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Synthesis and structure revision of tyroscherin, and bioactivities of its stereoisomers against IGF-1-dependent tumor cells

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ABSTRACT

Synthesis of the proposed structure of tyroscherin, a growth inhibitor of IGF-1-dependent cancer cells, was succeeded by one-pot Julia coupling. However, spectral data of the synthetic compound were not identical with those of natural tyroscherin. The stereochemistry of tyroscherin is revised to be 2*S*,3*R*,8*R*,10*R* by syntheses of stereoisomers. Synthetic tyroscherin showed more potent activity than its stereoisomers against IGF-1-dependent cancer cells.

1. Introduction

Recently, mechanism-based drugs have been remarkably noticed, since they will potentially provide selective treatments for various diseases such as infections and cancers. Insulin-like growth factor (IGF) plays a key role in human cancer progression,[1] and selective inhibitors of its signal transduction are thought to provide a selective treatment against IGF-dependent tumor cells. In 2004, Hayakawa et al. isolated tyroscherin from the mycelium of *Pseudallescheria* sp. as a potent and selective inhibitor of IGF-1-dependent growth of MCF-7 human breast cancer cell.[2] Then, we started the synthesis of tyroscherin with the intention of further research on its biological activity and structure–activity relationship. We have already published a rapid communication,[3] in which we revised the stereochemistry of tyroscherin as shown in Figure 1, by syntheses of stereoisomers. Here, we wish to report a full account of our work.

2. Results and discussion

Our retrosynthesis of the proposed structure (**1**) is shown in Scheme 1. We selected one-pot Julia coupling as a key step, which could provide an easy way to synthesize other stereoisomers. In our previous report,[3] *N*-methyl group was introduced at later stage of the synthesis, but now we decided to install *N*-methyl group at earlier stage to avoid complicated sequence for methylation and to revise total yield. Compound **1** would be obtained by one-pot Julia coupling of sulfone **A** and known aldehyde **B**.[4] Sulfone **A** would be prepared from ketone **C** via stereoselective reduction. This ketone would be given by C_3 elongation of Weinreb amide **D**, which would be able to be derived from D-tyrosine.

The synthesis of the proposed structure (**1**) is shown in Scheme 2. D-Tyrosine was protected in a usual manner to give ester **3**. This ester was converted to the corresponding Weinreb amide **4**, which was subjected to N-methylation[5] to give **5** without any racemization. C_3 elongation of the Weinreb amide **5** afforded amino ketone **6**. This ketone was subjected to stereoselective reduction[6] to give *syn*-amino alcohol **7**, whose stereochemistry was confirmed by NOE experiment after conversion into cyclic carbamate **13**. After protection and deprotection, the primary alcohol **7** was converted to PT-sulfone **10** via Mitsunobu reaction.[7] Then one-pot Julia coupling[8,9] of the sulfone **10** and known aldehyde **11**[7] gave (*E*)-olefin **12** selectively. Finally, deprotection of **12** gave the desired compound, the proposed structure of tyroscherin (**1**). However, ^{1}H NMR spectral data of the synthetic compound **1** were not identical with those reported for natural tyroscherin.[2] Chemical shifts of 1-H, 2-H, and 3-H were much different between natural tyroscherin and synthetic **1** as shown in Table 1. Hayakawa et al. have determined the relative configuration at C-2 and C-3 by analysis of ^{1}H–^{1}H and ^{1}H–^{13}C coupling constants,[2,10] after determination of the absolute configuration at C-3 by modified Mosher's method.[11] We supposed the appropriate relative stereochemistry of natural compound to be

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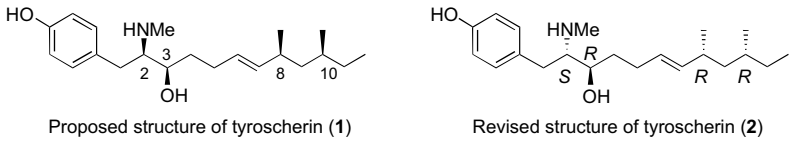
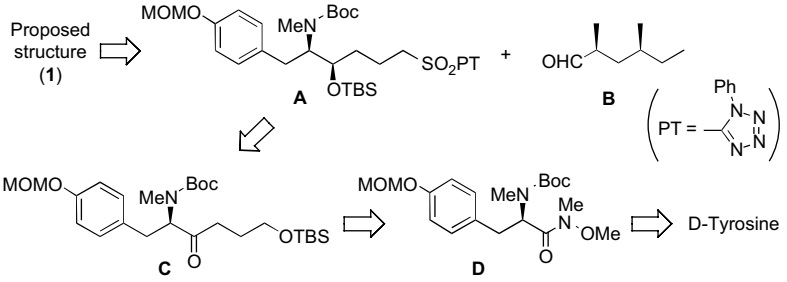


Figure 1. Proposed and revised structures of tyroscherin.



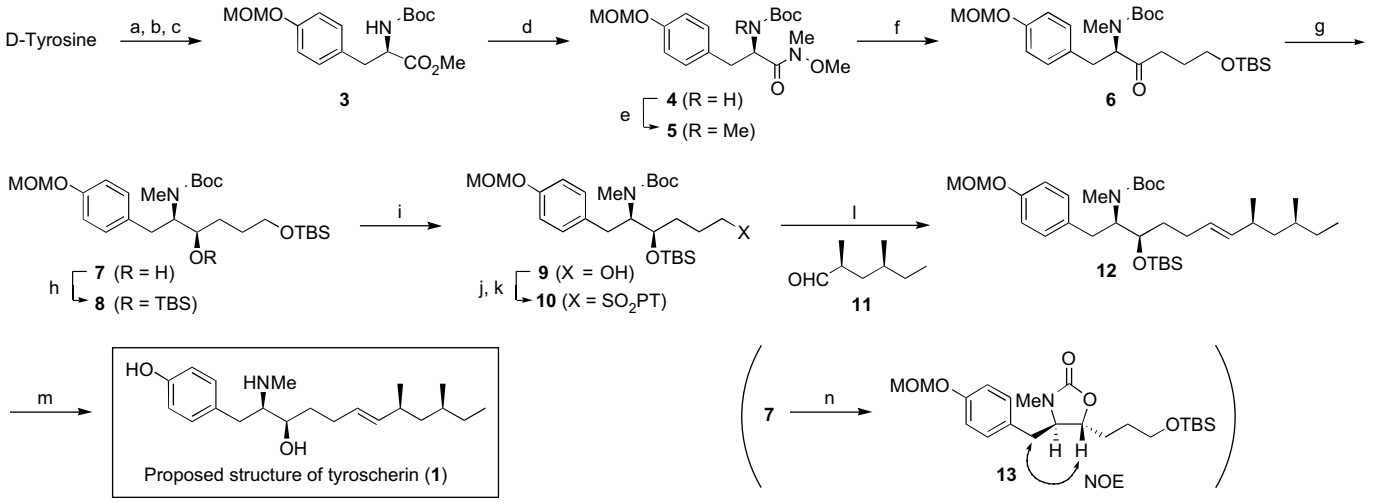
Scheme 1. Retrosynthetic analysis of the proposed structure of tyroscherin (**1**).

2,3-*anti*. To ascertain the correct structure of natural tyroscherin, we started synthesis of 2,3-*anti*-stereoisomers of **1**.

Four possible 2,3-*anti*-stereoisomers (**2**, **14**, *ent*-**2**, and *ent*-**14**) are shown in Figure 2. In our previous report,[3] a mixture of **14** and *ent*-**2** (2:1) was synthesized from enantiomerically poor material (33% ee) and the relative configuration of natural tyroscherin was determined to be same as the minor component (*ent*-**2**) by comparing ^{1}H NMR spectrum of the mixture (**14**/*ent*-**2**=2:1) with that of natural tyroscherin (Fig. 3 and Table 1). After that compounds **2** and *ent*-**2** were synthesized enantioselectively in order to determine the absolute configuration of tyroscherin. ^{1}H NMR spectra of these compounds were completely identical with that of natural tyroscherin (Fig. 3 and Table 1). The specific rotation of **2** was identical to that of natural tyroscherin, while *ent*-**2** showed opposite sign of specific rotation {**2**: $[\alpha]_D^{25}$ −21 (*c* 0.35, MeOH), *ent*-**2**: $[\alpha]_D^{24}$ +20 (*c* 0.35, MeOH), natural tyroscherin: $[\alpha]_D^{24}$ −21 (*c* 0.35, MeOH)[2]}. From these results, we finally concluded that the correct stereostructure of natural tyroscherin is **2**, that is, the absolute configuration of natural tyroscherin was revised to be 2*S*,3*R*,8*R*,10*R*.

In this paper, we describe the enantioselective syntheses of all of the four 2,3-*anti*-stereoisomers (**2**, **14**, *ent*-**2**, and *ent*-**14**) in order to examine the structure–activity relationship for tyroscherin.

