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ORIGINAL ARTICLE

Enantioselective total synthesis of naturally occurring eushearilide and evaluation of its antifungal activity

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The asymmetric total synthesis of a newly proposed structure of (3*S*,16*E*,20*E*,23*S*)-(+)-eushearilide was achieved primarily through an asymmetric Mukaiyama aldol reaction, Schlosser-modified Wittig reaction and 2-methyl-6-nitrobenzoic anhydride-mediated macrolactonization. Based on detailed spectroscopic analyses, the obtained synthetic compound was found to be identical to natural eushearilide. Therefore, we were able to determine the true structure of eushearilide. Moreover, the synthetic compound was found to exhibit significant *in vitro* antifungal activity against various fungi and bacteria.
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INTRODUCTION

Eushearilide was first isolated from a culture of the fungus *Eupenicillium shearii* and structurally characterized in 2006 by Hosoe *et al.*[1] (Figure 1, structure **1**). This was found to be a very useful natural product as it exhibits a broad range of antifungal activity against diverse fungi and yeasts, such as the human pathogens *Aspergillus fumigatus*, *Trichophyton* spp. and *Candida* spp. The originally proposed planar structure of eushearilide (**1**) was shown to consist of a 24-membered lactone framework as the main part bearing two stereogenic centers (C3 and C23), whose absolute configurations were not determined. Moreover, the present compound was proposed to have a nonconjugated diene system (16*Z* and 20*E*) and a phosphorylcholine group. This is in contrast to typical polyene macrolides, which generally contain a conjugated polyene structure and a 1,3-polyol section, such as in the antifungal drugs amphotericin B, nystatin and pimaricin. These unique structural characteristics and a promising structure–activity relationship (SAR) prompted us to stereoselectively synthesize **1** in order to determine the absolute stereostructure of naturally occurring eushearilide and accelerate its SAR studies. While researching the synthesis of eushearilide,[2–5] Higashiyama *et al.*[6] reported the first total synthesis of **1**, but they stated that the synthetic product was not identical to the natural compound. However, we achieved the asymmetric total synthesis of (3*R*,23*R*)-**1** and its diastereomer ((3*S*,23*R*)-**1**) mainly through an asymmetric aldol reaction and 2-methyl-6-nitrobenzoic anhydride (MNBA)[7–14] mediated macrolactonization in the presence of a nucleophilic catalyst such as 4-(dimethylamino)pyridine (DMAP) or 4-(dimethylamino)pyridine *N*-oxide.[15] Based on detailed NMR analyses of these synthetic compounds, we revealed that natural eushearilide does not possess a (16*Z*,20*E*) configuration but rather a (16*E*,20*E*) configuration. We also successfully synthesized (3*R*,16*E*,20*E*,23*R*)-(−)-eushearilide, whose optical rotation sense was found to be opposite to that of the natural product.[15] We report herein the enantioselective total synthesis of a newly proposed structure of (3*S*,16*E*,20*E*,23*S*)-eushearilide (**2**) and evaluation of its antimicrobial activity.

RESULTS AND DISCUSSION

In order to synthesize **2**, we first performed the transformation to desired seco acid **13** starting from aldehyde **3** with phosphonium salt **4** (Scheme 1; For experimental procedures of **3** and **4**, see Supplementary Information). Stereoselective formation of the (*E*)-olefin moiety at C16-C17 in **2** could be achieved via a *trans*-selective modified Wittig olefination, also known as the Schlosser modification.[16–18] Transformation of aldehyde **3** with phosphonium salt **4** via the modified Wittig reaction was performed to afford desired coupling product **5** in good yield exclusively in the *trans*-form. The configuration of the double bond (C8-C9) in **5** was assigned as *E* based on the downfield shifts of the allylic methylene (δ_C 32.6 (C7) and δ_C 32.6 (C10)), while the *Z*-configuration was deduced from the upfield chemical shifts of the allylic methylene (δ_C 27.3 (C7) and δ_C 27.2 (C10)).[19–22] Successive deprotection of the terminal TBS group in **5** and subsequent oxidation of obtained alcohol **6** afforded corresponding aldehyde **7** in high yield. Aldol product **9**, which possesses a (3*S*,23*S*) configuration, was stereoselectively synthesized via the asymmetric Mukaiyama aldol reaction[23–25] of 1-ethylthio-1-(trimethylsiloxy)ethene (**8**), which was derived from *S*-ethyl propanethioate, with obtained aldehyde **7** in the presence of an (*R*)-diamine-Sn(II) complex as the catalyst with $^{n}Bu_3SnF$. Next, the transesterification of thioester **9** with silver trifluoroacetate was performed to provide corresponding ethyl ester **10**. Consecutive *p*-methoxybenzyl (PMB) protection of the secondary hydroxyl group in **10** and deprotection of the BOM group followed by saponification of the ethyl ester moiety yielded desired seco acid **13**, a precursor of the ring-closing product, in good yield.

