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α-Pyrones and Diketopiperazine Derivatives from the Marine-Derived Actinomycete *Nocardiopsis dassonvillei* HR10-5

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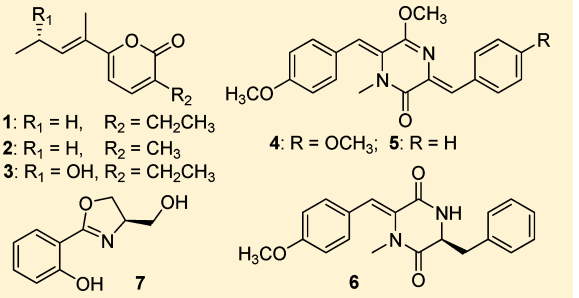
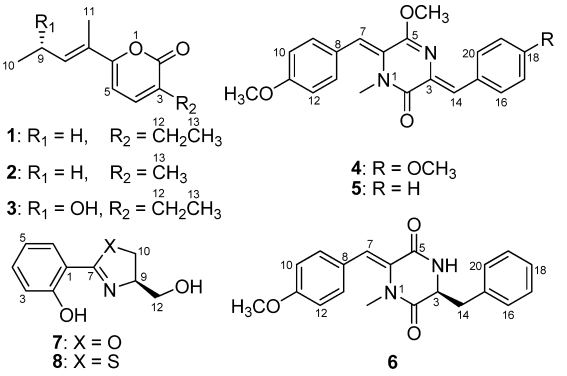
Supporting Information

ABSTRACT: Three new α-pyrones, nocapyrones E–G (**1**–**3**), and three new diketopiperazine derivatives, nocazines A–C (**4**–**6**), together with a new oxazoline compound, nocazoline A (**7**), were isolated from the marine-derived actinomycete *Nocardiopsis dassonvillei* HR10-5. The new structures of **1**–**7** were determined by spectroscopic analysis, X-ray single-crystal diffraction, CD spectra, and modified Mosher and Marfey methods. Compounds **1**–**3** showed modest antimicrobial activity against *Bacillus subtilis* with MIC values of 26, 14, and 12 μM, respectively.



With more than 13 000 bioactive metabolites described, cultured bacteria have been a prolific resource for drug discovery.[1] About 70% of these bioactive compounds have been isolated from cultured actinomycetes. The actinomycetes are best known as soil bacteria; thus, the majority of microbial drug discovery research has focused on terrestrial actinomycetes.[2] Unfortunately, beginning in the late 1980s, the rate of discovery of new drug candidates from terrestrial actinomycetes began to decrease,[3] which led many pharmaceutical researchers to abandon terrestrial actinomycetes in favor of alternate sources of chemical diversity such as the marine-derived actinomycetes.[4−9] The Yellow River delta located in the north of the Shandong peninsula is a typical coastal wetland, which possesses a complex ecosystem and rich biodiversity due to the interaction of land and sea.[10,11] Although the microorganisms in this special environment might be an important source for structurally new and bioactive compounds, very little research on secondary metabolites from these microbes has been undertaken. In order to search for new bioactive compounds, marine sediment samples were collected from the estuary of the Yellow River. From one sediment sample, the actinomycete strain HR10-5, which was identified as *Nocardiopsis dassonvillei*, was isolated and selected for chemical study because its secondary metabolites showed antibacterial activity against *Bacillus subtilis* at a concentration of 100 μg/mL. Other strains of *N. dassonvillei* had been reported to show antimicrobial activity[12] and produce a macrolide antibiotic.[13] A series of metabolites contained in the extract of strain HR10-5 showed UV absorptions similar to those of α-pyrones such as gibepyrones A–F by HPLC-UV analysis at 233 and 328 nm.[14] Additionally, α-pyrones are known to have antimicrobial activity.[14] Chemical investigation resulted in the isolation of three new α-pyrones (**1**–**3**), three new diketopiperazine derivatives (**4**–**6**), and a new oxazoline compound (**7**). These compounds were inactive against HL-60 and A549 tumor cell lines, but **1**–**3** display modest antimicrobial activity against *B. subtilis* with MIC values of 26, 14, and 12 μM, respectively.



