AN ASYMMETRIC TOTAL SYNTHESIS OF A POTENT IMMUNOSUPPRESSANT, MYCESTERICINS D AND F, THROUGH AN ALDOL REACTION USING L-THREONINE ALDOLASE

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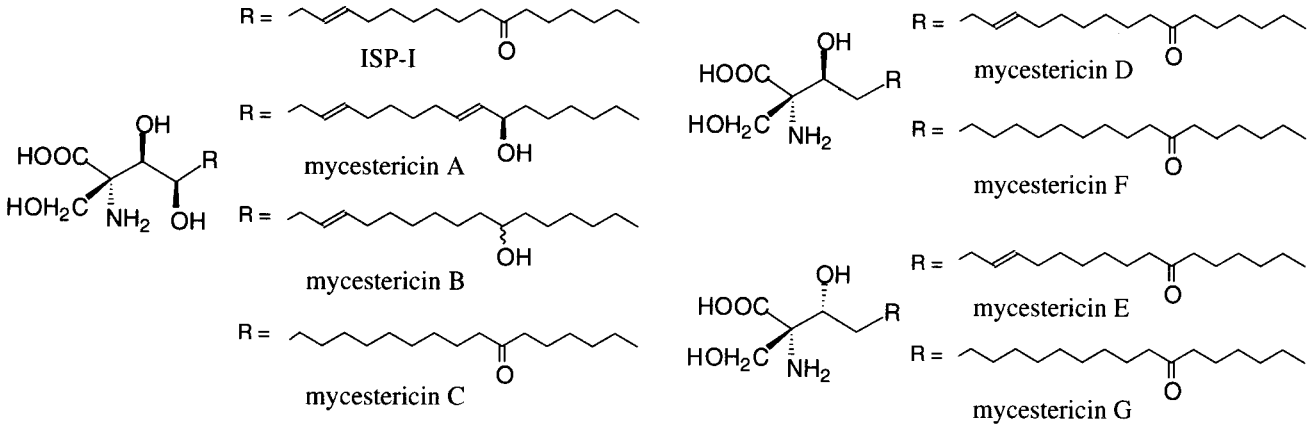
Abstract --- L-Threonine aldolase from *Candida humicola* catalyzed the aldol reaction of 4-benzyloxybutanal (**1**) with glycine to give β-hydroxy-α-amino acids (**2e,t**), whose *erythro* / *threo* ratio was controlled by using either kinetic or thermodynamic conditions. The *erythro* derivative (**4e**) was effectively converted to mycestericins D and F *via* a stereoselective hydroxymethylation of oxazoline derivative (**6**) as the key step.

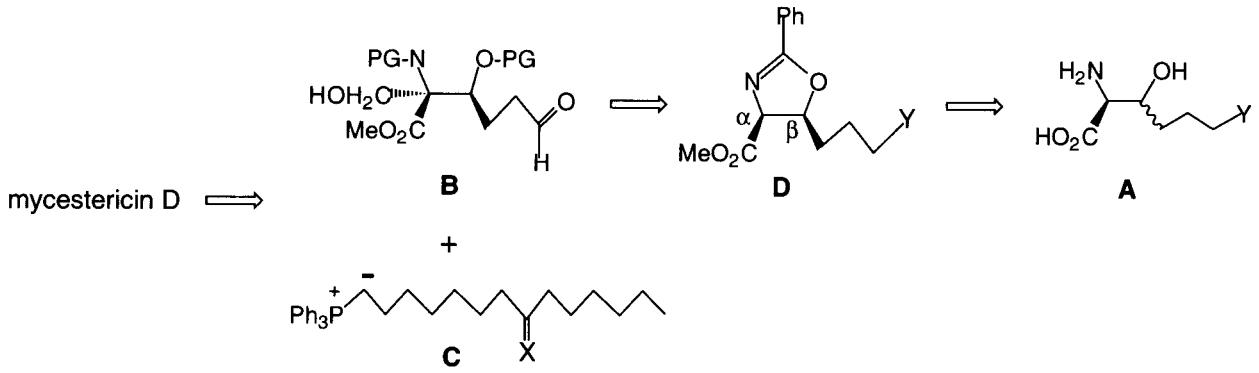
INTRODUCTION

The discovery of ISP-I[1] (myriocin,[2] thermozymocidin[3]) as a 10 to 100-fold more potent immunosuppressant than cyclosporins from the culture broth of *Isaria sinclairii* (ATCC 24400) has attracted many synthetic chemists to investigate its asymmetric total synthesis.[4] Immunosuppressant mycestericins A-G isolated from *Mycelia sterilia* (ATCC 20349) by Fujita *et al.* are very similar in structure to ISP-I.[5] Although the synthesis of mycestericins E and G has been reported by us,[6] little attention has been focused on the synthesis of mycestericins. A minor component, mycestericin D, has been also found to have almost the same potent immunosuppressive activity as ISP-I.[5] These features attracted our attention to the synthesis of mycestericin D using a chemicoenzymatic reaction.



Recently, it was found that L-threonine aldolase from *Candida humicola* (AKU 4586) catalyzes the aldol condensation of glycine with an aldehyde having an oxygen or a nitrogen atom at adjacent positions with high *erythro / threo* selectivity.[7] We expected that the above enzyme-catalyzed reaction of functionalized butanal could give the desired β-hydroxy-α-amino acid (**A**) in Scheme 1, which would be of great use for the asymmetric synthesis of mycestericins D, E, F and G. In the retro-synthetic analysis of mycestericin D, as shown in Scheme 1, the most crucial reaction would be an enantioselective hydroxymethylation at the α-carbon atom of α-amino acid (**A**). Since Wittig olefination of an aldehyde (**B**) with an ylide (**C**) would form the adequately functionalized carbon skeleton for the synthesis of mycestericin D, we planned an easy and diastereoselective introduction of a hydroxymethyl group using 2-phenyloxazoline derivative (**D**) as a suitable protective group for the 1,2-amino alcohol moiety.

Scheme 1. A Synthetic Strategy of Mycestericin D



RESULTS AND DISCUSSION

As functionalized butanal in aldolase-catalyzed reaction using glycine, we chose 4-benzyloxybutanal (**1**), because a selective removal of the protective group would be easy. The L-threonine aldolase-catalyzed aldol reaction of glycine with 4–benzyloxybutanal (**1**), as shown in Scheme 2, was conducted according to the previous method[7] to give the desired β-hydroxy-α-amino acids (**2e,t**). The L-(*S*)-absolute configuration of the α-position was determined by the complete reactivity difference of **2e,t** with L- and D-amine oxidases as reported.[7] The stereochemistry of the hydroxyl group in the aldol product was determined by the modified Mosher method[8] in the (*R*)- and (*S*)-MTPA esters (**3a,b**) of the *N-tert*-butoxycarbonyl methyl esters derived from a mixture of **2e** and **2t**. In the above aldol reaction, we found that the ratio of *erythro / threo* in the product (**2**) varied according to the change in the reaction time as shown in Table 1. Namely, the *erythro* isomer (**2e**) was obtained as a major product in a shorter reaction time (entries 1 and 2), while the *threo* isomer (**2t**) as a major product was formed in the reaction time of 15 h (entry 3). The former should be controlled kinetically, and the latter, thermodynamically. Since this aldol reaction must be an equilibrium reaction between the starting material (glycine and 4-benzyloxybutanal) and the aldol products (*erythro*- and *threo*-β-hydroxy-α-amino acids) one diastereomer might be afforded selectively when the products (**2e,t**) were subjected to the retro-aldol reaction. Indeed, the *threo* isomer (**2t**) was obtained as the sole product by the enzymatic treatment of the diastereomeric mixture, but the yield was not satisfactory (entry 4). Therefore, the diminished yield may be attributable to the digestion by the aldolase.