At first, syntheses of (2*R*,3*S*,8*S*,10*S*)-isomer (*ent*-**2**) and (2*R*,3*S*,8*R*,10*R*)-isomer (*ent*-**14**) are shown in Scheme 3. Weinreb amide (**5**) derived from D-tyrosine was reduced to aldehyde **15**, whose enantiomeric purity was determined to be 97% ee by chiral HPLC (Chiralpak AD-H, hex/*i*-PrOH=19:1). The aldehyde **15** was reacted with siloxypropyllithium in Et_2O to afford a mixture of *anti*- and *syn*-amino alcohols (**16** and **7**). After protection of the hydroxy group, *anti*- and *syn*-isomers were separated by silica gel chromatography (**17**/**8**=5:1). The stereochemistry of **16** at C-3 was confirmed by NOE experiment after conversion to cyclic carbamate **22**. The *anti*-isomer **17** was converted to the corresponding sulfone **19** and then it was subjected to one-pot Julia coupling with **11** to give (*E*)-olefin **20** selectively. Finally, deprotection was followed by



Scheme 2. Synthesis of the proposed structure (**1**) of tyroscherin. (a) $SOCl_2$, MeOH, reflux; (b) NaOH, H_2O, then $(Boc)_2O$, THF, 0 °C to rt; (c) MOMCl, DIPEA, CH_2Cl_2, rt, 93% in three steps; (d) MeNHOMe·HCl, *i*-PrMgBr, THF, −20 °C to rt, 82%; (e) NaH, MeI, DMF, −20 °C, 76%; (f) $TBSO(CH_2)_3I$, *t*-BuLi, Et_2O, −78 °C to rt, 90%; (g) $NaBH_4$, MeOH, EtOH, −20 °C, 96%, single isomer; (h) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, 83%; (i) Dowex-50, MeOH, H_2O, rt, 62%; (j) PTSH, DEAD, PPh_3, THF, 0 °C to rt; (k) 35% H_2O_2, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, EtOH, 0 °C to rt, 86% in two steps; (l) KHMDS, THF, −78 °C to rt, 67%; (m) TFA, THF, MeOH, H_2O, reflux, quant.; (n) NaH, THF, reflux, 76%.

Table 1
Selected ^{1}H NMR (500 MHz, CD_3OD) data and specific rotations for natural tyroscherin and synthetic stereoisomers

		^{1}H NMR (ppm, multiplicity, Hz)							$[\alpha]_D$ (c 0.35, MeOH)
		1-H_a	1-H_b	2-H	3-H	12-H	8-Me	10-Me	
Natural tyroscherin[2]		2.86, dd, 14.0, 7.0	2.91, dd, 14.0, 7.0	3.34, ddd, 7.0, 7.0, 3.0	3.83, dt, 10.0, 3.0	0.84, t, 7.0	0.91, d, 6.5	0.82, d, 6.5	−21
Proposed structure (**1**)		2.87, dd, 13.8, 8.3	**2.96**, dd, 13.8, 6.0	**3.22**, ddd, 8.3, 6.0, 4.7	**3.62**, ddd, 6.8, 5.3, 4.7	0.85, t, 70	0.89, d, 6.2	0.81, d, 6.5	+11
Mixture of *anti*-isomers (**14**/*ent*-**2**=2:1)	Major (**14**)	2.86, dd, 14.7, 7.9	**2.93**, dd, 14.7, 6.7	3.32–3.38, m, overlapped	3.81–3.87, m, overlapped	0.84, t, 7.3	**0.92**, d, 6.8	**0.81**, d, 6.5	—
	Minor (*ent*-**2**)	2.86, dd, 14.7, 7.9	2.91, dd, 14.7, 7.0	3.32–3.38, m, overlapped	3.81–3.87, m, overlapped	0.84, t, 7.3	0.91, d, 6.8	0.82, d, 6.5	—
(2*S*,3*R*,8*R*,10*R*)-Isomer (**2**)		2.86, dd, 14.7, 7.9	2.91, dd, 14.7, 7.0	3.34, ddd, 7.9, 7.0, 3.0	3.83, ddd, 9.4, 3.6, 3.0	0.84, t, 7.3	0.91, d, 6.8	0.82, d, 6.5	−21

recrystallization to afford (2*R*,3*S*,8*S*,10*S*)-isomer (*ent*-**2**) in a diastereomerically pure form. In a similar manner, (2*R*,3*S*,8*R*,10*R*)-isomer (*ent*-**14**) was also obtained via one-pot Julia coupling of the sulfone **19** with aldehyde *ent*-**11**.

On the other hand, syntheses of (2*S*,3*R*,8*R*,10*R*)-isomer (**2**) and (2*S*,3*R*,8*S*,10*S*)-isomer (**14**) are shown in Scheme 4. L-Tyrosine was converted to Weinreb amide *ent*-**5** in five steps. Reduction of *ent*-**5** with LAH gave aldehyde *ent*-**15** with no racemization and its enantiomeric purity was confirmed to be >99% ee by chiral HPLC. This aldehyde was subjected to C_3 elongation to afford *ent*-**16** wherein diastereoselectivity was improved by using THF as a solvent instead of Et_2O (*anti*/*syn*=13:1 after separation of *ent*-**17** and *ent*-**8**). In the same manner as Scheme 3, *ent*-**16** was transformed to give (2*S*,3*R*,8*R*,10*R*)-isomer (**2**) and (2*S*,3*R*,8*S*,10*S*)-isomer (**14**) as colorless crystals, respectively. The specific rotation, melting point, and spectroscopic data of **2** were reconfirmed to be fully identical to those of natural tyroscherin.[2]

Having succeeded in the total syntheses of all the four stereoisomers, our next interest came to structure–activity relationship of tyroscherin. We examined the effects of tyroscherin and its derivatives on IGF-1-dependent MCF-7 human breast cancer cells (Table 2). Tyroscherin (**2**) inhibited the growth of MCF-7 cells in a serum-free medium containing IGF-1with an IC_{50} of 3.4 nM. In the presence of fetal bovine serum instead of IGF-1, tyroscherin exhibited no activity against MCF-7 cells at less than 1 μM. As shown in Table 2, tyroscherin (**2**) showed more potent and selective activity than other stereoisomers. Interestingly, the isomer **14** and 8,10-didemethyl-derivative **23** (Fig. 4), which had (2*S*,3*R*)-stereochemistry, exhibited medium selectivities. The (2*S*,3*R*)-stereochemistry should have important role for selective inhibition of IGF-1-dependent growth of MCF-7 cells (Fig. 4).

In summary, we succeeded in the first synthesis of tyroscherin and its stereoisomers, in 5.2–20.0% overall yield from D- and L-tyrosines, and revised the absolute configuration of tyroscherin to be 2*S*,3*R*,8*R*,10*R*. Inhibitory activity against IGF-1-dependent growth of MCF-7 cells was evaluated using the synthesized tyroscherin and its stereoisomers, and it was found that (2*S*,3*R*)-stereochemistry was important for the selective inhibition. We wish our study on these compounds, which have potent and selective activities, would offer any useful information for further investigation on the related fields.

3. Experimental

3.1. General

Melting points were measured with a Yanaco micro-melting point apparatus and are uncorrected values. 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 were recorded on JEOL JNM AL300 or JEOL JNM GSX500. Chemical shifts (δ) were referenced to the residual solvent peaks as the internal standard ($CDCl_3$: δ_H=7.26; DMSO-d_6: δ_H=2.49; CD_3OD: δ_H=3.30, δ_C=49.0). Refractive indexes were measured with an Atago 1 T refractometer. Mass spectra were recorded on JEOL JMS SX102. Column chromatography was performed using Kanto silica gel 60N (0.060–0.200 mm). TLC was carried out on Merck glass plates precoated with silica gel 60 F_{254} (0.25 mm). HPLC was performed using Hitachi L-2130 and Hitachi L-2400 UV detector (254 nm).

3.2. Synthetic studies

*3.2.1. Methyl (R)-N-(tert-butoxycarbonyl)-O-(methoxymethyl)-tyrosinate (**3**)*

To a mixture of D-tyrosine (10.9 g, 60 mmol) and MeOH (37 ml) was added thionyl chloride (4.8 ml, 66 mmol) dropwise at 0 °C. The reaction mixture was refluxed for 4 h and concentrated in vacuo. Crude white solids (14.0 g) were used for next reaction without further purification.

To a solution of crude material (13.9 g) in water (30 ml) were added successively 6 M aqueous NaOH solution (10 ml), THF (100 ml), and di-(*tert*-butyl)dicarbonate (20.1 ml, 60 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The

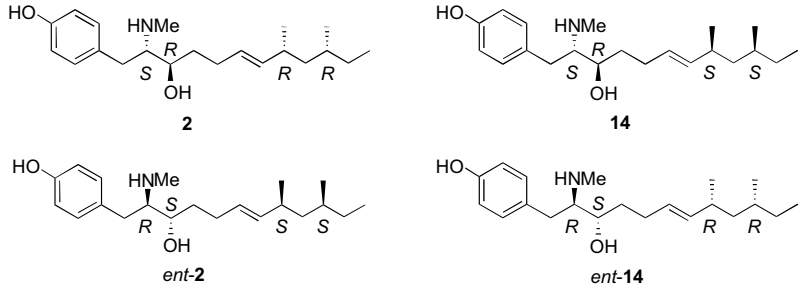


Figure 2. Structures of possible *anti*-isomers.

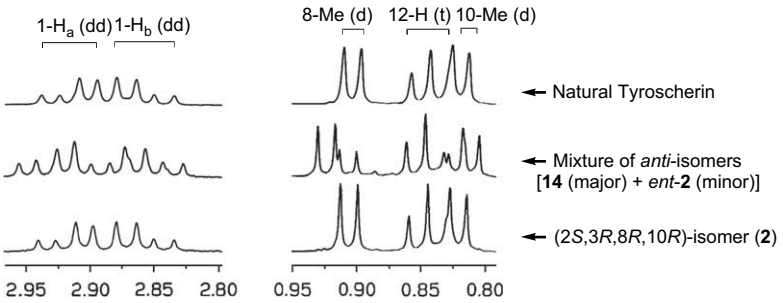


Figure 3. ^{1}H NMR signals of natural and synthesized compounds (1-H_2 and Me groups).

reaction mixture was filtered, poured into saturated aqueous ammonium chloride solution, and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, saturated aqueous sodium bicarbonate solution, and brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. Residual crystals (17.9 g) were used for next reaction without further purification.