Nocapyrone E (**1**) gave an HRESIMS peak at *m*/*z* 193.1237 $[M + H]^+$, corresponding to the molecular formula $C_{12}H_{16}O_2$. Its IR absorption at 1708 cm^{-1} and UV peaks at λ_{max} 233 and 328 nm are in accordance with an extended α-pyrone chromophore.[14] Analysis of the 1D NMR data for **1** revealed one carbonyl, three quaternary carbons, two methylenes, and three methyls (Table 1). The ^1H NMR spectrum showed two signals at δ_H 6.09 (d, *J* = 6.6 Hz) and 7.10 (d, *J* = 6.6 Hz), due to two vicinal protons in a 3,6-disubstituted α-pyrone system.

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Table 1. ^{1}H and ^{13}C NMR Data for 1−3 and 7 (600 and 150 MHz, $CDCl_3$, TMS, δ in ppm)

	1		2		3		7	
position	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1							110.5, C	
2	163.1, C		163.6, C		162.8, C		159.8, C	
3	128.6, C		123.1, C		129.8, C		116.8, CH	6.99, dd (8.2, 1.1)
4	138.4, CH	7.10, d (6.6)	140.1, CH	7.12, d (7.1)	138.0, CH	7.11, d (7.1)	133.7, CH	7.36, dt (8.2, 1.1)
5	100.8, CH	6.09, d (6.6)	100.8, CH	6.06, d (7.1)	102.1, CH	6.17, d (7.1)	118.9, CH	6.86, dt (7.7, 1.1)
6	159.7, C		159.9, C		158.8, C		128.3, CH	7.64, dd (8.3, 1.6)
7	125.4, C		125.4, C		126.6, C		166.9, C	
8	135.4, CH	6.54, dt (7.4, 1.1)	135.5, CH	6.54, t (7.4)	135.9, CH	6.50, d (8.2)		
9	21.9, CH_2	2.23, dq (7.4, 7.4)	22.0, CH_2	2.23, dq (7.4, 7.4)	64.9, CH	4.72, dq (8.1, 6.4)	67.0, CH	4.46, m
10	13.6, CH_3	1.06, t (7.4)	13.6, CH_3	1.06, t (7.7)	23.4, CH_3	1.34, d (6.4)	68.7, CH_2	4.44, dd (9.9, 1.8); 4.33, t (6.1)
11	12.4, CH_3	1.85, s	12.3, CH_3	1.85, s	12.7, CH_3	1.62, s		
12	23.5, CH_2	2.49, q (7.1)			23.6, CH_2	2.50, q (7.1)	64.0, CH_2	3.86, dd (11.5, 3.3); 3.68, dd (11.6, 3.8)
13	12.2, CH_3	1.17, t (7.1)	16.7, CH_3	2.09, s	12.4, CH_3	1.18, t (7.1)		
2-OH								11.96, brs

The ^{1}H−^{1}H COSY and HMBC correlations (Figure 1) further supported that a α-pyrone nucleus, a pent-2-en-2-yl residue, and an ethyl group were present in **1**, and the pent-2-en-2-yl and ethyl residues were linked to C-6 and C-3 of the α-pyrone nucleus, respectively. The NOESY correlation between H-9 and H_3-11 indicated the *E* configuration of the pent-2-en-2-yl residue (Figure 1). Thus, the structure of nocapyrone E (**1**) was determined to be (*E*)-3-ethyl-6-(pent-2-en-2-yl)-2*H*-pyran-2-one.

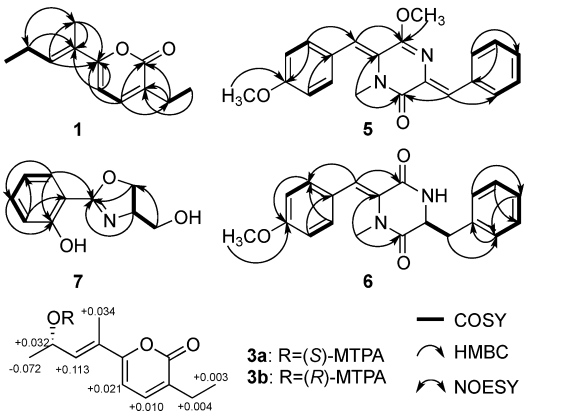

Figure 1. Selected 2D NMR correlations for **1** and **5**−**7** and $\Delta\delta$ ($=\delta_S - \delta_R$) values for (*S*)- and (*R*)-MTPA esters of **3**.

