# Lipoxazolidinones A, B, and C: Antibacterial 4-Oxazolidinones from a Marine Actinomycete Isolated from a Guam Marine Sediment

Venkat R. Macherla,* Jehnan Liu, Michelle Sunga, Donald J. White, Jennifer Grodberg, Sy Teisan, Kin S. Lam, and Barbara C. M. Potts

*Nereus Pharmaceuticals, Inc., 10480 Wateridge Circle, San Diego, California 92121*

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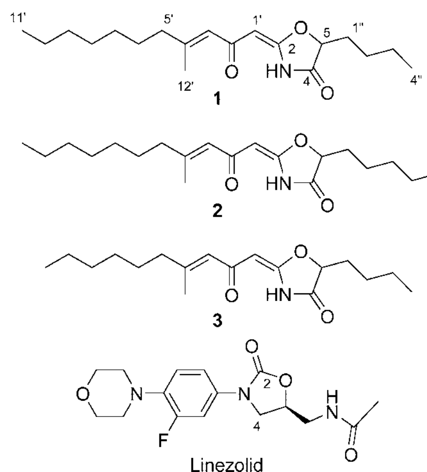
Marine actinomycete strain NPS008920, a member of the new genus *Marinispora*, was isolated from a sediment sample collected in Cocos Lagoon, Guam. In natural sea water containing media, the strain produced a series of novel 2-alkylidene-5-alkyl-4-oxazolidinones, lipoxazolidinone A (**1**), B (**2**), and C (**3**). Compounds **1–3** showed broad spectrum antimicrobial activity similar to that of the commercial antibiotic linezolid (Zyvox), a 2-oxazolidinone. Hydrolysis of the amide bond of the 4-oxazolidinone ring of **1** resulted in loss of antibacterial activity. The 2-alkylidene-4-oxazolidinone represents a new antibiotic pharmacophore and is unprecedented in nature.

Concern over the paucity of new antibiotics has raised questions regarding the next source of new chemical entities (NCEs) that can meet the challenge of continually emerging resistance.[1] Between 1981 and 2002, the vast majority of NCEs approved for use as antibiotics were natural-product derived,[2] indicating that nature (in particular microorganisms) offers highly relevant scaffolds for developing therapies in the infectious disease arena. While many of the NCEs approved for use at the end of the past century resulted from semisynthetic modifications to compounds discovered during the “Golden Age” of antibiotics, some recent discoveries indicate that alternative technologies are providing access to new antibiotic scaffolds.[3] One approach, which maintains credence in the historic success of microbial-derived NCEs, is to culture new microorganisms from unique natural environments as a source of novel chemistry. Despite the vastness of the earth’s oceans and their inherent biodiversity, the marine environment remains a largely untapped source of new microorganisms, yet evidence has emerged that focused exploration of the marine environment will yield unprecedented, chemically prolific species.[4] Here, we provide further evidence that the ocean offers new microorganisms with the potential to generate therapeutic agents for the treatment of infectious diseases.

## Results and Discussion

In 2002, we sampled a variety of marine sediments in Cocos Lagoon, Guam, from which we isolated marine actinomycete strain NPS008920. 16S rRNA sequence analysis indicated that the organism belongs to the newly proposed genus *Marinispora*.[4,5] In natural sea water-containing media, the strain produced a series of novel 2-alkylidene-5-alkyl-4-oxazolidinones, lipoxazolidinones A (**1**), B (**2**), and C (**3**). The crude ethyl acetate extract of the culture broth was fractionated on a standard C18 HPLC column with a $H_2O/CH_3CN$ gradient. Bioassay and UV data for resulting fractions indicated that growth inhibition of MSSA was localized to a subset of fractions, 27–29 (see Supporting Information). LC-MS analysis of these fractions indicated the presence of a major compound and two minor compounds with similar UV spectra and pseudomolecular ions (**1**: $m/z$ = 322 [M + H]$^+$; **2**: $m/z$ 336 [M + H]$^+$; and **3**: $m/z$ 308 [M + H]$^+$) that did not match any known compounds in our in-house database. Additional crude extract was generated by repeat fermentation, which was followed by fractionation via preparative HPLC using $CH_3CN/H_2O$ gradients. The active fractions were identified by their LC-MS fingerprints and further purified by semipreparative or preparative HPLC to yield the major active component, lipoxazolidinone A (**1**; isolated yield ~10 mg/L) and minor active components lipoxazolidinone B (**2**; isolated yield ~2 mg/L) and lipoxazolidine C (**3**; isolated yield ~1 mg/L) as a pure samples.