Scheme 2

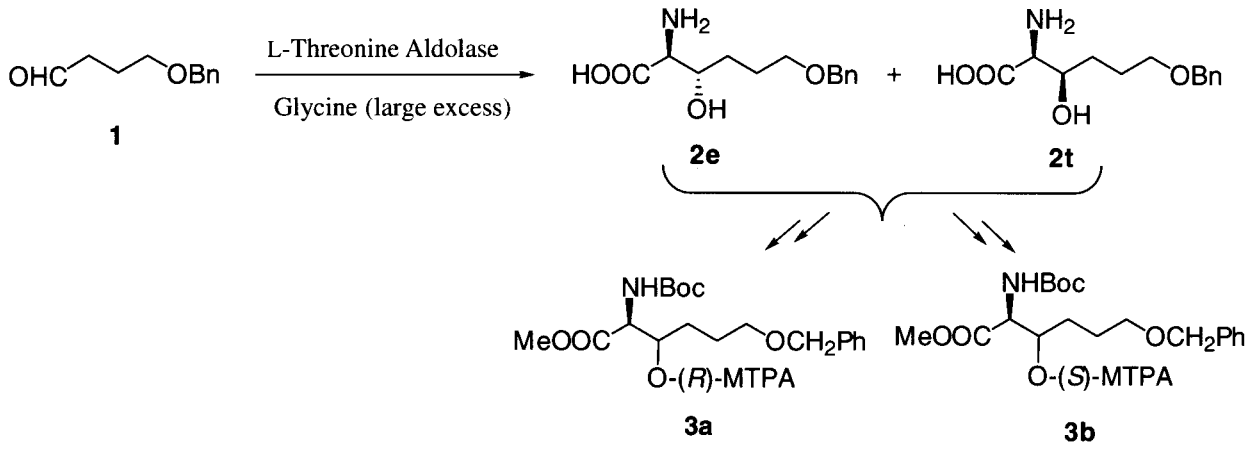


Table 1. L-Threonine Aldolase Catalyzed Reaction

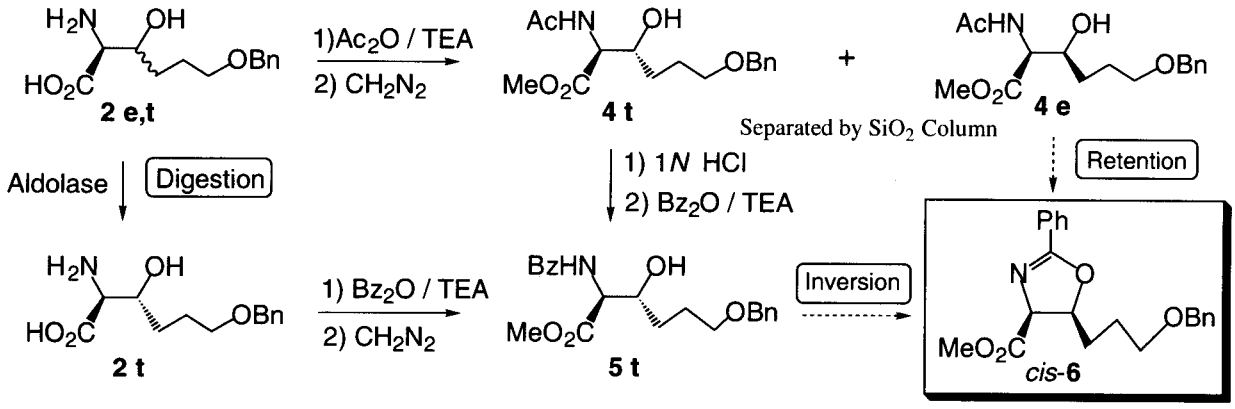
Entry	Substrate (mmol)	L-Threonine Aldolase (units) [a]	Reaction Time	Product	
				Yield (%)	Ratio (**2e** : **2t**) [b]
1	**1** (1.06 mmol)	133	5 min	18	90 : 10
2	**1** (1.06 mmol)	133	5 h	80	68 : 32
3	**1** (1.06 mmol)	133	15 h	70	40 : 60
4	**2e** + **2t** [c] (0.04 mmol)	20	1 h	50	0 : 100

a) Units / substrate (1 mmol) b) Determined by ^{1}H-NMR. c) A mixture (45 : 55) was used.

In order to separate the diastereomixture of the obtained β-hydroxy-α-amino acids (**2e,t**), we tried their derivatization because of the difficulty in their separation by both normal and reverse-phase silica gel chromatography. Fortunately, the amide ester derivatives (**4e,t**) obtained by the acetylation of an amino group and the subsequent methylation of carboxylic acid could be separated by silica gel column chromatography, though the benzoyl and *tert*-butoxycarbonyl derivatives of the amino group were not separated on silica gel chromatography. The hydroxymethylation at the α-carbon of oxazoline (**D**) in Scheme 1 will be introduced from the opposite direction to the alkyl substituent at the β-position by steric control. Because the α-carbon of the amino acid moiety in mycestericins is *S*-configuration, a *cis*-oxazoline derivative (*cis*-**6a**) as the substrate in the hydroxymethylation is required, and the formation from the separated **4e** and **4t** is considered feasible by cyclization through retention or inversion of the configuration of the β-hydroxyl group, as shown in Scheme 3. Hydrolysis of the *N*-acetyl group of *threo* isomer (**4t**) with 1*N* hydrochloric acid and the subsequent benzoylation gave *N*-benzoyl derivative (**5t**) in 72%, which could be also prepared from **2t** obtained by digestion with aldolase through benzoylation and following methylation in 87% yield. The observed facile hydrolysis of the *N*-acetyl group would be attributed to the neighboring group participation[9] of the hydroxyl group at the β-position.