The molecular formula of nocapyrone F (**2**) was determined to be $C_{11}H_{14}O_2$ on the basis of the HRESIMS peak at m/z 179.1073 $[M + H]^+$, with one less CH_2 than **1**. The ^{1}H and ^{13}C NMR data of **2** (Table 1) were similar to those of **1**, except that a methyl signal (δ_H/δ_C 2.09/16.7) replaced the 3-ethyl signals at δ_H/δ_C 2.49/23.5 and 1.17/12.2. In addition, C-3 and C-4 were shifted upfield and downfield, respectively. These data indicated **2** as the C-3 methyl analogue of **1**. The NOESY correlation between H-9 and H_3-11 indicated the *E* configuration of the pent-2-en-2-yl residue. Nocapyrone F (**2**) was accordingly elucidated as (*E*)-3-methyl-6-(pent-2-en-2-yl)-2*H*-pyran-2-one.

The molecular formula of nocapyrone G (**3**) was determined to be $C_{12}H_{16}O_3$ by HRESIMS (m/z 209.1170 $[M + H]^+$), with one more oxygen atom than **1**. The ^{1}H and ^{13}C NMR data of **3** were almost the same as those of nocapyrone E (**1**), except for differences in the pentenyl unit. The lack of methylene group signals at δ_H/δ_C 2.23/21.9 and an additional oxygenated methine group signal at δ_H/δ_C 4.72/64.9 indicated that compound **3** was the hydroxylated derivative of **1** at C-9. This was confirmed by the downfield shifts of CH_3-10 and C-7. The NOESY correlation between H-9 and H-11 revealed the *E* configuration of the pentenyl moiety in **3**. The absolute configuration of C-9 was determined as *S* by the modified Mosher method.[15] When it was reacted with (*R*)- and (*S*)-MTPA chloride, compound **3** gave the corresponding (*S*)- and (*R*)-MTPA esters **3a**,**b**, respectively. The observed chemical shift differences $\Delta\delta_{S-R}$ (Figure 1) clearly defined the *S* configuration. Therefore, nocapyrone G (**3**) was identified as (*S*,*E*)-3-ethyl-6-(4-hydroxypent-2-en-2-yl)-2*H*-pyran-2-one.

Nocazine A (**4**) was obtained as colorless needles with the molecular formula $C_{22}H_{22}N_2O_4$ from the ESIMS peak at m/z 379.1665 $[M + H]^+$. Its ^{1}H and ^{13}C NMR data (Table 2) corresponded to one *N*-methyl group (δ_H/δ_C 3.00/35.2), three methoxy groups (δ_H/δ_C 3.83/54.3, 3.85/54.3, and 4.04/53.3), ten sp^2 methines, seven sp^2 quaternary carbons, and one amidocarbonyl (δ_C 161.8). Two sets of coupled ^{1}H NMR signals at δ_H 7.18 (d, J = 8.4 Hz), 6.89 (d, J = 8.6 Hz) and δ_H 8.09 (d, J = 8.8 Hz), 6.93 (d, J = 8.8 Hz) revealed the presence of two 1,4-disubstituted benzene rings that were further identified as two 4-OCH_3 phenyl units from the HMBC correlations between 11-OCH_3 and C-11 and between 18-OCH_3 and C-18. Those HMBC correlations from the olefinic proton H-7 to C-9 and C-5, from 5-OCH_3 to C-5, and from H-14 to C-16 and amidocarboyl carbon (C-2) identified the specific diketopiperazine core formed from two *p*-MeO-Phe moieties. The proposed structure was confirmed by X-ray crystal diffraction analysis, which further revealed the *Z* configurations of both Δ^6 and $\Delta^{3(14)}$ double bonds (Figure 2). The structure of nocazine A (**4**) was determined as (3*Z*,6*Z*)-5-methoxy-3,6-bis(4-methoxybenzylidene)-1-methyl-1,6-dihydropyrazin-2(3*H*)-one.