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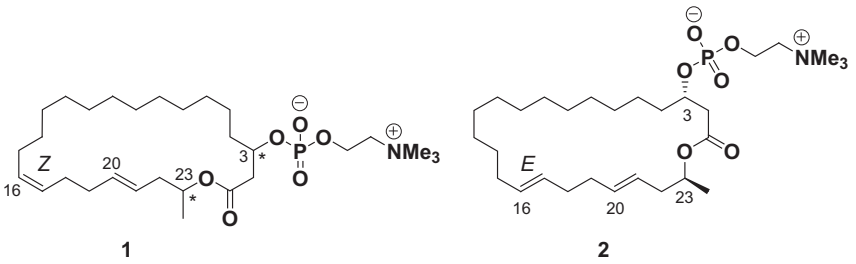
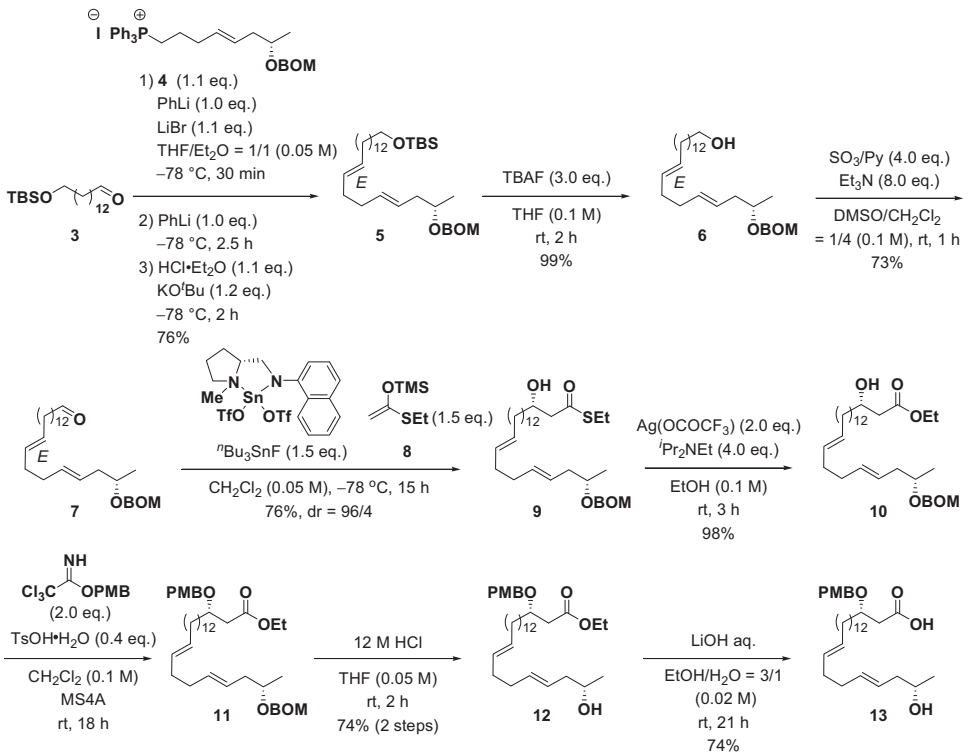
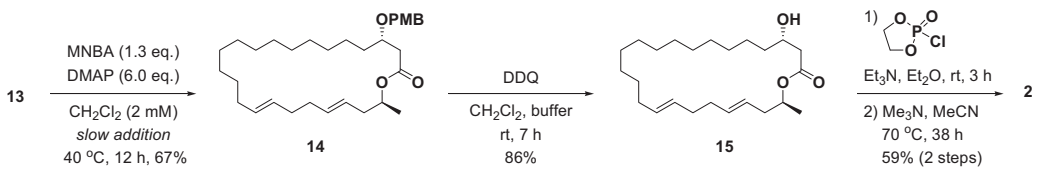


Figure 1 Original (**1**) and new (**2**) proposed structures of eushearilide.



Scheme 1 Transformation of **3** to seco acid **13**.

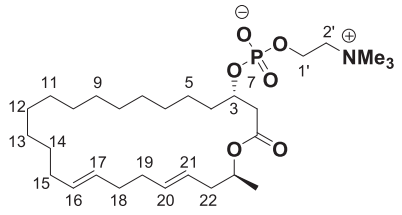


Scheme 2 Transformation of **13** to the naturally occurring eushearilide (**2**).

Next, we attempted the macrolactonization of seco acid **13** via the MNBA method in the presence of DMAP as a nucleophilic catalyst (Scheme 2).[7–15] An excess amount (6.0 eq.) of DMAP was employed with MNBA in dichloromethane at room temperature to afford 24-membered lactone **14** in 67% yield. After the successful macrolactonization using MNBA, a choline residue was attached to the obtained macrolactone framework. Following deprotection of the PMB group in **14** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, a phosphorylcholine moiety[26] was introduced on the secondary hydroxyl group in **15** to yield the desired (3*S*,16*E*,20*E*,23*S*)-**2**.

