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# Cytotoxic Bipyridines from the Marine-Derived Actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6

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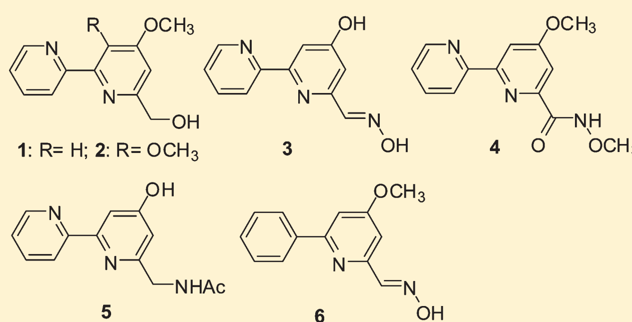
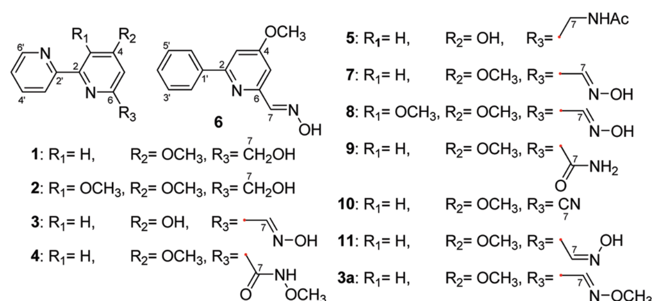
Supporting Information

**ABSTRACT:** Five new bipyridine alkaloids (**1**−**5**) and a new phenylpyridine alkaloid (**6**), which we name caerulomycins F−K, along with five known analogues (**7**−**11**), were isolated from the marine-derived actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6. The structures of **1**−**6** were established on the basis of spectroscopic analyses and chemical methods. Compounds **1**−**10** showed cytotoxicity against the HL-60, K562, KB, and A549 cell lines, with $IC_{50}$ values of 0.26 to 15.7 $\mu$M. Compounds **7** and **8** also showed antimicrobial activities against *Escherichia coli, Aerobacter aerogenes, Pseudomonas aeruginosa*, and *Candida albicans*, with MIC values of 9.7 to 38.6 $\mu$M.




The actinomycetes are a prolific source of structurally new and biologically active metabolites and are responsible for producing over 45% of all known microbial natural products.[1] Most research on actinomycetes has been carried out with terrestrial species.[2,3] However, the discovery of new lead compounds from common soil-derived actinomycetes has declined in the past two decades.[4] Examining the natural product profiles of the underexploited marine-derived actinomycetes has become a research hot spot in drug discovery. To date, nearly 400 new compounds with cytotoxicity and antimicrobial activity have been isolated from marine actinomycetes.[5−8] As part of our ongoing research on new antitumor compounds from marine-derived actinomycetes, strain WH1-2216-6, identified as *Actinoalloteichus cyanogriseus*, was isolated from marine sediments and was found to exhibit significant cytotoxic effects on the K562 cell line. A series of metabolites contained in the extract of strain WH1-2216-6 showed UV absorptions similar to those of bipyridine alkaloids such as caerulomycins A−E, caerulomycinonitrile, and caerulomycinamide in HPLC-UV analysis at 230 and 270 nm.[9−13] Bipyridine alkaloids are known to have antibiotic,[10,14] phytotoxic,[12] and immunosuppressant activity.[15] A chemical investigation resulted in the isolation of five new bipyridine alkaloids and a new phenylpyridine alkaloid, which we have named caerulomycins F−K (**1**−**6**). Five known analogues, caerulomycin A (**7**),[16,17] caerulomycin C (**8**),[18,19] caerulomycinamide (**9**),[11] caerulomycinonitrile (**10**),[11] and (*Z*)-4-methoxy-2,2′-bipyridine-6-carbaldehyde oxime (**11**), were also isolated.[17] Potent cytotoxicities were observed for compound **3** against HL-60, compound **4** against K562, compound **8** against K562, and compound **7** against HL-60 and A549 cells, with $IC_{50}$ values of 1.6, 0.37, 1.8, 0.71, and 0.26 $\mu$M, respectively. Compounds **7** and **8** also showed antimicrobial activities against *Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans* with MIC values of 10.9/9.7, 21.8/38.6, and 21.8/19.3 $\mu$M, respectively.