Nocazine B (**5**) was obtained as a yellow, amorphous powder. Its molecular formula was determined as $C_{21}H_{20}N_2O_3$ according to its HRESIMS peak at m/z 349.1546 $[M + H]^+$, with one less CH_2O unit than **4**. Its 1D NMR spectra were similar to those of **4**, except for the lack of a methoxy signal and

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Table 2. ^{1}H and ^{13}C NMR Data for 4−6 (600 and 150 MHz for 4 and 6, 500 and 125 MHz for 5, $CDCl_3$, TMS, δ in ppm)

	4		5		6	
position	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1-NCH_3	35.2, CH_3	3.00, s	36.1, CH_3	3.00, s	35.4, CH_3	2.90, s
2	161.8, C		162.6, C		167.5, C	
3	129.5, C		132.4, C		57.3, CH	4.33, m
4-NH						6.67, br s
5	155.0, C		156.7, C		164.4, C	
5-OCH_3	53.3, CH_3	4.04, s	54.4, CH_3	4.05, s		
6	126.2, C		128.6, C		129.5, C	
7	113.7, CH	6.79, s	115.3, CH	6.82, s	121.3, CH	7.03, s
8	125.9, C		126.8, C		125.7, C	
9/13	129.8, CH	7.18, d (8.4)	130.9, CH	7.19, d (8.4)	131.2, CH	7.11, d (8.8)
10/12	112.8, CH	6.89, d (8.6)	113.8, CH	6.89, d (8.7)	113.8, CH	6.89, d (8.8)
11	158.3, C		159.5, C		159.9, C	
11-OCH_3	54.3, CH_3	3.83, s	55.3, CH_3	3.83, s	55.4, CH_3	3.83, s
14	127.3, CH	7.24, s	127.3, CH	7.32, s	39.8, CH_2	3.33, dd (13.8, 3.9); 3.03, dd (13.7, 8.8)
15	127.3, C		135.5, C		135.4, C	
16/20	132.1, CH	8.09, d (8.8)	131.4, CH	8.10, d (8.6)	129.7, CH	7.24, d (7.1)
17/19	112.7, CH	6.93, d (8.8)	128.3, CH	7.39, dd (7.3, 8.2)	127.6, CH	7.31, dd (7.2, 7.7)
18	159.0, C		131.6, CH	7.31, t (7.3)	125.7, CH	7.22, t (7.7)
18-OCH_3	54.3, CH_3	3.85, s				

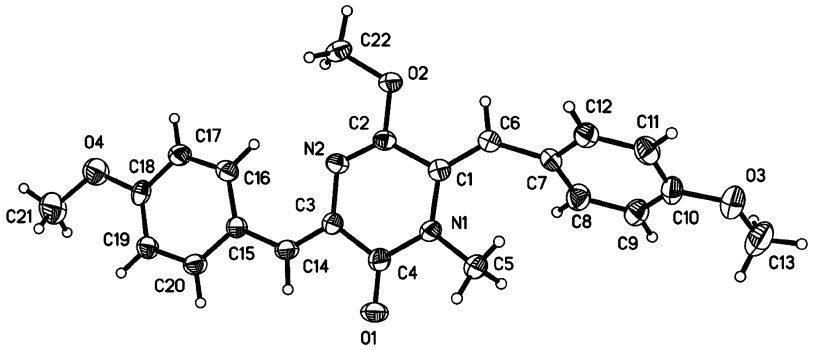

Figure 2. Final X-ray drawing of 4.

resonances for a monosubstituted benzene nucleus replaced those for a corresponding 1,4-disubstituted phenyl ring (Table 2). The HMBC correlations from the vinyl proton H-7 and 5-OCH_3 to C-5, from H-9/13 to C-7 and C-11, and from 11-OCH_3 to C-11, combined with the $^{1}H-^{1}H$ COSY correlations from H-16/20 (δ_H 8.10) to H-18 (δ_H 7.31) through H-17/19 (δ_H 7.39), indicated that the 18-OCH_3 of **4** was missing in **5** (Figure 1). Furthermore, the Z configurations of both Δ^{6} and $\Delta^{3(14)}$ double bonds in **5** could be deduced from the similarity of the chemical shifts of CH-7 and CH-14 to those of **4** (δ_H/δ_C 6.82/115.3 and 7.32/127.3 vs 6.79/113.7 and 7.24/127.3, respectively). Thus, the structure of **5** was elucidated as (3*Z*,6*Z*)-3-benzylidene-5-methoxy-6-(4-methoxybenzylidene)-1-methyl-1,6-dihydropyrazin-2(3*H*)-one.