Lipoxazolidinone A (**1**) was obtained as a colorless oil ([α]$_D$ −31 ($c$ 0.02, MeOH)). The molecular formula, $C_{19}H_{31}NO_3$, was derived from HRESIMS ($m/z$ 322.2372 [M + H]$^+$) and indicated five degrees of unsaturation. UV absorptions at 310 nm (log $\varepsilon$ 4.36) and 253 nm (3.96) indicated the presence of extended conjugation in the molecule. $^1$H and $^{13}$C NMR spectra displayed the presence of two carbonyl carbons ($\delta$ 189.4 (C-2′); 173.7 (C-4)), two substituted olefins ($\delta$ 166.7 (C-2); 84.0 (C-1′, $\delta_H$ 5.18, 1H, br s); 124.3 (C-3′, $\delta_H$ 5.87, 1H, s), and 157.7 (C-4′)), one methine substituted with oxygen ($\delta$ 78.6 (C-5, $\delta_H$ 4.59, 1H, dd, $J$ = 4.4, 6.6 Hz)), a methyl group ($\delta$ 19.2 (C-12′, $\delta_H$ 2.15, 3H, br s)), and two saturated alkyl chains (see Table 1). A trisubstituted olefin spin system was established as follows. The olefinic proton at $\delta$ 5.87 (H-3′) showed COSY correlations to a methyl group at $\delta$ 2.15 ($H_3$-12) and a methylene proton at $\delta$ 2.10 ($H_2$-5′), both of which represented allylic couplings, and HMBC correlations to $\delta$ 189.4 (C-2′), 157.7 (C-4′), 19.2 (C-12′), and 41.5 (C-5′) (see Figure 1). Together, these indicated the presence of a double bond in which one olefinic carbon (C-3′) was substituted with a carbonyl and the other (C-4′) with a methyl and an alkyl group. A second spin system was established by COSY correlations from the proton at $\delta$ 4.59 (H-5) to a methylene at $\delta$ 1.96 (1H, m, H-2a″) and 1.79 (1H, m, H-2b″) and HMBC correlations to $\delta$ 166.7 (C-2), 173.7 (C-4), 30.8

* To whom correspondence should be addressed. Phone: 858-200-8334. Fax: 858-587-4088. E-mail: vmacherla@nereuspharm.com.

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**Table 1.** $^{1}$H and $^{13}$C NMR Data for Compounds **1**, **2**, and **3**

| | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| C no. | $\delta_C$ mult | $\delta_H$ ($J$ in Hz) | $\delta_C$ mult | $\delta_H$ ($J$ in Hz) | $\delta_C$ mult | $\delta_H$ ($J$ in Hz) |
| 2 | 166.7, qC | | 166.5, qC | | 166.6, qC | |
| 4 | 173.7, qC | | 173.6, qC | | 173.7, qC | |
| 5 | 78.6, CH | 4.59, dd (4.4, 7) | 78.6, CH | 4.58, dd (4.4, 7) | 78.6, CH | 4.59, dd (4.4, 7) |
| 1′ | 84.0, CH | 5.18, s | 84.0, CH | 5.17, s | 84.1, CH | 5.18, s |
| 2′ | 189.4, qC | | 189.4, qC | | 189.4, qC | |
| 3′ | 124.3, CH | 5.87, s | 124.3, CH | 5.86, s | 124.3, CH | 5.87, s |
| 4′ | 157.7, qC | | 157.7, qC | | 157.7, qC | |
| 5′ | 41.5, $CH_2$ | 2.10, br t (7.6) | 41.4, $CH_2$ | 2.09, br t (7.6) | 41.5, $CH_2$ | 2.10, br t (7.6) |
| 6′ | 27.6, $CH_2$ | 1.44, m | 27.6, $CH_2$ | 1.44, m | 27.6, $CH_2$ | 1.44, m |
| 7′ | 29.1, $CH_2$[a] | 1.26, m | 29.1, $CH_2$[b] | 1.26, m | 28.9, $CH_2$ | 1.28, m |
| 8′ | 29.2, $CH_2$[a] | 1.26, m | 29.7, $CH_2$[b] | 1.26, m | 31.7, $CH_2$ | 1.24, m |
| 9′ | 31.8, $CH_2$ | 1.23, m | 31.7, $CH_2$ | 1.23, m | 22.5, $CH_2$ | 1.27, m |
| 10′ | 22.6, $CH_2$ | 1.26, m | 22.6, $CH_2$ | 1.26, m | 14.1, $CH_3$ | 0.86, t (7) |
| 11′ | 14.1, $CH_3$ | 0.87, t (7) | 14.0, $CH_3$ | 0.85, t (7) | | |
| 12′ | 19.2, $CH_3$ | 2.15, br s | 19.1, $CH_3$ | 2.14, br s | 19.1, $CH_3$ | 2.15, br s |
| 1″ | 30.8, $CH_2$ | 1.79, m | 31.8, $CH_2$ | 1.77, m | 30.8, $CH_2$ | 1.79, m |
| | | 1.96, m | | 1.94, m | | 1.96, m |
| 2″ | 26.2, $CH_2$ | 1.40, m | 23.7, $CH_2$ | 1.42, m | 26.1, $CH_2$ | 1.41, m |
| 3″ | 22.2, $CH_2$ | 1.36, m | 31.2, $CH_2$ | 1.28, m | 22.2, $CH_2$ | 1.35, m |
| 4″ | 13.7, $CH_3$ | 0.90, t (7) | 22.3, $CH_2$ | 1.28, m | 13.7, $CH_3$ | 0.90, t (7) |
| 5″ | | | 13.9, $CH_3$ | 0.90, t (7) | | |

[a,b] The assignments may be interchangeable.

**Table 2.** Antimicrobial Susceptibility Profile (MIC, $\mu$g/mL) for Compounds **1**, **2**, **3**, and **4** and Linezolid

| organism | 1 | 2 | 3 | 4 | linezolid |
|---|---|---|---|---|---|
| *Staphylococcus aureus* ATCC 29213 (MSSA) | 0.9 | 6.0 | 4.0 | 24 | 2.0 |
| *Staphylococcus aureus* ATCC 43300 (MRSA) | 1.0 | 1.5 | 3.0 | >32 | 1.5 |
| *Staphylococcus epidermidis* ATCC 700578 | 0.5 | 1.3 | NT[a] | NT | 1.0 |
| *Staphylococcus epidermidis* ATCC 700582 | 0.5 | 0.8 | NT | NT | 1.0 |
| *Streptococcus pneumoniae* ATCC 49619 (Pen S) | 2.6 | 8.0 | 10 | >32 | 1.0 |
| *Streptococcus pneumoniae* ATCC 51915 (Pen R) | 4.7 | 6.0 | NT | NT | 1.0 |
| *Enterococcus faecalis* ATCC 29212 (VSE) | 1.0 | 3.0 | 2 | 32 | 1.0 |
| *Enterococcus faecium* ATCC 700221 (VRE) | 1.8 | 1.5 | NT | NT | 4.0 |
| *Haemophilus influenzae* ATCC 49247 | 12 | 12 | 16 | >32 | 12 |
| *Haemophilus influenzae* ATCC 49766 | 12 | 16 | 5 | >32 | 12 |
| *Escherichia coli* ATCC 25922 | >32 | >32 | >32 | >32 | >32 |

[a] NT = not tested.

(C-1″), and 26.2 (C-2″), which indicated the presence of a 2-exocyclic-5-alkyl-4-oxazolidinone ring. The 4-carbon substituent at C-5 of the oxazolidinone ring was revealed by HMBC correlations from the methyl at $\delta$ 0.92 ($H_3$-4″) and methine at $\delta$ 4.59 (H-5) to $\delta$ 26.2 (C-2″). The exocyclic double bond was then linked to the C-2′ carbonyl via HMBC correlations from a proton singlet at $\delta$ 5.18 (H-1′) to 189.4 (C-2′) and 166.7 (C-2). Thus, the core structure of the molecule was established, leaving only the characterization of the side-chain substituent at C-4′, which was assigned as *n*-heptyl on the basis of $^{13}$C NMR signals for one methyl and six methylene carbons and molecular weight considerations. To evaluate the double-bond geometries, NOESY spectra were acquired. The NOESY correlation between H-1′ and H-3′ indicated that these two protons were in close proximity, and a correlation between $\delta$ 5.87 (H-3′) and 2.10 (H-5′) suggested the double-bond geometry between C-3′ and C-4′ is *E*. The NOESY studies in DMSO-$d_6$ indicated the absence of a correlation between NH and H-1′, which may support the *E* geometry of the exocyclic double bond.

