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Kawaguchipeptin A, a Novel Cyclic Undecapeptide from Cyanobacterium *Microcystis aeruginosa* (NIES-88)

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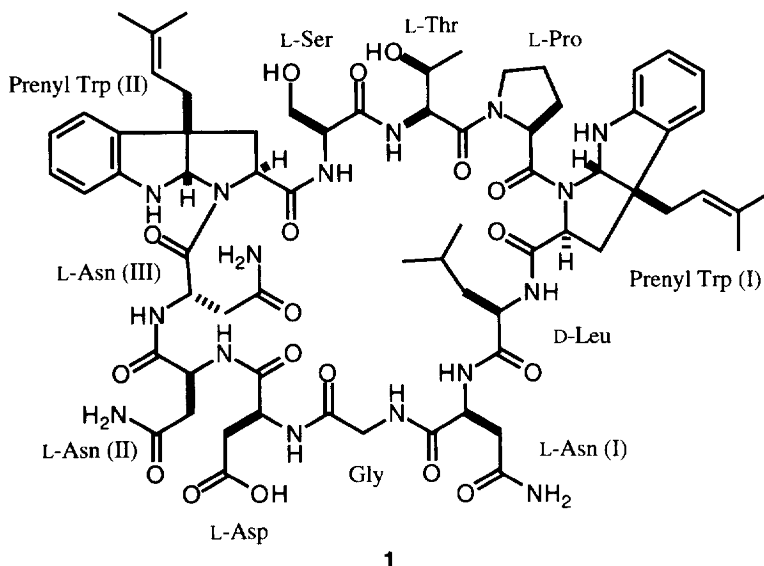
Abstract: Kawaguchipeptin A (**1**), a novel cyclic undecapeptide, was isolated from cyanobacterium, *Microcystis aeruginosa* (NIES-88) which produces microcystins. Its structure was determined by two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C NMR correlation experiments and confirmed by mass spectral and amino acid analyses. Its absolute stereochemistry was deduced by a combination of spectral and chemical studies. Copyright © 1996 Elsevier Science Ltd

Microcystis aeruginosa has been extensively studied from the environmental, toxicological, biological, and chemical points of view because they are responsible for water blooms that frequently produce potent hepatotoxins. These hepatotoxic cyclic peptides, microcystins, have been identified in a number of strains of *Microcystis* and some other cyanobacteria.[1] Recently, these peptides have been found to be a highly effective inhibitors of protein phosphatases 1 and 2A.[2] Toxic *M. aeruginosa* strains have been also shown to be a rich source of peptides besides microcystins such as aeruginopeptins,[3] microcystilide A,[4] cyanopeptolins,[5] aeruginosin 298-A[6] and microviridins.[7] We report here the isolation and structure elucidation of an unusual cyclic undecapeptide, kawaguchipeptin A (**1**), from *M. aeruginosa* (NIES-88) which produces microcystins.[8]

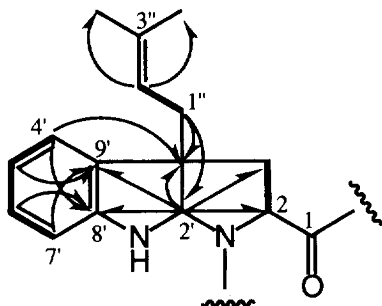
M. aeruginosa (NIES-88)[9] was isolated from a broom on Kawaguchi Lake and mass-cultured as previously described.[10] The 80% methanol extract of freeze-dried alga was partitioned between water and diethyl ether. The aqueous layer was further extracted with *n*-butanol and fractionated by ODS flash column chromatography (20-100%MeOH elution) followed by reversed-phase HPLC, using 0.05% TFA in MeCN/H_2O (1:1) to yield **1** (7.7 mg).