The molecular formula for nocazine C (**6**) was assigned to be $C_{20}H_{20}N_2O_3$ from the molecular ion peak at m/z 337.1564 $[M + H]^+$ in the positive-ion HRESIMS spectrum. Its 1D NMR spectra displayed two amidocarbonyl carbon signals of a diketopiperazine at δ_C 164.4 and 167.5 and one amide proton signal at δ_H 6.67. Careful comparison of its ^{1}H and ^{13}C NMR spectra (Tables 2) with those of **4** showed that two methoxy signals, 18-OCH_3 and 5-OCH_3, were absent. Moreover, resonances for a trisubstituted double bond were replaced by a methine at δ_H/δ_C 4.33/57.3 and a methylene at δ_H/δ_C 3.33 and 3.03/39.8, indicating that the $\Delta^{3(14)}$ double bond was hydrogenated. HMBC correlations from H_2-14 to C-16 and C-20 and from H-3 to C-15 established the phenylalanine (Phe) unit, whose absolute configuration was determined as L by Marfey's method.[16] The 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) derivative of the acid hydrolysates of **6** gave the same retention time as that prepared from an authentic L-Phe sample in HPLC analysis (Figure S37, Supporting Information). The Z configuration of the Δ^{6} double bond in **6** could be deduced from the relative downfield shift of H-7 (δ_H 7.03) and comparison with the vinyl proton chemical shifts of the 6-benzylidene-substituted derivatives of piperazine-2,5-dione in the literature. The (*Z*)-vinyl proton is farther downfield than the (*E*)-vinyl proton because of the deshielding effect of the 5-ketone in this kind of compound, such as $\delta_{H\text{-}7}$ 7.17 in *threo*-(*Z*)-6-benzylidene-3-(α-methoxybenzyl)-1,4-dimethylpiperazine-2,5-dione[17,18] and $\delta_{H\text{-}7}$ 6.54 in (3*S*,6*E*)-3-benzyl-6-benzylidenepiperazine-2,5-dione.[19] In addition, there was no NOESY correlation between H-7 and 1-NCH_3, further suggesting the Z configuration of the double bond in **6**. Therefore, the structure of **6** was assigned as (3*S*,6*Z*)-3-benzyl-6-(4-methoxybenzylidene)-1-methylpiperazine-2,5-dione. It is noted that the constitution of **6** has been previously proposed, but without experimental data.[20]

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Nocazoline A (7) was isolated as a yellow oil and assigned the molecular formula $C_{10}H_{11}NO_3$ on the basis of the HRESIMS peak at m/z 194.0810 $[M + H]^+$. The IR spectrum showed the presence of a hydroxy group (3329 cm^{-1}), an aromatic ring (1617, 1492 cm^{-1}), and an imine group (1642 cm^{-1}). The ^1H NMR signals at δ_H 6.99 (dd, J = 8.2, 1.1 Hz), 7.36 (dt, J = 8.2, 1.1 Hz), 6.86 (dt, J = 7.7, 1.1 Hz), and 7.64 (dd, J = 8.3, 1.6 Hz) indicated that a 1,2-disubstituted phenyl nucleus was present in 7. A survey of the literature revealed that the ^1H and ^{13}C NMR spectra of 7 were very similar to those of aerugine (8),[21] except for downfield shifts of C-7 and C-10 (Table 1), showing that the sulfur of the aerugine thiazoline was replaced by oxygen. This deduction was confirmed by $^1H-^1H$ COSY correlations of H-10/H-9/H-12 and the key HMBC connections from H-6, H-9, and H-10 to C-7 (Figure 1). The R configuration of 7 was deduced from CD Cotton effects similar to those for aerugine[22] at 304 nm ($\Delta\varepsilon_{max}$ −0.8) and 248 nm ($\Delta\varepsilon_{max}$ +1.5). The specific rotation of 7 ($[\alpha]^{25}_D$ = +15°) is similar to that of (*R*)-4-hydroxymethyl-2-phenyl-4,5-dihydrooxazole ($[\alpha]^{25}_D$ = +35°)[23] and has the opposite sign compared to the rotation of (*S*)-4-hydroxymethyl-2-phenyl-4,5-dihydrooxazole ($[\alpha]^{25}_D$ = −82°),[24] which further supports the *R* configuration of 7. Thus, nocazoline A (7) was identified as (*R*)-2-(2-hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole.

