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Structure Determination, Biosynthetic Origin, and Total Synthesis of Akazaoxime, an Enteromycin-Class Metabolite from a Marine-Derived Actinomycete of the Genus *Micromonospora*

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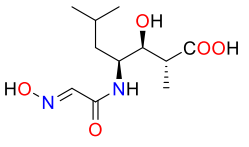
ABSTRACT: A new enteromycin-class antibiotic, akazaoxime (**1**), possessing an aldoxime functionality in place of *O*-methyl nitronic acid, was isolated from the cultured extract of a marine-derived actinomycete of the genus *Micromonospora*, along with known A-76356 (**2**). The structure of **1**, including the absolute stereochemistry of three chiral centers, was established by comprehensive analysis of nuclear magnetic resonance (NMR) and mass spectrometry data coupled with magnetic anisotropy analysis of its phenylglycine methyl ester derivatives. The stereochemistry of **2**, not determined previously, was proven to be the same as that of **1** on the basis of the similarity of their NMR and specific rotation data. Precursor feeding experiments using ^{13}C-labeled compounds elucidated that the carbon skeletons of **1** and **2** are constructed from propionate (methylmalonate), leucine, and glycine. Establishment of the concise and flexible synthetic route to **1** enabled us to implement biological evaluation of **1** and its unnatural analogues, demonstrating weak to moderate antimicrobial activities of **1** against Gram-positive *Kocuria rhizophila* [minimum inhibitory concentration (MIC) of 50 μg/mL] and those of synthetic analogues against a plant pathogen *Glomerella cingulata* (MIC of 50 μg/mL) and a human pathogen *Trichophyton rubrum* (MIC of 25−50 μg/mL).



✓isolation ✓structure determination ✓NMR calculation
✓biosynthesis ✓total synthesis ✓bioactivity

INTRODUCTION

The *O*-methyl nitronic acid functionality is quite rare in natural products. Enteromycin is the first natural compound that possesses this functional group.[1] To date, fewer than 10 related compounds have been isolated, exclusively from actinomycetes,[2−7] and most of them were described to show antibacterial activity against Gram-positive and/or Gram-negative bacteria. Despite the rarity of *O*-methyl nitronic acid, nothing is known about its biosynthesis and no total synthesis of this class of compounds has been described. Additionally, stereochemical assignment has not been made for any of the previously reported compounds with chiral centers.

Besides the terrestrial microorganisms that have been intensively screened for bioactive compounds, marine ecosystems have attracted attention as a reservoir for unexplored species of microorganisms and secondary metabolites thereof.[8] In the past several decades, the number of new natural products from marine microbes has been steadily increasing.[9,10] In particular, the marine environment is the potential niche for new species and/or genera of rare actinomycetes, from which several hundreds of new secondary metabolites were identified.[11,12] Our laboratory has been investigating secondary metabolites of marine actinomycetes to validate their biosynthetic potential as alternatives to terrestrial counterparts.[13−16] In the course of our continuous screening effort, one of the strains isolated from the seawater collected at a depth of 800 m in Sagami Bay, Japan, was found to produce two enteromycin-class metabolites. NMR- and MS-based structural analyses revealed that one was a known antibiotic A-76356 (**2**) and the other its new congener designated akazaoxime (**1**), bearing the aldoxime functionality instead of *O*-methyl nitronic acid (Figure 1). We herein describe the isolation, structure determination, and biosynthetic origin of **1** and **2** by ^{13}C labeling experiments. We also describe a total synthesis of **1** and its unnatural analogues, and the bioactivity of these compounds.

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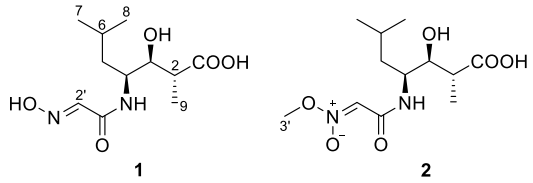

Figure 1. Structures of akazaoxime (**1**) and A-76356 (**2**).

RESULTS AND DISCUSSION

The producing strain *Micromonospora* sp. AKA109 was cultured in A16 medium, and the whole culture broth was extracted with 1-butanol. After evaporation, the extract was successively fractionated by silica gel and ODS column chromatographies. The final purification was performed by reverse-phase HPLC to yield akazaoxime (**1**) and A-76356 (**2**).

HRMS (ESI/TOF) analysis of **1** gave a molecular formula of $C_{11}H_{20}N_2O_5$, which was in good agreement with the ^{1}H and ^{13}C{^{1}H} NMR spectroscopic data. The IR spectrum displayed absorption bands for hydroxy and carbonyl groups (3268 and 1710 cm^{-1}). Analysis of ^{13}C{^{1}H} NMR and HSQC spectral data measured in CD_3OD allowed the assignment of 11 carbon resonances to two oxygenated sp^2 carbons (δ_C 179.5 and 164.8), one sp^2 methine (δ_C 144.0), four sp^3 methines (δ_C 75.9, 49.2, 45.4, and 26.1), one sp^3 methylene (δ_C 43.0), and three methyl groups (δ_C 23.6, 22.8 and 14.7) (Table 1). COSY analysis defined the connectivity among eight carbons, starting from the doublet methyl H9 to the isopropyl terminus comprising H6 methine and H7/H8 doublet methyls (Figure 2). An HMBC correlation from H9 to C1 (δ_C 179.5) indicated the presence of a carboxy group connecting to C2. ^{1}H and ^{13}C chemical shifts of H3/C3 and H4/C4 were suggestive of the connection of a hydroxy group at C3 and an amide functionality at C4, respectively. HMBC correlations from H4 and H2′ to C1′ connected the two-carbon unit (C1′−C2′) through the amide bond to C4. The remaining part was deduced to be an aldoxime group considering the chemical shift of C2′[17] and the remaining fragment HNO suggested by the high-resolution MS analysis, to complete the planar structure of **1** (Figure 2a).

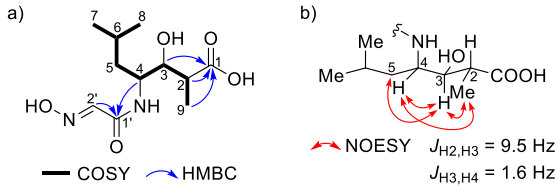

Figure 2. NMR analysis of **1**. (a) COSY and key HMBC correlations. (b) NOESY correlations and $^3J_{H,H}$ values supporting the relative configuration.

The geometry of the aldoxime group was determined to be *E* by comparison of the chemical shift of H2′ (δ_H 7.47) with literature values reported for *p*-chlorobenzaldoxime (*E*, δ_H 7.30; *Z*, δ_H 8.20) and *p*-nitrobenzaldoxime (*E*, δ_H 7.60; *Z*, δ_H 8.36).[18] The relative configurations of the contiguous asymmetric centers at C2−C4 were determined on the basis of $^3J_{HH}$ coupling constants and NOESY correlations. A large *J* value of 9.5 Hz for H2/H3 and a small value of 1.6 Hz for H3/H4 indicated an *anti*- and *syn*-relationship for these proton pairs, respectively (Figure 2b). Furthermore, NOEs were observed between H9 and H4, H9 and H3, H3 and H4, and H3 and H5, thereby establishing a 2*R**,3*S**,4*S** configuration. Next, the PGME method[19] was applied to determine the absolute configuration of C2. Compound **1** was reacted with (*R*)- or (*S*)-phenylglycine methyl ester (PGME) to prepare the corresponding PGME amides (**3a** and **3b**). Negative $\Delta\delta_{S-R}$ values for protons H3−H5 and a positive value for H9 unambiguously determined a 2*R* configuration (Figure 3), thereby establishing a 2*R*,3*S*,4*S* configuration.

