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Chemistry and biology of khafrefungin. Large-scale synthesis, design, and structure–activity relationship of khafrefungin, an antifungal agent †

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Large-scale synthesis, design, and structure–activity relationships of khafrefungin are reported. Khafrefungin is an antifungal agent that inhibits inositol phosphorylceramide (IPC) synthase, an enzyme involved in fungal sphingolipid biosynthesis. Unlike other inhibitors that inhibit the corresponding enzyme in fungi and mammals to the same extent, khafrefungin does not impair sphingolipid synthesis in mammals. We have developed an efficient method for large-scale synthesis of khafrefungin, and various khafrefungin derivatives were synthesized based on this method. While most of the khafrefungin derivatives lost antifungal activity, a lactone-type derivative had almost the same activity as khafrefungin. We also designed and synthesized derivatives which contain a five- or six-membered ring at the central part of the structure based on NOE experiments of khafrefungin. A macrocyclic khafrefungin derivative was also synthesized, but the antifungal activity was lost. These results suggest that the structure of khafrefungin might be strictly recognized in fungi.

Introduction

Khafrefungin (**1**), an antifungal agent isolated from the fermentation culture MF6020 by a Merck group,[1] has been shown to inhibit IPC (inositol phosphorylceramide) synthase, which catalyzes the fungal specific step in *Saccharomyces cerevisiae* and pathogenic fungi. Different from other inhibitors that inhibit the corresponding enzyme in fungi and mammals to the same extent, khafrefungin does not impair sphingolipid synthesis in mammals.[2] As regards the structure of khafrefungin, although the Merck group showed the plane structure, the stereochemistry remained unknown until we revealed the relative and absolute configuration of khafrefungin in 2001. We have already completed the first total synthesis of khafrefungin using chiral tin(II)-catalyzed aldol reaction, Sharpless asymmetric epoxidation, and Keck esterification as the key steps.[3] Furthermore, we have accomplished another convergent total synthesis of khafrefungin[4] using chiral zirconium(IV)-catalyzed aldol reaction and Suzuki–Miyaura coupling as the key steps. In the course of our further investigations on the chemistry and biology of khafrefungin, it was necessary to prepare khafrefungin on a hundreds-of-milligrams to gram scale. In this paper, we report an efficient method for large-scale synthesis of khafrefungin and its derivatives, and the structure–activity relationship. We designed various analogues of khafrefungin partially based on a metabolism experiment and NOE and HMBC experiments of khafrefungin. Especially, a macrocyclic khafrefungin analogue was designed as an analogue to rustmicin, which is also an IPC synthase inhibitor.[2d]

Results and discussion

Large-scale synthesis of khafrefungin

Recently, we have completed the first total synthesis of khafrefungin and also developed a convergent route to khafrefungin.[3,4] However, in order to investigate the structure–activity relationship of khafrefungin, a more efficient route to khafrefungin which tolerates hundreds-of-milligrams to gram scale preparation was necessary. One drawback of the previous convergent route was the use of a toxic thallium reagent in the Suzuki–Miyaura coupling reaction. Therefore, we tried to avoid the use of the thallium reagent in a newly developed convergent route.

Retrosynthetic analysis of khafrefungin is shown in Scheme 1. Khafrefungin (**1**) is divided into three fragments: alkyne **3**, alkenyliodide **4**, and protected alcohol **5** (aldonic acid part). In the previous report, **3** was connected with **4** first, and then **5** was introduced. Namely, Suzuki–Miyaura coupling of **3** with **4** followed by esterification with **5** gave khafrefungin. However, a problem is that synthesis of fragment **3** needs the most multiple transformations among those of the three fragments, and therefore, it would be more efficient if **4** was connected with **5** by esterification first, followed by Suzuki–Miyaura coupling with **3**. As mentioned above, another drawback of the previous convergent synthesis is the use of a thallium reagent as a base in the Suzuki–Miyaura coupling.

First, we decided to develop a new synthetic pathway to alcohol **10**, which was a key intermediate for the preparation of **3** (Scheme 2). Commercially available methyl (*R*)-(−)-3-hydroxy-2-methylpropionate (**8**) was protected as its benzyl ether, and successive reduction using lithium aluminium hydride and tosylation afforded **9** (91%, 3 steps). Benzyl ether **9** was then alkylated, and the benzyl group was deprotected using BCl_3 to produce chiral alcohol **10**. Alcohol **10** was then converted to alkyne **3** according to the reported procedure.[4] The whole transformations were conducted on a multigram scale. Since we have already synthesized units **4** and **5** on a multigram scale, we completed the synthesis of all the three units (**3**–**5**) also in multigram-scale quantities.[5] Ester **4** was converted to carboxylic acid **12**, which was coupled with alcohol **5** under Keck's esterification conditions[6] to afford the desired adduct (**13**) (Scheme 3). We then examined several conditions of the Suzuki–

† Electronic supplementary information (ESI) available: full experimental details. See http://www.rsc.org/suppdata/ob/b3/b305818b/

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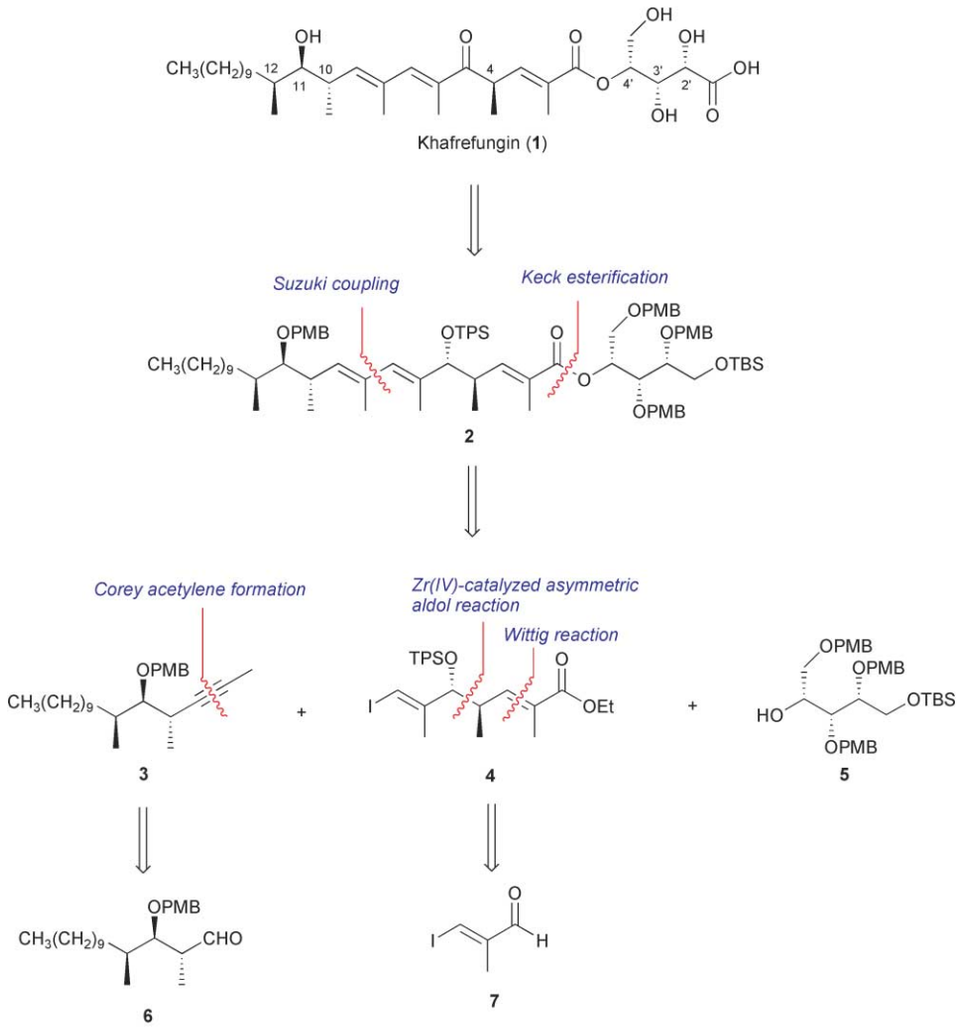
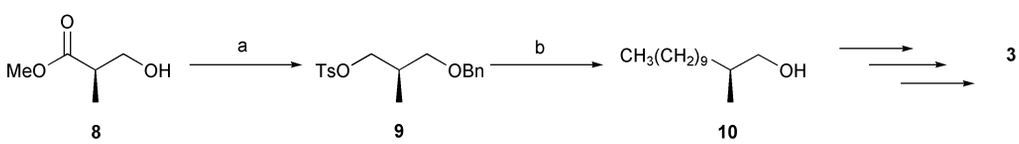
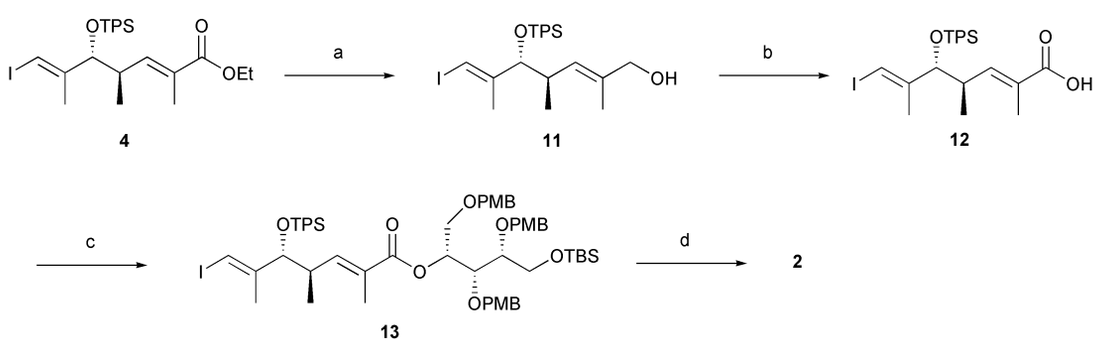


Scheme 1 Retrosynthetic analysis for the synthesis of khafrefungin.



Scheme 2 Synthesis of chiral alcohol **10**. Reagents and conditions: (a) i) $BnOC(=NH)CCl_3$, cat. TfOH, Et_2O, ii) $LiAlH_4$, THF, iii) TsCl, DABCO, CH_2Cl_2, 3 steps, 91%; (b) i) $(C_9H_{19})_2$ CuLi, Et_2O, ii) BCl_3, CH_2Cl_2, 2 steps, 84%.

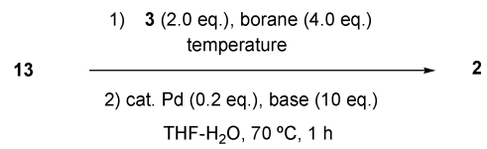


Scheme 3 Synthesis of **2**. Reagents and conditions: (a) DIBAL, CH_2Cl_2, −78 °C, 99%; (b) i) MnO_2, CH_2Cl_2, ii) $NaClO_2$ NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 87%; (c) **5**, DCC, DMAP, DMAP·HCl, CH_2Cl_2, reflux, 79%; (d) **3**, 9-BBN, THF, rt, then $PdCl_2(dppf)$, K_3PO_4, H_2O, 65 °C, 84%.

Miyaura coupling[7] of **13** with alkyne **3**, and the results are summarized in Table 1. In the previous synthesis, catecholborane and thallium ethoxide[8] were used as reagents for this coupling reaction; however, thallium ethoxide is not suitable for large-scale synthesis due to its high toxicity. After screening several reaction conditions, it was found that a one-pot procedure with 9-borabicyclo[3.3.1]nonane (9-BBN), $PdCl_2(dppf)$,[9] and K_3PO_4 gave **2** in high yield (76%, Table 1, entry 4). Moreover, the yield was improved to 84% in a larger-scale reaction (Scheme 3). After purification by column chromatography on silica gel, no regioisomer of **2** was observed by NMR analysis. It is noted that multigram-preparation of **2** has been successfully performed according to a novel synthetic strategy.

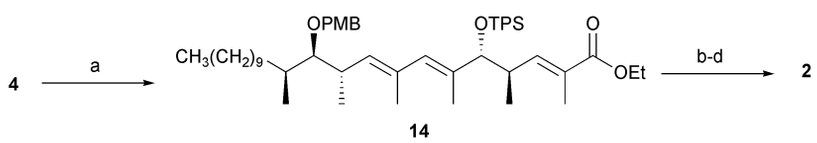
On the other hand, the Suzuki–Miyaura coupling of alkenyliodide **4** with alkyne **3** also proceeded smoothly under

Table 1 Suzuki coupling reaction

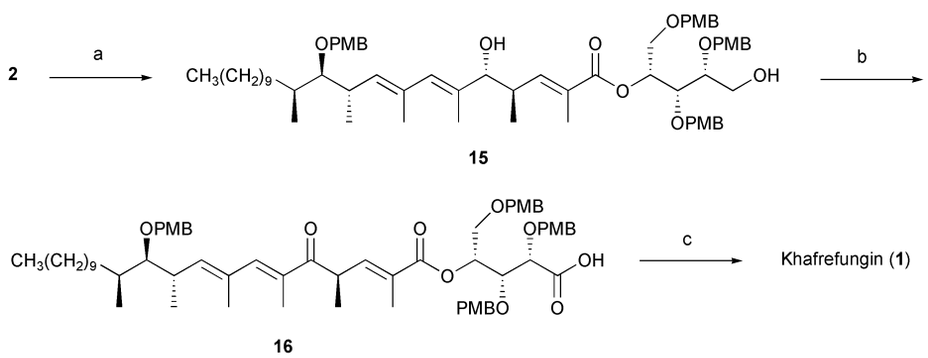


Entry	Borane	Temp.	Pd	Base	Yield (%)
1	catecholborane	50 °C	$Pd(PPh_3)_4$	TlOEt	42 (33)[a]
2	catecholborane	50 °C	$Pd(PPh_3)_4$	Na_2CO_3	27 (53)[a]
3	catecholborane	rt	$PdCl_2$(dppf)	K_3PO_4	50
4	9-BBN	rt	$PdCl_2$(dppf)	K_3PO_4	76

[a] Recovered **13** (%).



Scheme 4 Synthesis of **2**. Reagents and conditions: (a) **3**, 9-BBN, THF, rt, then $PdCl_2$(dppf), K_3PO_4, H_2O, 65 °C, 88%; (b) DIBAL, CH_2Cl_2, −78 °C, 94%; (c) i) MnO_2, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 73%; (d) **5**, DCC, DMAP, DMAP·HCl, CH_2Cl_2, reflux, 69%.



Scheme 5 Synthesis of khafrefungin **1**. Reagents and conditions: (a) HCl, THF, 84%; (b) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 55%; (c) BCl_3, CH_2Cl_2, −78 °C, 51%.

thallium-free conditions. Indeed, the coupling reaction of **4** with **3** proceeded smoothly to afford the desired product **14** in 88% yield (Scheme 4). After treatment with DIBAL, the resulted alcohol was oxidized to the corresponding aldehyde first, and then to the carboxylic acid by treatment with sodium chlorite[10] in 73% yield for two steps. Keck's esterification reaction of the carboxylic acid with alcohol **5** furnished the desired ester **2** in 69%.

The final stage of the total synthesis of khafrefungin is shown in Scheme 5. Deprotection of two silyl groups of ester **2** was conducted with 1 M aqueous hydrochloric acid in THF in 84% yield. Oxidation of the resulting diol **15** was successfully performed using Dess–Martin periodinane[11] to afford the keto-aldehyde, which was treated with sodium chlorite to furnish the ketocarboxylic acid **16** in good yields. Finally, four PMB groups were removed with BCl_3 to afford khafrefungin.