Compounds **1**−**7** were tested for cytotoxic effects on the HL-60 cell line using the MTT method[25] and on the A549 cell line using the SRB method.[26] These compounds did not show significant cytotoxic activities ($IC_{50} \geq 100$ μM). The antimicrobial activities against *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans* were also evaluated by an agar dilution method.[27] Compounds **1**−**3** showed modest antimicrobial activity against *Bacillus subtilis* with MIC values of 26, 14, and 12 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. CD spectra were measured on a JASCO J-715 spectropolarimeter. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer as KBr disks. NMR data of **1**−**4**, **6**, and 7 were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. NMR spectra of **5** were recorded on a Bruker Avance 500 spectrometer. ESIMS measurements were carried out on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4.0 mL/min). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory), and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) utilized silica gel H (Qingdao Marine Chemical Factory). The marinum salt used was made from the evaporation of seawater collected in Laizhou Bay (Weifang Haisheng Chemical Factory).

Actinomycete Material. The actinomycete *Nocardiopsis dassonvillei* HR10-5 was isolated from marine sediments collected from the estuary of Yellow River, Dongying, People's Republic of China. The marine sediments (2 g) were air-dried for 15 days in a 45 mL sterile centrifuge tube. The dried sediments were diluted to 10^{-3} g/mL, 100 μL of which was dispersed across a solid-phase agar plate (Gause's synthetic agar media) and incubated at 28 °C for 10 days. A single colony was transferred to Gause's synthetic agar media and was identified according to its morphological characteristics and 16S rRNA gene sequences (Supporting Information, GenBank accession No. JN253591). A reference culture is maintained in our laboratory at −80 °C. The producing strain was prepared on Gause's synthetic agar slants and stored at 4 °C.

Fermentation and Extraction. Spores were directly inoculated into 500 mL Erlenmeyer flasks containing 150 mL of fermentation media (glucose 20 g, beef extract 3 g, yeast extract 10 g, soluble starch 10 g, peptone 10 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.5 g, $CaCO_3$ 2 g, and marinum salt 33 g, dissolved in 1 L of tap water, pH 7.0). The flasks were incubated on a rotatory shaker at 180 rpm at 28 °C for 8 days. At harvest, 60 L of whole broth was extracted three times with EtOAc (90 L each). The EtOAc extract was concentrated under reduced pressure to give a dark brown gum (30 g).

Purification. The EtOAc extract (30 g) was separated into seven fractions on a silica gel VLC column using step gradient elution with CH_2Cl_2−petroleum ether (50%−100%) and then with MeOH−CH_2Cl_2 (0−50%). Fraction 1 (3.5 g) was further separated into two subfractions by Sephadex LH-20, with MeOH−CH_2Cl_2 (1:2) as eluent. Subfraction 1-2 (1.1 g) was further purified by semipreparative HPLC (80% MeOH−H_2O) to yield **2** (52 mg, t_R = 7.2 min) and **1** (310 mg, t_R = 8.6 min). Fraction 2 (2.4 g) was separated into four subfractions by Sephadex LH-20, with MeOH−CH_2Cl_2 (1:2) as eluent. Subfraction 2-2 (720 mg) was additionally separated by semipreparative HPLC (85% MeOH−H_2O) to yield **5** (3.9 mg, t_R = 11.6 min) and **4** (14.3 mg, t_R = 15.2 min). Fraction 3 (2.1 g) was separated into three subfractions by Sephadex LH-20, with MeOH−CH_2Cl_2 (1:1) as eluent. Subfraction 3-2 (670 mg) was separated by semipreparative HPLC (65% MeOH−H_2O, 4.0 mL/min) to yield **3** (12.5 mg, t_R = 7.8 min) and **6** (41.0 mg, t_R = 12.2 min). Subfraction 3-3 (520 mg) was further separated by semipreparative HPLC (60% MeOH−H_2O) to yield 7 (30.3 mg, t_R = 9.5 min).

