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Enantioselective synthesis and stereochemical determination of the highly reduced polyketide ishigamide

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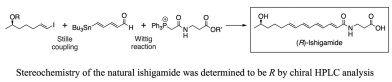
ABSTRACT

Ishigamide was isolated as a metabolite of a recombinant strain of *Streptomyces* sp. MSC090213JE08 and its unsaturated fatty acid moiety has been confirmed *in vitro* to be synthesized by a type II PKS. Biosynthesis of such a highly reduced polyketide by a type II PKS is worthy of note. However, absolute configuration of ishigamide remained unknown. (*R*)-Ishigamide was synthesized enantioselectively employing Stille coupling and Wittig reaction between three units, vinyl iodide, stannyldienal, and Wittig salt. Stereochemistry of natural ishigamide was determined to be *R* by chiral HPLC analysis comparing with the synthesized standard.

Graphical Abstract



Stereochemistry of the natural ishigamide was determined to be *R* by chiral HPLC analysis

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Keywords: ishigamide, determination of absolute configuration, tetraene

Even today, discovery of novel antibiotics is significant, because of unmet needs caused by drug-resistant bacteria. Diversity of secondary metabolites of microorganisms plays an important role as a source of new bioactive compounds. A number of secondary metabolites have been isolated from fermentation products in the past few decades (Newman and Cragg 2007), and synthetic studies of them are very important as well as elucidation of their biosynthesis pathways and their biological activities.

In the pathway that forms secondary metabolites, type II polyketide synthases (PKSs) are generally known as protein complexes to produce aromatic compounds (Hertweck *et al.* 2007; Hertweck 2009). However, a new subfamily of type II PKS, that is, highly reducing type II PKS, has been recently proposed

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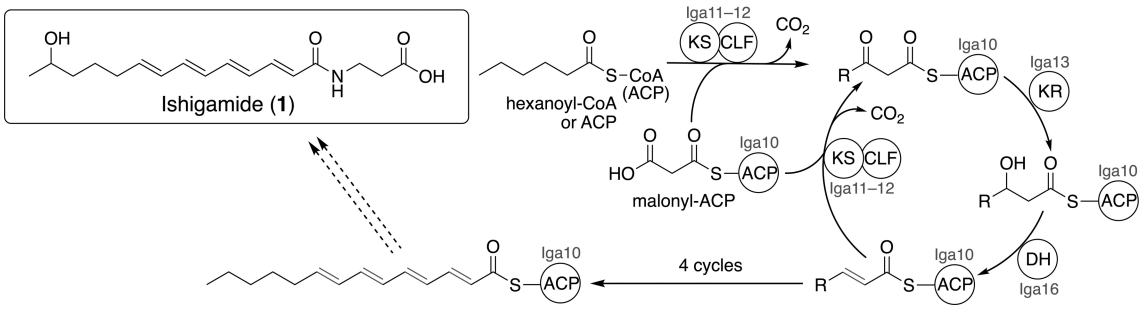
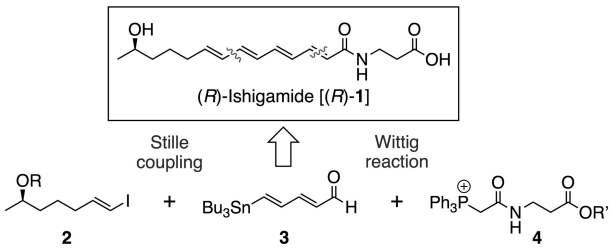


Figure 1. Predicted biosynthetic pathway of ishigamide (**1**) (Du *et al.* 2018). ACP: acyl carrier protein, KS: ketosynthase, KR: ketoreductase, DH: dehydratase, CLF: chain length factor.



