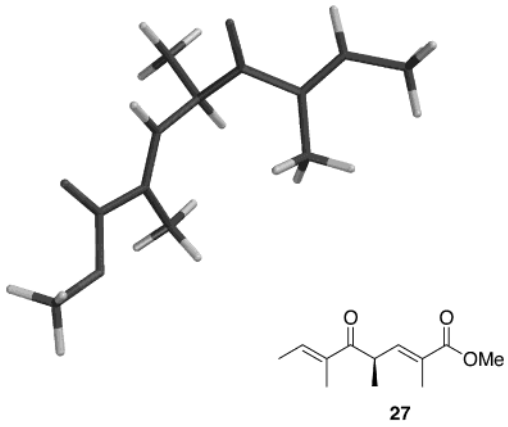
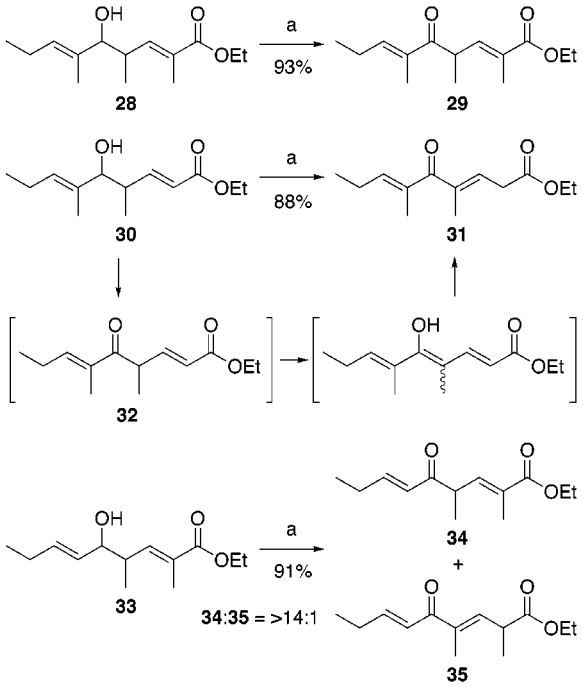


Figure 2. Lowest-energy conformer of **27**.

Scheme 6. Oxidation of Alcohols 28, 30, and 33[a]



[a] Reagents and conditions: (a) Dess−Martin periodinane, pyridine, CH_2Cl_2, rt, 1 h.

Structure and Activity Relationships of Khafrefungin. We next explored the structure and activity relationships of khafrefungin. Synthesized khafrefungin (**1**) and authentic natural khafrefungin showed antifungal activity with the same MIC values (7.4 μM) (Table 2). The MIC value was similar to the previously reported MIC value of natural khafrefungin on *S. cerevisiae* cells.[2] Removal of the aldonic acid group (**39,** Figure 3) completely inactivated the antifungal activity (Table 2). Thus, the aldonic acid group is essential for the activity of khafrefungin. The importance of the aldonic acid group is not simply due to the enhancement of miscibility in water, because the replacement of the aldonic acid group

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(19) Cf.: Evans, D. A.; Clark, J. S.; Metternich, R.; Novack, V.; Sheppard, G. S. *J. Am. Chem. Soc.* **1990**, *112*, 866.

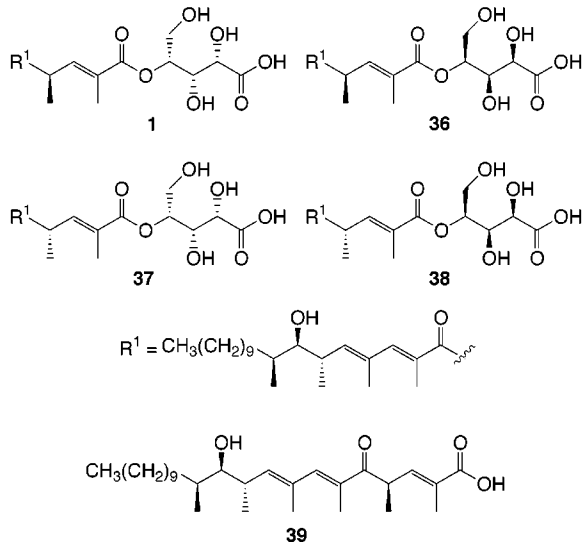

Figure 3. Khafrefungin derivatives.

Table 2. Antifungal Activities

compound	MIC/μM
1	7.4
36	>200
37	>200
38	>200
***ent*-1**	>200
***ent*-36**	>200
***ent*-37**	>200
***ent*-38**	>200
39	>200
authentic sample	7.4

by its enantiomer type (**36**) also resulted in suppressed activity (Table 2). To our surprise, isomerization of the methyl group at the C4 position (**37**) completely inactivated khafrefungin (Table 2). Moreover, another stereoisomer (**38**) and enantiomers of the compounds **1**, **36**, **37**, and **38** (***ent*-1**, ***ent*-36**, ***ent*-37**, and ***ent*-38**, respectively) exhibited no activity (Table 2). We further examined the effect of khafrefungin and its derivatives on the synthesis of sphingolipids on intact yeast cells by metabolic labeling of lipids with radioactive inositol. When the cells were exposed to 2.5 μM drugs, both synthesized and natural types of khafrefungin inhibited synthesis of sphingolipids by ~70%, whereas other synthesized isomers did not inhibit it at all (data not shown), in agreement with the results in terms of antifungal activity previously described. Therefore, the configuration of the methyl group at the C4 position in khafrefungin appears to be crucial for inhibiting the activity of IPC synthase, the target enzyme of khafrefungin, although it remains to be determined whether the presence of the methyl group at the C4 position is essential for the inhibition activity.

Conclusion

Total synthesis of khafrefungin has been achieved using catalytic asymmetric aldol reactions and the Suzuki coupling reaction as key steps. The synthesis is more convergent than our first total synthesis. For the structure of khafrefungin, strict conformational constraints to prevent enolization were indicated, and semiempirical calculations and synthesis have clarified the stability. Furthermore, khafrefungin derivatives were prepared, and their antifungal activities were measured to obtain information on the structure−activity relationships. Because we have established two types of stereoselective synthetic routes for khafrefungin in the previous and present studies, these established routes would allow us in the future to investigate the structure and activity relationships of this drug in more detail by using various derivatives of khafrefungin.

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

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