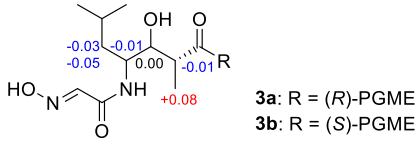

Figure 3. $\Delta\delta_{S-R}$ values for PGME derivatives (**3a** and **3b**) of **1**.

Table 1. ^{1}H and ^{13}C{^{1}H} NMR Data for Akazaoxime (1) and A-76356 (2)

	1					2		
	CD_3OD			acetone-d_6		$CDCl_3$		
position	δ_C[a]	δ_H mult [J (Hz)][b]	HMBC[b,c]	δ_C[a]	δ_H mult [J (Hz)][b]	δ_C[a]	δ_H mult [J (Hz)][b]	HMBC[b,c]
1	179.5, C			177.1		179.9, C		
2	45.4, CH	2.41 dq (9.5, 7.0)	1, 3, 4, 9	44.3	2.45 dq (9.5, 7.1)	42.9, CH	2.57 dq (8.6, 7.2)	1, 3, 4, 9
3	75.9, CH	3.69 dd (9.5, 1.6)	1, 2, 5	75.2	3.74 dd (9.5, 1.2)	74.5, CH	3.75 dd (8.8, 1.8)	1, 2, 5, 9
4	49.2, CH	4.24 ddd (8.9, 5.3, 1.6)	5, 6, 1′	48.2	4.25 br. dd (7.7, 5.5)	48.4, CH	4.28 dddd (9.5, 9.5, 5.5, 1.8)	5, 6, 1′
5	43.0, CH_2	1.37 m	3, 4, 6, 7, 8	42.7	1.39 m	41.6, CH_2	1.43 m	3, 4, 6, 7, 8
		1.59 m	3, 4, 6, 7, 8		1.63 m		1.67 m	3, 4, 6, 7, 8
6	26.1, CH	1.61 m	4, 5	25.5	1.63 m	24.8, CH	1.63 m	5, 7, 8
7	23.6, CH_3	0.946 d (6.3)	5, 6, 8	23.4	0.92 d (6.4)	22.9, CH_3	0.94 d (6.4)	5, 6, 8
8	22.8, CH_3	0.954 d (6.3)	5, 6, 7	22.6	0.94 d (6.3)	22.2, CH_3	0.95 d (6.4)	5, 6, 7
9	14.7, CH_3	1.17 d (7.0)	1, 2, 3	14.4	1.16 d (7.1)	14.1, CH_3	1.27 d (7.2)	1, 2, 3
1′	164.8, C			162.6		160.1, C		
2′	144.0, CH	7.47 s	1′	144.5	7.45 s	110.0, CH	6.83 s	1′
3′						54.3, CH_3	3.84 s	
NH					6.95 d (8.9)		8.42 d (9.5)	4, 1′

[a]Recorded at 125 MHz (referenced to δ_C 49.2 for CD_3OD, δ_C 29.8 for acetone-d_6, and δ_C 77.0 for $CDCl_3$). [b]Recorded at 500 MHz (referenced to δ_H 3.31 for CD_3OD, δ_H 2.05 for acetone-d_6, and δ_H 7.27 for $CDCl_3$). [c]HMBC correlations are from protons stated to the indicated carbon(s).

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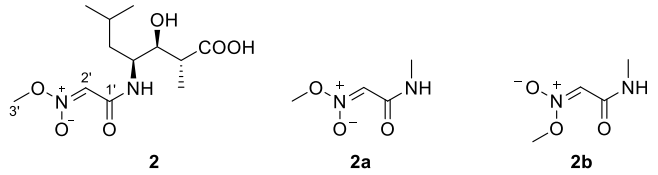
Table 2. Comparison of the Experimental and Calculated NMR Chemical Shifts of 2 and Truncated Structures 2a and 2b[a,b]



2 2a 2b

	A-76356 (2)		2a[a]		2b[a]		2a		2b	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	$\lvert\Delta\delta_C\rvert$[b]	$\lvert\Delta\delta_H\rvert$[b]	$\lvert\Delta\delta_C\rvert$[b]	$\lvert\Delta\delta_H\rvert$[b]
1′	160.1		159.0		158.4		1.1		1.7	
2′	110.0	6.83	111.1	6.53	111.3	6.14	1.1	0.30	1.3	0.69
3′	54.3	3.84	52.8	3.66	52.5	3.71	1.5	0.18	1.8	0.13
						MAE[c]	1.2	0.24	1.6	0.41

[a]NMR chemical shifts were calculated at the mPW1PW91/6-311+G(2d,p)-IEFPCM level of theory and corrected by the scaling facotors. See ref 22. [b]$\lvert\Delta\delta_C\rvert = \lvert\delta_C(\mathbf{2}) - \delta_C(\mathbf{2a}\text{ or }\mathbf{2b})\rvert$, and $\lvert\Delta\delta_H\rvert = \lvert\delta_H(\mathbf{2}) - \delta_H(\mathbf{2a}\text{ or }\mathbf{2b})\rvert$. [c]MAE is the mean absolute error of the calculated scaled shifts to experimental shifts.

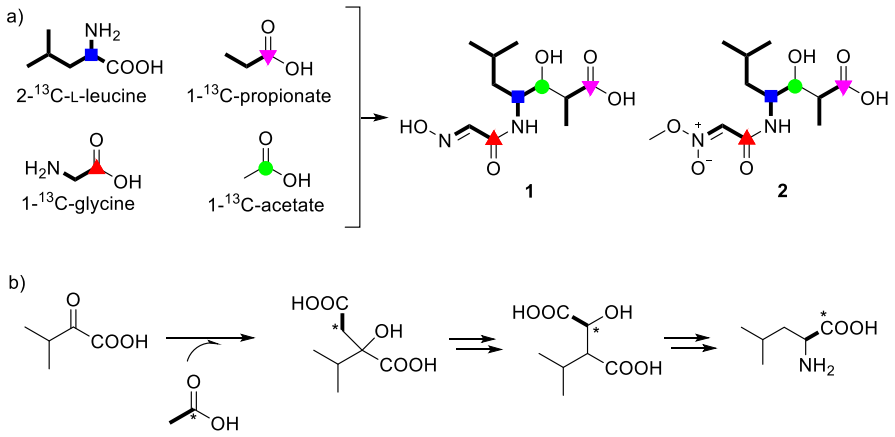


Figure 4. (a) Incorporation of ^{13}C-labeled precursors into akazaoxime (**1**) and A-76356 (**2**). (b) Biosynthesis of leucine from isovaleric acid and acetate.

The planar structure of **2**, determined by NMR and mass spectroscopic analyses (Table 1), was identical to A-76356, an antibacterial metabolite from *Micromonospora* described in a patent.[7] Because $J_{H,H}$ information was lacking in the literature and ^{1}H and ^{13}C chemical shifts were slightly different presumably due to different purification conditions, their identity was reasonably affirmed by the similarity in the producing strains and other physicochemical properties. Thus, **2** was concluded to be A-76356. Analysis of $^3J_{H,H}$ coupling constants (Table 1) and NOESY correlation data (Figure S21) supported the same relative configuration of **1**. The geometry of the *O*-methyl nitronate group was unclear, although a weak NOESY correlation between H2′ and H3′ was indicative of its *E* configuration. To gain further proof for this double bond geometry, NMR chemical shifts were calculated for the truncated model compounds, *E*-**2a** and *Z*-**2b**.[20,21] The experimental NMR data showed better matching to the simulated data for **2a**, with the MAE values being smaller than those of **2b** for both ^{1}H and ^{13}C{^{1}H} NMR chemical shifts, which supported the *E* geometry of the *O*-methyl nitronate group of **2** (Table 2).

