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SYNTHESIS OF BOTH ENANTIOMERS OF BREVIOXIME AND DETERMINATION OF ITS ABSOLUTE CONFIGURATION

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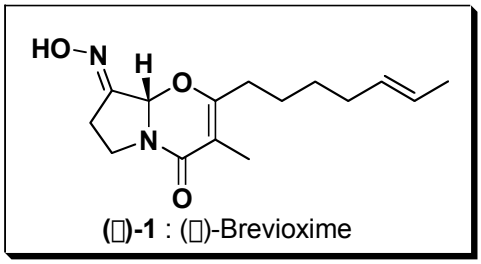
Abstract – Brevioxime is an inhibitor of juvenile hormone biosynthesis, isolated from *Penicillium brevicompactum*. We synthesized both enantiomers of brevioxime and determined the absolute configuration of natural product as *S*.

INTRODUCTION

Brevioxime (**1**) was isolated from *Penicillium brevicompactum* as anti-juvenile hormone (JH) agent by Primo- YÚfera and coworkers in 1997. [1] It is known that the enzymes involved in the final steps of JH biosynthesis are specific for insects. Brevioxime is thought to inhibit the final steps of JH biosynthesis, so, it is very attractive compound as a potential and selective insecticide. Racemic syntheses of brevioxime have already been reported by three groups including us.[2] Although Clark reported (□)-brevioxime, they couldn't determine its absolute configuration.[3] Now, we report the synthesis of both enantiomers of brevioxime and the determination of the absolute configurations of natural compound in this paper.



(□)-1 : (□)-Brevioxime

Figure 1

RESULTS AND DISCUSSION

Our synthetic approach is based on its probable biosynthetic route as shown in Figure 2. We had already synthesized racemic brevioxime in similar approach successfully.[2] The heterocyclic skeleton of brevioxime (**1**) will be constructed by intramolecular aminalization of **2** under acid condition. We considered that it is possible to control occurring stereochemistry by introduction of chiral center at the adjacent carbon. Even if the selectivity is not desirable, it will be no matter to separate those diastereomers.

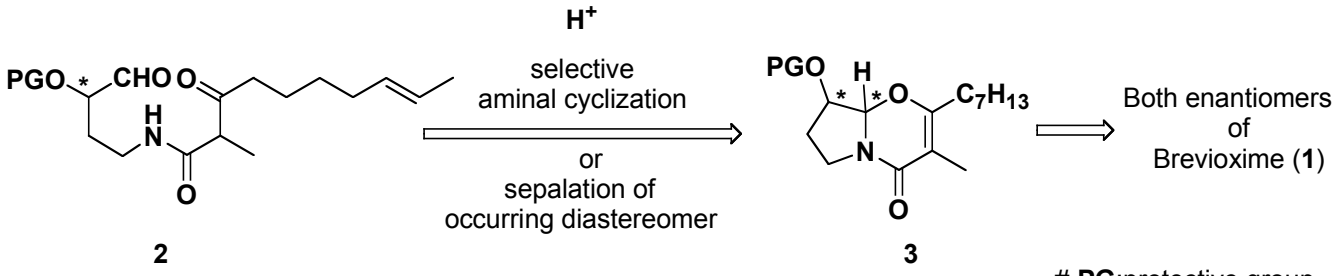
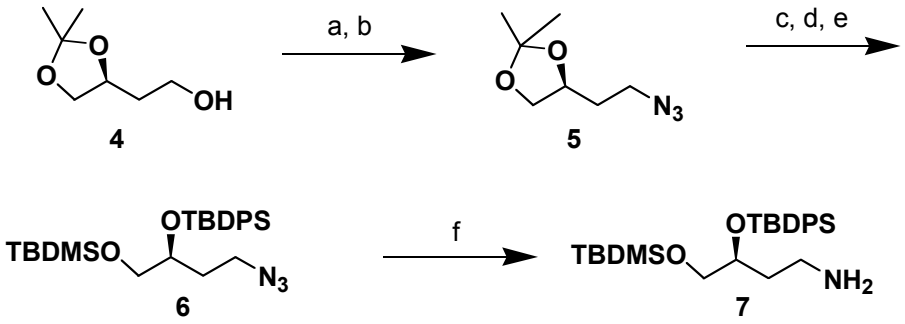