The positive FABMS of **1**, using glycerol as matrix displayed a molecular ion peak at *m/z* 1421 $[M+H]^+$ and a high resolution measurement established its molecular formula as $C_{68}H_{92}N_{16}O_{18}$. The peptidic nature of **1** was suggested by the ^{1}H and ^{13}C NMR spectra (Table 1), and the amino acid analysis of the hydrolyzate of **1** giving Asp, Gly, Leu, Pro Thr and Ser. The extensive two-demensional NMR analyses including ^{1}H-^{1}H COSY, HOHAHA, HMQC[11] and HMBC[12] spectra in DMSO-d_6 established the eleven partial structures. Nine of them were common α-amino acid units, *viz.*, aspartyl, asparaginyls (3 moles), glycyl, leucyl, prolyl, threonyl and seryl units. The presence of three asparaginyls was confirmed by the HMBC (Asn (I), Asn (III)) and NOSEY (Asn (II)) correlations, from the primary amide protons. The remaining two units were prenyl



tryptophanyls (Prenyl Trp), an unusual aromatic imino acid that relates to amauromine isolated from *Amauroascus* sp.[13]

The structure of Prenyl Trp (I) (**2**) was determined as follows. Six ^{13}C signals in NMR at δ 149.6 (s, C-8'), 132.1 (s, C-9'), 127.9 (d, C-6'), 122.9 (d, C-4'), 118.4 (d, C-5') and 109.8 (d, C-7'), and the UV absorption (λ max: 240 and 293 nm)[13] indicated the presence of indoline moiety.[14] The four consecutive aromatic protons (H-4' to H-7'), which were readily assigned by ^{1}H-^{1}H COSY spectrum, also supported the presence of the indoline moiety. A methine proton (δ_H; 5.42) of **1** was assigned to position 2' by comparison with ^{1}H NMR spectra of aszonalenine[14] and amauromine.[13] From this proton, five HMBC correlations shown in Scheme 1 were observed. The prenyl group, which was identified by ^{1}H-^{1}H COSY, HMQC and HMBC spectra, was sequentially connected to C-3' by the HMBC correlation between H-1" and C-3'. Moreover, hydrolized products of **1**, with 1% phenol in 6 N HCl at 110°C for 4 hr, yielded L-Trp[15]. ^{1}H and ^{13}C NMR spectra of Prenyl Trp (II) were very similar to those of Prenyl Trp (I). From these results, the structures of Prenyl Trp (I) and (II) were unambiguously determined.



Scheme 1. ^{1}H-^{1}H COSY (bold lines) and HMBC (half arrows) correlations of Prenyl Trp(I) in kawaguchipeptin A

The sequence of **1** was determined by HMBC and NOESY spectra. HMBC correlations were observed between Leu NH/Prenyl Trp (I) C=O, Asn (I) NH/Leu C=O, Gly NH/Asn C=O, Asp NH/Gly C=O, Asn (II) NH/Asp C=O, Asn (III) NH/Asn (II) C=O and Thr NH/Ser C=O. NOEs were observed between Asn (I) NH/Gly NH, Asn (I) H-2/Gly NH, Asn (III) H-2/Prenyl Trp (II) H-2', Prenyl Trp (II) H-2/Ser NH, Thr H-2/Pro H-5 and Pro H-2/Prenyl Trp (I) H-2'.

Chiral GC analysis of *N*-trifluoroacetyl isopropyl ester derivatives of the acid hydrolyzate (6N HCl, 110°C, 20 hr) of **1** indicated that the Asn (3 moles), Asp, Pro, Thr