## RESULTS AND DISCUSSION

The bioactive EtOAc extract of *A. cyanogriseus* WH1-2216-6 was chromatographed on a silica gel column, and extensive reversed-phase preparative HPLC was carried out to give compounds **1**−**11**. Among them, **7** was the major compound isolated (4 g from a 50 L culture, compared to all other compounds, which were isolated in quantities less than 50 mg). Its molecular formula was tentatively assigned as $C_{12}H_{11}N_3O_2$ on the basis of a molecular ion peak at *m/z* 230 $[M + H]^+$ observed by ESIMS. Analysis of the 1D $^{13}C$ NMR data for **7** revealed four quaternary carbons, seven methylene carbons, and one methoxyl carbon. The $^1H$ NMR spectrum (Table S1) showed four signals at 8.40

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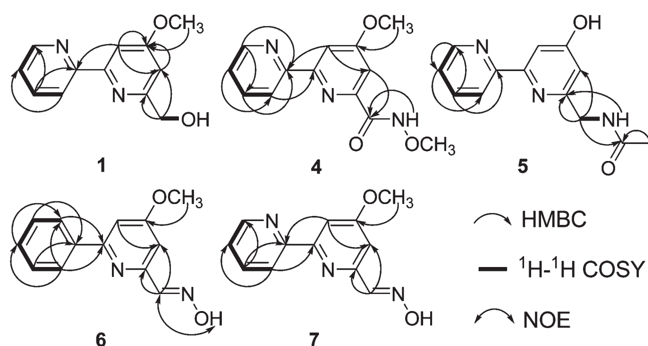


**Figure 1.** Key HMBC, $^1$H−$^1$H COSY, and NOE correlations for **1** and **4**−**7**.

(d, $J$ = 7.7 Hz), 7.96 (td, $J$ = 7.7, 1.9 Hz), 7.48 (ddd, $J$ = 7.7, 4.5, 1.3 Hz), and 8.70 (d, $J$ = 3.9 Hz), which were assigned to a 2-disubstituted pyridine ring system, and two signals at 7.92 (d, $J$ = 2.6 Hz) and 7.34 (d, $J$ = 2.6 Hz) assigned to a 2,4, 6-trisubstituted pyridine ring system. HMBC correlations (Figure 1) from H-3′ to C-2 and from H-3 to C-2′ identified a bipyridine structure. Further comparison of NMR spectra (Table S1) with reported data[16,17] indicated that compound **7** was caerulomycin A.

Caerulomycin F (**1**) was obtained as a white, amorphous powder. Its molecular formula was assigned as $C_{12}H_{12}N_2O_2$ from the HRESIMS peak at $m/z$ 217.0976 [M + H]$^+$, which requires eight degrees of unsaturation. The IR spectrum showed the presence of a hydroxy group (3450 cm$^{-1}$) and an aromatic system (1652 cm$^{-1}$). With the exception that an oxygenated methylene signal ($\delta_{C/H}$ 64.2/4.62) in **1** replaced an oxime methine signal ($\delta_{C/H}$ 148.8/8.13) in **7**, the $^1$H and $^{13}$C NMR spectra of **1** (Table 1) were very similar to those of **7** (Table S1).[16,17] This suggested that the same 4-methoxy-2,2′-bipyridine skeleton found in **7** was present in **1**. Upfield shifts for C-2 to C-6 indicated that **1** was the hydroxymethyl-substituted derivative of **7**. 2D NMR correlations (Figure 1) also support the structure of **1**. Thus, the structure of compound **1** was determined to be 4-methoxy-2,2′-bipyridine-6-methanol.

Caerulomycin G (**2**) was obtained as colorless needles. The molecular formula was determined to be $C_{13}H_{14}N_2O_3$ from the [M + H]$^+$ peak at $m/z$ 247.1079 in the HRESIMS spectrum. The IR spectrum established the presence of hydroxy groups (3622 cm$^{-1}$) and an aromatic system (1553 cm$^{-1}$). The $^1$H and $^{13}$C NMR data of **2** (Table 1) were almost identical to those of **1** except that the aromatic methine signal at $\delta_{C/H}$ 106.4/7.08 in **1** was replaced by a methoxy signal at $\delta_{C/H}$ 61.5/3.70 and an aromatic quaternary carbon signal at $\delta_C$ 142.6. In addition, upfield shifts were observed for C-2 to C-4, supporting the postulation that **2** was the 3-methoxy-substituted derivative of **1**. Therefore, compound **2** was identified as 3,4-dimethoxy-2,2′-bipyridine-6-methanol.