Scheme 1. Synthetic plan for ishigamide (**1**) via coupling reaction of three units.

to biosynthesize highly reduced polyketides, such as polyene structures (Pohle *et al.* 2011; Bilyk *et al.* 2016). In 2016, a novel natural product, ishigamide (**1**), was isolated as a metabolite of a recombinant *Streptomyces* sp. MSC090213JE08 strain, in which a transcriptional activator (SARP-7) was overproduced, by Ohnishi and Katsuyama's group (Du *et al.* 2016). The unsaturated fatty acid moiety of ishigamide was confirmed *in vitro* to be synthesized by a type II PKS, IgaPKS (Du *et al.* 2018). In addition, the mechanism of polyene biosynthesis catalyzed by IgaPKS was proposed by the X-ray crystallography and biochemical analysis (Du *et al.* 2020). Predicted biosynthetic pathway of ishigamide is shown in Figure 1. IgaPKS assembles the polyene moiety of **1** through the repeating cycle of condensation, reduction, and dehydration, catalyzed by ketosynthase–chain length factor (KS–CLF), ketoreductase (KR), and dehydratase (DH), respectively. Biosynthesis of the highly reduced polyketide **1** by a new subfamily of type II PKS is worthy of note. However, its absolute configuration remained to be elucidated because of the low productivity of **1**. So, we aimed for enantioselective synthesis of **1** in order to determine the absolute configuration of natural ishigamide. In this paper, we report the concise synthesis of **1** and the determination of stereochemistry of natural **1**.

Results and discussion

Our synthetic plan for (*R*)-ishigamide is depicted in Scheme 1. We planned to access to tetraene structure of (*R*)-**1** by Stille coupling and Wittig reaction, between three units (vinyl iodide **2**, stannyldienal **3**, and Wittig salt **4**), which would be concisely prepared in several steps.

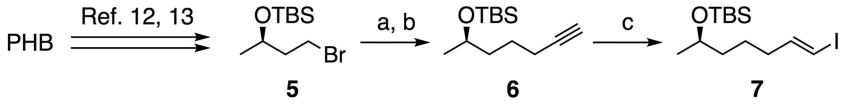
Preparation of vinyl iodide **2** is shown in Scheme 2. Several methods for synthesizing vinyl iodide **2** have been previously reported (Bernardas *et al.* 1995; Lauzon *et al.* 2008; Dermenci *et al.* 2011; Gadakh and Sudalai 2016), but we selected another concise method using poly[(*R*)-3-hydroxybutyrate] (PHB), a readily available biopolymer, as a starting material. According to the reported manner, PHB was ethanolyzed to ethyl (*R*)-3-hydroxybutyrate (Mori and Watanabe 1984), which was converted to the corresponding bromide **5** after reduction (Gadakh and Sudalai 2016). Three carbons were introduced by a reaction with 1-trimethylsilylpropargyllithium (Huang *et al.* 2007; Zhao *et al.* 2018) to give terminal alkyne **6** after deprotection. Treatment of **6** with Schwartz's reagent (Hart and Schwartz 1974) followed by NIS afforded **7** as the (*E*)-vinyl iodide **2**, the first unit for the coupling reaction, selectively.

The second unit, stannyldienal **3**, was prepared based on the reported procedure (Scheme 3). Pyridine was transformed into a pyridinium salt by Zincke reaction (Zincke 1904) with 2,4-dinitrochlorobenzene. The pyridine ring was then opened by treatment with dimethylamine to give Zincke aldehyde (Zincke and Würker 1904), which was converted to stannyldienal **3** via 1,6-addition-elimination reaction of stannyl anion (Michels *et al.* 2008; Yamamoto *et al.* 2017).

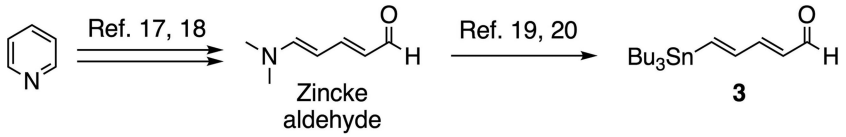
The third unit, Wittig salt, was prepared from β-alanine **8** (Scheme 4). Carboxy group of **8** was protected as a 2-(trimethylsilyl)ethyl (TMSE) ester **9** (Kobayashi *et al.* 1994; Yoshida *et al.* 2014). Acylation of the primary amine using bromoacetyl bromide afforded α-bromoamide **10**, which was treated with triphenylphosphine to give **11** as the desired Wittig salt **4**.