Derivatization of **2** with PGME was not successful because of the instability under the reaction conditions, but it is highly likely that **1** and **2** possess the same absolute configuration considering their similar negative specific rotation values as well as the production from the same organism.

Enteromycin-class antibiotics are characterized by a rare nitrogen-containing functional group, *O*-methyl nitronic acid. To date, only fewer than 10 related compounds are known and all but **2** are produced by actinomycetes of the genus *Streptomyces*.[7] Among them, U-15774[4] has an aldoxime group. Akazaoxime (**1**) is the second to have this functionality in this class.

Despite the rarity of aldoxime and *O*-methyl nitronic acid in microbial metabolites, none of the compounds in this class have yet to become a subject of biosynthetic studies, which motivated us to conduct feeding experiments with ^{13}C-labeled leucine, propionate, acetate, and glycine, as the plausible precursors.

For the measurement of ^{13}C{^{1}H} NMR peak areas, acetone-d_6 was used as the solvent, because the C4 (δ_C 49.2) resonance in **1** (Table 1) was overlapped by a CD_3OD peak. Feeding of [1-^{13}C]propionate resulted in ^{13}C enrichment at C1, indicating the incorporation of methylmalonate into C1, C2, and C9 (Figure 4a). On the contrary, a DL-[2-^{13}C]leucine-fed culture produced a C4-labeled **1**, which suggested leucine to be the origin of the C_6 unit from C3 to C8. This was supported by labeling of C3 by [1-^{13}C]acetate feeding, because leucine is biosynthesized from α-ketoisovaleric acid and acetic acid in four steps (Figure 4b).[23] The aldoxime functionality was not labeled by any of the precursors described above except [1-^{13}C]glycine, which caused 2.5-fold signal enhancement at C1′ (Table 3). Similarly, the same labeling patterns were

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Table 3. Incorporation of ^{13}C-Labeled Precursors into Akazaoxime (1)

		relative enrichment[a]				
position	δ_C[b]	no label	DL-[2-^{13}C] leucine	[1-^{13}C] propionate	[1-^{13}C] acetate	[1-^{13}C] glycine
1	177.1	0.48	ND[c]	**6.75**	ND[c]	1.15
2	44.3	0.93	0.75	0.71	1.38	1.11
3	75.2	0.95	0.98	1.01	**3.56**	1.19
4	48.2	1.15	**2.61**	1.17	1.12	0.97
5	42.7	1.31	0.87	1.09	1.26	1.10
6	25.5	1.24	1.10	0.98	1.68	1.02
7	23.4	1.41	1.10	1.05	1.09	1.04
8	22.6	1.60	1.08	1.00	1.32	1.23
9	14.4	1.15	1.12	0.85	1.46	1.39
1′	162.6	0.50	0.86	0.84	0.58	**2.53**
2′	144.5	1.00	1.00	1.00	1.00	1.00

[a]^{13}C signal intensity of each peak in labeled **1** divided by that of the corresponding signal in the unlabeled, normalized to the peak area of C2′ to give an enrichment ratio for the enriched peak. The bold values indicate ^{13}C-enriched atoms from ^{13}C-labeled precursors. [b]Recorded at 125 MHz (referenced to δ_C 29.84 for acetone-d_6). [c]Not determined due to a low signal intensity.

obtained for **2** (Figure 4a and Table 4). Altogether, **1** was thought to be biosynthesized from propionate (methylmalonate), leucine, and glycine, and further modification at the aldoxime terminus should give nitronic acid **2**.

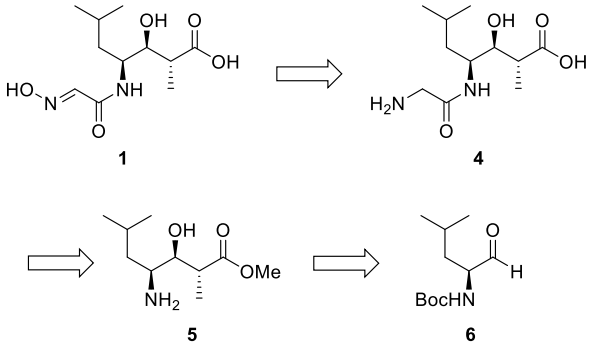
Table 4. Incorporation of ^{13}C-Labeled Precursors into A-76356 (2)

		relative enrichment[a]				
position	δ_C[b]	no label	L-[2-^{13}C] leucine	[1-^{13}C] propionate	[1-^{13}C] acetate	[1-^{13}C] glycine
1	179.9	0.70	0.36	**4.93**	0.43	0.44
2	42.9	0.99	0.91	1.06	0.64	1.17
3	74.5	0.96	0.89	0.98	**4.14**	1.05
4	48.4	0.94	**2.81**	1.10	0.91	1.10
5	41.6	1.09	1.31	1.18	0.93	1.49
6	24.8	1.02	1.34	1.29	1.34	1.81
7	22.9	1.26	1.62	1.45	1.84	2.13
8	22.2	1.16	1.48	1.30	1.58	2.05
9	14.1	0.98	1.05	1.21	1.30	1.17
1′	160.1	0.57	0.52	0.47	0.25	**2.95**
2′	110.0	1.00	1.00	1.00	1.00	1.00
3′	54.3	0.88	0.84	0.93	0.73	1.03

[a]^{13}C signal intensity of each peak in labeled **2** divided by that of the corresponding signal in the unlabeled, normalized to the peak area of C2′ to give an enrichment ratio for the enriched peak. The numbers in bold type indicate ^{13}C-enriched atoms from ^{13}C-labeled precursors. [b]Recorded at 125 MHz (referenced to δ_C 77.0 for $CDCl_3$).

The scarcity of **1** from the producing strain, *Micromonospora* sp. AKA109, hampered us from fully evaluating its biological activity. Thus, we decided to establish the synthetic route to **1** to obtain sufficient quantities for biological evaluation. The synthetic route was planned on the basis of the biosynthetic pathway (Scheme 1). We envisioned that **1** would be synthesized from **4** by oxidation of the amine to aldoxime at the final stage. Amide **4** would be obtained from amine **5** by condensation with Boc-glycine. The propionate part in **1** could be stereoselectively constructed through Brown's crotylation.

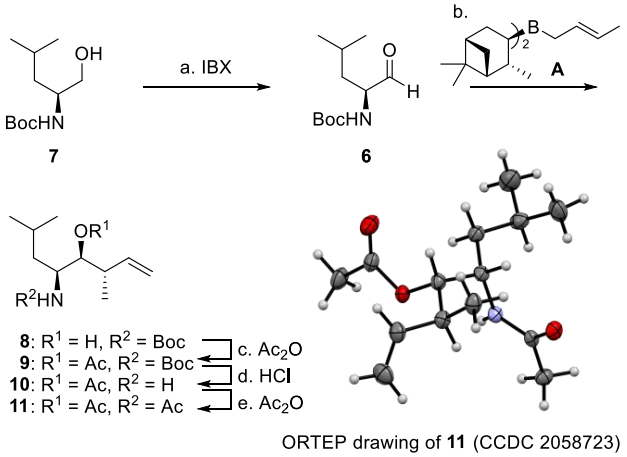
Scheme 1. Synthetic Plan of Akazaoxime (1)



Accordingly, we chose known *N*-Boc-L-leucinal (**6**) as a starting compound for the total synthesis.

