DOI: 10.1002/asia.200700233

Miuraenamides: Antimicrobial Cyclic Depsipeptides Isolated from a Rare and Slightly Halophilic Myxobacterium

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Abstract: Marine myxobacteria are rare culture-resistant microorganisms, several strains of which have been identified by research groups in Asia. *Paraliomyxa miuraensis*, a slightly halophilic myxobacterium discovered in Japan, produces the cyclic hybrid polyketide–peptide antibiotics known as miuraenamides A and B, whose taxonomical and biological characteristics have been reported previously. Herein, we describe the chemical characterization of these two miuraenamides and introduce four new members of the miuraenamide family. We carried out the complete structural analysis of miuraenamides A and B on the basis of NMR spectroscopic analysis and elucidated the absolute configuration of miuraenamide A by chemical derivatization and subsequent use of the modified Mosher method or the Marfey method. Miuraenamides C–F were isolated from the same strain of the bacterium as miuraenamides A and B. The structure–antimicrobial-activity relationships of the six natural metabolites and four chemically derived compounds demonstrated the importance of both the macrocyclic structure and the β-methoxyacrylate moiety.

Keywords: antibiotics · peptides · polyketides · structure elucidation · structure–activity relationships

Introduction

Myxobacteria have been recognized as a rich source of novel antibiotics that are not found in actinomycetes or fungi.[1] A prominent example of myxobacterial secondary metabolites is the epothilone family: derivatives of these non-taxane microtubule-stabilizing anticancer agents[2] are currently in clinical trials.[3] The unique Gram-negative bacteria had been regarded as terrestrial microorganisms until some myxobacterial strains were isolated a decade ago from marine environments and characterized phylogenetically.[4] Since then, only a few strains of halophilic or halotolerant myxobacteria have been discovered by Asian researchers.[5] *Haliangium ochraceum*, which was discovered in Japan and produces the potent antimicrobial polyenes named haliangicins, is perhaps the most interesting of these species.[6] More recently, a slightly halophilic species, *Paraliomyxa miuraensis* strain SMH-27-4, was discovered in near-shore soil in Japan and shown to produce the novel halogen-containing depsipeptides miuraenamides A (**1**) and B (**2**). The biological properties of these natural products were found to include antimicrobial activity and inhibitory activity against NADH oxidase.[7] Herein, we report full details of the structural analysis of **1** and **2**, the determination of the absolute configuration of **1**, and the isolation and characterization of four additional members of this family of antibiotics, miuraenamides C–F (**3**–**6**). We also report structure–antimicrobial-activity relationships of the miuraenamides and related derivatives.

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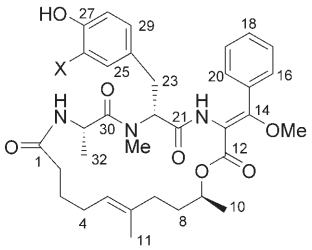
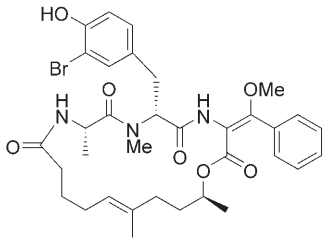
Results and Discussion

Production and Isolation

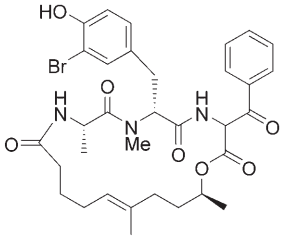
The myxobacterium *P. miuraensis* strain SMH-27-4 was isolated from a soil sample collected near the seashore on the Miura Peninsula in Kanagawa, Japan.[7] The strain required an optimum NaCl concentration of approximately 0.5–1.0% for growth; that is, the strain was slightly halophilic. Unlike many bacteria, the growth rate of this species was so slow



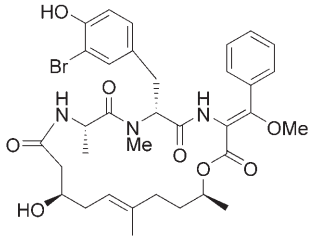
miuraenamide A (**1**): X = Br
miuraenamide B (**2**): X = I
miuraenamide C (**3**): X = Cl



miuraenamide D (**4**)



miuraenamide E (**5**)



miuraenamide F (**6**)

that the production of secondary metabolites required a long cultivation time (18 days). A total of 20 L of culture broth was used for the extraction and isolation. The bacterial cells, including the resin, were extracted with acetone, and the extract was partitioned between EtOAc and water. The fraction in EtOAc was subjected to column chromatography on silica gel and then purified by reversed-phase HPLC to afford the major compound miuraenamide A (**1**; 19.6 mg) and the minor congeners miuraenamides B (**2**; 0.4 mg) and E (**5**; 1.9 mg). Other relevant HPLC fractions were combined with the corresponding fractions obtained from additional cultures (total 33 L) and purified by TLC on silica gel to give miuraenamides C (**3**; 0.1 mg) and D (**4**; 0.4 mg). A fraction obtained by silica-gel column chromatography that was slightly more polar than those containing **1**–**5** was also separated by HPLC to afford another minor congener, miuraenamide F (**6**; 0.5 mg).

Abstract in Japanese:

海洋性粘液細菌は希少な難培養性微生物で、アジアの研究者によるわずかな例が知られるのみである。Miuraenamide A, B は低塩濃度要求性粘液細菌 *Paraliomyxa miuraensis* から単離された環状のポリケチドーペプチド複合型抗生物質で、分類および生物作用の研究が報告されている。今回、miuraenamide とその関連物質に関して化学的研究を報告する。構造解析の詳細は NMR に基づき行い、miuraenamide A の絶対立体配置は化学変換と改良 Mosher 法または Marfey 法により決定した。また、同菌より微量成分として 4 種の類縁体 miuraenamide C－F を発見し構造を解明した。これら miuraenamide 類とその誘導体の植物疫病菌に対する抗菌活性を評価した結果、環状構造とα-amino-β-methoxyacrylate 構造が活性に重要であることが判明した。

Structural Elucidation

The physicochemical properties of miuraenamides A (**1**) and B (**2**) were summarized previously.[7] Miuraenamide A (**1**) has the molecular formula $C_{34}H_{42}N_3O_7Br$, as determined by high-resolution MS analysis. Its IR spectrum indicated the presence of amide bonds and thus suggested the peptide nature of the molecule. We investigated the structure further by two-dimensional NMR spectroscopy. ^1H–^1H COSY correlations revealed the partial structures $-CH_2CH_2CH_2CH=$, $CH_2CH_2CH(O)CH_3$, $-CHCHCH-$ (part of a phenyl group), $-CHCH_2-$, a 1,2,4-trisubstituted phenyl ring, and $-CH(NH)CH_3$. ^1H NMR spectroscopy showed the presence of three isolated methyl groups, CCH_3, NCH_3, and OCH_3 (Table 1). The direct proton–carbon connectivities were determined by heteronuclear multiple quantum coherence (HMQC) experiments. Although most of the partial structures could be connected in the usual way on the basis of heteronuclear multiple bond correlation (HMBC) data (Scheme 1 and Table 2), the connectivity of the phenyl group and an alkene moiety (C13–C14) remained unclear. This problem was then solved by an HMBC experiment in [D_6]dimethyl sulfoxide. The additional correlations 13-NH/C12, 13-NH/C14, H16/C14, and H17/C15 observed in this solvent led to the elucidation of the planar structure of **1**. Ester formation between C9 and C12 was interpreted from the chemical shift of 9-H and the HMBC correlation 9-H/C12. The geometry of the two double bonds, C5=C6 and C13=C14, was determined from the NOESY correlations 5-H/7-H and 16-H, 20-H/13-NH. The absolute configuration of **1** is discussed below.

