Supporting Information

Kasumigamide: an Antialgal Peptide from the Cyanobacterium *Microcystis aeruginosa*

Keishi Ishida and Masahiro Murakami*

Laboratory of Marine Biochemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

**Experimental Section.**

**General Methods.** Ultraviolet spectrum was measured on a Hitachi 330 spectrophotometer. Optical rotation was determined with a JASCO DIP-1000 polarimeter. $^1$H and $^{13}$C NMR spectra were measured on either Bruker A300, JEOL JNM-A500 or A600 NMR spectrometers. 2D-NMR spectra were recorded on a JEOL JNM-A500 spectrometer equipped with a VAXserver 4000-200 computer. Mass spectra, including high resolution mass measurements, were measured on a JEOL SX-102 mass spectrometer.

**Culture Conditions.** *Microcystis aeruginosa* (NIES-87) was obtained from the NIES-collection (Microbial Culture Collection, the National Institute for Environmental Studies, Japan) and cultured in 10 L glass bottles containing MA medium [Ca(NO$_3$)$_2$·4H$_2$O 5 mg, KNO$_3$ 10 mg, NaNO$_3$ 5 mg, Na$_2$SO$_4$ 4 mg, MgCl$_2$·6H$_2$O 5 mg, β-Na$_2$glycerophosphate 10 mg, Na$_2$EDTA·2H$_2$O 0.5 mg, FeCl$_3$·6H$_2$O 0.05 mg, MnCl$_2$·4H$_2$O 0.5 mg, ZnCl$_2$ 0.05 mg, CoCl$_2$·6H$_2$O 0.5 mg, Na$_2$MoO$_4$·2H$_2$O 0.08 mg, H$_3$BO$_3$ 2 mg, BICINE 50 mg, distilled water 100 mL, pH 8.6] with aeration (filtered air, 0.3 L/min) at 25 °C under illumination of 250 μE/m$^2$-s on a 12L:12D cycle. Cells were harvested by continuous centrifugation at 10,000 rpm after incubation for 14-21 days. Harvested alga was lyophilized and kept in a freezer at -20 °C until extraction.

**Extraction and Isolation.** Freeze-dried alga (18.2 g from 100 L of culture) was extracted with 80% MeOH (2.0 L × 1) and MeOH (2.0 L × 1). Combined 80% MeOH and MeOH
extracts were concentrated to an aqueous suspension which was then extracted with ether. The aqueous layer was extracted with n-BuOH. The n-BuOH layer was evaporated under reduced pressure to green dry solid (2.04 g), which was subjected to flash chromatography on ODS (YMC-ODS, AM120Å, 5 × 10 cm) with aqueous MeOH (20, 40, 60, 80 and 100%) followed by CH₂Cl₂.

The 40% and 60% MeOH fractions (87.0 mg) were combined and subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 32% MeCN containing 0.05% TFA; flow rate 2.0 mL/min; UV detection at 210 nm) to yield kasumigamide (1, 10.0 mg).

**Acid Hydrolysis.** For amino acid analysis, 100 µg of 1 was dissolved in 0.5 mL of 6 N HCl and heated at 110 °C for 16 h. After evaporation, the residue was dissolved in 0.6 mL of 0.02 N HCl and subjected to a Hitachi L-8500A amino acid analyzer. Retention times in the standard amino acids (min): β-Ala (58.2) and Arg (108.4). Retention times in the acid hydrolysates of 1 (min): β-Ala (58.2) and Arg (108.4).

**HPLC Analysis of the Marfey Derivatives.** To acid hydrolysate of a 100 µg portion of 1, 50 µL of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (l-FDAA) in acetone (10 mg/mL) and 100 µL of 1 M NaHCO₃ were added, and the mixture was kept at 80 °C for 3 min. To the reaction mixture, 50 µL of 2 N HCl and 300 µL of 50% MeCN were added and analyzed by reversed-phase HPLC (Cosmosil 5C18-MS, 4.6 × 250 mm; gradient elution from H₂O/TFA (100:0.1) to MeCN/H₂O/TFA (60:40:0.1) in 60 min; UV-detection 340 nm; flow rate 1.0 mL/min). Retention times in the derivatives of the standard amino acids (min): D-Arg (42.0), L-Arg (43.0). Retention time in the derivatives of the acid hydrolysates of 1 (min): D-Arg (42.0).