Lipoxazolidinone B (**2**) was also obtained as a colorless oil. The molecular formula $C_{20}H_{33}NO_3$ was established from HRESIMS (*m*/*z* 336.2527 [M + H]$^+$). $^1$H and $^{13}$C NMR data indicated that compound **2** is similar to **1** but with an additional methylene group on the short aliphatic chain at C-5, which was confirmed by HSQC and HMBC experiments. Both $H_2$-1″ ($\delta_H$ 1.77 and 1.94) and $H_3$-5″ ($\delta_H$ 0.90) protons had HMBC correlations with C-3″ ($\delta$ 31.2), which suggested the five-carbon alkyl substituent at the C-5 carbon of the oxazolidinone ring system.

Lipoxazolidinone C (**3**) was also obtained as a colorless oil. The molecular formula $C_{18}H_{29}NO_3$ was established from HRESIMS (*m*/*z* 308.2238 [M + H]$^+$). $^1$H and $^{13}$C NMR data indicated that compound **3** is similar to **1** with loss of a methylene group. $^1$H and $^{13}$C NMR data for the four-carbon aliphatic chain of **3** is identical to that of **1**, indicating that **3** contains one less methylene unit in the long aliphatic chain compared to **1**.

Lipoxazolidinones A–C (**1**–**3**) are subject to pH-dependent hydrolysis, with slow conversion of **1** to **4** in buffered solutions at

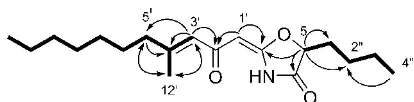


**Figure 1.** COSY (thick lines) and HMBC (arrows) correlations of compound **1**.

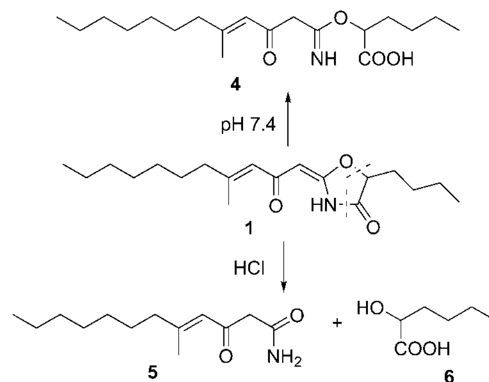


**Figure 2.** Degradation of lipoxazolidinone A (**1**) to **4**, **5**, and **6**.

low to neutral pH values, and rapid hydrolysis to **5** in concentrated acid (see Figure 2). Lipoxazolidinone A (**1**) was completely converted to compound **4** at pH values between 4 and 7 within 6 days. This conversion was very slow at higher pH (20% conversion after 6 days at pH 9). A pure sample of compound **4** was prepared by hydrolysis of **1** in an aqueous/organic solution at pH 7.4. The LRESIMS data (*m/z* 340; $[M + H]^+$) clearly indicated the addition of one molecule of water. The $\lambda_{max}$ shift from 310 to 245 nm indicated that the conjugation in the molecule was broken. Analysis of the NMR spectra indicated the presence of a new isolated methylene group ($\delta_C$ 50.3; $\delta_H$ 3.67 and 3.56, each 1H, d, $J = 16.4$ Hz) between the C-2′ ($\delta$ 193.5) carbonyl carbon and C-2 ($\delta$ 166.6), which is in agreement with a double-bond shift to the nitrogen. The remaining structural elements were confirmed by COSY, HSQC, and HMBC data. Lipoxazolidinone A (**1**) was completely degraded in the presence of strong acid (6 N HCl) at 40 °C. The major degradation product (**5**) was characterized by LC-MS, from which the observed molecular ion (LRESIMS *m/z* 226 $[M + H]^+$) suggested that the oxazolidinone ring had been completely hydrolyzed; the expected byproduct of this reaction is **6**.