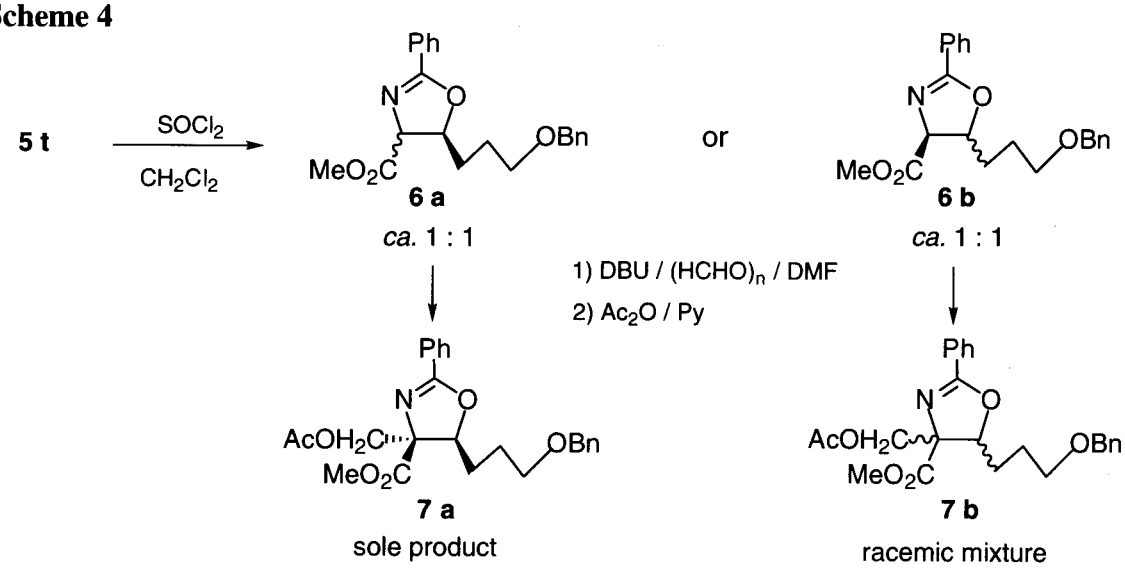
Since Elliot and Schmidt have reported that the treatment of an optically active *N*-benzoyl threonine ester with thionyl chloride gave an oxazoline in the inversion manner,[10] we investigated the oxazoline ring

Scheme 3. Synthesis of β-Hydroxyamino Acid Part



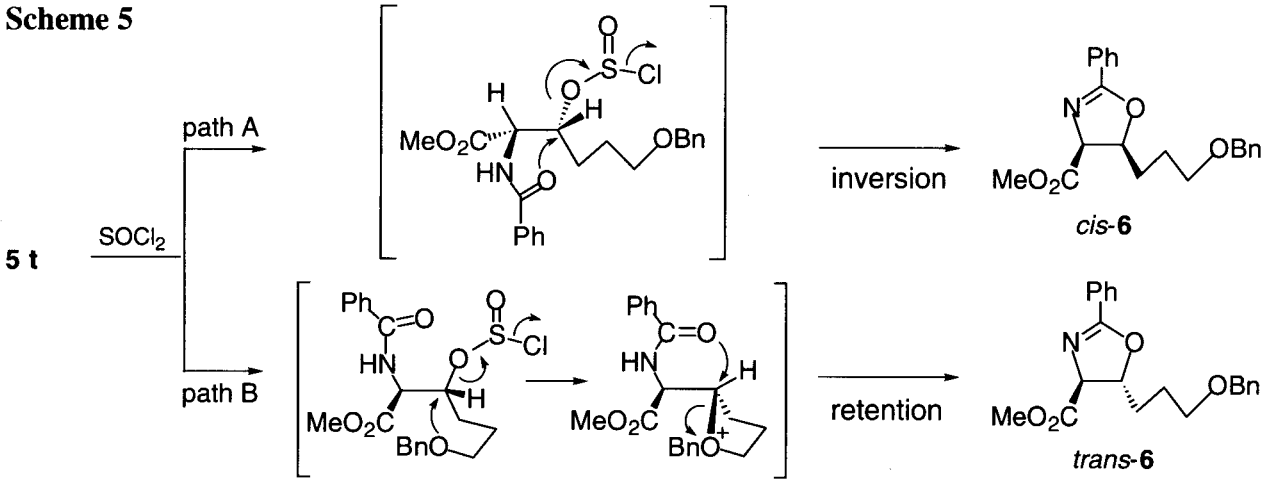
formation in this inversion manner, as shown in Scheme 4. The reaction of alcohol (**5t**) with thionyl chloride gave unexpectedly a mixture of *trans*-**6** and *cis*-**6** in the ratio *ca.* 1 : 1 in 42% yield, whose structure must be **6a** or **6b**, in which the (*S*)-configuration on the β-carbon or the α-carbon is fixed, respectively. If the structure of these diastereomixture is **6a**, the stereoselective hydroxymethylation will give **7a** as an enantiomerically pure compound. On the other hand, the stereoselective hydroxymethylation will give **7b** as a racemic compound, if the structure of these diastereomixture is **6b**. The hydroxymethylation of a mixture of *trans*- and *cis*-**6** gave indeed **7b** in 14% enantiomeric excess. It is obvious that the configuration of the alcoholic β-carbon of **5t** is not inverted completely. We could conclude that a double-inversion process involving the participation of the benzyloxy group (path B) as well as the inversion (path A) took part in this oxazoline-forming reaction, as shown in Scheme 5. Therefore, we discarded the route for the formation of the oxazoline ring through the inversion process.

Scheme 4



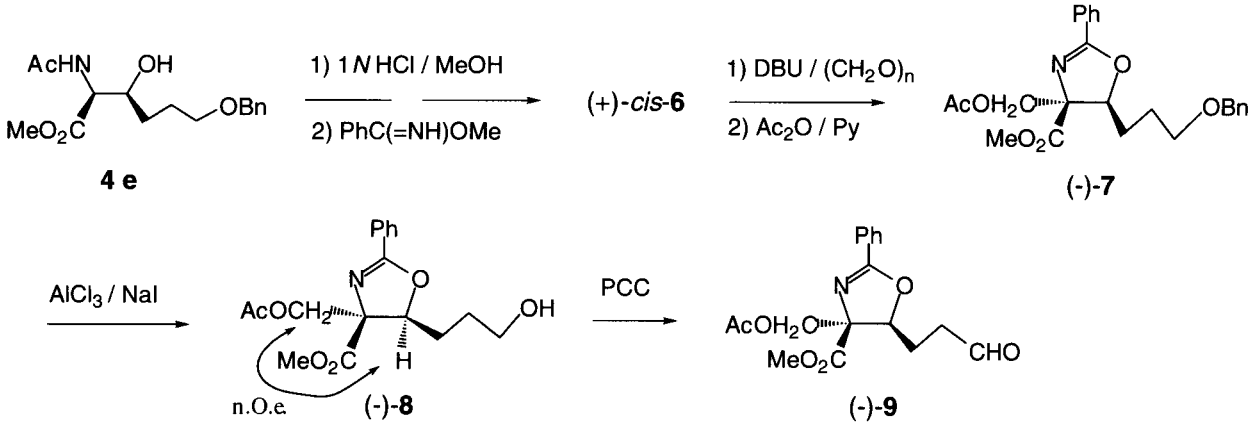
Next, we examined the retention process as shown in Scheme 6. Namely, the hydrolysis of an acetamide group of **4e** with 1*N* hydrochloric acid and the subsequent formation of an oxazoline ring with methyl benzimidate[11] afforded *cis*-**6** in 76% yield, whose enantiomeric excess was determined to be >99% by a

Scheme 5



chiral HPLC analysis using a Daicel CHIRALCEL OD.