N-Boc-L-leucinal (**6**) was synthesized by the IBX oxidation of *N*-Boc-L-leucinol (**7**) in refluxing EtOAc (Scheme 2).[24] The stereochemically unstable **6** was immediately subjected to Brown's crotylation[25,26] using BF_3·OEt_2 and **A**, which was prepared from (*E*)-2-butene and (−)-B(Ipc)$_2$OMe, affording **8** with the requisite three consecutive stereocenters as a single isomer. The structure of **8** was determined by the X-ray crystallographic analysis of **11**, which was derivatized from **8** in three steps, acetylation of the hydroxy group (**8** → **9**), removal of the Boc group (**9** → **10**), and acetamide formation (**10** → **11**).

Scheme 2[a]



ORTEP drawing of **11** (CCDC 2058723)

[a]Reagents and conditions: (a) IBX, EtOAc, reflux; (b) **A**, BF_3·OEt_2, THF, −78 °C; (c) Ac_2O, pyridine, DMAP, 53% (three steps); (d) HCl, MeOH; (e) Ac_2O, pyridine, Et_3N, DMAP, 67% (two steps).

Akazaoxime (**1**) was synthesized from **9** in eight steps (Scheme 3). First, the vinyl group of **9** was oxidatively cleaved with $KMnO_4$ to provide carboxylic acid **12**. After the acetyl group of **12** was removed under basic conditions, the carboxylic acid was protected as a methyl ester by the treatment with $TMSCHN_2$. Then, removal of the Boc group in TFA gave amine **5**, which was condensed with *N*-Boc-glycine by the action of EDCI·HCl and DMAP, constructing the full carbon skeleton of **1**. Prior to the final aldoxime formation, the methyl ester was hydrolyzed with NaOH, and the Boc group on the glycine residue was removed with TFA. Although the aldoxime was not obtained by the standard oxidation of **4** with oxone or MMPP, the use of *m*-CPBA in EtOAc[27] enabled the

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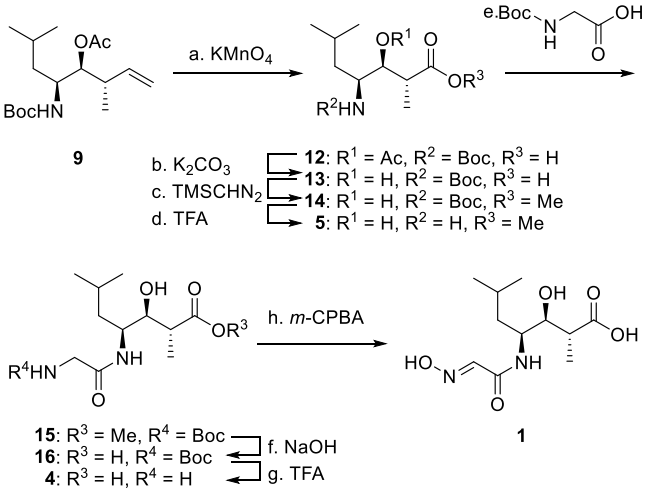
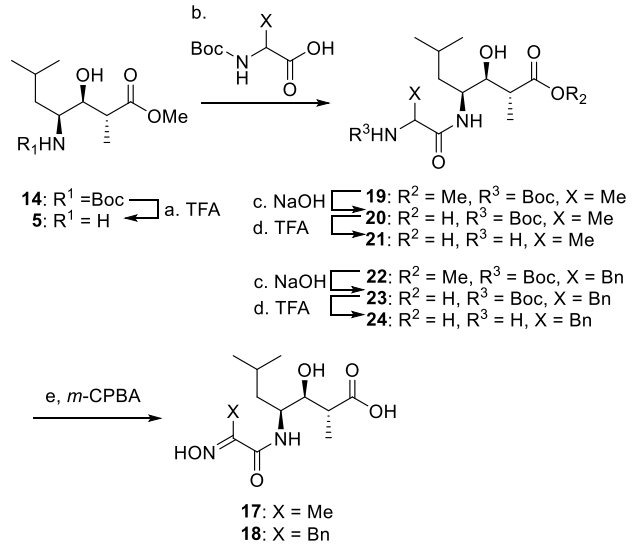
Scheme 3[a]



[a]Reagents and conditions: (a) $KMnO_4$, $NaIO_4$, $NaHCO_3$, *t*-BuOH, H_2O; (b) K_2CO_3, MeOH; (c) $TMSCHN_2$, MeOH, benzene, 42% (three steps); (d) TFA, CH_2Cl_2; (e) *N*-Boc-glycine, EDCI·HCl, Et_3N, DMAP, CH_2Cl_2, 75% (two steps); (f) aqueous NaOH, THF; (g) TFA, CH_2Cl_2; (h) *m*-CPBA, Na_2HPO_4, EtOAc, 33% (three steps).

Scheme 4[a]



[a]Reagents and conditions: (a) TFA, CH_2Cl_2; (b) *N*-Boc-DL-alanine/*N*-Boc-DL-phenylalanine, EDCI·HCl, Et_3N, DMAP, CH_2Cl_2; (c) aqueous NaOH, THF; (d) TFA, CH_2Cl_2; (e) *m*-CPBA, Na_2HPO_4, EtOAc, 35% for **17**, 52% for **18** (five steps).

desired oxidation, giving rise to **1**. All of the spectroscopic data of synthesized **1** ($[\alpha]_D$, IR, and ^1H and $^{13}C\{^1H\}$ NMR) were identical with those of natural **1**.

Establishment of the concise and flexible route of **1** motivated us to synthesize its unnatural analogues for biological evaluation. Focusing on the effect of the unique structure of the oxidized amino acid residue on bioactivity, we newly designed **17** and **18** as the synthetic target compounds, which possess oxime moieties on alanine and phenylalanine residues instead of the aldoxime of **1**.

After an acid treatment of **14**, the resulting amine **5** was condensed with *N*-Boc-DL-alanine and *N*-Boc-DL-phenylalanine to provide **19** and **22**, respectively (Scheme 4). Removal of the acetyl and Boc groups of **19** and **22**, followed by *m*-CPBA oxidations in EtOAc, afforded **17** and **18**, respectively.

The bioactivity of **1**, **17**, and **18**, as well as synthetic intermediate **4** for **1**, was examined in antimicrobial and cytotoxic assays (Table 5). Compounds **1** and **17** showed weak to moderate antimicrobial activities against Gram-positive *Kocuria rhizophila*, while these compounds were inactive against other Gram-positive and -negative bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Rhizobium radiobacter*), a yeast (*Candida albicans*), and filamentous fungi *Glomerella cingulata* and *Trichophyton rubrum*. Intriguingly, compounds **4** and **18** showed different antimicrobial spectra with selective activities against filamentous fungi *G. cingulata* and *T. rubrum* with MIC values of 25−50 μg/mL. Compound **2** was weakly active against *G. cingulata* with an MIC of 100 μg/mL but inactive against *T. rubrum*. These results indicated that the substituents of the C4-amino group of **1** affect the antimicrobial activity.