Now that all of the three units (**3**, **7**, and **11**) were obtained successfully, the coupling reaction between them was examined. At first, aldehyde **3** was subjected to Wittig reaction with **11**, and then the resulting trienylstannane was subjected to Stille coupling (Stille 1985) with vinyl iodide **7**. The Wittig reaction proceeded in moderate yield, however, reactivity of the subsequent Stille coupling was found to be very low, and separation of the substrate and the product was found to be difficult. On the other hand, as shown in Scheme 5, Stille coupling of the vinyl iodide **7** and stannyldienal **3** proceeded very smoothly to give trienal **12**, and subsequent Wittig reaction with **11** gave tetraene **13** in sufficient yield. In this coupling sequence, the trienal **12** was stereoselectively obtained as a single isomer and the tetraene **13** was obtained together with a small amount of (2′*Z*)-isomer, which was easily removed by silica gel chromatography (*E*/*Z* = ca. 6:1).

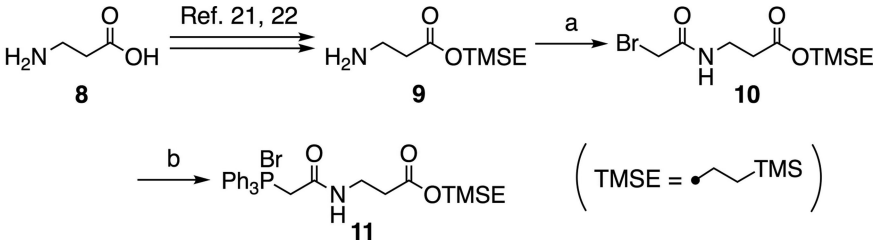
Having succeeded in the construction of carbon skeleton of ishigamide (**1**), our remaining task toward the synthesis of **1** was removal of the two silicon-protecting groups from the tetraene **13**. We expected that both protecting groups of **13** could be removed at the same time. However, it was found that only the TMSE group was smoothly removed with TASF or TBAF, while only the TBS group was smoothly removed with HF. So, the tetraene **13** was treated stepwise with TASF and HF to give (*R*)-ishigamide (**1**). The ^{1}H and ^{13}C NMR spectra of the synthetic **1** well supported its structure, however slight differences were observed in chemical shifts between the



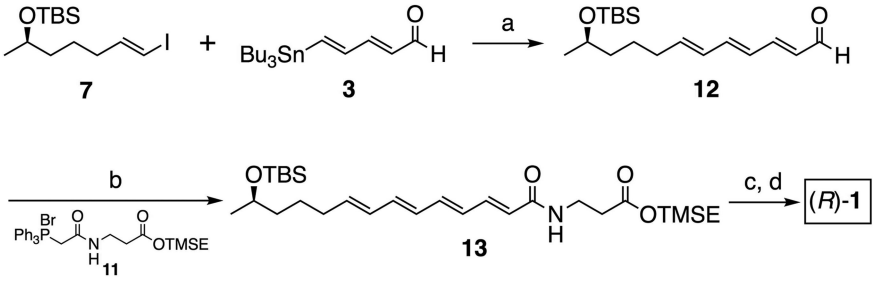
Scheme 2. Preparation of vinyl iodide **7** from PHB. *Reagents and conditions*: (a) TMSC≡CCH$_3$, *n*-BuLi, THF, −20 to 0 °C; (b) K_2CO_3, MeOH, rt, 62% in two steps; (c) Schwartz's reagent, THF, 0 °C to rt, then NIS, −78 °C to rt, 72%.



Scheme 3. Preparation of stannyldienal **3** via Zincke aldehyde.



Scheme 4. Preparation of Wittig salt **11** from *β*-alanine. *Reagents and conditions*: (a) BrCH$_2$COBr, pyridine, THF, −20 °C, 52%; (b) Ph_3P, toluene, reflux, 75%.



Scheme 5. Synthesis of (*R*)-ishigamide by coupling reaction between three units, **3**, **7**, and **11**. *Reagents and conditions*: (a) $PdCl_2(PPh_3)_2$, DMF, rt, 74%; (b) **11**, *n*-BuLi, −20 °C to rt, 57%; (c) TASF, DMF, rt; (d) HF, MeCN, rt, 45% in two steps.

natural product and the synthetic compound (see Table S1). Data of the natural product were measured in the presence of a small amount of formic acid, which was used for purification (Du *et al*. 2016). As shown in Figure 2, it was confirmed that both compounds were identical by LC–MS analysis, so it was considered that the differences in chemical shifts would be due to acidity.