Thus, new synthetic routes to khafrefungin, which tolerate large-scale preparation, have been developed. Synthetic khafrefungin was used for the following investigations to prepare khafrefungin-based derivatives.

Structure–activity relationship

Design. We examined the structure–activity relationship of khafrefungin.[12] Our purpose is not only enhancement of the antifungal activity of khafrefungin but also the conformational study of khafrefungin as well as improvement of metabolism stability for therapeutic agents.

First, we focused on the Merck group's suggestion that strong NOE between H4 and H7 of khafrefungin was observed.[13] We conducted the conformational analysis of khafrefungin in detail once again using HMBC and NOE experiments in CD_3OD (Fig. 1). The analysis suggested that the conformation of khafrefungin seemed to bend around C4 and play an important role in the antifungal activity. To examine the effect of this unique structure on the antifungal activity, we designed rigid derivatives **17** and **18** and dimethyl derivative **19**. Compared to rustmicin which is also known as an IPC synthase inhibitor, macrocyclic khafrefungin analogue **20** was also planned to be synthesized with both antifungal activity and synthetic interest.

Second, from the initial metabolism study of khafrefungin, the ester bond was easily metabolized to afford carboxylic acids **22** and **23**, and oxidation of the lipophilic side chain was observed in mouse plasma, rat hepatocytes, and liver microsomes from mice and humans (Fig. 2). To elucidate the function of this linkage, we designed representative linkage derivatives, for example, amides **24**, thioester **25**, ether **26**, and methylene ketone **27** (Fig. 3). To decrease the lipophilicity of khafrefungin, we also designed *anti*-derivatives **28** and **29**, in which their stereochemistry would be constructed using asymmetric aldol reactions in the presence of a chiral zirconium catalyst. Furthermore, a derivative without two methyl groups (**30**) and a derivative having a shorter alkyl chain (**31**) were designed. The aldonic acid part of khafrefungin has the characteristic structure (three stereogenic centers and three hydroxy groups). To investigate the effect of this aldonic acid part, derivatives **22**, **32**–**36** were designed (Fig. 4).

Finally, as mentioned above, we had synthesized large quantities of khafrefungin in hand. We then planned to modify

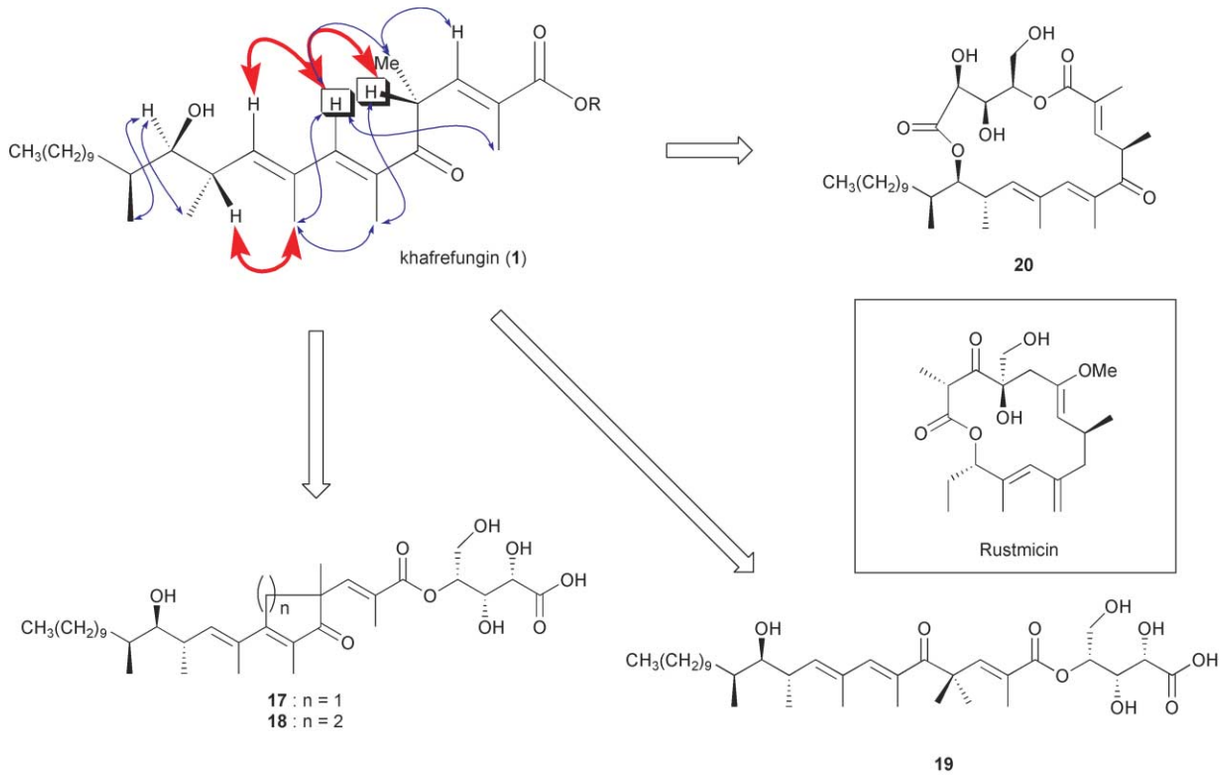


Fig. 1 NOE experiment study of khafrefungin in CD_3OD and designed derivatives from conformational analysis.

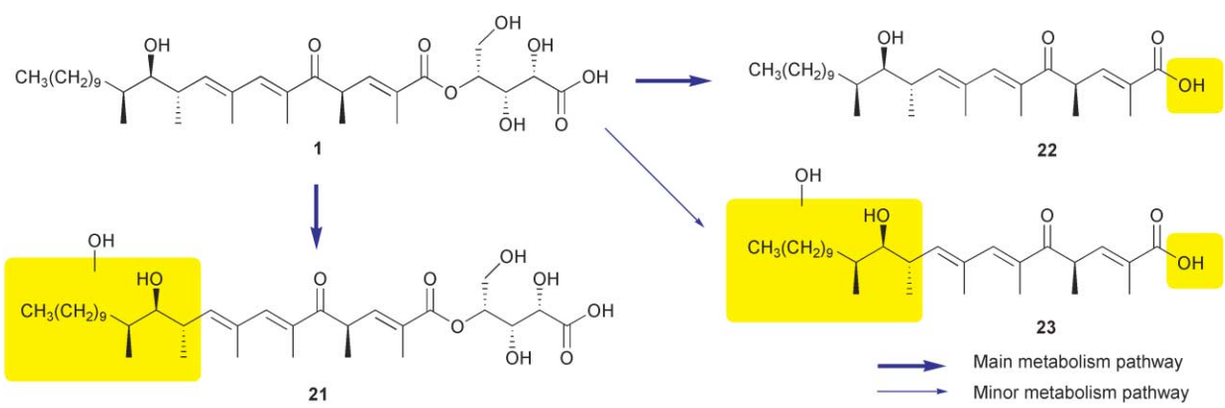


Fig. 2 Proposed metabolites of khafrefungin in mouse plasma, rat hepatocytes, and liver microsomes from mice and humans.

khafrefungin in order to investigate several functional groups of khafrefungin.

Synthesis. The synthesis of 5-membered derivative **17** is summarized in Scheme 6. Iodide **37**, which was prepared according to the literature,[14] was converted into fragment **38** and then **39**. After successive connection with alkyne **3** using Suzuki–Miyaura coupling reaction and with alcohol **5** by modified Keck esterification, 5-membered derivative **17** was obtained according to the procedures shown in our total synthesis of khafrefungin. 6-Membered derivative **18** was also prepared as shown in Scheme 6. Dimethyl derivative **19** was synthesized from alcohol **47** as shown in Scheme 7.

Synthesis of macrocyclic khafrefungin **20** was performed starting from fragments **3**, **4**, and **5** (Scheme 8). Replacement of the *p*-methoxybenzyl (PMB) group into the acetyl (Ac) group at the C11 position was conducted first, and then one-pot Suzuki–Miyaura coupling reaction with iodide **13** and selective desilylation by cooled HF in an MeCN solution afforded fully functionalized adduct **52**. Seco acid **53** was furnished by oxidation into the carboxylic acid and deprotection of the acetyl group by DIBAL. Yamaguchi macrolactonization[15] was then applied to afford the desired macrocycle **54** in moderate yield. According to the usual protocol, macrocyclic khafrefungin analogue **20** was obtained.

The synthesis of the amide derivative is shown in Scheme 9. The amino group was introduced *via* protection of primary alcohol **55** with trityl chloride (TrCl), inversion of the secondary alcohol using Mitsunobu reaction,[16] further inversion using sodium azide, and reduction using triphenylphosphine and H_2O. Amine **58** was converted to protected amine **59**, which was coupled with **62** using bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBrop) as a coupling reagent to afford **60**. The 2,4-dimethoxybenzyl (DMB) group was removed to afford lactone **61**. Compound **24** was not observed under these conditions. In a similar way, thioester derivative **25** was obtained as shown in Scheme 10.

The synthesis of ether derivative **26** is shown in Scheme 11. After examination of several reaction conditions, the ether bond was formed from **68** and **5** in moderate yield using AgOTf in the presence of MS 4A and 2,6-dibutylpyridine. After conversion of iodide **69** into **70**, alcohol **70** was oxidized *via* two steps, followed by deprotection of the PMB groups according to the previous procedures to afford ether derivative **26**.

Methylene ketone derivatives **27a** and **27b** (diastereomers at C4) were synthesized according to Scheme 12. Aldehyde **71**

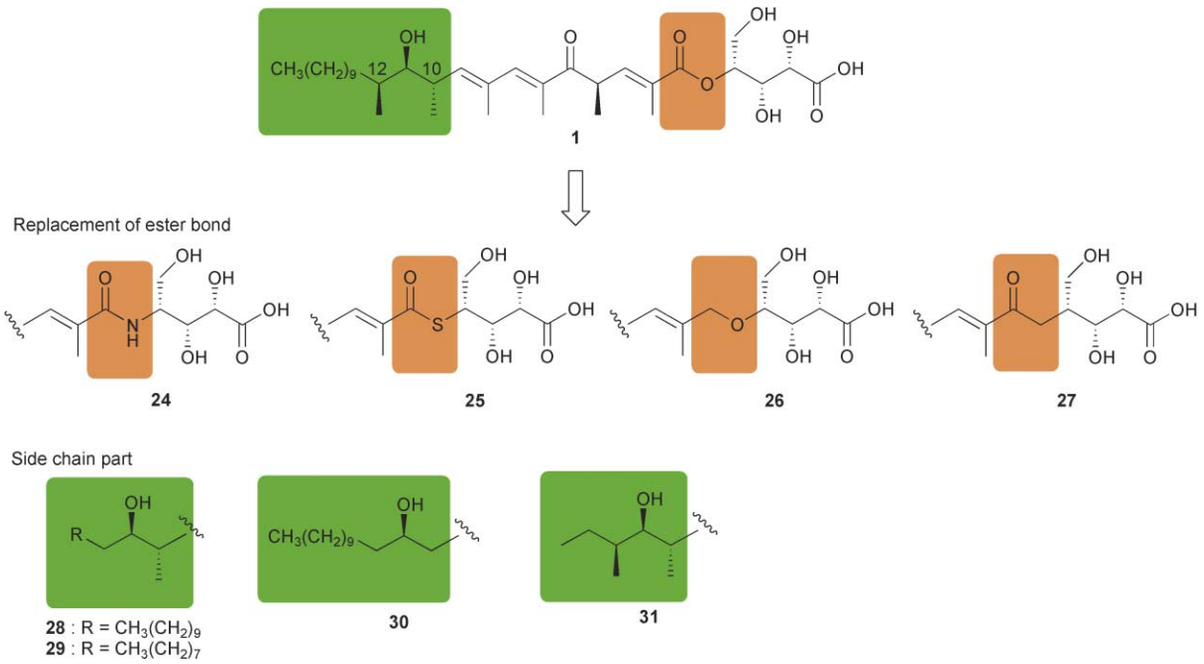


Fig. 3 Designed derivatives for study of metabolism stability.

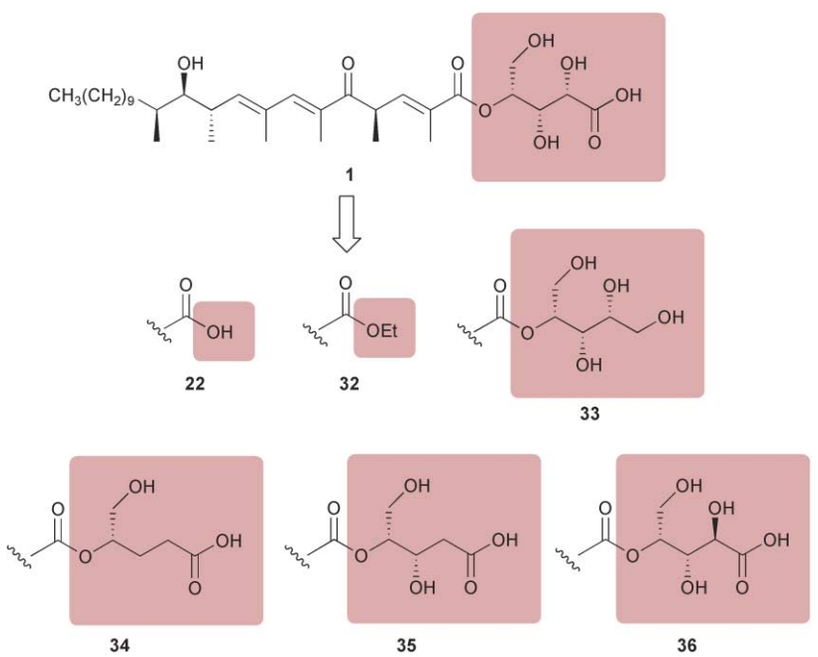
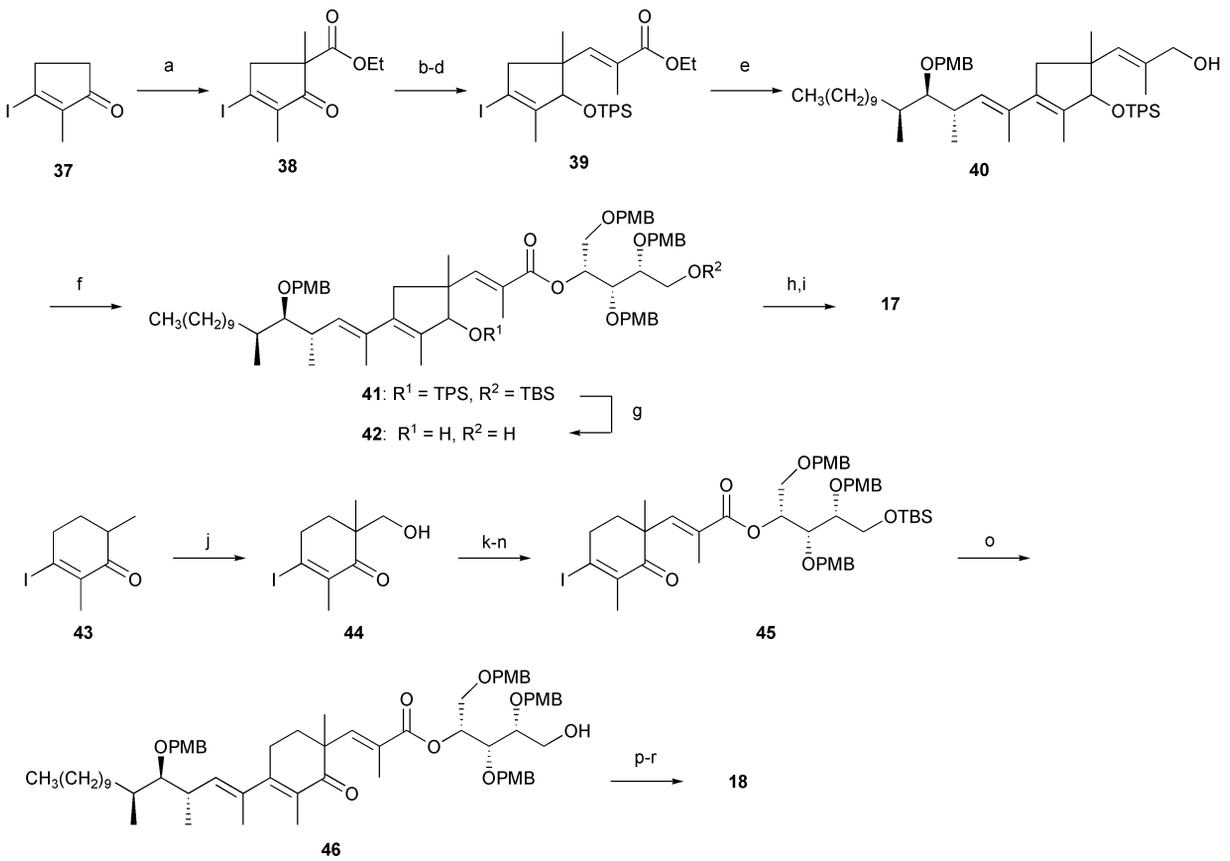


Fig. 4 Khafrefungin derivatives containing modified aldonic acid parts.