The ^{1}H and ^{13}C NMR spectra of the desired compound **2** were then compared with those of the natural product (Table 1). Owing to the newly stereoselective construction of the (*E*)-olefin moiety at C16-C17 in **2**, the chemical shifts of all carbons in **2** were in complete agreement with those of the natural product reported in the literature,[1] as clearly shown in Figure 2. Moreover, the optical rotation sense of **2** ($[\alpha]_D^{25}$+11.3 (*c* 0.85, MeOH)) was consistent with that reported for naturally occurring eushearilide ($[\alpha]_D^{25}$+12.8 (*c* 0.75, MeOH)).[1] From these results, we were able to determine the complete stereostructure of eushearilide.

Table 1 ^{1}H and ^{13}C NMR shifts (p.p.m.) of natural and synthetic eushearilide (2) in $CDCl_3$



2

	Natural		***2***	
Position	δ_H (J *in Hz*)	δ_C	δ_H (J *in Hz*)	δ_C (J *in Hz*)
1	—	171.8	—	171.8
2	2.54 dd (8.2, 14.2) 2.82 dd (4.2, 14.2)	41.9	2.54 dd (8.7, 14.5) 2.82 dd (4.3, 14.5)	41.9
3	4.54 m	74.1	4.54 m	74.1 d (5.8)
4	1.64 m	36.1	1.65 ddt (14.5, 14.5, 7.5)	36.1 d (5.7)
5	1.41 m; 1.46 m	25.4	1.40–1.50 m	25.4
6	1.30 br s	30.1	1.30 br s	30.1
7	1.30 br s	29.2[a]	1.30 br s	29.2[a]
8	1.30 br s	29.4[a]	1.30 br s	29.4[a]
9	1.30 br s	29.6[a]	1.30 br s	29.5[a]
10	1.30 br s	29.7[a]	1.30 br s	29.6[a]
11	1.30 br s	29.7[a]	1.30 br s	29.7[a]
12	1.30 br s	29.8[a]	1.30 br s	29.8[a]
13	1.30 br s	28.5	1.30 br s	28.5
14	1.36 m	29.5	1.36 m	29.5
15	2.00 br dd (6.0, 11.5)	32.8	2.00 br dd (5.8, 9.0)	32.8
16	5.37 br d (7.8)	131.8	5.37 br s	131.8
17	5.36 br d (7.8)	131.2	5.37 br s	131.2
18	2.06 m	33.6	2.07 br s	33.6
19	2.07 m	33.9	2.07 br s	33.9
20	5.50 m	134.7	5.50 m	134.7
21	5.39 dt (7.3, 15.1)	126.5	5.39 m	126.5
22	2.22 ddd (6.4, 7.3, 14.0) 2.28 ddd (6.9, 7.3, 14.0)	40.0	2.22 ddd (6.8, 6.8, 13.5) 2.28 ddd (6.8, 6.8, 13.5)	40.0
23	4.87 br q (6.4)	72.3	4.88 tq (6.8, 6.5)	72.3
23-Me	1.19 d (6.4)	19.7	1.19 d (6.5)	19.7
1′	4.26 m	60.3	4.26 m	60.3 d (4.3)
2′	3.62 m	67.5	3.63 m	67.5 d (3.6)
NMe_3	3.21 s	54.7	3.22 s	54.7 d (4.3)

[a]Carbon chemical shifts might be interchanged.

Finally, the *in vitro* antimicrobial activities of synthetic compound **2** and (3*R*,23*R*)-**1**, a stereoisomer of the originally proposed structure **1**, against various fungi and bacteria were assayed using the disk diffusion method according to the Clinical and Laboratory Standards Institute.[27,28] The inhibition zones were measured in millimeters against each microorganism, as shown in Table 2. The inhibition diameters of **2** against pathogenic bacteria *Staphylococcus aureus* (NBRC 12732) and pathogenic filamentous fungi *Aspergillus niger* (NBRC 105649) were 1.3 and 4.3 mm, respectively, at a concentration of 50 μg per disk. Moreover, the antimicrobial activity level of **2** against pathogenic filamentous fungi *Trichophyton mentagrophytes* (NBRC 5466) was relatively high (17.3 mm diameter) compared with the above two microorganisms. These results are well in line with the reported data in the literature[1] and exhibit the same or higher level of activity compared with the general antimicrobial agents, such as trichlosan, commonly used as a chemical drug for sterilization.[29] Moreover, (3*R*,23*R*)-**1** was also found to show the same tendency as in the case of compound **2**. Therefore, the geometrical isomeric structure of the olefin moiety at C16-C17 might not affect antimicrobial activity of eushearilide. Thus synthetic compound **2** was found to clearly exhibit antifungal activity against various microorganisms, as described in the literature.[1]

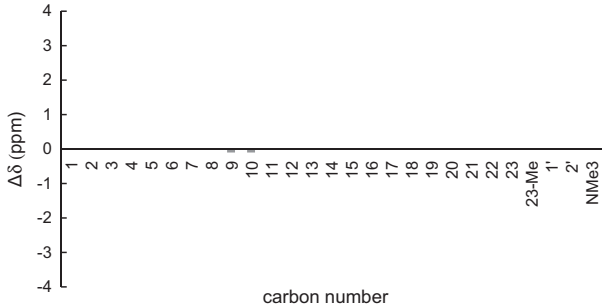


Figure 2 $\Delta\delta$ (p.p.m.) of the ^{13}C NMR chemical shifts of (3*S*,16*E*,20*E*,23*S*)-**2** in $CDCl_3$. $\Delta\delta$ corresponds to the difference in the chemical shift of the natural and synthetic products ($\Delta\delta = \delta$ (synthetic) $- \delta$ (natural)).

