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Piericidins C_5 and C_6: New 4-Pyridinol Compounds Produced by *Streptomyces* sp. and *Nocardioides* sp.

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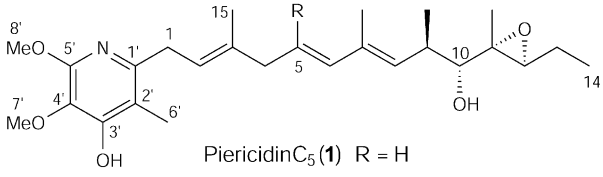
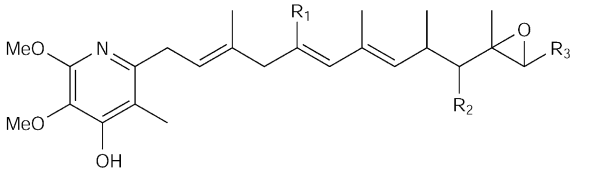
Abstract—Piericidins C_5 (**1**) and C_6 (**2**), two new members of the piericidin family, were isolated from a *Streptomyces* sp. and a *Nocardioides* sp., together with known piericidins C_1 (**3**), C_2 (**4**), C_3 (**5**), C_4 (**6**), D_1 (**7**), and A_3 (**8**). The structures were determined on the basis of their spectroscopic data. Both new compounds inhibited cell division of fertilized starfish (*Asterina pectinifera*) eggs at the minimum inhibitory concentration of 0.09 μg/mL.

Introduction

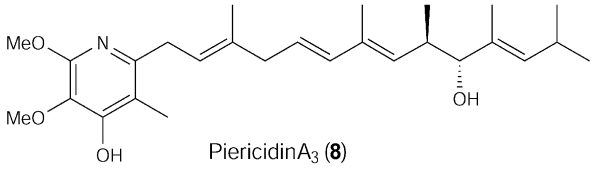
In the course of our search for biologically active compounds using the bioassay system of starfish embryogenesis,[1–5] we have found that *i*-PrOH extracts of two broths of a *Streptomyces* sp. and a *Nocardioides* sp. showed potent inhibitory activity against cell division of fertilized starfish (*Asterina pectinifera*) eggs. Bioassay-guided purification of the crude extracts resulted in the isolation of two new compounds belonging to the piericidin family designated piericidin C_5 (**1**) and piericidin C_6 (**2**), together with known piericidins C_1 (**3**), C_2 (**4**), C_3 (**5**), C_4 (**6**), D_1 (**7**), and A_3 (**8**)[6,7] which had previously been reported as insecticidal substances produced by *Streptomyces* strains.[8,9] In this paper, we report the isolation, structure elucidation, and biological activities of these new compounds.



Piericidin C_5 (**1**) R = H
Piericidin C_6 (**2**) R = Me



Piericidin C_1 (**3**) R_1= H,R_2= OH,R_3= Me
Piericidin C_2 (**4**) R_1= Me,R_2= OH,R_3= Me
Piericidin C_3 (**5**) R_1= H,R_2= OH,R_3= *i*-Pr
Piericidin C_4 (**6**) R_1 = Me,R_2 = OH,R_3 = *i*-Pr
Piericidin D_1 (**7**) R_1 = H,R_2 = OMe,R_3 = Me



Piericidin A_3 (**8**)

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Results and Discussion

The fermentation broth of a *Streptomyces* sp. was extracted with *i*-PrOH. The *i*-PrOH extract was partitioned between EtOAc and H_2O. The EtOAc extract was subjected to column chromatography on silica gel using 20–80% EtOAc in hexane as eluent. The bioactive fractions were individually chromatographed on ODS using MeOH–H_2O (9:1) as eluent to afford **1**, **3**, **5**, **7**, and **8**. In the same manner, the fractionation and purification of the broth of a *Nocardioides* sp. afforded **2**, **4**, and **6**.

