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Microginins 299-A and -B, Leucine Aminopeptidase Inhibitors from the Cyanobacterium *Microcystis aeruginosa* (NIES-299)

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Abstract: Microginins 299-A (**1**) and -B (**2**) have been isolated from the cyanobacterium *Microcystis aeruginosa* (NIES-299). Their structures were determined by two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C NMR correlation experiments and confirmed by mass spectral and amino acid analyses. Their absolute stereochemistries were deduced by a combination of spectral and chemical studies. **1** and **2** inhibited leucine aminopeptidase with IC$_{50}$'s of 4.6 and 6.5 μg/mL, respectively.
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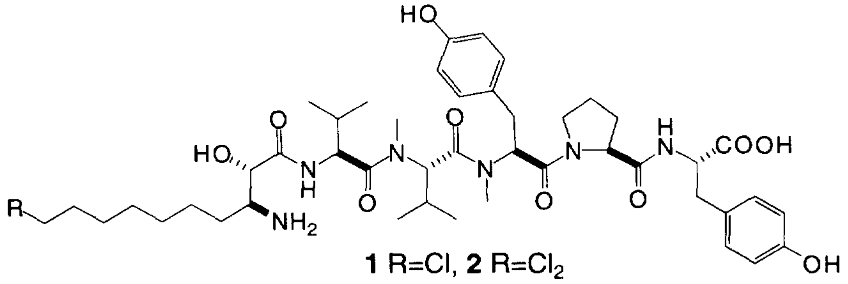
Microcystis aeruginosa produces some unique and interesting bioactive peptides.[1] Noteworthy examples are hepatotoxic cyclicpeptides microcystins[2] and nontoxic cyclic depsipeptides micropeptins[3] and microviridins,[4] which inhibit serine proteases such as plasmin, trypsin, elastase or chymotrypsin. As part of continuing search for protease inhibitors from cyanobacteria, we have described a novel linear pentapeptide microginin, which inhibits angiotensin-converting enzymes from *M. aeruginosa* (NIES-100).[5] We report here the isolation and structure elucidation of congeners to microginin, microginins 299-A (**1**) and -B (**2**) (Fig. 1), which inhibit leucine aminopeptidase, from *M. aeruginosa* (NIES-299).

M. aeruginosa (NIES-299)[6] was isolated from a bloom in Lake Kasumigaura and mass-cultured in our laboratory as previously described.[7] The 80% methanol extract of freeze-dried alga was partitioned between water and diethyl ether. The aqueous layer was further extracted with *n*-butanol and fractionated by ODS flash column chromatography (20-100% MeOH elution) followed by reversed-phase HPLC, using 0.05% TFA in 35% MeCN to yield microginins 299-A (**1**, 70 mg) and -B (**2**, 144 mg) as colorless amorphous powders.



1 R=Cl, **2** R=Cl$_2$

The pseudomolecular ion at *m/z* 885/887 [M - H]$^-$ in the negative FABMS using glycerol as matrix of microginin 299-A (**1**) revealed the presence of one chlorine atom. The molecular formula of microginin 299-A (**1**) was established as $C_{45}H_{67}N_6O_{10}Cl$ by the high resolution FABMS and NMR spectral data (Table 1). It's peptidic nature was suggested by the ^1H and ^{13}C NMR spectra of **1** (Table 1), and the amino acid analysis of the hydrolyzate gave Pro, Val and Tyr. The ^1H-^1H COSY and HMQC[8] spectra also indicated the presence of those amino acids Pro, Val and Tyr residues. Two *N*-methyl amino acids *N*-Me Val and *N*-Me Tyr residues' were assigned from the ^1H-^1H COSY, HMQC and HMBC[9] spectra, which showed correlations from *N*-methyl protons (*N*-Me Val δ 2.37, *N*-Me Tyr δ 2.77) to α-carbons (*N*-Me Val δ 57.9, *N*-Me Tyr δ 54.4). The structure of 10-chloro-3-amino-2-hydroxydecanoic acid (chloro Ahda), a novel β-amino acid, containing chlorine was deduced as follows. In the ^1H-^1H COSY spectrum, the connectivities from H-2 (δ 4.23) to H-4 (δ 1.34, 1.49) and from H-7 (δ 1.22) to H-10 (δ 3.60) were determined, and the HMBC correlations between H-4 and C-6 (δ 28.7), H-6 (δ 1.20) and C-5 (δ 24.6), H-7 and C-6 and H-8 (δ 1.345) and C-6 connected H-4 to H-7 (Fig. 2).