Table 2 Antimicrobial activities of synthetic eushearilide (2) and original structure of eushearilide (3*R*,23*R*)-1

		Inhibition zone[a] *(mm)*	
Microorganisms		***2***	*(3R,23R)-**1***
Staphylococcus aureus	NBRC 12732	1.3	2.2
Aspergillus niger	NBRC 105649	4.3	3.7
Trichophyton mentagrophytes	NBRC 5466	17.3	6.3

[a]The clear zone of inhibition (mm) around a paper disk impregnated with an antimicrobial agent at a concentration of 50 μg per disk.

In conclusion, we successfully achieved the first asymmetric total synthesis of naturally occurring eushearilide (**2**) and elucidated its true structure through detailed spectroscopic analyses; that is, (3*S*,16*E*,20*E*,23*S*)-(+)-eushearilide. Moreover, we confirmed that (3*S*,16*E*,20*E*,23*S*)-**2** demonstrated the expected antimicrobial activity against various fungi and bacteria.

MATERIALS AND METHODS

General experimental procedures

IR spectra were obtained using a Horiba FT-300 Fourier transform IR spectrometer (Horiba Ltd., Kyoto, Japan). NMR spectra were recorded on the following instrument: JEOL JNM-AL500 (^{1}H at 500 MHz and ^{13}C at 125 MHz; JEOL Ltd., Tokyo, Japan). Optical rotations were determined using a Jasco P-1020 polarimeter (JASCO Ltd., Tokyo, Japan). Mass spectra were determined by a Bruker Daltonics micrOTOF focus (ESI-TOF) mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA). HPLC was performed with a Hitachi LaChrom Elite system composed of the Organizer (Hitachi High-Technologies Corporation, Tokyo, Japan), L-2400 UV Detector and L-2130 Pump. All reactions were carried out under argon atmosphere in dried glassware unless otherwise noted.

Experimental procedures and characterization data

Experimental procedures of all new compounds are summarized in Supplementary Information.

(2S,4E,8E)-2-Benzyloxymethoxy-22-tert-butyldimethylsilyloxy-docosa-4,8-diene (5). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.33 (m, 5H, BOM), 5.51–5.38 (m, 4H, 4-H, 5-H, 8-H, 9-H), 4.79 (dd, J = 6.9, 14.6 Hz, 2H, BOM), 4.62 (dd, J = 12.0, 15.5 Hz, 2H, BOM), 3.79 (ddd, J = 6.3, 12.3, 12.5 Hz, 1H, 2-H), 3.59 (t, J = 6.3 Hz, 2H, 22-H), 2.22 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 3-H), 2.17 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 3-H), 2.05 (br s, 4H, 6-H, 7-H), 1.95 (dd, J = 6.9, 12.3 Hz, 2H, 10-H), 1.51 (tt, J = 6.9 Hz, 2H, 21-H), 1.31–1.25 (m, 20H, 11-H to 20-H), 1.17 (d, J = 6.3 Hz, 3H, 1-H), 0.897 (s, 9H, TBS), 0.056 (s, 6H, TBS). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 138.0 (BOM), 132.7 (C5), 130.8 (C9), 129.5 (C8), 128.4 (BOM), 127.8 (BOM), 127.6 (BOM), 126.2 (C4), 92.8 (BOM), 73.0 (C2), 69.3 (BOM), 63.4 (C22), 40.1 (C3), 32.9 (C21), 32.8 (C6), 32.6 (C7), 32.6 (C10), 29.7, 29.6, 29.6, 29.5, 29.2, 25.8 (C11 to C20), 26.0 (TBS), 19.9 (C1), 18.4 (TBS), −5.23 (TBS). HR-MS (ESI-TOF): *m/z* calcd for $C_{36}H_{64}O_3SiNa$ $[M+Na]^+$ 595.4517, found 595.4494. IR (neat): 2923, 2854 cm^{-1}. $[\alpha]_D^{23}$ −3.63 (*c* 1.00, $CHCl_3$).