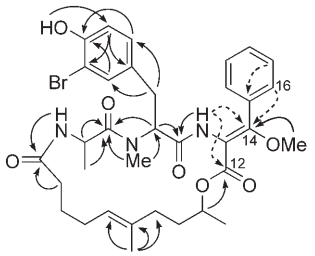
Miuraenamide B (**2**) was deduced to be a congener of **1** on the basis of its similar ^1H NMR spectrum (Table 1) and its molecular formula $C_{34}H_{42}N_3O_7I$, which contains iodine in place of the bromine atom in **1**. The ^1H–^1H COSY data of **2** indicated the presence of carbon frameworks identical to those in **1**. The only notable difference between the NMR spectroscopic data of the two compounds lies in the chemical shifts of the halogenated phenol moiety. The chemical shifts of the halogen-bearing carbon atom (C26) and 25-H are shifted significantly from $\delta_C = 110.0$ ppm and $\delta_H = 7.28$ ppm in **1** to $\delta_C = 85.8$ ppm and $\delta_H = 7.47$ ppm in **2**. The chemical shifts of the neighboring hydrogen and carbon atoms are also shifted to some extent. On the other hand, the chemical shifts of the other parts of **2** were superimposable with those of **1** ($\Delta\delta_H \leq \pm 0.01$, $\Delta\delta_C \leq \pm 0.2$). These data indicated that the bromine atom of the tyrosine residue in **1** is replaced with an iodine atom in **2** and that the two structures are otherwise completely identical.

The ^1H NMR spectroscopic data of miuraenamide C (**3**; Table 1) are also similar to those of **1**. The amount of the sample was insufficient to obtain ^{13}C NMR spectroscopic data. The isotope peaks at *m/z* 640.3 and 642.3 (≈3:1) in the mass spectrum indicated the presence of a chlorine atom in the molecule. The molecular formula of $C_{34}H_{42}N_3O_7Cl$ determined by high-resolution MS suggested that **3** is the congener with a chlorine atom instead of the bromine atom in

Table 1. ^{1}H NMR spectroscopic data for miuraenamides A–F in $CDCl_3$ (600 MHz).[a]

Position	1	2	3	4	5	6
2-H	2.32 (dt, 13.2, 8.4)	2.33 (dt, 13.8, 8.4)	2.32 (dt, 13.8, 8.4)	2.28–2.24 (m)	2.15–2.21 (m)	2.60 (dd, 15.3, 2.4)
	2.11–2.17 (m)	2.12–2.18 (m)	2.12–2.18 (m)	2.08–2.14 (m)		2.28 (dd, 15.3, 8.4)
3-H	1.79–1.85 (m)	1.79–1.85 (m)	1.79–1.85 (m)	1.83–1.89 (m)	1.63–1.69 (m)	3.92–3.98 (m)
	1.67–1.74 (m)	1.67–1.74 (m)	1.67–1.74 (m)	1.56–1.63 (m)		
4-H	2.05–2.10 (m)	2.05–2.10 (m)	2.05–2.10 (m)	1.99–2.03 (m)	1.99–2.05 (m)	2.35–2.41 (m)
					1.92–1.98 (m)	2.16–2.22 (m)
5-H	5.04–5.10 (m)	5.05–5.11 (m)	5.05–5.11 (m)	4.95 (t, 7.0)	4.80 (t, 7.2)	5.13 (t, 7.8)
7-H	2.15–2.20 (m)	2.15–2.20 (m)	2.15–2.20 (m)	1.82–1.88 (m)	1.65–1.71 (m)	2.14–2.20 (m)
	1.94–2.00 (m)	1.94–2.00 (m)	1.94–2.00 (m)	1.65–1.71 (m)	1.46–1.52 (m)	1.95–2.00 (m)
8-H	1.84–1.90 (m)	1.83–1.89 (m)	1.83–1.89 (m)	1.34–1.40 (m)	1.42–1.48 (m)	1.83–1.89 (m)
	1.61–1.67 (m)	1.60–1.66 (m)	1.60–1.66 (m)	1.03–1.08 (m)	1.35–1.40 (m)	1.60–1.66 (m)
9-H	5.09–5.13 (m)	5.09–5.13 (m)	5.08–5.12 (m)	4.60–4.66 (m)	4.85 (sext, 6.2)	5.02–5.08 (m)
10-H	1.33 (d, 6.6)	1.33 (d, 6.6)	1.33 (d, 6.0)	0.67 (d, 6.6)	1.20 (d, 6.2)	1.33 (d, 6.6)
11-H	1.60 (s)	1.60 (s)	1.60 (s)	1.52 (s)	1.46 (s)	1.64 (s)
13-H	–	–	–	–	6.11 (d, 8.4)	–
16-H, 20-H	7.17–7.21 (m)	7.17–7.21 (m)	7.17–7.21 (m)	7.32 (d, 7.0)	8.07 (d, 8.0)	7.16–7.20 (m)
17-H, 19-H	7.17–7.21 (m)	7.17–7.21 (m)	7.17–7.21 (m)	7.40 (t, 7.0)	7.50 (t, 7.5)	7.16–7.20 (m)
18-H	7.29–7.33 (m)	7.29–7.33 (m)	7.29–7.33 (m)	7.43 (t, 7.0)	7.64 (t, 7.5)	7.32 (t, 6.9)
22-H	5.05–5.09 (m)	5.05–5.09 (m)	5.05–5.09 (m)	5.49 (dd, 8.4, 7.2)	5.46 (dd, 8.4, 7.2)	5.05 (dd, 10.7, 4.8)
23-H	3.24 (dd, 13.2, 10.8)	3.23 (dd, 13.5, 10.8)	3.25 (dd, 13.5, 10.5)	3.36 (dd, 14.4, 8.4)	3.28 (dd, 14.4, 8.4)	3.27 (dd, 13.8, 10.7)
	2.61 (dd, 13.2, 5.0)	2.60 (dd, 13.5, 5.0)	2.61 (dd, 13.5, 5.4)	2.85 (dd, 14.4, 7.2)	2.83 (dd, 14.4, 7.2)	2.62 (dd, 13.8, 4.8)
25-H	7.28 (d, 1.5)	7.47 (d, 1.5)	7.14 (d, 1.5)	7.38 (d, 1.5)	7.33 (d, 1.5)	7.30 (d, 2.0)
28-H	6.88 (d, 8.4)	6.85 (d, 8.2)	6.88 (d, 8.4)	6.94 (d, 8.4)	6.91 (d, 8.2)	6.91 (d, 8.4)
29-H	6.96 (dd, 8.4, 1.5)	6.99 (dd, 8.2, 1.5)	6.93 (dd, 8.4, 1.5)	7.12 (dd, 8.4, 1.5)	7.07 (dd, 8.2, 1.5)	7.00 (dd, 8.4, 2.0)
31-H	4.79 (dq, 8.0, 6.0)	4.79 (dq, 7.8, 6.0)	4.80 (dq, 8.0, 6.6)	4.84 (dq, 7.5, 6.6)	4.97 (dq, 8.4, 6.7)	4.84 (dq, 7.8, 7.2)
32-H	1.29 (d, 6.0)	1.30 (d, 6.0)	1.30 (6.6)	1.33 (d, 6.6)	1.32 (d, 6.7)	1.32 (d, 7.2)
OMe	3.49 (s)	3.49 (s)	3.49 (s)	3.42 (s)	–	3.48 (s)
NMe	2.86 (s)	2.86 (s)	2.86 (s)	2.92 (s)	2.97 (s)	2.90 (s)
13-NH	7.06 (s)	7.05 (s)	7.06 (s)	7.34 (s)	7.47 (d, 8.4)	7.02 (s)
31-NH	7.17–7.21 (m)	7.17 (d, 7.8)	7.18 (d, 8.0)	7.29 (d, 7.5)	6.53 (d, 8.4)	7.14 (d. 7.8)
OH	5.72 (s)	5.33 (br s)	5.49 (br s)	5.48 (s)	5.49 (br s)	3.65 (br s)
						5.51 (br s)