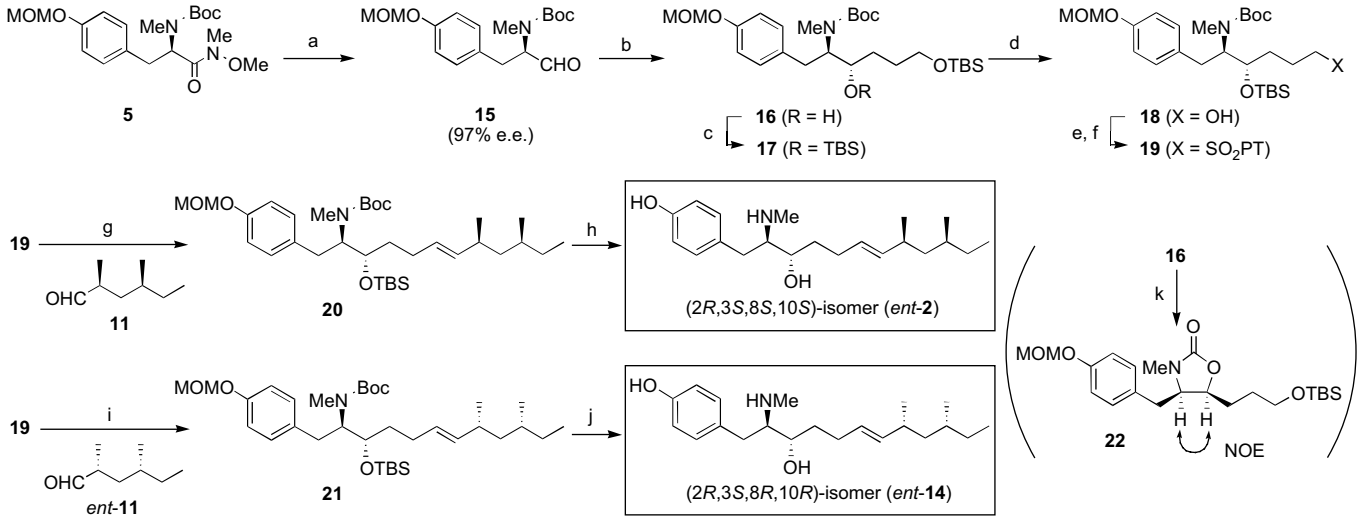
To a solution of crude material (17.7 g) in dichloromethane (200 ml) were added *N*,*N*-diisopropylethylamine (31.4 ml, 180 mmol) and chloromethyl methyl ether (13.7 ml, 120 mmol) at 0 °C and the mixture was stirred at room temperature for 24 h. The reaction mixture was poured into saturated aqueous ammonium chloride solution and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, saturated aqueous sodium bicarbonate solution, and brine, and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (3:1) gave **3** (19.0 g, 93% in three steps) as colorless prisms. Mp=67–69 °C. $[\alpha]_D^{25}$ −48 (*c* 1.0, $CHCl_3$). IR (Nujol): ν=3378, 1739, 1700, 1610, 1226 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ=1.42 (9H, br s), 3.01 (1H, dd, *J*=14.1, 6.3 Hz), 3.04 (1H, dd, *J*=14.1, 6.0 Hz), 3.47 (3H, s), 3.72 (3H, s), 4.55 (1H, m), 4.96 (1H, br d, *J*=7.2 Hz), 5.15 (2H, s), 6.96 (2H, d, *J*=8.4 Hz), 7.03 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{17}H_{25}NNaO_6$ $[M+Na]^+$ 362.1574, found 362.1591.

3.2.2. (R)-N-α-(tert-Butoxycarbonyl)-N-methoxy-O-(methoxymethyl)-N-methyltyrosinamide (*4*)

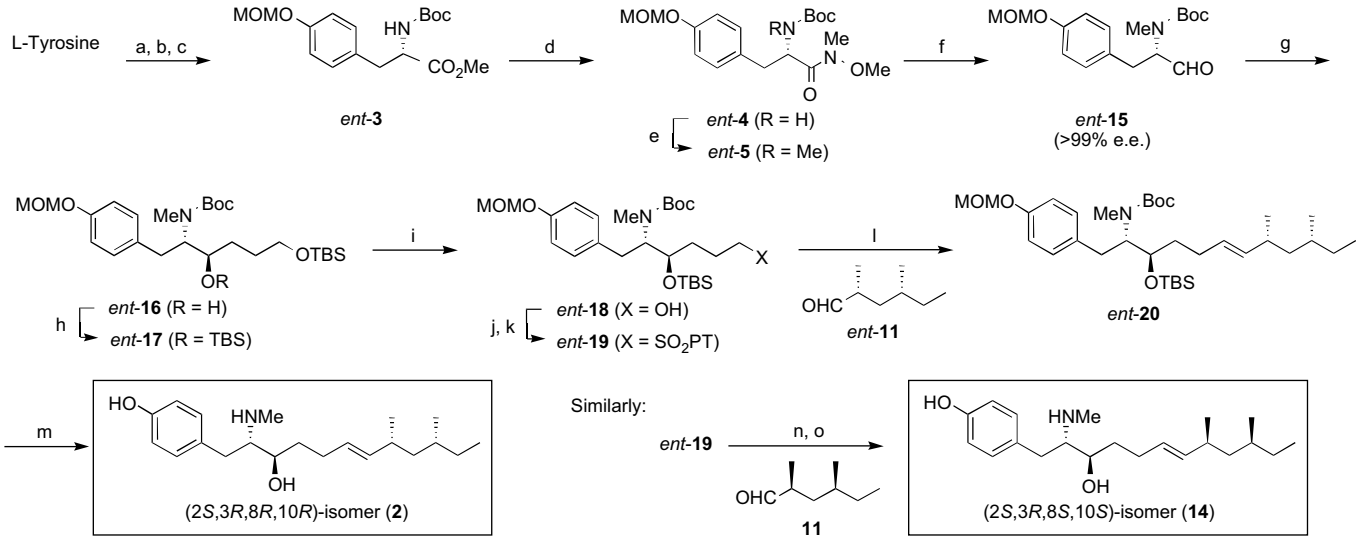
To a mixture of magnesium turnings (2.19 g, 90.1 mmol) and ether (5 ml) was added isopropyl bromide (1.0 g, 8.1 mmol) at room temperature and the mixture was stirred for 5 min. A solution of isopropyl bromide (10.1 g, 82.1 mmol) solution in ether (45 ml) was slowly added to the reaction mixture at room temperature and it was stirred for 1 h at the same temperature. The resultant solution was added dropwise to a mixture of ester **3** (6.78 g, 20.0 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (4.29 g, 44.0 mmol), and THF (200 ml) at −20 °C under argon atmosphere, and this mixture was stirred for 30 min at room temperature. The reaction mixture was poured into saturated aqueous ammonium chloride solution and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, saturated aqueous sodium bicarbonate solution, and brine, and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (3:1 to 1:1) gave **4** (6.05 g, 82%) as colorless prisms. Mp=69–72 °C. $[\alpha]_D^{25}$ −18 (*c* 1.0, $CHCl_3$). IR (Nujol): ν=3335, 1698, 1649, 1510, 1150 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.39 (9H, br s), 2.82 (1H, dd, *J*=13.5, 6.9 Hz), 2.99 (1H, dd, *J*=13.5, 6.0 Hz), 3.17 (3H, br s), 3.46 (3H, s), 3.68 (3H, s), 4.91 (1H, m), 5.14 (1H, m,), 5.14 (2H, s), 6.95 (2H, d, *J*=8.4 Hz), 7.07 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{18}H_{28}N_2NaO_6$ $[M+Na]^+$ 391.1840, found 391.1827.

3.2.3. (R)-N-α-(tert-Butoxycarbonyl)-N-methoxy-O-(methoxymethyl)-N,N-α-dimethyltyrosinamide (*5*)

To a solution of Weinreb amide **4** (4.42 g, 12.0 mmol) in DMF (50 ml) was added portionwise a suspension of NaH (0.30 g, 12.6 mmol) in THF (5 ml) at −20 °C under argon atmosphere. After stirring for 2 h at same temperature, MeI (1.12 ml, 18.0 mmol) was added dropwise to the reaction mixture. After stirring for 3 h, the reaction mixture was poured into brine, and extracted with ether.



Scheme 3. Synthesis of (2*R*,3*S*,8*S*,10*S*)-isomer (*ent*-**2**) and (2*R*,3*S*,8*R*,10*R*)-isomer (*ent*-**14**). (a) DIBAL, Et_2O, −20 °C to rt, 97% ee; (b) $TBSO(CH_2)_3I$, *t*-BuLi, Et_2O, −78 °C to rt; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 61% in three steps, dr=5:1; (d) Dowex-50, MeOH, H_2O, rt, 75%; (e) PTSH, DEAD, PPh_3, THF, 0 °C to rt,; (f) 35% H_2O_2, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, EtOH, 0 °C to rt, 83% in two steps; (g) KHMDS, THF, −78 °C to rt, 73%; (h) TFA, THF, MeOH, H_2O, 50 °C, 86%; (i) KHMDS, THF, −78 °C to rt, 56%; (j) TFA, THF, MeOH, H_2O, 50 °C, 89%; (k) NaH, THF, reflux, 68%.