Nocapyrone E (1): colorless oil; UV (MeOH) λ_{max} (log ε) 233 (3.62), 328 (3.60) nm; IR (KBr) ν_{max} 2973, 2937, 2879, 1708, 1638, 1577, 1459, 1379, 1273, 1122, 1092, 1025, 960 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS m/z 193.1237 $[M + H]^+$ (calcd for $C_{12}H_{17}O_2$ 193.1229).

Nocapyrone F (2): colorless oil; UV (MeOH) λ_{max} (log ε) 233 (3.54), 328 (3.51) nm; IR (KBr) ν_{max} 2928, 1706, 1641, 1579, 1456, 1377, 1345, 1278, 1122, 1090, 994 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS m/z 179.1073 $[M + H]^+$ (calcd for $C_{11}H_{15}O_2$ 179.1072).

Nocapyrone G (3): colorless oil; $[\alpha]^{25}_D$ = −37° (c 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (3.89), 321 (3.84) nm; IR (KBr) ν_{max} 3426, 2968, 2928, 2868, 1686, 1645, 1559, 1449, 1252, 1121, 1062, 1024 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS m/z 209.1170 $[M + H]^+$ (calcd for $C_{12}H_{17}O_3$ 209.1178).

Nocazine A (4): colorless needles (MeOH); mp 120 °C; UV (MeOH) λ_{max} (log ε) 242 (3.18), 371 (3.62) nm; IR (KBr) ν_{max} 2921, 2851, 1664, 1601, 1508, 1463, 1253, 1175, 1102, 1033, 800 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 2; HRESIMS m/z 379.1665 $[M + H]^+$ (calcd for $C_{22}H_{23}N_2O_4$ 379.1658).

Nocazine B (5): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 242 (3.35), 367 (3.85) nm; IR (KBr) ν_{max} 3409, 2919, 2851, 1665, 1604, 1465, 1347, 1252, 1175, 1106, 1033 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 2; HRESIMS m/z 349.1546 $[M + H]^+$ (calcd for $C_{21}H_{21}N_2O_3$ 349.1552).

Nocazine C (6): yellow, amorphous powder; $[\alpha]^{25}_D$ = −307° (c 1.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 221 (3.66), 306 (3.83) nm; IR (KBr) ν_{max} 3438, 2953, 1684, 1627, 1501, 1370, 1253, 1176, 1110, 1029, 832 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 2; HRESIMS m/z 337.1564 $[M + H]^+$ (calcd for $C_{20}H_{21}N_2O_3$ 337.1552).

Nocazoline A (7): yellow oil; $[\alpha]^{25}_D$ = +15° (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (4.05), 234 (3.98), 295 (3.70) nm; CD (MeOH) λ_{max} (Δε) 304 (−0.8), 248 (+1.5), 233 (+0.5), 219 (+2.6), 210 (−0.7), 199 (+2.9) nm; IR (KBr) ν_{max} 3329, 2930, 1642, 1617, 1492, 1368, 1309, 1260, 1233, 1159, 1130, 1063, 958, 829, 756 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS m/z 194.0810 $[M + H]^+$ (calcd for $C_{10}H_{12}NO_3$ 194.0817).

Preparation of the (*S*)-and (*R*)-MTPA Esters of 3 by the Modified Mosher Method. Compound **3** (2 mg) was dissolved in 500 μL of pyridine, and dimethylaminopyridine (3 mg) and (*R*)-MTPACl (8 μL) were then added in sequence. The reaction mixture