Figure 2

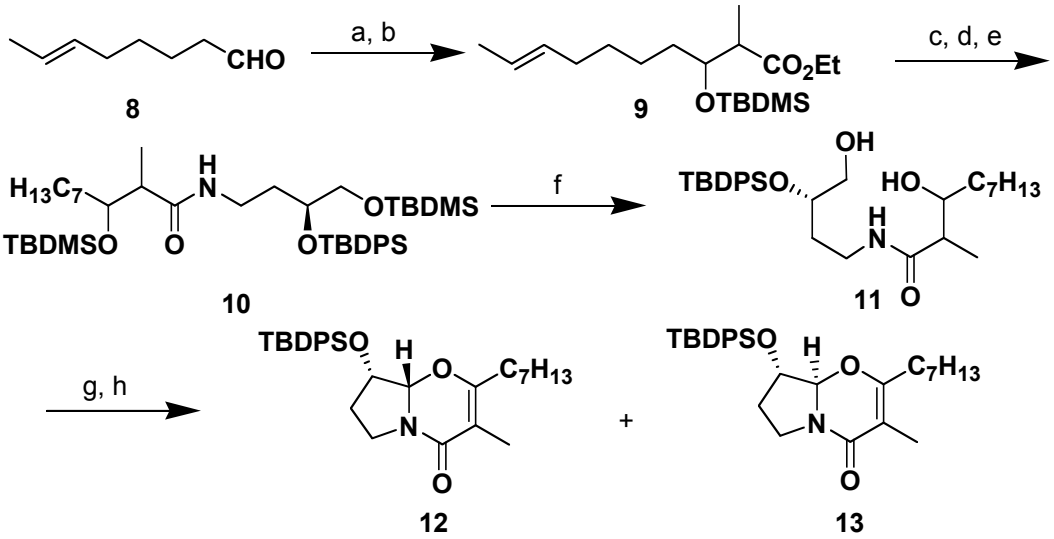
Synthesis of the amine moiety of the key intermediate (**2**) was achieved as shown in Scheme 1. We employed alcohol (**4**) as starting material prepared from (*S*)-malic acid in 4 steps easily by Moriwake's procedure.[4] Alcohol (**4**) was converted to azide (**5**), which was treated with hydrochloric acid to afford diol. Then primary and secondly hydroxy groups were selectively protected using *tert*-butyldimethylsilyl chloride (TBDMSCl) and *tert*-butyldiphenylsilyl chloride (TBDPSCl) respectively in 2 steps to give **6**. Finally the desired amine (**7**) was obtained by hydrogenatin of the azide (**6**).



Scheme 1. a) MsCl, pyridine, CH_2Cl_2. b) NaN_3, DMF, 100 ^oC, 87% in 2 steps. c) conc.HCl, MeOH, quant. d) TBDMSCl, imidazole, DMF, 93%. e) TBDPSCl, imidazole, DMF, 92%. f) H_2, 10% Pd-C, EtOAc, quant.

The carboxylic acid moiety of **2** was prepared from 2-octenal (**8**)[5] as shown in Scheme 2. The aldehyde (**8**) was reacted with ethyl □-bromopropionate under Reformatsky's condition to give corresponding hydroxy ester, whose hydroxyl group was protected as TBDMS ether (**9**). Hydrolysis of this ester

followed by the coupling reaction with the amine (**7**) gave amide (**10**). TBDMS groups were removed to afford **11**, which was oxidized by Swern's method to give corresponding hemiaminal. Concerning to this oxdation, we tried other conditions, but no better result was obtained than that by Swern oxdation.[2] Treatment of the resulting hemiaminal substrate with *p*-toluenesulfonic acid promoted cyclization which afforded a mixture of oxazinones (**12** and **13**, *ca*. 1:1 ratio) with bicyclic. These diastereomers were separated easily by flash colum chromatography.



Scheme 2. a) ethyl □-bromopropionate, Zn, benzene, 91%. b) TBDMSCl, imidazole, 84%. c) LiOH, H_2O, THF, MeOH, 92%. d) $(COCl)_2$, benzene. e) **7**, Et_3N, CH_2Cl_2, 97% in 2 steps. f) TsOH, MeOH, 86%. g) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2. h) TsOH, CH_2Cl_2, **12**:32% in 2 steps, **13**:30% in 2 steps.