Caerulomycin H (**3**) was obtained as a white, amorphous powder, and the molecular formula $C_{11}H_9N_3O_2$ was assigned on the basis of the HRESIMS peak at $m/z$ 216.0781 [M + H]$^+$. Careful comparison of its $^1$H and $^{13}$C NMR spectra (Table 1) with those of **7** (Table S1) revealed that **3** contained the same 2,2′-bipyridine-6-carbaldehyde oxime skeleton, but that the $CH_3O$-4 of **7** was replaced by HO-4 in **3**. To confirm the structure of compound **3**, both HO-8 and HO-4 of **3** and HO-8 of **7** were methylated, which resulted in the production of a common product as expected (**3a**). 1D NMR data of the oxime moiety in **3** were very similar to those of **7**, which indicated that the oxime moiety of **3** was also of the $E$ configuration. Thus, the structure of compound **3** was determined to be ($E$)-4-hydroxy-2,2′-bipyridine-6-carbaldehyde oxime.

Caerulomycin I (**4**) was obtained as colorless needles, and the molecular formula $C_{13}H_{13}N_3O_3$ was assigned on the basis of the HRESIMS peak at $m/z$ 282.0855 [M + Na]$^+$. The $^1$H and $^{13}$C NMR spectra (Table 1) of **4** were very similar to those of **9** (Table S1),[11] with the exception that an amide proton signal at $\delta_H$ 8.45 (1H, brs) was replaced by a methoxy signal at $\delta_{C/H}$ 63.9/3.78. Furthermore, another amide proton signal was shifted downfield to $\delta$ 12.15, suggesting that **4** was the methoxy-substituted derivative of **9** at the amide nitrogen. The nearly identical NMR data of the methoxy to those of $O$-methylbenzohydroxamic acid ($\delta_{C/H}$ 63.9/3.78 vs 63.1/3.71)[20,21] established the placement of the methoxy group on the amido nitrogen atom, and the assignment was further supported by key cross-peaks from H-3 ($\delta_H$ 8.04) and the amide group proton ($\delta_H$ 12.15) to C-7 ($\delta_C$ 161.3) in the HMBC spectrum (Figure 1). Therefore, the structure of compound **4** was identified as $N$,4-dimethoxy-2,2′-bipyridine-6-carboxamide.

The molecular formula of caerulomycin J (**5**, isolated as a yellow oil) was determined as $C_{13}H_{13}N_3O_2$ on the basis of the molecular ion peak at $m/z$ 266.0905 [M + Na]$^+$ in the HRESIMS spectrum. With the exception of the oxime moiety, the $^1$H and $^{13}$C NMR spectra of **5** were similar to those of **3** (Table 1), indicating the same molecular skeleton. Compared with **3**, the $^1$H and $^{13}$C NMR spectra (Table 1) of **5** showed additional signals for a methylene ($\delta_{C/H}$ 44.6/4.30) and an acetyl group ($\delta_{C/H}$ 170.1 and 23.2/1.92), while the oxime methine signals ($\delta_{C/H}$ 149.6/8.09) in **3** were not observed. These observations suggested that the oxime group of **3** was reduced to the corresponding aminomethyl in **5**. This postulation was confirmed by a key $^1$H−$^1$H COSY correlation between the NH ($\delta_H$ 8.44) and the $CH_2$ ($\delta_H$ 4.30) and key HMBC correlations between the NH and C-6 ($\delta_C$ 159.9) and between the $CH_2$ and C-5 ($\delta_C$ 109.3) and C-6 (Figure 1). HMBC correlations observed between the $CH_2$ and the acetyl carbon indicated that the acetyl was connected to the NH. Thus, the structure of **5** was elucidated as $N$-[(4-hydroxy-2,2′-bipyridine-6-yl)methyl]-acetamide.

Caerulomycin K (**6**) was isolated as colorless needles. Its molecular formula was assigned as $C_{13}H_{12}N_2O_2$ on the basis of a HRESIMS peak at $m/z$ 229.0968 [M + H]$^+$. The IR spectrum showed the presence of hydroxy groups and aromatic rings. Two sets of coupled $^1$H NMR signals at $\delta_H$ 8.10 (2H, d, $J$ = 8.3 Hz), 7.49 (2H, t, $J$ = 7.8 Hz), 7.44 (t, $J$ = 7.3 Hz) and 7.48 (br s), 7.26 (d, $J$ = 2.3 Hz) revealed the presence of a monosubstituted benzene ring and a 2,4,6-trisubstituted pyridine ring, and these assignments were also supported by the $^{13}$C NMR spectrum (Table 1). A methoxy signal at $\delta_{C/H}$ 55.6/3.93 and an oxime signal at $\delta_{C/H}$ 149.0/8.13 and $\delta_H$ 11.72 were also observed. These data were very similar to those of **7** (Table S1). Further comparison of their 1D NMR spectra indicated that one pyridine ring of **7** was replaced by a benzene ring in **6**. This deduction was confirmed by cross-peaks in the $^1$H−$^1$H COSY spectrum from H-2′/6′ ($\delta_H$ 8.10) to H-4′ ($\delta_H$ 7.44) through H-3′/5′ ($\delta_H$ 7.49) and key HMBC correlations from H-2′/6′ to C-2 ($\delta_C$ 157.6), from H-7 ($\delta_H$ 8.13) to C-5 ($\delta_C$ 103.8) and C-6 ($\delta_C$ 153.5), and from H-3 ($\delta_H$ 7.48) to C-5 (Figure 1). Meanwhile, an NOE correlation between H-7 and HO-8 indicated that the oxime