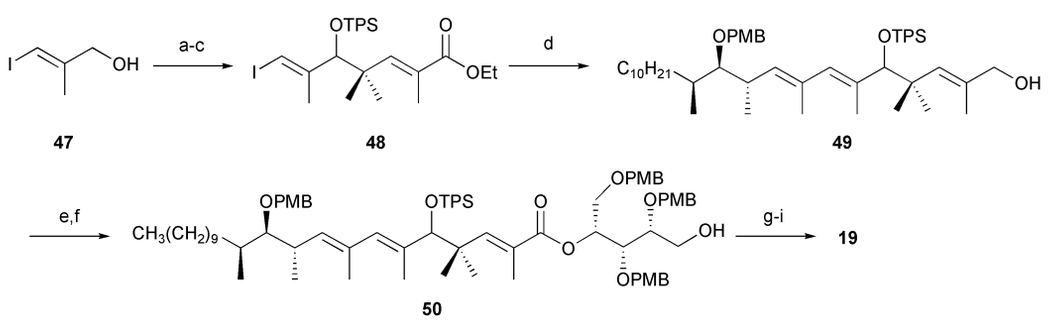
was readily prepared from alcohol **5** using Swern oxidation, Wittig reaction, 1,4-reduction, and DIBAL reduction. Two diastereomers with the ratio of 3 : 5 were separated by silica gel column chromatography. *Anti*-adduct **72** was converted into **73**, and zirconation using Cp_2ZrHCl[17] followed by iodination gave iodide **75**. After Nozaki–Hiyama–Kishi coupling[18] with the vinyl iodide, two silyl groups were deprotected to afford triols **76ac**–**76bd**. According to the similar protocol of the total synthesis of khafrefungin, methylene ketones **27a** and **27b** were obtained.

We have already reported that *anti* aldol adducts were obtained stereoselectively using a novel chiral zirconium catalyst.[19] Thus, catalytic asymmetric aldol reactions of aldehydes **77** and **78** with silyl enol ether **79** using a chiral zirconium catalyst prepared from $Zr(O\textit{t}\text{-}Bu)_4$, (*S*)-3,3′,6,6′-tetraiodo-1,1′-binaphthyl-2,2′-diol ((*S*)-3,3′,6,6′-I_4BINOL), propanol, and H_2O proceeded smoothly to afford the corresponding aldol adducts **80** and **81**, respectively, in high yields with high diastereo- and enantioselectivities. Each adduct was converted to an alkyne according to the previous synthesis of fragment **3**. After oxidation and deprotection, derivatives **28** and **29** were obtained as shown in Scheme 13. On the other hand, derivatives **30** and **31** were synthesized based on the first synthetic route to natural khafrefungin (**1**) (Schemes 14 and 15).[3] Similarly, derivatives **22**, **32**–**36** were prepared (Schemes 16–19). In the synthesis of the sugar parts of derivatives **34**–**36**, the corresponding carboxylic acid or monosaccharides were used as starting materials (Scheme 19).

From synthetic khafrefungin, which was supplied in the large-scale synthesis, several derivatives were prepared as shown in Scheme 20. Treatment of khafrefungin under acidic conditions gave 6-membered lactone **111**. It was found that the lactone was stable during the course of purification by work-up and silica gel chromatography. To investigate the effect of the aldonic acid part, some other derivatives were obtained (Scheme 20).



Scheme 6 Synthesis of fused derivatives. Reagents and conditions: (a) KHMDS, toluene, −78 °C, $ClCO_2Et$ then CH_3I, rt, 65%; (b) $NaBH_4$, MeOH, 0 °C, 70%; (c) TPSCl, imidazole, DMF, quant.; (d) i) DIBAL, CH_2Cl_2, −78 °C, ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, iii) $PPh_3{=}C(CH_3)CO_2Et$, toluene, reflux, 3 steps, 93%; (e) i) **3**, 9-BBN, THF, rt then $PdCl_2(dppf)$, K_3PO_4, DMF, H_2O, 65 °C, ii) DIBAL, CH_2Cl_2, −78 °C, 2 steps, 67%; (f) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, iii) **5**, EDCI·HCl, DMAP, CH_2Cl_2, reflux, 3 steps, 57%; (g) TBAF, AcOH, THF, 50 °C, 93%; (h) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 44%; (i) BCl_3, CH_2Cl_2, −78 °C, 68%; (j) HCHO aq, NaOH, dioxane, 62%; (k) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 92%; (l) $PPh_3{=}C(CH_3)CO_2Et$, THF, reflux, 97%; (m) $(CH_3)_3SiI$, 80 °C, 54%; (n) **5**, EDCI·HCl, DMAP, CH_2Cl_2, reflux, 44%; (o) i) **3**, 9-BBN, THF, rt then $PdCl_2(dppf)$, K_3PO_4, DMF, H_2O, 65 °C, ii) HCl, THF, 2 steps, 44%; (p) Dess–Martin periodinane, pyridine, CH_2Cl_2, 91%; (q) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 85%; (r) BCl_3, CH_2Cl_2, −78 °C, 47%.

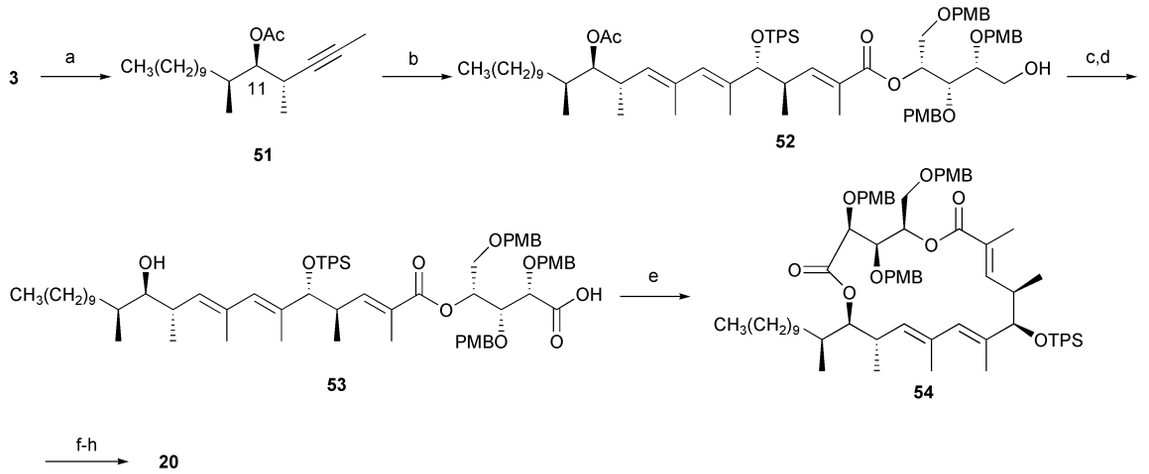


Scheme 7 Synthesis of **19**. Reagents and conditions: (a) i) MnO_2, CH_2Cl_2, ii) methyl isobutyrate, LDA, THF, −78 °C, 2 steps, 80%; (b) i) TPSCl, imidazole, DMF, ii) DIBAL, CH_2Cl_2, −78 °C, 2 steps, 97%; (c) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) $PPh_3{=}C(CH_3)CO_2Et$, toluene, reflux, 2 steps, 91%; (d) i) **3**, 9-BBN, THF, rt then $PdCl_2(dppf)$, K_3PO_4, DMF, H_2O, 65 °C, ii) DIBAL, CH_2Cl_2, −78 °C, 2 steps, 86%; (e) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 86%; (f) i) **5**, EDCI·HCl, DMAP, CH_2Cl_2, reflux, ii) HCl, THF, 2 steps, 65%; (g) TBAF, AcOH, THF, 50 °C, 89% (h) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 41%; (i) BCl_3, CH_2Cl_2, −78 °C, 50%.

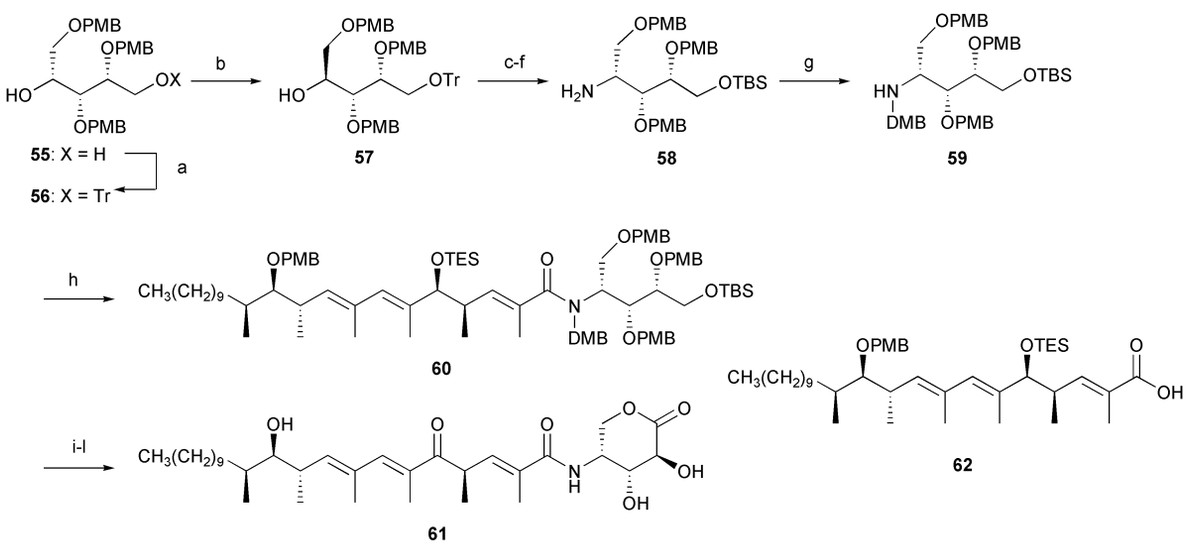
Biological evaluation. We next explored the structure–activity relationship of khafrefungin. The MIC values of khafrefungin on the liquid culture growth of *Saccharomyces cereviciae* cells was ~10 μM, being consistent with previous studies.[4]. Lactone-type derivative **111** showed almost the same MIC (~10 μM) as natural khafrefungin. However, other novel derivatives synthesized in the present study showed far less or no antifungal activity.The derivatives **28**, **29** and **30**, in which the C10 or C12 methyl group was removed, inhibited the yeast cell growth by 30–60% at 0.2 mM, but did not reach MIC value even at 0.2 mM, the highest concentration we examined. For other derivatives, no inhibition of the yeast cell growth was observed up to 0.2 mM.

Discussion

In the structure of khafrefungin, the ester part is assumed to be important because it was found to be easily metabolized in mouse plasma, rat hepatocytes, and liver microsomes in a metabolic stability study. The fact that carboxylic acid **22**, which lacks the aldonic acid part, does not have the antifungal activity suggests that cleavage of the ester bond results in disappearance of the activity. We synthesized various derivatives that have other functional groups instead of the ester group. To our surprise, in the cases of other functional groups such as amide, ether, thioester, and methylene ketone, the activity disappeared. These results suggest that the ester group is essential



Scheme 8 Synthesis of macrocyclic khafrefungin. Reagents and conditions: (a) i) TFA, CH_2Cl_2, 0 °C, ii) Ac_2O, pyridine, DMAP, CH_2Cl_2, 2 steps, 81%; (b) i) **13**, 9-BBN, THF, rt then $PdCl_2$(dppf), K_3PO_4, DMF, H_2O, 65 °C, ii) HF aq., CH_3CN, −10 °C, 2 steps, 42%; (c) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 66%; (d) DIBAL, CH_2Cl_2, −78 °C, 48%; (e) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF then DMAP, toluene, reflux, 40%; (f) HF aq., CH_3CN, 0 °C, 54%; (g) Dess–Martin periodinane, pyridine, CH_2Cl_2, 93%; (h) BCl_3, CH_2Cl_2, −78 °C, 73%.



Scheme 9 Synthesis of amide derivative. Reagents and conditions: (a) TrCl, Et_3N, CH_2Cl_2, 86%; (b) i) *m*-nitrobenzoic acid, DEAD, PPh_3, THF, ii) DIBAL, CH_2Cl_2, −78 °C, 2 steps, 55%; (c) i) MsCl, Et_3N, CH_2Cl_2, ii) NaN_3, DMF, 100 °C, 2 steps, 55%; (d) TsOH, MeOH, 62%; (e) TBSCl, imidazole, DMF, 90%; (f) PPh_3, H_2O, THF, 94%; (g) i), 2,4-$(MeO)_2C_6H_3CHO$, MS 4A, toluene, ii) $NaBH_4$, MeOH, 2 steps, 92%; (h) **62**, PyBrop, *i*-Pr_2NEt, CH_2Cl_2, 45%; (i) HCl, THF, 88%; (j) Dess–Martin periodinane, pyridine, CH_2Cl_2, 60%; (k) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O; (l) TFA, anisole, CH_2Cl_2, 40%.

for the activity, possibly because the ester carbonyl group might be attacked by a nucleophile, which is an important step for the IPC synthase inhibition.

The derivatives in which the methyl group at the C12 position was removed (**28**, **29**) showed far lower antifungal activity than khafrefungin. Derivative **31** with a shorter alkyl chain lost the activity. The aldonic acid part seems to have an important role in the antifungal activity. Lactone-type derivative **111** has almost the same activity as that of khafrefungin, which suggests that khafrefungin and its lactone derivative may have the same active species under equilibrium in the aqueous phase. The hydroxy groups in the aldonic acid part also seem to play an important role for the activity. The antifungal activity disappears upon protecting one of the hydroxy groups as its acetyl ester. The stereochemistry of the hydroxy groups is also crucial for the biological activity.

The 5-membered derivative **17** and 6-membered derivative **18** which were designed based on NOE data of khafrefungin exhibited no activity. The active conformation may be different from that of khafrefungin. The macrocyclic khafrefungin derivative also lost the activity. The structure of khafrefungin might be strictly recognized in fungi.