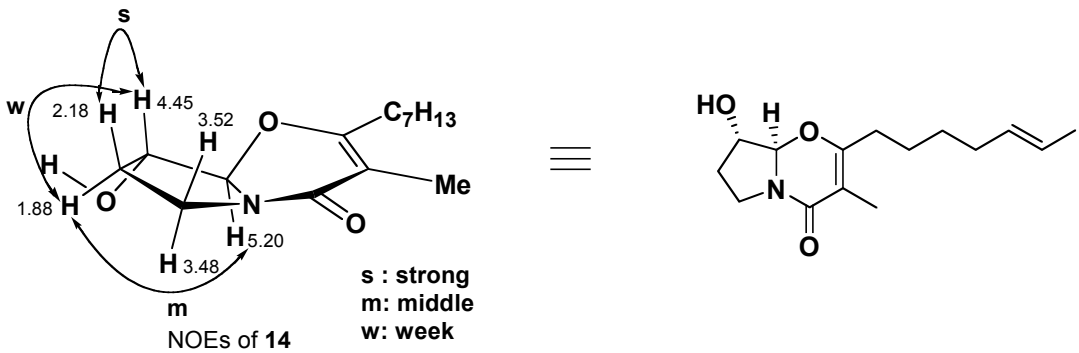
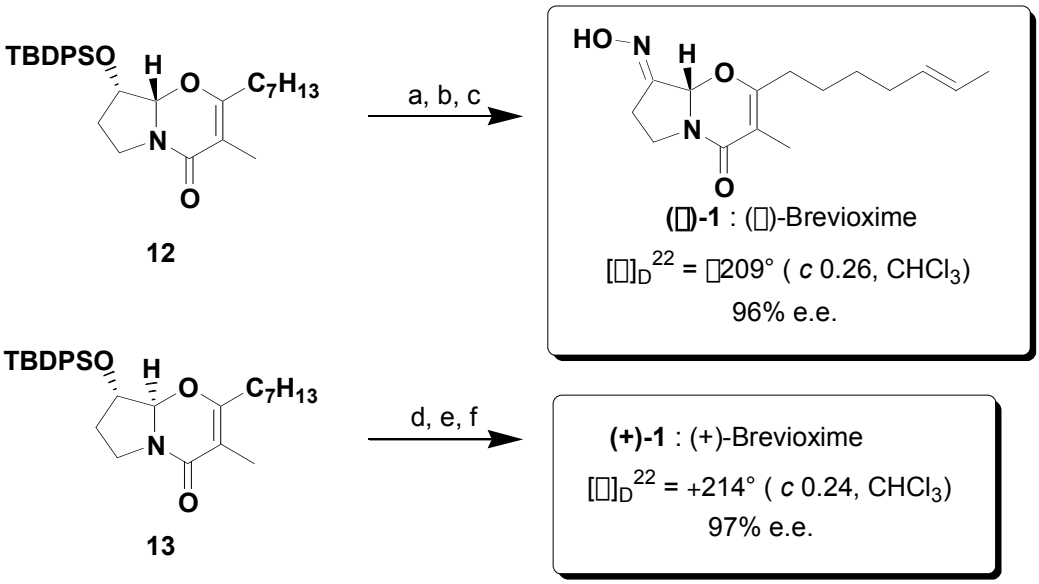


Figure 3

In order to determine the relative stereochemistry of **12** and **13**, the alcohol (**14**) derived from **13** was subjected to NOE experiments. Its stereochemistry was unambiguously assigned by NOE observations as shown in Figure 3. Then, these diastereoisomers (**12** and **13**) were comverted to (+)- and (□)-brevioxime respectively as follows (Scheme 3). After the removal of silyl group of **12**, hydroxyl group was oxidized with Dess-Martin periodinane to yield ketone. Finally, this ketone was reacted with hydroxylamine[6] to

afford (−)-brevioxime ((−)-**1**) as colorless crystal ($[\alpha]_D^{22}$ = −209° (*c* 0.26, $CHCl_3$)). Although this reaction gave both geometrical isomers in a ratio of *ca*. 2:1, these were easily separable by flash column chromatography. The *Z*-isomer was convertible to *E*-isomer according to the reported procedure.[2] In the same manner as above, **13** was converted to (+)-**1** ($[\alpha]_D^{22}$ = +214° (*c* 0.26, $CHCl_3$)). IR, ^{1}H NMR and ^{13}C-NMR spectra of synthetic (−)-**1** and (+)-**1** were identical with those of natural brevioxime.

Thus, the stereochemistry of natural brevioxime was determined to be *S*. However $[\alpha]_D$ values described in previous reports were –39 (natural product) and –129, respectively,[1,3] which were much different from that of the present study. So, we examined the enantiomeric purity of our synthesized brevioximes. Both (−)-**1** and (+)-**1** were converted to the corresponding (*R*)-MTPA ester and analyzed by HPLC. It was found that the optical purity of (−)-**1** was 96% e.e. and that of (+)-**1** was 97% e.e. We suppose that the reported natural and synthetic brevioxime were not optically or chemically pure.



Scheme 3. a) TBAF, THF, 91%. b) Dess-Martin ox., 59%. c) $HONH_2$·HCl, NaOAc, MeOH, *E*-isomer 46%, *Z*-isomer 24%. d) TBAF, THF, 89%. e) Dess-Martin ox., 94%. f) $HONH_2$HCl, NaOAc, MeOH, *E*-isomer 49%, *Z*-isomer 27%. .