Table 1. ^{1}H and ^{13}C NMR Data for Kawaguchipeptin A in DMSO-d_6

Position	^{1}H J (Hz)	^{13}C	HMBC(^{1}H)
Prenyl Trp (I) (PTI)			
1		169.9 (s)	PTI 2, 3, Leu 2, NH
2	3.98 (dd, 11.2,6.2)	60.4 (d)	PTI 3, 2'
3	2.03 (dd, 11.5,11.2)	39.9 (t)	PTI 2, 2'
	2.28 (dd, 11.5,6.2)		
2'	5.42 (s)	80.5 (d)	PTI 3, 1''
3'		57.0 (s)	PTI 3, 2', 4', 1''
4'	7.12 (d, 7.3)	122.9 (d)	PTI 6'
5'	6.62 (dd, 7.7,7.3)	118.4 (d)	PTI 7'
6'	6.85 (dd, 7.7,7.7)	127.9 (d)	PTI 4'
7'	6.67 (d, 7.7)	109.8 (d)	PTI 5'
8'		149.6 (s)	PTI 4', 6'
9'		132.1 (s)	PTI 5', 7'
1''	2.42 (m)	35.4 (t)	
2''	5.08 (t, 6.5)	119.5 (d)	PTI 1'', 4'', 4'''
3''		133.6 (s)	PTI 1'', 4'', 4'''
4''	1.47 (s)	17.8 (q)	PTI 2'', 4'''
4'''	1.61 (s)	25.7 (q)	PTI 2'', 4''
Leu			
1		171.57 (s)	Leu 2, Asn(I) NH
2	4.21 (d, 11.2,8.5,4.2)	50.3 (d)	
3	1.32 (m)	39.8 (t)	Leu 5, 5'
	1.59 (m)		
4	2.49 (m)	24.2 (d)	Leu 5, 5'
5	0.77 (d, 6.2)	21.0 (q)	Leu 5'
5'	0.84 (d, 6.5)	23.0 (q)	Leu 5
NH	8.37 (d, 8.5)		
Asn (I)			
1		171.3 (s)	Gly NH
2	4.50 (ddd, 8.1,7.3,6.6)	50.4 (d)	
3	2.45 (dd, 14.2,7.3)	38.0 (t)	Asn(I) 2, NH_2
	2.50 (dd, 14.2,6.6)		
4		171.63 (s)	Asn(I) 3, NH_2
NH	7.62 (d, 8.1)		
NH_2	6.95 (br)		
	7.14 (br)		
Gly			
1		168.7 (s)	Gly 2, Asp NH
2	3.62 (dd, 11.2,5.4)	42.8 (t)	
	3.68 (dd, 11.2,5.0)		
NH	7.94 (dd, 5.4,5.0)		
Asp			
1		170.9 (s)	Asp 3, Asn(II) 2, NH
2	4.49 (ddd, 7.7,7.5,5.2)	49.5 (d)	Asp 3
3	2.60 (dd, 16.5,7.5)	36.2 (t)	Asp 2
	2.68 (dd, 16.5,5.2)		
4		171.9 (s)	Asp 2, 3
NH	8.01 (d, 7.7)		
Asn (II)			
1		171.2 (s)	Asn(II) 2, Asn(III) NH
2	4.31 (ddd, 7.9,6.9,5.4)	50.0 (d)	Asn(II) 3
3	2.24 (dd, 15.6,5.4)	35.4 (t)	Asn(III) 2
	2.90 (dd, 15.6,7.9)		
4		171.63 (s)	Asn(II) 2, 3
NH	8.39 (d, 7.9)		
NH_2	6.87 (br)		
	7.38 (br)		