was stirred for 24 h at room temperature, and 1 mL of H_2O was then added. The solution was extracted by 5 mL of CH_2Cl_2 and the organic phase was concentrated under reduced pressure. Then the residue was purified by semipreparative HPLC (83% MeOH−H_2O) to yield (*S*)-MTPA ester **3a** (1.0 mg, t_R = 9.2 min). By the same procedure, (*R*)-MTPA ester **3b** (1.7 mg, t_R 9.2 min) was obtained from the reaction of **3** (2 mg) with (*S*)-MTPACl (8 μL). (*S*)-MTPA ester (**3a**): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.12 (1H, d, *J* = 7.1 Hz, H-4), 6.47 (1H, d, *J* = 9.4 Hz, H-8), 6.22 (1H, d, *J* = 7.1 Hz, H-5), 5.94 (1H, m, H-9), 2.51 (2H, q, *J* = 7.7 Hz, H-12), 2.00 (3H, s, H-11), 1.40 (3H, d, *J* = 6.6 Hz, H-10), 1.18 (3H, t, *J* = 7.7 Hz, H-13); ESIMS *m/z* 425 $[M + H]^+$. (*R*)-MTPA ester (**3b**): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.11 (1H, d, *J* = 7.1 Hz, H-4), 6.36 (1H, d, *J* = 8.8 Hz, H-8), 6.20 (1H, d, *J* = 7.1 Hz, H-5), 5.91 (1H, m, H-9), 2.51 (2H, q, *J* = 7.7 Hz, H-12), 1.97 (3H, s, H-11), 1.47 (3H, d, *J* = 6.1 Hz, H-10), 1.18 (3H, t, *J* = 7.7 Hz, H-13); ESIMS *m/z* 425 $[M + H]^+$.

X-ray Structure Determination of Nocazine A (4). Compound **4** was obtained as a colorless monoclinic crystal: molecular formula $C_{22}H_{22}N_2O_4$, space group $P2_1/n$, *a* = 7.1550(8) Å, *b* = 25.307(2) Å, *c* = 12.83410(11) Å, α = 90.00°, β = 123.880(2)°, γ = 90.00°, *V* = 1929.3(3) Å^3, *Z* = 4, D_{calcd} = 1.303 Mg/m^3, μ = 0.090 mm^{-1}, *F*(000) = 800, crystal size 0.28 × 0.17 × 0.15 mm, *T* = 298(2) K. A total of 3413 unique reflections (2θ < 50°) were collected on a CCD area detector diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques (SHELXL-97). The final cycle of full-matrix least-squares refinement was based on 3413 unique reflections (2θ < 50°) and 257 variable parameters and converged with unweighted and weighted agreement factors of R1 = 0.1468, wR2 = 0.0882, and *R* = 0.0525 for I > 2σ(*I*) data. Crystallographic data (excluding structure factors) for structure **4** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 809170. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax, +44 (0)-1223-336033; e-mail, deposit@ccdc.cam.ac.uk).

Absolute Configuration Determination of Phenylalanine of 6 by Marfey's Method.[16] A solution of **6** (1.5 mg) in 6 M HCl (1 mL) was heated to 105 °C for 19 h. The solution was then evaporated to dryness and the residue redissolved in H_2O (250 μL). A 50 μL portion of the acid hydrolysate solution was then placed in a 1 mL reaction vial and treated with a 1% solution of FDAA (200 μL) in acetone followed by 1.0 M $NaHCO_3$ (40 μL). The reaction mixture was heated at 45 °C for 1 h, cooled to room temperature, and then acidified with 2.0 M HCl (20 μL). In a similar fashion, standard D- and L-Phe were derivatized separately. The derivatives of the hydrolysates and standard amino acids were subjected to HPLC analysis (YMC C_{18} column; 5 μm, 4.6 × 250 mm; 1.0 mL/min) at 30 °C using the following gradient program: solvent A, water + 0.2% TFA; solvent B, MeCN; linear gradient 0 min 25% B, 40 min 60% B, 45 min 100% B; UV detection at 340 nm. The retention times for the FDAA derivatives of hydrolysates of **6**, standard L-Phe, and D-Phe were 21.0, 21.0, and 24.6 min, respectively (Figure S34, Supporting Information).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Text giving bioassay protocols used and 16S rRNA gene sequences of *Nocardiopsis dassonvillei* HR10-5, figures giving NMR spectra of compounds **1**−**7** and HPLC profiles of acidic hydrolysate of **6**, and a CIF file giving crystallographic data for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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