(21S,14E,18E)-21-Benzyloxymethoxydocosa-14,18-dien-1-ol (6). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36–7.27 (m, 5H, BOM), 5.51–5.34 (m, 4H, 14-H, 15-H, 18-H, 19-H), 4.79 (dd, J = 7.5, 14.3 Hz, 2H, BOM), 4.61 (dd, J = 11.5, 15.5 Hz, 2H, BOM), 3.79 (ddd, J = 6.3, 12.3, 12.6 Hz, 1H, 21-H), 3.64 (t, J = 6.9 Hz, 2H, 1-H), 2.28 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 20-H), 2.16 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 20-H), 2.04 (br s, 4H, 16-H,17-H), 1.95 (dd, J = 6.9, 12.3 Hz, 2H, 13-H), 1.56 (tt, J = 6.9, 6.9 Hz, 2H, 2-H), 1.31–1.25 (m, 20H, 3-H to 12-H), 1.17 (d, J = 6.3 Hz, 3H, 22-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 138.0 (BOM), 132.7 (C18), 130.8 (C14), 129.5 (C15), 128.4 (BOM), 127.9 (BOM), 127.6 (BOM), 126.2 (C19), 92.8 (BOM), 73.0 (C21), 69.3 (BOM), 63.1 (C1), 53.9 (C2), 40.1 (C20), 32.8 (C17), 32.6 (C16), 32.6 (C13), 29.6, 29.6, 29.5, 29.4, 29.4, 29.2, 29.1, 25.7, 20.8 (C4 to C12), 14.1 (C3), 19.9 (C22). HR-MS (ESI-TOF): *m/z* calcd for $C_{30}H_{50}O_3Na$ $[M+Na]^+$ 481.3652, found 481.3659. IR (neat): 3402, 2923, 2854 cm^{-1}. $[\alpha]_D^{24}$ −3.28 (*c* 1.01, $CHCl_3$).

(21S,14E,18E)-21-Benzyloxymethoxydocosa-14,18-dienal (7). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.65 (t, J = 1.7 Hz, 1H, 1-H), 7.27–7.18 (m, 5H, BOM), 5.43–5.30 (m, 4H, 14-H, 15-H, 18-H, 19-H), 4.70 (dd, J = 6.9, 14.3 Hz, 2H, BOM), 4.53 (dd, J = 12.0, 15.5 Hz, 2H, BOM), 3.71 (ddd, J = 6.3, 12.3, 12.5 Hz, 1H, 21-H), 2.31 (ddd, J = 2.3, 7.2, 7.3 Hz, 2H, 3-H), 2.19 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 20-H), 2.07 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 20-H), 1.97 (br s, 4H, 16-H, 17-H), 1.88 (dd, J = 6.9, 12.9 Hz, 2H, 13-H), 1.53 (tt, J = 7.5, 7.5 Hz, 2H, 2-H), 1.25–1.18 (m, 18H, 4-H to 12-H), 1.09 (d, J = 6.3 Hz, 3H, 22-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 202.7 (C1), 138.0 (BOM), 132.6 (C18), 130.7 (C14), 129.4 (C15), 128.3 (BOM), 127.8 (BOM), 127.5 (BOM), 126.2 (C19), 92.7 (BOM), 72.9 (C21), 69.2 (BOM), 43.8 (C2), 40.0 (C20), 32.7 (C17), 32.5 (C16), 32.5 (C13), 29.6, 29.5, 29.5, 29.4, 29.3, 29.1 (C4 to C12), 22.0 (C3), 19.8 (C22). HR-MS (ESI-TOF): *m/z* calcd for $C_{30}H_{48}O_3Na$ $[M+Na]^+$ 479.3496, found 479.3514. IR (neat): 2924, 2854, 1727 cm^{-1}. $[\alpha]_D^{21}$ −3.91 (*c* 1.00, $CHCl_3$).

(3S,16E,20E,23S)-Ethyl 23-benzyloxymethoxy-3-hydroxytetracosa-16,20-dienthioate (9). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36–7.27 (m, 5H, BOM), 5.51–5.34 (m, 4H, 16-H, 17-H, 20-H, 21-H), 4.80 (dd, J = 7.5, 14.3 Hz, 2H, BOM), 4.61 (ddd, J = 11.5, 15.5 Hz, 2H, BOM), 4.05 (brs, 1H, OH), 3.80 (ddd, J = 6.3, 10.3, 12.6 Hz, 1H, 3-H), 2.90 (q, J = 7.5 Hz, 2H, Et), 2.74 (dd, J = 2.9, 15.5 Hz, 1H, 23-H), 2.65 (m, 2H, 2-H), 2.28 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 22-H), 2.17 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 22-H), 2.04 (br s, 4H, 18-H, 19-H), 1.95 (qn, J = 7.5 Hz, 2H, 15-H), 1.55–1.25 (m, 25H, 4-H to 14-H, Et), 1.17 (d, J = 6.3 Hz, 3H, 24-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 199.7 (C1), 138.0 (BOM), 132.7 (C20), 130.8 (C16), 129.4 (C17), 128.4 (BOM), 127.8 (BOM), 127.6 (BOM), 126.2 (C21), 92.8 (BOM), 73.0 (C23), 69.2 (BOM), 68.7 (C3), 50.6 (C2), 40.0 (C22), 36.5 (C4), 32.8 (C19), 32.6 (C18), 32.5 (C15), 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.1, 25.4 (C6 to C14, Et), 23.3 (C5), 19.9 (C24), 14.6 (Et). HR-MS (ESI-TOF): *m/z* calcd for $C_{34}H_{56}O_4Na$ $[M+Na]^+$ 583.3792, found 583.3778. IR (neat): 2924, 2854, 1727 cm^{-1}. $[\alpha]_D^{21}$ +4.87 (*c* 1.00, $CHCl_3$).