Position	^{1}H J (Hz)	^{13}C	HMBC(^{1}H)
Asn (III)			
1		171.8 (s)	Asn(III) 2, 3
2	4.90 (ddd, 8.5,7.9,6.0)	48.0 (d)	Asn(III) 3
3	2.39 (m)	38.2 (t)	Asn(III) 2, NH_2
	2.77 (dd, 15.2,7.9)		
4		171.63 (s)	Asn(III) 2, NH_2
NH	7.51 (d, 8.5)		
NH_2	7.22 (br)		
	7.61 (br)		
Prenyl Trp (II) (PTII)			
1		170.5 (s)	PTII 2, 3
2	3.97 (dd, 10.5,6.9)	61.6 (d)	PTII 3, 2'
3	2.16 (dd, 11.9,10.5)	39.6 (t)	PTII 2, 2'
	2.54 (dd, 11.9,6.9)		
2'	5.60 (s)	81.3 (d)	PTII 1''
3'		56.8 (s)	PTII 2', 4', 1''
4'	7.16 (d, 7.3)	122.9 (d)	PTII 6'
5'	6.70 (dd, 7.7,7.3)	118.3 (d)	PTII 7'
6'	7.02 (dd, 7.7,7.3)	128.3 (d)	PTII 4'
7'	6.56 (d, 7.3)	109.1 (d)	PTII 5'
8'		148.6 (s)	PTII 2', 4', 6'
9'		131.4 (s)	PTII 2', 5',7'
1''	2.40 (m)	35.8 (t)	
2''	5.10 (t, 6.5)	119.1 (d)	PTII 1'', 4'', 4'''
3''		133.9 (s)	PTII 4'', 4'''
4''	1.48 (s)	17.8 (q)	PTII 2'', 4'''
4'''	1.63 (s)	25.7 (q)	PTII 2'', 4''
Ser			
1		169.3 (s)	Ser 3, Thr 2, NH
2	4.05 (br)	56.7 (d)	
3	3.70 (dd, 10.8,7.7)	60.2 (t)	
	3.81 (dd, 10.8,3.8)		
NH	8.27 (br)		
Thr			
1		168.8 (s)	Thr 2, 3
2	4.45 (dd, 7.7,6.2)	56.8 (d)	Thr 4
3	3.92 (qd, 6.2,6.2)	67.2 (d)	Thr 2, 4
4	1.11 (d, 6.2)	19.3 (q)	
NH	7.37 (d, 7.7)		
Pro			
1		171.0 (s)	Pro 3
2	4.67 (dd, 7.7,7.3)	58.2 (d)	Pro 2
3	1.72 (m)	28.7 (t)	
	2.27 (m)		
4	1.82 (m)	25.0 (t)	
	1.97 (m)		
5	3.58 (m)	47.9 (t)	
	3.82 (m)		

and Ser units were L-form and Leu was D-form.

The relative and absolute stereochemistry of two Prenyl Trp's (I) (**2**) and (II) were deduced by a combination of spectral and chemical studies. First of all, the Marfey analysis[16] of the amino acids in the acid hydrolyzate (1% phenol in 6N HCl, 110°C, 4 hr) of **1** yielded L-Trp as a degradation product and therefore C-2 of **2** was determined to be S configuration. Secondly, NOESY spectrum of **1** suggested that B and C rings of **2** had *cis* relation, since a cross peak was noted between H-2' and H-1" of **2**. Finally, as shown in Table 2, the coupling constants between H-2 and H-3 (J=11.2 and 6.2) of **2** were very similar to those (J=11.0 and 6.0) of Trp derivative (**3**)[13] but not to those of Trp derivative (**4**),[13] indicating that the configurations of C-2, C-2' and C-3' of **2** are S . These data suggested that the absolute stereochemistry of **2** to be 2S, 2'S and 3'S and that those of Prenyl Trp (II) had the same stereochemistry.

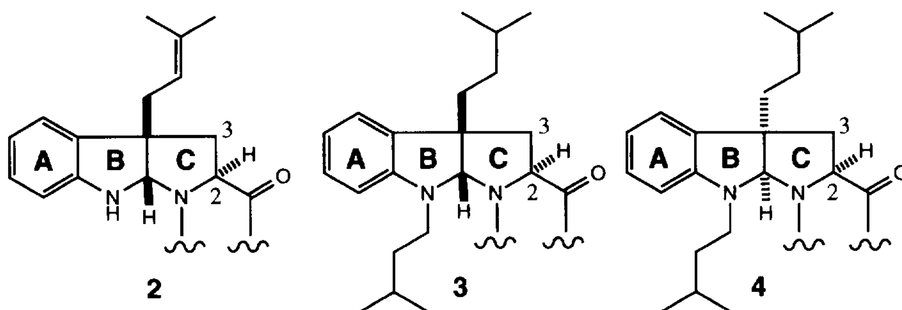


Table 2. Chemical shifts and coupling constants of Prenyl Trp (I) and analogs

	^{1}H-NMR (δ)	
	H-2	H-3
2[a]	3.98 (dd, J=11.2, 6.2)	2.03 (dd, J=11.5, 11.2)
		2.28 (dd, J=11.5, 6.2)
3[b]	4.11 (dd, J=11.0, 6.0)	2.10 (dd, J=11.0, 11.0)
		2.66 (dd, J=11.6, 6.0)
4[b]	4.38 (t, J=9.0)	2.37 (d, J=9.0)

a: DMSO-d_6, b: $CDCl_3$

Cyclic undecapeptides have been also isolated from the terrestrial and marine cyanobacterium *Anabaena laxa* [17] and *Hormothamnion entermorphoides*,[18] respectively, although the structure of these peptides were not related to those of **1**.