Lipoxazolidinones A (**1**), B (**2**), and C (**3**) and hydrolysis product **4** were screened against a panel of various Gram-positive and Gram-negative bacteria (Table 2). Compound **1** showed broad spectrum activity, with minimum inhibitory concentration (MIC) values ranging from 0.5 to 5 $\mu$g/mL against Gram-positive bacteria and 12 $\mu$g/mL against two strains of *Haemophilus influenzae*. The antibacterial spectrum and potency of **1** were similar to those of the commercially available antibiotic linezolid (Zyvox).[6] Compounds **2** and **3** also showed broad spectrum antibacterial activity, albeit with lesser overall potency than **1**. In contrast, hydrolysis product **4** showed only weak activity against MSSA, indicating the importance of an intact oxazolidinone ring system. While the oxazolidinone heterocycle is a common structural motif shared by the lipoxazolidinones and linezolid, the compounds are clearly distinguished as 4- and 2-oxazolidinones, respectively, and each class is uniquely substituted. Thus, the 4-oxazolidinones offer a unique scaffold with antibiotic therapeutic potential.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Rudolph Autopol III polarimeter using a 4 × 100 mm sample cell. UV spectra were obtained with a Beckman Coulter DU 640 spectrophotometer or from analytical HPLC analysis of the purified compound using an Agilent HP1100 HPLC equipped with an Agilent PDA detector; the mobile phase was a mixture of $CH_3CN$ and $H_2O$. NMR spectra were collected using a 500 MHz Bruker Avance NMR spectrometer with an inverse probe, except for the $^{13}C$ NMR spectrum, which was acquired with a broad-band observe probe. All NMR data were acquired at 298 K in $CDCl_3$ or DMSO-$d_6$, referenced to 7.24 and 2.50 ppm in the proton spectra and 77.00 and 39.00 ppm in carbon spectra, respectively. High-resolution mass spectra were acquired using a Micromass Q-Tof2 mass spectrometer with ES+. HRESI spectra were referenced using a poly(ethylene glycol) polymer mixture, which was coinjected during acquisition as an internal accurate mass standard. Additional ESI-MS and crude extract analysis experiments were collected using an Agilent HP1100 HPLC equipped with an Agilent PDA detector and an 1100 series MSD Agilent mass spectrometer. HPLC was performed on a Gilson HPLC equipped with a Gilson 215 fraction collector and an Agilent PDA detector. For RP HPLC separations, an Eclipse XDB-C18, 5 $\mu$m, 150 × 21 mm i.d. column was used at a flow rate of 14.5 mL/min. HPLC solvents were obtained from Fisher Scientific and VWR, and $H_2O$ used for HPLC was filtered through a NANOpure Infinity ultrafilter. Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc.

**Microorganism.** The producing culture, NPS008920, was collected from Cocos Lagoon, Guam, in January 2002. Strain NPS008920 was deposited with the American Type Culture Collection and assigned the accession number PTA-6527. The 16S rRNA sequence was submitted to GenBank and assigned the accession number EF470589. The 16S rRNA sequence of strain NPS008920 matched closely to those of the newly proposed genus *Marinispora*.[4,5]

**Media and Culturing Conditions for 1, 2, and 3.** Frozen stock cultures of strain NPS008920 were inoculated in 100 mL of seed medium and allowed to grow for 3–4 days at 28 °C on a rotary shaker operating at 250 rpm. The seed medium consisted of the following per liter of sea water: starch, 10 g; yeast extract, 4 g; and peptone, 2 g. This seed culture was then divided (5 mL each) between nine 500 mL flasks containing 100 mL of the same seed medium. After 2–3 days of growth, 5 mL aliquots of this seed culture were inoculated into 500 mL flasks containing 100 mL of production medium having the same composition of the seed medium. The number of production flasks inoculated depended on the desired size of fermentation. After 5 days of growth, the culture broth was extracted with equal volume of EtOAc, and the extract was concentrated under reduced pressure.