Table 1. $^1$H and $^{13}$C NMR Data for 1−6 (600 MHz, 150 MHz, DMSO-$d_6$, TMS, $\delta$ ppm)

| | 1 | | 2 | | 3 | | 4 | | 5[a] | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
| 2 | 155.0, C | | 150.8, C | | 157.3, C | | 157.0, C | | 149.7, C | | 157.6, C | |
| 3 | 106.4, CH | 7.80, d (2.3) | 142.6, C | | 107.5, CH | 7.79, d (2.2) | 108.7, CH | 8.04, d (2.3) | 107.2, CH | 7.61, brs | 106.9, CH | 7.48, s |
| 3-OCH$_3$ | | | 61.5, CH$_3$ | 3.70, s | | | | | | | | |
| 4 | 167.0, C | | 160.2, C | | 165.7, C | | 167.9, C | | 166.5, C | | 166.6, C | |
| 4-OH (4-OCH$_3$) | 55.3, CH$_3$ | 3.92, s | 56.4, CH$_3$ | 3.94, s | | 10.95, brs | 56.3, CH$_3$ | 3.96, s | | 11.09, brs | 55.6, CH$_3$ | 3.93, s |
| 5 | 104.1, CH | 7.08, d (2.3) | 105.2, CH | 7.22, s | 108.9, CH | 7.21, d (2.2) | 108.9, CH | 7.54, d (2.3) | 109.3, CH | 6.65, brs | 103.8, CH | 7.26, d (2.3) |
| 6 | 163.7, C | | 157.0, C | | 153.9, C | | 151.5, C | | 159.9, C | | 153.5, C | |
| 7 | 64.2, CH$_2$ | 4.62, s | 64.7, CH$_2$ | 4.51, d (6.7) | 149.6, CH | 8.09, s | 161.3, C | | 44.6, CH$_2$ | 4.30, d (5.9) | 149.0, CH | 8.13, s |
| 7-OH (NH) | | 5.52, brs | | 5.47, d (6.6) | | | | 12.15, s | | 8.44, t (5.9) | | |
| N-OH (N-OCH$_3$) | | | | | | 11.67, s | 63.9, CH$_3$ | 3.78, s | | | | 11.72, s |
| 1′ | | | | | | | | | | | 138.2, C | |
| 2′ | 156.2, C | | 158.7, C | | 155.3, C | | 154.5, C | | 155.7, C | | 128.6, CH | 8.10, d (8.3) |
| 3′ | 120.7, CH | 8.37, d (7.7) | 124.5, CH | 7.64, d (7.5) | 121.2, CH | 8.36, d (7.7) | 122.4, CH | 8.86, d (8.2) | 121.2, CH | 8.34, d (8.3) | 126.8, CH | 7.49, t (7.8) |
| 4′ | 137.2, CH | 7.93, td (7.8, 1.9) | 136.9, CH | 7.87, t (7.5) | 137.8, CH | 7.93, t (6.6) | 137.7, CH | 7.96, td (7.8, 1.8) | 137.7, CH | 7.91, td (7.8, 1.8) | 129.3, CH | 7.44, t (7.3) |
| 5′ | 124.3, CH | 7.45, ddd (7.5, 4.9, 1.3) | 123.5, CH | 7.40, dd (7.5, 4.4) | 124.9, CH | 7.45, t (6.6) | 125.3, CH | 7.47, ddd (7.3, 4.6, 0.9) | 124.6, CH | 7.42, dd (6.4, 4.6) | 126.8, CH | 7.49, t (7.8) |
| 6′ | 148.1, CH | 8.68, d (4.1) | 149.2, CH | 8.64, d (5.5) | 149.8, CH | 8.67, d (4.6) | 149.6, CH | 8.67, d (4.1) | 149.7, CH | 8.64, d (4.1) | 128.6, CH | 8.10, d (8.3) |

[a] The NMR data for the acetyl are $\delta_H$ 1.92 (s, 3H) and $\delta_C$ 23.2 (CH$_3$) and 170.1 (C).