While specific rotation of natural **1** had not been measured because of its low productivity, absolute value of the specific rotation of synthetic (*R*)-**1** ($[\alpha]_D^{24} = -2$ (*c* 0.7, CH_3OH)) was too small to unambiguously determine the absolute configuration of the natural product by comparison of those of the synthetic and natural **1**. Furthermore, using CD spectrum was also thought to be difficult because of a long distance between the chiral center and the chromophore. So the absolute configuration of the natural product was determined by chiral HPLC. In addition to (*R*)-**1**, racemic form was synthesized in the same manner, and subjected to chiral HPLC analysis together with (*R*)- and natural **1** (Figure 3). As a result, natural **1** showed the same retention time as the synthesized (*R*)-**1**, and the stereochemistry of the natural product was successfully determined to be *R*.

Conclusion

In summary, we have succeeded in an enantioselective synthesis of (*R*)-ishigamide employing Stille coupling and Wittig reaction between three units, vinyl iodide, stannyldienal, and Wittig salt. The absolute configuration of ishigamide was unambiguously determined to be *R* by chiral HPLC analysis comparing with the synthesized standard. We wish our study on this compound would offer some useful information for further investigation on its biosynthesis and the related fields.

Experimental

General procedures

Optical rotations were recorded with a JASCO P-2100 polarimeter. Melting points were measured with a Yanaco micro-melting point apparatus and were uncorrected values. IR spectra were measured with a JASCO FT/IR-4100 spectrophotometer. ^{1}H and ^{13}C NMR were recorded on a JEOL JNM ECX-400 spectrometer. Chemical shifts (δ ppm) were referenced to the residual solvent peaks as the internal standard ($CDCl_3$: $\delta_H = 7.26$, $\delta_C = 77.0$, CD_3OD: $\delta_H = 3.30$, $\delta_C = 49.0$, DMSO-d_6: $\delta_H = 2.49$, $\delta_C = 39.5$). Mass

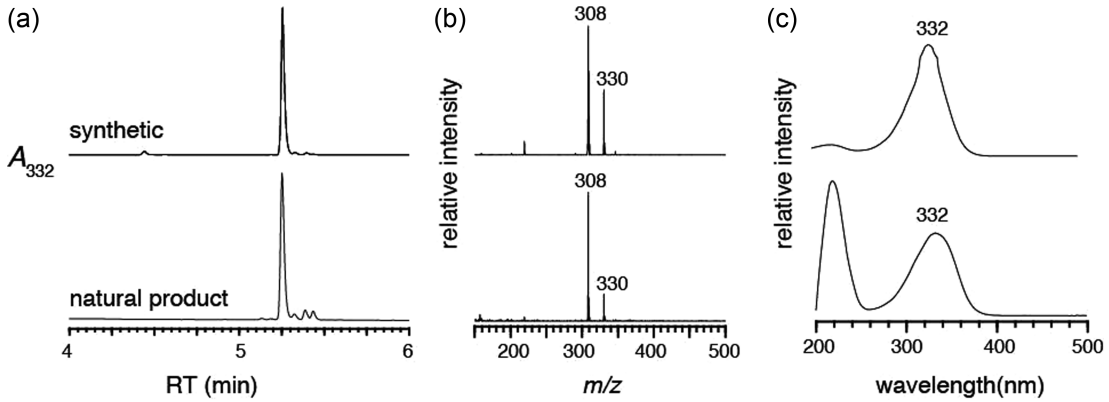


Figure 2. LC–MS analysis of synthetic and natural ishigamide (**1**). (a) UV chromatograms; (b) mass spectra in positive ion mode; (c) UV spectra. *Conditions*: column: COSMOCORE 2.6 C_{18} column (2.1D × 150 mm; Nacalai Tesque, Kyoto, Japan), eluent: linear gradient of water and acetonitrile both containing 0.1% formic acid, flow rate: 0.6 mL/min, detection: UV (330 nm) and Quadrupole mass spectrometer LCMS-8030 (Shimadzu Corp., Kyoto, Japan).