**Purification of Compounds 1 and 2.** The crude extract (0.38 g) of NPS008920 obtained as described above was dissolved in MeOH (19 mL) and injected in 950 $\mu$L aliquots (19 mg each) on preparative reversed-phase HPLC (column: C18-HL, 5 um, 21 mm i.d. × 15 cm; detection: DAD) using a solvent gradient of 20% $CH_3CN/H_2O$ to 80% $CH_3CN/H_2O$ in 12 min; 80–100% $CH_3CN/H_2O$ in 1 min, and then 9 min at 100% $CH_3CN$ with a flow rate of 14.5 mL/min.

Compounds **1** and **2** eluted at 21 and 23 min, respectively. These compounds were further purified by RP semipreparative HPLC using a Hamilton Polymeric RP (PRP-1) column (10 mm i.d. × 25 cm; 10 um) using the solvent gradient of 40–80% $CH_3CN/H_2O$ in 8 min; 80–100% $CH_3CN/H_2O$ in 1 min, and then 15 min at 100% $CH_3CN$ with a flow rate of 3 mL/min.

**Lipoxazolidinone A (1):** colorless oil, $[\alpha]^{21}_D$ −31 (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 310 (4.36), 253 (3.96) nm; $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$ 5.98 (1H, br s, H-3′), 5.28 (1H, s, H-1′), 4.88 (1H, dd, $J = 4.7$, 6.6 Hz, H-5), 2.11 (3H, br s, H-12′), 2.08 (2H, br t, $J = 7.5$ Hz, H-5′), 1.84 (1H, m, H-1a″), 1.71 (1H, m, H-1b″), 1.43 (2H, m, H-6′), 1.31 and 1.30 (2H, m, H-2″ and H-3″), 1.28 (2H, m, H-10′), 1.25 (6H, m, H-7′ to H-9′), 0.87 (3H, t, $J = 6.6$ Hz, H-4″), 0.86 (3H, t, $J = 6.6$ Hz, H-11′); $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$ 187.9 (C, C-2′), 173.8 (C, C-4), 165.3 (C, C-2), 155.4 (C, C-4′), 124.6 (CH, C-3′), 83.6 (CH, C-1′), 77.6 (CH, C-5), 40.4 ($CH_2$, C-5′), 31.1 (CH2, C-9′), 29.9 ($CH_2$, C-1″), 28.6 ($CH_2$, C-8′), 28.4 ($CH_2$, C-7′), 27.0 ($CH_2$, C-6′), 25.6 ($CH_2$, C-2″), 22.0 ($CH_2$, C-10′), 21.6 ($CH_2$, C-3″), 18.4 ($CH_3$, C-12′), 13.9 ($CH_3$, C-11′), 13.6 ($CH_3$, C-4″); see Table 1 for $^1H$ and $^{13}C$ NMR data in $CDCl_3$; HRESIMS *m/z* 322.2372 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_3$, 322.2382; $\Delta = 3.3$ ppm).

**Lipoxazolidinone B (2):** colorless oil, UV ($CH_3CN/H_2O$) $\lambda_{max}$ 310, 255 nm; see Table 1 for $^1H$ and $^{13}C$ NMR data; HRESIMS *m/z* 336.2527 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_3$, 336.2539; $\Delta = 3.5$ ppm).

**Purification of Compound 3.** The crude extract (1.2 g) of NPS008920 obtained as described above was dissolved in $H_2O$ (100 mL) and extracted with hexanes (3 × 100 mL). The combined organic layer was concentrated to yield about 570 mg of crude material enriched in **1**–**3**, which was purified by preparative scale RP HPLC (C18-HL, 5 $\mu$m, 21 mm i.d. × 15 cm; DAD detection) using a solvent gradient of 50% $CH_3CN/H_2O$ to 100% $CH_3CN$ in 12 min and then 13 min at 100% $CH_3CN$ with a flow rate of 14.5 mL/min. Compound **3** eluted at about 18 min as a minor compound. This compound was further purified by RP preparative HPLC (C18-HL, 5 $\mu$m, 21 mm i.d. × 15 cm; detection: DAD) using a solvent gradient of 20–80% MeOH/$H_2O$ in 12 min; 80% MeOH/$H_2O$ to 100% MeOH in 1 min, and then 9 min at 100% MeOH with a flow rate of 14.5 mL/min. Lipoxazolidine C eluted as a pure compound at about 18.5 min.