**Scheme 1. Plausible Biogenetic Pathways of 1−5 and 7−10**

* [Me]: methylation

**Table 2. Cytotoxicities against Tumor Cells for 1−10 and 3a ($IC_{50}$, $\mu M$)**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 3a |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HL-60 | >50 | >50 | 1.6 | >50 | >50 | >50 | 0.71 | >50 | >50 | >50 | >50 |
| K562 | 15.7 | NT[a] | NT | 0.37 | 15.0 | >50 | >50 | 1.8 | NT | 15.0 | 31.8 |
| A549 | >50 | >50 | 8.4 | >50 | >50 | >50 | 0.26 | >50 | >50 | >50 | >50 |
| KB | >50 | NT | NT | 5.2 | 25.7 | >50 | >50 | 3.1 | NT | >50 | >50 |

[a] NT = not tested.

moiety had the *E* configuration (Figure 1). Thus, the structure of compound **6** was elucidated as (*E*)-4-methoxy-2-phenylpyridine-6-carbaldehyde oxime.

It was also established that **11** could be formed from **7** by direct photoisomerization, indicating that *E*-aldoximes can be transformed into *Z*-aldoximes using light (Figure S32). Even though **11** was present in the extract before chromatography (Figure S33), it is still possible that **11** could be formed nonenzymatically during the extraction process. The HPLC profile of the EtOAc extract (Figure S33) also showed **4** is a natural product.

Compounds **1**−**5** and **7**−**10** are probably biosynthesized from amino acid and polyketide pathways (Scheme 1).[13,22] Intramolecular reactions of lysine can produce picolinic acid. Picolinic acid (likely as its CoA ester) may condense with acetyl CoA to form picolinoylacetyl CoA, which undergoes condensation with 2-aminoacrylic acid derived from serine to produce 4-hydroxybipyridine-6-carboxylic acid. The bipyridine carboxylic acid can be converted to the corresponding bipyridine amide intermediate that would yield **9** upon methylation. Compound **10** can be produced by the dehydration of **9**. The bipyridine amide or acid can undergo reduction to yield the bipyridine carbaldehyde, which through methylation, reduction, and oxidation steps can yield **1** and **2**. The bipyridine carbaldehyde is also postulated to form the oxime by incorporation of ammonia and oxidation. The oxime can be further processed to yield compounds **3**, **3a**, **4**, and **7** (Scheme 1). Compound **5** is probably formed from the carbaldehyde by ammonia incorporation, reduction, and acylation.[13] Compound **6** may be formed by the same biogenetic pathway as **7** except starting with benzoylacetyl CoA in place of picolinoylacetyl CoA.

Compounds **1**−**10** were evaluated for their cytotoxicity against the K562, HL-60, and KB cell lines using the MTT method,[23] and the A549 cell line using the SRB method (Table 2).[24] The new compounds **1** and **3**−**5** were found to be active against K562, HL-60, A549, and KB cells with $IC_{50}$ values ranging from 0.37 to 31.8 $\mu M$. Compound **4** had the most potent activity, with an $IC_{50}$ value of 0.37 $\mu M$ against K562 cells. Caerulomycin A (**7**) showed potent cytotoxicity against the HL-60 and A549 cell lines, with $IC_{50}$ values of 0.71 and 0.26 $\mu M$, respectively. Caerulomycin C (**8**) showed moderate cytotoxicity against K562 and KB cells, with $IC_{50}$ values of 1.8 and 3.1 $\mu M$, respectively. Weak cytotoxicities for caerulomycinonitrile (**10**) against A549 cells, and **3a** against K562 cells, were also observed, with $IC_{50}$ values of 15.0 and 31.8 $\mu M$, respectively. The other compounds isolated were found to be inactive ($IC_{50} > 50$ $\mu M$) against the cell lines tested. Antimicrobial activities against *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, and *Candida albicans* were also evaluated for compounds **1**, **4**−**8**, **10**, and **3a** using an agar dilution method.[25] Compound **7** showed weak antimicrobial activities against *E. coli*, *P. aeruginosa*, and *C. albicans* with MIC values of 10.9, 21.8, and 21.8 $\mu M$, respectively. Compound **8** showed weak antimicrobial activities against *E. coli*, *A. aerogenes*, *P. aeruginosa*, and *C. albicans* with MIC values of 9.7, 19.3, 38.6, and 19.3 $\mu M$, respectively. Compound **3a** selectively inhibited the growth of *E. coli* with a MIC value of 41.1 $\mu M$. The other compounds did not show antimicrobial activity (MIC $\geq$ 100 $\mu M$). The results showed that the antimicrobial activity of bipyridines is not due to general metal chelation, but more importantly might be related to the oxime moiety.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer as KBr discs. $^{1}$H NMR, $^{13}$C NMR, and DEPT spectra of compounds **1**−**6** and 2D NMR spectra of compounds **4** and **5** were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as an internal standard, and chemical shifts were recorded as $\delta$ values. 2D NMR spectra of compound **6** were recorded on a Bruker Avance 500 spectrometer. 1D NOE spectra were obtained on a Varian INOVA 400 spectrometer. ESIMS utilized a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pak ODS-A, 10 × 250 mm, 5 $\mu$m, 4 mL/min]. TLC and column chromatography (CC) were performed on plates precoated with silica gel $GF_{254}$ (10−40 $\mu$m) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory). Marinum salt used is made from the evaporation of seawater collected in Laizhou Bay (Weifang Haisheng Chemical Factory).