**Derivatization and HPLC Analysis of Pla.** l-Menthol (10.0 mg) and MeCN (50 µL) were added to the acid hydrolysate of 1 (100 µg). After cooling, TMSCl (20 µL) was added, and the mixture was sealed in a test tube with screw cap, and heated at 100 °C for 10 min. Then the mixture was evaporated in a stream of nitrogen and diluted with MeCN (100 µL), and analyzed by reversed-phase HPLC (Cosmosil 5C18-AR, 10 × 250 mm, 65% MeCN containing 0.05%TFA, UV detection 210 nm, flow rate 1.0 mL/min). Retention times (min) of standards:
D-Pla (21.6 min), L-Pla (22.6 min). Retention time (min) of the acid hydrolysate of 1: D-Pla (21.6 min).

**Ethyl α-Phenylazobenzoylacetaet.** Aniline (470 mg) in a mixture of conc. HCl (2 mL) and ice-cold H₂O (5 mL), was diazotized by the slow addition of ice-cold solution of 350 mg of NaNO₃ in 2 mL of H₂O. The diazonium salt solution was added over the course of 1 h to a well-stirred mixture of sodium acetate trihydrate (4.5 g), H₂O (5 mL), ethyl benzoylacetaet (1 g) and EtOH (18 mL) held at 0 °C. After the addition of the diazonium salt was complete, the volume of the reaction mixture was made up to 40 mL with ice-cold water and stirring was continued for another hour. The product was collected by filtration and thoroughly washed with H₂O. After drying in vacuo, it was washed with hexane, concentrated and subjected to silica gel column chromatography (Kieselgel 60, 3.5 × 6 cm) using Hexane/EtOAc as eluant to yield ethyl α-phenylazobenzoylacetaet (835 mg; 54%): HRFABMS (Positive), m/z 297.1254 [M+H]+ (C₁₇H₁₇N₂O₃, Δ +1.4 mmu); ¹H NMR (CDCl₃; 600 MHz) δ 1.29 (t, 6.8, 3H), 4.31 (m, 2H), 7.01 (t, 7.3, 1H), 7.10 (d, 6.8, 2H), 7.24 (t, 7.7, 2H), 7.40 (t, 7.7, 2H), 7.50 (t, 7.3, 1H), 7.87 (d, 7.3, 2H).

**Erythro-N-Acetyl-β-Phenyl-D,L-Serine.** A solution of ethyl α-phenylazobenzoylacetaet (400 mg) in glacial acetic acid (1 mL) was added to a well-stirred mixture of zinc powder (600 mg), glacial acetic acid (2 mL) and acetic anhydride (430 µL) at 0 °C. After stirring for 10 min at 0 °C, the mixture was further stirred at room temperature for 2 h and excess zinc powder were removed by filtration and thoroughly washed with glacial acetic acid. The combined filtrates were concentrated and subjected to silica gel column chromatography (Kieselgel 60, 3.5 × 6 cm) using hexane/EtOAc as eluant to yield ethyl α-acetamidobenzoylacetaet (317 mg; 94%): HRFABMS (Positive), m/z 250.1098 [M+H]+ (C₁₃H₁₆NO₄, Δ +1.9 mmu).

To ethyl α-acetamidobenzoylacetaet (300 mg) dissolved in glacial acetic acid (3 mL), 10% palladium carbon (50 mg) was added and stirred at room temperature under hydrogen atmosphere. After stirring for 6 h, the catalyst was removed by filtrarion and the filtrate was concentrated and lyophilized to yield erythro-N-acetyl-β-phenyl-D,L-serine as acetate salt (324 mg; 90%): HRFABMS (Positive), m/z 252.1253 [M+H]+ (C₁₃H₁₈NO₄, Δ +1.7 mmu); ¹H NMR (DMSO-d₆; 600 MHz) δ 1.11 (t, 6.8, 3H), 1.71 (s, 3H), 4.03 (m, 2H), 4.45 (t, 8.1,
1H), 4.72 (d, 7.7, 1H), 5.03 (d, 3.8, 1H), 5.74 (br, 1H), 7.23 (t, 7.3, 1H), 7.29 (t, 7.3, 2H), 7.35 (d, 7.7, 2H), 8.12 (d, 8.6, 1H), 8.19 (d, 9.0, 1H).