CONCLUSION

Our chemical investigation of a marine-derived *Micromonospora* AKA109 led to the isolation of a new enteromycin-class secondary metabolite akazaoxime (**1**) along with its known congener, A-76356 (**2**). The absolute configurations of **1** and **2** were determined by spectrochemical analyses, and the *E* geometry of *O*-methyl nitronic acid of **2** was confirmed by the NOESY analysis and NMR chemical shift calculation of truncated model compounds. The biosynthetic origin of their aldoxime/nitronic acid unit was elucidated to be glycine by precursor feeding experiments. The total synthesis of **1** was achieved on the basis of the biosynthetic pathway in 11 steps from *N*-Boc-L-leucinol (**7**). The synthetic route enabled us to supply sufficient amounts of **1** and its unnatural analogues for biological evaluation. Compound **1** was found to be weakly active against Gram-positive bacterium *K. rhizophila*, while compounds **4** and **18** possess antimicrobial spectra different from those of **1**, showing selective activity against filamentous fungi.

Enteromycin-class metabolites make up a very rare class of natural products possessing *O*-methyl nitronic acid functionality. Our ^{13}C labeling experiments suggested that this functional group originated from glycine via an aldoxime functionality through oxidation of the primary amino group.[28] Discovery of **1** validates the significance of marine environments as yet untapped habitats for unstudied microbial species to obtain new chemical entities.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a JASCO P-1030 polarimeter and an ATAGO AP-300 polarimeter at room temperature using the sodium D line. UV and IR spectra were recorded on a Shimadzu UV-1800 spectrophotometer and a PerkinElmer Spectrum 100 instrument, respectively. ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker AVANCE II 400 spectrometer and a Bruker ANANCE NEO 500 spectrometer at room temperature, unless otherwise noted. Chemical shifts are reported in parts per million on the δ scale relative to $CHCl_3$ (δ 7.27 for ^1H NMR), $CDCl_3$ (δ 77.0 for $^{13}C\{^1H\}$ NMR), CD_2HOD (δ 3.31 for ^1H NMR), CD_3OD (δ 49.15 for $^{13}C\{^1H\}$ NMR), C_6D_5H (δ 7.16 for ^1H NMR), C_6D_6 (δ 128.06 for $^{13}C\{^1H\}$ NMR), CD_2HCOCD_3 (δ 2.05 for ^1H NMR), and CD_3COCD_3 (δ 29.84 for $^{13}C\{^1H\}$ NMR), as internal references. Structural assignments were made with additional information from gCOSY, gHSQC, gHMBC, and gNOESY experiments. High-resolution mass spectra (HRMS) were recorded by ESI

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Table 5. Antimicrobial Activity of 1 and Its Synthetic Analogues

	MIC (μg/mL)					
microorganism	**1**	**4**	**17**	**18**	kanamycin	amphotericin B
Kocuria rhizophila ATCC9341	50	25	50	100	0.39	NT
S. aureus FDA209P JC-1	>100	>100	>100	>100	0.78	NT
E. coli NIHJ JC-2	>100	>100	>100	>100	1.25	NT
R. radiobacter NBRC14554	>100	>100	>100	>100	1.25	NT
C. albicans NBRC0197	>100	>100	>100	>100	NT	0.39
G. cingulata NBRC5907	>100	50	>100	50	NT	1.25
T. rubrum NBRC5467	>100	25	>100	50	NT	0.78

using a TOF-MS analyzer on a Bruker micrOTOF focus mass spectrometer. An Agilent HPLC system equipped with a diode array detector and a Shimazu HPLC system were used for analysis and purification. Melting points were measured on Yanaco micro melting point apparatus and are uncorrected. The X-ray crystallographic analysis was performed with a Bruker D8 QUEST instrument with a PHOTON II CPAD detector and with an IμS DIAMOND Microfocus CuKα radiation source (λ = 1.54178 Å) at 100 K.

All reactions sensitive to air or moisture were carried out in dry solvents under an argon atmosphere, unless otherwise noted. THF and CH_2Cl_2 (dehydrated, stabilizer free) were purchased from Kanto Chemical Co., Inc. All other reagents were used as supplied. Analytical thin-layer chromatography (TLC) was performed using Merck TLC Silica Gel 60 F254 (0.25 mm). An oil bath was used as the heat source for reactions that required heating. Preparative thin-layer chromatography (PTLC) was performed using Merck PLC Silica Gel 60 F254 (0.50 mm). Flash chromatography was performed using 63−210 μm Silica Gel 60N (Kanto Chemical Co., Inc.).

The conformational search was performed with MacroModel implemented in the Maestro 12.3 software package.[29,30] All DFT-based calculations were performed with Gaussian 16 revision B.01.[31] Some of these computations was conducted using the Super-Computer System (Institute for Chemical Research, Kyoto University). Molecular structures were visualized using the Maestro 12.3 software package.

Microorganism. *Micromosnospora* sp. AKA109 was isolated from a seawater sample collected at a depth of 800 m in Sagami Bay, Japan, as previously described.[13] The isolated strain was identified as *Micromonospora* on the basis of 99.4% similarity in the 16S rRNA gene sequence (1402 nucleotides; DDBJ accession number LC476779) to *Micromonospora rifamycinica* AM105^T (accession number LRMV01000349).

Fermentation. Strain AKA109 growing on a Bn-2 agar plate was inoculated into 500 mL K-1 flasks each containing 100 mL of seed medium consisting of 1% soluble starch, 0.5% glucose, 0.3% NZ-case (Sigma-Aldrich, Co.), 0.2% yeast extract (Kyokuto Pharmaceutical Industrial, Co., Ltd.), 0.5% Tryptone (Difco Laboratories), 0.1% K_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$, and 0.3% $CaCO_3$ (pH 7.0). The flasks were shaken at 30 °C for 4 days on a rotary shaker (200 rpm). The seed culture (3 mL) was transferred into 500 mL K-1 flasks each containing 100 mL of A-16 production medium consisting of 2.0% glucose, 1.0% Pharmamedia (Traders Protein), 0.5% $CaCO_3$, and 1.0% Diaion HP-20 (Mitsubishi Chemical Co.) (pH 7.0). The inoculated flasks were placed on a rotary shaker (200 rpm) at 30 °C for 7 days.

Extraction and Isolation. At the end of the fermentation period, 100 mL of 1-butanol was added to each flask, which was then agitated on a rotary shaker for 1 h. The mixture was centrifuged at 6000 rpm for 10 min, and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the organic layer gave approximately 3.4 g of extract from 2 L of culture. This crude material was subjected to silica gel column chromatography with a step gradient of $CHCl_3$/MeOH [1/0, 20/1, 10/1, 4/1, 2/1, 1/1, and 0/1 (v/v)]. Concentration of fraction 4 (4/1) provided 309 mg of dark brown oil, which was further purified by ODS column chromatography with a step gradient of a MeCN/0.1% HCO_2H solution [2/8, 3/7, 4/6, 5/5, 6/4, 7/3, and 8/2 (v/v)]. Fraction 2 (3/7) was evaporated to give 15 mg of yellow oil. Final purification was achieved by HPLC purification (COSMOSIL 5C_{18}-AR-II, Nacalai Tesque, 10 mm × 250 mm) with a MeOH/0.1% HCO_2H solution (38/62) at a rate of 4 mL/min. The collected fraction was evaporated under reduced pressure, and the resulting aqueous layer was extracted with ethyl acetate. Concentration of the organic layer gave akazaoxime (**1**, 8.9 mg, t_R = 14.1 min). Concentration of silica gel column fraction 3 (10/1) provided 0.32 g of brown oil, which was further purified by ODS column chromatography with a step gradient of a MeCN/0.1% HCO_2H solution [2/8, 3/7, 4/6, 5/5, 6/4, 7/3, and 8/2 (v/v)]. Fraction 3 (4/6) was concentrated to give 39 mg of yellow oil, which was subjected to HPLC purification (COSMOSIL 5C_{18}-AR-II, Nacalai Tesque, 10 mm × 250 mm) with a MeCN/0.1% HCO_2H solution (24/76) at a rate of 4 mL/min, in a manner similar to that described for **1**, to yield A-76356 (**2**, 8.0 mg, t_R = 20.8 min).