[a] Chemical shifts are in ppm; multiplicities and coupling constants (Hz) are given in parentheses. The signals were assigned by 2D NMR spectroscopy as described in the text.



Scheme 1. HMBC correlations of **1** measured in $CDCl_3$ (dashed arrows: in [D_6]dimethyl sulfoxide).

1. The ^{1}H NMR spectroscopic data were superimposable with those of **1**, except for the chemical shift of 25-H, which was shifted from $\delta_H = 7.28$ ppm in **1** to $\delta_H = 7.14$ ppm in **3**. These data indicated that the bromine atom of the tyrosine residue in **1** is replaced with a chlorine atom in **3** and that the two structures are otherwise completely identical.

The NMR spectroscopic data of miuraenamide D (**4**), which has the same molecular formula as **1**, are similar to those of **1** (Tables 1 and 2). Thus, **4** appeared to be an isomer of **1**. The chemical shifts observed for the polyketide moiety (5-H–11-H) appeared at higher field ($\Delta\delta = -0.08$ to -0.66 ppm) in the ^{1}H NMR spectrum of **4** than in the spectrum of **1**, and the chemical shifts for the peptide moiety (13-NH, 22-H–31-H) appeared at lower field ($\Delta\delta = 0.05$ to 0.42 ppm). This finding suggested that these protons are affected differently by the anisotropic effect of the phenyl group at C14 as a result of *E*/*Z* isomerism of the C13–C14 double bond. The *Z* geometry of this double bond was then established by NOE correlations between 16-H/20-H and 10-H and between 17-H/19-H and 10-H. Therefore, we concluded that miuraenamide D (**4**) is the 13*Z* isomer of **1**.

Miuraenamide E (**5**) has the molecular formula $C_{33}H_{40}N_3O_7Br$, which is smaller than that of **1** by CH_2. The NMR spectroscopic data (Tables 1 and 2) indicated the lack of the methyl enol ether moiety (C13=C14–OMe) and the presence of a ketone ($\delta_C = 191.0$ ppm) and a methine group ($\delta_H = 6.11$ ppm, $\delta_C = 57.6$ ppm). The other NMR signals corresponded to those of **1** according to ^{1}H–^{1}H COSY analysis. The modified substructure around the ketone group was analyzed by an HMBC experiment. The HMBC correlations summarized in Scheme 2 revealed the structure **5**, in which the enol ether moiety had been hydrolyzed to the corresponding ketone. The existence of a benzoyl group (C14–C20) was also supported by the downfield shifts of the signals for the aromatic hydrogen atoms (H16–H20) relative to those of the equivalent atoms in **1** ($\Delta\delta = 0.31$–0.88 ppm). As the differences in the proton chemical shifts observed for **5** and **1** were analogous to those found for **4** and **1** (upfield

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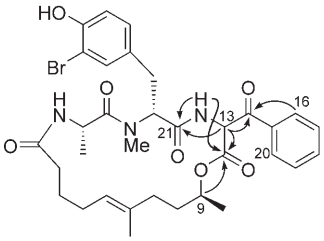
Table 2. ^{13}C NMR spectroscopic data (ppm) for miuraenamides A–F in $CDCl_3$ (150 MHz).

Carbon atom	1	2	4	5	6
1	173.4	173.4	173.3	172.9	172.7
2	34.9	34.9	34.5	35.4	39.7
3	25.9	25.9	25.7	25.8	69.0
4	26.5	26.5	26.1	26.5	35.8
5	125.4	125.4	125.2	125.8	121.3
6	134.7	134.7	134.7	133.7	136.6
7	35.0	35.0	35.0	34.5	35.1
8	33.3	33.3	32.6	32.4	33.0
9	70.3	70.2	70.1	72.1	70.2
10	19.7	19.7	18.6	20.0	19.4
11	16.3	16.3	16.2	15.5	16.4
12	165.8	165.8[a]	165.3[c]	166.2	166.2
13	111.1	n.d.[b]	107.8	57.6	n.d.[b]
14	165.0	165.0[a]	165.4[c]	191.0	164.0[a]
15	131.7	131.7	132.8	134.0	131.7
16, 20	128.6	128.5	128.1	129.7	128.6
17, 19	128.2	128.3	129.2	128.8	128.3
18	130.1	130.1	129.5	134.7	130.2
21	168.8	168.8	167.9	169.5	168.8
22	56.7	56.7	56.6	56.3	56.7
23	31.8	31.6	31.6	31.5	31.8
24	130.4	131.0	130.7	130.3	n.d.[b]
25	132.5	138.6	132.3	132.3	132.5
26	110.0	85.8[a]	110.0[a]	110.1	110.0[a]
27	151.1	153.6	151.1	151.1	151.1
28	116.1	115.0	116.1	116.1	116.1
29	130.0	131.1	129.8	129.8	130.2
30	172.8	172.8	173.7	174.1	172.4
31	46.1	46.1	46.1	45.3	45.8
32	17.2	17.2	17.4	17.9	17.1
OMe	58.6	58.6	57.4	–	58.6
NMe	30.2	30.2	30.1	30.4	30.3

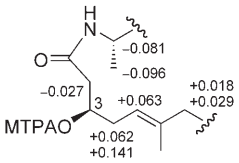
[a] The chemical shift of the signal was determined from HMBC spectroscopic data. [b] Not determined. [c] Interchangeable signal assignments.