**Erythro-β-Phenyl-D,L-Serine.** Erythro-N-acetyl-β-phenyl-D,L-serine (200 mg) was dissolved in 6 N HCl (6 mL) and hydrolyzed at 100 °C for 10 h. After solvent was removed by evaporation and lyophyllization, the acid hydrolysate was subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 x 250 mm; 6 x 10⁻³ M HCl; UV detection at 210 nm; flow rate 2.0 mL/min) to yield erythro-β-phenyl-D,L-serine (119 mg; 83%): ¹H NMR (DMSO-d₆, 300 MHz), δ 3.85 (d 4.3, 1H), 5.00 (d 4.3, 1H), 7.28-7.40 (m, 5H). [Threo-β-phenyl-D,L-serine: ¹H NMR (DMSO-d₆, 300 MHz), δ 4.00 (d 4.4, 1H), 5.07 (d 4.4, 1H), 7.28-7.45 (m, 5H)].

**Benzoyl-Glycyl-threo-β-Phenyl-D,L-Serine Methyl Ester.** To a solution of dried MeOH (6 mL) and SOCl₂ (2 mL), threo-β-phenyl-D,L-serine (1 g) was added at 0 °C and stirred at room temperature for 15 h under argon. After the solvent was removed by evaporation and lyophyllization, the reaction mixture was subjected to silicagel column chromatography (Kieselgel 60, 3.5 x 6 cm) using CHCl₃/MeOH as elutant to yield threo-β-phenyl-D,L-serine methyl ester (1.06 g; 99%).

To a solution of threo-β-phenyl-D,L-serine methyl ester (100 mg) in DMF (1.5 mL), benzoyl glycine (100 mg), PyBOP (300 mg), HOBT (80 mg) and N-methylmorphorine (150 µL) were added at 0 °C. After stirring at room temperature for 5 h, the reaction mixture was lyophyllized and then poured into ice-cold 1 N HCl. The aqueous phase was extracted with EtOAc, and the combined organic extracts were washed with saturated aqueous NaHCO₃, saturated aqueous NaCl, dried over MgSO₄, and subjected to silicagel column chromatography (Kieselgel 60, 3.5 x 5 cm) using hexane/EtOAc as elutant to yield benzoyl-glycyl-threo-β-phenyl-D,L-serine methyl ester (154.9 mg; 85%): HRFABMS (Positive), m/z 357.1414 [M+H]⁺ (C₁₉H₂₁N₂O₅, Δ -3.6 mmu); ¹H NMR (DMSO-d₆, 600 MHz) δ 3.61 (s, 3H), 3.80 (dd, 16.6, 5.6, 1H), 3.93 (dd, 16.6, 5.1, 1H), 4.52 (dd, 8.6, 3.4, 1H), 5.08 (t, 3.8, 1H), 5.91 (d, 4.7, 1H), 7.21 (t, 7.7, 1H), 7.23 (t, 7.7, 2H), 7.31 (d, 6.8, 2H), 7.47 (t, 7.7, 2H), 7.53 (t, 6.8, 1H), 7.84 (d, 8.1, 2H), 8.11 (d, 8.6, 1H), 8.67 (t, 6.0, 1H).
**Benzoyl-Glycyl-threo-β-Phenyl-D,L-Serine.** To a solution of benzoyl-glycyl-threo-β-phenyl-D,L-serine methyl ester (100 mg) in MeOH (10 mL), 1 N NaOH (1 mL) was added and the solution was stirred at room temperature. After stirring at room temperature for 2 h, the reaction mixture was concentrated and then neutralized by addition of 1 N HCl. The resulting mixture was subjected to ODS column chromatography (YMC-ODS, 2 x 5 cm) using aqueous MeOH as eluant to yield benzoyl-glycyl-threo-β-phenyl-D,L-serine (64 mg; 67%); HR-FABMS (Negative), m/z 341.1125 [M-H]- (C_{18}H_{17}N_{2}O_{5}, Δ -1.3 mmu); 1H NMR (DMSO-d6; 600 MHz) δ 3.78 (dd, 16.2, 5.6, 1H), 3.91 (dd, 16.2, 5.1, 1H), 4.41 (brd, 7.7, 1H), 5.10 (s, 1H), 7.19 (t, 6.8, 1H), 7.21 (t, 7.3, 2H), 7.32 (d, 7.3, 2H), 7.47 (t, 7.3, 2H), 7.53 (t, 7.3, 1H), 7.84 (d, 8.1), 8.69 (t, 6.0, 1H).