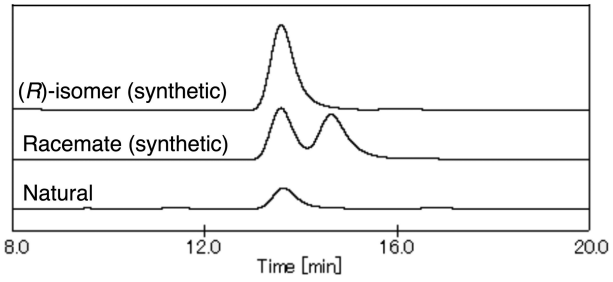


Figure 3. Chiral HPLC analysis of synthetic and natural ishigamide (**1**). *Conditions*: column: CHIRALPAK® ID (4.6 × 250 mm), eluent: MeCN/MeOH/TFA (95:5:0.1), flow rate: 0.8 mL/min, detection: UV (330 nm), R_t: 13.6 min [(*R*)-isomer]; 14.6 min [(*S*)-isomer].

spectra were recorded on a JEOL JMS-T100LP. HPLC was performed using HITACHI Chromaster (5110 and 5410). LC–MS was performed using LC-2040C 3D Plus (Shimadzu Corp.) coupled with LCMS-8030 (Shimadzu Corp.) Column chromatography was performed using Kanto silica gel 60 N (0.060–0.200 mm). Natural ishigamide was re-isolated from the culture of the recombinant *Streptomyces* sp. MSC090213JE08 strain in which the SARP-7 gene was forcedly expressed as described previously (Du *et al.* 2016).

(*R*)-2-(*tert*-butyldimethylsilyloxy)hept-6-yne (6)

Under Ar atmosphere, to a solution of TMS-propyne (768 µL, 5.13 mmol) in THF (5 mL) was added a solution of *n*-butyllithium in hexane (1.60 M, 3.21 mL, 5.13 mmol) at −20 °C. After stirring for 30 min, bromide **5** (456 mg, 1.71 mmol) was added to this solution and the reaction mixture was stirred for 17 h at 0 °C. The reaction mixture was poured into water and extracted with ethyl acetate. Combined organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (Hex/EtOAc = 10:1) to give crude TMS-alkyne.

This material was dissolved in methanol (15 mL) and K_2CO_3 (339 mg, 2.46 mmol, 1.5 eq.) was added to the solution. After stirring for 22 h at room temperature, the reaction mixture was concentrated *in vacuo* and the residue was suspended in ethyl acetate. The resultant suspension was washed with saturated NH_4Cl solution and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (Hex/EtOAc = 20:1) to give alkyne **6** (240 mg, 62% in 2 steps) as a slightly brown oil.

$[\alpha]_D^{24} = -13$ (*c* 1.0, $CHCl_3$). IR (film): ν_{max} (cm^{-1}) = 3314, 2957, 2858, 1463, 1374, 1255, 1030, 775. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.05 (6H, s), 0.89 (9H, s), 1.13 (3H, d, J = 6.0 Hz), 1.50-1.64 (4H, m), 1.94 (1H, t, J = 2.6 Hz), 2.17-2.21 (2H, m), 3.81 (1H, sext, J = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = −4.7, −4.4, 18.1, 18.5, 23.8, 24.7, 25.9, 38.6, 68.1, 68.2, 84.6. HRMS (DART-TOFMS): *m*/*z* calcd. for $C_{13}H_{27}OSi$ $[M + H]^+$ 227.1826, found 227.1845.

(*R*, *E*)-6-(*tert*-butyldimethylsilyloxy)-1-iodohept-1-ene(7)

Under Ar atmosphere and light-shielding conditions, to a solution of zirconocene dichloride (1.16 g, 3.97 mmol) in THF (6 mL) was added a solution of DIBAL in hexane (1.00 M, 3.97 mL, 3.97 mmol) at 0 °C. After stirring for 30 min, a solution of alkyne **6** (600 mg, 2.65 mmol) in THF (3 mL) was added and the mixture was stirred for 2 h at room temperature. After the reaction mixture was cooled down to −78 °C, a solution of NIS (1.19 g, 5.30 mmol) in THF (3 mL) was added at −78 °C and the mixture was stirred overnight while slowly warming to room temperature. After addition of saturated Rochelle's salt solution the mixture was extracted with ether. Combined organic layer was washed with distilled water, saturated $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution and brine, then dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (Hex/EtOAc = 40:1) to give vinyl iodide **7** (677 mg, 72%) as a slightly yellow oil.