The sequence of the six amino acids was determined by interpretation of FABMS fragmentations and the HMBC and ROESY experiments. The positive FABMS using glycerol as matrix of microginin 299-A (**1**) revealed the sequence from chloro Ahda to *N*-Me Tyr (Fig. 1). The HMBC correlations obtained from α-protons and amide protons to carbonyl carbons and from *N*-methyl protons to carbonyl carbons and α-carbons supported its sequence (Fig. 2, Table 1). The ROESY correlation between the α-proton *N*-Me Tyr and the δ-proton of Pro connected *N*-Me Tyr to Pro (Fig. 2).

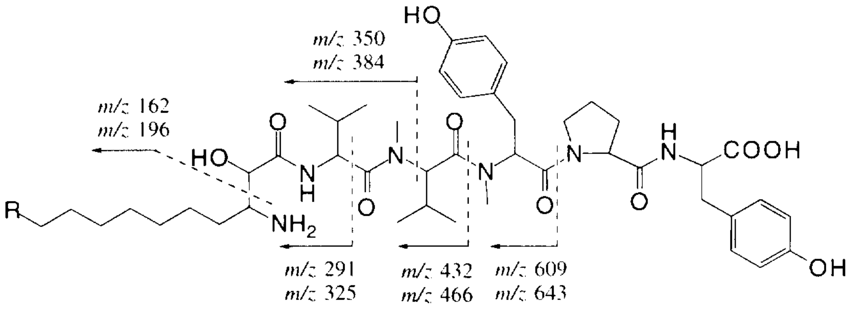


Fig. 1. Positive FABMS fragmentations of **1** (above) and **2** (below)

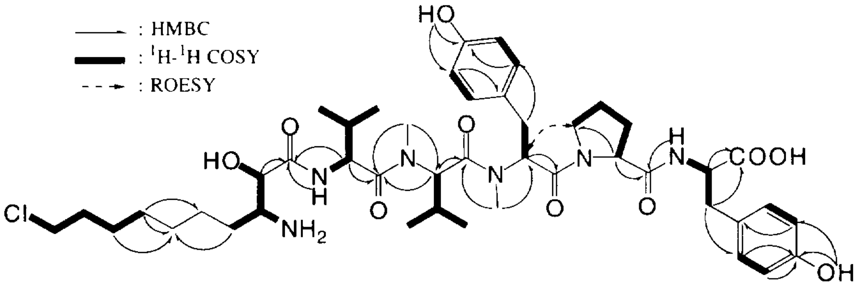


Fig. 2. ^1H-^1H COSY, HMBC and ROESY correlations of microginin 299-A (**1**)

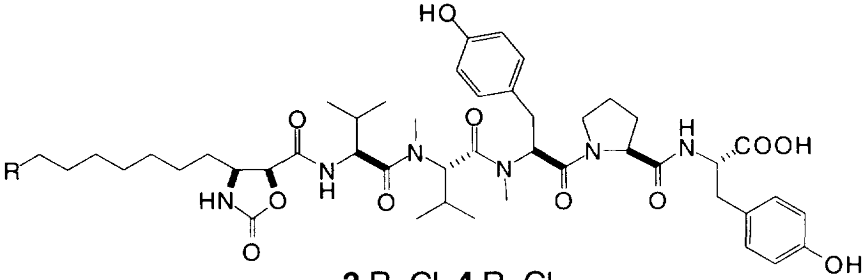
Table 1. ^{1}H and ^{13}C NMR Data for Microginin 299-A (**1**) in DMSO-d_6