Akazaoxime (1). Colorless amorphous; $[\alpha]^{25}_D$ −23 (*c* 0.10, MeOH); IR (ATR) ν_{max} 3268, 2957, 1710, 1657, 1535 cm^{-1}; UV (MeOH) λ_{max} (log ε) 213 (3.56) nm; for ^{1}H and ^{13}C{^{1}H} NMR data, see Table 1; HRMS (ESI/TOF) *m*/*z* 283.1256 [M + Na]$^+$ (calcd for $C_{11}H_{20}N_2NaO_5$, 283.1264).

A-76356 (2). Light brown amorphous; $[\alpha]^{25}_D$ −18 (*c* 0.10, MeOH) [lit.[7] $[\alpha]^{25}_D$ −27 (*c* 0.70, MeOH)]; IR (ATR) ν_{max} 3324, 2955, 1710, 1640, 1538 cm^{-1}; UV (MeOH) λ_{max} (log ε) 251 (4.07) nm; for ^{1}H and ^{13}C{^{1}H} NMR data, see Table 1; HRMS (ESI/TOF) *m*/*z* 313.1364 [M + Na]$^+$ (calcd for $C_{12}H_{22}N_2NaO_6$, 313.1370).

Preparation of PGME Amides (3a and 3b) of 1. To a solution of **1** (1.0 mg, 3.9 μmol) in dry DMF (100 μL) and dry triethylamine (50 μL) were added (*R*)-PGME (4.1 mg, 15 μmol), PyBOP (4.1 mg, 7.9 μmol), and HOBt (2.0 mg, 15 μmol) at room temperature. After being stirred for 1 h, the reaction mixture was concentrated by lyophilization. This crude mixture was directly purified by preparative HPLC (COSMOSIL 5C_{18}-PAQ, Nacalai Tesque, 10 mm × 250 mm) with a gradient of a MeCN/0.1% HCO_2H solution (MeCN concentration of 34% from 0 to 5 min and 34% to 100% from 5 to 71 min) to afford (*R*)-PGME amide (**3a**, 1.2 mg, t_R = 26.3 min): ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (s, 1H, H2′), 7.40−7.32 (m, 5H, -C_6H_5), 5.50 (s, 1H, −C*H*Ph), 4.24 (m, 1H, H4), 3.70 (s, 3H, -OCH_3), 3.69 (overlapped, 1H, H3), 2.54 (dq, 1H, *J* = 6.0, 8.6 Hz, H2), 1.61 (m, 2H, H5 and H6), 1.37 (m, 1H, H5), 1.09 (d, 3H, *J* = 6.9 Hz, H9), 0.94 (d, 6H, *J* = 6.3 Hz, H7 and H8); HRMS (ESI/TOF) *m*/*z* 430.1943 [M + Na]$^+$ (calcd for $C_{20}H_{30}N_3NaO_6$ 430.1954).

(*S*)-PGME amide (**3b**, 1.4 mg, t_R = 25.6 min) was obtained by the reaction of **1** (1.0 mg, 3.9 μmol) and (*S*)-PGME in the same manner as described for (*R*)-PGME amide (**3a**): ^{1}H NMR (400 MHz, CD_3OD) δ 7.43 (s, 1H, H2′), 7.41−7.32 (m, 5H, -C_6H_5), 5.50 (s, 1H, −C*H*Ph), 4.23 (m, 1H, H4), 3.70 (s, 3H, -OCH_3), 3.69 (overlapped, 1H, H3), 2.53 (m, 1H, H2), 1.58 (m, 1H, H6), 1.56 (m, 1H, H5), 1.34 (m, 1H, H5), 1.17 (d, 3H, *J* = 6.9 Hz, H9), 0.93 (d, 6H, *J* = 6.5 Hz, H7 and H8); HRMS (ESI/TOF) *m*/*z* 430.1942 [M + Na]$^+$ (calcd for $C_{20}H_{30}N_3NaO_6$ 430.1954).

Feeding Experiments. Feeding experiments were performed with DL-[2-^{13}C]leucine, sodium [1-^{13}C]propionate, sodium [1-^{13}C]-acetate, and [1-^{13}C]glycine. Inoculation, cultivation, and purification were carried out in the same manner as described above. Addition of

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^{13}C-labeled precursors was initiated 48 h after inoculation and periodically carried out every 24 h four times. After being incubated for a further 24 h, the cultures were extracted with 1-butanol and subjected to the same purification protocol described above.

(1) DL-[2-^{13}C]Leucine. After feeding of DL-[2-^{13}C]leucine (Sigma-Aldrich, total of 80 mg, 2 mg × 10 flasks × 4 days), ^{13}C-labeled **1** (1.9 mg) and **2** (2.9 mg) were isolated from 1 L of culture. The ^{13}C{^{1}H} NMR spectra showed an enriched signal at δ 48.2 for **1** and δ 48.4 for **2**.

(2) *Sodium [1-^{13}C]Propionate*. After feeding of sodium [1-^{13}C]-propionate (Cambridge Isotope Laboratories Inc., total of 800 mg, 20 mg × 10 flasks × 4 days), ^{13}C-labeled **1** (9.0 mg) and **2** (3.4 mg) were obtained from 1 L of culture. The ^{13}C{^{1}H} NMR spectra showed an enriched signal at δ 177.1 for **1** and δ 179.9 for **2**.

(3) *Sodium [1-^{13}C]Acetate*. After feeding of sodium [1-^{13}C]acetate (Cambridge Isotope Laboratories Inc., total of 800 mg, 20 mg × 10 flasks × 4 days), ^{13}C-labeled **1** (3.8 mg) and **2** (1.5 mg) were obtained from 1 L of culture. The ^{13}C{^{1}H} NMR spectrum showed an enriched signal at δ 75.2 for **1** and δ 74.5 for **2**.

(4) *[1-^{13}C]Glycine*. After feeding of [1-^{13}C]glycine (Cambridge Isotope Laboratories Inc., total of 80 mg, 2 mg × 10 flasks × 4 days), ^{13}C-labeled **1** (9.9 mg) and **2** (5.5 mg) were obtained from 1 L of culture. The ^{13}C{^{1}H} NMR spectrum showed an enriched signal at δ 162.6 for **1** and δ 160.1 for **2**.