Scheme 6

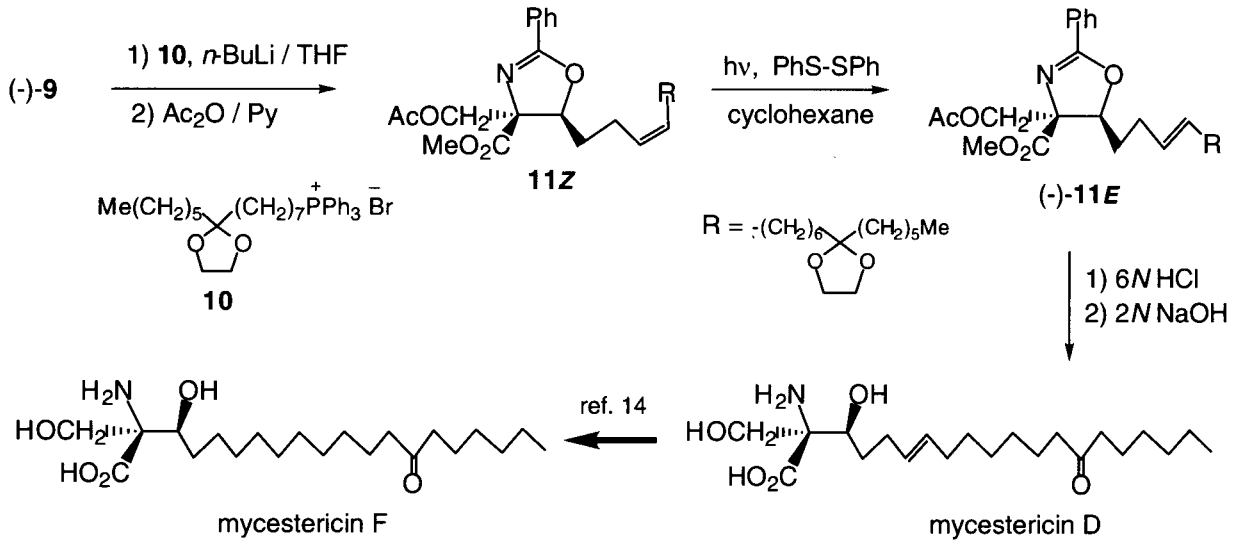


With an enantiomerically pure oxazoline [(+)-*cis*-**6**] in hand, we attempted the stereoselective hydroxymethylation. Among several trials, the following procedure using tertiary amines as a base gave the best results. Treatment of (+)-*cis*-**6** with paraformaldehyde and DBU in DMF and the subsequent acetylation gave the desired acetate [(-)-**7**] stereoselectively in 83% yield. The benzyl ether [(-)-**7**], coexisting methyl ester and acetate moieties in the molecule, was selectively debenzylated by our combination reagent[12] of aluminum chloride and sodium iodide to give an alcohol [(-)-**8**] in 88% yield. The *cis* stereochemistry of (-)-**8** was confirmed by the nuclear Overhauser effect between the methine proton and the methylene protons, as shown in Scheme 6. Oxidation of alcohol (-)-**8** with PCC proceeded smoothly to give aldehyde [(-)-**9**] which served as the substrate for the next Wittig olefination.

The trials of the Schlosser modification[13] of the Wittig reaction of (-)-**9** gave a mixture of **11*Z*** and **11*E***; therefore, the Wittig reaction of the aldehyde (**9**) with the phosphonium salt (**10**) prepared by the Nagao procedure[4i] and the subsequent re-acetylation of the hydrolyzed alcohol afforded a (*Z*)-olefin (**11*Z***) in 82% yield, as shown in Scheme 7. Next, we conducted the photochemical isomerization of **11*Z*** to **11*E*** by the irradiation of a high-pressure mercury lamp in the presence of diphenyl disulfide to give the desired (-)-**11*E*** quantitatively.[4e,f] The deprotection of the oxazoline to 1,2-amino alcohol with 6*N* hydrochloric acid and the subsequent hydrolysis of the two esters moieties with 2*N* sodium hydroxide gave mycestericin D as

a powder in 63% yield, whose specific rotation {$[\alpha]_D^{23}$ -7.05° (0.09, MeOH), lit.,[5a] $[\alpha]_D$ -7.5° (0.16, MeOH)} and melting point (mp 164-170 °C, lit.,[5a] mp 162-167 °C) as well as spectroscopic data were identical with the natural mycestericin D.[5] An asymmetric synthesis of mycestericin D implies a formal synthesis of mycestericin F, because the conversion of mycestericin D into mycestericin F has been reported by Fujita and colleagues.[14]

Scheme 7



CONCLUSION

We found that the L-threonine aldolase-catalyzed aldol reaction of glycine with 4-benzyloxybutanal gave kinetically-controlled *erythro*-β-hydroxy-α-amino acid (**2e**) predominantly in a short reaction time, or thermodynamically-controlled *threo*-β-hydroxy-α-amino acid (**2t**) predominantly in the longer reaction time. Also, we were able to elucidate that the oxazoline ring-formation reaction of the *threo*-ε-benzyloxy-β-hydroxy-α-benzamido ester (**5t**) with thionyl chloride resulted in the loss of the chirality of the β-alcoholic carbon. The *erythro* derivative (**4e**) was effectively used for the asymmetric synthesis of mycestericins D and F *via* the stereoselective hydroxymethylation to the α-carbon based on the chirality of the β-carbon in the oxazoline derivative.

ACKNOWLEDGMENT

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EXPERIMENTAL

General: Melting points are taken with a micro hot-stage apparatus (Yanagimoto) and are uncorrected. The IR spectra are recorded with a JASCO IR-810 or a Shimadzu FT IR-8100 diffraction grating infrared spectrophotometer. ^{1}H-NMR spectra are obtained with a JEOL JNM-GX-270, a Varian XL-300, or G Omega-600 NMR spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard, or in D_2O or CD_3OD as a standard of the remaining protons. MS are determined on a JEOL JMS-DX300 or a JEOL JMS-HX-110 mass spectrometer. Specific rotation was recorded with a Horiba Sepa-200 polarimeter.

Combustion analysis was done with a PERKIN ELMER Series II CHNS/O Analyzer 2400. The HPLC analyses were performed with a Shimadzu LC-9A and LC-10A Liquid Chromatograph series using Daicel chiral column (CHIRALCEL OD). Their data were recorded with a Shimadzu C-R6A Chromatopac. Wakogel C-200 (silica gel) (100-200 mesh, Wako), Wakogel C-300 (Wako Pure Chemical), or Kieselgel 60 Art9385 (Merck) was used for open-column chromatography. Flash column chromatography was performed with Silica Gel 60H (nakalai tesque). Kieselgel 60 F-254 plates (Merck) was used for TLC. Preparative TLC (PTLC) was done with Kieselgel 60 F-254 plates (0.25mm, Merck) or Silica gel 60 F-254 plates (0.5mm, Merck). Reverse-phase column chromatography was performed with Bondapak C18 125Å Part No. 20739 (Waters).