Position		^{1}H	*J* (Hz)	^{13}C		HMBC (^{1}H)
Chloro Ahda	1			170.2	(s)	Chloro Ahda 2, Val 2, NH
	2	4.23	(br)	70.6	(d)	
	3	3.37	(m)	53.0	(d)	Chloro Ahda 2, 4, 2-OH
	4	1.34	(m)	26.9	(t)	
		1.49	(m)			
	5	1.19	(m)	24.6	(t)	Chloro Ahda 7
		1.350	(m)			
	6	1.20	(m)	28.7	(t)	Chloro Ahda 4, 7, 8
	7	1.22	(m)	27.8	(t)	Chloro Ahda 5, 9
	8	1.345	(m)	26.1	(t)	Chloro Ahda 7, 9, 10
	9	1.68	(tt, 7.5, 6.6)	32.0	(t)	Chloro Ahda 8, 10
	10	3.60	(t, 6.6)	45.3	(t)	Chloro Ahda 8, 9
	2-OH	6.48	(br)			
	3-NH_2	7.96	(br)			
Val	1			170.9	(s)	Val 2, *N*-Me Val 2, *N*-Me
	2	4.47	(dd, 8.8, 5.5)	53.2	(d)	Val 4, 4', NH
	3	1.75	(qqd, 7.0, 6.6, 5.5)	24.1	(d)	
	4	0.75	(d, 6.6)	16.7	(q)	Val 2, 3, 4'
	4'	0.83	(d, 7.0)	19.9	(q)	Val 2, 3, 4
	NH	7.77	(8.8)			
N-Me Val	1			168.9	(s)	*N*-Me Val 2, *N*-Me Tyr *N*-Me
	2	4.88	(d, 10.6)	57.9	(d)	*N*-Me Val 3, 4, 4', *N*-Me
	3	2.10	(dqq, 10.6, 6.6, 6.6)	26.3	(d)	*N*-Me Val 2, 4, 4'
	4	0.60	(d, 6.6)	17.7	(q)	*N*-Me Val 2, 4'
	4'	0.75	(d, 6.6)	19.5	(q)	*N*-Me Val 2, 3, 4
	N-Me	2.37	(s)	29.2	(q)	Val 2
N-Me Tyr	1			168.3	(s)	*N*-Me Tyr 2, *N*-Me
	2	5.58	(dd, 11.0, 4.8)	54.4	(d)	*N*-Me Tyr 3, *N*-Me
	3	2.81	(dd, 14.6, 11.0)	33.0	(t)	*N*-Me Tyr 2, 5, 9
		2.90	(dd, 14.6, 4.8)			
	4			126.9	(s)	*N*-Me Tyr 3, 6, 8
	5, 9	6.98	(d, 8.4)	129.8	(d)	*N*-Me Tyr 3, 5, 9
	6, 8	6.64	(d, 8.4)	114.9	(d)	*N*-Me Tyr 6, 8, 7-OH
	7			155.9	(s)	*N*-Me Tyr 5, 6, 8, 9, 7-OH
	N-Me	2.77	(s)	29.9	(q)	*N*-Me Tyr 2
	7-OH	9.21	(br)			
Pro	1			171.3	(s)	Pro 2, Tyr NH
	2	4.32	(dd, 8.3, 2.7)	59.5	(d)	
	3	1.79	(m)	28.8	(t)	
		1.97	(m)			
	4	1.79	(m)	29.7	(t)	
	5	3.40	(m)	46.8	(t)	Pro 2, 3, 4
		3.55	(m)			
Tyr	1			172.9	(s)	Tyr 2, 3
	2	4.31	(ddd, 8.1, 8.0, 5.5)	53.8	(d)	Tyr 3, NH
	3	2.79	(m)	35.9	(t)	
		2.91	(m)			
	4			127.4	(s)	Tyr 6, 8
	5, 9	7.02	(d, 8.4)	130.1	(d)	Tyr 3, 5, 9
	6, 8	6.65	(d, 8.4)	114.9	(d)	Tyr 6, 8, 7-OH
	7			155.9	(s)	Tyr 5, 6, 8, 9, 7-OH
	NH	7.93	(d, 8.1)			
	7-OH	9.21	(br)			