Piericidin C_5 (**1**), $[\alpha]_D^{25}$ +7° (*c* 0.12, MeOH), was obtained as a pale yellow viscous oil. The FABMS showed an $[M+H]^+$ ion at *m*/*z* 446 in the positive mode and an $[M-H]^-$ ion at *m*/*z* 444 in the negative mode. The molecular formula, $C_{26}H_{39}NO_5$, was determined by HRFABMS (*m*/*z* 446.2909 $[M+H]^+$, Δ +0.3 mmu). The IR (ν_{max}^{film} 3400, 1607 sh, 1588, 1472 cm^{-1}) and UV [λ_{max}^{MeOH} 231 (ε 32,000), 236 (ε 32,300), 267 (ε 5,100) nm] spectra showed the presence of OH groups and an aromatic ring. The ^1H NMR (Table 1), ^{13}C NMR (Table 2), ^1H–^1H COSY and HMQC spectra of **1** revealed the presence of 26 carbon atoms and allowed the presence of the partial structures of C-1 to C-2, C-4 to C-6, C-8 to C-10, and C-12 to C-14. In the HMBC experiment, correlations were observed from H_3-15 to C-2, C-3, C-4, from H_3-16 to C-6, C-7, C-8, from H_3-18 to C-10, C-11, C-12, thereby allowing the connection of these partial structures. Further HMBC correlations from H_3-6′ to C-1′, C-2′, C-3′, from 3′-OH to C-2′, C-3′, C-4′, from H_3-7′ to C-4′, and H_3-8′ to C-5′ defined a full substituted pyridine ring. HMBC correlations from H_2-1 to C-1′ and C-2′ revealed that the C-1–C-14 chain connected to the pyridine ring. ^{13}C-chemical shifts (δ_C 62.6 s and 64.1 d) suggested that the terminal structure of the side chain (C-11 and C-12) contained an epoxide ring. This was supported by the similarity of ^1H and ^{13}C NMR signals between **1** and the both known compounds, piericidins C_1 (**3**) and C_3 (**5**). The geometry of the disubstituted olefin Δ^5 was determined to be *E* from the large vicinal ^1H coupling constant ($J_{5,6}$ = 15.6 Hz). The geometry of the trisubstituted olefins Δ^2 and Δ^7 was determined by NOE difference experiments (Fig. 1). Irradiation of the H_2-1 and H-2 resulted in 6.3% enhancement of H_3-15 and 2.0% enhancement of H_2-4, respectively. The H-6 and H_3-16 signals were enhanced upon irradiation of H-8 and H_3-17 signals, respectively. These findings indicate that the geometry of Δ^2 and Δ^7 is 2*E*,7*E*. Irradiation of the H-10 and H_3-18 resulted in 6.2% enhancement of H-12 and 2.4–2.8% enhancements of H_2-13, respectively, indicating that C-10 and C-13 on the epoxide ring are in a *trans* disposition. Consequently, the gross structure of piericidin C_5 was elucidated as **1**.

Table 1. ^1H NMR data for **1** and **2** in $CDCl_3$[a]

No.	1	2
1	3.36 d (2H, 6.8)	3.37 d (2H, 6.8)
2	5.40 dt (6.8, 1.0)	5.40 dt (6.8, 1.2)
4	2.77 d (2H, 7.1)	2.69 s (2H)
5	5.59 dt (15.5, 7.1)	—
6	6.07 d (15.5)	5.68 br s
8	5.22 br d (9.3)	5.08 br d (9.5)
9	2.67 ddq (9.3, 8.9, 6.8)	2.61 ddq (9.5, 9.0, 6.8)
10	2.90 d (8.9)	2.88 d (9.0)
12	2.79 t (6.4)	2.79 t (6.4)
13a	1.65 dq (7.6, 6.4)	1.67 dq (7.6, 6.4)
13b	1.56 dq (7.6, 6.4)	1.56 dq (7.6, 6.4)
14	1.05 t (3H, 7.6)	1.05 t (3H, 7.6)
15	1.74 br s (3H)	1.67 br s (3H)
16	1.79 br d (3H, 0.8)	1.66 d (3H, 1.2)
17	0.94 d (3H, 6.8)	1.75 d (3H, 1.1)
18	1.30 s (3H)	0.95 d (3H, 6.8)
19	—	1.31 s (3H)
6′	2.09 s (3H)	2.09 s (3H)
7′	3.85 s (3H)	3.86 s (3H)
8′	3.94 s (3H)	3.94 s (3H)
10-OH	2.00 br s	2.02 br s
3′-OH	6.22 br s	6.20 br s

[a]Coupling constants, $J_{H\text{-}H}$ (in Hz), are given in parentheses.

Table 2. ^{13}C NMR data for **1** and **2** in $CDCl_3$[a]

No.	1	2
1	34.4 t	34.5 t
2	122.2 d	123.5 d
3	134.8 s	133.9 s
4	43.1 t	51.1 t
5	126.7 d	134.5 s
6	135.6 d	130.1 d
7	135.1 s	134.8 s
8	132.4 d	131.1 d
9	36.2 d	36.4 d
10	81.8 d	81.9 d
11	62.6 s	62.5 s
12	64.1 d	64.0 d
13	21.4 t	21.4 t
14	10.4 q	10.4 q
15	16.6 q	15.8 q
16	13.0 q	17.3 q
17	17.1 q	17.5 q
18	11.0 q	17.1 q
19	—	11.0 q
1′	150.8 s	151.0 s
2′	111.9 s	111.9 s
3′	153.9 s	153.9 s
4′	127.8 s	127.8 s
5′	153.5 s	153.5 s
6′	10.5 q	10.5 q
7′	60.6 q	61.0 q
8′	53.0 q	53.0 q

[a]Multiplicities were determined by DEPT experiments.