(3S,16E,20E,23S)-Ethyl 23-benzyloxymethoxy-3-hydroxytetracosa-16,20-dienoate (10). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.35–7.27 (m, 5H, BOM), 5.52–5.34 (m, 4H, 16-H, 17-H, 20-H, 21-H), 4.80 (dd, J = 7.5, 14.3 Hz, 2H, BOM), 4.61 (ddd, J = 12.0, 15.5 Hz, 2H, BOM), 4.17 (q, J = 6.9 Hz, 2H, Et), 4.00 (brs, 1H, OH), 3.80 (ddd, J = 6.3, 10.3, 12.6 Hz, 1H, 3-H), 2.94 (d, J = 4.0 Hz, 1H, 23-H), 2.51–2.48 (m, 2H, 2-H), 2.28 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 22-H), 2.15 (ddd, J = 6.3, 6.9, 10.0 Hz, 1H, 22-H), 2.04 (br s, 4H, 18-H, 19-H), 1.95 (m, 2H, 15-H), 1.63–1.25 (m, 25H, 4-H to 14-H, Et), 1.17 (d, J = 6.3 Hz, 3H, 24-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 173.1 (C1), 138.0 (BOM), 132.7 (C20), 130.8 (C16), 129.5 (C17), 128.4 (BOM), 127.8 (BOM), 127.6 (BOM), 126.2 (C21), 92.8 (BOM), 73.0 (C23), 69.3 (BOM), 68.0 (C3), 60.6 (Et), 41.3 (C2), 40.0 (C22), 36.5 (C4), 32.8 (C19), 32.6 (C18), 32.6 (C15), 29.6, 29.6, 29.6, 29.5, 29.2 (C6 to C14), 25.5 (C5), 19.9 (C24), 14.2 (Et). HR-MS (ESI-TOF): *m/z* calcd for $C_{34}H_{56}O_5Na$ $[M+Na]^+$ 567.4020, found 567.4000. IR (neat): 3464, 2924, 2854, 1728 cm^{-1}. $[\alpha]_D^{20}$ +4.27 (*c* 1.02, $CHCl_3$).

(3S,16E,20E,23S)-Ethyl 23-hydroxy-3-(p-methoxybenzyloxy)tetracosa-16,20-dienoate (12). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.24 (m, 2H, PMB), 6.86 (m, 2H, PMB), 5.58–5.34 (m, 4H, 16-H, 17-H, 20-H, 21-H), 4.46 (dd, J = 10.9, 15.8 Hz, 2H, PMB), 4.14 (dq, J = 1.7, 7.5 Hz, 2H, Et), 3.88–3.76 (m, 5H, 3-H, 23-H, PMB), 2.58 (q, J = 7.5 Hz, 1H, 22-H), 2.44 (dd, J = 5.7, 14.5 Hz, 1H, 22-H), 2.23–2.17 (m, 2H, 2-H), 2.13–2.04 (m, 4H, 18-H, 19-H), 1.96 (q, J = 6.9 Hz, 2H, 15-H), 1.63–1.49 (m, 2H, 4-H), 1.43–1.24 (m, 23H, 5-H to 14-H, Et), 1.18 (d, J = 6.3 Hz, 3H, 24-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.9 (C1), 159.1 (PMB), 134.2 (C20), 131.1 (C16), 130.7 (C17), 129.4 (PMB), 129.3 (PMB), 126.2 (C21), 113.7 (PMB), 75.8 (PMB), 71.2 (C23), 67.0 (C3), 60.4 (Et), 55.3 (PMB), 42.5 (C2), 40.0 (C22), 34.5 (C4), 32.7 (C19), 32.6 (C18), 32.5 (C15), 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.2 (C6 to C14), 25.2 (C5), 22.6 (C24), 14.2 (Et). HR-MS (ESI-TOF): *m/z* calcd for $C_{34}H_{56}O_5Na$ $[M+Na]^+$ 567.4020, found 567.4010. IR (neat): 3464, 2924, 2854, 1728 cm^{-1}. $[\alpha]_D^{20}$ +2.65 (*c* 1.00, $CHCl_3$).