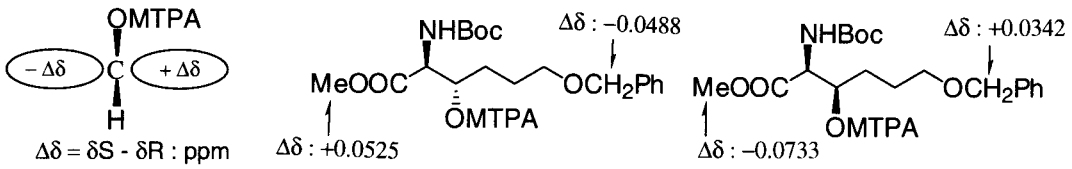
Materials: THF and ether were distilled from sodium benzophenone ketyl, and dichloromethane (after washing ten times with water to remove methanol contaminated), triethylamine, acetonitrile and DMF were distilled from CaH_2, before use. *n*-BuLi (hexane solution) was purchased from Wako Pure Chemical Industries.

4-Benzyloxybutanal (1) After sodium hydride (60% oil suspension 5.1 g, 106.5 mmol) was washed with dry ether (15 mL) under nitrogen atmosphere, dry DMF (70 mL) was added, and followed by the addition of 1,4-butanediol (7.8 mL, 88.8 mmol) at 0 °C. Benzyl bromide (7 mL, 58.5 mmol) was added dropwise to the reaction mixture, and the resultant mixture was stirred for 1 h at rt. The reaction mixture was poured into water, then extracted with ether. The ethereal extract was washed with water, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (elute, AcOEt : Hexane = 1 : 1) gave 4-benzyloxybutanol (9.76 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.27 (m, 5H), 4.52 (s, 2H), 3.64 (q, *J* = 5.5 Hz, 2H), 3.52 (t, *J* = 5.8 Hz, 2H), 2.30 (t, *J* = 5.5 Hz, 1H), 1.74-1.66 (m, 4H); IR ($CHCl_3$): 3625, 3420, 3010, 2940, 2870, 1497, 1428, 1362, 1100, 1050, 1023, 615 cm^{-1}; EI-MS *m/z* 180 (M^+, 4), 107 (62), 91 (100); HRMS calcd for $C_{11}H_{16}O_2$ (M^+): 180.1146, found: 180.1166.

To a dichloromethane (90 mL) solution of oxalyl chloride (3.7 mL, 42.1 mmol) was added dropwise dimethyl sulfoxide (6.0 mL, 84 mmol) at -78 °C under nitrogen atmosphere. After being stirred for 30 min, a dichloromethane (10 mL) solution of 4-benzyloxybutanol (5.0 g, 28 mmol) was added dropwise; the reaction mixture was stirred for 1 h, then followed by the addition of triethylamine (11.7 mL, 84 mmol). After additional stirring for 2 h, the reaction mixture was poured into a saturated ammonium chloride solution, then extracted with dichloromethane. The extract was washed with water, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (elute, AcOEt : Hexane = 2 : 1) gave 4-benzyloxybutanal (**1**) (4.8 g, 96%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.78 (t, *J* = 1.6 Hz, 1H), 7.38-7.25 (m, 5H), 4.49 (s, 2H), 3.50 (t, *J* = 6.1 Hz, 2H), 2.55 (dt, *J* = 1.6, 7.1 Hz, 2H), 1.95 (tt, *J* = 7.1, 6.1 Hz, 2H); IR ($CHCl_3$): 3000, 2930, 2960, 2725, 1720, 1495, 1450, 1405, 1385, 1355, 1090, 1040, 1020, 605 cm^{-1}; FAB-MS *m/z* 179 $[M+H]^+$; HRMS calcd for $C_{11}H_{15}O_2$ $[M+H]^+$: 179.1068, found: 179.1064.

(2*S*,3*S* and 3*R*)-2-Amino-6-benzyloxy-3-hydroxyhexanoic Acid (2e,t) (Table 1) A mixture of (2*S*,3*S* and 3*R*)-2-amino-6-benzyloxy-3-hydroxyhexanoic acids (**2e,t**) was prepared using 4-benzyloxybutanal (**1**) and L-threonine aldolase indicated in Table 1, according to the reported procedure. [7a,c] **2e,t**: white powder; ^{1}H NMR (D_2O, 300 MHz) δ 7.40-7.30 (m, 5H), 4.494 and 4.486 (s, 2H, $PhCH_2O$), 4.01-3.97 (m, 1H, β-proton), 3.75 (d, *J* = 3.7 Hz, *erythro* α-proton), 3.57-3.52 (m, *threo* α-proton and γ-protons), 1.74-1.48 (m, 4H); IR (KBr): 3400, 3100, 2857, 2365, 1665, 1640-1540, 1497, 1455, 1402, 1348, 1110, 1003, 733, 696 cm^{-1}; FAB-MS *m/z* 254; HRMS calcd for $C_{13}H_{20}NO_4$ $[M+H]^+$: 254.1387, found: 254.1409.

The absolute configurations of the β-position were determined by the modified Mosher's method [8] in the compounds (**3a,b**) derived from **2e** and **2t**, respectively, as shown in the following Scheme.



Methyl (2S,3*S* and 3*R*)-2-Acetamido-6-benzyloxy-3-hydroxyhexanoate (4e,t) To a suspension of **2e, 2t** (96.8 mg, 0.384 mmol) in dichloromethane (5 mL) were added triethylamine (0.267 mL, 1.918 mmol) and acetic anhydride (36

μL, 0.384 mmol) at 0 °C under nitrogen atmosphere, and the resultant mixture was stirred for 30 min. Methanol was added to the reaction mixture, then the solvent was evaporated under reduced pressure. The obtained residue was dissolved in ether / methanol, then a ethereal solution of diazomethane was added dropwise at 0 °C. After the excess diazomethane was excluded by bubbling of nitrogen gas, the solvent was evaporated under reduced pressure. Purification of the residue by silica gel column chromatography (elute, AcOEt : Hexane = 3:1) gave *erythro* (**4e**, polar, 30 mg) and *threo* (**4t**, less polar, 58 mg) (total, 88 mg, 75%).

Methyl (2*S*,3*S*)-2-Acetamido-6-benzyloxy-3-hydroxyhexanoate (**4e**): colorless oil; $[\alpha]_D^{19}$ +34.8° (c 1.82, $CHCl_3$), >99% e.e. [HPLC (DAICEL CHIRALCEL OD, Hexane : *i*-PrOH = 9 : 1, flow rate; 1 mL / min, retention time; (2*R*,3*R*)-**4e**: 29 min; (2*S*,3*S*)-**4e**: 44 min]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38-7.28 (m, 5H), 6.49 (br d, *J* = 7.6 Hz, 1H), 4.65 (dd, *J* = 7.6, 3.4 Hz, 1H), 4.51 (s, 2H), 3.92-3.88 (m, 1H), 3.89 (d, *J* = 2.0 Hz, 1H), 3.76 (s, 3H), 3.54-3.50 (m, 2H), 2.05 (s, 3H), 1.80-1.57 (m, 4H); IR ($CHCl_3$): 3430, 3000, 2950, 2860, 1735, 1670, 1500, 1435, 1365, 1090 cm^{-1}; FAB-MS *m/z* 310 $[M+H]^+$; HRMS calcd for $C_{16}H_{23}NO_5$ $[M+H]^+$: 310.1648, found: 310.1638; Anal. Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.09; H, 7.57; N, 4.51.