**Actinomycete Material.** The actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6 was isolated from marine sediments collected from the seashore of Weihai, China. The marine sediments (2 g) were air dried for 15 days in a 45 mL sterile centrifuge tube. The dried sediments were diluted into $10^{-3}$ g/mL, 100 $\mu$L of which was dispersed across a solid-phase agar plate (2216 media) and incubated at 28 °C for 10 days. Then a single colony was transferred on Gause's synthetic agar media. It was identified according to its morphological characteristics and 16S rRNA gene sequences (see Supporting Information; GenBank EU596461) and has been preserved in the China Center for Type Culture Collection (patent depository number CCTCC M 209277). Working stocks were 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 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 140 rpm and 28 °C. After 11 days of cultivation, 50 L of whole broth was extracted three times with EtOAc (50 L each). The EtOAc extract was concentrated under reduced pressure to give a dark brown gum (28.0 g).

**Purification.** The gum (28.0 g) was separated into 10 fractions on a silica gel VLC column using stepwise gradient elution, with $CH_2Cl_2$/petroleum ether (50−100%) followed by MeOH/$CH_2Cl_2$ (0−50%). Fraction 4 (5.1 g, $CH_2Cl_2$/MeOH, 50:1) was recrystallized from MeOH to give compound **7** (4.0 g), and the mother liquor of the recrystallization was then purified by semipreparative HPLC (45% MeOH/$H_2O$) to afford compound **11** (3.3 mg, $t_R$ 22 min). Fraction 3 (4.2 g) was separated into four subfractions by VLC on RP-18 silica using stepwise gradient elution with 5−90% MeOH/$H_2O$. Subfraction 3-2 (650 mg) was further purified by semipreparative HPLC (70% MeOH/$H_2O$) to yield **10** (6.0 mg, $t_R$ 7.2 min). Fraction 5 (4.5 g) was separated into nine subfractions by VLC on RP-18 using stepwise gradient elution with 5−90% MeOH/$H_2O$. Subfraction 5-1 (1.3 g) was separated into six subfractions on Sephadex LH-20, eluting with MeOH/$CH_2Cl_2$ (1:1). Subfractions 5-1-2 (96 mg) and 5-1-3 (260 mg) were further purified by semipreparative HPLC (45% MeOH/$H_2O$) to yield **2** (3.1 mg, $t_R$ 11.5 min) and **8** (13.6 mg, $t_R$ 13.1 min), respectively. Subfraction 5-3 (1.1 g) was separated into five further subfractions by Sephadex LH-20, eluting with MeOH/$CH_2Cl_2$ (1:1). Subfractions 5-3-3 (330 mg) and 5-3-4 (235 mg) were further separated by semipreparative HPLC to yield **4** (53.0 mg, $t_R$ 14.2 min) and **1** (12.0 mg, $t_R$ 9.7 min), eluting with 47% and 60% MeOH/$H_2O$, respectively. Subfraction 5-4 (940 mg) was further separated into seven subfractions by Sephadex LH-20, eluting with MeOH. Subfraction 5-4-5 (107 mg) was further purified by semipreparative HPLC (60% MeOH/$H_2O$) to yield **6** (3.5 mg, $t_R$ 13.3 min). Fraction 6 (1.1 g) was separated into three subfractions by Sephadex LH-20, eluting with MeOH/$CH_2Cl_2$ (1:1). Subfraction 6-2 (370 mg) and 6-3 (240 mg) were further purified by semipreparative HPLC to yield **9** (10.0 mg, $t_R$ 10.8 min) and **3** (3.0 mg, $t_R$ 13.7 min), eluting with 40% and 30% MeOH/$H_2O$, respectively. Fraction 7 (1.5 g) was subjected to CC using a stepwise gradient elution of EtOAc/petroleum ether (25−100%) to afford four subfractions. Subfraction 7-5 (250 mg) was further purified by semipreparative HPLC (35% MeOH/$H_2O$) to yield **5** (3.2 mg, $t_R$ 8.9 min).