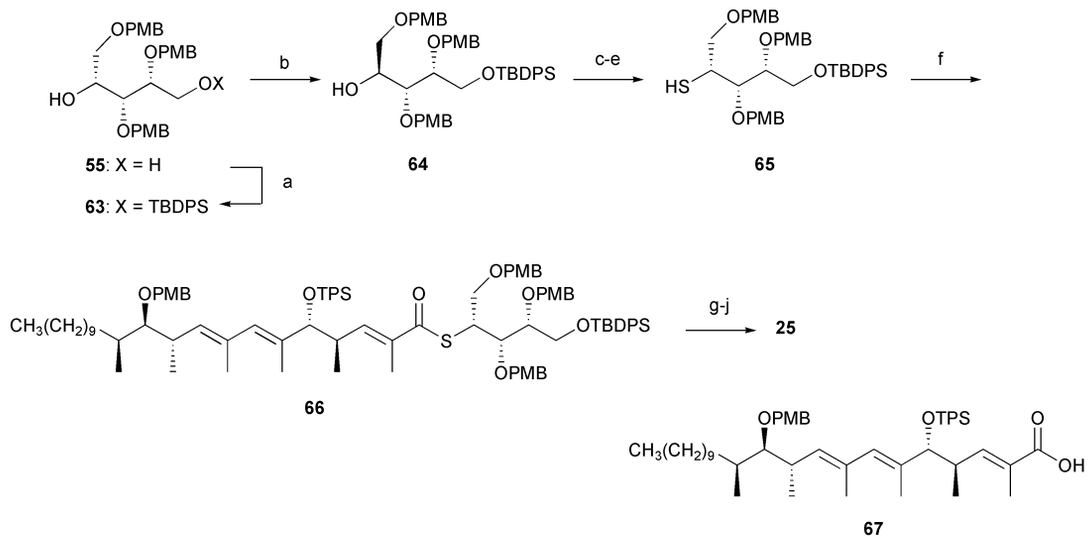
Conclusion

In conclusion, we have developed an efficient synthetic route to khafrefungin, and prepared three key units for the synthesis on a gram scale. We designed various derivatives of khafrefungin and synthesized them by applying the efficient synthetic route to khafrefungin. Study of the structure–activity relationship has revealed that most of the stereochemistry and functional groups of khafrefungin are crucial for the antifungal activity. A lactone-type derivative has almost the same antifungal activity, suggesting that there may be an equilibrium between natural khafrefungin and its lactone-type derivative. It was also revealed that the structure of khafrefungin was strictly distinguished in fungi. Further design and synthesis of khafrefungin derivatives are now under investigation.

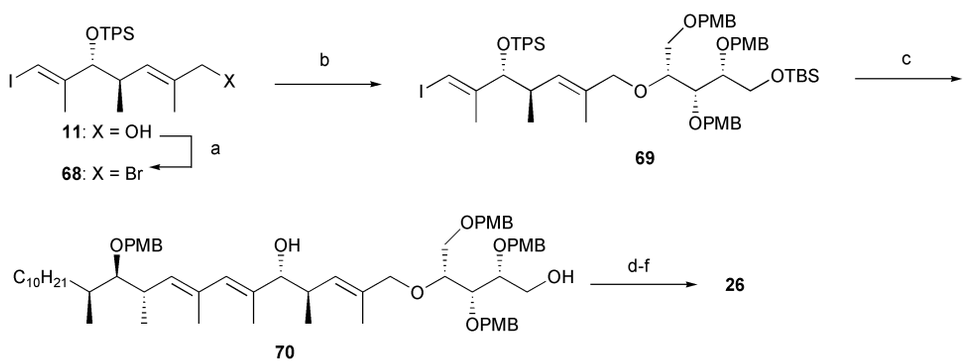
Experimental

General

IR spectra were recorded on a JASCO FT/IR-610. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA300,



Scheme 10 Synthesis of thioester **25**. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, 81%; (b) i) *m*-nitrobenzoic acid, DIAD, Ph_3P, THF, ii) DIBAL, CH_2Cl_2, −78 °C, 2 steps, 57%; (c) MsCl, Et_3N, CH_2Cl_2, 85%; (d) AcSK, toluene, DMF, 140 °C, 57%; (e) $LiAlH_4$, Et_2O, 88%; (f) **67**, EDCI·HCl, DMAP, CH_2Cl_2, reflux, 62%; (g) TBAF, AcOH, THF, 50 °C, 79%; (h) Dess–Martin periodinane, pyridine, CH_2Cl_2, 31%; (i) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 59%; (j) BCl_3, CH_2Cl_2, −78 °C, 30%.



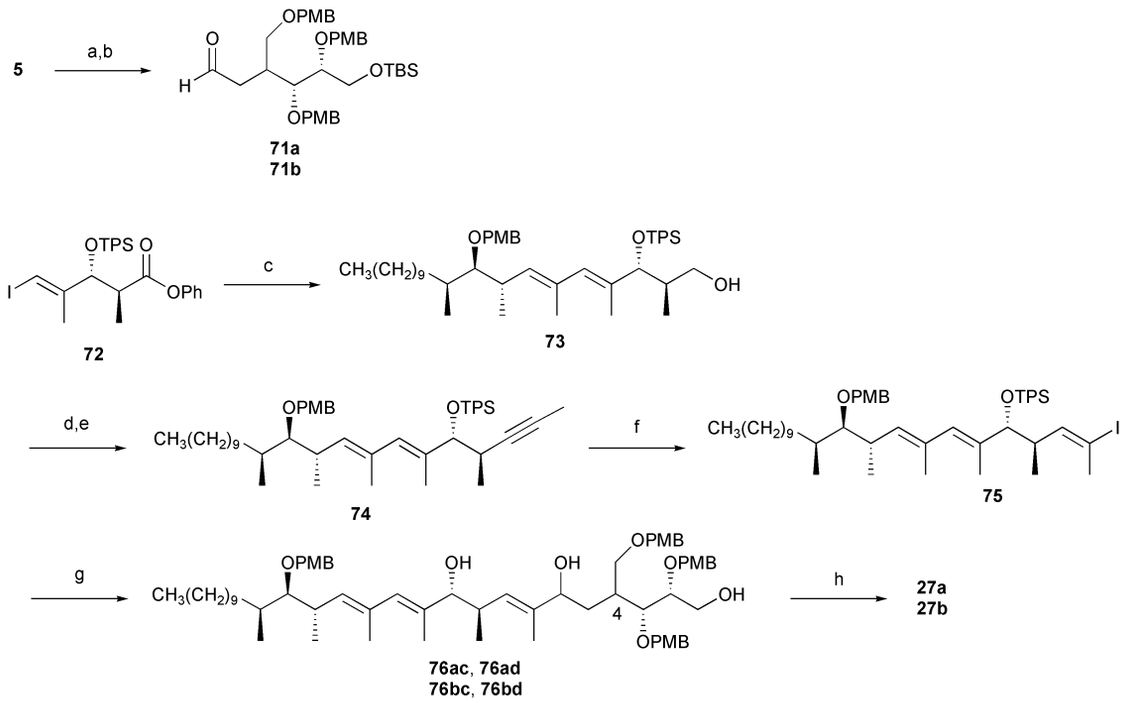
Scheme 11 Synthesis of **26**. Reagents and conditions: (a) CBr_4, PPh_3, THF, 98%; (b) **5**, AgOTf, 2,6-dibutylpyridine, MS 4A, CH_2Cl_2, 0 °C, 41%; (c) i) **3**, 9-BBN, THF, rt then $PdCl_2$(dppf), K_3PO_4, DMF, H_2O, 65 °C, ii) HCl, THF, 2 steps, 40%; (d) Dess–Martin periodinane, pyridine, CH_2Cl_2, 34%; (e) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 73%; (f) BCl_3, CH_2Cl_2, −78 °C, 17%.

JNM-LA400 or a JNM-LA500 spectrometer in $CDCl_3$ unless otherwise noted. Tetramethylsilane (TMS) served as internal standard (δ 0) for ^{1}H NMR, and $CDCl_3$ was used as internal standard (δ 77.0) for ^{13}C NMR. When CD_3OD was used, CD_3OD served as internal standard (δ 3.3) for ^{1}H NMR, and (δ 49.0) for ^{13}C NMR. HPLC was carried out using a Shimadzu C-R6A chromatopac, SPD-10A, and LC-10AT. Optical rotations were recorded on a JASCO P-1010. Column chromatography was performed on Silica gel 60 (Merck). Mass spectra were recorded on a JEOL JMS-SX102A mass spectrometer or a micromass Quattro II and Q-Toff-2 instrument. Preparative thin layer chromatography was performed on Wakogel B5F or using Silica gel 60 F254 (Merck). All non-aqueous reactions were performed under an oxygen-free atmosphere of argon with rigid exclusion of moisture from reagents and glassware. All solvents were purified according to standard procedures.

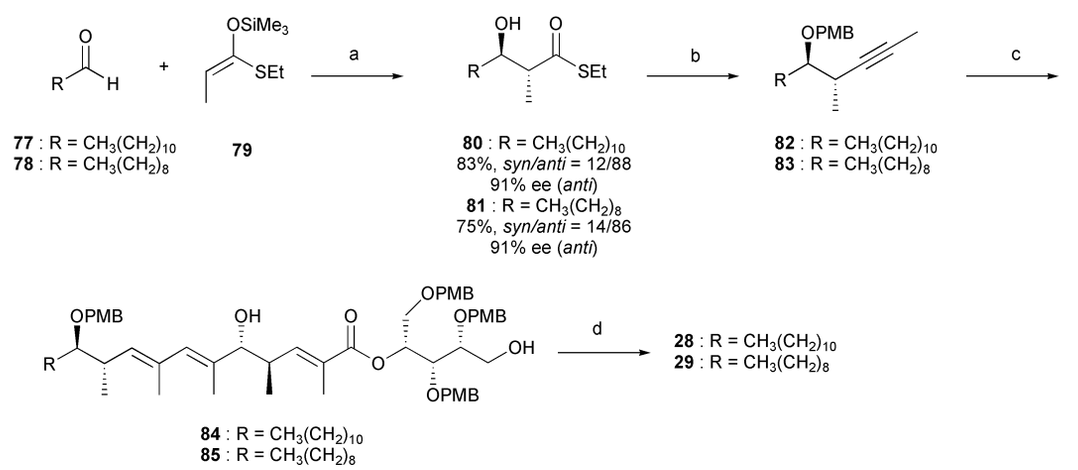
Growth inhibition was determined according to the procedure shown in ref. 4.

Synthesis of khafrefungin (1)

(2*R*)-2-Methyl-3-(benzyloxy)propyl 4-methylbenzenesulfonate 9. To a solution of (*R*)-(−)-3-hydroxy-2-methylpropionate (10.0 mL, 90.2 mmol) and benzyl 2,2,2-trichloroacetimidate (21.1 mL, 108 mmol) in diethyl ether (300 mL) was added trifluoromethanesulfonic acid (1.20 mL, 12.6 mmol) dropwise. After stirring at room temperature for 3 h, the reaction mixture was cooled to 0 °C and quenched with saturated $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with diethyl ether. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was filtered through a short column of silica gel (hexane : EtOAc = 10 : 1) to give the corresponding benzylate as a colorless oil, which was used without further purification. To a suspension of lithium aluminium hydride (8.56 g, 226 mmol) in THF (200 mL) was added the benzylate in THF (100 mL) at −10 °C slowly. After stirring at this temperature for 2 h, the reaction mixture was quenched with MeOH and then added aqueous Rochelle salt and diethyl ether. The mixture was warmed to room temperature and stirred vigorously for 5 h. The organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was filtered through a column of silica gel (hexane : EtOAc = 3 : 1) to give the corresponding alcohol as a colorless oil, which was used without further purification. To a solution of the alcohol in dichloromethane (200 mL) was added 1,4-diazabicyclo[2.2.2]octane (26.8 g, 239 mmol) and TsCl (33.7 g, 177 mmol). After stirring at room temperature for 1.5 h, the reaction mixture was poured into saturated $NaHCO_3$ and then extracted with diethyl ether. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 6 : 1) to give the tosylate (**9**) (27.4 g, 3 steps, 91%) as a colorless oil (Found: C, 64.89; H 6.47. $C_{18}H_{22}O_4S$ requires C, 64.64; H, 6.63%); $[a]_D^{25.5}$ −4.74 (*c* 1.50 in



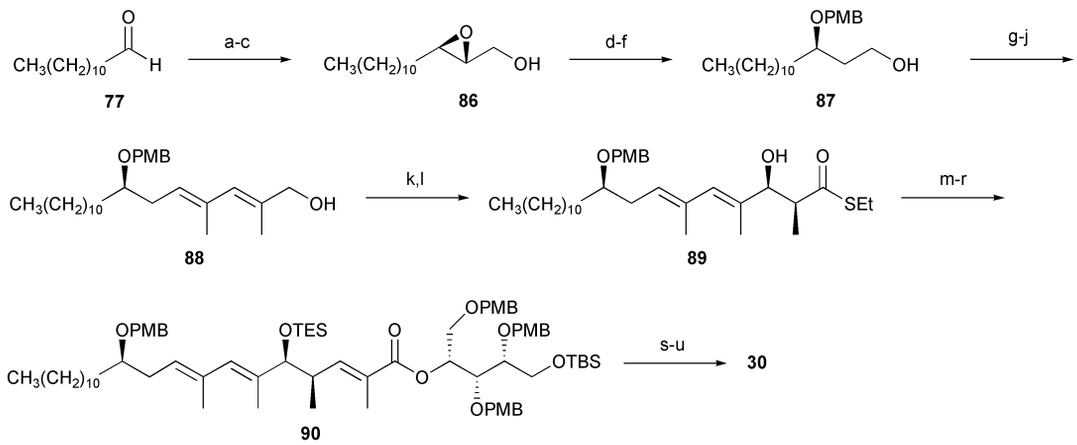
Scheme 12 Synthesis of **27**. Reagents and conditions: (a) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) PPh_3=$CHCO_2$ Et, THF, reflux, iii) $NiCl_2$, $NaBH_4$, THF, EtOH, 3 steps, 77%; (b) DIBAL, CH_2Cl_2, 36% (**71a**), 57% (**71b**); (c) i) **3**, 9-BBN, THF then $PdCl_2$(dppf), K_3PO_4, DMF, H_2O, 65 °C, ii) DIBAL, CH_2Cl_2 −78 °C, 2 steps, 78%; (d) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) PPh_3, CBr_4, CH_2Cl_2, 2 steps, 92%; (e) *n*-BuLi, THF, −78 °C,CH_3I, rt, 91%; (f) Cp_2ZrHCl, THF then I_2, 80%; (g) i) **71a** or **71b**, $NiCl_2$, $CrCl_2$, DMSO, ii) HCl, THF, 2 steps, 13% (**76ac**), 20% (**76ad**), 15% (**76bc**), 10% (**76bd**); (h) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, iii) BCl_3, CH_2Cl_2, −78 °C.