In conclusion, we have accomplished the synthesis of both enantiomers of brevioxime and determined the absolute configuration of natural (−)-brevioxime to be *S* as shown in Scheme 3. It seems that natural brevioxime is not optically pure and its enantiomeric excess is estimated at 17%, if chemically pure. Biological study of these enantiomers and related analogs was conducted and the results will be reported elsewhere.

EXPERIMENTAL

Optical rotations were recorded with a JASCO DIP-1000 polarimeter. IR spectra were measured with a

JASCO FT/IR-230 spectrophotometer. ^{1}H and ^{13}C NMR and NOESY were recorded on JEOL JNM EX90, AL300 and A500. MS spectra were recorded on JEOL JMS SX102. Column chromatography was performed using Merck silica gel 60 (0.060-0.200 mm) and Kanto Silica gel 60N (40-100 μm). TLC was carried out on Merck glass plates precoated with silica gel 60 F_{254} (0.25 mm). HPLC was performed using Senshu Pak Silica-1301-N (4.6ø x 300 mm).

(S)-4-(2-Azidoethyl)-2,2-dimethyl-1,3-dioxolane (5)

To an ice-cooled solution of **4** (34.2 g, 234 mmol) and pyridine (57 mL, 36.6 mmol) in CH_2Cl_2 (300 mL) was added MsCl (32.6 mL, 421 mmol) and stirred at 4 °C for 8 h. The reaction mixture was poured into ice-cooled water and extracted with Et_2O. The organic layer was washed with saturated cupric sulfate solution, water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was employed in the next step without further purification.

To a solution of crude mesylate in DMF (350 mL) was added NaN_3 (22.8 g, 351 mmol) and stirred at 100°C for 2.5 h. After dilution with H_2O at 0 °C the reaction mixture was extracted with hexane and combined organic layers were washed with H_2O, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 1:20) to afford **5** (34.8 g, 87% in 2 steps) as a colorless oil: $[\alpha]_D^{19} = -16.1°$ (*c* 0.82, $CHCl_3$); IR (film, cm^{-1}) ν 2100 (N_3); ^{1}H NMR (90 MHz, $CDCl_3$) δ = 1.40 (3H, s), 1.35 (3H, s), 1.7-1.9 (2H, m), 3.3-3.7 (1H, m), 3.34 (2H, t, *J* = 7.1 Hz), 4.0-4.3 (2H, m).

(S)-4-Azido-1-tert-butyldimethylsilyloxy-2-tert-butyldiphenylsilyloxybutane (6)

To a solution of **5** (22.3 g, 118 mmol) in MeOH (150 mL) was added 3 N HCl (20 mL) and the mixture was stirred at rt for 3 days. Then the reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (EtOAc only) to afford (*S*)-4-azidobutane-1,2-diol (15.6 g, quant.) as a colorless oil: IR (film, cm^{-1}) ν 3440 (OH), 2100 (N_3); ^{1}H NMR (90 MHz, $CDCl_3$) δ = 1.5-1.8 (2H, m), 2.4-4.0 (7H, m).

To an ice-cooled solution of (*S*)-4-azidobutane-1,2-diol (21.1 g, 157 mmol) and imidazole (16.1 g, 236 mmol) in DMF (200 mL) was added *tert*-butyldimethylsilyl chloride (28.4 g, 188 mmol) carefully and the reaction mixture was stirred at 4 °C for 10 h. After the addition of water, the reaction mixture was stirred 30 min and then extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 1:10) to afford (*S*)-4-azido-1-*tert*-butyldimethylsilyloxybutan-2-ol (36.0 g, 93%) as a colorless oil: $[\alpha]_D^{21} = -10.6°$ (*c* 1.37, $CHCl_3$); IR (film, cm^{-1}) ν 3420 (OH), 2100 (N_3), 1260 (SiMe); ^{1}H NMR (90 MHz, $CDCl_3$) δ = 0.08 (6H, s), 0.90 (9H, s), 1.67 (2H, q, *J* = 6.6 Hz), 3.47 (2H, t, *J* = 6.6 Hz),

3.3-3.9 (3H, m).