Piericidin C_6 (**2**), $[\alpha]_D^{25}$ +34° (*c* 0.03, MeOH), was obtained as a pale yellow viscous oil. The molecular formula, $C_{27}H_{41}NO_5$, was determined by HRFABMS (*m*/*z* 460.3034 $[M+H]^+$, Δ −2.9 mmu). The IR and UV spectra of **2** closely resembled those of **1**, suggesting that **2** is an analogue of **1**. The ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectra of **2** were almost identical to

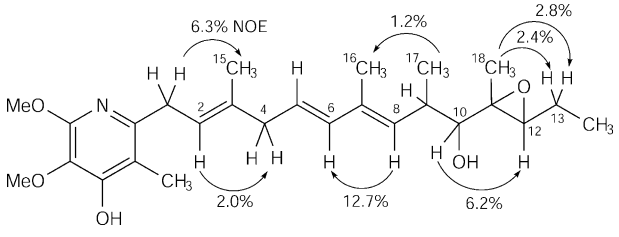


Figure 1. Key NOEs observed for **1**.

those of piericidin C_5 (**1**) except that they contained an additional methyl signal and lacked an olefinic proton signal. In the HMBC experiment, correlations from the additional methyl protons (H_3-16) to C-4, C-5, and C-6 were observed, suggesting that the replacement of H-5 in **1** by a methyl group gives rise to **2**. The 2D NMR spectral data including ^1H–^1H COSY, HMQC, and HMBC were compatible with the plane structure for **2**. The geometry of the trisubstituted olefins was determined to be 2*E*,5*E*,7*E* by the following NOESY correlations: H_2-1 with H_3-15; H_2-4 with H-2 and H-6; H-6 with H-8; H_3-17 with H-9 and H_3-16 (Fig. 2). The following NOESY correlations were also observed: H-10 with H-12; H_2-13 with H_3-19. These data indicated that C-10 and C-13 on the epoxide ring are in a *trans* disposition. Thus, the gross structure of piericidin C_6 was elucidated as **2**.

The absolute stereochemistry at C-10 in the side chain of **1** and **2** was elucidated by the application of the modified Mosher's method[10] for 10-*O*-MTPA esters. Two aliquots of **1** were treated with (−)- and (+)-MTPA chlorides in pyridine to afford (*S*)- and (*R*)-MTPA esters **9a** and **9b**, respectively. The signs of the $\Delta\delta$ ($\delta_S-\delta_R$) values for protons of H-2, H_2-4, H-5, H-6, H-8, H-9, H_3-16 (7-Me), H_3-17 (9-Me) were positive, while those for protons of H-12, H_2-13, H_3-14, and H_3-18 (11-Me) were negative, as shown in Figure 3. In accordance with the modified Mosher model, the absolute configuration at C-10 was assigned as *R*.

In the same manner, **2** was converted into the MTPA esters **10a** and **10b**, and the $\Delta\delta$ ($\delta_S-\delta_R$) value for each proton of the esters (Fig. 4) demonstrated that the absolute configuration at C-10 is *R*.

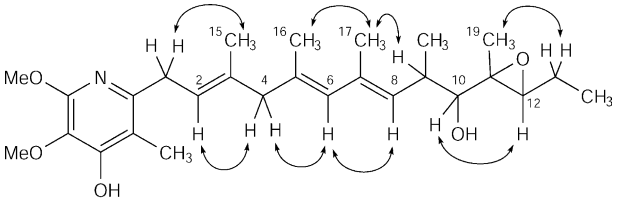


Figure 2. Key NOESY correlations observed for **2**.

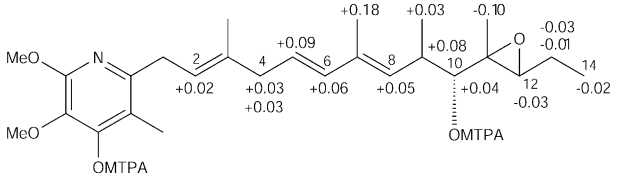


Figure 3. $\Delta\delta$ values for the MTPA esters **9a** and **9b**; $\Delta\delta$ (ppm) = $\delta_S-\delta_R$.

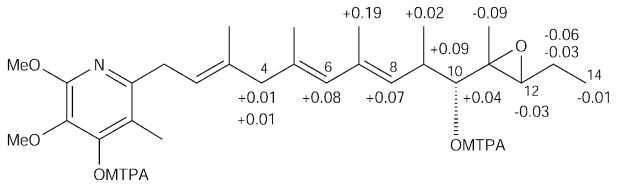


Figure 4. $\Delta\delta$ values for the MPTA esters **10a** and **10b**; $\Delta\delta$ (ppm) = $\delta_S-\delta_R$.

The stereochemistry at C-9 of **1** was elucidated by analysis of the coupling constants and NOE difference experiments. The relatively large (8.9 Hz) coupling constant between H-9 and H-10 and the lack of an NOE between these signals indicated the absence of free rotation about C-9–C-10 bond. Two possible structures **1A** (9*R*,10*R*) and **1B** (9*S*,10*R*) could be proposed based on these data (Fig. 5). NOE difference experiments were employed to discriminate between the two possibilities. Irradiation at H_3-17 resulted in 2.6% enhancement of H_3-18, while no NOE was observed between H-8 and H_3-18. These findings indicate that **1** has 9*R*,10*R* configuration (**1A**), which is consistent with the previously known stereochemistry of piericidin A_1.[11] In the similar manner, the *R* configuration at C-9 of **2** was suggested on the basis of the relatively large (9.0 Hz) coupling constant between H-9 and H-10 and the NOESY correlation between H_3-18 and H_3-19 of **2** (Fig. 6).