Table 2. ^{1}H and ^{13}C NMR Data for Microginin 299-B (**2**) in DMSO-d_6

Position		^{1}H	*J* (Hz)	^{13}C		HMBC (^{1}H)
Dichloro Ahda	1			170.2	(s)	Dichloro Ahda 2, Val 2, NH
	2	4.25	(brd, 2.9)	70.6	(d)	
	3	3.37	(m)	52.9	(d)	Dichloro Ahda 2, 3-NH_2
	4	1.33	(m)	26.9	(t)	Dichloro Ahda 2
		1.49	(m)			
	5	1.19	(m)	24.6	(t)	Dichloro Ahda 7
		1.35	(m)			
	6	1.20	(m)	28.6	(t)	Dichloro Ahda 7, 8
	7	1.26	(m)	27.5	(t)	Dichloro Ahda 8, 9
	8	1.45	(m)	25.2	(t)	Dichloro Ahda 9, 10
	9	2.12	(m)	42.8	(t)	Dichloro Ahda 8, 10
	10	6.28	(t, 5.9)	74.8	(t)	Dichloro Ahda 8, 9
	2-OH	6.48	(br)			
	3-NH_2	7.95	(br)			
Val	1			170.9	(s)	Val 2, *N*-Me Val 2, *N*-Me
	2	4.47	(dd, 8.8, 5.1)	53.2	(d)	Val 3, 4, 4', NH
	3	1.75	(qqd, 7.0, 7.0, 5.1)	24.1	(d)	
	4	0.75	(d, 7.0)	16.7	(q)	Val 2, 3, 4'
	4'	0.83	(d, 7.0)	19.9	(q)	Val 2, 3, 4
	NH	7.78	(d, 8.8)			
N-Me Val	1			168.9	(s)	*N*-Me Val 2, *N*-Me Tyr 2.*N*-Me
	2	4.88	(d, 10.6)	57.9	(d)	*N*-Me Val 3, 4, 4', *N*-Me
	3	2.10	(dqq, 10.6, 7.0, 7.0)	26.3	(d)	*N*-Me Val 2, 3, 4, 4'
	4	0.59	(d, 7.0)	17.7	(q)	*N*-Me Val 2, 3, 4'
	4'	0.75	(d, 7.0)	19.5	(q)	*N*-Me Val 2, 3, 4
	N-Me	2.37	(s)	29.2	(q)	Val 2
N-Me Tyr	1			168.3	(s)	*N*-Me Tyr 2
	2	5.59	(dd, 11.0, 5.1)	54.4	(d)	*N*-Me Tyr *N*-Me Tyr *N*-Me
	3	2.81	(dd, 14.3, 11.0)	33.0	(t)	*N*-Me Tyr 2, 5, 9
		2.90	(dd, 14.3, 5.1)			
	4			126.9	(s)	*N*-Me Tyr 3, 6, 8
	5, 9	6.98	(d, 8.1)	129.8	(d)	*N*-Me Tyr 3, 5, 9
	6, 8	6.64	(d, 8.1)	114.9	(d)	*N*-Me Tyr 6, 8
	7			155.9	(s)	*N*-Me Tyr 5, 6, 8, 9
	N-Me	2.77	(s)	29.9	(q)	*N*-Me Tyr 2
	7-OH	9.21	(br)			
Pro	1			171.3	(s)	Pro 2, 3, Tyr NH
	2	4.32	(dd, 8.7, 2.7)	59.5	(d)	
	3	1.79	(m)	28.8	(t)	
		1.97	(m)			
	4	1.79	(m)	29.7	(t)	Pro 2
	5	3.40	(m)	46.8	(t)	
		3.55	(m)			
Tyr	1			172.9	(s)	Tyr 2, 3
	2	4.31	(ddd, 8.4, 7.7, 5.5)	53.8	(d)	Tyr 3, NH
	3	2.79	(m)	35.9	(t)	Tyr 2, 5, 9
		2.91	(m)			
	4			127.4	(s)	Tyr 2, 3, 6, 8
	5, 9	7.02	(d, 8.4)	130.1	(d)	Tyr 3, 5, 9
	6, 8	6.65	(d, 8.4)	114.9	(d)	Tyr 6, 8, 7-OH
	7			155.9	(s)	Tyr 5, 6, 8, 9, 7-OH
	NH	7.93	(d, 7.7)			
	7-OH	9.21	(br)			