Scheme 2. HMBC correlations around the keto group (C14) of miuraenamide E (**5**).

shifts for the polyketide moiety and downfield shifts for the peptide moiety), we concluded that the orientation of the phenyl group is similar in **5** to that observed in **4**. The absolute configuration at C13 is unclear; however, the NMR spectroscopic data showed that **5** is a single isomer. Miuraenamides D (**4**) and E (**5**) could be artifacts that result from the isomerization and hydrolysis of the enol ether moiety of **1** during the isolation procedure.

Miuraenamide F (**6**) has the molecular formula $C_{34}H_{42}N_3O_8Br$, which is larger than that of **1** by an oxygen atom. Therefore, this compound appeared to be a hydroxylated derivative of **1**. The NMR spectroscopic data (Tables 1 and 2) indicated that the methylene group at C3 in **1** is replaced with an oxymethine functionality in **6** (δ_H = 3.95 ppm, δ_C = 69.0 ppm). The signals for 2-H and 4-H were shifted downfield with respect to those observed for **1** ($\Delta\delta_{2\text{-}H}$ = 0.28/0.14 and $\Delta\delta_{4\text{-}H}$ = 0.11/0.30 ppm, $\Delta\delta_{C2}$ = 4.8 and $\Delta\delta_{C4}$ = 9.3 ppm). The NMR signals for the other parts of the molecule corresponded to those of **1** on the basis of 2D NMR spectroscopic analysis. Therefore, **6** was determined to be 3-hydroxymiuraenamide A. The absolute configuration at C3 was determined to be *R* by comparing the ^1H NMR spectroscopic data of the (*S*)- and (*R*)-MTPA esters **7s** and **7r** derived from **6** by the modified Mosher method (Scheme 3).

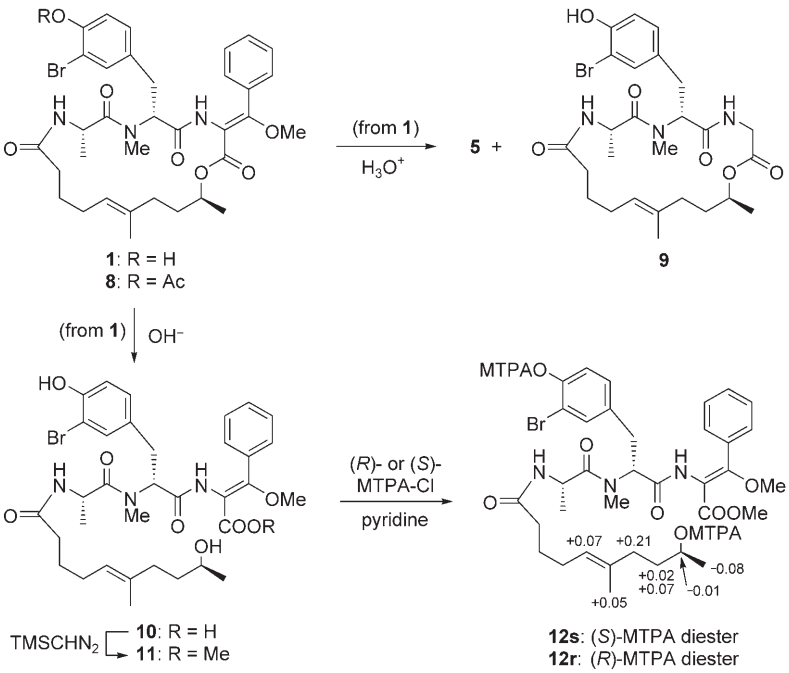


Scheme 3. A portion of the methoxy(trifluoromethyl)phenylacetic acid (MTPA) esters **7s** and **7r** derived from miuraenamide F (**6**) with $\Delta\delta$ values ($\delta_{7s}-\delta_{7r}$) in ppm.

Absolute Stereostructure

To determine the absolute stereostructure of the natural products, we attempted to prepare crystalline derivatives of **1** (Scheme 4). Acetate **8** was first prepared from **1** under standard conditions. The acid hydrolysis of **1** in 1 N HCl afforded ketone **5**, which was identical to the natural derivative **5** in all respects. On the other hand, the treatment of **1** with 2 N HCl also promoted a retroaldol reaction to furnish **9** as well as **5**. None of the derivatives, including the natural metabolites, afforded suitable single crystals for X-ray analysis.

Next, we applied the modified Mosher method and the Marfey method to determine the absolute configuration of the stereogenic carbon atoms of the ester group (C9) and the peptide moiety (C22 and C31), respectively. The basic hydrolysis of **1** in 2 N NaOH gave seco acid **10**, which was converted into methyl ester **11** with $TMSCHN_2$. Methyl ester **11** was then converted into bis(MTPA) diesters **12s** and **12r**. The chemical-shift differences ($\delta_{12s}-\delta_{12r}$; Scheme 4), which were determined on the basis of ^1H–^1H COSY experiments, revealed the 9*S* configuration.[8] To determine the absolute configuration of the two amino acids, miuraenamide A (**1**) was heated in 12 N HCl, and a portion of the hydrolysate was treated with 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide or the corresponding 5-D-leucinamide (L- or D-FDLA) to afford a mixture of FDLA derivatives of the amino acids.[9] Derivatization with L-FDLA afforded the *N*-methyl-D-tyrosine and L-alanine derivatives, as determined by comparison with authentic samples (Figure 1A), whereas derivatization with D-FDLA afforded the diastereomeric compounds (Figure 1B). The results demonstrated that the alanine and *N*-methyltyrosine units in **1** have the L and D configuration, respectively. The complete stereostructure of



Scheme 4. Derivatization of miuraenamide A (**1**). For the MTPA esters **12s** and **12r**, $\Delta\delta$ values ($\delta_{12s}-\delta_{12r}$) are given in ppm. TMS = trimethylsilyl.

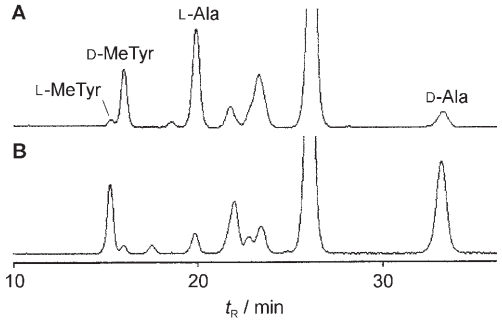


Figure 1. HPLC traces of L-FDLA (A) and D-FDLA (B) derivatives of the acid hydrolysate of miuraenamide A (**1**). MeTyr = *N*-methyltyrosine.

1 was therefore determined as shown. The similarity of their spectra to those of **1** and chemical correlations suggest that the other congeners **2**–**5** have the same stereostructure.