$[\alpha]_D^{24} = -8.6$ (*c* 1.0, $CHCl_3$). IR (film): ν_{max} (cm^{-1}) = 2929, 2857, 1472, 1374, 1255, 1035, 837, 774. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.04 (3H, s), 0.05 (3H, s), 0.88 (9H, s), 1.11 (3H, d, J = 6.0 Hz), 1.33-1.51 (4H, m), 2.02-2.08 (2H, m), 3.78 (1H, sext, J = 6.0 Hz), 5.98 (1H, d, J = 14.8 Hz), 6.50 (1H, dt, J = 14.8, 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = −4.7, −3.7, 23.8, 24.5, 25.9, 36.1, 38.9, 68.3, 74.4, 146.6. HRMS (DART-TOFMS): *m*/*z* calculated for $C_{13}H_{28}IOSi$ $[M + H]^+$ 355.0949, found 355.0944.

2-(Trimethylsilyl)ethyl 3-(2-bromoacetamido) propanoate (10)

Under Ar atmosphere, to a solution of amine **9** (587 mg, 3.10 mmol) and pyridine (375 µL, 4.65 mmol, 1.5 eq.) in THF

(10 mL) was added bromoacetyl bromide (354 µL, 4.03 mmol) at −20 °C. After stirring for 1 h at the same temperature, saturated $NaHCO_3$ solution was added to the reaction mixture at 0 °C and the resulting mixture was extracted with ethyl acetate. Combined organic layer was washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (Hex/EtOAc = 50:1) to give bromide **10** (497 mg, 52%).

IR (film): ν_{max} (cm^{-1}) = 3298, 2954, 2898, 1660, 1541, 1251, 1176, 1062, 937, 839, 764, 695, 551. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.05 (9H, s), 1.00 (2H, t, J = 8.8 Hz), 2.55 (2H, t, J = 6.0 Hz), 3.56 (2H, q, J = 6.0 Hz), 3.86 (2H, s), 4.22 (2H, t, J = 8.8 Hz), 7.09 (1H, br). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = −1.5, 17.4, 29.1, 33.8, 35.5, 63.2, 165.4, 172.5. HRMS (DART-TOFMS): m/z calculated for $C_{10}H_{21}BrNO_3Si$ $[M + H]^+$ 310.0469, found 310.0482.

(2-Oxo-2-((3-oxo-3-(2-(trimethylsilyl)ethoxy)propyl) amino)ethyl)tri-phenylphosphonium bromide (11)

Under Ar atmosphere, a solution of bromide **10** (497 mg, 1.60 mmol) and triphenylphosphine (462 mg, 1.76 mmol, 1.1 eq.) in toluene (13 mL) was refluxed for 24 h. After cooling down to room temperature, hexane was added to the reaction mixture. The resulting mixture was further cooled down to 4 °C to precipitate phosphonium salt. After filtration, the precipitate was washed with hexane to give phosphonium salt **11** (689 mg, 75%) as a white solid.

^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.02 (9H, s), 0.95 (2H, t, J = 8.4 Hz,), 2.38 (2H, t, J = 8.0 Hz), 3.38 (2H, dt, J = 6.0, 8.0 Hz), 4.12 (2H, t, J = 8.4 Hz), 5.03 (2H, d, J = 14.8 Hz), 7.63–7.70 (6H, m), 7.75–7.87 (9H, m), 9.61 (1H, brt, J = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = −1.5, 17.2, 32.3 (d, J = 55 Hz), 33.6, 35.7, 62.7, 118.3 (d, J = 88 Hz), 130.1 (d, J = 13 Hz), 134.1 (d, J = 11 Hz), 135.0 (d, J = 4 Hz), 162.5 (d, J = 6 Hz), 171.1.

(*R*,2*E*,4*E*,6*E*)-11-(*tert*-Butyldimethylsilyloxy)dodeca-2,4,6-trienal (12)

Under Ar atmosphere, to a solution of bis (triphenylphosphine)palladium (II) dichloride (3 mg, 4.27 µmol) in DMF (2 mL) was added a solution of vinyl iodide **7** (50.0 mg, 141 µmol) in DMF (2 mL) at room temperature. After stirring for 15 min, a solution of stannyldienal **3** (62.9 mg, 169 µmol, 1.2 eq.) in DMF (2 mL) was added and the resulting mixture was stirred for overnight at room temperature. Then saturated $NaHCO_3$ solution was added to the reaction mixture at 0 °C and the mixture was extracted with hexane. Combined organic layer was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (Hex/EtOAc = 25:1) to give trienal **12** (32 mg, 74%) as a slightly yellow oil.