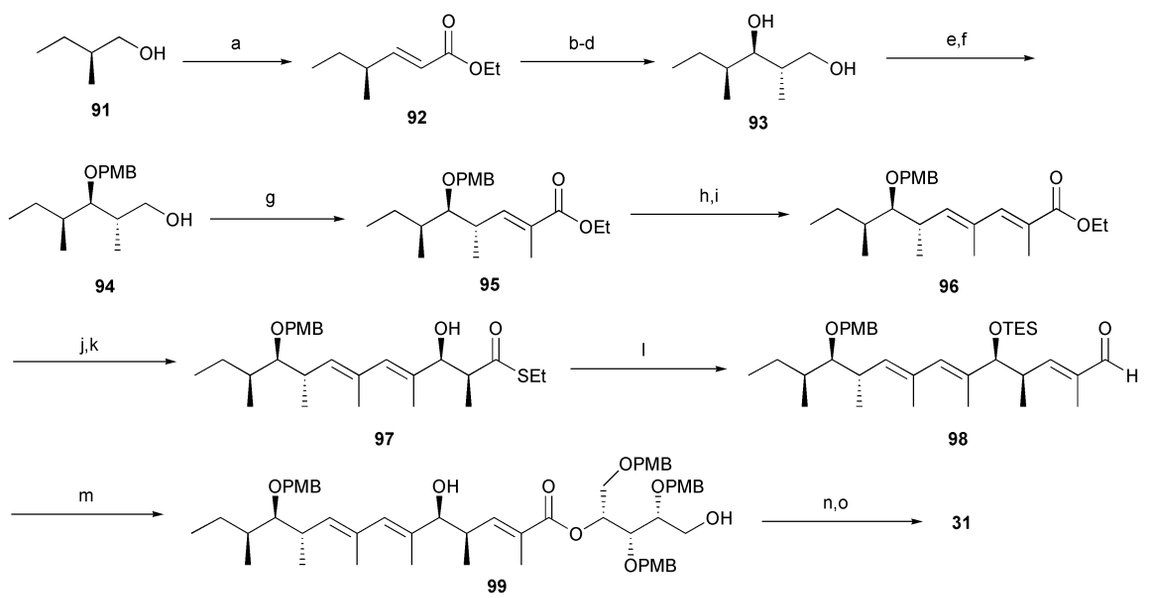
Scheme 13 Synthesis of **28** and **29**. Reagents and conditions: (a) (*S*)-3,3,6,6-I_4BINOL, Zr(O*t*-Bu)$_4$, PrOH, H_2O, toluene, 0 °C; (b) i) $LiAlH_4$, THF, ii) $PMPCH(OMe)_2$, *p*-TsOH, CH_2Cl_2, iii) DIBAL, CH_2Cl_2, 0 °C, iv) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, v) CBr_4, PPh_3, CH_2Cl_2, 0 °C, vi) *n*-BuLi, MeI, THF, 6 steps, 41% (**82**), 86% (**83**); (c) i) **13**, 9-BBN, THF, then $PdCl_2(dppf)_2$, K_3PO_4, DMF, H_2O, 65 °C, ii) HCl, THF, 2 steps, 53% (**84**), 58% (**85**); (d) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, iii) BCl_3, CH_2Cl_2, −78 °C.

$CHCl_3$); ν_{max}(film)/cm^{-1} 1598, 1362, 1176, 1095, 973, 812, 666; δ_H (400 MHz, $CDCl_3$) 7.78 (2 H, d, *J* 8.3 Hz), 7.35–7.20 (7 H, m), 4.39 (2 H, s), 4.05 (1 H, dd, *J* 9.5, 5.6 Hz), 3.98 (1 H, dd, *J* 9.5, 5.8 Hz), 3.55 (1 H, dd, *J* 9.3, 5.4 Hz), 3.52 (1 H, dd, *J* 9.3, 6.8 Hz), 2.41 (3 H, s), 2.17–2.05 (1 H, m), 0.94 (3 H, d, *J* 7.1 Hz); δ_C (100 MHz, $CDCl_3$) 144.6, 138.1, 132.9, 129.7, 128.2, 127.8, 127.5, 127.3, 72.9, 72.1, 71.0, 33.6, 21.5, 13.5; *m*/*z* (ESI) 335 $[M + H]^+$.

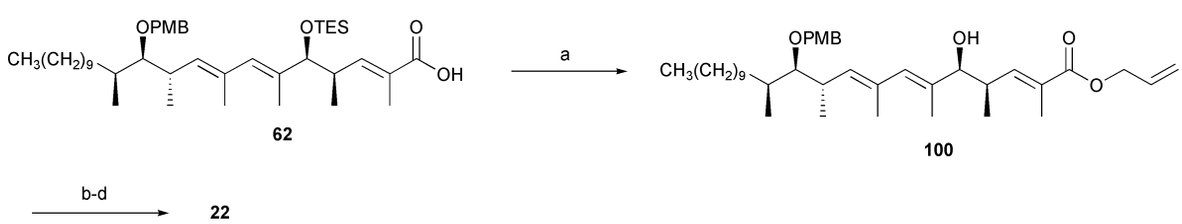
(2*S*)-2-Methyl-1-dodecanol 10. To a suspension of CuI (41.7 g, 219 mmol) in diethyl ether (100 mL) was added nonyllithium (93.4 mL, 93.4 mmol, 1.0 M in diethyl ether) at −23 °C slowly. After stirring at this temperature for 10 min, the reaction mixture was cooled to −78 °C. The tosylate (**9**) (29.3 g, 87.5 mmol) in diethyl ether (100 mL) was added, and the mixture was stirred at −23 °C for 30 min. The reaction mixture was quenched with NH_4Cl and then diethyl ether was added. The organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was filtered through a short column of silica gel (hexane : EtOAc = 30 : 1) to give the corresponding adduct as a colorless oil, which was used without further purification. To a solution of the adduct in CH_2Cl_2 (300 mL) was added BCl_3 (100 mL, 100 mmol, 1.0 M in heptane) at −78 °C. After stirring at 0 °C for 30 min, the reaction mixture was quenched with MeOH and saturated $NaHCO_3$, and diethyl ether was added. The organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 10 : 1) to give the alcohol (**10**) (14.7 g, 2 steps, 84%) as a colorless oil (Found: C, 77.69; H 14.03. $C_{13}H_{28}O$ requires C, 77.93; H, 14.09%); $[a]_D^{26}$ −10 (*c* 0.50



Scheme 14 Synthesis of **30**. Reagents and conditions: (a) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, 97%; (b) DIBAL, CH_2Cl_2, −78 °C, 88%; (c) Ti(O*i*-Pr)$_4$, (D)-DET, TBHP, CH_2Cl_2, −23 °C, 98%; (d) Red-Al, THF, 91%; (e) $PMPCH(OMe)_2$, TsOH, CH_2Cl_2, 90%; (f) DIBAL, CH_2Cl_2, −78 °C, 98%; (g) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 84%; (h) i) $Ph_3P{=}C(CH_3)CO_2Et$, THF, ii) DIBAL, CH_2Cl_2, −78 °C, 2 steps, 84%; (i) TPAP, NMO, MS 4A, CH_2Cl_2, 89%; (j) i) $Ph_3P{=}C(CH_3)CO_2Et$, THF, i) DIBAL, CH_2Cl_2, −78 °C, 2 steps, 78%; (k) MnO_2, CH_2Cl_2, 95%; (l) chiral Sn(OTf)$_2$-catalyzed aldol reaction (see ref. 3), 78%; (m) TESOTf, 2,6-lutidine, CH_2Cl_2, 85%; (n) DIBAL, CH_2Cl_2, −78 °C, 95%; (o) $Ph_3P{=}(CH_3)CO_2Et$, CH_2Cl_2, 98%; (p) DIBAL, CH_2Cl_2, −78 °C, 87%; (q) i) MnO_2, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps; 91%; (r) **5**, DCC, DMAP, DMAP·HCl, CH_2Cl_2, reflux, 65%; (s) HCl, THF, 84%; (t) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2 steps, 36%; (u) BCl_3, CH_2Cl_2, −78 °C.



Scheme 15 Synthesis of **31**. Reagents and conditions: (a) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, 2 steps, 70%; (b) DIBAL, CH_2Cl_2, −78 °C, 72%; (c) Ti(O*i*-Pr)$_4$, (D)-DET, TBHP, CH_2Cl_2, −23 °C, 79%; (d) MeLi, CuI, Et_2O, −23 °C, 73%; (e) $PMPCH(OMe)_2$, TsOH, CH_2Cl_2, 87%; (f) DIBAL, CH_2Cl_2, −78 °C, 91%; (g) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) $Ph_3P{=}C(CH_3)CO_2Et$, CH_2Cl_2, 2 steps, 82%; (h) DIBAL, CH_2Cl_2, −78 °C, 93%; (i) i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, ii) $Ph_3P{=}C(CH_3)CO_2Et$, CH_2Cl_2, 2 steps, 81%; (j) i) DIBAL, CH_2Cl_2, −78 °C, ii) TPAP, NMO, MS 4A, CH_2Cl_2, 2 steps, 81%; (k) chiral Sn(OTf)$_2$-catalyzed aldol reaction (see ref. 3), 75%; (l) (i) TESOTf, lutidine, CH_2Cl_2, ii) DIBAL, CH_2Cl_2, −78 °C, iii) $Ph_3P{=}C(CH_3)CO_2Et$, CH_2Cl_2, iv) DIBAL, CH_2Cl_2, −78 °C, (v) TPAP, NMO, MS 4A, CH_2Cl_2, 5 steps, 57%; (m) i) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, ii) **5**, DCC, DMAP, DMAP·HCl, CH_2Cl_2, reflux, iii) HCl, THF, 3 steps, 39%; (n) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, (o) BCl_3, CH_2Cl_2, −78 °C.



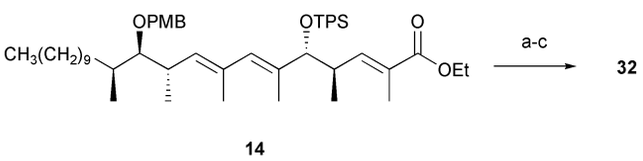
Scheme 16 Synthesis of **22**. Reagents and conditions: (a) i) allyl bromide, Cs_2CO_3, DMF, ii) HCl, EtOH, 2 steps, 72%; (b) Dess–Martin periodinane, pyridine, CH_2Cl_2, 70%; (c) DDQ, CH_2Cl_2, H_2O, 71%; (d) cat. $Pd(PPh_3)_4$, morpholine, THF, 51%.

in EtOH); ν_{max}(film)/cm^{-1} 3360, 2920; δ_H (400 MHz, $CDCl_3$) 3.51 (1 H, dd, *J* 10.5, 5.9 Hz), 3.41 (1 H, dd, *J* 10.5, 6.6 Hz), 1.64–1.53 (1 H, m), 1.47 (1 H, br s), 1.40–1.05 (18 H, m), 0.91 (3 H, d, *J* 6.6 Hz), 0.88 (3 H, t, *J* 6.8 Hz); δ_C (100 MHz, $CDCl_3$) 68.4, 35.7, 33.1, 31.9, 29.9, 29.7, 29.6, 29.3, 27.0, 22.7, 16.6, 14.1; *m*/*z* (EI) 199 $[M]^+$.

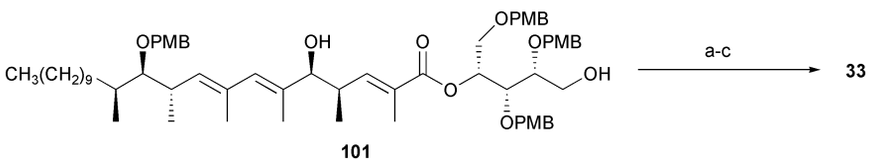
(2*E*,4*R*,5*R*,6*E*)-2,4,6-Trimethyl-5-(triphenylsiloxy)-7-iodo-2,6-heptadien-1-ol 11. To a solution of the ester (**4**) (9.00 g, 15.1 mmol) in CH_2Cl_2 (60 mL) at −78 °C was added dibutylaluminium hydride (40.5 mL, 37.7 mmol, 0.93 M in hexane) dropwise. After stirring at this temperature for 30 min, the reaction mixture was quenched with MeOH and then aqueous

Rochelle salt and EtOAc. The mixture was warmed to room temperature and stirred vigorously for 5 h. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 6 : 1) to give the alcohol (**11**) (8.06 g, 99%) as a colorless oil (Found: C, 60.60; H 5.67. $C_{28}H_{31}IO_2Si$ requires C, 60.65; H, 5.63%); $[a]_D^{26.6}$ +33.8 (*c* 0.895 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3367, 3062, 2965, 1428; δ_H (400 MHz, $CDCl_3$) 7.54 (6 H, dt, *J* 8.0, 1.5 Hz), 7.42 (3 H, tt, *J* 7.4, 1.5 Hz), 7.36 (6 H, dd, *J* 8.0, 7.4 Hz), 5.86 (1 H, s), 5.03 (1 H, dq, *J* 9.8, 1.0 Hz), 4.04 (1 H, d, *J* 8.0 Hz), 3.82 (3 H, d, *J* 1.0 Hz), 1.66 (3 H, d, *J* 1.0 Hz), 0.71 (3 H, d, *J* 6.8 Hz); δ_C (100 MHz, $CDCl_3$) 148.1, 135.5, 135.3, 134.1, 130.0, 128.8, 127.7, 82.8, 80.5, 68.6, 37.0, 19.5, 16.9, 14.0; *m*/*z* (ESI) 577 [M + Na]$^+$.

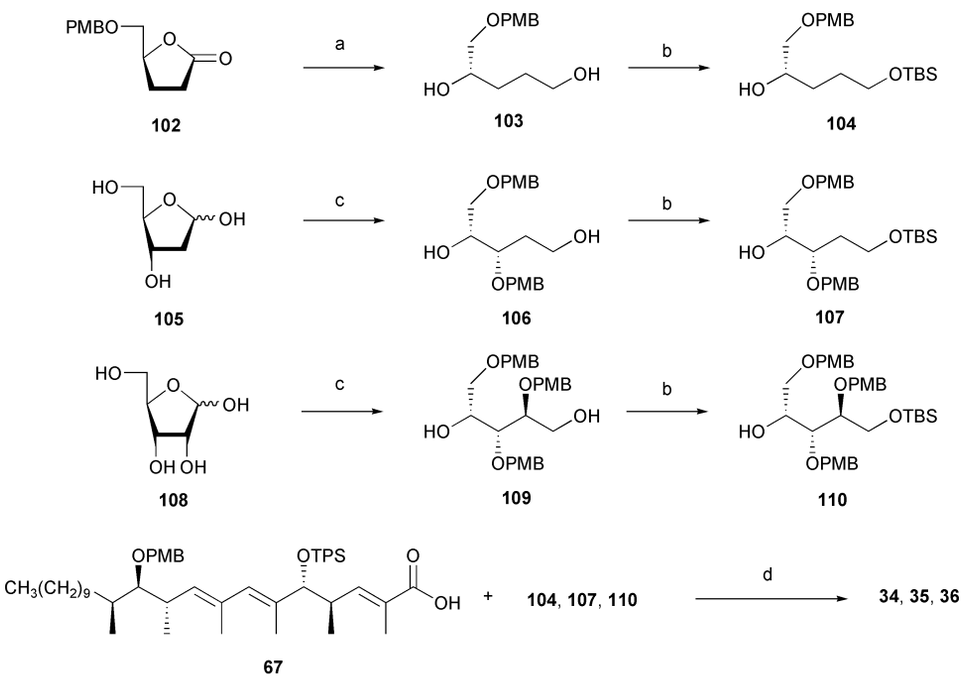
(2*E*,4*R*,5*R*,6*E*)-2,4,6-Trimethyl-5-(triphenylsiloxy)-7-iodo-2,6-heptadienoic acid 12. To a suspension of manganese dioxide (2.24 g, 25.8 mmol) in dichloromethane (13 mL) was added a solution of the alcohol (**11**) (1.39 g, 2.58 mmol) in dichloromethane (5 mL) at room temperature. After stirring at this temperature for 12 h, the reaction mixture was filtered through Celite and evaporated to give the crude aldehyde, which was used without further purification. To a solution of the crude aldehyde in *tert*-butyl alcohol (30 mL) and 2-methyl-2-butene (18 mL) was added a mixture of sodium chlorite (5.16 g) and sodium dihydrogenphosphate (5.16 g) in water (30 mL). After stirring for 20 h at room temperature, the reaction mixture was diluted with EtOAc and 0.5 M $KHSO_4$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with 10% $NaHSO_3$ and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 5 : 1) to give the acid (**12**) (1.27 g, 2 steps, 87%) as a colorless oil (Found: C, 58.92; H 5.32. $C_{28}H_{29}IO_3Si$ requires C, 59.15; H, 5.14%); $[a]_D^{23.5}$ +38.8 (*c* 1.37 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3441, 2972, 1683, 1645, 1426, 1271, 1068, 746, 706; δ_H (400 MHz, $CDCl_3$) 7.57 (6 H, dd, *J* 7.8, 1.2 Hz), 7.40–7.25 (9 H, m), 7.23 (2 H, d, *J* 8.6 Hz), 6.84 (2 H, d, *J* 8.6 Hz), 6.62 (1 H, dd, *J* 10.0, 1.3 Hz), 5.50 (1 H, s), 5.17 (1 H, d, *J* 9.8 Hz), 4.46 (1 H, d, *J* 10.7 Hz), 4.43 (1 H, d, *J* 10.7 Hz), 4.12 (2 H, q, *J* 7.2 Hz), 3.91 (1 H, d, *J* 6.6 Hz), 3.78 (3 H, s), 3.04 (1 H, dd, *J* 5.4, 5.1 Hz), 2.93–2.81 (1 H, m), 2.75–2.64 (1 H, m), 1.88 (3 H, d, *J* 1.3 Hz), 1.68 (3 H, s), 1.55 (3 H, s), 1.42–1.17(19 H, m), 1.22 (3 H, t, *J* 7.2 Hz), 0.98 (3 H, d, *J* 6.6 Hz), 0.94 (3 H, d, *J* 6.8 Hz), 0.88 (3 H, t, *J* 6.7 Hz), 0.78 (3 H, d, *J* 6.8 Hz); δ_C (100 MHz, $CDCl_3$) 168.1, 158.8, 145.9, 135.6, 134.5, 133.8, 133.7, 133.2, 131.6, 131.1, 129.7, 128.9, 127.7, 127.5, 113.5, 87.2, 84.6, 74.4, 60.3, 55.2, 38.2, 36.1, 35.7, 34.2, 31.9, 30.0, 29.7, 29.7, 29.6, 29.3, 27.3, 22.7, 18.4, 16.8, 16.4, 14.7, 14.2, 14.1, 13.1, 12.8; *m*/*z* (ESI) 591 [M + Na]$^+$; HRMS (ESI) calcd for $C_{28}H_{33}NO_3SiI$ (M + NH_4)$^+$ 586.1274, found 586.1273.