Antifungal Activity

Miuraenamide A (**1**) inhibited selectively the funguslike phytopathogen *Phytophthora capsici* at a minimum dose of 0.025 μg per disk and had no effect on bacteria.[7] We evaluated the anti-*Phytophthora* activity of the other miuraenamides and related compounds synthesized in this study relative to that of **1** (Table 3).

Table 3. Minimum doses of miuraenamides and derivatives for anti-*Phytophthora* activity.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**9**	**10**	**11**
Dose [μg per disk]	0.025	0.025	0.025	1	10	0.13	5	2	>50	50

The type of halogen present in the tyrosine residue in miuraenamides A–C (**1**–**3**) is not important for their activity. The decrease in activity observed for miuraenamide E (**5**) and **9** could be attributable to the lack of an appropriately oriented β-methoxyacrylate moiety (C12–C14), the well-known pharmacophore of the fungicides that target the mitochondrial cytochrome bc_1 complex, such as the strobilurins,[10] myxothiazole A,[11] and the cystothiazoles.[12] The low activity of miuraenamide D (**4**) shows that even a change in the geometry of the β-methoxyacrylate group affects the activity of these compounds. Indeed, the conformation of the β-methoxyacrylate group is known to be important for the antimicrobial activity of these fungicides.[13] The lipophilicity of the polyketide moiety and the free phenol group on the peptide portion of the structure appear to be important for the activity of these compounds on the basis of the low activity observed for miuraenamide F (**6**) and acetate **8**, respectively. The anti-*Phytophthora* activity is lost completely in the case of the ring-opened derivatives **10** and **11**, although they contain the β-methoxyacrylate moiety. Thus, the macrocyclic structure of the miuraenamides appears to be essential. Several polyketide–peptide hybrid-type metabolites that resemble the miuraenamides have been isolated from marine sponges and a mollusk (e.g., the geodiamolides,[14] the seragamides,[15] and doliculide[16]). The true producers of these metabolites could be unknown halophilic myxobacteria and/or related microorganisms.

Conclusions

Four antimicrobial depsipeptides, miuraenamides C–F (**3**–**6**), were isolated from the unique slightly halophilic myxobacterium *P. miuraensis* strain SMH-27-4 together with the previously isolated miuraenamides A (**1**) and B (**2**). We carried out the complete structural analysis of **1**–**6**, including the assignment of the absolute stereostructure of **1**, by a combination of spectral analysis and chemical derivatization. The major metabolite, miuraenamide A (**1**), was found to be a potent inhibitor of the growth of the phytopathogenic microorganism *P. capsici* at a minimum dose of 25 ng per disk. Structure–activity-relationship

studies, for which the natural products and chemically derived derivatives were examined, revealed the importance of the macrocyclic structure and the α-amino-β-methoxyacrylate moiety.

Experimental Section

General

Flash chromatography was carried out with a low-pressure gradient system equipped with an 880-PU HPLC pump and an 880-02 gradient unit (Jasco, Tokyo). Silica gel 60 F_{254} (0.25-mm thickness; Merck, Rahway, NJ) was used for both analytical and preparative TLC. Preparative HPLC was performed with a high-pressure gradient system equipped with PU-1586 and PU-2086 pumps and a UV-1570 detector (Jasco). Specific rotation was measured by using a DIP-370 digital polarimeter (Jasco). FTIR spectra were recorded on an FTIR-7000S spectrometer (Jasco). UV/Vis spectra were recorded on a Ubest-50 UV/Vis spectrophotometer (Jasco). Mass spectra (MS) were recorded on a Mariner Biospectrometry Workstation (Applied Biosystems, Foster City, CA) in the positive-ESI mode. A peptide mixture (angiotensin I, bradykinin, and neurotensin) was used as an internal standard for high-resolution MS analysis. NMR spectra were recorded at 27 °C on an AMX2 600 (600 MHz) or an ARX 400 (400 MHz) spectrometer (Bruker, Rheinstetten, Germany). The NMR chemical shifts (ppm) were referenced to the solvent peaks at $\delta_H = 7.26$ ppm and $\delta_C = 77.0$ ppm (residual $CHCl_3$) for samples in $CDCl_3$.

Isolation

The culture conditions used were the same as those reported previously.[7] The bacterial cells and the absorber resin were obtained from 20 L of the culture broths by centrifugation and extracted three times with acetone (2 L) at room temperature. (Each time, the cells were exposed to acetone for 1 day.) The extracts were combined and concentrated to give an aqueous mixture (400 mL), which was extracted three times with EtOAc (400 mL). The extracts in EtOAc were concentrated, and the residue (878 mg) was subjected to flash column chromatography on silica gel (Hi-Flash SI-40W-M (12 g, 20 i.d. × 60 mm; Yamazen, Osaka, Japan), 30–100 % (35 min) EtOAc in hexane, then 0–40 % (20 min) MeOH in EtOAc, 6 mL min^{-1}). The fractions eluted with 66–94 % EtOAc in hexane were combined and concentrated. The residue (61 mg) was subjected to reversed-phase HPLC (YMC-Pack D-ODS-5 (20 i.d. × 250 mm; YMC, Kyoto, Japan), 65–80 % MeOH (90 min, linear gradient), 8 mL min^{-1}, detection at 225 nm) to give **1** (19.6 mg, $t_R = 46.3$ min), **2** (0.4 mg, $t_R = 50.5$ min), and **5** (1.9 mg, $t_R = 63.0$ min).

To purify other minor congeners, the fractions of interest were combined with the corresponding fractions obtained from additional culture broths (total 33 L). The fraction with a peak at 43.0 min (0.4 mg) in the last HPLC separation was combined with the new fraction (0.3 mg) containing the same substance. The mixture was separated by silica-gel TLC (20 × 10 cm) with benzene/EtOAc (1:2). The UV-positive band ($R_f = 0.4$) was collected, and the product was purified further by TLC (20 × 10 cm) with the eluent mixture $CHCl_3$/acetone/MeOH (86:10:4) to give **3** (0.1 mg; $R_f = 0.8$). The fraction with the peak at 57.9 min (0.2 mg) in the last HPLC separation was combined with the corresponding fraction (0.9 mg) from the additional culture broths. The mixture was separated by silica-gel TLC (20 × 10 cm) with $CHCl_3$/acetone/MeOH (90:10:5) to give **4** (0.4 mg; $R_f = 0.85$).

The fractions eluted with 94–100 % EtOAc in hexane and 0–12 % MeOH in EtOAc by flash chromatography were combined and concentrated. The residue (17.0 mg) was subjected to ODS chromatography (Cosmosil 75C18-OPN (6 g; Nacalai Tesque, Kyoto, Japan), 50–100 % MeOH in water (50 min, linear gradient), 2 mL min^{-1}). The fractions eluted with 62–74 % MeOH in water were combined and concentrated, and the residue (4.6 mg) was subjected to reversed-phase HPLC (Develosil ODS-UG-5 (10 i.d. × 250 mm; Nomura Chemical, Aichi, Japan), 60–70 % MeOH in water (30 min, linear gradient), 4 mL min^{-1}, detection at 250 nm, $t_R = 18.5$ min, or 40 % MeCN, 4 mL min^{-1}, detection at 250 nm, $t_R = 18.5$ min) to give **6** (0.5 mg).