**Caerulomycin F (1):** white, amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 203 (2.41), 253 (1.16) nm; IR (KBr) $\nu_{max}$ 3449, 2924, 1652, 1464, 1381, 1113, 1099, 1056 cm$^{-1}$; $^{1}$H and $^{13}$C NMR, see Table 1; HRESIMS $m/z$ 217.0976 $[M + H]^+$ (calcd for $C_{12}H_{13}N_2O_2$, 217.0977).

**Caerulomycin G (2):** colorless needles (MeOH); mp 127 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 214 (3.16), 277 (2.62) nm; IR (KBr) $\nu_{max}$ 3749, 3622, 2906, 2846, 1779, 1692, 1553, 1454, 1069, 670 cm$^{-1}$; $^{1}$H and $^{13}$C NMR, see Table 1; HRESIMS $m/z$ 247.1079 $[M + H]^+$ (calcd for $C_{13}H_{15}N_2O_3$, 247.1083).

**Caerulomycin H (3):** white, amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 240 (2.91), 280 (2.75) nm; IR (KBr) $\nu_{max}$ 3171, 2953, 1651, 1502, 1378, 1302, 1016, 980, 797, 738 cm$^{-1}$; $^{1}$H and $^{13}$C NMR, see Table 1; HRESIMS $m/z$ 216.0781 $[M + H]^+$ (calcd for $C_{11}H_{10}N_3O_2$, 216.0773).

**Caerulomycin I (4):** colorless needles (MeOH); mp 101 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 230 (3.66), 278 (3.23) nm; IR (KBr) $\nu_{max}$ 3257, 3098, 3065, 2939, 1676, 1584, 1480, 1426, 1356, 1219, 1034, 875, 780, 671 cm$^{-1}$; $^{1}$H and $^{13}$C NMR, see Table 1; HRESIMS $m/z$ 282.0855 $[M + Na]^+$ (calcd for $C_{13}H_{13}N_3O_3Na$, 282.0855).

**Caerulomycin J (5):** yellow oil; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 230 (3.83), 273 (3.63) nm; IR (KBr) $\nu_{max}$ 3288, 2926, 1633, 1537, 1442, 1372, 1027, 794, 621 cm$^{-1}$; $^{1}$H and $^{13}$C NMR, see Table 1; HRESIMS $m/z$ 266.0905 $[M + Na]^+$ (calcd for $C_{13}H_{13}N_3O_2Na$, 266.0905).

**Caerulomycin K (6):** colorless needles (MeOH); mp 153 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 210 (2.24), 242 (3.34) nm; IR (KBr) $\nu_{max}$ 3748, 3167, 3062, 2925, 2852, 1593, 1562, 1463, 1359, 1052, 987 cm$^{-1}$; $^{1}$H and $^{13}$C NMR, see Table 1; HRESIMS $m/z$ 229.0968 $[M + H]^+$ (calcd for $C_{13}H_{13}N_2O_2$, 229.0977).

**Chemical Transformations of 3 and 7.** A suspension of NaH (1.0 mg) in 1.0 mL of anhydrous DMF was treated with a solution of compound **3** (1.0 mg) in 1.0 mL of DMF at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and then $CH_3I$ (10 $\mu$L) was added. After 30 min, a saturated solution of $NH_4Cl$ (5.0 mL) was added to quench the reaction. The product was extracted with EtOAc (4 × 10 mL) and purified by VLC on RP-18 silica to give **3a** (1.0 mg, 94%). Using the same procedure, compound **7** also afforded **3a** (0.9 mg, 85%). Product **3a** was identified as *O*-methyl-(*E*)-4-methoxy-2,2′-bipyridine-6-carbaldehyde oxime by comparison of its $^{1}$H NMR spectrum with that reported in the literature.[11] Compound **3a**: white, amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 231 (3.63), 257 (3.41) nm; $^{1}$H NMR ($CDCl_3$, 600 MHz) $\delta$ 3.98 (s, 3H, $OCH_3$-8), 4.04 (s, 3H, $OCH_3$-4), 7.32 (ddd, 1H, $J$ = 7.1, 4.9, 1.1 Hz, H-5′), 7.38 (d, 1H, $J$ = 2.8 Hz, H-5), 7.82 (td, 1H, $J$ = 7.7, 1.7 Hz, H-4′), 7.95 (d, 1H, $J$ = 2.8 Hz, H-3), 8.21 (s, 1H, H-7), 8.43 (d, 1H, $J$ = 7.7, H-3′), 8.67 (d, 1H, $J$ = 4.1 Hz, H-6′); ESIMS $m/z$ 244 $[M + H]^+$.