Scheme 4. Synthesis of (2*S*,3*R*,8*R*,10*R*)-isomer (**2**). (a) $SOCl_2$, MeOH, reflux; (b) NaOH, H_2O, then $(Boc)_2O$, THF, 0 °C to rt; (c) MOMCl, DIPEA, CH_2Cl_2, rt, 99% in three steps; (d) MeNHOMe·HCl, *i*-PrMgBr, THF, −20 °C to rt, 87%; (e) NaH, MeI, DMF, −20 °C, 95%; (f) $LiAlH_4$, ether, 0 °C, >99% ee; (g) $TBSO(CH_2)_3I$, *t*-BuLi, THF, −78 °C; (h) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 55% in three steps, dr=13:1; (i) Dowex-50, MeOH, H_2O, rt, 82%; (j) PTSH, DEAD, PPh_3, THF, 0 °C to rt; (k) 35% H_2O_2, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, EtOH, 0 °C to rt, 83% in two steps; (l) KHMDS, THF, −78 °C to rt, 70%; (m) TFA, THF, MeOH, H_2O, 50 °C, 87%; (n) KHMDS, THF, −78 °C to rt, 58%; (o) TFA, THF, MeOH, H_2O, 50 °C, 71%.

The organic layer was washed with water and brine and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/acetone (8:1) gave **5** (3.48 g, 76%) as colorless prisms. Mp=41–44 °C. $[\alpha]_D^{26}$ +93 (*c* 1.0, $CHCl_3$). IR (Nujol): ν=1682, 1613, 1514, 1233, 1151 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=1.27 (9H, br s), 2.73 (3H, s), 2.83 (1H, dd, *J*=14.1, 9.3 Hz), 2.92 (1H, dd, *J*=14.1, 6.0 Hz), 3.11 (3H, s), 3.37 (3H, s), 3.63 (3H, s), 5.11 (2H, s), 5.18 (1H, m), 6.92 (2H, d, *J*=7.8 Hz), 7.10 (2H, d, *J*=7.8 Hz). ESI-TOFMS *m*/*z* calcd for $C_{19}H_{30}N_2NaO_6$ $[M+Na]^+$ 405.1996, found 405.1994.

3.2.4. *tert*-Butyl (*R*)-[5-(*tert*-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-2-oxopentyl]methylcarbamate (**6**)

Under argon atmosphere, a solution of *t*-BuLi in pentane (1.59 M, 29.4 ml, 46.8 mmol) was diluted with ether (35 ml). To this solution was added dropwise a solution of 1-(*tert*-butyldimethylsilyloxy)-3-iodopropane (7.04 g, 23.4 mmol) in ether (15 ml) at −78 °C. After stirring for 30 min, the resultant lithium reagent was added slowly to a solution of amide **5** (4.98 g, 13.0 mmol) in ether (100 ml) at −78 °C under argon. After stirring for 30 min, the reaction mixture was allowed to warm to room temperature and stirred for 15 min. This mixture was poured into ice-cold brine and extracted with ether. The organic layer was washed with water and brine and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/acetone (10:1) gave **6** (5.83 g, 90%) as a colorless oil. n_D=1.4788. $[\alpha]_D^{25}$ +130 (*c* 1.0, $CHCl_3$). IR (film): ν=1696, 1612, 1511, 1253, 1153, 1008 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.03 (6H, s), 0.87 (9H, s), 1.26–1.40 (2H, m), 1.32 (9H, br s), 1.65–1.77 (2H, m), 2.59 (3H, s), 2.79 (1H, dd, *J*=14.4, 8.4 Hz), 3.07 (1H, dd, *J*=14.4, 4.8 Hz), 3.37 (3H, s), 3.58 (2H, t, *J*=6.3 Hz), 4.52 (1H, dd, *J*=8.4, 4.8 Hz), 5.11 (2H, s), 6.91 (2H, d, *J*=8.4 Hz), 7.09 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{26}H_{45}NNaO_6Si$ $[M+Na]^+$ 518.2908, found 518.2901.

3.2.5. *tert*-Butyl (1*R*,2*R*)-[5-(*tert*-butyldimethylsilyloxy)-2-hydroxy-1-[4-(methoxymethoxy)benzyl]pentyl]methylcarbamate (**7**)

To a solution of ketone **6** (3.74 g, 7.54 mmol) in EtOH (30 ml) and MeOH (100 ml) was added sodium borohydride (571 mg, 15.1 mmol) at −20 °C and the mixture was stirred for 3 h at the same temperature. The reaction mixture was poured into saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (7:1) gave **7** (3.62 g, 96%) as a colorless oil. n_D=1.4781. $[\alpha]_D^{20}$ +41 (*c* 1.0, $CHCl_3$). IR (film): ν=3439, 1668, 1613, 1512, 1234, 1151, 1010 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.02 (6H, s), 0.87 (9H, s), 1.14–1.68 (4H, m), 1.25 (9H, br s), 2.70–2.85 (2H, m), 2.73 (3H, s), 3.37 (3H, s), 3.59 (2H, t, *J*=6.3 Hz), 4.09 (1H, m), 4.38 (1H, m), 5.10 (2H, s), 6.89 (2H, d, *J*=8.7 Hz), 7.08 (2H, d, *J*=8.7 Hz). ESI-TOFMS *m*/*z* calcd for $C_{26}H_{47}NNaO_6Si$ $[M+Na]^+$ 520.3065, found 520.3092.

3.2.6. *tert*-Butyl (1*R*,2*R*)-[2,5-bis(*tert*-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]pentyl]methylcarbamate (**8**)

To a solution of alcohol **7** (2.01 g, 4.04 mmol) in dichloromethane (40 ml) were added 2,6-lutidine (1.18 ml, 10.1 mmol) and TBSOTf (1.11 ml, 4.85 mmol) at 0 °C. The reaction mixture was

Table 2
Selective cytotoxicity of tyroscherin and its derivatives against IGF-1-dependent MCF-7 cells

Compound	IC_{50} (nM)		Selectivity index
	+IGF-1	+FBS	
Tyroscherin (**2**)	3.4	6000	1800
8,10-Didemethyltyroscherin (**23**)	75	23,000	310
(2*S*,3*R*,8*S*,10*S*)-Isomer (**14**)	13	3900	300
(2*R*,3*S*,8*R*,10*R*)-Isomer (*ent*-**14**)	98	4000	41
(2*R*,3*R*,8*S*,10*S*)-Isomer (**1**)	290	7700	27
(2*R*,3*S*,8*S*,10*S*)-Isomer (*ent*-**2**)	220	4500	21

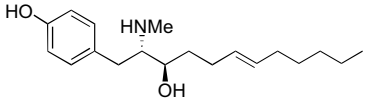


Figure 4. 8,10-Didemethyl-derivative for bioassay.

warmed to room temperature and stirred for 2 h. It was poured into saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was washed with water and brine and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (15:1) gave **8** (2.05 g, 83%) as a colorless oil. n_D=1.4741. $[\alpha]_D^{25}$ +24 (*c* 1.0, $CHCl_3$). IR (film): ν=1692, 1612, 1512, 1254, 1151, 1011 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.03 (6H, s), 0.09 (6H, s), 0.88 (9H, s), 0.91 (9H, s), 1.21 (9H, br s), 1.45–1.65 (4H, m), 2.60–2.90 (2H, m), 2.72 (3H, s), 3.36 (3H, s), 3.50–3.65 (2H, m), 3.91 (1H, m), 4.25 (1H, m), 5.10 (2H, s), 6.89 (2H, d, *J*=8.4 Hz), 7.06 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{32}H_{61}NNaO_6Si_2$ $[M+Na]^+$ 634.3930, found 634.3966.

3.2.7. *tert-Butyl (1R,2R)-[2-(tert-butyldimethylsilyloxy)-5-hydroxy-1-[4-(methoxymethoxy)benzyl]pentyl]methylcarbamate (**9**)*

Dowex-50 (0.75 g) was washed with deionized water three times. To a solution of bis-TBS ether **8** (1.98 g, 3.24 mmol) in MeOH (60 ml) was added a suspension of Dowex-50 in deionized water (5 ml) and stirred for 36 h at room temperature. After filtration, triethylamine (200 μl) was added to the filtrate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (4:1 to 2:1) gave **9** (1.00 g, 62%) as a colorless oil. n_D=1.4817. $[\alpha]_D^{21}$ +29 (*c* 1.0, $CHCl_3$). IR (film): ν=3449, 1739, 1690, 1612, 1512, 1253, 1152 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.09 (6H, s), 0.91 (9H, s), 1.21 (9H, br s), 1.45–1.65 (4H, m), 2.70 (1H, m), 2.71 (3H, s), 2.81 (1H, dd, *J*=14.1, 11.1 Hz), 3.35 (3H, s), 3.35–3.45 (2H, m), 3.90 (1H, m), 4.25 (1H, m), 5.10 (2H, s), 6.90 (2H, d, *J*=8.7 Hz), 7.07 (2H, d, *J*=8.7 Hz). ESI-TOFMS *m*/*z* calcd for $C_{26}H_{47}NNaO_6Si$ $[M+Na]^+$ 520.3065, found 520.3092.