The physicochemical properties of **1** and **2**, except for NMR spectroscopic data, were reported in reference [7].

3: Colorless powder. $[\alpha]_D^{27} = +24$ ($c = 0.005$ M, MeOH); UV/Vis (MeOH): λ_{max} (ε) = 202 (61 000), 258 nm (11 000 M^{-1} cm^{-1}); IR (film): $\tilde{\nu}$ = 1698, 1653, 1636, 1508, 1260, 1116, 1057 cm^{-1}; MS (ESI): *m/z* (%) = 640.3 and 642.3 (23 and 8) $[M+H]^+$, 662.3 and 664.3 (54 and 28) $[M+Na]^+$; HRMS: *m/z* calcd for $C_{34}H_{43}N_3O_7{}^{35}Cl$: 640.2785; found: 640.2760.

4: Colorless powder. $[\alpha]_D^{27} = -37$ ($c = 0.03$ M, MeOH); UV/Vis (MeOH): λ_{max} (ε) = 203 (58 000), 258 nm (13 000 M^{-1} cm^{-1}); IR (film): $\tilde{\nu}$ = 3340, 1683, 1662, 1635, 1297, 1218, 1124, 757 cm^{-1}; MS (ESI): *m/z* (%) = 684.2 and 686.2 (17 and 24) $[M+H]^+$, 706.2 and 708.2 (92 and 100) $[M+Na]^+$; HRMS: *m/z* calcd for $C_{34}H_{43}N_3O_7{}^{79}Br$: 684.2279; found: 684.2256.

5: Colorless powder. $[\alpha]_D^{26} = +14$ ($c = 0.13$ M, $CHCl_3$); UV/Vis (MeOH): λ_{max} (ε) = 208 (26 000), 250 (13 000), 280 nm (4100 M^{-1} cm^{-1}); IR (KBr): $\tilde{\nu}$ = 3421, 1749, 1673, 1635, 1508, 1228, 1134, 1045, 816, 689 cm^{-1}; MS (ESI): *m/z* (%) = 670.2 and 672.2 (22 and 27) $[M+H]^+$, 692.2 and 694.2 (59 and 73) $[M+Na]^+$; HRMS: *m/z* calcd for $C_{33}H_{41}N_3O_7H^{79}Br$: 670.2122; found: 670.2125.

6: Colorless powder. $[\alpha]_D^{26} = +17$ ($c = 0.067$ M, MeOH); UV/Vis (MeOH): λ_{max} (ε) = 210 nm (27 000), 278 (sh, 12 000 M^{-1} cm^{-1}); IR (KBr): $\tilde{\nu}$ = 3326, 1679, 1652, 1508, 1217, 1119, 757 cm^{-1}; HRMS: *m/z* calcd for $C_{34}H_{42}N_3O_8{}^{79}BrNa$: 722.2052; found: 722.2048.

7s: Miuraenamide F (**6**; 0.2 mg, 0.3 μmol) was treated with (*R*)-MTPA chloride (4 μL, 21 μmol) in dry pyridine (0.1 mL) for 20 h at room temperature. The product was isolated under standard conditions and purified by HPLC (Develosil ODS-UG-5 (10 × 250 mm), MeOH/H_2O (9:1), 4 mL min^{-1}, detected at 210 nm, $t_R = 9$ min) to give **7s** (0.3 mg, 93 %). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.74–7.70 (m, 2H), 7.57–7.51 (m, 2H), 7.50–7.44 (m, 3H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.40–7.35 (m, 3H), 7.32–7.23 (m, 5H), 7.13 (dd, *J* = 1.8, 8.1 Hz, 1H), 7.07 (s, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.27 (d, *J* = 7.8 Hz, 1H), 5.59–5.55 (m, 1H), 5.15 (dd, *J* = 6.6, 10.2 Hz, 1H), 5.17–5.11 (m, 1H), 5.03–4.87 (m, 1H), 4.77 (dq, *J* = 6.6, 7.8 Hz, 1H), 3.80 (s, 3H), 3.54 (s, 3H), 3.49 (s, 3H), 3.34 (dd, *J* = 10.2, 14.4 Hz, 1H), 2.83 (s, 3H), 2.67 (dd, *J* = 6.6, 14.4 Hz, 1H), 2.60–2.50 (m, 3H), 2.46–2.40 (m, 1H), 2.25–2.19 (m, 1H), 2.01–1.95 (m, 1H), 1.83–1.77 (m, 1H), 1.71–1.65 (m, 1H), 1.64 (s, 3H), 1.31 (d, *J* = 6.6 Hz, 3H), 1.07 ppm (d, *J* = 6.6 Hz, 3H); MS (ESI): *m/z* = 1132.3 and 1134.3 $[M+H]^+$, 1154.3 and 1156.3 $[M+Na]^+$, 1170.3 and 1172.3 $[M+K]^+$.

7r: Compound **6** (0.2 mg) was converted under the same conditions with (*S*)-MTPA chloride (4 μL, 21 μmol) into **7r** (0.2 mg, 62 %). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.74–7.70 (m, 2H), 7.58–7.52 (m, 2H), 7.50–7.44 (m, 4H), 7.41–7.36 (m, 3H), 7.31–7.20 (m, 5H), 7.14 (dd, *J* = 1.8, 8.1 Hz, 1H), 7.06 (s, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 6.34 (d, *J* = 7.8 Hz, 1H), 5.57–5.51 (m, 1H), 5.12 (dd, *J* = 6.0, 9.6 Hz, 1H), 5.10–5.04 (m, 1H), 5.04–4.98 (m, 1H), 4.89 (dq, *J* = 6.6, 7.8 Hz, 1H), 3.75 (s, 3H), 3.55 (s, 3H), 3.48 (s, 3H), 3.34 (dd, *J* = 9.6, 13.5 Hz, 1H), 2.88 (s, 3H), 2.69 (dd, *J* = 6.0, 13.5 Hz, 1H), 2.62–2.56 (m, 2H), 2.50–2.44 (m, 1H), 2.29–2.23 (m, 1H), 2.22–2.16 (m, 1H), 1.99–1.93 (m, 1H), 1.82–1.76 (m, 1H), 1.72–1.66 (m, 1H), 1.62 (s, 3H), 1.31 (d, *J* = 6.6 Hz, 3H), 1.17 ppm (d, *J* = 6.6 Hz, 3H); MS (ESI): *m/z* = 1132.3 and 1134.2 $[M+H]^+$, 1154.3 and 1156.3 $[M+Na]^+$, 1170.3 and 1172.3 $[M+K]^+$.