Experimental Section

General Information. 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 AM600 or JEOL JNM-A500 NMR spectrometers. Two-dimensional NMR spectra, except HMQC spectrum, were recorded on a Bruker AM600 NMR spectrometer equipped with an ASPECT 1000 computer. HMQC spectrum was recorded on a JEOL JNM-A500 NMR spectrometer equipped with a VAXserver 4000-200 computer. All two-dimensional homonuclear spectra were recorded in pure-phase absorption mode. HOHAHA and NOESY spectra were recorded with mixing times of 80 and 400 ms,

respectively. HMBC and HMQC spectra were recorded essentially as described in the literature.[11,12] Mass spectra, including high resolution mass measurements, were measured on a JEOL SX-102 mass spectrometer.

Cultivation of Alga. *Microcystis aeruginosa* f. *aeruginosa* (NIES-88) 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 [$Ca(NO_3)_2{\cdot}4H_2O$ 5 mg, KNO_3 10 mg, $NaNO_3$ 5 mg, Na_2SO_4 4 mg, $MgCl_2{\cdot}6H_2O$ 5 mg, β-Na_2glycerophosphate 10 mg, $Na_2EDTA{\cdot}2H_2O$ 0.5 mg, $FeCl_3{\cdot}6H_2O$ 0.05 mg, $MnCl_2{\cdot}4H_2O$ 0.5 mg, $ZnCl_2$ 0.05 mg, $CoCl_2{\cdot}6H_2O$ 0.5 mg, $Na_2MoO_4{\cdot}2H_2O$ 0.08 mg, H_3BO_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 $\mu E/m^2{\cdot}s$ on a 12L:12D cycle. Cells were harvested by continuous flow 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.

Isolation of Kawaguchipeptin A. Freeze-dried alga (121.7 g from 590 L of culture) was extracted with 80% MeOH (2 L × 2) 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 (7.0 g), which was subjected to flash chromatography on ODS (YMC-GEL, 120Å, 10 × 12 cm) with aqueous MeOH followed by CH_2Cl_2. A solution containing 200 mg of the solid out of the MeOH fraction (2.34 g) was purified by MPLC on ODS (YMC-GEL, 120Å, 4 × 17 cm, UV-detection at 210 nm, flow rate 8.0 mL/min) with 50% MeCN containing 0.05% TFA. Final purification was achieved by HPLC on ODS (Cosmosil MS C18, 10 × 250 mm, UV-detection 210 nm, flow rate 2.0 mL/min) with 50% MeCN containing 0.05% TFA to yield 7.7 mg of **1**.

Kawaguchipeptin A (1). $[\alpha]_D$ -103.1° (*c* 0.1, MeOH); UV (MeOH) λmax 240 nm (ε 13300), 293 nm (ε 4870); HRFABMS *m/z* 1421.6895 ($C_{68}H_{93}N_{16}O_{18}$, Δ +4.1 mmu).

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 hr. 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. Under this condition, Thr and allo-Thr were not separated. The presence of Thr in the acid hydrolyzate was verified by HPLC analysis of the Marfey derivatives. Retention times in the amino acid analysis (min): Asp (10.58), Thr (15.33), Ser (17.01), Gly (33.92), Leu (48.53), Pro (32.10).