Methyl (2*S*,3*R*)-2-Acetamido-6-benzyloxy-3-hydroxyhexanoate (**4t**): colorless oil; $[\alpha]_D^{23}$ +32.8° (c 0.57, $CHCl_3$), >99% e.e. [HPLC (DAICEL CHIRALCEL OD, Hexane : *i*-PrOH = 9 : 1, flow rate; 1 mL / min, retention time; (2*R*,3*S*)-**4t**: 33 min; (2*S*,3*R*)-**4t**: 43 min]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38-7.28 (m, 5H), 6.38 (br d, *J* = 9.1 Hz, 1H), 4.63 (dd, *J* = 9.1, 2.0 Hz, 1H), 4.52 (s, 2H), 4.17-4.13 (m, 1H), 3.75 (s, 3H), 3.74 (d, *J* = 4.1 Hz, 1H), 3.57-3.43 (m, 2H), 2.06 (s, 3H), 1.78-1.53 (m, 4H); IR ($CHCl_3$): 3440, 3375, 3000, 2950, 2860, 1740, 1670, 1510, 1430, 1365, 1280, 1140, 1095, 1050 cm^{-1}; FAB-MS *m/z* 310 $[M+H]^+$; HRMS calcd for $C_{16}H_{23}NO_5$ $[M+H]^+$: 310.1648, found: 310.1632.

Methyl (2*S*,3*R*)-2-Benzamido-6-benzyloxy-3-hydroxyhexanoate (5t) Method A: To a suspension of **2t** (28 mg, 0.11 mmol) in dichloromethane (2 mL) were added triethylamine (46 μL, 0.33 mmol) and benzoic anhydride (28 mg, 0.12 mmol) at 0 °C under nitrogen atmosphere. After being stirred for 1 h, methanol was added to the reaction mixture. The reaction mixture was evaporated under reduced pressure, then acidified with 1*N* HCl solution to pH 2. The mixture was extracted with ethyl acetate by salting-out techniques. The extract was washed with water, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was dissolved in methanol and ether, and followed by addition of diazomethane ethereal solution. Excess diazomethane was excluded by bubbling of nitrogen gas, then the solvent was evaporated under reduced pressure. Purification of the residue by silica gel column chromatography (elute, AcOEt : hexane = 1 : 2) gave **5t** (36 mg, 87 %).

Method B: A mixture of **4t** (440 mg, 1.43 mmol) in 1 *N* HCl-MeOH (10 mL) was stirred for 4 h at 60 °C. The reaction mixture was concentrated *in vacuo* to give a residue (amine hydrochloride). To a suspension of the residue in dry dichloromethane (10 mL) were added triethylamine (0.99 mL, 7.14 mmol) and then benzoic anhydride (355.1 mg, 1.57 mmol) at 0 °C under nitrogen atmosphere. After being stirred for 3 h, the reaction mixture was poured into a saturated ammonium chloride solution, then extracted with dichloromethane. The extract was washed with water, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (elute, AcOEt : hexane = 1 : 2) gave **5t** (381 mg, 72%). colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.86-7.81 (m, 2H), 7.55-7.42 (m, 3H), 7.36-7.25 (m, 5H), 6.99 (br d, *J* = 8.8 Hz, 1H, NH), 4.87 (dd, *J* = 8.8, 2.3 Hz, 1H), 4.52 (s, 2H), 4.27 (dt, *J* = 9.3, 2.3 Hz, 1H), 3.78 (s, 3H), 3.59-3.42 (m, 2H), 1.86-1.58 (m, 4H); IR ($CHCl_3$): 3440, 3000, 2950, 2870, 1740, 1660, 1580, 1515, 1480, 1440, 1360, 1300-1250, 1100 cm^{-1}; EI-MS *m/z* 371 (M^+, 1), 327 (6), 294 (4), 262 (7), 247 (10), 222 (5), 193 (54), 179 (5), 161 (38), 127 (20), 105 (100), 91 (63), 57 (49), 43 (24); HRMS calcd for $C_{21}H_{25}NO_5$ (M^+): 371.1726, found: 371.1705.

Methyl (4*S*,5*R*)-5-(3-Benzyloxypropyl)-2-phenyl-Δ²-oxazoline-4-carboxylate ((+)-*trans*-6) **5** (1.28 g, 3.446 mmol) was dissolved in thionyl chloride (5 mL, 57.7 mmol) at 0 °C under nitrogen atmosphere, and the mixture was stirred for 60 h at rt. The reaction mixture was concentrated *in vacuo*. The residue was basified with saturated sodium bicarbonate solution, then extracted with dichloromethane. The extract was washed with water, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (elute, AcOEt : hexane = 1 : 3) gave a mixture of **6** (771 mg, 63%), which were separated by a preparative HPLC. (+)-*trans*-**6**: colorless oil; $[\alpha]_D^{17}$ +75.5° (c 1.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.99-7.96 (m, 2H), 7.52-7.27 (m, 8H), 4.92 (dt, *J* = 7.3, 6.4 Hz, 1H), 4.53 (d, *J* = 7.3 Hz, 1H), 4.52 (s, 2H), 3.80 (s, 3H), 3.57-3.53 (m, 2H), 1.91-1.79 (m, 4H); IR ($CHCl_3$): 3075, 3000, 2950, 2860,

1740, 1645, 1580, 1500, 1455, 1440, 1360, 1280, 1095, 1070, 1030 cm^{-1}; EI-MS *m/z* 353 (M^+, 2), 294 (29), 262 (40), 230 (15), 212 (3), 188 (5), 176 (2), 141 (12), 140 (5), 105 (44), 91 (100), 77 (15), 57 (6), 39 (2); HRMS calcd for $C_{21}H_{23}NO_4$ (M^+): 353.1621, found: 353.1636.

Methyl (4*S*,5*S*)-5-(3-Benzyloxypropyl)-2-phenyl-Δ^2-oxazoline-4-carboxylate [(+)-*cis*-6] A mixture of **4e** (78.3 mg, 0.25 mmol) in 1 *N* HCl-MeOH (5 mL) was heated at 60 °C for 4 h. The reaction mixture was concentrated *in vacuo* to give the hydrochloride. The obtained hydrochloride was dissolved in water (0.5 mL), and then a ether (3 mL) solution of methyl benzimidate (58.1 mg, 0.43 mmol) was added. After being stirred for 19 h at rt, the reaction mixture was extracted with ether, and the extract was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by PTLC (AcOEt : Hexane = 1 : 1) gave (+)-*cis*-**6** (67 mg, 76%).