**Lipoxazolidinone C (3):** colorless oil; UV ($CH_3CN/H_2O$) $\lambda_{max}$ 310, 255 nm; for $^1H$ and $^{13}C$ NMR data, see Table 1; HRESIMS *m/z* 308.2234 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_3$, 308.2226; $\Delta = 2.8$ ppm).

**Preparation of Compound 4 from 1.** Compound **4** was obtained by adding pH 7.4 buffer (2 mL; 75 mM Tris buffer) into a solution of **1** (4.2 mg) in $CH_3CN$ (2 mL) and letting it stand at room temperature for about 4 days. The $CH_3CN$ was removed by evaporation under reduced pressure, and the remaining aqueous layer was extracted with $CH_2Cl_2$ (3 × 20 mL). The combined organic layer was concentrated to yield **4** (3.2 mg).

**Compound 4:** colorless oil; UV ($CH_3CN/H_2O$) $\lambda_{max}$ 245 nm; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 6.02 (1H, br s, H-3′), 5.24 (1H, dd, $J =$ 3.8, 8.5 Hz, H-5), 3.67 (1H, d, $J = 16.4$ Hz, H-1′a), 3.56 (1H, d, $J =$ 16.4 Hz, H-1′b), 2.15 (2H, br t, $J = 7.6$ Hz, H-5′), 2.15 (3H, br s, H-12′), 1.96 (1H, m, H-1″a), 1.82 (1H, m, H-1″b), 1.46 (2H, m, H-6′), 1.34–1.31 (4H, m, H-2″ and H-3″), 1.26–1.23 (8H, m, H-7′ to H-10′), 0.88 (3H, t, $J = 7$ Hz, H-4″), 0.87(3H, t, $J = 7$ Hz, H-11′); $^{13}C$ NMR

($CDCl_3$, 125 MHz) $\delta$ 193.5 (C, C-2′), 172.8 (C, C-4), 166.6 (C, C-2), 164.9 (C, C-4′), 121.5 (CH, C-3′), 74.3 (CH, C-5), 50.3 ($CH_2$, C-1′), 41.5 ($CH_2$, C-5′), 31.7 ($CH_2$, C-9′), 31.2 ($CH_2$, C-1″), 29.0 and 29.2 (2 $\times$ $CH_2$, C-7′ and C-8′), 27.5 ($CH_2$, C-6′), 27.2 ($CH_2$, C-2″), 22.6 ($CH_2$, C-10′), 22.3 ($CH_2$, C-3″), 20.0 ($CH_3$, C-12′), 14.0 ($CH_3$, C-11′), 13.9 ($CH_3$, C-4″); LRESIMS *m/z* 340 $[M + H]^+$.

**Acid Hydrolysis of Compound 1 with HCl.** To a solution of **1** (1 mg) in $CH_3CN$ (1 mL) was added 1 mL of 6 N HCl, and the reaction was allowed to stir at 40 °C for about 10 min. The products were analyzed by LC-MS. Compound **5**: *m/z* 226 $[M + H]^+$.

**Antibiotic Activity Assay.** The minimum inhibitory concentration (MIC) of compounds **1**–**4** and linezolid were obtained against a variety of bacteria (Table 2) using a conventional broth dilution assay in accordance with standards recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[7] The serial microbroth dilution method used BBL Mueller-Hinton broth (cation adjusted), except for the *Streptococcus pneumoniae* and *Haemophilus influenzae*. BBL Mueller-Hinton broth II (cation adjusted) with lysed horse blood and Haemorphilus Test Medium were used for *S. pneumonaie* and *H. influenzae*, respectively. *S. pneumonaie* and *H. influenzae* were incubated in a $CO_2$ incubator, whereas the others were not (standard NCCLS). The final bacterial inoculum contained $\sim 5 \times 10^5$ CFU/mL, and the plates were incubated at 35 °C for 14–16 h. The MIC was defined as the lowest drug concentration that prevented visible growth of the bacterium. Compound **2** was not soluble in DMSO; however, it was possible to screen the compound by using a trace amount of MeOH.

**Supporting Information Available:** Chromatogram of crude extract eluted into fractions and corresponding biological activity for active fractions. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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