**Photoisomerization.** The isomerization of **7** (1 mg) was carried out under the following experimental conditions: reaction times, 1 h, 4 h, 40 h; solvent (2 mL), $Me_2CO$; temperature, 25 °C; light source, mercury vapor lamp or no light (control). The reaction mixtures obtained from each set of reaction conditions were analyzed by HPLC (50% MeOH) (Figure S32). Compound **7** was found to isomerize to **11** when light was

applied, while it was stable in the absence of light. The proportion of **7** that had isomerized with the application of light after 1, 4, and 40 h was 8.4%, 14%, and 33%, respectively.

## ■ ASSOCIATED CONTENT

**Supporting Information.** NMR spectra for compounds **1**−**6**, NMR data for compounds **7**−**11**, HPLC analysis of the product of photoisomerization of **7**, a description of the bioassay protocols used, and 16S rRNA sequences of *A. cyanogriseus* WH1-2216-6 are available free of charge via the Internet at http://pubs.acs.org.

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## ■ REFERENCES

(1) Bérdy, J. *J. Antibiot.* **2005**, *58*, 1–26.
(2) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **2009**, *74*, 675–684.
(3) Lam, K. S. *Curr. Opin. Microbiol.* **2006**, *9*, 245–251.
(4) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Am. Chem. Soc.* **2006**, *128*, 1622–1632.
(5) Proksch, P.; Muller, W. E. G. *Frontiers in Marine Biotechnology*; Routledge: Norfolk, 2006; pp 225−288.
(6) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673.
(7) Blunt, J. W.; Copp, B. R.; Hu, W.; Munro, M. H. G.; Northcote, P. T.; Rrinsep, M. R. *Nat. Prod. Rep.* **2009**, *26*, 170–244.
(8) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Rrinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165–237.
(9) Mcinnes, A. G.; Smith, D. G.; Walter, J. A.; Wright, J. L. C.; Vining, L. C.; Arsenault, G. P. *Can. J. Chem.* **1978**, *56*, 1836–1841.
(10) Funk, A.; Divekar, P. V. *Can. J. Microbiol.* **1959**, *5*, 317–321.
(11) Divekar, P. V.; Read, G.; Vining, L. C. *Can. J. Chem.* **1967**, *45*, 1215–1223.
(12) Chandren, R. R.; Sankaran, R.; Diverker, P. V. *J. Antibiot.* **1968**, *3*, 243.
(13) Vining, L. C.; Mcinnes, A. G.; Mcculloch, A. W.; Smith, D. G.; Walter, J. A. *Can. J. Chem.* **1988**, *66*, 191–194.
(14) Chatterjee, D. K.; Raether, W.; Iyer, N.; Ganguli, B. N. *Z. Parasitenkunde* **1984**, *70*, 569–573.
(15) Singla, A. K.; Agrewala, J. N.; Vohra, R. M.; Singh, J. R. World Patent WO 2007/031832, 2007.
(16) Trecourt, F.; Gervais, B.; Mongin, O.; Gal, C. L.; Mongin, F.; Queguiner, G. *J. Org. Chem.* **1998**, *63*, 2892–2897.
(17) Alreja, B. D.; Kattige, S. L.; Lal, B.; Souza, N. J. D. *Heterocycles* **1986**, *24*, 1637–1640.
(18) Trecourt, F.; Gervais, B.; Mallet, M.; Queguiner, G. *J. Org. Chem.* **1996**, *61*, 1673–1676.
(19) Mcinnes, A. G.; Smith, D. G.; Wright, J. L. C.; Vining, L. C. *Can. J. Chem.* **1977**, *55*, 4159–4165.
(20) Brown, D. A.; Glass, W. K.; Mageswaran, R.; Mohammed, S. A. *Magn. Reson. Chem.* **1991**, *29*, 40–45.
(21) Zhang, Z.; Yu, Y.; Liebeskind, L. S. *Org. Lett.* **2008**, *10*, 3005–3008.
(22) Mcinnes, A. G.; Smith, D. G.; Walter, J. A.; Vining, L. C.; Wright, J. L. C. *Can. J. Chem.* **1979**, *57*, 3200–3204.
(23) Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55–63.
(24) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
(25) Zaika, L. L. *J. Food Safety* **1988**, *9*, 97–118.