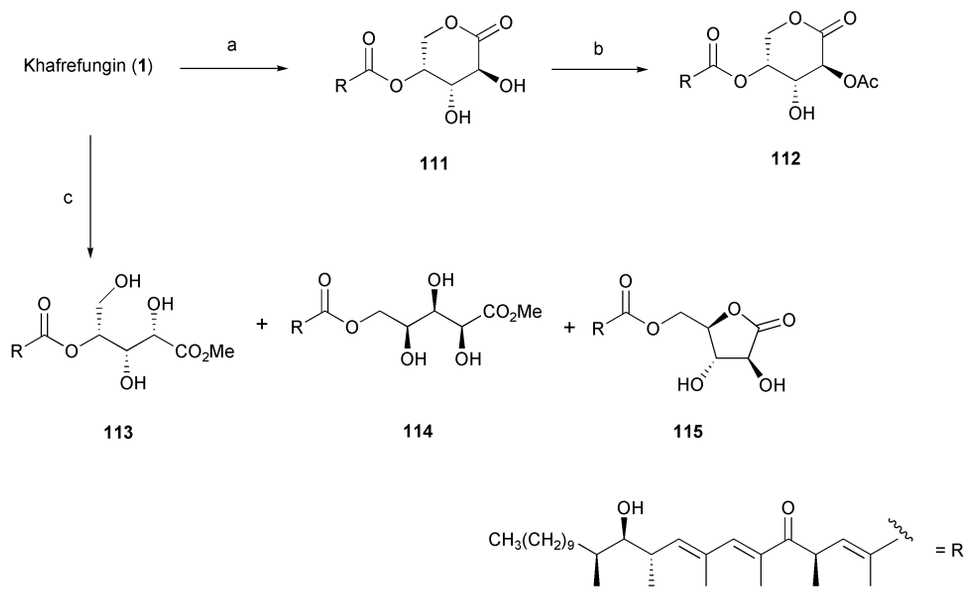
Scheme 17 Synthesis of **32**. Reagents and conditions: (a) HCl, THF, 80%; (b) Dess–Martin periodinane, pyridine, CH_2Cl_2, 60%; (c) TFA, CH_2Cl_2, 0 °C, 74%.



Scheme 18 Synthesis of **33**. Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 63%; (b) i) Dess–Martin periodinane, pyridine, CH_2Cl_2, ii) HCl, THF, 2 steps, 58%; (c) BCl_3, CH_2Cl_2, −78 °C, 46%.



Scheme 19 Synthesis of **34**, **35**, and **36**. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C, 97%; (b) TBSCl, imidazole, 70% (**104**), 90% (**107**), 77% (**110**); (c) i) Allyl alcohol, H_2SO_4, ii) PMBCl, TBAB, KOH, THF, H_2O, iii) DIBAL, $NiCl_2$(dppp), Et_2O, 20% (**107**), 47% (**110**); (d) i) EDCI·HCl, DMAP, CH_2Cl_2, reflux, ii) HCl, THF, 50 °C, iii) Dess–Martin periodinane, pyridine, CH_2Cl_2, iv) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, v) BCl_3, CH_2Cl_2, −78 °C.



Scheme 20 Modification of khafrefungin. Reagents and conditions: (a) TFA, CH_2Cl_2, rt, 41%; (b) Ac_2O, pyridine, CH_2Cl_2, rt, 10%; (c) $TMSCHN_2$, MeOH, 0 °C, 12% (**113**), 15% (**114**), 12% (**115**).

(1*R*,2*S*,3*R*)-1-[(4-Methoxybenzyloxy)methyl]-2,3-bis(4-methoxybenzyloxy)-4-(*tert*-butyldimethylsiloxy)butyl(2*E*,4*R*,5*R*,6*E*)-2,4,6-trimethyl-5-(triphenylsiloxy)-7-iodo-2,6-heptadienoate 13. To a solution of the acid (**12**) (1.90 g, 3.35 mmol) and the alcohol (**5**) (2.93 g, 4.68 mmol) in dichloromethane (35 mL) was added *N*,*N*-dimethylaminopyridine (1.23 g, 10.1 mmol), *N*,*N*-dimethylaminopyridine hydrogenchloride (1.06 g, 6.68 mmol), and dicyclohexylcarbodiimide (1.38 g, 6.69 mmol). The reaction mixture was heated at reflux for 6 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc. The organic layer was washed with 0.5 M $KHSO_4$, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 4 : 1) to give the adduct (**13**) (3.10 g, 79%) as a colorless oil (Found: C, 64.56; H 6.63. $C_{63}H_{77}IO_{10}Si_2$ requires C, 64.27; H, 6.59%); $[\alpha]_D^{23.1}$ +20.3 (*c* 0.995 in $CHCl_3$); ν_{max}(film)/cm^{-1} 1711, 1613, 1510, 1251, 1088, 837, 707; δ_H (300 MHz, $CDCl_3$) 7.62–7.53 (6 H, m), 7.45–7.31 (9 H, m), 7.22 (2 H, d, *J* 8.6 Hz), 7.18 (2 H, d, *J* 8.6 Hz), 7.13 (2 H, d, *J* 8.6 Hz), 6.82 (2 H, d, *J* 8.6 Hz), 6.79 (4 H, d, *J* 8.6 Hz), 6.73 (1 H, d, *J* 10.1 Hz), 5.93 (1 H, s), 5.33 (1 H, m), 4.55 (2 H, d, *J* 11.0 Hz), 4.46 (2 H, d, *J* 11.0 Hz), 4.45 (1 H, d, *J* 11.7 Hz), 4.34 (1 H, d, *J* 11.7 Hz), 4.20 (1 H, d, *J* 6.8 Hz), 3.94–3.73 (3 H, m), 3.78 (3 H, s), 3.78 (3 H, s), 3.75 (3 H, s), 3.73–3.57 (3 H, m), 2.88–2.78 (1 H, m), 1.85 (3 H, s), 1.66 (3 H, s), 0.89 (9 H, s), 0.86 (3 H, d, *J* 6.8 Hz), 0.02 (6 H, s); δ_C (75 MHz, $CDCl_3$) 167.2, 159.1, 159.1, 159.0, 147.4, 144.6, 135.5, 133.7, 130.7, 130.5, 130.4, 130.0, 129.8, 129.1, 129.1, 128.4, 127.8, 113.6, 113.6, 113.6, 81.8, 81.1, 79.6, 77.7, 74.0, 73.4, 73.3, 72.7, 68.3, 63.0, 55.2, 55.2, 55.2, 38.5, 25.9, 20.1, 18.2, 16.2, 12.9, −5.4, −5.4; *m*/*z* (ESI) 1199 (M + Na)$^+$; HRMS (ESI) calcd for $C_{63}H_{81}NO_{10}Si_2I$ (M + NH_4)$^+$ 1194.4444, found 1194.4437.

(1*R*,2*S*,3*R*)-1-[(4-Methoxybenzyloxy)methyl]-2,3-bis(4-methoxybenzyloxy)-4-(*tert*-butyldimethylsiloxy)butyl (2*E*,4*R*,5*R*,6*E*,8*E*,10*S*,11*R*,12*S*)-2,4,6,8,10,12-hexamethyl-5-(triphenylsiloxy)-11-(4-methoxybenzyloxy)-2,6,8-docosatrienoate 2. To a solution of alkyne (**3**) (1.09 g, 2.82 mmol) in THF (12 mL) was added 9-BBN (12.0 mL, 6.00 mmol, 0.5 M in THF) at 0 °C. After stirring at room temperature for 12 h, a solution of iodide (**13**) (2.76 g, 2.34 mmol) in THF (12 mL), H_2O (12 mL), K_3PO_4 (2.50 g, 11.8 mmol), and $PdCl_2$(dppf) (384 mg, 0.470 mmol) were added, and the reaction mixture was stirred at 65 °C for 30 min. The reaction mixture was cooled to room temperature, diluted with EtOAc, and filtered through Celite. The filtrate was washed with saturated $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 6 : 1) to give the adduct (**2**) (2.92 g, 84%) as a colorless oil; $[\alpha]_D^{26.0}$ −8.17 (*c* 0.655 in $CHCl_3$); ν_{max}(film)/cm^{-1} 1710, 1615, 1510, 1462, 1249, 1088; δ_H (400 MHz, $CDCl_3$) 7.56 (6 H, dd, *J* 7.8, 1.4 Hz), 7.38–7.23 (9 H, m), 7.22 (2 H, d, *J* 8.5 Hz), 7.21 (2 H, d, *J* 8.5 Hz), 7.16 (2 H, d, *J* 8.8 Hz), 7.10 (2 H, d, *J* 8.8 Hz), 6.84 (2 H, d, *J* 8.8 Hz), 6.80 (2 H, d, *J* 8.8 Hz), 6.77 (2 H, d, *J* 8.5 Hz), 6.77 (2 H, d, *J* 8.5 Hz), 6.75 (1 H, m), 5.52 (1 H, s), 5.31 (1 H, m), 5.14 (1 H, d, *J* 9.6 Hz), 4.56–4.38 (7 H, m), 4.32 (1 H, d, *J* 11.7 Hz), 3.98 (1 H, d, *J* 8.1 Hz), 3.90 (1 H, dd, *J* 5.4, 3.4 Hz), 3.85 (1 H, dd, *J* 11.3, 3.2 Hz), 3.81–3.75 (1 H, m), 3.78 (3 H, s), 3.76 (3 H, s), 3.76 (3 H, s), 3.73 (3 H, s), 3.68–3.56 (3 H, m), 3.03 (1 H, t, *J* 5.3 Hz), 2.93–2.82 (1 H, m), 2.74–2.62 (1 H, m), 1.90 (3 H, s), 1.64 (3 H, s), 1.54 (3 H, s), 1.41–1.20 (19 H, m), 0.96 (3 H, d, *J* 6.8 Hz), 0.93 (3 H, d, *J* 6.8 Hz), 0.88 (9 H, s), 0.82 (3 H, t, *J* 6.8 Hz), 0.81 (3H, d, *J* 6.8 Hz), 0.00 (6 H, s); δ_C (100 MHz, $CDCl_3$) 167.4, 159.1, 159.1, 159.0, 158.8, 146,6, 144.6, 135.6, 134.4, 133.8, 133.2, 131.6, 131.1, 130.8, 130.6, 130.4, 129.8, 129.8, 129.7, 129.1, 128.9, 127.7, 127.6, 113.6, 113.6, 113.6, 113.5, 87.1, 84.3, 79.7, 77.7, 74.4, 74.0, 73.4, 72.6, 68.4, 63.2, 55.2, 55.2, 55.2, 55.1, 38.5, 36.0, 35.7, 34.2, 31.9, 30.0, 29.7, 29.7, 29.6, 29.3, 27.3, 25.9, 22.7, 18.3, 18.3, 16.8, 16.5, 14.7, 14.1, 13.2, 13.0, −5.4, −5.5; *m*/*z* (ESI) 1459 (M + Na)$^+$.

Ethyl (2*E*,4*R*,5*R*,6*E*,8*E*,10*S*,11*R*,12*S*)-2,4,6,8,10,12-hexamethyl-5-(triphenylsiloxy)-11-(4-methoxybenzyloxy)-2,6,8-docosatrienoate 14. To a solution of alkyne (**3**) (3.49 g, 9.04 mmol) in THF (36 mL) was added 9-BBN (36.0 mL, 18.0 mmol, 0.5 M in THF) at 0 °C. After stirring at room temperature for 12 h, a solution of iodide (**4**) (4.50 g, 7.54 mmol) in DMF (36 mL), H_2O (36 mL), K_3PO_4 (7.64 g, 36.0 mmol), and $PdCl_2$(dppf) (1.23 g, 1.51 mmol) were added and the mixture was stirred at 65 °C for 30 min. The reaction mixture was cooled to room temperature, diluted with EtOAc, and filtered through Celite. The filtrate was washed with saturated $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 7 : 1) to give the adduct (**14**) (5.66 g, 88%) as a colorless oil; $[\alpha]_D^{23.2}$ −12.4 (*c* 0.605 in $CHCl_3$); ν_{max}(film)/cm^{-1} 1710, 1516, 1459, 1247, 1113, 706; δ_H (400 MHz, $CDCl_3$) 7.57 (6 H, dd, *J* 7.8, 1.2 Hz), 7.40–7.25 (9 H, m), 7.23 (2 H, d, *J* 8.6 Hz), 6.84 (2 H, d, *J* 8.6 Hz), 6.62 (1 H, dd, *J* 10.0, 1.3 Hz), 5.50 (1 H, s), 5.17 (1 H, d, *J* 9.8 Hz), 4.46 (1 H, d, *J* 10.7 Hz), 4.43 (1 H, d, *J* 10.7 Hz), 4.12 (2 H, q, *J* 7.2 Hz), 3.91 (1 H, d, *J* 6.6 Hz), 3.78 (3 H, s), 3.04 (1 H, dd, *J* 5.4, 5.1 Hz), 2.93–2.81 (1 H, m), 2.75–

2.64 (1 H, m), 1.88 (3 H, d, *J* 1.3 Hz), 1.68 (3 H, s), 1.55 (3 H, s), 1.42–1.17 (19 H, m), 1.22 (3 H, t, *J* 7.2 Hz), 0.98 (3 H, d, *J* 6.6 Hz), 0.94 (3 H, d, *J* 6.8 Hz), 0.88 (3 H, t, *J* 6.7 Hz), 0.78 (3 H, d, *J* 6.8 Hz); δ_C (100 MHz, $CDCl_3$) 168.1, 158.8, 145.9, 135.6, 134.5, 133.8, 133.7, 133.2, 131.6, 131.1, 129.7, 128.9, 127.7, 127.5, 113.5, 87.2, 84.6, 74.4, 60.3, 55.2, 38.2, 36.1, 35.7, 34.2, 31.9, 30.0. 29.7, 29.7, 29.6, 29.3, 27.3, 22.7, 18.4, 16.8, 16.4, 14.7, 14.2, 14.1, 13.1, 12.8; *m*/*z* (ESI) 879 $(M + Na)^+$. HRMS (ESI) calcd for $C_{56}H_{80}NO_5Si$ $(M + NH_4)^+$ 874.5806, found 874.5797.