(+)-*cis*-**6**: colorless oil; $[\alpha]_D^{20}$ +26.3° (c 1.31, $CHCl_3$), >99% e.e. [HPLC (DAICEL CHIRALCEL OD, Hexane : *i*-PrOH = 9 : 1, flow rate; 1 mL / min, retention time; (4*S*,5*S*)-*cis*-**6**: 16 min; (4*R*,5*R*)-*cis*-**6**: 51 min)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.00-7.96 (m, 2H), 7.52-7.26 (m, 8H), 5.00 (d, *J* = 10.2 Hz, 1H), 4.93-4.86 (m, 1H), 4.51 (s, 2H), 3.76 (s, 3H), 3.57-3.46 (m, 2H), 1.93-1.72 (m, 4H); IR ($CHCl_3$): 3000, 2950, 2860, 1740, 1640, 1580, 1490, 1450, 1435, 1355 cm^{-1}; EI-MS *m/z* 353 (M^+,2), 294 (60), 262 (17), 247 (9), 230 (7), 193 (4), 188 (11), 160 (4), 142 (5), 117 (5), 105 (53), 91 (100), 77 (19), 57 (5), 43 (3); HRMS calcd for $C_{21}H_{23}NO_4$ (M^+): 353.1621, found: 353.1636.

The same reaction of **5t** (22.3 mg, 0.06 mmol) with thionyl chloride (44 μL, 0.6 mmol) in dichloromethane (2 mL) gave a 1 : 1 mixture of (+)-*trans*-**6** and (+)-*cis*-**6** (8.9 mg, 42%). Hydroxymethylation of the products as described below gave **7b** whose ee (14% e.e.) was determined by a chiral HPLC analysis (DAICEL CHIRALCEL OD, Hexane : *i*-PrOH = 9 : 1).

Methyl (4*S*,5*S*)-4-Acetoxymethyl-5-(3-benzyloxypropyl)-2-phenyl-Δ^2-oxazoline-4-carboxylate [(-)-7] To a DMF (0.5 mL) solution of (+)-**6**-*cis* (4.3 mg, 0.012 mmol) were added DBU (2 μL, 0.012 mmol) and paraformaldehyde (4 mg) at 0 °C under nitrogen atmosphere, and the mixture was stirred for 1 h at rt. The reaction mixture was poured into a saturated ammonium chloride solution, then extracted with ether. The ethereal extract was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The obtained residue was dissolved in pyridine (0.5 mL, 6.2 mmol) and acetic anhydride (0.5 mL, 5.3 mmol) at 0 °C, and the resultant solution was stirred overnight at rt. Methanol was added to the reaction mixture, and the solvents was evaporated under reduced pressure to give a crude product, which was purified by PTLC (AcOEt : Hexane = 1 : 1) to afford (-)-**7** (4.3 mg, 83%). colorless oil; $[\alpha]_D^{18}$ -50.4° (c 1.83, $CHCl_3$), 98% e.e. [HPLC (DAICEL CHIRALCEL OD, Hexane : *i*-PrOH = 9 : 1, flow rate; 1 mL / min, retention time; (4*S*,5*S*)-**7**: 17 min; (4*R*,5*R*)-**7**: 33 min)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.00-7.97 (m, 2H), 7.54-7.26 (m, 8H), 4.57 (dd, *J* = 4.6, 8.8 Hz, 1H), 4.54 (A part of AB, *J* = 11.3 Hz, 1H), 4.51 (s, 2H), 4.39 (B part of AB, *J* = 11.3 Hz, 1H), 3.77 (s, 3H), 3.56-3.49 (m, 2H), 2.03 (s, 3H), 1.94-1.71 (m, 4H); IR ($CHCl_3$): 3000, 2960, 2870, 1740, 1650, 1580, 1495, 1450, 1360, 1085, 1065, 1040, 1025 cm^{-1}; EI-MS *m/z* 425 (M^+, 1), 366 (11), 352 (44), 334 (7), 306 (22), 259 (4), 246 (2), 230 (3), 185 (4), 172 (11), 149 (7), 105 (30), 91 (100), 77 (14), 43 (17); HRMS calcd for $C_{24}H_{27}NO_6$ (M^+): 425.1831, found: 425.1830.

Methyl (4*S*,5*S*)-4-Acetoxymethyl-5-(3-hydroxypropyl)-2-phenyl-Δ^2-oxazoline-4-carboxylate [(-)-8] Aluminum chloride (695.8 mg, 5.218 mmol) and sodium iodide (782.2 mg, 5.218 mmol) were dissolved in acetonitrile (7 mL) at 0 °C under nitrogen atmosphere. To this solution was added a acetonitrile (3 mL) solution of (-)-**7** (222 mg, 0.522 mmol), and the resultant solution was stirred for 2.5 h at rt. The reaction mixture was poured into ice water, and extracted with ether. The ethereal extract was washed with a solution of sodium hyposulfate, then with brine, and dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue with silica gel column chromatography (elute; AcOEt : Hexane = 2 : 1) gave (-)-**8** (154 mg, 88%). colorless oil; $[\alpha]_D^{22}$ -66.4° (c 0.87, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.01-7.97 (m, 2H), 7.55-7.40 (m, 3H), 4.59 (dd, *J* = 9.2, 4.6 Hz, 1H), 4.55 (A part of AB, *J* = 11.2 Hz, 1H), 4.42 (B part of AB, *J* = 11.2 Hz, 1H), 3.79 (s, 3H), 3.76-3.70 (m, 2H), 2.05 (s, 3H), 1.90-1.71 (m, 4H); IR ($CHCl_3$): 3630, 3450, 2960, 1740, 1645, 1580, 1495, 1445, 1360, 1130, 1060, 970-950 cm^{-1}; EI-MS *m/z* 335 (M^+, 1), 276 (37), 262 (97), 230 (71), 216 (42), 186 (38), 172 (65), 160 (9), 130 (6), 105 (100), 103 (30), 77 (53), 51 (8), 43 (53), 39 (3); HRMS calcd for $C_{17}H_{21}NO_6$ (M^+): 335.1363, found: 335.1383.

Methyl (4*S*,5*S*)-4-Acetoxymethyl-5-(2-formylethyl)-2-phenyl-Δ^2-oxazoline-4-carboxylate [(-)-9] To a dichloromethane (10 mL) solution of (-)-**8** (60.8 mg, 0.181 mmol) was added pyridinium chlorochromate (PCC, 117.4 mg, 0.544 mmol) at 0 °C, and the resultant solution was stirred for 2 h at rt. The inorganic oxidant was removed by short column

chromatography (elute; AcOEt) on silica gel, then the obtained organic residue was purified by silica gel column chromatography (elute; AcOEt : Hexane = 2 : 1) to give (-)-**9** (56 mg, 92%). colorless oil; $[\alpha]_D^{21}$ -62.8° (c 1.79, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.83 (t, *J* = 1.1 Hz, 1H), 7.98-7.95 (m, 2H), 7.56-7.41 (m, 3H), 4.62 (dd, *J* = 10.3, 3.6 Hz, 1H), 4.54 (A part of AB, *J* = 11.3 Hz, 1H), 4.44 (B part of AB, *J* = 11.3 Hz, 1H), 3.81 (s, 3H), 2.78-2.72 (m, 2H), 2.04 (s, 3H), 2.06-1.94 (m, 2H); IR ($CHCl_3$): 2950, 2830, 2725, 1730, 1640, 1580, 1490, 1445, 1380, 1360, 1125, 1060, 600 cm^{-1}; FAB-MS *m/z* 334 $[M+H]^+$; HRMS calcd for $C_{17}H_{19}NO_6$ $[M+H]^+$: 334.1285, found: 334.1297.