To an ice-cooled solution of (*S*)-4-azido-1-*tert*-butyldimethylsilyloxybutane-2-ol (1.99 g, 8.11 mmol), imidazole (1.38 g, 20.3 mmol) and *N,N*-dimethylaminopyridine (10 mg) in DMF (20 mL) was added *tert*-butyldiphenylsilyl chloride (3.2 mL, 12 mmol) and the reaction mixture was stirred at rt for 10 h. After the addition of water, the reaction mixture was stirred 30 min and then extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 1:50) to afford **6** (3.77 g, 92%) as a colorless oil: $[\alpha]_D^{21} = -9.63°$ (*c* 0.50, $CHCl_3$); IR (film, cm^{-1}) ν 2100 (N_3), 1260 (SiMe); ^1H NMR (300 MHz, $CDCl_3$) δ = –0.16 (3H, s), 0.12 (3H, s), 0.78 (9H, s), 1.03 (9H, s), 1.7-1.9 (2H, m), 3.2-3.5 (4H, m), 3.79 (1H, qui, *J* = 5.4 Hz).

(S)-4-tert-Butyldimethylsilyloxy-3-tert-butyldiphenylsilyloxybutylamine (7)

Under hydrogen, a suspension of the azide (**6**) (10.4 g, 20.5 mmol) and 10% Pd-C (350 mg) in EtOAc (50 mL) was stirred at rt for 3 h. The reaction mixture was filtered through Celite and concentrated in vacuo to afford crude **7** (9.35 g, quant.) as a colorless oil: IR (film, cm^{-1}) ν 3380 and 3310 and 1600 (NH_2), 1260 (SiMe). This amine (**7**) was employed in the next reaction without further purification.

(E)-3-tert-Butyldimethylsilyloxy-2-methyldec-8-enoic acid ethyl ester (9)

Under argon, to the suspension of activated zinc (168 mg, 4.10 mmol) in benzene (1 mL) was added dropwise a solution of ethyl α-bromopropionate (706 mg, 3.90 mmol) and aldehyde (**8**) (500 mg, 3.90 mmol) in benzene (10 mL) at 60 °C and the suspension was refluxed for 2 h. To the reaction mixture was added 20% sulfuric acid and the reaction mixture was stirred at rt for 10 min and then poured into water. The mixture was extracted with EtOAc and the organic layer was washed with diluted sulfuric acid, water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 1:50) to afford hydroxy ester (813 mg, 91%) as a colorless oil: IR (film, cm^{-1}) ν 3480 (OH), 1730 and 1710 (CO_2Et); ^1H NMR (300 MHz, $CDCl_3$) δ = 1.1-1.7 (15H, m), 1.9-2.1 (2H, m), 2.5-2.7 (1H, m), 3.65 and 3.89 (1H, m), 4.1-4.3 (2H, m), 5.3-5.5 (2H, m).

To an ice-cooled solution of hydroxy ester (813 mg, 3.56 mmol) and imidazole (363 mg, 5.43 mmol) in DMF (6 mL) was added *tert*-butyldimethylsilyl chloride (968 mg, 4.63 mmol) and the reaction mixture was stirred at rt for 10 h. After the addition of water, the reaction mixture was stirred for 30 min and then extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 1:100) to afford **9** (1.03 g, 85%) as a colorless oil: IR (film, cm^{-1}) ν = 1740 (CO_2Et), 1260 (SiMe); ^1H NMR (300 MHz, $CDCl_3$) δ = 0.0-0.5 (6H, m), 0.86 (9H, s), 1.07 and 1.10 (3H, d, *J* = 7.0), 1.25 and 1.25

(3H, t, *J* = 7.1), 1.2-1.5 (6H, m), 1.6-1.65 (3H, m), 1.9-2.0 (2H, m), 2.45-2.7 (1H, m), 3.9-4.0 (1H, m), 4.05-4.15 (2H, m), 5.3-5.5 (2H, m).

(E)-N-[(S)-4-tert-Butyldimethylsilyloxy-3-trert-butyldiphenylsilyloxybutyl]-3-tert-butyldimethylsilyloxy-2-methyldec-8-enamide (10)

To an ice-cooled solution of **9** (5.83 g, 17.0 mmol) in THF (70 mL), MeOH (30 mL) and water (10 mL) was added a solution of 3 N lithium hydroxide (35 mL, 105 mmol) and the reaction mixture was stirred at rt for 3 days. The resulting solution was quenched with 1 N hydrochloric acid carefully at 0 °C and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 1:50) to afford corresponding carboxylic acid (4.92 g, 92%) as a colorless oil: IR (film, cm^{-1}) □ 3100 (CO_2H), 1260 (SiMe).