Chiral GC Analysis. Compound **1** (100 μg) was dissolved in 0.5 mL of 6 N HCl and heated at 110°C for 16 hr. The reaction mixture was evaporated in a stream of nitrogen, dissolved in 10% HCl in *i*-PrOH (200 μL) and heated at 100°C for 30 min. The product was evaporated, dissolved in trifluoro-*N*-acetic anhydride (200 μL) and CH_2Cl_2 (200 μL), reacted at 100°C for 10 min and evaporated. The residue was dissloved in CH_2Cl_2 and analyzed by GC with a Chirasil-L-Val capillary column (Chrompak, 0.25 mm × 25 m). The oven temparature was maintained for 5 min at 60°C and raised to 200°C at 4°C/min, which was maintained for 3 min. Retention times of the amino acid residues (min): D-Thr (13.133), L-Thr (14.567), D-Pro (15.82), L-Pro (16.01), D-Leu (16.892), D-Ser (17.125), L-Ser (17.918), L-Leu (18.813), D-Asp (23.158), L-Asp (23.550). Retention times of derivatives of the acid hydrolyzate of **1** (min): L-Thr (14.517), L-Pro (16.117), D-Leu (16.975), L-Ser (17.992), L-Asp (23.592).

Hplc Analysis of the Marfey Derivatives. Compound **1** (100 μg) was dissolved in 0.5 mL of 1% phenol in 6 N HCl and heated at 110°C for 4 hr. The reaction mixture was evaporated in a stream of nitrogen, added with 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 $NaHCO_3$, and the mixture was kept at 80 °C for 3 min. To the reaction mixture were added 50 μL of 2 N HCl and 300 μL of 50% MeCN, and products were analyzed by reversed-phase ODS-HPLC, respectively: column Cosmosil MS (4.6 x 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 in the amino acid residues (min): L-Thr (37.2), L-allo-Thr (37.6), D-allo-Thr (38.8), D-Thr (42.0), L-Trp (50.8), D-Trp (52.9). Retention times in the amino acid of **1** (min): L-Thr (37.2), L-Trp (50.8).