Methyl (4*S*,5*S*)-4-Acetoxymethyl-5-{(3*Z*)-11,11-ethylenedioxyheptadec-3-enyl}-2-phenyl-Δ²-oxazoline-4-carboxylate [11*Z*] To a THF (10 mL) solution of **10**[4i] (665.3 mg, 1.115 mmol) was added dropwise *n*-butyllithium (1.64 M hexane solution, 0.618 mL, 1.014 mmol) at -78 °C under nitrogen atmosphere, then the mixture was stirred for 30 min at 0 °C. Then, the reaction mixture was cooled to -20 °C. A solution of aldehyde (-)-**9** (112.5 mg, 0.338 mmol) in THF (3 mL) was added dropwise to the reaction mixture, and the resultant mixture was stirred for 2 h. The reaction mixture was poured into saturated ammonium chloride solution, then extracted with ether. The ethereal extract was washed with brine, dried ($MgSO_4$), filtered, then concentrated *in vacuo*. The obtained crude product was dissolved in pyridine (2 mL, 25.7 mmol) and acetic anhydride (2 mL, 21.2 mmol) at 0 °C, and the mixture was stirred overnight. The excess methanol was added to the reaction mixture, then the solvent was removed under reduced pressure. Purification of the residue by silica gel column chromatography (elute, AcOEt : hexane = 1 : 3) gave **11*Z*** (159 mg, 82%). colorless oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 8.04-8.01 (m, 2H), 7.56-8.88 (m, 3H), 5.48 (dt, *J* = 10.8, 7.2 Hz, 1H), 5.34 (dt, *J* = 10.8, 7.3 Hz, 1H), 4.59 (dd, *J* = 3.3, 10.5 Hz, 1H), 4.56 (A part of AB, *J* = 11.3 Hz, 1H), 4.40 (B part of AB, *J* = 11.3 Hz, 1H), 3.92 (s, 4H), 3.79 (s, 3H), 2.30 (q, *J* = 7.0 Hz, 2H), 2.10-2.03 (m, 2H), 2.03 (s, 3H), 1.73-1.55 (m, 6H), 1.36-1.24 (m, 16H), 0.88 (br t, *J* = 7.0 Hz, 3H); IR ($CHCl_3$): 2930, 2850, 1740, 1640, 1445, 1360, 1130, 1085, 1060, 1040, 940, 920 cm^{-1}; EI-MS *m/z* 571 (M+, 24), 512 (13), 486 (92), 415 (13), 157 (100), 105 (34), 57 (27), 43 (25); HRMS calcd for $C_{33}H_{49}NO_7$ (M^+): 571.3496, found: 571.3519.

Methyl (4*S*,5*S*)-4-Acetoxymethyl-5-{(3*E*)-11,11-ethylenedioxyheptadec-3-enyl}-2-phenyl-Δ²-oxazoline-4-carboxylate [(-)-11*E*] The solution of **10*Z*** (86.2 mg, 0.151 mmol) and diphenyl disulfide (65.8 mg, 0.302 mmol) in cyclohexane (15 mL) was degassed in a quartz flask under nitrogen atmosphere by the freeze-and-thaw technique. The reaction mixture was irradiated by high-pressure mercury lamp for 5 h. The reaction mixture was concentrated *in vacuo*, the residue was purified by silica gel column chromatography (elute, AcOEt : hexane = 1 : 2) to give (-)-**11*E*** (84 mg, 98%). colorless oil: $[\alpha]_D^{19}$ -49.25° (c 0.46, CHCl3), 96% e.e. [HPLC (DAICEL CHIRALCEL OD, flow rate; 1 mL / min, retention time; (4*S*,5*S*)-**11*E***: 6 min; (4*R*,5*R*)-**11*E***: 8 min)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.02-7.99 (m, 2H), 7.55-7.40 (m, 3H), 5.50 (dt, *J* = 15.4, 6.4 Hz, 1H), 5.38 (dt, *J* = 15.4, 6.5 Hz, 1H), 4.55 (dd, *J* = 10.0, 3.6 Hz, 1H), 4.54 (A part of AB, *J* = 11.2 Hz, 1H), 4.39 (B part of AB, *J* = 11.2 Hz, 1H), 3.92 (s, 4H), 3.78 (s, 3H), 2.30-2.16 (m, 2H), 2.05 (s, 3H), 1.99 (br q, *J* = 6.7 Hz, 2H), 1.73-1.56 (m, 6H), 1.29-1.24 (br m, 16H), 0.88 (t, *J* = 6.7 Hz, 3H); IR ($CHCl_3$): 2925, 2850, 1740, 1640, 1445, 1360, 1125, 1080, 1060, 1040, 965, 940 cm^{-1}; EI-MS *m/z* 571 (M^+, 23), 486 (81), 415 (11), 157 (100), 105 (30), 43 (17); HRMS calcd for $C_{33}H_{49}O_7N$ (M^+): 571.3496, found: 571.3516.

(2*S*,3*S*)-(*E*)-2-Amino-3-hydroxy-2-hydroxymethyl-14-oxoicos-6-enoic Acid (mycestericin D) (-)-**11*E*** (41.5 mg, 0.073 mmol) was heated in 6*N* hydrochloric acid (1 mL) at 100 °C for 6 h. The reaction mixture was neutralized with 2*N* NaOH to pH 7. After condensation of the solvent, the residue was dissolved in MeOH (1 mL) and 2*N* NaOH (1 mL), then heated at 60 °C for 2.5 h. The reaction mixture was neutralized with 1*N* HCl to pH 5-6, then concentrated *in vacuo*. The obtained crude product was dissolved in water and allowed to stand overnight. The precipitated powder was collected by filtration as mycestericin D (18 mg, 63%). Further purification was performed by HPLC (YMC-ODS SH-343-5, MeOH : H_2O = 70 : 30). white powder: mp 164-170 °C (H_2O); $[\alpha]_D^{23}$ -7.05° (c 0.09, CH_3OH); ^1H NMR (CD_3OD, 600 MHz) δ 5.45 (dt, *J* = 15.5, 5.8 Hz, 1H), 5.41 (dt, *J* = 15.5, 5.8 Hz, 1H), 3.97 (A part of AB, *J* = 11.3 Hz, 1H), 3.84-3.83 (m, 1H), 3.81 (B part of AB, *J* = 11.3 Hz, 1H), 2.43 (t, *J* = 7.5 Hz, 2H), 2.43 (t, *J* = 7.5 Hz, 2H), 2.27-2.20 (m, 1H), 2.06-1.99 (m, 1H), 1.96 (q, *J* = 6.6 Hz, 2H), 1.56-1.50 (m, 6H), 1.36-1.25 (m, 12H), 0.89 (t, *J* = 7.1 Hz, 3H); IR (KBr): 3400, 3100, 2928, 2855, 1707, 1676, 1636, 1561, 1518, 1500, 1460, 1049, 968 cm^{-1}; FAB-MS *m/z* 386 $[M+H]^+$; HRMS calcd for $C_{21}H_{39}NO_5$ $[M+H]^+$: 386.2896, found: 386.2924.

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