The absolute configuration at C-11 and C-12 of **1** and **2** was elucidated by chemical conversions and NOE difference experiments. To determine the stereochemistry of C-11 in **1** and **2**, MTPA esters of **1** (**9a** and **9b**) and MTPA esters of **2** (**10a** and **10b**) were reduced to 10,11,3′-triols **11** and **12**, respectively, which were then converted into acetonides **13** and **14**, respectively. NOE difference experiments revealed that H-10 is located on the opposite face of the dioxolane ring with respect to H_3-18 and H_3-19 of **13** and **14**, respectively, as shown in Figure 7. These findings indicate the 11*S* configuration in **1** and **2**.

Since the relative configuration around the epoxide ring of **1** and **2** was defined by NOE difference experiments as described above, **1** and **2** have the 12*S* configuration. Consequently, the absolute stereochemistry of piericidins C_5 (**1**) and C_6 (**2**) was elucidated to be 9*R*,10*R*,11*S*,12*S*.

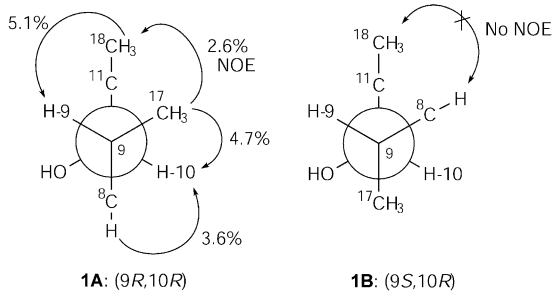


Figure 5. Key NOEs and two possible structures for **1**.

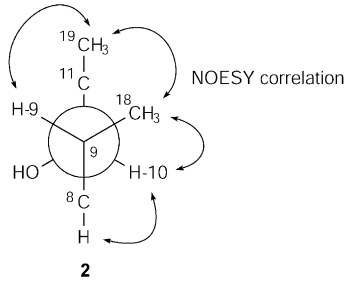


Figure 6. Key NOESY correlations and the absolute configuration at C-9 of **2**.

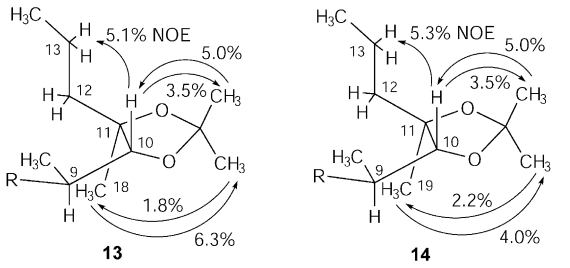
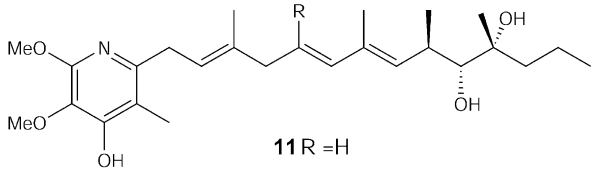
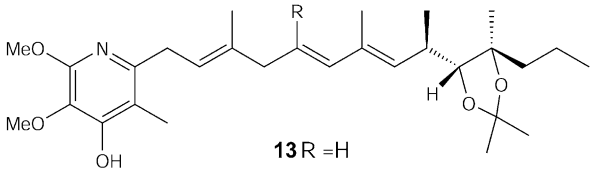


Figure 7. Key NOEs and relative stereochemistry of the dioxolane ring moiety in **13** and **14**.





In this study, all piericidins (**1**, **3**, **5**, **7**, and **8**) isolated from a *Streptomyces* have an olefinic hydrogen at C-5, while all piericidins (**2**, **4**, and **6**) isolated from a *Nocardioides* sp. have a methyl group at C-5. The species-specific difference in the structures could be arisen from the difference in the metabolism, namely, the fifth biogenetic unit to construct the C-4 to C-5 unit during the biosynthesis of piericidins[12–15] was acetate in the case of the *Streptomyces* sp. and propionate in the case of the *Nocardioides* sp.

Natural products **1–8** and their derivatives **11–14** were examined for the cell-division inhibitory activity during starfish embryogenesis. Minimum inhibitory concentrations (MIC) of **1–8** and **11–14** are given in Table 3. Piericidins C_5 (**1**), C_6 (**2**), C_1 (**3**), C_2 (**4**), C_3 (**5**), C_4 (**6**), and piericidin A_3 (**8**) were potently active (MIC = 0.07–0.10 μg/mL). Piericidin D_1 (**7**), triol **11**, and triol **12** exhibited relatively weak activity (MIC = 0.70–0.80 μg/mL). Acetonide derivatives **13** and **14** did not affect cell division. Compounds **1–6** and **8** inhibited cell division of fertilized eggs and embryos at the morula and the blastula stages within 10 min after the treatment. It is suggested that the presence of a hydroxyl group at C-10 and the presence of an epoxide or a double bond between C-11 and C-12 are important for the potent inhibitory activity. The methyl substituent at C-5 or the sort of the terminal alkyl group (Me, Et, or *i*-Pr) at C-12 is not an essential structural factor required for the inhibitory activity. It is known that piericidin A_1 inhibits respiration through the inhibition of mitochondrial NADH-ubiquinone oxidoreductases (Complex I) as a quinone antagonist.[16,17] To the best of our knowledge, this study is the first demonstration that piericidins are potent inhibitors of cell division during starfish embryogenesis.