(3S,16E,20E,23S)-23-Hydroxy-3-(p-methoxybenzyloxy)tetracosa-16,20-dienoic acid (13). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.25 (m, 2H, PMB), 6.87 (m, 2H, PMB), 5.56–5.34 (m, 4H, 16-H, 17-H, 20-H, 21-H), 4.51 (dd, J = 10.9, 14.3 Hz, 2H, PMB), 3.86–3.76 (m, 5H, 3-H, 23-H, PMB), 2.58 (dq, J = 5.2, 14.9 Hz, 2H, 22-H), 2.19 (dt, J = 5.2, 16.3 Hz, 2H, 2-H), 2.19–2.05 (m, 4H, 18-H, 19-H), 1.96 (dd, J = 6.3, 13.8 Hz, 2H, 15-H), 1.63 (m, 1H, 4-H), 1.55 (m, 1H, 4-H), 1.35–1.25 (m, 20H, 5-H to 14-H), 1.18 (d, J = 6.3 Hz, 3H, 24-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.0 (C1), 159.4 (PMB), 134.3 (C20), 131.1 (C16), 129.8 (C17), 129.6 (PMB), 129.4 (PMB), 126.1 (C21), 113.9 (PMB), 75.3 (PMB), 71.2 (C23), 67.1 (C3), 55.3 (PMB), 42.5 (C2), 39.0 (C22), 33.9 (C4), 32.7 (C19), 32.6 (C18), 32.5 (C15), 29.7, 29.6, 29.6, 29.5, 29.5, 29.1 (C6 to C14), 25.0 (C5), 22.5 (C24). HR-MS (ESI-TOF): *m/z* calcd for $C_{32}H_{52}O_5Na$ $[M+Na]^+$ 539.3707, found 539.3727. IR (neat): 3464, 2924, 2854, 1728 cm^{-1}. $[\alpha]_D^{20}$ +15.1 (*c* 1.00, $CHCl_3$).

(3S,16E,20E,23S)-3-(p-Methoxybenzyloxy)-23-methyltricosa-16,20-dienolide (14). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.26 (m, 2H, PMB), 6.87 (m, 2H, PMB), 5.47–5.29 (m, 4H, 16-H, 17-H, 20-H, 21-H), 4.91 (dt, J = 6.0, 6.5 Hz, 1H, 3-H), 4.47 (dd, J = 11.5, 40.4 Hz, 2H, PMB), 3.83–3.77 (m, 4H, 23-H, PMB), 2.65 (dd, J = 5.4, 14.0 Hz, 1H, 2-H), 2.43 (dd, J = 7.7, 14.0 Hz, 1H, 2-H), 2.24 (ddd, J = 5.7, 6.3, 7.5 Hz, 2H, 22-H), 2.04 (br s, 4H, 18-H, 19-H), 1.99 (dd, J = 6.3, 11.7 Hz, 2H, 15-H), 1.39–1.27 (m, 22 H, 4-H to 14-H), 1.19 (d, J = 6.0 Hz, 3H, 24-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.9 (C1), 159.1 (PMB), 133.5 (C20), 130.8 (C16), 130.6 (C17), 129.8 (PMB), 129.3 (PMB), 125.2 (C21), 113.7 (PMB), 75.5 (PMB), 70.7 (C23), 70.6 (C3), 55.3 (PMB), 40.0 (C2), 39.0 (C22), 33.9 (C4), 32.7 (C19), 32.4 (C18), 31.8 (C15), 28.8, 28.6, 28.5, 28.4, 28.2, 28.2, 27.5 (C6 to C14), 24.4 (C5), 19.4 (C24). HR-MS (ESI-TOF): *m/z* calcd for $C_{32}H_{50}O_4Na$ $[M+Na]^+$ 521.3601, found 521.3600. IR (neat): 2924, 2854, 1728 cm^{-1}. $[\alpha]_D^{20}$ −4.63 (*c* 1.00, $CHCl_3$).

(3S,16E,20E,23S)-3-Hydroxy-23-methyltricosa-16,20-dienolide (15). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.51–5.34 (m, 4H, 16-H, 17-H, 20-H, 21-H), 5.00 (dt, J = 1.5, 6.0 Hz, 1H, 3-H), 3.94 (m, 1H, 23-H), 2.54–2.39 (m, 2H, 2-H), 2.31–2.17 (m, 2H, 22-H), 2.05 (br s, 4H, 18-H, 19-H), 2.00 (d, J = 5.5 Hz, 2H, 15-H), 1.58–1.27 (m, 22 H, 4-H to 14-H), 1.24 (d, J = 6.0 Hz, 3H, 24-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.2 (C1), 133.7 (C20), 130.8 (C16), 129.8 (C17), 125.0 (C21), 70.8 (C23), 68.2 (C3), 41.4 (C2), 39.0 (C22), 36.1 (C4), 32.9 (C19), 32.5 (C18), 31.9 (C15), 28.7, 28.6, 28.4, 28.2, 28.2, 28.2, 28.1, 28.0, 27.5 (C6 to C14), 24.7 (C5), 19.6 (C24). HR-MS (ESI-TOF): *m/z* calcd for

$C_{24}H_{42}O_3Na$ $[M+Na]^+$ 401.3026, found 401.3008. IR (neat): 3410, 2924, 2854, 1728 cm^{-1}. $[\alpha]_D^{22}$ −13.5 (*c* 0.87, $CHCl_3$).