**Enzymatic Hydrolysis of Benzoyl-Glycyl-threo-β-Phenyl-L-Serine.** To reaction vial was added: 2.4 mL of 1 M Tris HCl, 0.5 M NaCl, pH 7.5; 0.8 mL DMSO solution containing of benzoyl-glycyl-threo-β-phenyl-D,L-serine (40 mg), 0.4 mL of 10% LiCl containing 100 units carboxypeptidase A (Sigma Chem Co.). The vial was incubated at 37 °C for 6 h and then the reaction mixture was lyophilized. The reaction mixture was subjected to ODS column chromatography (YMC-ODS, 2 x 5 cm) using H2O and 50% MeOH as eluant. H2O fraction was subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 6 × 10^{3} M HCl; UV-detection 210 nm; flow rate 2.0 mL/min) to yield threo-β-phenyl-L-serine (11.5 mg); [α]_{25}^{25} -47.4° (c 0.575, 6 N HCl).

The 50% MeOH fraction was concentrated, dissolved in 6 N HCl (5 mL) and heated at 100 °C for 12 h. After the solvent was removed by evaporation and lyophilization, the residue was subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 6 × 10^{3} M HCl; UV-detection 210 nm; flow rate 2.0 mL/min) to yield threo-β-phenyl-D-serine (7.5 mg); [α]_{25}^{25} 48.1° (c 0.5, 6 N HCl).

**Benzoyl-Glycyl-erythro-β-Phenyl-D,L-Serine Methyl Ester.** To a solution of dried MeOH (2 mL) and SOCl₂ (0.5 mL), erythro-β-phenyl-D,L-serine (100 mg) was added at 0 °C and stirred at room temperature for 12 h under argon. After the solvent was removed by evaporation and lyophilization, the reaction mixture was subjected to silicagel column
chromatography (Kieselgel 60, 3.5 × 6 cm) using CHCl₃/MeOH as eluant to yield erythro-β-phenyl-D,L-serine methyl ester (44.7 mg; 41%).

To a solution of threo-β-phenyl-D,L-serine methyl ester (44.7 mg) in DMF (1 mL), benzoyl glycine (50 mg), PyBOP (150 mg), HOBT (40 mg) and N-methylmorphorine (70 μL) were added at 0 °C. After stirring at room temperature for 10 h, the reaction mixture was lyophilized and then poured into ice-cold 1 N HCl. The aqueous phase was extracted with EtOAc, and the combined organic extracts were washed with saturated aqueous NaHCO₃, saturated aqueous NaCl, dried over MgSO₄, and subjected to silicagel column chromatography (Kieselgel 60, 3.5 × 5 cm) using hexane/EtOAc as eluant to yield benzoyl-glycyl-erythro-β-phenyl-D,L-serine methyl ester (38.3 mg; 47%): HRFABMS (Positive), m/z 357.1454 [M+H]+ (C₁₉H₂₁N₂O₅, Δ +0.3 mmu); ¹H NMR (DMSO-d₆; 600 MHz) δ 3.59 (s, 3H), 3.70 (dd, 16.2, 5.1, 1H), 3.88 (dd, 16.2, 5.6, 1H), 4.51 (t, 8.1, 1H), 4.78 (dd, 7.3, 5.6, 1H), 5.84 (d, 4.7, 1H), 7.23 (t, 7.7, 1H), 7.26 (t, 7.7, 2H), 7.34 (d, 7.7, 2H), 7.46 (t, 7.3, 2H), 7.53 (t, 7.3, 1H), 7.83 (d, 8.1, 2H), 8.22 (d, 9.0, 1H), 8.62 (t, 6.0, 1H).

**Benzoyl-Glycyl-erythro-β-Phenyl-D,L-Serine.** To a solution of benzoyl-glycyl-erythro-β-phenyl-D,L-serine methyl ester (30 mg) in MeOH (3 mL), 1 N NaOH (0.5 mL) was added and the solution was stirred at room temperature. After stirring at room temperature for 1 h, the reaction mixture was concentrated and then neutralized by addition of 1 N HCl. The resulting mixture was subjected to ODS column chromatography (YMC-ODS, 2 × 5 cm) using aqueous MeOH as eluant to yield benzoyl-glycyl-erythro-β-phenyl-D,L-serine (25.0 mg; 87%): HRFABMS (Negative), m/z 341.1130 [M-H]- (C₁₈H₁₇N₂O₅, Δ -0.7 mmu); ¹H NMR (DMSO-d₆; 600 MHz) δ 3.71 (dd, 16.7, 5.6), 3.90 (dd, 16.7, 5.6, 1H), 4.48 (t, 7.3, 1H), 4.80 (d, 6.8), 7.21 (t, 6.8, 1H), 7.23 (t, 6.8, 2H), 7.32 (d, 8.1, 2H), 7.46 (t, 7.7, 2H), 7.53 (t, 7.3, 1H), 7.83 (d, 8.6, 2H), 7.94 (d, 8.6, 1H), 8.65 (t, 5.6, 1H).