Table 3. Inhibitory effects of **1–8** and **11–14** on cell division of starfish embryos

Compd		Minimum inhibitory concentration (μg/mL)
Piericidin C_5	(**1**)	0.09
Piericidin C_6	(**2**)	0.09
Piericidin C_1	(**3**)	0.09
Piericidin C_2	(**4**)	0.08
Piericidin C_3	(**5**)	0.08
Piericidin C_4	(**6**)	0.10
Piericidin D_1	(**7**)	0.75
Piericidin A_3	(**8**)	0.07
Triol of **1**	(**11**)	0.80
Triol of **2**	(**12**)	0.70
Acetonide of **1**	(**13**)	>20.0
Acetonide of **2**	(**14**)	>20.0

Experimental

General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter at the sodium D line (589 nm). CD spectra were recorded with a JASCO J-600 circular dichroic spectropolarimeter. UV and IR spectra were recorded on a Shimadzu UV-160A and a JASCO FT/IR-5300 spectrometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX500 spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C) at 25 °C. ^1H and ^{13}C NMR chemical shifts were referenced to solvent peaks: δ_H 7.26 and δ_C 77.0 for $CDCl_3$. FABMS and HRFABMS were measured on a JEOL SX102A spectrometer. Silica gel (Wacogel C-300) and ODS (Wacogel LP40C18) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) and RP_{18} F_{254} plates (Merck).

Fermentation

The *Streptomyces* sp. and the *Nocardioides* sp. were obtained from a soil of a province of east Kalimantan, Indonesia, according to the method of Hayakawa et al.[18] and they were cloned to afford strains. The fermentation of the microorganisms was carried out at 28 °C for 5 days under agitation at 220 rpm in a 300-mL Erlenmeyer flask containing 50 mL of a production medium (soluble starch 4.0%, soybean meal 1.0%, corn steep liquor 0.5%, dry yeast 0.5%, KH_2PO_4 0.5%, $ZnSO_4{\cdot}7H_2O$ 0.001%, $CoCl_2{\cdot}6H_2O$ 0.0001%, $NiSO_4$ 0.0001%, and $Mg_3(PO_4)_2{\cdot}8H_2O$ 0.05% adjusted to pH 7.0). The medium was inoculated with 5.0% of the volume of a seed culture prepared as follows. The organisms, *Streptomyces* sp. and *Nocardioides* sp., were first cultured for 4 days and 11 days, respectively, at 28 °C with vigorous shaking in a test tube (21 mm i.d.×200 mm) containing 10 mL of a seed medium [glucose 1.0%, soluble starch 1.0%, beef extract 0.3%, yeast extract 0.5%, bactotryptone 0.5%, KH_2PO_4 0.1%, $Mg_3(PO_4)_2{\cdot}8H_2O$ 0.05% adjusted to pH 7.0], and the culture (3.0%) was then inoculated into 50 mL of a seed medium in a 300-mL Erlenmeyer flask and cultured for 3 days at 28 °C on a rotary shaker.

Bioassay

Adult individuals of *Asterina pectinifera* were collected from the coastal waters off Japan during their breeding season and kept in seawater at 15 °C in laboratory aquaria. Experiments were performed at 20 °C and filtered seawater diluted to 90% (v/v) with distilled water was used throughout. Oocytes and sperm were removed from ovarian and testicular fragments, respectively. Oocyte maturation was induced by the treatment with 1 μM 1-methyladenine (Sigma, St. Louis, Missouri, USA).[19] Maturing oocytes were fertilized by the addition of the diluted sperm suspension, at 40 min after the start of the 1-methyladenine treatment. Fertilized eggs were washed three times with seawater. The MeOH solution of sample to be tested was added to the suspensions of embryos to give final concentrations of MeOH less than 0.2% in seawater. MeOH at the concentrations used had no effect on embryonic development. The embryos were periodically observed for any cytological changes.

Extraction and isolation

To the fermentation broth (600 mL) of a *Streptomyces* sp. was added equal volume of *i*-PrOH. The *i*-PrOH suspension was separated to the mycelial cake and supernatant by centrifugation. The supernatant was filtered and the filtrate was concentrated to afford an aqueous solution, which was extracted three times with EtOAc. The EtOAc layer was concentrated to a small volume under reduced pressure below 30 °C and dried over anhydrous Na_2SO_4. The crude extract (630 mg) was subjected to silica gel column chromatography (1.5 cm i.d.×85 cm) using 20–80% EtOAc in hexane. The biologically active fractions which showed the same R_f value on TLC were combined. Further purification of the individual fractions by column chromatography on ODS (MeOH–H_2O, 9:1) afforded **1** (13 mg), **3** (120 mg), **5** (120 mg), **7** (3.7 mg), and **8** (5.5 mg). In the same manner, the fermentation broth (500 mL) of a *Nocardioides* sp. was partitioned to afford the crude EtOAc extract (390 mg) and the subsequent purification by chromatography afforded **2** (4 mg), **4** (34 mg), and **6** (3 mg).