(3S,16E,20E,23S)-Eushearilide (2). ^{1}H-NMR (500 MHz, CD_3OD): δ 5.50 (m, 2H, 20-H), 5.39 (m, 2H, 21-H), 5.37 (br s, 4H, 16-H, 17-H), 4.88 (tq, *J* = 6.8, 6.5 Hz, 1H, 23-H), 4.54 (m, 1H, 3-H), 4.26 (m, 2H, 1′-H), 3.63 (m, 2H, 2′-H), 3.22 (s, 9H, NMe_3), 2.82 (dd, *J* = 4.3, 14.5 Hz, 1H, 2-H), 2.54 (dd, *J* = 8.7 14.5 Hz, 1H, 2-H), 2.28 (ddd, *J* = 6.8, 6.8, 13.5 Hz, 1H, 22-H), 2.22 (ddd, *J* = 6.8, 6.8, 13.5 Hz, 1H, 22-H), 2.07 (br s, 4H, 18-H, 19-H), 2.00 (br dd, *J* = 5.8, 9.0 Hz, 2H, 15-H), 1.65 (ddt, *J* = 14.5, 14.5, 7.5 Hz, 2H, 4-H), 1.50–1.40 (m, 2H, 5-H), 1.36 (m, 2H, 14-H), 1.30 (br s, 16H, 6-H to 13-H), 1.19 (d, *J* = 6.5 Hz, 3H, 23-Me). ^{13}C-NMR (125 MHz, CD_3OD): δ 171.8 (C1), 134.7 (C20), 131.8 (C16), 131.2 (C17), 126.5 (C21), 74.1 (C3), 72.3 (C23), 67.5 (C2′), 60.3 (C1′), 54.7 (C27 (NMe_3)), 41.9 (C2), 40.0 (C22), 36.1 (C4), 33.9 (C19), 33.6 (C18), 32.8 (C15), 30.1 (C6), 29.5 (C14), 29.8, 29.7, 29.6, 29.5, 29.4, 29.2 (C7 to C12), 28.5 (C13), 25.4 (C5), 19.7 (C24). HR-MS (ESI-TOF): *m/z* calcd for $C_{29}H_{54}NO_6PNa$ $[M+Na]^+$ 566.3586, found 566.3592. IR (neat): 3432, 2954, 2537, 2090, 1728 cm^{-1}. $[\alpha]_D^{25}$ +11.3 (*c* 0.85, CH_3OH).

Synthesis of 5 by using Schlosser-modified Wittig reaction

To a cooled (−78 °C) solution of **4** (16 mg, 0.048 mmol) in tetrahydrofuran (1.0 ml) were added LiBr (45 mg, 0.052 mmol) and PhLi (1.6 M in butyl ether, 0.03 ml, 0.048 mmol). After the solution was stirred at that temperature for 30 min, then the solution was added dropwise via cold cannula to a solution of **3** (30 mg, 0.048 mmol) in diethyl ether (1.0 ml) at − 78 °C. After completion of the dripping, PhLi (1.6 M in butyl ether, 0.03 ml, 0.048 mmol) was added to the mixture, and the solution was stirred for 3 h. After successive addition of HCl (1.0 M in Et_2O, 0.08 ml, 0.080 mmol) and KO^{t}Bu (1.0 M in Et_2O, 0.06 ml, 0.057 mmol), the reaction mixture was stirred for 1 h at − 78 °C. The reaction mixture was quenched with water, and the organic layer was separated and the aqueous layer was extracted with diethyl ether three times. The combined organic layer was washed with water and brine and dried over sodium sulfate. After evaporation of the solvent, the crude product was purified by TLC on silica gel (hexane/ethyl acetate = 10/1) to afford **5** (21 mg, 76%).

Synthesis of 14 by using MNBA-mediated macrolactonization with DMAP

To a solution of MNBA (20 mg, 57.9 μmol) and DMAP (33 mg, 0.27 mmol) in dichloromethane (18.0 ml) at room temperature was slowly added a solution of the seco acid **13** (23 mg, 44.5 μmol) in dichloromethane (4.0 ml) with a mechanically driven syringe over a 12 h period. After cooling to 0 °C, saturated aqueous sodium hydrogen carbonate was added. The organic layer was separated, the aqueous layer was extracted with dichloromethane, and the organic layer was washed with brine and water and dried over sodium sulfate. The combined organic layer was washed with water and brine and dried over sodium sulfate. After evaporation of the solvent, the crude product was purified by TLC on silica gel (hexane/ethyl acetate = 6/1) to afford **14** (15 mg, 67%).

Antimicrobial assay

The antimicrobial assay against *S. aureus* (NBRC 12732), *A. niger* (NBRC 105649) and *T. mentagrophytes* (NBRC 5466) was performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute.[13] Details of the assay method are described in Supplementary Information.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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