**Enzymatic Hydrolysis of Benzoyl-Glycyl-erythro-β-Phenyl-L-Serine.** To reaction vial was added: 1.5 mL of 1 M Tris HCl, 0.5 M NaCl, pH 7.5; 0.5 mL DMSO solution containing of benzoyl-glycyl-threo-β-phenyl-D,L-serine (20 mg), 0.2 mL of 10% LiCl containing 50 units of carboxypeptidase A (Sigma Chem Co.). The vial was incubated at 37 °C for 10 h and then the reaction mixture was lyophilized. The reaction mixture was subjected to
ODS column chromatography (YMC-ODS, 2 × 5 cm) using H2O and 50% MeOH as eluant. The H2O fraction was subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 6 × 10^{-3} M HCl; UV-detection 210 nm; flow rate 2.0 mL/min) to yield erythro-β-phenyl-L-serine (2.2 mg); [α]^{25}_D 81.2° (c 0.1, 6 N HCl).

The 50% MeOH fraction was concentrated, dissolved in 6 N HCl (5 mL) and heated at 100 °C for 12 h. After the solvent was removed by evaporation and lyophylization, the residue was subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 6 × 10^{-3} M HCl; UV-detection 210 nm; flow rate 2.0 mL/min) to yield erythro-β-phenyl-D-serine (2.0 mg); [α]^{25}_D -82.9° (c 0.1, 6 N HCl).

**HPLC Analysis and Isolation of β-Phenylserine.** Compound 1 (20.0 mg) was dissolved in 5 mL of 6 N HCl and heated at 105 °C for 4 h. After removal of the solvent by evaporation, the acid hydrolysates were subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 2% MeCN containing 6 × 10^{-3} M HCl; UV detection at 210 nm; flow rate 2.0 mL/min) to yield β-phenylserine (1.8 mg); [α]^{25}_D -79.7° (c 0.12, 6 N HCl). 1H NMR (DMSO-d$_6$, 600 MHz), δ 3.85 (br, 1H), 5.00 (d 4.3, 1H), 7.25-7.35 (m, 5H).

β-Phenylserine was analyzed by reversed-phase HPLC (Cosmosil 5C18-MS, 4.6 × 250 mm; 2% MeCN containing 0.05% TFA; UV detection 210 nm; flow rate 1.0 mL/min). Retention times (min) in standards: erythro-β-phenyl-D,L-serine (8.0) and threo-β-phenyl-D,L-serine (9.8). Retention time (min) in β-phenylserine of 1: (8.0).

**HPLC Analysis of the Marfey Derivatives of β-Phenylserine.** β-Phenylserine was derivatized by above mentioned procedure and analyzed by reversed-phase HPLC (Cosmosil 5C18-MS, 4.6 × 250 mm; 35% MeCN containing 0.1% TFA; UV-detection 340 nm; flow rate 1.0 mL/min). Retention times in the derivatives of the standards (min): erythro-β-phenyl-L-serine (14.8), threo-β-phenyl-L-serine (15.6), erythro-β-phenyl-D-serine (19.2) and threo-β-phenyl-D-serine (24.8). Retention time in the derivatives of β-phenylserine of 1 (min): 19.2.

**β-Keto Ester Formation.** To a solution of 200 mg of Boc L-Trp (For) in 5 mL of dried THF was added N,N'-carbonyldiimidazole (130 mg). The resulting mixture was stirred for 1 h at 0 °C followed by at room temperature for 3 h. Distilled hydrogen ethyl malonate (132 mg; prepared from the potassium salt by treatment of an aqueous solution with excess of
concentrated hydrochloric acid at 0 °C and extraction of the free acid with ether) dissolved in 3 mL of dried THF was reacted with isopropyl magnesium chloride (1 mL of a 2 M solution in THF) at 0 °C for 30 min and then at room temperature for 45 min and at 45 °C for 30 min under argon. This solution of the magnesium enolate was added dropwise with stirring via cannulae to the cooled imidazole solution. The resulting mixture, after warming to room temperature, was stirred for 3 h and then poured into ice-cold 1 N HCl. The aqueous phase was extracted with EtOAc, and the combined organic extracts were washed with saturated NaCl, dried over Na₂SO₄, and concentrated to give the crude β-keto esters as oils. These were purified by column chromatography on silica gel (Kieselgel 60; 3 × 10 cm) using n-hexane/EtOAc as eluant to yield (4S)-4-[(tert-butyloxycarbonyl)amino]-5-[1-(formyl)indolyl]-3-oxopentanoic acid ethyl ester (240 mg; 99%). (4R)-4-[(tert-butyloxycarbonyl)amino]-5-[1-(formyl)indolyl]-3-oxopentanoic acid ethyl ester was obtained from 200 mg of Boc D-Trp (For) in 77% yield.