Piericidin C_5 (1). Pale yellow oil; $[\alpha]_D^{25}$ +7° (*c* 0.12, MeOH); UV (MeOH) λ_{max} 231 (ε 32,000), 236 (ε 32,300), 267 (ε 5,100) nm; IR (film) ν_{max} 3400, 1607 sh, 1588, 1472 cm^{-1}; ^1H NMR data: see Table 1; ^{13}C NMR data: see Table 2; (+)HRFABMS *m*/*z* 446.2909 $[M+H]^+$ (calcd for $C_{26}H_{40}NO_5$, 446.2906); (+)ESIMS *m*/*z* 446 $[M+H]^+$, 468 $[M+Na]^+$, 484 $[M+K]^+$; (−)ESIMS *m*/*z* 444 $[M-H]^-$; CD $\Delta\varepsilon_{236}$ −2.3±0.3 (4.5×10^{-5} M, MeOH).

Piericidin C_6 (2). Pale yellow oil; $[\alpha]_D^{25}$ +34° (*c* 0.03, MeOH); UV (MeOH) λ_{max} 225 (ε 20,600), 267 (ε 6,000) nm; IR (film) ν_{max} 3400, 1588, 1466 cm^{-1}; ^1H NMR data: see Table 1; ^{13}C NMR data: see Table 2; (+)HRFABMS *m*/*z* 460.3034 $[M+H]^+$ (calcd for $C_{27}H_{42}NO_5$, 460.3063); (+)FABMS *m*/*z* 460 $[M+H]^+$; (−)FABMS *m*/*z* 458 $[M-H]^-$; CD $\Delta\varepsilon_{236}$ −1.6±0.1 (3.2×10^{-5} M, MeOH).

Preparation of MTPA ester of 1. To a solution of **1** (3.4 mg) in dry pyridine (200 μL), was added (−)-MTPA chloride (15 μL) and stored at 4 °C overnight. The reaction mixture was purified by short silica gel column chromatography (EtOAc–hexane, 3:7) to afford 10,3′-bis-(*S*)-MTPA ester **9a** (5.8 mg). In the same way, by using (+)-MTPA chloride, **1** (3.4 mg) was converted into 10,3′-bis-(*R*)-MTPA ester **9b** (5.5 mg). **9a**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 1.02 (3H, d, *J* = 6.8 Hz, H_3-17), 1.05 (3H, t, *J* = 7.6 Hz, H_3-14), 1.25 (3H, s, H_3-18), 1.56 (1H, dq, *J* = 7.6, 6.4 Hz, H-13a), 1.63 (1H, dq, *J* = 7.6, 6.4 Hz, H-13b), 1.72 (6H, br s, H_3-15 and H_3-16), 1.90 (3H, s, H_3-6′), 2.80 (2H, d, *J* = 7.1 Hz, H_2-4), 2.88 (1H, t, *J* = 6.4 Hz, H-12), 2.91 (1H, ddq, *J* = 9.3, 8.6, 6.8 Hz, H-9), 3.40 (2H, d, *J* = 6.8 Hz, H_2-1), 3.52 (3H, s, 10-MTPA OCH_3), 3.72 (3H, s, 3′-MTPA OCH_3), 3.73 (3H, s, H_3-7′), 3.96 (3H, s, H_3-8′), 4.64 (1H, d, *J* = 8.9 Hz, H-10), 5.24 (1H, d, *J* = 6.3 Hz, H-8), 5.40 (1H, dt, *J* = 6.8, 1.0 Hz, H-2), 5.61 (1H, dt, *J* = 15.5, 7.1 Hz, H-5), 6.05 (1H, d, *J* = 15.5 Hz, H-6), 7.29, 7.42, 7.43, 7.75 (10H, m, MTPA phenyl protons); (+)FABMS *m*/*z* 878 $[M+H]^+$. **9b**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.99 (3H, d, *J* = 6.8 Hz, H_3-17), 1.07 (3H, t, *J* = 7.6 Hz, H_3-14), 1.35 (3H, s, H_3-18), 1.54 (3H, d, *J* = 0.8 Hz, H_3-16), 1.57 (1H, dq, *J* = 7.6, 6.4 Hz, H-13a), 1.66 (1H, dq, *J* = 7.6, 6.4 Hz, H-13b), 1.72 (3H, br s, H_3-15) 1.90 (3H, s, H_3-6′), 2.77 (2H, d, *J* = 7.1 Hz, H_2-4), 2.83 (1H, ddq, *J* = 9.3, 8.6, 6.8 Hz, H-9), 2.91 (1H, t, *J* = 6.4 Hz, H-12), 3.40 (2H, d, *J* = 6.8 Hz, H_2-1), 3.55 (3H, s, 10-MTPA OCH_3), 3.71 (3H, s, 3′-MTPA OCH_3), 3.73 (3H, s, H_3-7′), 3.97 (3H, s, H_3-8′), 4.60 (1H, d, *J* = 8.9 Hz, H-10), 5.19 (1H, br d, *J* = 6.3 Hz, H-8), 5.38 (1H, dt, *J* = 6.8, 1.0 Hz, H-2), 5.52 (1H, dt, *J* = 15.5, 7.1 Hz, H-5), 5.99 (1H, d, *J* = 15.5 Hz, H-6), 7.29, 7.42, 7.49, 7.75 (10H, m, MTPA phenyl protons); (+)FABMS *m*/*z* 878 $[M+H]^+$.