(1*R*,2*S*,3*R*)-1-[(4-Methoxybenzyloxy)methyl]-2,3-bis(4-methoxybenzyloxy)-4-hydroxybutyl (2*E*,4*R*,5*R*,6*E*,8*E*,10*S*,11*R*,12*S*)-2,4,6,8,10,12-hexamethyl-5-hydroxy-11-(4-methoxybenzyloxy)-2,6,8-docosatrienoate 15. To a solution of the adduct (**2**) (2.95 g, 2.03 mmol) in THF (70 mL) was added a 1 M aqueous HCl solution (20 mL) at room temperature. After stirring at this temperature for 2 days, the reaction mixture was quenched with saturated $NaHCO_3$, and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 2 : 1) to give the diol (**15**) (1.82 g, 84%) as a colorless oil. The physical data of the diol (**15**) were completely consistent with those of an authentic sample.[4]

(2*S*,3*S*,4*R*)-2,3,5-Tris(4-methoxybenzyloxy)-4-[[(2*E*,4*R*,6*E*,8*E*,10*S*,11*R*,12*S*)-1,5-dioxo-2,4,6,8,10,12-hexamethyl-11-(4-methoxybenzyloxy)-2,6,8-docosatrienyl]oxy]pentanoic acid 16. To a solution of the diol (**15**) (1.58 g, 1.48 mmol) and pyridine (0.60 mL, 7.42 mmol) in dichloromethane (20 mL) was added Dess–Martin periodinane (2.51 g, 5.92 mmol) at 0 °C. After stirring at room temperature for 2.5 h, the reaction mixture was diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, 10% $NaHSO_3$, and brine, dried over Na_2SO_4 and evaporated. The residue was filtered through a short column of silica gel (hexane : EtOAc = 3 : 1) to give the ketoaldehyde as a colorless oil, which was used without further purification. To a solution of the ketoaldehyde in *tert*-butyl alcohol (18 mL) and 2-methyl-2-butene (11 mL) was added a mixture of sodium chlorite (2.96 g) and sodium dihydrogenphosphate (2.96 g) in water (18 mL). After stirring for 20 h at room temperature, the reaction mixture was diluted with EtOAc and 0.5 M $KHSO_4$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with 10% $NaHSO_3$ and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : acetone = 2 : 1) to give the ketoacid (**16**) (847 mg, 2 steps, 55%) as a colorless oil. The physical data of the ketoacid (**16**) were completely consistent with those of an authentic sample.[3]

Khafrefungin (1). To a solution of the ketoacid (**16**) (305 mg, 0.283 mmol) in dichloromethane (24 mL) was added BCl_3 (1.41 mL, 1.41 mmol, 1.0 M in heptane) at −78 °C. After stirring at this temperature for 5 min, the reaction mixture was quenched with saturated $NaHCO_3$ and poured into aqueous citric acid and EtOAc. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 10 : 1 : 0 to 4 : 1 : 0.1) and then freeze-dried by benzene to give khafrefungin (**1**) (86.1 mg, 51%) as a colorless amorphous powder. The physical data of the synthetic khafrefungin (**1**) were completely consistent with those of an authentic sample.[3]

Synthesis of derivative 20

(4*S*,5*R*,6*S*)-4,6-Dimethyl-5-acetoxy-2-hexadecyne 51. To a solution of fragment (**3**) (1.45 g, 3.76 mmol) in dichloromethane (4.0 mL) was added trifluoroacetic acid (2.0 mL) at 0 °C. After stirring at this temperature for 1 h, the reaction mixture was quenched with saturated $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was filtered through a short column of silica gel (hexane : EtOAc = 9 : 1) to give the crude alcohol as a colorless oil, which was used without further purification. To a solution of the crude alcohol in dichloromethane (3.6 mL) at 0 °C was added pyridine (3.6 mL) and acetic anhydride (3.6 mL). After stirring at room temperature for 3 h, 4-dimethylaminopyridine (22 mg, 0.18 mmol) was added, and the mixture was further stirred for 1 h. The reaction mixture was evaporated, and the residue was diluted with EtOAc. The organic layer was washed with saturated NH_4Cl, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : Et_2O = 40 : 1) to give the corresponding acetate (**51**) (938 mg, 2 steps, 81%) as a colorless oil. δ_H (300 MHz, $CDCl_3$) 4.73 (1 H, dd, *J* 6.4, 5.9 Hz), 2.74 (1 H, m), 2.11 (3 H, s), 1.78 (1 H, d, *J* 2.4 Hz), 1.40–1.18 (19 H, m), 1.10 (3 H, d, *J* 7.0 Hz), 0.88 (3 H, t, *J* 6.7 Hz), 0.86 (3 H, d, *J* 6.6 Hz); δ_C (75 MHz, $CDCl_3$) 170.9, 79.7, 79.0, 77.0, 34.5, 33.1, 31.9, 29.7, 29.6, 29.6, 29.6, 29.3, 28.6, 26.1, 22.7, 21.0, 17.9, 14.4, 14.1, 3.5.

(1*R*,2*S*,3*R*)-1-[(4-Methoxybenzyloxy)methyl]-2,3-bis(4-methoxybenzyloxy)-4-hydroxybutyl (2*E*,4*R*,5*R*,6*E*,8*E*,10*S*,11*R*,12*S*)-2,4,6,8,10,12-hexamethyl-5-(triphenylsiloxy)-11-acetoxy-2,6,8-docosatrienoate 52. To a solution of the alkyne (**51**) (450 mg, 1.46 mmol) in THF (5.8 mL) was added 9-BBN (5.84 mL, 2.92 mmol, 0.5 M in THF) at 0 °C. After stirring at room temperature for 12 h, a solution of the iodine (**13**) (1.56 g, 1.33 mmol) in DMF (6.0 mL), H_2O (6.0 mL), K_3PO_4 (1.41 g, 6.64 mmol), and $PdCl_2$(dppf) (217 mg, 0.266 mmol) were added, and the mixture was stirred at 65 °C for 30 min. The reaction mixture was cooled to room temperature, diluted with EtOAc, and filtered through Celite. The filtrate was washed with saturated $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and evaporated. The resulting residue was filtered through a short column of silica gel (hexane : EtOAc = 7 : 1) to give the corresponding adduct as a colorless oil, which was used without further purification. To a solution of the adduct in acetonitrile (70 mL) at −10 °C was added aqueous 46% hydrogen fluoride (0.7 mL) dropwise. After stirring at this temperature for 3 h, the reaction mixture was quenched with saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 2 : 1) to give the corresponding alcohol (**52**) (692 mg, 2 steps, 42%) as a colorless oil and diol (310 mg, 2 steps, 24%) as a colorless oil. ν_{max}(film)/cm^{-1} 3498, 2927, 1710, 1615, 1515, 1460, 1247, 1039, 823, 707; δ_H (400 MHz, $CDCl_3$) 7.56 (6 H, d, *J* 7.5 Hz), 7.40–7.23 (9 H, m), 7.23–7.06 (6 H, m), 6.88–6.73 (7 H, m), 5.46 (1 H, s), 5.29 (1 H, m), 4.98 (1 H, d, *J* 9.5 Hz), 4.77 (1 H, dd, *J* 6.1, 5.4 Hz), 4.54 (1 H, *J* 10.5 Hz), 4.48 (1 H, d, *J* 10.9 Hz), 4.46 (1 H, d, *J* 11.7 Hz), 4.43 (1 H d, *J* 11.7 Hz), 4.43 (1 H, d, *J* 10.5 Hz), 4.39 (1 H, d, *J* 10.9 Hz), 3.98 (1 H, d, *J* 7.3 Hz), 3.99–3.50 (6 H, m), 3.77 (3 H, s), 3.77 (3 H, s), 3.75 (3 H, s), 2.88 (1 H, m), 2.70 (1 H, m), 1.98 (3 H, s), 1.90 (3 H, s), 1.60 (3 H, s), 1.53 (3 H, s), 1.40–1.15 (18 H, m), 1.09(1 H, m), 0.90 (3 H, d, *J* 6.8 Hz), 0.88 (3 H, t, *J* 6.8 Hz), 0.82 (3 H, d, *J* 6.6 Hz); δ_C (100 MHz, $CDCl_3$) 170.7, 167.4, 159.2, 159.2, 159.1, 146.6, 135.6, 134.5, 134.4, 134.4, 132.5, 132.1, 131.7, 130.7, 130.3, 130.2, 129.9, 129.8, 129.6, 129.2, 127.6, 113.7, 113.7, 113.7, 84..0, 80.0, 79.1, 79.0, 74.0, 73.2, 72.8, 72.8, 68.0, 61.9, 55.2, 55.2, 55.2, 38.7, 34.5, 34.4, 33.6, 31.9, 29.9, 29.6, 29.6, 29.6, 29.3, 26.9, 22.6, 20.9, 17.5, 16.7, 16.5, 14.1, 14.0, 13.2, 12.9.

(2*S*,3*S*,4*R*)-2,3,5-Tris(4-methoxybenzyloxy)-4-[[(2*E*,4*R*,6*E*,-8*E*,10*S*,11*R*,12*S*)-1-oxo-2,4,6,8,10,12-hexamethyl-5-(triphenylsiloxy)-11-hydroxy-2,6,8-docosatrienyl]oxy]pentanoic acid 53. To a solution of the alcohol (**52**) (692 mg, 0.556 mmol) and pyridine (0.180 mL, 2.23 mmol) in dichloromethane (10.0 mL) was added Dess–Martin periodinane (472 mg, 1.11 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, 10% $NaHSO_3$, and brine, dried over Na_2SO_4, and evaporated. The residue was filtered through a short column of silica gel (hexane : EtOAc = 4 : 1) to give the corresponding aldehyde as a colorless oil, which was used without further purification. To a solution of the aldehyde in *tert*-butyl alcohol (5.5 mL) and 2-methyl-2-butene (3.3 mL) was added a mixture of sodium chlorite (1.1 g) and sodium dihydrogenphosphate (1.1 g) in water (5.5 mL). After stirring for 20 h at room temperature, the reaction mixture was diluted with EtOAc and 0.5 M $KHSO_4$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with 10% $NaHSO_3$ and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : acetone = 3 : 1) to give the corresponding acid (462 mg, 2 steps, 66%) as a colorless oil. ν_{max}(film)/cm^{-1} 3497, 2927, 2857, 1712, 1614, 1515, 1248, 1037, 823, 744, 707; δ_H (400 MHz, $CDCl_3$) 7.55 (6 H, d, *J* 7.1 Hz), 7.38–7.25 (9 H, m), 7.16 (2 H, d, *J* 8.5 Hz), 7.14 (2 H, d, *J* 8.5 Hz), 7.06 (2 H, d, *J* 8.5 Hz), 6.79 (2 H, d, *J* 8.5 Hz), 6.78 (2 H, d, *J* 8.5 Hz), 6.78 (1 H, m), 6.76 (2 H, d, *J* 8.5 Hz), 5.50 (1 H, s), 5.26 (1 H, ddd, *J* 7.6, 3.8, 3.1 Hz), 4.99 (1 H, d, *J* 9.5 Hz), 4.76 (1 H, dd, *J* 6.1, 5.9 Hz), 4.46 (1 H, d, *J* 10.3 Hz), 4.44 (1 H, d, *J* 11.4 Hz), 4.40 (1 H, d, *J* 10.3 Hz), 4.36–4.21 (6 H, m), 4.04–3.97 (2 H, m), 3.76 (3 H, s), 3.74 (3 H, s), 3.74 (3 H, s), 2.94–2.81 (1 H, m), 2.75–2.65 (1 H, m), 1.98 (3 H, s), 1.88 (3 H, s), 1.67 (1 H, m), 1.60 (3 H, s), 1.52 (3 H, s), 1.37–1.17 (17 H, m), 1.09 (1 H, m), 0.90 (3 H, d, *J* 6.8 Hz), 0.88 (3 H, d, *J* 6.8 Hz), 0.88 (3 H, t, *J* 6.8 Hz), 0.83 (3 H, d, *J* 6.8 Hz): δ_C (100 MHz, $CDCl_3$) 172.2, 170.8, 167.0, 159.7, 159.4, 159.2, 147.0, 135.6, 134.5, 134.4, 132.6, 132.1, 131.8, 130.3, 130.0, 129.8, 129.5, 129.3, 128.2, 127.7, 127.6, 127.5, 114.0, 113.8, 113.7, 84.3, 80.1, 78.0, 77.6, 74.5, 74.1, 72.9, 71.6, 67.7, 55.2, 55.2, 55.2, 38.7, 34.5, 34.4, 33.6, 31.9, 29.9, 29.6, 29.6, 29.6, 29.3, 27.0, 22.7, 20.9, 17.6, 16.7, 16.5, 14.1, 14.0, 13.2, 12.9.

To a solution of the acid (20.0 mg, 0.0159 mmol) in CH_2Cl_2 (0.6 mL) at −78 °C was added dibutylaluminium hydride (0.060 mL, 0.056 mmol, 0.93 M in hexane) dropwise. After stirring at this temperature for 10 min, the reaction mixture was quenched with MeOH and then added aqueous Rochelle salt and EtOAc. The mixture was warmed to room temperature and stirred vigorously for 5 h. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : acetone = 2 : 1) to give the corresponding seco acid (**53**, 9.3 mg, 48%) as a colorless oil. ν_{max}(film)/cm^{-1} 3497, 2926, 1711, 1615, 1515, 1461, 1249, 1037, 822, 744, 707; δ_H (400 MHz, $CDCl_3$) 7.56 (6 H, d, *J* 7.6 Hz), 7.35–7.27 (9 H, m), 7.18 (2 H, d, *J* 8.5 Hz), 7.13 (2 H, d, *J* 8.5 Hz), 7.07 (2 H, d, *J* 8.5 Hz), 6.78 (2 H, d, *J* 8.5 Hz), 6.78 (2 H, d, *J* 8.5 Hz), 6.77 (1 H, m), 6.76 (2 H, d, *J* 8.5 Hz), 5.58 (1 H, s), 5.24 (1 H, ddd, *J* 8.3, 4.9, 3.4 Hz), 4.85 (1 H, d, *J* 10.0 Hz), 4.50–4.38 (6 H, m), 4.35–4.22 (3 H, m), 4.07–3.99 (2 H, m), 3.75 (3 H, s), 3.75 (3 H, s), 3.74 (3 H, s), 8.18 (1 H, dd, *J* 8.0, 3.7 Hz), 2.95–2.80 (1 H, m), 2.58–2.47 (1 H, m), 1.86 (3 H, s), 1.59 (3 H, s), 1.55 (1 H, m), 1.35–1.18 (9 H, m), 0.88 (3 H, d, *J* 6.8 Hz), 0.93–0.85 (9 H, m); δ_C (100 MHz, $CDCl_3$) 172.6, 167.0, 159.6, 159.3, 159.1, 146.7, 135.6, 134.6, 134.3, 133.7, 132.6, 132.4, 130.3, 130.0, 129.9, 129.4, 129.3, 128.3, 127.7, 127.7, 127.5, 113.9, 113.7, 113.6, 83.6, 78.2, 77.6, 77.5, 74.4, 73.8, 72.9, 71.6, 67.7, 55.2, 55.2, 55.2, 38.6, 36.3, 34.5, 34.4, 31.9, 29.9, 29.7, 29.7, 29.6, 29.3, 27.4, 22.7, 17.1, 17.1, 16.3, 14.1, 13.7, 12.9, 12.8.