The stereochemistries of the usual amino acids and *N*-methyl amino acids were determined as L by HPLC analysis of the derivatives of the acid hydrolyzate with L- or D-Marfey's reagent.[10,11] The relative stereochemistry of chloro Ahda was determined by conversion to microginin 299-A oxazolidinone (**3**) using carbonyl diimidazole (CDI). The coupling constant of oxazolidinone ring protons ($J_{4,5}$ = 8.8) in **3** confirmed the *cis* stereochemistry.[12] The ^{1}H and ^{13}C NMR spectra and an optical rotation value of chloro Ahda isolated from the acid hydrolyzate indicated the *2S, 3S* stereochemistry.[13]



3 R=Cl, **4** R=Cl$_2$

The high resolution FABMS spectrum and NMR data established that microginin 299-B (**2**) had a molecular formula of $C_{45}H_{66}N_6O_{10}Cl_2$. The ^{1}H and ^{13}C NMR spectra of **2** resembled those of **1**, but an isotopic cluster at *m/z* 919/921 [M - H]$^-$ in the ratio 3:2, consistent with two chlorine atoms, was observed and the ^{1}H and ^{13}C chemical shifts shifted from chloro Ahda H-10 (δ 3.60) and C-10 (δ 45.3) to dichloro Ahda H-10 (δ 6.28) and C-10 (δ 74.8) (Table 2). Therefore, the Ahda moiety of **2** was determined to be 10-dichloro-3-amino-2-hydroxydecanoic acid (dichloro Ahda). The sequence (Fig 1, Table 2) and the stereochemistries of the ususal amino acids and *N*-methyl amino acids of **2** were also determined by the above-mentioned procedures. The relative stereochemistry of dichloro Ahda was determined by conversion to microginin 299-B oxazolidinone (**4**) using CDI. The coupling constant of oxazolidinone ring protons ($J_{4,5}$ = 9.0) in **4** confirmed the *cis* stereochemistry.[12] The similarity of ^{1}H and ^{13}C NMR spectra between dichloro Ahda in **2** and chloro Ahda in **1** indicated the *2S, 3S* stereochemistry for dichloro Ahda.[13]

Microginins 299-A (**1**) and B(**2**) inhibited leucine aminopeptidase with IC_{50}'s of 4.6 and 6.5 µg/mL, respectively. These peptides did not inhibit angiotensin-converting enzyme, papain, trypsin, thrombin, plasmin, chymotrypsin and elastase at 100 µg/mL.

Experimental Section

General Information. Ultraviolet spectra were measured on a Hitachi 330 spectrophotometer. Optical rotations were measured on a JASCO DIP-1000 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on JEOL JNM-A500 NMR spectrometer. 2D-NMR spectra were recorded on a JEOL JNM-A500 NMR spectrometer equipped with a VAXserver 4000-200 computer. The HMBC spectra were acquired with an evolution time of 60 ms. ROESY spectra were recorded with mixing time of 200 ms. FAB mass spectra, including high resolution mass measurements, were measured on a JEOL SX-102 mass spectrometer. Amino acid analyses were carried out with a Hitachi L-8500A amino acid analyzer.