3.2.8. *tert-Butyl (1R,2R)-[2-(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-5-[(1-phenyl-1H-tetrazol-5-yl)sulfonyl]pentyl]methylcarbamate (**10**)*

The solution of alcohol **9** (920 mg, 1.85 mmol) in THF (80 ml) were added diethyl azodicarboxylate (387 mg, 2.22 mmol), PPh_3 (582 mg, 2.22 mmol), and 5-mercapto-1-phenyl-1*H*-tetrazole (396 mg, 2.22 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature and concentrated in vacuo. The residue was dissolved in 95% EtOH (40 ml), and ammonium molybdate tetrahydrate (487 mg, 0.39 mmol) and 35% aqueous hydrogen peroxide solution (3 ml) were added to the solution at 0 °C. This mixture was stirred for 24 h at room temperature, poured into 10% aqueous $Na_2S_2O_3$ solution, and extracted with EtOAc. Organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (4:1) gave **10** (1.10 g, 86% in two steps) as a colorless oil. n_D=1.5049. $[\alpha]_D^{25}$ +5 (*c* 1.0, $CHCl_3$). IR (film): ν=1685, 1611, 1510, 1341, 1233, 1152, 1009 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.08 (3H, s), 0.09 (3H, s), 0.89 (9H, s), 1.16 (9H, br s), 1.60–1.70 (2H, m), 1.82–2.00 (2H, m), 2.65 (1H, m), 2.70 (3H, s), 2.81 (1H, dd, *J*=14.4, 11.1 Hz), 3.36 (3H, s), 3.78 (2H, m), 3.92 (1H, q, *J*=5.4 Hz), 4.21 (1H, m), 5.10 (2H, s), 6.90 (2H, d, *J*=8.4 Hz), 7.07 (2H, d, *J*=8.4 Hz), 7.60–7.75 (5H, m). ESI-TOFMS *m*/*z* calcd for $C_{33}H_{51}N_5NaO_7SSi$ $[M+Na]^+$ 712.3171, found 712.3182.

3.2.9. *tert-Butyl (1R,2R,5E,7S,9S)-[2-(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-7,9-dimethylundec-5-enyl]methylcarbamate (**12**)*

Under argon atmosphere, to a solution of sulfone **10** (300 mg, 0.43 mmol) in THF (8 ml) was added KHMDS solution in toluene (0.5 M, 870 μl, 0.44 mmol) at −78 °C. After stirring for 2 min, the solution of aldehyde **11** (111 mg, 0.87 mmol) in THF (2 ml) was added to the reaction mixture. The resulting mixture was then allowed to warm to room temperature and poured into saturated aqueous ammonium chloride solution. The mixture was extracted with ether, and organic layer was washed with water and brine. After drying over anhydrous magnesium sulfate, the solution was concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (8:1) gave **12** (173 mg, 67%) as a colorless oil. n_D=1.4794. $[\alpha]_D^{25}$ +17 (*c* 1.0, $CHCl_3$). IR (film) ν=1692, 1612, 1512, 1253, 1152, 1011 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.09 (3H, s), 0.09 (3H, s), 0.82 (3H, d, *J*=6.6 Hz), 0.82 (3H, t, *J*=7.5 Hz), 0.91 (9H, s), 0.92 (3H, d, *J*=6.9 Hz), 1.02 (1H, ddd, *J*=13.2, 7.8, 5.1 Hz), 1.05–1.45 (4H, m), 1.22 (9H, br s), 1.48–1.61 (2H, m), 1.93–2.22 (3H, m), 2.66 (1H, m), 2.73 (3H, s), 2.84 (1H, dd, *J*=14.1, 10.8 Hz), 3.36 (3H, s), 3.88 (1H, ddd, *J*=10.8, 5.7, 5.7 Hz), 4.28 (1H, m), 5.10 (2H, s), 5.27 (1H, dd, *J*=15.3, 7.2 Hz), 5.34 (1H, dt, *J*=15.3, 6.3 Hz), 6.90 (2H, d, *J*=8.4 Hz), 7.07 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{34}H_{61}NNaO_5Si$ $[M+Na]^+$ 614.4211, found 614.4251.

3.2.10. *(2R,3R,6E,8S,10S)-1-(4-Hydroxyphenyl)-8,10-dimethyl-2-(methylamino)dodec-6-en-3-ol (**1**, proposed structure of tyroscherin)*

To a solution of **12** (135 mg, 0.23 mmol) in THF (2 ml), MeOH (1 ml), and water (1 ml) was added trifluoroacetic acid (400 μl). The reaction mixture was refluxed for 10 h and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with chloroform/MeOH (7:1) gave **1** (76 mg, quant.) as a colorless oil. n_D=1.4714. $[\alpha]_D^{25}$ +11 (*c* 0.35, CH_3OH). IR (KBr): ν=3382, 2958, 1681, 1517, 1202, 800 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ=0.81 (3H, d, *J*=6.5 Hz), 0.85 (3H, t, *J*=7.0 Hz), 0.89 (3H, d, *J*=6.2 Hz), 0.97 (1H, ddd, *J*=13.0, 8.6, 4.2 Hz), 1.13 (1H, m), 1.2–1.35 (2H, m), 1.21 (1H, ddd, *J*=13.0, 9.4, 4.7 Hz), 1.45–1.6 (2H, m), 1.95–2.15 (3H, m), 2.67 (3H, s), 2.87 (1H, dd, *J*=13.8, 8.3 Hz), 2.96 (1H, dd, *J*=13.8, 6.0 Hz), 3.22 (1H, ddd, *J*=8.3, 6.0, 4.7 Hz), 3.62 (1H, ddd, 6.8, 5.3, 4.7 Hz), 5.16 (1H, dd, *J*=15.1, 8.1 Hz), 5.27 (1H, dt, *J*=15.1, 6.5 Hz), 6.77 (2H, quasi d, *J*=8.1 Hz), 7.11 (2H, quasi d, *J*=8.1 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ=11.7, 19.3, 22.3, 29.3, 31.1, 31.8, 33.1, 34.1, 35.4, 35.7, 45.5, 65.9, 68.7, 116.8, 127.6, 128.3, 131.4, 138.6, 157.9. ESI-TOFMS *m*/*z* calcd for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2741, found 334.2773.

3.2.11. *(4R,5R)-5-[3-(tert-Butyldimethylsilyloxy)propyl]-4-[4-(methoxymethoxy)benzyl]-3-methyl-1,3-oxazolidin-2-one (**13**)*

To a solution of amino alcohol **7** (98 mg, 0.20 mmol) in THF (5 ml) was added NaH (13 mg, 0.32 mmol) at room temperature and the resulting mixture was stirred for 16 h under reflux. The reaction mixture was poured into saturated aqueous ammonium chloride solution, and extracted with ethyl acetate. The organic layer was washed with water and brine and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/acetone (3:1 to 1:1) gave **13** (63 mg, 76%) as a colorless oil. n_D=1.4978. $[\alpha]_D^{21}$ +3.5 (*c* 1.0, $CHCl_3$). IR (Nujol): ν=1752, 1612, 1512, 1236, 1152 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=0.00 (6H, s), 0.85 (9H, s), 1.35–1.6 (4H, m), 2.66 (1H, dd, *J*=13.8, 7.8 Hz), 2.86 (3H, s), 3.01 (1H, dd, *J*=13.8, 4.5 Hz), 3.42–3.58 (3H, m), 3.48 (3H, s), 4.15 (1H, m), 5.16 (2H, s), 6.99 (2H, d, *J*=8.7 Hz), 7.06 (2H, d, *J*=8.7 Hz). ESI-TOFMS *m*/*z* calcd for $C_{22}H_{37}NNaO_5Si$ $[M+Na]^+$ 446.2333, found 446.2338.

3.2.12. *tert-Butyl (1R,2S)-[2,5-bis(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]pentyl]methylcarbamate (**17**)*

To a solution of amide **5** (3.36 g, 8.79 mmol) in ether (100 ml) was added dropwise a solution of DIBAL in hexane (1.02 M, 9.0 ml, 9.18 mmol) at −20 °C and the resulting mixture was stirred for 2 h at 20 °C. The reaction mixture was poured into ice-cold saturated aqueous potassium sodium tartrate solution and stirred for 30 min at 0 °C. Then the mixture was allowed to warm to room temperature and extracted with ether. The organic layer was washed with water

and brine. After drying over anhydrous magnesium sulfate, the solution was concentrated in vacuo. The residue was solved in ether and filtered through silica gel. After washing with ether, the combined solution was concentrated to give crude aldehyde **15** (2.44 g) as a colorless oil. This material was used for next reaction without further purification. Small amount of crude aldehyde was analyzed by HPLC [column: Daicel Chiralpak AD-H (0.46 cm×25 cm), eluent: hexane/*i*-PrOH (19:1), flow rate: 0.8 ml/min, detection: UV (254 nm), (*S*)-isomer: t_R=11.8 min, (*R*)-isomer: t_R=12.9 min] and the enantiomeric purity was determined to be 97% ee.

To a solution of 1-(*tert*-butyldimethylsilyloxy)-3-iodopropane (3.75 g, 12.5 mmol) in ether (50 ml) was slowly added a solution of *t*-BuLi in pentane (1.58 M, 16.2 ml, 25.8 mmol) at −78 °C and the resulting mixture was stirred for 1 h at same temperature. The resultant solution of lithium reagent was added to a solution of crude aldehyde (2.25 g) in ether (80 ml) at −78 °C and the reaction mixture was slowly warmed to room temperature. After stirring for 1 h, it was poured into saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was washed with water and brine, and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (5:1) gave an inseparable mixture of **16** and **7** (3.04 g, 75% in two steps) as a slightly yellow oil. This mixture was used for next reaction without further purification.