Preparation of MTPA ester of 2. Following the method similar to that used in the preparation of MTPA ester of **1**, by using (−)-MTPA chloride, **2** (1.5 mg) was converted into 10,3′-bis-(*S*)-MTPA ester **10a** (2.5 mg). In the same way, by using (+)-MTPA chloride, **2** (1.5 mg) was converted into 10,3′-bis-(*R*)-MTPA ester **10b** (2.5 mg). **10a**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 1.03 (3H, d, *J* = 6.8 Hz, H_3-18), 1.06 (3H, t, *J* = 7.6 Hz, H_3-14), 1.26 (3H, s, H_3-19), 1.56 (1H, dq, *J* = 7.6, 6.4 Hz, H-13a), 1.62 (3H, d, *J* = 1.2 Hz, H_3-16), 1.63 (1H, dq, *J* = 7.6, 6.4 Hz, H-13b), 1.64 (3H, br s, H_3-15), 1.73 (3H, d, *J* = 1.1 Hz, H_3-17), 1.90 (3H, s, H_3-6′), 2.69 (2H, s, H_2-4), 2.86 (1H, ddq, *J* = 9.5, 9.0, 6.8 Hz, H-9), 2.88 (1H, t, *J* = 6.4 Hz, H-12), 3.41 (2H, d, *J* = 6.8 Hz, H_2-1), 3.54 (3H, s, 10-MTPA OCH_3), 3.72 (3H, s, 3′-MTPA OCH_3), 3.74 (3H, s, H_3-7′), 3.97 (3H, s, H_3-8′), 4.65 (1H, d, *J* = 9.0 Hz, H-10), 5.15 (1H, d, *J* = 9.5 Hz, H-8), 5.38 (1H, dt, *J* = 6.8, 1.2 Hz, H-2), 5.63 (1H, s, H-6), 7.29, 7.35, 7.42, 7.43, 7.75 (10H, m, MTPA phenyl protons); (+)FABMS *m*/*z* 892 $[M+H]^+$. **10b**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 1.01 (3H, d, *J* = 6.8 Hz, H_3-18), 1.07 (3H, t, *J* = 7.6 Hz, H_3-14), 1.35 (3H, s, H_3-19), 1.54 (3H, d, *J* = 1.1 Hz, H_3-17), 1.59 (1H, dq, *J* = 7.6, 6.4 Hz, H-13a), 1.62 (3H, d, *J* = 1.2 Hz, H_3-16), 1.64 (3H, br s, H_3-15) 1.68 (1H, dq, *J* = 7.6, 6.4 Hz, H-13b), 1.90 (3H, s, H_3-6′), 2.68 (2H, s, H_2-4), 2.79 (1H, ddq, *J* = 9.5, 9.0, 6.8 Hz,

H-9), 2.91 (1H, t, $J=6.4$ Hz, H-12), 3.41 (2H, d, $J=6.8$ Hz, H_2-1), 3.55 (3H, s, 10-MTPA OCH_3), 3.72 (3H, s, 3′-MTPA OCH_3), 3.73 (3H, s, H_3-7′), 3.97 (3H, s, H_3-8′), 4.61 (1H, d, $J=9.0$ Hz, H-10), 5.08 (1H, br d, $J=9.5$ Hz, H-8), 5.38 (1H, dt, $J=6.8$, 1.2 Hz, H-2), 5.55 (1H, br s, H-6), 7.29, 7.42, 7.49, 7.75 (10H, m, MTPA phenyl protons); (+)FABMS m/z 892 $[M+H]^+$.

Reduction of 9a and 9b. To a suspension of $LiAlH_4$ (10 mg) in dry ether (2 mL), was added a mixed solution of **9a** and **9b** (11.3 mg) in dry ether (0.5 mL) at 0 °C. The mixture was stirred at room temperature for 2 h, cooled at 0 °C, treated with EtOAc (2 mL) and then MeOH (1 mL), poured into ice-cold water (10 mL), and extracted with EtOAc (10 mL). Aqueous layer was neutralized with 0.1 M HCl, and extracted with EtOAc again. Combined organic layer was washed with 5% $NaHCO_3$, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by short silica gel column chromatography (EtOAc–hexane, 3:7) to afford 10,11,3′-triol **11** (3.6 mg). **11**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.93 (3H, t, $J=7.1$ Hz, H_3-14), 1.09 (3H, d, $J=6.8$ Hz, H_3-17), 1.11 (3H, s, H_3-18), 1.35 (2H, m, H_2-13), 1.47 (1H, m, H-12a), 1.49 (1H, m, H-12b), 1.73 (3H, br s, H_3-15), 1.76 (3H, br s, H_3-16), 2.09 (3H, s, H_3-6′), 2.23 (1H, br s, 10-OH), 2.74 (1H, ddq, $J=10.3$, 6.8, 2.7 Hz, H-9), 2.77 (2H, d, $J=8$ Hz, H_2-4), 3.36 (1H, br s, H-10), 3.37 (2H, d, $J=6.8$ Hz, H_2-1), 3.86 (3H, s, H_3-7′), 3.95 (3H, s, H_3-8′), 5.41 (1H, br t, $J=6.8$ Hz, H-2), 5.50 (1H, br d, $J=10.3$ Hz, H-8), 5.58 (1H, dt, $J=15.6$, 6.8 Hz, H-5), 6.05 (1H, d, $J=15.6$ Hz, H-6), 6.19 (1H, br s, 3′-OH); (+)FABMS m/z 448 $[M+H]^+$.