(4S)-4-[(tert-butyloxycarbonyl)amino]-5-[1-(formyl)indolyl]-3-oxopentanoic acid ethyl ester: [α]D²² -46.7° (c 0.5, MeOH); ¹H NMR (CDCl₃; 600 MHz) δ 1.23 (t, 7.0, 3 H, CH₂CH₃), 1.39 (s, 9 H), 3.10 (dd, 15.1, 6.6, 1H, CH₂Ar), 3.30 (dd, 15.1, 5.7, 1H, CH₂Ar), 3.52 (d, 16.0, 2 H, COCH₂), 4.14 (q, 7.0, 2 H, CH₂CH₃), 4.70 (br, 1 H, CH), 5.10 (d, 8.1, 1 H, NH), 7.19 (s, 1 H, ind. H), 7.33-7.40 (m, 2 H, ind. H), 8.37 (d, 6.6, 1 H, ind. H), 9.00 (s, 1 H, CHO).

(4R)-4-[(tert-butyloxycarbonyl)amino]-5-[1-(formyl)indolyl]-3-oxopentanoic acid ethyl ester: [α]D²² +45.0° (c 0.7, MeOH); ¹H NMR (CDCl₃; 600 MHz) δ 1.23 (t, 7.0, 3 H, CH₂CH₃), 1.39 (s, 9 H), 3.10 (dd, 15.1, 6.6, 1H, CH₂Ar), 3.30 (dd, 15.1, 5.7, 1H, CH₂Ar), 3.52 (d, 16.0, 2 H, COCH₂), 4.14 (q, 7.0, 2 H, CH₂CH₃), 4.70 (br, 1 H, CH), 5.10 (d, 8.1, 1 H, NH), 7.19 (s, 1 H, ind. H), 7.30-7.40 (m, 2 H, ind. H), 8.37 (d, 7.7, 1 H, ind. H), 9.03 (s, 1 H, CHO).

Reduction of β-Keto Ester. To a stirred solution of β-keto ester (50 mg) in 3 mL of dried THF at -20 °C was added NaBH₄ (2 mol equiv.). The reaction mixture was further stirred for 1.5 - 3 h. The reaction was quenched by pouring the mixture into ice-cold 1 N HCl covered with EtOAc. The aqueous phase was extracted three times with EtOAc, and the combined organic layers were washed with saturated NaCl, dried over Na₂SO₄ and MgSO₄, concentrated
and subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm, 70% MeOH, flow-rate 2.0 mL/min, UV-detection 210 nm) to yield (3S,4S)- and (3S,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester (3S,4S: 41%, 3S,4S: 25%) with high enantiomeric purity (99% ee), respectively. (3S,4R)- and (3R,4R)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester were also obtained from 50 mg of β-keto ester in 39% and 26% yield with high enantiomeric purity (96% ee), respectively.

(3S,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester. $^1$H NMR (CDCl$_3$; 600 MHz) $\delta$ 1.22 (t, 7.0, 3 H, CH$_2$CH$_3$), 1.42 (s, 9 H), 2.36 (brd, 17.1, 1 H, COCH$_2$), 2.59 (dd, 17.1, 10.3, 1 H, COCH$_2$), 3.02 (brd, 5.6, 2 H, CH$_2$Ar), 3.86 (br, 1 H, CH), 4.04 (brd, 9.8, 1 H, CHO), 5.02 (d, 9.4, 1 H, NH), 7.19 (s, 1 H, ind.), 7.33-7.36 (m, 2 H, ind.), 7.69 (d, 7.3, 1 H, ind.), 8.37 (d, 6.8, 1 H, ind.), 9.00 (s, 1 H, CHO).