Cultivation of Alga. Culture conditions were the same as used for producing kawaguchipeptin A.[14]

Isolation of Microginins 299-A and -B. Freeze-dried alga (91 g from 360 L of culture) was extracted with 80% MeOH (2 L × 3) and MeOH (2 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 (12.9 g), which was subjected to flash chromatography on ODS (YMC-GEL, 120Å, 10 cm × 12 cm) with aqueous MeOH followed by CH_2Cl_2. The 60% MeOH fraction was subjected to reversed-phase HPLC (Capcell pak C18 UG, 20 × 250 mm, UV-detection 210 nm, flow rate 6.0 mL/min) with 35% MeCN containing 0.05% TFA to yield **1** (70.3 mg) and **2** (144.1 mg).

Microginin 299-A (1). $[\alpha]_D$ -89.8° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 279 nm (ε 2950); ^{1}H and ^{13}C NMR see Table 1; HRFABMS *m/z* 885.4512 $[M - H]^-$ ($C_{45}H_{66}N_6O_{10}Cl$, Δ -1.7 mmu).

Microginin 299-B (2). $[\alpha]_D$ -51.4° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 278 nm (ε 2090); ^{1}H and ^{13}C NMR see Table 2; HRFABMS *m/z* 919.4164 $[M - H]^-$ ($C_{45}H_{65}N_6O_{10}Cl_2$, Δ +2.5 mmu).

Acid Hydrolysis. For amino acid analysis, 100 μg each of **1** and **2** in 0.5 mL of 6 N HCl was heated at 110 °C for 16 h. The reaction mixture was dried, dissolved in 0.6 mL of 0.02 N HCl and subjected to amino acid analysis. Retention times (min) in the amino acid analysis of **1** and **2**: Pro (31.97, 32.02), Val (40.66, 40.72), Tyr (50.21, 50.26).

HPLC Analysis of the Marfey Derivatives.[10] To the acid hydrolyzate of a 100 μg portion of the peptides, 50 μL of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in acetone (L-FDAA) (10 mg/mL) and 100 μL of 1 M $NaHCO_3$ 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 MS, (4.6 × 250 mm); gradient elution from H_2O/TFA (100:0.1) to MeCN/H_2O/TFA (60:40:0.1) in 60 min; UV-detection 340 nm; flow rate 1.0 mL/min). Retention times (min) of the amino acid residues: L-Pro (41.8), D-Pro (43.0), L-Val (47.4), D-Val (51.4), L-Tyr (57.4), D-Tyr (60.6), *N*-Me L-Val (50.4), *N*-Me D-Val (52.8). Retention times (min) of the amino acid derivatives of **1** and **2**: L-Pro (42.0, 42.0), L-Val (47.2, 47.2), L-Tyr (57.6, 57.4), *N*-Me L-Val (50.4, 50.2).

N-Me L-Tyr was derivatized with D- and L-FDAA as described above, respectively.[11] The derivatives were analyzed by reversed-phase ODS-HPLC: column Cosmosil MS (4.6 × 250 mm); mobile phase MeCN/H_2O/TFA (40: 60: 0.1); UV (340 nm). Retention times of standards (min): *N*-Me L-Tyr-(*N*, *O*)-L-FDAA (25.2), *N*-Me L-Tyr-(*N*, *O*)-D-FDAA (26.8). Retention times (min) of *N*-MeTyr-(*N*, *O*)-L-FDAA in the acid hydrolyzate of **1** and **2**: 25.2 and 25.0.