Reduction of 10a and 10b. Following the method similar to that used in the reduction of **9a** and **9b**, a mixed solution of **10a** and **10b** (5.0 mg) was converted into 10,11,3′-triol **12** (1.8 mg). **12**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.93 (3H, t, $J=7.1$ Hz, H_3-14), 1.11 (3H, d, $J=6.8$ Hz, H_3-18), 1.15 (3H, s, H_3-19), 1.37 (2H, m, H_2-13), 1.46 (1H, m, H-12a), 1.50 (1H, m, H-12b), 1.66 (6H, br s, H_3-15 and H_3-16), 1.77 (3H, br s, H_3-17), 2.09 (3H, s, H_3-6′), 2.19 (1H, br s, 10-OH), 2.68 (2H, br s, H_2-4), 2.71 (1H, ddq, $J=10.3$, 6.8, 2.7 Hz, H-9), 3.34 (1H, dd, $J=6.4$, 2.7 Hz, H-10), 3.38 (2H, d, $J=6.8$ Hz, H_2-1), 3.86 (3H, s, H_3-7′), 3.95 (3H, s, H_3-8′), 5.40 (1H, br t, $J=6.8$ Hz, H-2), 5.38 (1H, br d, $J=10.3$ Hz, H-8), 5.66 (1H, br s, H-6), 6.16 (1H, br s, 3′-OH); (+)FABMS m/z 462 $[M+H]^+$.

Preparation of acetonide of 11. To a solution of **11** (3.1 mg) dissolved in 2,2-dimethoxypropane (1.0 mL), was added a catalytic amount of *p*-toluenesulfonic acid and stirred for 30 min at room temperature. The reaction mixture was diluted with $CHCl_3$, washed with 5% $NaHCO_3$, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was purified by short silica gel column chromatography (EtOAc–hexane, 3:7) to give acetonide **13** (3.1 mg). **13**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.92 (3H, t, $J=7.1$ Hz, H_3-14), 0.96 (3H, d, $J=6.8$ Hz, H_3-17), 1.13 (3H, s, H_3-18), 1.29 (3H, s, isopropylideneoxide CH_3a), 1.39 (3H, s, isopropylideneoxide CH_3b), 1.47 (2H, m, H_2-13), 1.58 (2H, m, H_2-12), 1.75 (6H, br s, H_3-15 and H_3-16), 2.09 (3H, s, H_3-6′), 2.68 (1H, m, H-9), 2.78 (2H, d, $J=6.8$ Hz, H_2-4), 3.37 (2H, d, $J=6.8$ Hz, H_2-1), 3.59 (1H, d, $J=8.1$ Hz, H-10), 3.85 (3H, s, H_3-7′), 3.95 (3H, s, H_3-8′), 5.40 (1H, br d, $J=9.0$ Hz, H-8), 5.41 (1H, br t, $J=6.8$ Hz, H-2), 5.54 (1H, dt, $J=15.6$, 6.8 Hz, H-5), 6.08 (1H, d, $J=15.6$ Hz, H-6), 6.16 (1H, br s, 3′-OH); (+)FABMS m/z 488 $[M+H]^+$.

Preparation of acetonide of 12. Following the method similar to that used in the preparation of acetonide of **11**, **12** (1.0 mg) was converted into acetonide **14** (1.0 mg). **14**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.92 (3H, t, $J=7.1$ Hz, H_3-14), 0.98 (3H, d, $J=6.8$ Hz, H_3-18), 1.15 (3H, s, H_3-19), 1.28 (3H, s, isopropylideneoxide CH_3a), 1.38 (3H, s, isopropylideneoxide CH_3b), 1.48 (2H, m, H_2-13), 1.57 (2H, m, H_2-12), 1.67 (3H, br s, H_3-16), 1.68 (3H, br s, H_3-15), 1.73 (3H, br s, H_3-17), 2.09 (3H, s, H_3-6′), 2.63 (1H, m, H-9), 2.69 (2H, s, H_2-4), 3.38 (2H, d, $J=6.8$ Hz, H_2-1), 3.57 (1H, d, $J=8.3$ Hz, H-10), 3.86 (3H, s, H_3-7′), 3.95 (3H, s, H_3-8′), 5.23 (1H, br d, $J=9.0$ Hz, H-8), 5.40 (1H, br t, $J=6.8$ Hz, H-2), 5.68 (1H, br s, H-6), 6.15 (1H, br s, 3′-OH); (+)FABMS m/z 502 $[M+H]^+$.

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