To a solution of crude mixture of **16** and **7** (1.59 g) in dichloromethane (50 ml) was added 2,6-lutidine (0.93 ml, 8.0 mmol) followed by TBSOTf (0.88 ml 3.8 mmol) at 0 °C. After stirring for 3 h, the reaction mixture was poured into saturated aqueous ammonium chloride solution, and extracted with ether. The organic layer was washed with 1 N HCl, saturated aqueous sodium bicarbonate solution, water, and brine, and then dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (20:1) gave **17** (1.30 g, 50% in three steps) and **8** (0.28 g, 10.8% in three steps) as colorless oils. n_D=1.4741. $[\alpha]_D^{22}$ +21 (*c* 1.0, $CHCl_3$). IR (film): ν=1695, 1613, 1514, 1254 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.02 (6H, s), 0.12 (3H, s), 0.12 (3H, s), 0.87 (9H, s), 0.93 (9H, s), 1.22 (9H, br s), 1.40–1.65 (4H, m), 2.59 (3H, s), 2.67 (1H, m), 2.95 (1H, m), 3.36 (3H, s), 3.57 (2H, t, *J*=6.0 Hz), 3.94 (1H, m), 3.98 (1H, m), 5.10 (2H, s), 6.90 (2H, d, *J*=8.4 Hz), 7.03 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{32}H_{61}NNaO_6Si_2$ $[M+Na]^+$ 634.3930, found 634.3934.

3.2.13. *tert-Butyl (1R,2S)-[2-(tert-butyldimethylsilyloxy)-5-hydroxy-1-[4-(methoxymethoxy)benzyl]pentyl]methylcarbamate (**18**)*

Dowex-50 (0.25 g) was washed with deionized water three times. To a solution of TBS ether **17** (1.10 g, 1.80 mmol) in MeOH (50 ml) was added a suspension of Dowex-50 in deionized water (5 ml) and stirred for 36 h at room temperature. After filtration, triethylamine (300 μl) was added to the filtrate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (5:1 to 1:1) gave **18** (0.67 g, 75%) as a colorless oil. n_D=1.4882. $[\alpha]_D^{24}$ +34 (*c* 1.0, $CHCl_3$). IR (film): ν=3444, 1694, 1613, 1512, 1233, 1153 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.12 (6H, s), 0.94 (9H, s), 1.23 (9H, br s), 1.36–1.62 (4H, m), 2.59 (3H, s), 2.63 (1H, m), 2.86 (1H, m), 3.30–3.44 (2H, m), 3.36 (3H, s), 3.93 (1H, m), 4.02 (1H, m), 5.10 (2H, s), 6.90 (2H, d, *J*=8.4 Hz), 7.07 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{26}H_{47}NNaO_6Si$ $[M+Na]^+$ 520.3065, found 520.3090.

3.2.14. *tert-Butyl (1R,2S)-[2-(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-5-[(1-phenyl-1H-tetrazol-5-yl)sulfonyl]pentyl]methylcarbamate (**19**)*

To a solution of alcohol **18** (660 mg, 1.33 mmol) in THF (30 ml) was added a solution of diethyl azodicarboxylate in toluene (1.98 M, 804 μl, 1.59 mmol), PPh_3 (417 mg, 1.59 mmol), and 5-mercapto-1-phenyl-1*H*-tetrazole (283 mg, 1.59 mmol) at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was chromatographed over silica gel and elution with hexanes/ethyl acetate (4:1) gave crude sulfide (1.3 g). To a solution of this crude material in 95% EtOH (30 ml) were added ammonium molybdate tetrahydrate (160 mg, 0.13 mmol) and 35% hydrogen peroxide solution (1.5 ml) at 0 °C. The mixture was stirred for 24 h at room temperature and poured into 10% $Na_2S_2O_3$ solution. It was extracted with EtOAc and the organic layer was washed with brine. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (4:1) gave **19** (759 mg, 83% in two steps) as a colorless oil. n_D=1.5010. $[\alpha]_D^{26}$ +6.6 (*c* 1.0, $CHCl_3$). IR (film): ν=1690, 1612, 1510, 1345, 1233, 1152, 1081, 1010 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.11 (3H, s), 0.13 (3H, s), 0.91 (9H, s), 1.19 (9H, br s), 1.51–1.75 (2H, m), 1.84–2.00 (2H, m), 2.59 (3H, s), 2.61 (1H, m), 2.96 (1H, m), 3.36 (3H, s), 3.74 (2H, t, *J*=7.5 Hz), 3.87–4.03 (2H, m), 5.10 (2H, s), 6.90 (2H, d, *J*=8.4 Hz), 7.03 (2H, d, *J*=8.4 Hz), 7.55–7.77 (5H, m). ESI-TOFMS *m*/*z* calcd for $C_{33}H_{51}N_5NaO_7SSi$ $[M+Na]^+$ 712.3171, found 712.3169.

3.2.15. *tert-Butyl (1R,2S,5E,7S,9S)-[2-(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-7,9-dimethylundec-5-enyl]methylcarbamate (**20**)*

Under argon atmosphere, to a solution of sulfone **19** (321 mg, 0.47 mmol) in THF (10 ml) was added a solution of KHMDS in toluene (0.5 M, 1.1 ml, 0.56 mmol) at −78 °C. After stirring for 2 min, a solution of aldehyde **11** (119 mg, 0.93 mmol) in THF (5 ml) was added to the reaction mixture and it was stirred for another 5 min at same temperature. The reaction mixture was then allowed to warm to room temperature. This solution was poured into saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was washed with water and brine. After drying over anhydrous magnesium sulfate, the solution was concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (8:1) gave **20** (200 mg, 73%) as a colorless oil. n_D=1.4791. $[\alpha]_D^{23}$ +37 (*c* 1.0, $CHCl_3$). IR (film): ν=1694, 1613, 1513, 1234, 1153, 1011 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.12 (6H, s), 0.81 (3H, d, *J*=6.6 Hz), 0.81 (3H, t, *J*=7.2 Hz), 0.91 (3H, d, *J*=6.6 Hz), 0.93 (9H, s), 1.00 (1H, ddd, *J*=13.2, 7.4, 5.4 Hz), 1.04–1.40 (4H, m), 1.22 (9H, br s), 1.48 (1H, m), 1.58 (1H, m), 2.03–2.21 (3H, m), 2.60 (3H, s), 2.61 (1H, m), 2.95 (1H, m), 3.36 (3H, s), 3.90 (1H, ddd, *J*=8.4, 4.2, 4.2 Hz), 4.02 (1H, m), 5.10 (2H, s), 5.24 (1H, dd, *J*=15.3, 7.5 Hz), 5.34 (1H, dt, *J*=15.3, 6.3 Hz), 6.90 (2H, d, *J*=8.4 Hz), 7.03 (2H, d, *J*=8.4 Hz). ESI-TOFMS *m*/*z* calcd for $C_{34}H_{61}NNaO_5Si$ $[M+Na]^+$ 614.4211, found 614.4246.

3.2.16. *(2R,3S,6E,8S,10S)-1-(4-Hydroxyphenyl)-8,10-dimethyl-2-(methylamino)dodec-6-en-3-ol (ent-**2**)*

To a solution of **20** (58 mg, 0.13 mmol) in THF (2 ml), MeOH (1 ml), and water (1 ml) was added trifluoroacetic acid (100 μl) and the mixture was stirred for 48 h at room temperature. The reaction mixture was concentrated at 50 °C in vacuo. The residue was chromatographed over silica gel. Elution with chloroform/MeOH (7:1) followed by recrystallization from hexanes/ether gave *ent*-**2** (28 mg, 86%) as colorless needles. Mp=122–127 °C. $[\alpha]_D^{25}$ +20 (*c* 0.35, CH_3OH). IR (KBr): ν=3239, 2961, 1671, 1203, 1185, 1146 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ=0.82 (3H, d, *J*=6.5 Hz), 0.84 (3H, t, *J*=7.3 Hz), 0.91 (3H, d, *J*=6.8 Hz), 0.99 (1H, ddd, *J*=13.5, 9.7, 4.8 Hz), 1.13 (1H, m), 1.22 (1H, ddd, *J*=13.5, 9.7, 4.8 Hz), 1.25–1.35 (2H, m), 1.45–1.6 (2H, m), 1.99 (1H, m), 2.1–2.25 (2H, m), 2.62 (3H, s), 2.86 (1H, dd, *J*=14.7, 7.9 Hz), 2.91 (1H, dd, *J*=14.7, 7.0 Hz), 3.34 (1H, ddd, *J*=7.9, 7.0, 3.0 Hz), 3.83 (1H, ddd, *J*=9.4, 3.6, 3.0 Hz), 5.22 (1H, dd, *J*=15.5, 8.3 Hz), 5.33 (1H, dt, *J*=15.5, 6.7 Hz), 6.78 (2H, quasi d,

J=8.5 Hz), 7.10 (2H, quasi d, J=8.5 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ=11.7, 19.4, 22.3, 29.9, 31.1, 32.4, 33.1, 33.2, 35.8, 45.6, 66.8, 68.7, 116.9, 127.6, 128.4, 131.3, 138.8, 158.0. ESI-TOFMS m/z calcd for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2741, found 334.2763.