Conversion to 3 using CDI from 1. A solution of **1** (2.5 mg, 2.8 μmol) in dried THF (1.0 mL) was treated with solid carbonyldiimidazole (1.0 mg, 6.1 μmol) and stirred at room temperature for 5 h under argon. The solvent was removed by evaporation and freeze-dried. The reaction mixture was subjected to reversed-phase HPLC (Cosmosil MS, 10 × 250 mm; 37-57% MeCN containing 0.05% TFA; flow rate, 2.0 mL/min; UV detection at 210 nm) to yield microginin 299-A oxazolidinone **3** (1.0 mg, 39%); HRFABMS *m/z* 913.4403 $[M + H]^+$ ($C_{46}H_{66}N_6O_{11}Cl$, Δ -7.5 mmu); ^{1}H and ^{13}C NMR (DMSO-d_6), 5-carboxy-(7'-chloro)-4-heptyl-2-oxazolidinone, NH (δ_H 7.93, br), 4 (δ_H 3.87, brdd 9.2, 8.8, δ_C 53.8), 5 (δ_H 5.03, d 8.8, δ_C 76.0), 1' (δ_H 1.10, m and 1.36, m, δ_C 30.5), 2' (δ_H 1.14, m and 1.35, m, δ_C 25.3), 3' (δ_H 1.15, m, δ_C 28.6), 4' (δ_H 1.23, m, δ_C 27.8), 5' (δ_H 1.33, m, δ_C 26.1), 6' (δ_H 1.69, m, δ_C 32.0), 7' (δ_H 3.59, t 6.6, δ_C 45.2), Val NH (δ_H 8.16, d 8.8), 2 (δ_H 4.49, dd 8.8, 5.9, δ_C 53.2), 3 (δ_H 1.75, m, δ_C 24.1), 4 (δ_H 0.75, d 6.6, δ_C 19.5), 4' (δ_H 0.81, d 6.6, δ_C 19.7), *N*-Me Val *N*-Me (δ_H 2.37, s, δ_C 29.2), 2 (δ_H 4.88, dd 10.4, 3.9, δ_C 57.9), 3 (δ_H

2.10, m, δ_C 26.3), 4 (δ_H 0.60, d 7.0, δ_C 17.7), 4' (δ_H 0.75, d 7.7, δ_C 17.0), *N*-Me Tyr *N*-Me (δ_H 2.78, s, δ_C 29.9), 2 (δ_H 5.57, dd 11.8, 4.6, δ_C 54.4), 3 (δ_H 2.79, m and 2.88, m, δ_C 33.0), 5 and 9 (δ_H 6.97, d 8.1, δ_C 129.8), 6, 8 (δ_H 6.62, d 8.1, δ_C 114.9), Pro 2 (δ_H 4.32, m, δ_C 59.5), 3 (δ_H 1.76, m, 1.96, m, δ_C 27.8), 4 (δ_H 1.76, m, δ_C 29.4), 5 (δ_H 3.37, m and 3.52, m, δ_C 46.8), Tyr NH (δ_H 7.93, d 8.4), 2 (δ_H 4.30, m, δ_C 53.7), 3 (δ_H 2.76, m and 2.91, m, δ_C 36.0), 5 and 9 (δ_H 6.98, d 8.1, δ_C 129.8), 6 and 8 (δ_H 6.63, d 8.1, δ_C 114.9).