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REFERENCES AND NOTES

1. a) Carmichael, W. W.; Beasley, V. R.; Bunner, D. L.; Eloff, J. N.; Falconer, I.; Gorham, P. Harada, K.-I.; Krishnamurthy, T.; Yu, M.-J.; Moore, R. E.; Rinehart, K. L.; Runnegar, M.; Skulberg, O. M.; Watanabe, M. F. *Toxicon* **1988**, 26, 971-973. b) Namikoshi, M.; Rinehart, K. L.;Sakai, R.; Stotts, R. R.; Dahlem, A. M.; Beasley, V. R.; Carmichael, W. W.; Evans, W. R. *J. Org.Chem.* **1992**, 57, 866-872. c) Sivonen, K.; Namikoshi, M.; Evans, W. R.; Färdig, M.; Carmichael, W. W.; Rinehart, K. L. *Chem. Res. Toxicol.* **1992**, 5, 464-469. d) Kiviranta, J.; Namikoshi, M.; Sivonen, K.; Evans, W. R.; Carmichael, W. W. Rinehart, K. L. *Toxicon* **1992**, 30,1093-1098. e) Namikoshi, M.; Sivonen, K.; Evans, W, R,; Carmichael, W. W.; Rouhiainen, L.; Luukkainen, R.; Rinehart, K. L.*Chem. Res. Toxicol.* **1992**, 5, 661-666. f) Namikoshi, M.; Sivonen, K.; Evans, W. R.; Carmichael, W. W.; Sun, F.; Rouhiainen, L.; Luukkainen, R.; Rinehart, K. L. *Toxicon* **1992**, 30, 1457-1464. g) Sivonen, K.; Skulberg, O. M.; Namikoshi, M.; Evans, W. R.; Carmichael, W. W.; Rinehart, K. L. *Toxicon* **1992**, 30, 1465-1471. h) Namikoshi, M.; Sivonen, K.; Evans, W. R.; Sun, F.; Carmichael, W. W.; Rinehart, K. L. *Toxicon* **1992**, 30, 1473-1479. i) Sivonen, K.; Namikoshi, M.; Evans, W. R.; Gromov, B. V.; Carmichael, W. W.; Rinehart, K. L. *Toxicon* **1992**, 30, 1481-1485.
2. a) Mackintosh, C.; Beattie, K. A.; Klumpp, S.; Cohen, P; Codd, G. A. *FEBS Lett.* **1990**, 264, 187-192. b) Yoshizawa, S.; Matsushima, R.; Watanabe, M. F.; Harada, K.-I.; Ishihara, A.; Carmichael, W. W.; Fujiki, H. *J. Cancer Res. Clin. Oncol.* **1990**, 116, 609-614. c) Matsushima, R.; Yoshizawa, S.; Watanabe, M. F.; Harada, K.-I.; Furusawa, M.; Carmichael, W. W.; Fujiki, H. *Biochem. Biophys. Res. Commun.* **1990**, 171, 867-874. d) Fujiki, H.; Suganuma, M. *Adv. in Cancer Res.* **1993**, 61, 143-194. e) Mackintosh, R. W.; Dalby, K. N.; Campbell, D. G.; Cohen, P. T. W.; Cohen, P.; Mackintosh, C. *FEBS Lett.* **1995**, 371, 236-240. f) Goldberg, J.; Huang, H.-B.; Kwon, Y.-G.; Greengard, P.; Nairn, A. C.; Kuriyan, J. *Nature* **1995**, 376, 745-753.
3. Harada, K.-I.; Mayumi, T.; Shimada, T.; Kondo, F.; Watanabe, M. F. *Tetrahedron Lett.* **1993**, 34, 6091-6094.
4. Tsukamoto, S.; Painuly, P.; Young, A.; Yang, X.; Shimizu, Y. *J. Am. Chem. Soc.* **1993**, 115, 11046-11047.
5. Martin, C.; Oberer, L.; Ino, T.; König, W. A.; Busch, M.; Weckesser, J. *J. Antibiot.* **1993**, 46, 1550-1556.
6. Murakami, M.; Okita, Y.; Matsuda, H.; Okino, T.; Yamaguchi, K. *Tetrahedron Lett.* **1994**, 35, 3129-3132.
7. Okino, T.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron* **1995**, 51, 10679-10686.
8. In the course of isolation of kawaguchipeptin A from *M. aeruginosa* (NIES-88) (121.7 g, freeze-dried), we isolated seven microcystins; microcystins RR (91.3 mg), LR (182.1 mg), DR (11.8 mg), LY (8.7 mg), LW (6.3 mg), LF (3.5 mg) and HtyR (20.8 mg).
9. Watanabe, M. M.; Kasai, F.; Hiwatari, T.; Suda, S.; Nei, T. *Jpn. J. Freez. Dry.* **1984**, 30, 23-26.
10. Watanabe, M. M.; Satake, K. N. In *NIES-Collection List of Strains Third Edition Microalgae and Protozoa Microbial Culture Collection, Natl. Inst. Environ. Stud.*; Tsukuba, Japan, **1991**, p32.
11. Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, 647, 565-569.
12. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, 108, 2093-2094.
13. Takase, S.; Kawai, Y.; Uchida, I.; Tanaka, H.; Aoki, H. *Tetrahedron Lett.* **1984**, 25, 4673-4676.
14. Kimura, Y.; Hamasaki, T.; Nakajima, H.; Isogai, A.; *Tetrahedron Lett.* **1982**, 23, 225-228.
15. Muramoto, K.; Kamiya, H. *Anal. Biochem.* **1990**, 189, 223-230.
16. Marfey, P. *Carlsberg Res. Commun.* **1984**, 49, 591-596.
17. a) Frankmölle, W. P.; Larsen, L. K.; Caplan, F. R.; Patterson, G. M. L.; Knübel, G.; Levine, I. A.; Moore, R. E. *J. Antibiot.* **1992**, 45, 1451-1457. b) Frankmölle, W. P.; Knübel, Moore, R. E.; Patterson, G. M. L. *J. Antibiot.* **1992**, 45, 1458-1466.
18. Gerwick, W. H.; Jiang, Z. D.; Agarwal, S. K.; Farmer, B. T. *Tetrahedron* **1992**, 48, 2313-2324.

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