8: Miuraenamide A (**1**; 2.3 mg) was treated with a mixture of pyridine (0.5 mL) and acetic anhydride (0.5 mL) at room temperature for 3.5 h. The mixture was then concentrated in vacuo to give **8** (2.7 mg, 100 %) as a colorless powder. $[\alpha]_D^{26} = +130$ ($c = 0.13$ M, $CHCl_3$); UV/Vis (MeOH): λ_{max} (ε) = 208 (25 000), 261 nm (1000 M^{-1} cm^{-1}); IR (KBr): $\tilde{\nu}$ = 3351, 1772, 1695, 1645, 1529, 1209, 1189, 1117, 1047, 778, 705 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, *J* = 2.0 Hz, 1H),7.36–7.30 (m, 1H), 7.25–7.20 (m, 4H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.09 (s, 1H), 7.06 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 5.16–5.06 (m, 3H), 4.80 (quint, *J* = 7.2 Hz, 1H), 3.50 (s, 3H), 3.32 (dd, *J* = 13.6, 10.4 Hz, 1H), 2.86 (s, 3H), 2.65 (dd, *J* = 13.6, 5.2 Hz, 1H), 2.37 (s, 3H), 2.36–2.30 (m, 1H), 2.20–2.14 (m, 2H), 2.11–2.05 (m, 2H), 1.95–2.01 (m, 1H), 1.92–1.77 (m, 2H), 1.75–1.58 (m, 2H), 1.61 (s, 3H), 1.33 (d, *J* = 6.0 Hz, 3H), 1.29 ppm (d, *J* = 6.4 Hz, 3H); MS (ESI): *m/z* (%) = 382.6 and 383.6 (72 and 100) [*M*+H+

K]$^{2+}$, 726.2 and 728.2 (48 and 66) $[M+H]^+$, 748.2 and 750.2 (38 and 35) $[M+Na]^+$; HRMS: *m/z* calcd for $C_{36}H_{45}N_3O_8{}^{79}Br$: 726.2497; found: 726.2392.

Hydrolysis of **1** to **5** and **9**: Miuraenamide A (**1**; 2.6 mg, 3.8 μmol) was stirred in a mixture of THF (0.9 mL), water (0.1 mL), and HCl (12 N, 0.2 mL; final concentration: 2 N) at room temperature for 9 h. The mixture was then diluted with saturated $NaHCO_3$ (5 mL) and water (5 mL) and extracted three times with diethyl ether. The combined ethereal extracts were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by HPLC (Develosil ODS UG-5 (10 i.d.×250 mm), 70–80% MeOH in water (30 min), 3 mL min^{-1}, detection at 215 nm) to give **5** (0.3 mg, 12%, t_R=25.2 min) and **9** (0.3 mg, 14%, t_R=13.3 min), each as a colorless powder. The treatment of **1** with HCl at lower concentration (a final concentration of 1 N) for 4 h afforded only **5** (43%).

9: ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.32 (d, J=2.0 Hz, 1H), 7.07 (dd, J=8.4, 2.0 Hz, 1H), 6.92 (d, J=8.4 Hz, 1H), 6.83 (d, J=8.0 Hz, 1H), 6.74 (br d, J=9.6 Hz, 1H), 5.41 (br s, 1H), 5.34 (dd, J=8.4, 7.2 Hz, 1H), 5.05 (br t, J=7.0 Hz, 1H), 4.97–4.93 (m, 1H), 4.83 (dq, J=7.6, 6.4 Hz, 1H), 4.42 (dd, J=18.0, 9.6 Hz, 1H), 3.40 (dd, J=18.0, 3.6 Hz, 1H), 3.29 (dd, J=14.4, 8.4 Hz, 1H), 2.92 (s, 3H), 2.81 (dd, J=14.4, 7.2 Hz, 1H), 2.27–2.10 (m, 2H), 2.09–2.03 (m, 2H), 1.95–1.89 (m, 1H), 1.85–1.40 (m, 5H), 1.58 (s, 3H), 1.30 (d, J=6.4 Hz, 3H), 1.24 ppm (d, J=6.4 Hz, 1H); MS (ESI): *m/z* (%)=566.0 and 568.0 (84 and 100) $[M+H]^+$, 587.9 and 589.9 (50 and 63) $[M+Na]^+$; HRMS: *m/z* calcd for $C_{26}H_{37}N_3O_6{}^{79}Br$: 566.1806; found: 566.1850.

10: Miuraenamide A (**1**; 1.9 mg, 2.8 μmol) was stirred in a mixture of EtOH (0.8 mL) and NaOH (5 N, 0.4 mL) at room temperature for 24 h under nitrogen atmosphere. The mixture was then diluted with saturated NH_4Cl (3 mL), HCl (1 N, 1 mL) was added, and the resulting mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel (Wako gel C-300, 2.8 g; eluent: $CHCl_3$/MeOH=9:1–8:1) to give **10** (1.0 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.44–7.40 (m, 1H), 7.35 (t, J=7.2 Hz, 2H), 7.32–7.27 (m, 3H), 7.17 (d, J=1.4 Hz, 1H), 6.91 (br d, J=8.4 Hz, 1H), 6.84 (d, J=8.4 Hz, 1H), 6.49 (br d, J=6.8 Hz, 1H), 5.22 (t, J=8.0 Hz, 1H), 5.19–5.15 (m, 1H), 4.65 (quint, J=6.8 Hz, 1H), 3.79 (sext, J=6.4 Hz, 1H), 3.63 (s, 3H), 3.12 (dd, J=14.8, 7.0 Hz, 1H), 2.68 (dd, J=14.8, 9.0 Hz, 1H), 2.63 (s, 3H), 2.19 (t, J=7.2 Hz, 2H), 2.13–2.00 (m, 4H), 1.70–1.64 (m, 2H), 1.61 (s, 3H), 1.58–1.52 (m, 2H), 1.19 (d, J=6.4 Hz, 3H), 1.03 ppm (d, J=6.4 Hz, 3H).