3.2.17. *tert*-Butyl (1R,2S,5E,7R,9R)-[2-(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-7,9-dimethylundec-5-enyl]methylcarbamate (**21**)

Under argon atmosphere, to a solution of sulfone **19** (126 mg, 0.18 mmol) in THF (5 ml) was added a solution of KHMDS in toluene (0.5 M, 438 μl, 0.22 mmol) at −78 °C. After stirring for 2 min, a solution of aldehyde *ent*-**11** (47 mg, 0.37 mmol) in THF (2 ml) was added to the reaction mixture and it was stirred for another 5 min at same temperature. The reaction mixture was then allowed to warm to room temperature. This mixture was poured into saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was washed with water and brine. After drying over anhydrous magnesium sulfate, the solution was concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (8:1) gave **21** (60 mg, 56%) as a colorless oil. n_D=1.4779. $[\alpha]_D^{24}$ +14 (c 1.0, $CHCl_3$). IR (film): ν=1694, 1613, 1512, 1234, 1154, 1010 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6, 90 °C): δ=0.12 (6H, s), 0.81 (3H, d, J=6.0 Hz), 0.82 (3H, t, J=7.2 Hz), 0.91 (3H, d, J=7.2 Hz), 0.93 (9H, s), 1.00 (1H, ddd, J=13.2, 8.4, 5.7 Hz), 1.04–1.39 (4H, m), 1.23 (9H, br s), 1.42–1.56 (2H, m), 2.01–2.22 (3H, m), 2.60 (3H, s), 2.61 (1H, m), 2.95 (1H, m), 3.36 (3H, s), 3.91 (1H, ddd, J=8.4, 3.6, 3.6 Hz), 4.05 (1H, m), 5.10 (2H, s), 5.25 (1H, dd, J=15.6, 7.5 Hz), 5.34 (1H, dt, J=15.6, 6.3 Hz), 6.90 (2H, d, J=8.4 Hz), 7.03 (2H, d, J=8.4 Hz). ESI-TOFMS m/z calcd for $C_{34}H_{61}NNaO_5Si$ $[M+Na]^+$ 614.4211, found 614.4234.

3.2.18. (2R,3S,6E,8R,10R)-1-(4-Hydroxyphenyl)-8,10-dimethyl-2-(methylamino)dodec-6-en-3-ol (*ent*-**14**)

To a solution of **21** (40 mg, 0.07 mmol) in THF (1 ml), MeOH (0.5 ml), and water (0.5 ml) was added trifluoroacetic acid (300 μl) and it was stirred for 48 h at room temperature. The reaction mixture was concentrated at 50 °C in vacuo. The residue was chromatographed over silica gel. Elution with chloroform/MeOH (7:1) followed by recrystallization from hexanes/ether gave *ent*-**14** (20 mg, 89%) as colorless needles. Mp=95–98 °C. $[\alpha]_D^{24}$ −13 (c 0.35, CH_3OH). IR (KBr): ν=3232, 2961, 1672, 1241, 1186, 1146 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ=0.81 (3H, d, J=6.5 Hz), 0.85 (3H, t, J=7.3 Hz), 0.92 (3H, d, J=6.8 Hz), 0.99 (1H, ddd, J=13.5, 9.0, 5.3 Hz), 1.13 (1H, m), 1.24 (1H, ddd, J=13.5, 9.6, 4.3 Hz), 1.24–1.35 (2H, m), 1.45–1.6 (2H, m), 2.00 (1H, m), 2.11–2.24 (2H, m), 2.62 (3H, s), 2.86 (1H, dd, J=14.7, 7.9 Hz), 2.93 (1H, dd, J=14.7, 7.0 Hz), 3.35 (1H, ddd, J=7.9, 7.0, 2.8 Hz), 3.84 (1H, ddd, J=9.0, 4.3, 2.8 Hz), 5.23 (1H, dd, J=15.5, 8.2 Hz), 5.35 (1H, dt, J=15.5, 6.7 Hz), 6.78 (2H, quasi d, J=8.5 Hz), 7.11 (2H, quasi d, J=8.5 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ=11.7, 19.4, 22.3, 29.9, 31.1, 32.4, 33.1, 33.2, 35.8, 45.6, 66.8, 68.7, 116.9, 127.6, 128.4, 131.3, 138.8, 158.0. ESI-TOFMS m/z calcd for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2741, found 334.2756.

3.2.19. (4R,5S)-5-[3-(tert-Butyldimethylsilyloxy)propyl]-4-[4-(methoxymethoxy)benzyl]-3-methyl-1,3-oxazolidin-2-one (**22**)

To a solution of amino alcohol **16** (40 mg, 0.08 mmol) in THF (3 ml) was added NaH (5 mg, 0.13 mmol) at room temperature and it was stirred for 16 h under reflux. The reaction mixture was poured into saturated aqueous ammonium chloride solution, and extracted with ethyl acetate. The organic layer was washed with water and brine and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/acetone (3:1 to 1:1) gave **22** (23 mg, 68%) as a colorless oil. n_D=1.4978. $[\alpha]_D^{18}$ +5.9 (c 1.0, $CHCl_3$). IR (Nujol): ν 1747, 1611, 1512, 1234, 1152 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.02 (6H, s), 0.87 (9H, s), 1.45–1.85 (4H, m), 2.64 (3H, s), 2.85 (1H, dd, J=14.4, 7.2 Hz), 2.86 (1H, dd, J=14.4, 7.2 Hz), 3.38 (3H, s), 3.52–3.72 (2H, m), 3.97 (1H, q, J=7.2 Hz), 4.50 (1H, m), 5.16 (2H, s), 6.99 (2H, d, J=8.4 Hz), 7.14 (2H, d, J=8.4 Hz). ESI-TOFMS m/z calcd for $C_{22}H_{37}NNaO_5Si$ $[M+Na]^+$ 446.2333, found 446.2347.

3.2.20. Methyl (S)-N-(tert-butoxycarbonyl)-O-(methoxymethyl)-tyrosinate (*ent*-**3**)

In the same manner as the synthesis of **3** described above, L-tyrosine (25.5 g, 140 mmol) was subjected to esterification, Boc-protection, and MOM-protection to give *ent*-**3** (40.9 g, 99% in three steps) as colorless prisms. Mp=67–70 °C. $[\alpha]_D^{21}$ +46 (c 1.0, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical with those of **3**. ESI-TOFMS m/z calcd for $C_{17}H_{25}NNaO_6$ $[M+Na]^+$ 362.1574, found 362.1612.

3.2.21. (S)-N-α-(tert-Butoxycarbonyl)-N-methoxy-O-(methoxymethyl)-N-methyltyrosinamide (*ent*-**4**)

In the same manner as the synthesis of **4** described above, ester *ent*-**3** (8.48 g, 25.0 mmol) was treated with *N*,*O*-dimethylhydroxylamine hydrochloride (5.36 g, 55.0 mmol) in THF (250 ml) in the presence of isopropyl magnesium bromide to give *ent*-**4** (7.97 g, 87%) as colorless prisms. Mp=68–71 °C. $[\alpha]_D^{21}$ +19 (c 1.0, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical with those of **4**. ESI-TOFMS m/z calcd for $C_{18}H_{28}N_2NaO_6$ $[M+Na]^+$ 391.1840, found 391.1890.

3.2.22. (S)-N-α-(tert-Butoxycarbonyl)-N-methoxy-O-(methoxymethyl)-N,N-α-dimethyltyrosinamide (*ent*-**5**)

In the same manner as the synthesis of **4** described above, *ent*-**4** (6.91 g, 18.8 mmol) was treated with NaH (0.79 g, 19.7 mmol), MeI (1.75 ml, 28.2 mmol) in DMF (80 ml), and THF (5 ml) to give *ent*-**5** (6.78 g, 95%) as colorless prisms. Mp=40–44 °C. $[\alpha]_D^{21}$ −103 (c 1.0, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical with those of **5**. ESI-TOFMS m/z calcd for $C_{19}H_{30}N_2NaO_6$ $[M+Na]^+$ 405.1996, found 405.1989.

3.2.23. tert-Butyl (1S,2R)-[2,5-bis(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]pentyl]methylcarbamate (*ent*-**17**)

To a solution of amide *ent*-**5** (3.83 g, 10.0 mmol) in THF (75 ml) was added lithium aluminum hydride (0.38 g, 10.0 mmol) at 0 °C. After stirring for 1 h at same temperature, the reaction mixture was cooled down to −70 °C. Saturated aqueous ammonium chloride solution (30 ml) was added to the reaction mixture with vigorous stirring, and the mixture was allowed to warm to room temperature. Dark gray precipitates were filtered off through Celite and washed with ether. After concentration, the residue was dissolved in ether and filtered through silica gel. Concentration in vacuo gave crude aldehyde *ent*-**15** (3.26 g) as a colorless oil. This material was used for next reaction without further purification. Small amount of crude aldehyde was analyzed by HPLC [column: Daicel Chiralpak AD-H (0.46 cm×25 cm), eluent: hexane/*i*-PrOH (19:1), flow rate: 0.8 ml/min, detection: UV (254 nm), (*S*)-isomer: t_R=11.8 min, (*R*)-isomer: t_R=12.9 min] and the enantiomeric purity was determined to be >99% ee.

To a solution of 1-(*tert*-butyldimethylsilyloxy)-3-iodopropane (6.34 g, 21.1 mmol) in ether (100 ml) was slowly added a solution of *t*-BuLi in pentane (1.58 M, 26.5 ml, 42.1 mmol) at −78 °C and the resulting mixture was stirred for 1 h at same temperature. The resultant solution of lithium reagent was added to a solution of crude aldehyde (3.25 g) in ether (50 ml) at −78 °C and the reaction mixture was slowly warmed to room temperature. After stirring for 1 h, it was poured into saturated aqueous ammonium chloride solution and extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (5:1) gave inseparable mixture of *ent*-**16** and *ent*-**7** (3.47 g) as slightly yellow oil. This mixture was used for next reaction without further purification.

To a solution of crude mixture of *ent*-**16** and *ent*-**7** (1.00 g) in dichloromethane (30 ml) was added 2,6-lutidine (0.94 ml, 8.0 mmol) followed by TBSOTf (0.92 ml 4.0 mmol) at 0 °C. After stirring for 3 h, the reaction mixture was poured into saturated aqueous ammonium chloride solution, and extracted with ether. The organic layer was washed with 1 N HCl, saturated aqueous sodium bicarbonate solution, water and brine, and then dried over anhydrous magnesium sulfate. After concentration in vacuo, the residue was chromatographed over silica gel. Elution with hexanes/ethyl acetate (20:1) gave *ent*-**17** (0.89 g, 51% in three steps) and *ent*-**8** (0.07 g, 4% in three steps) as colorless oils. n_D=1.4728. $[\alpha]_D^{26}$ −27 (*c* 1.0, $CHCl_3$). IR and ^1H NMR spectra of *ent*-**17** were identical with those of **17**. ESI-TOFMS *m*/*z* calcd for $C_{32}H_{61}NNaO_6Si_2$ $[M+Na]^+$ 634.3930, found 634.3949.