A solution of carboxylic acid (1.38 g, 4.39 mmol) in benzene (18 mL) was treated with oxalyl chloride (5 mL, 57 mmol) at 0 °C and stirred at rt for 2 h. After concentration, the residue was solved in CH_2Cl_2 (30 mL). This solution was added dropwise to a solution of amine (**7**) (3.01 g, 6.57 mmol) and triethylamine (3 mL, 32 mmol) in CH_2Cl_2 (30 mL) at 0 °C and the reaction mixture was stirred at rt for 3 h. Then the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 1:20) to afford **10** (3.21 g, 97%) as a colorless oil: $[\square]_D^{20}$ = –8.40° (*c* 1.11, $CHCl_3$); IR (film, cm^{-1}) □ 3550 and 1660 (NHCO), 1260 (SiMe); ^1H NMR (300 MHz, $CDCl_3$) □ = –0.2-0.7 (12H, m), 0.79 (9H, s), 0.88 (9H, s), 1.05 (9H, s), 1.2-1.5 (7H, m), 1.6-1.8 (7H, m), 1.9-2.0 (2H, m), 2.2 (1H, m), 3.1-3.5 (4H, m), 3.7-3.8 (2H, m), 5.3-5.5 (2H, m), 6.13, 6.24 and 6.40 (1H, m), 7.3-7.5 (6H, m), 7.60 (4H, d, *J*=7.4 Hz). Anal. Calcd for $C_{43}H_{75}NO_4Si_3$: C, 68.47; H, 10.02; N, 1.86. Found: C, 67.70; H, 9.99; N 1.83.

(E)-N-[(S)-4-Hydroxy-3-tert-butyldiphenylsilyloxybutyl]-3-hydroxy-2-methyldec-8-enamide (11)

A solution of **10** (2.10 g, 2.78 mmol) and *p*-TsOH (600 mg) in CH_2Cl_2 (150 mL) was stirred at 40 °C for 1 day. Then the reaction mixture was poured into saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc:hexane = 2:1- EtOAc only) to afford **11** (1.26 g, 86%) as a colorless oil: $[\square]_D^{21}$ = –0.69° (*c* 0.35, $CHCl_3$); IR (film, cm^{-1}) □ 3550 (OH), 1660-1630 (NCO); ^1H NMR (300 MHz, $CDCl_3$) □ = 1.08 (9H, s), 1.2-1.5 (7H, m), 1.6-1.8 (7H, m), 1.9-2.0 (2H, m), 2.5 (1H, m), 3.0-3.9 (6H, m), 5.3-5.6 (2H, m), 7.3-7.5 (6H, m), 7.6-7.7 (4H, m); HRMS m/z [MH^+] 526.3353 (calcd for $C_{31}H_{48}NO_4Si$ 525.3340).

(E)-2-Hept-5-enyl-8-hydroxy-3-methyl-6,7,8,8a-tetrahydro-8-tert-butyldiphenylsilyloxy-3-methyl-4H-pyrrolo[2,1-b][1,3]oxazin-4-one [(8S,8aS)-12 and (8S, 8aR)-13]

To a solution of oxalyl chloride (1.99 mL, 22.8 mmol) in CH_2Cl_2 (180 mL) was added DMSO (1.78 mL, 25.1 mmol) at –78 °C. After 5 min, a solution of diol (**11**) (3.00 g, 5.71 mmol) in CH_2Cl_2 (20 mL) was added to the reaction mixture and it was stirred at –40 °C for 1 h. After addition of triethyl amine (20 mL, 143 mmol), the reaction mixture was poured into water and extracted with Et_2O. The organic layer was washed with water, saturated NH_4Cl and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was filtered through silica gel (EtOAc only) to afford hemiaminal as a colorless oil: IR (film, cm^{-1}) □ 3550 (OH), 1660-1630 (NCO). This compound was employed in the next step without further purification.

A solution of hemiaminal and *p*-TsOH (300 mg) in CH_2Cl_2 (180 mL) was stirred at 0 °C for 3 h. Then the reaction mixture was poured into saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (EtOAc:hexane = 1:4-1:3) to afford **12** (943 mg, 32%) and **13** (865 mg, 30%) as a colorless oil:

12: $[\square]_D^{28}$ = –115° (*c* 1.08, $CHCl_3$); IR (film, cm^{-1}) □ 3450, 1670, 1660 and 1650 (NCO); ^{1}H NMR (300 MHz, $CDCl_3$) □ = 1.07 (9H, s), 1.3-1.5 (4H, m), 1.63 (3H, d, J = 4.2 Hz), 1.79 (3H, s), 1.8-2.1 (5H, m), 2.23 (1H, m), 3.45 (1H, dt, *J* = 3.8, 7.5 Hz), 3.78 (1H, dt, *J* = 7.9, 10.8 Hz), 4.43 (1H, dd, *J* = 3.8, 7.5 Hz), 4.94 (1H, d, *J* = 3.8 Hz), 5.3-5.5 (2H, m), 7.3-7.5 (6H, m), 7.6-7.8 (4H, m); HRMS m/z [MH^+] 504.2947 (calcd for $C_{31}H_{42}NO_3Si$ 504.2934).