(3R,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester. $^1$H NMR (CDCl$_3$; 600 MHz) $\delta$ 1.25 (t, 7.0, 3 H, CH$_2$CH$_3$), 1.35 (s, 9 H), 2.54 (dd, 16.2, 9.0, 1 H, COCH$_2$), 2.61 (brd, 16.2, 1 H, COCH$_2$), 2.96 (m, 1 H, CH$_2$Ar), 3.08 (brd, 14.5, 1 H, CH$_2$Ar), 3.95 (br, 1 H, CH), 3.99 (br, 1 H, CHO), 4.60 (br, 1 H, NH), 7.19 (s, 1 H, ind.), 7.32-7.36 (m, 2 H, ind.), 7.57 (d, 7.7, 1 H, ind.), 8.37 (d, 7.7, 1 H, ind.), 9.03 (s, 1 H, CHO).

($R$,$S$)-MTPA esterification. A solution of (3R,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester (10.0 mg) in dried pyridine (250 μL) was added with excess (S)-MTPA chloride, and the mixture was stirred at room temperature for 24 h under argon. Water was added to the reaction mixture, and the solvent was removed by lyophylization. The residue was subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 80% MeOH; UV-detection at 210 nm; flow rate 2.0 mL/min) to yield O-(R)-MTPA-(3R,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester (40-74%). O-(S)-MTPA-(3R,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester was obtained from 10 mg of (3R,4S)-4-
[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester in 40-75% yield.

**O-(S)-MTPA-(3R,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester.** $^1$H NMR (CDCl$_3$; 600 MHz) δ 1.220 (t, 7.3, 3 H, CH$_2$CH$_3$), 1.310 (s, 9 H), 2.555 (br, 1 H, CH$_2$Ar), 2.735 (d, 5.5, 2 H, COCH$_2$), 2.805 (m, 1 H, CH$_2$Ar), 3.583 (s, 3 H, OMe), 4.125 (m, 2H, CH$_2$CH$_3$), 4.220 (br, 1 H, CH), 4.345 (br, 1 H, NH), 5.660 (br, 1 H, CHO, CHOH), 7.065 (s, 1 H, ind.), 7.292 (br, 1 H, phenyl), 7.350 (br, 1 H, ind.), 7.398 (br, 2 H, phenyl), 7.403 (m, 1 H, ind.), 7.545 (m, 1 H, ind.), 7.582 (m, 2 H, phenyl), 8.348 (d, 7.4, 1 H, ind.), 8.990 (br, 1 H, CHO).

**O-(R)-MTPA-(3R,4S)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester.** $^1$H NMR (CDCl$_3$; 600 MHz) δ 1.190 (t, 7.3, 3 H, CH$_2$CH$_3$), 1.306 (s, 9 H), 2.715 (m, 1 H, CH$_2$Ar), 2.700 (d, 5.5, 2 H, COCH$_2$), 2.945 (dd, 15.4, 4.3, 1 H, CH$_2$Ar), 3.870 (s, 3 H, OMe), 4.070 (m, 2 H, CH$_2$CH$_3$), 4.335 (br, 1 H, CH), 4.460 (br, 1 H, NH), 5.605 (br, 1 H, CHO), 7.195 (s, 1 H, ind.), 7.310 (t, 7.7, 1 H, phenyl), 7.360 (m, 1 H, ind.), 7.400 (m, 2 H, phenyl), 7.410 (m, 1 H, ind.), 7.545 (d, 7.7, 2 H, phenyl), 7.550 (d, 7.6, 1 H, ind.), 8.365 (d, 7.3, 1 H, ind.), 9.150 (br, 1 H, CHO).

**O-(S)-MTPA-(3S,4R)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester.** $^1$H NMR (CDCl$_3$; 600 MHz) δ 1.192 (t, 7.3, 3 H, CH$_2$CH$_3$), 1.308 (s, 9 H), 2.702 (d, 5.5, 2 H, COCH$_2$), 2.709 (br, 2 H, CH$_2$Ar), 2.945 (dd, 15.4, 4.3, 1 H, CH$_2$Ar), 3.490 (s, 3 H, OMe), 4.075 (m, 2 H, CH$_2$CH$_3$), 4.340 (br, 1 H, CH), 4.460 (br, 1 H, NH), 5.610 (m, 1 H, CHO), 7.195 (s, 1 H, ind.), 7.308 (t, 7.3, 1 H, phenyl), 7.367 (t, 8.6, 1 H, ind.), 7.400 (m, 2 H, phenyl), 7.412 (m, 1 H, ind.), 7.548 (m, 1 H, phenyl), 7.580 (m, 2 H, ind.), 8.366 (d, 7.7, 1 H, ind.), 9.015 (br, 1 H, CHO).