$[\alpha]_D^{24}$ = −9.8 (c 1.0, $CHCl_3$). IR (film): ν_{max} (cm^{-1}) = 2928, 2857, 1683, 1614. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.045 (3H, s), 0.049 (3H, s), 0.89 (9H, s), 1.12 (3H, d, J = 6.0 Hz), 1.35–1.54 (4H, m), 2.17 (2H, q, J = 6.8 Hz), 3.79 (1H, sext, J = 6.0 Hz), 6.02 (1H, dt, J = 15.2, 6.8 Hz), 6.13 (1H, dd, J = 8.0, 15.2 Hz), 6.18 (1H, dd, J = 11.2, 15.2 Hz), 6.35 (1H, dd, J = 11.2, 14.8 Hz), 6.65 (1H, dd, J = 10.8, 14.8 Hz), 7.12 (1H, dd, J = 10.8, 15.2 Hz), 9.55 (1H, d, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = −4.7, −4.4, 18.1, 23.8, 25.0, 25.9, 33.1, 39.1, 68.3, 127.8, 129.9, 130.7, 142.4, 143.2, 152.4, 193.6. HRMS (DART-TOFMS): m/z calculated for $C_{18}H_{33}O_2Si$ $[M + H]^+$ 309.2244, found 309.2242.

2-(Trimethylsilyl)ethyl 3-((*R*,2*E*,4*E*,6*E*,8*E*)-13-(*tert*-butyldimethylsilyl-oxy)tetradeca-2,4,6,8-tetraenamido) propanoate (13)

Under Ar atmosphere, to a suspension of phosphonium salt **11** (61 mg, 107 µmol) in THF (1 mL) was added a solution of *n*-butyl lithium in hexane (1.60 M, 122 µL, 194 µmol) at −20 °C. After stirring for 30 min, a solution of trienal **12** (30 mg, 97.2 µmol) in THF (1 mL) was added at the same temperature and the resulting mixture was stirred for 2 h at room temperature. Then saturated NH_4Cl solution was added to the reaction mixture at 0 °C and the mixture was extracted with ethyl acetate. Combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (Hex/EtOAc = 3:1) to give tetraene **13** (29.0 mg, 57%) as a slightly yellow solid together with a small amount of (2'Z)-isomer (5.3 mg, 10%).

13: $[\alpha]_D^{24}$ = −7.0 (c 1.0, $CHCl_3$). IR (film): ν_{max} (cm^{-1}) = 3301, 2954, 2857, 1731, 1652, 1252, 1175, 1062, 695, 505. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.04 (15H, s), 0.88 (9H, s), 0.99 (2H, t, J = 8.6 Hz), 1.11 (3H, d, J = 6.0 Hz), 1.34-1.52 (4H, m), 2.09-2.17 (2H, m), 2.55 (2H, t, J = 6.0 Hz), 3.60 (2H, q, J = 6.0 Hz), 3.77 (1H, sext, J = 6.0 Hz), 4.19 (2H, t, J = 8.6 Hz), 5.79 (1H, d, J = 14.8 Hz), 5.82 (1H, dt, J = 14.8, 7.2 Hz), 6.07 (1H, m), 6.10 (1H, dd, J = 10.4, 15.2 Hz), 6.18 (1H, dd, J = 11.4, 14.8 Hz), 6.25 (1H, dd, J = 12.0, 15.2 Hz), 6.34 (1H, dd, J = 10.4, 14.8 Hz), 6.53 (1H, dd, J = 11.4, 14.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = −4.7, −4.4, −1.5, 17.4, 18.2, 23.8, 25.3, 25.9, 27.8, 34.2, 34.9, 39.2, 63.0, 68.4, 122.6, 129.2, 129.8, 130.4, 136.8, 138.0, 140.0, 141.1, 166.1, 173.0. HRMS (DART-TOFMS): m/z calculated for $C_{28}H_{52}NO_4Si_2$ $[M + H]^+$ 522.3429, found 522.3419.