Procedure for the Optimization, Energy Evaluation, and Simulation of the NMR Spectra for Structures 2a and 2b. The conformational search on structure **2a** began upon application of 100000 steps of the Monte Carlo Multiple Minimum (MCMM) method with PRCG energy minimization using the OPLS3e force field (gas phase) to obtain 11 conformational isomers with energies within 35.85 kcal/mol. The next optimizations were performed at the B3LYP/6-31+G(d,p) level of theory. Frequency calculations were carried out at the same level of theory to confirm the absence of imaginary frequencies and to obtain thermal corrections to the Gibbs free energies at 1 atm and 298.15 K. After single-point energies were calculated at the B3LYP/6-311+G(d,p) level of theory with the IEF-PCM solvation model ($CHCl_3$), the thermal corrections at the B3LYP/6-31+G(d,p) level were added to obtain the Gibbs free energies. The shielding tensors of the most stable geometry were calculated by the GIAO method at the mPW1PW91/6-311G+(2d,p) level of theory with solvation of the IEF-PCM ($CHCl_3$) model. Because the other 10 conformers were located at significantly higher energies (>2.5 kcal/mol), these conformers were not subjected to the calculation. The shielding tensors (σ) were converted the chemical shifts (δ_{calc}) using the equation δ_{calc} = (intercept − σ)/−slope, where slope and intercept are scaling factors, −1.0719 and 31.8733 for ^{1}H and −1.0420 and 186.3567 for ^{13}C, respectively, reported by Pierens.[22] The NMR spectra of structure **2b** were similarly simulated by using seven conformers as the OPLS3e-minimized structures and DFT-optimized structures and one conformer as the geometry for the NMR calculation.

Synthetic Procedures for Synthesis of Akazaoxime (1) and Its Analogues. *Boc-L-leucinol 7*. Boc_2O (5.9 mL, 25.6 mmol) was added to a solution of a commercially available L-leucinol (2.50 g, 21.3 mmol) and Et_3N (4.4 mL, 32.0 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was stirred at room temperature for 20 h, and then H_2O (110 mL) was added. After being stirred for 1.5 h, the mixture was separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel twice (70 g, hexane/EtOAc 10/1 to 5/1 to 3/1 to EtOAc; 100 g, hexane/EtOAc 20/1 to 15/1 to 10/1 to 5/1 to 2/1 to 1/2 to 1/10) to afford Boc-L-leucinol **7** (3.79 g, 17.4 mmol) in 82% yield as a colorless oil. Boc-L-leucinol **7** is a known compound, and the ^{1}H NMR spectrum of synthesized **7** was identical to that reported previously.[32]

Acetate 9. IBX (39% purity, 9.87 g, 13.7 mmol) was added to a solution of Boc-L-leucinol **7** (3.00 g, 13.8 mmol) in EtOAc (55 mL). The reaction mixture was heated to reflux temperature and stirred for 3.5 h. After being cooled to room temperature, the reaction mixture was filtered through a short pad of Celite with Et_2O (100 mL). Saturated aqueous $NaHCO_3$ (30 mL) was added. After separation, the resulting solution was extracted with Et_2O (3 × 30 mL), and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (200 g, hexane/EtOAc 10/1 to 5/1) to afford crude **6**, which was used in the next reaction without further purification.

A balloon charged with *trans*-2-butene was connected to an ice-cooled flask at −78 °C, and *trans*-2-butene (~6 mL, ~60 mmol) was released. After the balloon was removed, *t*-BuOK (3.10 g, 27.6 mmol) in THF (15 mL) and *n*-BuLi (2.5 M in hexanes, 11 mL, 28 mmol) were added. The reaction mixture was warmed to −45 °C and stirred for 10 min. The solution was cooled to −78 °C, and then a solution of (−)-methoxydiisopinocampheylborane (8.12 g, 25.7 mmol) in THF (10 mL) was added. After the resulting mixture was stirred at −78 °C for 30 min, BF_3·OEt_2 (3.5 mL, 28 mmol) and the above crude **6** in THF (2 × 5 mL) were added. The reaction mixture was stirred at −78 °C for 6 h, and then a NaOH solution (3 M in H_2O, 10 mL) and 30% aqueous H_2O_2 (5 mL) were added. The mixture was warmed to room temperature and stirred vigorously for 13 h. The resulting solution was extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic layers were washed with H_2O (2 × 30 mL) and brine (30 mL), dried over Na_2SO_4, filtered, and concentrated to afford crude **8**, which was used in the next reaction without further purification.

Ac_2O (10 mL) and DMAP (16.1 mg, 132 μmol) were added to a solution of crude **8** in pyridine (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 19 h. After toluene was added, the resulting solution was concentrated. Et_2O (30 mL) was added, and the combined organic layers were washed with a HCl solution (1 M in H_2O, 2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL), and brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (500 g, hexane/EtOAc 50/1 to 10/1) to afford acetate **9** (2.30 g, 7.33 mmol) in 53% yield over three steps as a colorless oil: $[\alpha]^{27}_{D}$ −49.5 (*c* 1.01, $CHCl_3$); IR (ATR) ν_{max} 3359, 2950, 1704, 1519, 1366, 1248, 1169, 1025 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6, 336 K) δ 5.72 (m, 1H), 4.93 (br d, 1H, *J* = 16.9 Hz), 4.91 (dd, 1H, *J* = 10.1, 1.7 Hz), 4.84 (br d, 1H, *J* = 5.6 Hz), 4.41 (br s, 1H), 4.19 (br s, 1H), 2.47 (m, 1H), 1.70 (s, 3H), 1.75−1.65 (m, 1H), 1.44 (s, 9H), 1.17 (m, 2H), 1.06 (d, 3H, *J* = 6.3 Hz), 0.95 (d, 3H, *J* = 6.3 Hz), 0.86 (d, 3H, *J* = 6.7 Hz); ^{13}C{^{1}H} NMR (125 MHz, C_6D_6, 336 K) δ 169.7, 155.8, 140.6, 115.3, 78.9, 78.7, 49.5, 43.1, 40.8, 28.6, 25.1, 23.2, 22.3, 20.4, 17.2; HRMS (ESI/TOF) *m*/*z* 336.2144 [M + Na]$^{+}$ (calcd for $C_{17}H_{31}NNaO_4$, 336.2145).

Acetamide 11. A HCl solution (3 M in H_2O, 0.10 mL) was added to a solution of acetate **9** (100 mg, 319 μmol) in MeOH (1 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h, and then a HCl solution (3 M in H_2O, 0.4 mL) was added. The reaction mixture was stirred at room temperature for 1 h, and then a HCl solution (6 M in H_2O, 0.4 mL) was added. The reaction mixture was stirred at room temperature for 18 h, and then a HCl solution (6 M in H_2O, 0.4 mL) was added. The reaction mixture was stirred at room temperature for 2 h, and then a HCl solution (6 M in H_2O, 0.4 mL) was added. The reaction mixture was stirred at room temperature for 25 h. The reaction mixture was warmed to 50 °C and stirred for 40 min. After the reaction mixture was cooled to room temperature, EtOH was added. The resulting solution was concentrated to afford crude **10**, which was used in the next reaction without further purification.