13: $[\square]_D^{19}$ = +28.9° (*c* 0.22, $CHCl_3$); IR (film, cm^{-1}) □ 3450, 1660 (NCO); ^{1}H NMR (300 MHz, $CDCl_3$) □ = 1.08 (9H, s), 1.3-1.5 (4H, m), 1.64 (3H, d, *J* = 4.2 Hz), 1.76 (3H, s), 1.8-2.0 (4H, m), 2.1-2.3 (2H, m), 3.45-3.65 (2H, m), 4.42 (1H, dd, *J* = 3.7, 6.8 Hz), 5.09 (1H, d, *J* = 3.7 Hz), 5.3-5.5 (2H, m), 7.3-7.5 (6H, m), 7.6-7.8 (4H, m); HRMS m/z [MH^+] 504.2948 (calcd for $C_{31}H_{42}NO_3Si$ 504.2934).

(S)-[(E,E) and (E,Z)]-2-(5-Heptenyl)-6,7-dihydro-3-methyl-4H-pyrrolo[2,1-b][1,3]oxazine-4,8(8aH)-dione 8-oxime [(–)-Brevioxime (1) and Z-Isomer of (–)-Brevioxime]

To an ice-cooled solution of **12** (325 mg, 0.65 mmol) in THF (7 mL) was added TBAF-hydrate (350 mg) and the reaction mixture was stirred at rt for 1 h and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (EtOAc:hexane = 1:1-2:1) to afford corresponding alcohol (156 mg, 91%) as a colorless oil: $[\square]_D^{19}$ = –60.8° (*c* 1.04, $CHCl_3$); IR (film, cm^{-1}) □ 3400 and 1650 (NCO); ^{1}H NMR (300 MHz, $CDCl_3$) □ = 1.3-1.45 (2H, m), 1.4-1.45 (2H, m), 1.64 (3H, d, *J* = 4.6 Hz), 1.80 (3H, s), 1.9-2.15 (4H, m), 2.15-2.4 (2H, m), 3.57 (1H, ddd, *J* = 2.7, 8.4, 11.1 Hz), 3.73 (1H, ddd, *J* = 7.2, 9.3, 11.1 Hz), 4.45 (1H, dd, *J* = 3.8, 6.0 Hz), 5.21 (1H, d, *J* = 3.5 Hz), 5.35-5.5 (2H, m).

To an ice-cooled solution of alcohol (400 mg, 1.50 mmol) in CH_2Cl_2 (15 mL) was added Dess-Martin periodinane (1.91 g, 4.49 mmol) and the mixture was stirred at rt for 6 h. The reaction mixture was poured into saturated Na_2SO_3 and extracted with CH_2Cl_2. The organic layer was washed with water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (EtOAc:hexane = 1:1) to afford ketone (236 mg, 60%) as a colorless oil: $[\alpha]_D^{28}$ = –203° (*c* = 1.37, $CHCl_3$); IR (film, cm^{-1}) ν 1780 (s, C=O), 1630-1680 (s, NCO); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.3-1.45 (2H, m), 1.5-1.7 (2H, m), 1.64 (3H, d, *J* = 4.8 Hz), 1.84 (3H, s), 1.95-2.05 (2H, m), 2.2-2.4 (2H, m), 2.65-2.8 (2H, m), 3.61 (1H, ddd, *J* = 7.2, 8.7, 11.4 Hz), 4.12 (1H, m), 5.03 (1H, s), 5.3-5.5 (2H, m).

To an ice-cooled solution of the corresponding ketone (165 mg, 0.63 mmol) in MeOH (10 mL) were added NaOAc (129 mg, 1.57 mmol) and NH_2OH•HCl (87.1 mg, 1.25 mmol) and the mixture was stirred at rt for 3 h. Then the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (EtOAc:hexane = 1:1) to afford **(–)-1** [*E*:*Z* = 46% (81 mg) : 24% (42 mg)] as colorless crystal.

(–)-Brevioxime (1): mp 147-149ºC; $[\alpha]_D^{22}$ = –209° (*c* 0.26, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.3-1.4 (2H, m), 1.5-1.6 (2H, m), 1.63 (3H, d, *J* = 4.5 Hz), 1.83 (3H, s), 1.85-2.0 (2H, m), 2.2-2.4 (2H, m), 2.8-3.0 (2H, m), 3.48 (1H, dt, *J* = 8.1, 11.4 Hz), 4.07 (1H, ddd, *J* = 3.6, 9.3, 11.4 Hz), 5.3-5.4 (2H, m), 5.56 (1H, s), 8.79 (1H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ =10.1, 17.9, 23.8, 26.2, 29.1, 30.6, 32.2, 41.7, 84.1, 107.0, 125.2, 130.8, 157.7, 163.4, 163.9; HRMS m/z [MH^+] 279.1669 (calcd for $C_{15}H_{23}N_2O_3$ 279.1708).