**O-(R)-MTPA-(3S,4R)-4-[(tert-butyloxy carbonyl)amino]-5-[1-(formyl)indolyl]-3-hydroxypentanoic acid ethyl ester.** $^1$H NMR (CDCl$_3$; 600 MHz) δ 1.221 (t, 7.3, 3 H, CH$_2$CH$_3$), 1.308 (s, 9 H), 2.557 (m, 1 H, CH$_2$Ar), 2.737 (d, 6.4, 2 H, COCH$_2$), 2.805 (dd, 15.4, 3.6, 1H, CH$_2$Ar), 3.582 (s, 3 H, OMe), 4.125 (m, 2 H, CH$_2$CH$_3$), 4.217 (m, 1 H, CH), 4.278 (br, 1 H, NH), 5.662 (br, 1 H, CHO), 7.063 (s, 1 H, ind.), 7.291 (br, 1 H, CH).
phenyl), 7.347 (m, 1 H, ind.), 7.397 (m, 2 H, phenyl), 7.407 (m, 1 H, ind.), 7.562 (m, 2 H, phenyl), 7.582 (m, 1 H, ind.), 8.346 (d, 8.1, 1 H, ind.), 8.992 (br, 1 H, CHO).

Saponification and Acid Hydrolysis of hydroxy acid. Each of hydroxy acids (10.0 mg) was dissolved in 1.1 mL of THF/1 N NaOH (10:1) and stirred at room temperature for 3 h. After removal of THF by evaporation, 1 N HCl (0.1 mL) was added to the reaction mixture and extracted three times with EtOAc, and the combined organic extracts were washed with saturated NaCl, dried over Na₂SO₄ and MgSO₄, and concentrated. This reaction mixture, which was used without further purification, was dissolved in 1 mL of HCOOH and one drop of H₂O and stirred at room temperature for 1.5 h. After removal of the solvent by evaporation and lyophylization, the reaction mixture was subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 30% MeOH; UV detection at 210 nm; flow rate 2.0 mL/min) to yield 4-amino-3-hydroxy-5-indolypentanoic acid [(3R,4S) Ahipa 2.7 mg; 45%), (3S,4S) Ahipa (2.0 mg; 33%), (3S,4R) Ahipa (2.3 mg; 38%) and (3R,4R) Ahipa (2.2 mg; 37%)].

Isolation of Ahipa from 1. Compound 1 (20.0 mg) was dissolved in 5 mL of 6 N HCl containing 1% phenol and heated at 110°C for 16 h. After removal of the solvent by evaporation, the acid hydrolysates were subjected to reversed-phase HPLC (Cosmosil 5C18-MS, 10 × 250 mm; 20-60% MeOH; UV detection at 210 nm; flow rate 2.0 mL/min) to yield Ahipa (1.7 mg).

Anti-green algal assay. Chlamydomonas neglecta (NIES-439) was obtained from the NIES-collection and grown in test tube containing 5 mL of C medium [Ca(NO₃)₂·4H₂O 15 mg, KNO₃ 10 mg, β-Na₂glycerophosphate 5 mg, MgSO₄·7H₂O 4 mg, Vitamin B₁₂ 0.01 μg, Biotin 0.01 μg, Thiamine HCl 1 μg, PIV metals (FeCl₃·6H₂O 19.6 mg, MnCl₂·4H₂O 3.6 mg, ZnSO₄·7H₂O 2.2 mg, CoCl₂·6H₂O 0.4 mg, Na₂MoO₄·2H₂O 0.25 mg, Na₂EDTA·2H₂O 100 mg, Distilled water 100 mL) 0.3 mL, Tris (hydroxymethyl) aminomethane 50 mg, Distilled water 99.7 mL, pH 7.5] at 25°C under illumination of 90 μEm⁻²s⁻¹ on a 12L:12D cycle. After 9 days-incubation, a MeOH solution (1-10 μL) of kasumigamide was added to test tube and further incubated for 2 weeks.
\textsuperscript{1}H NMR Spectrum of Kasumigamide in DMSO-$d_6$ at 300K
1H-1H COSY Spectrum of Kasumigamide in DMSO-$d_6$ at 300K
HMBC Spectrum of Kasumigamide in DMSO-\textit{d}_6 at 300K