(3*S*,4*S*,5*R*,8*E*,10*R*,11*R*,12*E*,14*E*,16*S*,17*R*)-3,4-Bis(4-methoxybenzyloxy)-5-[(4-methoxybenzyloxy)methyl]-8,10,12,14,16-pentamethyl-11-(triphenylsiloxy)-17-[(1*S*)-1-methylundecyl]-1,6-dioxa-8,12,14-cycloheptadecatriene-2,7-dione 54. To a solution of the seco acid (**53**, 106.2 mg, 0.0873 mmol) and triethylamine (0.057 mL, 0.411 mmol) in THF (3.0 mL) was added 2,4,6-trichlorobenzoyl chloride (0.035 mL, 0.23 mmol) at room temperature. After stirring at this temperature for 2 h, DMAP (150 mg, 1.23 mmol) in toluene (78 mL) was added, and the mixture was stirred for further 15 h at reflux then cooled to room temperature. The reaction mixture was washed with 0.5 M $KHSO_4$, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 4 : 1) to give the corresponding macrocycle (**54**, 42.0 mg, 40%) as a colorless oil. $[a]_D^{23.1}$ +40.0 (*c* 0.25 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3503, 2926, 2856, 1748, 1710, 1614, 1513, 1248, 1096, 1036, 823, 712; δ_H (400 MHz, $CDCl_3$) 7.63 (6 H, d, *J* 8.1 Hz), 7.45–7.32 (9 H, m), 7.18 (2 H, d, *J* 8.8 Hz), 7.14 (2 H, d, *J* 8.8 Hz), 7.08 (2 H, d, *J* 8.8 Hz), 6.79 (2 H, d, *J* 8.8 Hz), 6.76 (2 H, d, *J* 8.8 Hz), 6.73 (2 H, d, *J* 8.8 Hz), 6.43 (1 H, d, *J* 10.2 Hz), 5.58 (1 H, s), 5.17 (1 H, d, *J* 7.8 Hz), 4.95 (1 H, d, *J* 9.8 Hz), 4.85 (1 H, m), 4.72 (1 H, d, *J* 10.5 Hz), 4.59 (1 H, d, *J* 11.5 Hz), 4.45 (1 H, d, *J* 12.2 Hz), 4.38 (1 H, m), 4.17 (1 H, m), 4.12 (1 H, m), 3.80–3.70 (2 H, m), 3.77 (3 H, s), 3.76 (3 H, s), 3.69 (3 H, s), 2.89–2.79 (1 H, m), 2.75–2.53 (1 H, m), 1.81 (3 H, s), 1.76 (3 H, s), 1.75–1.57 (2 H, m), 1.51 (3 H, s), 1.41 (1 H, m), 1.35–1.10 (16 H, m), 1.10 (3 H, d, *J* 5.8 Hz), 1.01 (3 H, d, *J* 6.1 Hz), 0.95 (3 H, d, *J* 7.1 Hz), 0.88 (3 H, d, *J* 6.4 Hz); δ_C (100 MHz, $CDCl_3$) 171.5, 166.3, 159.2, 159.1, 159.1, 143.9, 135.4, 134.5, 134.4, 134.3, 134.3, 134.1, 132.9, 132.6, 130.0, 130.0, 129.1, 129.0, 127.8, 127.8, 127.0, 113.7, 113.6, 113.5, 82.6, 80.1, 77.2, 73.4, 72.8, 72.6, 72.6, 71.4, 67.6, 55.2, 55.2, 55.1, 38.7, 40.2, 35.2, 34.7, 34.0, 31.9, 29.8, 29.7, 29.7, 29.6, 29.3, 27.4, 22.7, 18.8, 16.8, 16.2, 14.4, 14.2, 14.1, 12.3. *m/z* (ESI) 1121 $(M + Na)^+$.

(3*S*,4*S*,5*R*,8*E*,10*R*,12*E*,14*E*,16*S*,17*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-8,10,12,14,16-pentamethyl-17-[(1*S*)-1-methylundecyl]-1,6-dioxa-8,12,14-cycloheptadecatriene-2,7,11-trione 20. To a solution of the macrocycle (**54**, 62.5 mg, 0.0521 mmol) in acetonitrile (5.0 mL) was added 46% hydrogen fluoride in water (0.2 mL) at 0 °C. After stirring at this temperature for 10 h, the reaction mixture was quenched with saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 3 : 1) to give the corresponding alcohol (26.6 mg, 54%) as a colorless oil and recovered starting material (5.8 mg, 9%). $[a]_D^{25.1}$ + 26.9 (*c* 1.33 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3506, 2927, 1745, 1712, 1613, 1515, 1465, 1248, 1093, 1038, 820, 746; δ_H (400 MHz, $CDCl_3$) 7.25 (2 H, d, *J* 8.5 Hz), 7.21 (2 H, d, *J* 8.5 Hz), 7.12 (2 H, d, *J* 8.5 Hz), 6.83 (2 H, d, *J* 8.5 Hz), 6.80 (2 H, d, *J* 8.5 Hz), 6.79 (2 H, d, *J* 8.5 Hz), 6.54 (1 H, dq, *J* 9.5, 1.2 Hz), 5.84 (1 H, s), 5.27 (1 H, ddd, *J* 9.3, 3.4, 2.9 Hz), 5.00 (1 H, d, *J* 9.8 Hz), 4.84 (1 H, t, *J* 5.1 Hz), 4.77 (1 H, d, *J* 10.5 Hz), 4.60 (1 H, d, *J* 10.5 Hz), 4.47 (1 H, d, *J* 11.7 Hz), 4.43 (2 H, d, *J* 11.4 Hz), 4.42 (1 H, d, *J* 11.4 Hz), 4.41 (1 H, d, *J* 11.7 Hz), 4.32 (1 H, dd, *J* 9.3, 2.2 Hz), 4.05 (1 H, d, *J* 3.4 Hz), 3.94 (1 H, d, *J* 2.2 Hz), 3.78 (1 H, m), 3.78 (3 H, s), 3.77 (3 H, s), 3.77 (3 H, s), 3.73 (1 H, dd, *J* 11.3 Hz), 2.91–2.78 (2 H, m), 1.84 (3 H, d, *J* 1.2 Hz), 1.81 (3 H, d, *J* 0.7 Hz), 1.73 (1 H, m), 1.68 (3 H, s), 1.63 (1 H, m), 1.43 (1 H, m), 1.36–1.08 (16 H, m), 1.10 (3 H, d, *J* 7.1 Hz), 0.99 (3 H, d, *J* 6.8 Hz), 0.93 (3 H, d, *J* 6.8 Hz), 0.88 (3 H, t, *J* 6.8 Hz); δ_C (100 MHz, $CDCl_3$) 171.3, 166.6, 159.3, 159.1, 159.1, 142.6, 135.0, 133.1, 132.3, 130.7, 130.4, 130.3, 130.2, 129.3, 129.2, 129.1, 127.7, 113.7, 113.7, 113.5, 82.9, 77.7, 77.5, 75.7, 73.5, 72.6, 72.5, 71.4, 67.8, 55.2, 55.2, 55.2, 38.3, 35.6, 34.5, 33.7,

31.9, 29.8, 29.7, 29.7, 29.6, 29.3, 27.3, 22.7, 19.3, 16.9, 15.5, 14.9, 14.7, 14.1, 12.6.

To a solution of the alcohol (9.7 mg, 0.010 mmol) and pyridine (0.004 mL, 0.05 mmol) in dichloromethane (0.5 mL) was added Dess–Martin periodinane (8.7 mg, 0.021 mmol) at 0 °C. After stirring at room temperature for 30 min, the reaction mixture was diluted with EtOAc The organic layer was washed with saturated $NaHCO_3$, 10% $NaHSO_3$, and brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 3 : 1) to give the corresponding ketone (9.0 mg, 93%) as a colorless oil. δ_H (400 MHz, $CDCl_3$) 7.25 (2 H, d, *J* 8.8 Hz), 7.16 (2 H, d, *J* 8.8 Hz), 7.10 (2 H, d, *J* 8.8 Hz), 6.81 (2 H, d, *J* 8.8 Hz), 6.81 (2 H, d, *J* 8.8 Hz), 6.80 (1 H, s), 6.78 (2 H, d, *J* 8.8 Hz), 6.67 (1 H, dq, *J* 9.3, 1.2 Hz), 5.30 (1 H, m), 4.87 (1 H, dq, *J* 7.0, 1.2 Hz), 4.78 (1 H, d, *J* 11.8 Hz), 4.53 (1 H, d, *J* 10.5 Hz), 4.45 (1 H, d, *J* 11.8 Hz), 4.43 (1 H, d, *J* 11.8 Hz), 4.39 (1 H, d, *J* 11.8 Hz), 4.36 (1 H, d, *J* 10.5 Hz), 4.18 (1 H, dd, *J* 9.5, 1.2 Hz), 3.78 (3 H, s), 3.77 (3 H, s), 3.77 (3 H, s), 3.75 (1 H, m), 3.73 (2 H, m), 3.68 (1 H, d, *J* 2.4 Hz), 3.64 (1 H, m), 2.84 (1 H, m), 1.90 (3 H, d, *J* 1.2 Hz), 1.83 (3 H, d, *J* 1.0 Hz), 1.79 (3 H, d, *J* 1.2 Hz), 1.44–1.10 (19 H, m), 1.22 (3 H, d, *J* 6.9 Hz), 1.01 (3 H, d, *J* 7.1 Hz), 0.88 (3 H, d, *J* 6.4 Hz), 0.87 (3 H, t, *J* 6.7 Hz).

To a solution of the ketone (14.5 mg, 0.0154 mmol) in CH_2Cl_2 (2.0 mL) was added BCl_3 (0.070 mL, 0.070 mmol, 1.0 M in heptane) at −78 °C. After stirring at this temperature for 5 min, the reaction mixture was quenched with saturated $NaHCO_3$ and poured into aqueous citric acid. After addition of EtOAc, the organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : EtOAc = 1 : 1) to give macrocyclic khafrefungin (**20**, 6.5 mg, 73%) as a colorless oil. $[\alpha]_D^{25.1}$ +84 (*c* 0.16 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3466, 2926, 2856, 1743, 1714, 1652, 1457, 1384, 1229, 1126, 1079, 1028, 741; δ_H (400 MHz, $CDCl_3$) 6.79 (1 H, dq, *J* 9.8, 1.2 Hz), 6.76 (1 H, d, *J* 1.2 Hz), 5.28 (1 H, d, *J* 10.3 Hz), 5.08 (1 H, dt, *J* 9.3, 3.5 Hz), 4.76 (1 H, dq, *J* 7.8, 1.2 Hz), 4.17 (1 H, m), 4.10 (1 H, m), 3.98 (2 H, d, *J* 3.5 Hz), 3.73 (1 H, dq, *J* 9.8, 6.8 Hz), 3.26 (1 H, br s), 2.97 (1 H, br s), 2.88 (1 H, dq, *J* 6.8, 10.3 Hz), 2.56 (1 H, br s), 1.99 (3 H, d, *J* 1.2 Hz), 1.93 (3 H, d, *J* 1.2 Hz), 1.86 (3 H, d, *J* 1.2 Hz), 1.56 (1 H, m), 1.67 (18 H, m), 1.23 (3 H, d, *J* 6.8 Hz), 1.00 (3 H, d, *J* 6.8 Hz), 0.88 (3 H, d, *J* 6.8 Hz), 0.81 (3 H, d, *J* 6.8 Hz); δ_C (100 MHz, $CDCl_3$) 202.3, 172.1, 166.9, 143.6, 142.5, 142.4, 133.9, 132.2, 128.5, 85.1, 74.0, 69.8, 68.8, 62.5, 42.8, 36.0, 34.1, 33.0, 31.9, 29.9, 29.6, 29.6, 29.6, 29.3, 26.7, 22.7, 19.6, 16.1, 15.2, 14.5, 14.1, 14.0, 12.6; HRMS (ESI) calcd. for $C_{33}H_{53}O_8$ $(M - H)^-$ 577.3741, found 577.3748.

Synthesis of derivative 111

(3*R*,4*S*,5*S*)-4,5-Dihydroxy-6-oxotetrahydro-2*H*-pyran-3-yl (2*E*,4*R*,6*E*,8*E*,10*S*,11*R*,12*S*)-5-oxo-2,4,6,8,10,12-hexamethyl-11-hydroxy-2,6,8-docosatrienoate 111. To a solution of synthetic khafrefungin (**1**) (50.0 mg, 0.0839 mmol) in dichloromethane (1.0 mL) was added trifluoroacetic acid (0.25 mL) at 0 °C. After stirring at this temperature for 1 h, the reaction mixture was warmed to room temperature and further stirred for 2 h. The mixture was concentrated under reduced pressure. The resulting residue was purified by preparative thin layer chromatography on silica gel (hexane : EtOAc = 2 : 3) to give the derivative (**111**) (21.1 mg, 41%) as a colorless oil and recovered khafrefungin (**1**) (24.7 mg, 49%). δ_H (400 MHz, CD_3OD) 7.12 (1 H, s), 6.79 (1 H, dd, *J* 9.8, 1.4 Hz), 5.75 (1 H, d, *J* 9.8 Hz), 5.40 (1 H, ddd, *J* 3.2, 2.7, 2.2 Hz), 4.46 (1 H, dd, *J* 12.7, 2.2 Hz), 4.41 (1 H, dq, *J* 9.8, 6.8 Hz), 4.36 (1 H, dd, *J* 12.7, 2.7 Hz), 4.34 (1 H, d, *J* 10.0 Hz), 4.12 (1 H, dd, *J* 10.0, 3.2 Hz), 3.25 (1 H, dd, *J* 5.9, 5.6 Hz), 2.75 (1 H, m), 1.96 (3 H, d, *J* 1.5 Hz), 1.94 (3 H, s), 1.91 (3 H, d, *J* 1.0 Hz), 1.53 (1 H, m), 1.40–1.15 (18 H, m), 1.21 (3 H, d, *J* 6.6 Hz), 1.01 (3 H, d, *J* 6.8 Hz), 0.91 (3 H, d, *J* 6.8 Hz), 0.89 (3 H, t, *J* 7.1 Hz); HRMS (ESI) calcd. for $C_{33}H_{53}O_8$ $(M - H)^-$ 577.3741, found 577.3727.

Antifungal activity

Growth inhibition was determined by microtiter broth dilution assay in Difco yeast nitrogen base medium containing 2% glucose, 20 mg l^{-1} uracil, and 100 mg l^{-1} leucine with *Saccharomyces cerevisiae* (KA311A strain) inoculated at A_{600nm} = 0.01. Serial 3-fold dilutions of drugs were made from 200 μM. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration, which prevented visible growth after 24 hours at 30 °C.

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