***Z*-Isomer of (–)-Brevioxime:** ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.35-1.4 (2H, m), 1.5-1.6 (2H, m), 1.6-1.65 (3H, m), 1.83 (3H, s), 1.9-2.0 (3H, m), 2.15-2.45 (2H, m), 2.70 (1H, ddd, *J* = 1.8, 7.5, 9.6 Hz), 2.84 (1H, m), 3.34 (1H, ddd, *J* = 7.5, 9.6, 11.1 Hz), 5.3-5.5 (2H, m), 5.82 and 5.83 (1H, s), 9.0 (1H, brs); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 10.0, 17.9, 26.3, 27.1, 29.0, 30.5, 32.2, 41.5, 79.8, 106.7, 125.1, 130.9, 156.2, 163.3, 164.2.

(R)-[(E,E) and (E,Z)]-2-(5-Heptenyl)6,7-dihydro-3-methyl-4H-pyrrolo[2,1-b][1,3]oxazine-4,8(8aH)-dione 8-oxime [(+)-Brevioxime (1) and Z-Isomer of (+)-Brevioxime]

In the same manner as above, **13** (3.30 g, 6.53 mmol) was treated with TBAF to afford alcohol (**14**, 1.54 g, 89%) as colorless oil: $[\alpha]_D^{23}$ = +110° (*c* 1.06, $CHCl_3$); IR (film, cm^{-1}) ν = 1780 (s, C=O), 1630-1680 (s, NCO); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.5-1.6 (2H, m), 1.25-1.4 (2H, m), 1.65 (3H, m), 1.79 (3H, s), 1.5-2.0 (3H, m), 2.15-2.35 (3H, m), 3.6 (2H, m), 4.48 (1H, m), 5.03 (1H, d, *J* = 3.6 Hz), 5.3-5.5 (2H, m).

In the same manner as above, **14** (175 mg, 0.67 mmol) was oxidized to afford ketone (233 mg, 94%) as

colorless oil: $[\alpha]_D^{25}$ = +225° (*c* 1.06, $CHCl_3$); IR (film, cm^{-1}) ν = 1780 (s, C=O), 1630-1680 (s, NCO); ^1H NMR (300 MHz, $CDCl_3$) δ = 1.3-1.45 (2H, m), 1.5-1.7 (2H, m), 1.64 (3H, d, *J* = 4.8 Hz), 1.84 (3H, s), 1.95-2.05 (2H, m), 2.2-2.4 (2H, m), 2.65-2.8 (2H, m), 3.61 (1H, ddd, *J* = 7.2, 8.7, 11.4 Hz), 4.12 (1H, m), 5.03 (1H, s), 5.3-5.5 (2H, m).

In the same manner as above, ketone (250 mg, 0.94 mmol) was treated with NH_2OH•HCl to afford **(+)-1** [*E*:*Z* = 49% (92 mg) : 27% (51 mg)] as colorless crystal.

(+)-Brevioxime: mp 149-152ºC; $[\alpha]_D^{22}$ = +214° (*c* 0.26, $CHCl_3$); ^1H NMR and ^{13}C-NMR spectra are identical with those of (–)-brevioxime (**1**); HRMS m/z [MH^+] 279.1695 (calcd for $C_{15}H_{23}N_2O_3$ 279.1708).

***Z*-Isomer of (+)-Brevioxim:** ^1H NMR and ^{13}C-NMR spectra are identical with those of *Z*-isomer of (–)-Brevioxime.

Determination of enantiomeric purity of (–)-1 and (+)-1

Both enantiomers of Brevioxime were converted to the corresponding (*R*)-MTPA esters and their enantiomeric purities were determined by HPLC analysis on a Senshu Pak® [Silica-1301-N 4.6X300 mm hexane/THF (10:1), 1.0 mL/min]: t_R = 12 min [(*R*)-MTPA ester of (+)-Brevioxime **(+)-1**]]: t_R = 15 min [(*R*)-MTPA ester of (–)-brevioxime **(–)-1**].

(*R*)-MTPA ester of (–)-brevioxime **(–)-1**-----[**(–)-1**:**(+)-1** = 98.2:1.8]-----96% e.e.

(*R*)-MTPA ester of (+)-brevioxime **(+)-1**-----[**(–)-1**:**(+)-1** = 1.4:98.6]-----97% e.e.

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