(2'Z)-isomer: ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.04 (15H, s), 0.88 (9H, s), 0.94–1.02 (2H, m), 1.11 (3H, d, J = 6.0 Hz), 1.33–1.52 (4H, m), 2.08–2.17 (2H, m), 2.55 (2H, t, J = 6.0 Hz), 3.57 (2H, q, J = 6.0 Hz), 3.77 (1H, m), 4.16-4.22 (2H, m), 5.48 (1H, d, J = 11.2 Hz), 5.79 (1H, dt, J = 15.2, 6.8 Hz), 6.04–6.47 (5H, m), 7.62 (1H, dd, J = 11.2, 14.0 Hz).

3-((*R*,2*E*,4*E*,6*E*,8*E*)-13-Hydroxytetradeca-2,4,6,8-tetraenamido)pro-panoic acid (ishigamide, 1)

Under Ar atmosphere, to a solution of tetraene **13** (50 mg, 96 µmol) in DMF (1.25 mL) was added TASF reagent (27 mg, 98 µmol) at room temperature. After stirring for 30 min, the reaction mixture was poured into water at 0 °C and the mixture was extracted with ether. Combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To the residue was added a solution of 50% hydrofluoric acid (90 µL, 2.5 mmol) in acetonitrile (910 µL), and the mixture was stirred for 2 h at room temperature. Then water was added to the reaction mixture and the resulting mixture was extracted with ethyl acetate. Combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography ($CHCl_3$/MeOH = 5:1) to give ishigamide (**1**, 13.3 mg, 45%) as a slightly yellow solid.

$[\alpha]_D^{24}$ = −2 (c 0.7, CH_3OH). mp = 125 °C (decomp.). IR (KBr): ν_{max} (cm^{-1}) = 3296, 2926, 1695, 1646, 1131, 1000, 505. ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 1.14 (3H, d, J = 6.0 Hz), 1.28-1.65 (4H, m), 2.11-2.19 (2H, m), 2.52 (2H, t, J = 6.8 Hz), 3.49 (2H, t, J = 6.8 Hz), 3.71 (1H, sext, J = 6.0 Hz), 5.84 (1H, dt, J = 15.2, 7.2 Hz), 5.95 (1H, d, J = 14.8 Hz), 6.14 (1H, dd J = 10.8, 15.2 Hz), 6.23 (1H, dd, J = 11.2, 14.8 Hz), 6.31 (1H, dd, J = 11.6, 14.8 Hz), 6.38 (1H, dd, J = 10.8, 14.8 Hz), 6.58 (1H, dd, J = 11.2, 14.8 Hz), 7.17 (1H, dd, J = 11.6, 14.8 Hz). ^1H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.02

(3H, d, J = 6.0 Hz), 1.21-1.48 (4H, m), 2.08 (2H, brq, J = 7.2 Hz), 2.38 (2H, t, J = 6.4 Hz), 3.23-3.33 (2H, m), 3.56 (1H, sext, J = 6.0 Hz), 5.83 (1H, dt, J = 15.2, 7.2 Hz), 5.98 (1H, d, J = 15.2 Hz), 6.13 (1H, dd, J = 10.8, 15.2 Hz), 6.25 (1H, dd, J = 11.2, 15.2 Hz), 6.34 (1H, dd, J = 11.2, 14.8 Hz), 6.38 (1H, dd, J = 10.8, 15.2 Hz), 6.59 (1H, dd, J = 11.2, 14.8 Hz), 7.04, (1H, dd, J = 11.2, 15.2 Hz), 8.07 (1H, brt, J = 5.2 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) = 23.5, 26.5, 33.9, 35.0, 36.6, 39.7, 68.4, 123.8, 130.6, 131.2, 131.9, 138.1, 138.6, 141.3, 142.1, 169.0, 175.7. ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 23.6, 25.0, 32.4, 34.1, 35.0, 38.5, 65.6, 124.4, 129.8, 130.1, 130.4, 136.0, 137.4, 138.8, 138.9, 165.1, 173.1. HRMS (DART-TOFMS): m/z calculated for $C_{17}H_{26}NO_4[M + H]^+$ 308.1856, found 308.1859.

Supplementary material

Supplementary material is available at *Bioscience, Biotechnology, and Biochemistry* online.

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Author contribution

K.I. designed this study; M.S. carried out the experiments with meaningful discussions with all authors; M.S. and K.I. contributed to analytical works; D.D., Y.K. and Y.O. contributed to re-isolation of natural product and LC/MS analysis; M.S., Y.K. and K.I. wrote the manuscript with assistance from all authors; and K.I. supervised the research.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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