11: A solution of $TMSCHN_2$ (2 M) in diethyl ether (4 μL, 8 μmol) was added to **10** (1.0 mg, 1.4 μmol) in a mixture of benzene (1.4 mL) and MeOH (0.2 mL), and the resulting mixture was stirred at room temperature for 2 h. The mixture was then concentrated, and the residue was subjected to HPLC (Develosil ODS UG-5 (10 i.d.×250 mm), 60–80% MeOH in water (60 min), 3.0 mL min^{-1}, detection at 218 nm) to give **11** (0.8 mg, 78%, t_R=29.3 min) as a colorless powder. $[\alpha]_D^{26}$=+22 (c=0.06 M, $CHCl_3$); IR (KBr): $\tilde{\nu}$=3281, 1703, 1635, 1507, 1264, 1224, 1115, 981, 818, 757, 704, 666 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): δ=7.37 (t, J=7.2 Hz, 1H), 7.32 (t, J=7.2 Hz, 2H), 7.27 (d, J=7.2 Hz, 2H), 7.21 (d, J=2.0 Hz, 1H), 7.14 (s, 1H), 6.97 (dd, J=8.4, 2.0 Hz, 1H), 6.88 (d, J=8.4 Hz, 1H), 6.30 (d, J=6.6 Hz, 1H), 5.22 (dd, J=9.0, 6.6 Hz, 1H), 5.15 (t, J=6.6 Hz, 1H), 4.59 (quint, J=6.9 Hz, 1H), 3.81–3.75 (m, 1H), 3.78 (s, 3H), 3.52 (s, 3H), 3.19 (dd, J=14.4, 7.2 Hz, 1H), 2.75 (dd, J=14.4, 9.6 Hz, 1H), 2.66 (s, 3H), 2.14 (t, J=7.2 Hz, 2H), 2.10–2.04 (m, 2H), 2.02 (q, J=7.2 Hz, 2H), 1.68–1.62 (m, 2H), 1.60 (s, 3H), 1.58–1.51 (m, 2H), 1.19 (d, J=6.0 Hz, 3H), 0.96 ppm (d, J=7.2 Hz, 3H); MS (ESI): *m/z* (%)=738.2 and 740.2 (93 and 100) $[M+Na]^+$; HRMS: *m/z* calcd for $C_{35}H_{46}N_3O_8{}^{79}BrNa$: 738.2361; found: 738.2361.

12s: Methyl ester **11** (0.4 mg, 0.6 μmol) was treated with (*R*)-MTPA chloride (4 μL, 21 μmol) in dry pyridine (80 μL) under the standard conditions. The crude product was purified by HPLC (Develosil ODS UG-5 (10 i.d.×250 mm), 90% MeOH in water, 4.0 mL min^{-1}, detection at 220 nm) to give **12s** (0.5 mg, 78%, t_R=10.4 min) as a colorless powder. ^{1}H NMR (major conformer; 600 MHz, $CDCl_3$): δ=7.71–7.67 (m, 3H), 7.55–7.51 (m, 2H), 7.48–7.43 (m, 4H), 7.42–7.38 (m, 5H), 7.35–7.31 (m, 2H), 7.16 (s, 1H), 7.11 (dd, J=1.8, 8.4 Hz, 1H), 6.94 (d, J=8.4 Hz, 1H), 6.29 (d, J=6.6 Hz, 1H), 5.31 (d, J=9.0, 7.2 Hz, 2H), 5.13–5.04 (m, 2H), 4.56–4.50 (m, 1H), 3.78, 3.72, 3.64, 3.50, 3.55, and 3.53 (s, total 12H), 3.30 (dd, J=14.4, 7.2 Hz, 1H), 2.81 (dd, J=14.4, 9.6 Hz, 1H), 2.65 (s, 3H), 2.13–2.06 (m, 2H), 2.05–1.95 (m, 4H), 1.70–1.50 (m, 3H), 1.70–1.64 (m, 1H), 1.56 (s, 3H), 1.26 (d, J=6.0 Hz, 3H), 0.96 ppm (d, J=6.6 Hz, 3H); MS (ESI): *m/z* (%)=1148.4 and 1150.4 (13 and 18) $[M+H]^+$, 1170.3 and 1172.3 (90 and 100) $[M+Na]^+$.

12r: Methyl ester **11** (0.5 mg, 0.7 μmol) was converted under the same conditions with (*S*)-MTPA chloride (4 μL, 21 μmol) into **12r** (0.8 mg, 100%, t_R=9.95 min), which was obtained as a colorless powder. ^{1}H NMR (major conformer; 600 MHz, $CDCl_3$): δ=7.70–7.66 (m, 3H), 7.55–7.51 (m, 2H), 7.48–7.44 (m, 4H), 7.41–7.37 (m, 5H), 7.36–7.30 (m, 2H), 7.17 (s, 1H), 7.11 (dd, J=8.4, 1.8 Hz, 1H), 6.95 (d, J=8.4 Hz, 1H), 6.27 (d, J=6.6 Hz, 1H), 5.32 (dd, J=9.0, 7.2 Hz, 1H), 5.13–5.08 (m, 1H), 5.03–4.98 (m, 1H), 4.53 (quint, J=6.6 Hz, 1H), 3.78, 3.72, 3.64, 3.55, and 3.52 (s, total 12H), 3.30 (dd, J=14.4, 7.2 Hz, 1H), 2.81 (dd, J=9.6, 14.4 Hz, 1H), 2.65 (s, 3H), 2.14–2.06 (m, 2H), 1.94–2.02 (m, 2H), 1.93–1.85 (m, 2H), 1.74–1.68 (m, 1H) 1.65–1.55 (m, 3H), 1.51 (s, 3H), 1.34 (d, J=6.0 Hz, 3H), 0.97 ppm (d, J=6.6 Hz, 3H); MS (ESI): *m/z* (%)=1148.4 and 1150.4 (11 and 15) $[M+H]^+$, 1170.3 and 1172.3 (84 and 100) $[M+Na]^+$.

Hydrolysis of **1** and derivatization by the Marfey method: A solution of **1** (1.0 mg, 1.5 μmol) in HCl (12 N, 1 mL) was heated at 115–120 °C (oil-bath temperature) in a sealed glass vial under nitrogen atmosphere for 15 h. The hydrochloric acid was then removed by flushing with nitrogen, and the residue was dissolved in water and washed twice with diethyl ether. The aqueous layer was dried by flushing the vial with nitrogen. The residue was lyophilized and redissolved in water (150 μL). Aqueous $NaHCO_3$ (1 M, 20 μL) and 1% L- or D-FDLA (Tokyo Chemical Industry Co., Ltd., Tokyo) in acetone (30 μL, 1 μmol) were added to a portion (30 μL) of the resulting solution, and the mixture was incubated at 37 °C for 1 h. The reaction was then quenched by adding HCl (1 M, 20 μL), and the mixture was diluted with acetonitrile (100 μL) to give a solution (200 μL) containing L- or D-FDLA derivatives of amino acids. A portion (3 μL) of each solution was analyzed by HPLC (Develosil ODS UG-5 (4.6×250 mm), 45–50% A in water (A=MeCN/MeOH (3:1)–0.1% trifluoroacetic acid (TFA); 40 min, linear gradient), 1 mL min^{-1}, detection at 305 nm). The authentic FDLA derivatives of *N*-methyltyrosine (Bachem, Switzerland) and alanine were prepared and analyzed by the same method.

Anti-Phytophthora Assay

Detailed conditions for the assay were reported previously.[12] The phytopathogen *P. capsici* was cultured on a synthetic agar medium in a 9-cm dish at 25 °C for 2 days in the dark until the colony had grown to a size of about 3–4 cm in diameter. Each paper disk (8 mm in diameter) impregnated with a sample was placed 1 cm away from the front of the colony. After incubation for 1 day, the distance between the edge of the colony and the paper disk was measured (control: 0 mm).

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Received: July 30, 2007
Published online: November 19, 2007