Conversion to 4 using CDI from 2. Compound **2** (2.8 mg, 3.0 μmol) in dried THF (1.0 mL) was treated with solid CDI (1.0 mg, 6.1 μmol) and separated as above to yield microginin 299-B oxazolidinone **4** (1.8 mg, 62%); HRFABMS *m/z* 947.4084 $[M + H]^+$ ($C_{46}H_{65}N_6O_{11}Cl_2$, Δ -0.4 mmu); ^1H and ^{13}C NMR (DMSO-d_6), 5-carboxy-(7'-dichloro)-4-heptyl-2-oxazolidinone, NH (δ_H 7.93, br), 4 (δ_H 3.87, brdd 9.0, 8.6, δ_C 53.8), 5 (δ_H 5.03, d 9.0, δ_C 76.0), 1' (δ_H 1.10, m and 1.35, m, δ_C 30.5), 2' (δ_H 1.13, m and 1.35, m, δ_C 25.4), 3' (δ_H 1.15, m, δ_C 28.7), 4' (δ_H 1.26, m, δ_C 27.5), 5' (δ_H 1.43, m, δ_C 25.2), 6' (δ_H 2.12, m, δ_C 42.8), 7' (δ_H 6.28, t 6.0, δ_C 74.8), Val NH (δ_H 8.17, d 8.6), 2 (δ_H 4.49, dd 8.6, 5.6, δ_C 53.2), 3 (δ_H 1.75, m, δ_C 24.1), 4 (δ_H 0.75, d 6.6, δ_C 19.5), 4' (δ_H 0.81, d 6.6, δ_C 19.7), *N*-Me Val *N*-Me (δ_H 2.37, s, δ_C 29.2), 2 (δ_H 4.88, dd 10.4, 3.7, δ_C 57.9), 3 (δ_H 2.10, m, δ_C 26.3), 4 (δ_H 0.60, d 7.0, δ_C 17.7), 4' (δ_H 0.75, d 7.7, δ_C 17.0), *N*-Me Tyr *N*-Me (δ_H 2.78, s, δ_C 29.9), 2 (δ_H 5.57, dd 11.8, 4.6, δ_C 54.4), 3 (δ_H 2.79, m and 2.88, m, δ_C 33.0), 5 and 9 (δ_H 6.97, d 8.5, δ_C 129.8), 6 and 8 (δ_H 6.62, d 8.5, δ_C 114.9), Pro 2 (δ_H 4.32, m, δ_C 59.5), 3 (δ_H 1.76, m and 1.96, m, δ_C 27.8), 4 (δ_H 1.76, m, δ_C 29.4), 5 (δ_H 3.37, m and 3.52, m, δ_C 46.8), Tyr NH (δ_H 7.93, d 8.4), 2 (δ_H 4.29, ddd 8.6, 8.3, 5.6, δ_C 53.7), 3 (δ_H 2.76, dd 13.7, 8.3 and 2.91, dd 13.7, 5.6, δ_C 36.0), 5 and 9 (δ_H 6.98, d 7.7, δ_C 129.8), 6 and 8 (δ_H 6.63, d 7.7, δ_C 114.9).

Isolation of 10-Chloro-3-amino-2-hydroxydecanoic acid (chloro Ahda). Compound **1** (8.0 mg) was dissolved in 5 mL of 6 N HCl and heated at 110 °C for 16 h. The reaction mixture was removed the solvent by evaporation, freeze-dried, and subjected to reversed-phase HPLC (Cosmosil MS, 10 × 250 mm, 0-60%MeOH; flow rate, 2.0 mL/min; UV detection at 210 nm) to yield chloro Ahda (700 μg); $[\alpha]_D^{23}$ -14.2° (*c* 0.05, MeOH); HRFABMS, *m/z* 238.1195 $[M + H]^+$ ($C_{10}H_{21}NO_3Cl$, Δ -1.5 mmu); ^1H and ^{13}C NMR (DMSO-d_6), 2 (δ_H 3.37, brd 9.0, δ_C 69.8), 3 (δ_H 2.85, m, δ_C 53.5), 4 (δ_H 1.43, m and 1.69, m, δ_C 29.8), 5 (δ_H 1.27, m and 1.37, m, δ_C 24.6), 6 (δ_H 1.28, m, δ_C 28.9), 7 (δ_H 1.35, m, δ_C 27.9), 8 (δ_H 1.34, m and 1.38, m, δ_C 29.3), 9 (δ_H 1.70, m, δ_C 32.0), 10 (δ_H 3.61, t 6.4, δ_C 45.3).

Leucine Aminopeptidase Inhibitory Assay. Leucine aminopeptidase inhibitory activity was determined by the modification of the method of Umezawa *et al.*[15] The reaction mixture containing 20 μL of L-leucine *p*-nitroanilide (2 mM in 0.1 M Tris-HCl buffer (pH 7.0)), 50 μL of 0.1 M Tris-HCl buffer (pH 7.0) and 20 μL of test solution was added to each microtiter plate well. The mixture was incubated at 37 °C for 3 min, and 10 μL of leucine aminopeptidase (5.5 μL in 100 mL 3.5 M $(NH_4)_2SO_4$) was added. After incubation at 37 °C for 30 min, the enzymatic reaction was terminated by the addition of 100 μL of 1 M acetate buffer solution (pH 4.2). The absorbance of the well was read at 410 nm.

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