3.2.24. tert-Butyl (1S,2R)-[2-(tert-butyldimethylsilyloxy)-5-hydroxy-1-[4-(methoxymethoxy)benzyl]pentyl]methylcarbamate (*ent*-***18***)

In the same manner as the synthesis of **18** described above, *ent*-**17** (820 mg, 1.34 mmol) was treated with Dowex-50 (0.2 g) in MeOH (25 ml) and water (3 ml) to give *ent*-**18** (548 mg, 82%) as a colorless oil. n_D=1.4863. $[\alpha]_D^{26}$ −36 (*c* 1.0, $CHCl_3$). Its IR and ^1H NMR spectra were identical with those of **18**. ESI-TOFMS *m*/*z* calcd for $C_{26}H_{47}NNaO_6Si$ $[M+Na]^+$ 520.3065, found 520.3089.

3.2.25. tert-Butyl (1S,2R)-[2-(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-5-[(1-phenyl-1H-tetrazol-5-yl)sulfonyl]pentyl]methylcarbamate (*ent*-***19***)

In the same manner as the synthesis of **19** described above, *ent*-**18** (447 mg, 0.90 mmol) was reacted with 5-mercapto-1-phenyl-1*H*-tetrazole (241 mg, 1.35 mmol) in THF (30 ml) under Mitsunobu condition to give crude sulfide (0.6 g). This crude sulfide (0.6 g) was treated with ammonium molybdate tetrahydrate (250 mg, 0.20 mmol) and 35% aqueous hydrogen peroxide solution (2 ml) in 95% EtOH (30 ml) to give *ent*-**19** (512 mg, 83% in two steps) as a colorless oil. n_D=1.5033. $[\alpha]_D^{26}$ −5.7 (*c* 1.0, $CHCl_3$). Its IR and ^1H NMR spectra were identical with those of **19**. ESI-TOFMS *m*/*z* calcd for $C_{33}H_{51}N_5NaO_7SSi$ $[M+Na]^+$ 712.3171, found 712.3147.

3.2.26. tert-Butyl (1S,2R,5E,7R,9R)-[2-(tert-butyldimethylsilyloxy)-1-[4-(methoxymethoxy)benzyl]-7,9-dimethylundec-5-enyl]methylcarbamate (*ent*-***20***)

In the same manner as the synthesis of **20** described above, *ent*-**19** (195 mg, 0.28 mmol) was treated with a solution of KHMDS in toluene (0.5 M, 678 μl, 0.34 mmol) and aldehyde *ent*-**11** (73 mg, 0.57 mmol) in THF (10 ml) to give *ent*-**20** (117 mg, 70%) as colorless oil. n_D=1.4789. $[\alpha]_D^{25}$ −41 (*c* 1.0, $CHCl_3$). Its IR and ^1H NMR spectra were identical with those of **20**. ESI-TOFMS *m*/*z* calcd for $C_{34}H_{61}NNaO_5Si$ $[M+Na]^+$ 614.4211, found 614.4234.

3.2.27. (2S,3R,6E,8R,10R)-1-(4-Hydroxyphenyl)-8,10-dimethyl-2-(methylamino)dodec-6-en-3-ol (***2***, revised structure of tyroscherin)

In the same manner as the synthesis of *ent*-**2** described above, the solution of *ent*-**20** (98 mg, 0.17 mmol) in THF (2 ml), MeOH (1 ml), and water (1 ml) was treated with trifluoroacetic acid (100 μl) to give **2** (48 mg, 87%) as a colorless needle. Mp=122–126 °C. $[\alpha]_D^{25}$ −21 (*c* 0.35, CH_3OH). IR (KBr): ν=3239, 2961, 1671, 1203, 1185, 1146 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ=0.82 (3H, d, *J*=6.5 Hz), 0.84 (3H, t, *J*=7.3 Hz), 0.91 (3H, d, *J*=6.8 Hz), 0.99 (1H, ddd, *J*=13.5, 9.7, 4.8 Hz), 1.13 (1H, m), 1.22 (1H, ddd, *J*=13.5, 9.7, 4.8 Hz), 1.25–1.35 (2H, m), 1.45–1.6 (2H, m), 1.99 (1H, m), 2.1–2.25 (2H, m), 2.62 (3H, s), 2.86 (1H, dd, *J*=14.7, 7.9 Hz), 2.91 (1H, dd, *J*=14.7, 7.0 Hz), 3.34 (1H, ddd, *J*=7.9, 7.0, 3.0 Hz), 3.83 (1H, ddd, *J*=9.4, 3.6, 3.0 Hz), 5.22 (1H, dd, *J*=15.5, 8.3 Hz), 5.33 (1H, dt, *J*=15.5, 6.7 Hz), 6.78 (2H, quasi d, *J*=8.5 Hz), 7.10 (2H, quasi d, *J*=8.5 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ=11.7, 19.4, 22.3, 29.9, 31.1, 32.4, 33.1, 33.2, 35.8, 45.6, 66.8, 68.7, 116.9, 127.6, 128.4, 131.3, 138.8, 158.0. ESI-TOFMS *m*/*z* calcd for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2741, found 334.2740.

3.2.28. (2S,3R,6E,8S,10S)-1-(4-Hydroxyphenyl)-8,10-dimethyl-2-(methylamino)dodec-6-en-3-ol (***14***)

In the same manner as the synthesis of **21** described above, *ent*-**19** (154 mg, 0.22 mmol) was treated with a solution of KHMDS in toluene (0.5 M, 536 μl, 0.27 mmol) and aldehyde **11** (58 mg, 0.45 mmol) in THF (8 ml) to give *ent*-**21** (76 mg, 58%) as colorless oil. n_D=1.4789. $[\alpha]_D^{24}$ −15 (*c* 1.0, $CHCl_3$). Its IR and ^1H NMR spectra were identical with those of **21**. ESI-TOFMS *m*/*z* calcd for $C_{34}H_{61}NNaO_5Si$ $[M+Na]^+$ 614.4211, found 614.4227.

In the same manner as the synthesis of *ent*-**14** described above, the solution of *ent*-**21** (60 mg, 0.10 mmol) in THF (2 ml), MeOH (1 ml), and water (1 ml) was treated with trifluoroacetic acid (300 μl) to give **14** (24 mg, 71%) as colorless needles. Mp=97–99 °C. $[\alpha]_D^{25}$ +11 (*c* 0.35, CH_3OH). Its IR and ^1H NMR spectra were identical with those of *ent*-**14**. ESI-TOFMS *m*/*z* calcd for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2741, found 334.2758.

3.2.29. (2S,3R,6E)-1-(4-Hydroxyphenyl)-2-(methylamino)dodec-6-en-3-ol (***23***)

In a similar manner to the synthesis of *ent*-**2** via **20**, *ent*-**19** (70 mg, 0.10 mmol) was treated with a solution of KHMDS in toluene (0.5 M, 243 μl, 0.12 mmol) and 1-hexanal (25 μl, 0.20 mmol) in THF (5 ml) to give crude coupled product (20 mg) as a slightly yellow oil. A solution of crude coupled product (20 mg) in THF (2 ml), MeOH (1 ml), and water (1 ml) was treated with trifluoroacetic acid (300 μl) to give **23** (3.5 mg, 11% in two steps) as a colorless oil. ^1H NMR (500 MHz, CD_3OD): δ=0.89 (3H, t, *J*=7.0 Hz), 1.24–1.37 (6H, m), 1.43–1.60 (2H, m), 1.93–2.04 (3H, m), 2.19 (1H, m), 2.58 (3H, s), 2.83 (1H, dd, *J*=14.7, 7.9 Hz), 2.87 (1H, dd, *J*=14.7, 7.0 Hz), 3.24 (1H, m), 3.80 (1H, dt, *J*=9.0, 3.5 Hz), 5.38 (1H, dt, *J*=15.5, 6.2 Hz), 5.44 (1H, dt, *J*=15.5, 6.0 Hz), 6.77 (2H, quasi d, *J*=8.5 Hz), 7.09 (2H, quasi d, *J*=8.5 Hz). ESI-TOFMS *m*/*z* calcd for $C_{19}H_{32}NO_2$ $[M+H]^+$ 306.2428, found 306.2462.

3.3. Biological studies

MCF-7 human breast cancer cells were provided by Dr. Shin-Ichiro Takahashi (Departments of Animal Sciences and Applied Biological Chemistry, Graduate School of Agriculture and Life Sciences, The University of Tokyo). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and were grown at 37 °C in a humidified atmosphere of 5% CO_2. Cell proliferation assays were carried out in serum-free DMEM containing 0.1% bovine serum albumin (Sigma) and 30 ng/ml recombinant human insulin-like growth factor-1 (IGF-1, Sigma) and DMEM containing 0.5% FBS. The cells were plated in each well of 96-well plates at the density of 5×10^4 cells/ml. After incubation with various concentrations of samples at 37 °C for 72 h, the cells were treated with 0.5 μg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 37 °C for 4 h. Relative cell number was measured with formazan formation at 540 nm using a microplate reader (SUNRISE Remote, TECAN Inc.). IC_{50} values were calculated by linear interpolation between the two drug concentrations above and below the 50% inhibition line.

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