Et_3N (0.10 mL, 0.73 mmol) was added to a solution of crude **10** in a mixture of pyridine (20 mL) and Ac_2O (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h, and then DMAP (3.0 mg, 20 μmol) was added. The reaction mixture was stirred at room temperature for 15 h, and then Et_3N (0.20 mL, 1.4 mmol) was added. The reaction mixture was warmed to 50 °C and stirred for 2 h. After the reaction mixture was cooled to room temperature, toluene was added. The resulting solution was concentrated. The residue was dissolved in Et_2O (30 mL), and the

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organic layer was washed with a HCl solution (1 M in H_2O, 2 × 30 mL), $NaHCO_3$ (2 × 30 mL), and brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (10 g, hexane/EtOAc 10/1 to 3/1) to afford acetamide **11** (54.8 mg, 215 μmol) in 67% yield over two steps as colorless crystals. Acetamide **11** was recrystallized by a slow evaporation method from a mixture of Et_2O and hexane for the X-ray crystallographic analysis: mp 99−100 °C; $[\alpha]^{28}_D$ −42 (*c* 0.71, $CHCl_3$); IR (ATR) ν_{max} 3272, 2966, 2934, 2873, 1738, 1642, 1553, 1366, 1234 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.62 (ddd, 1H, *J* = 17.1, 10.2, 9.1 Hz), 5.45 (br d, 1H, *J* = 9.7 Hz), 4.98 (br d, 1H, *J* = 17.1 Hz), 4.95 (dd, 1H, *J* = 10.2, 1.6 Hz), 4.74 (dd, 1H, *J* = 8.3, 3.0 Hz), 4.40−4.31 (m, 1H), 2.33 (m, 1H), 2.02 (s, 3H), 1.98 (s, 3H), 1.58 (m, 1H), 1.25−1.18 (m, 2H), 1.04 (d, 3H, *J* = 6.8 Hz), 0.88 (d, 3H, *J* = 6.3 Hz), 0.87 (d, 3H, *J* = 6.5 Hz); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 170.4, 169.4, 139.7, 115.4, 78.2, 47.3, 42.1, 40.3, 24.5, 23.3, 23.0, 22.1, 20.8, 17.1; HRMS (ESI/TOF) *m*/*z* 278.1724 $[M + Na]^+$ (calcd for $C_{14}H_{25}NNaO_3$, 278.1727).

Methyl Ester ***14***. $NaHCO_3$ (1.41 g, 16.8 mmol), $NaIO_4$ (4.32 g, 20.2 mmol), and $KMnO_4$ (266 mg, 1.68 mmol) were added to a solution of acetate **9** (527 mg, 1.68 mmol) in a mixture of *t*-BuOH (19 mL) and H_2O (8.4 mL) at room temperature. The reaction mixture was stirred at room temperature for 30 min, and then saturated aqueous $NaHSO_3$ (20 mL) was added at 0 °C. A HCl solution (1 M in H_2O, 100 mL) was added, and the resulting solution was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (60 g, hexane/EtOAc/AcOH 5/1/0.03 to 1/1/0.01) to afford crude carboxylic acid **12**, which was used in the next reaction without further purification.

K_2CO_3 (1.66 g, 12.0 mmol) was added to a solution of crude **12** in MeOH (11 mL) at room temperature. The reaction mixture was stirred at room temperature for 22 h, and then a HCl solution (1 M in H_2O, 10 mL) was added. The resulting solution was extracted with EtOAc (3 × 20 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford crude **13**, which was used in the next reaction without further purification.

$TMSCHN_2$ (2.0 M in Et_2O, 1.8 mL, 3.6 mmol) was added to a solution of crude **13** in a mixture of benzene (11 mL) and MeOH (1.1 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h, and then AcOH (0.2 mL) was added. The resulting solution was concentrated. The residue was purified by flash column chromatography on silica gel (10 g, hexane/EtOAc 10/1 to 5/1) to afford methyl ester **14** (127 mg, 419 μmol) in 42% yield over three steps as a colorless oil: $[\alpha]^{24}_D$ −48.8 (*c* 1.02, $CHCl_3$); IR (ATR) ν_{max} 3447, 3389, 2957, 2931, 2872, 1713, 1502, 1366, 1250, 1163 cm^{-1}; ^1H NMR (500 MHz, C_6D_6, 333 K) δ 4.69 (br s, 1H), 3.97 (br s, 1H), 3.59 (br d, 1H, *J* = 8.0 Hz), 3.34 (s, 3H), 2.69 (m, 1H), 1.64 (m, 1H), 1.52 (m, 1H), 1.42 (s, 9H), 1.27−1.20 (m, 1H), 1.20 (d, 3H, *J* = 6.6 Hz), 0.95 (d, 3H, *J* = 6.4 Hz), 0.88 (d, 3H, *J* = 6.6 Hz); $^{13}C\{^1H\}$ NMR (125 MHz, C_6D_6, 333 K) δ 176.7, 156.2, 78.8, 75.6, 51.3, 49.8, 43.6, 42.9, 28.5, 25.2, 23.2, 22.5, 14.1; HRMS (ESI/TOF) *m*/*z* 326.1938 $[M + Na]^+$ (calcd for $C_{15}H_{29}NNaO_5$, 326.1935).

Amide ***15***. TFA (0.67 mL) was added to a solution of methyl ester **14** (265 mg, 873 μmol) in CH_2Cl_2 (2.5 mL) at room temperature. The reaction mixture was stirred at room temperature for 12 h, and then toluene was added. The resulting solution was concentrated to afford crude amine **5**, which was used in the next reaction without further purification.

Et_3N (0.36 mL, 2.6 mmol), EDCI·HCl (443 mg, 2.31 mmol), *N*-Boc-glycine (183 mg, 1.04 mmol), and DMAP (32.6 mg, 267 μmol) were added to a solution of crude **5** in CH_2Cl_2 (8.7 mL) at 0 °C. The reaction mixture was stirred for 4.5 h at room temperature, and then saturated aqueous NH_4Cl (5.0 mL) and H_2O (3.0 mL) were successively added. The resulting solution was extracted with EtOAc (5 × 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel twice (25 g, hexane/EtOAc 2/1 to 1/1; 25 g, hexane to hexane/EtOAc 1/1). to afford amide **15** (235 mg, 652 μmol) in 75% yield over two steps as a yellow oil: $[\alpha]^{28}_D$ −46 (*c* 0.93, $CHCl_3$); IR (ATR) ν_{max} 3342, 2956, 2497, 1714, 1652, 1367, 1250, 1166, 1049, 860 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.20 (d, 1H, *J* = 9.5 Hz), 4.15 (m, 1H), 3.69 (br s, 2H), 3.66 (s, 3H), 3.68−3.62 (m, 1H), 2.51 (m, 1H), 1.66−1.51 (m, 2H), 1.46 (s, 9H), 1.34−1.28 (m, 1H), 1.12 (d, 3H, *J* = 7.0 Hz), 0.94−0.92 (m, 6H); $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD) δ 178.0, 172.54, 172.47, 158.5, 81.0, 75.9, 52.3, 45.3, 45.1, 43.01, 42.97, 28.9, 25.9, 23.7, 22.8, 14.6; HRMS (ESI/TOF) *m*/*z* 383.2153 $[M + Na]^+$ (calcd for $C_{17}H_{32}N_2NaO_6$, 383.2131).

*Akazaoxime (****1****)*. A NaOH solution (1 M in H_2O, 590 μL) was added to a solution of amide **15** (169 mg, 469 μmol) in THF (5.8 mL) at room temperature. The reaction mixture was stirred at room temperature for 3.5 h, and then a HCl solution (1 M in H_2O, 5 mL) was added. The resulting solution was extracted with EtOAc (5 × 20 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford crude carboxylic acid **16**, which was used in the next reaction without further purification.

TFA (1.0 mL) was added to a solution of crude **16** in CH_2Cl_2 (4.6 mL) at room temperature for 2 h, and then toluene was added. The resulting solution was concentrated to afford crude amine **4**, which was used in the next reaction without further purification.

Na_2HPO_4 (336 mg, 2.36 mmol) and *m*-CPBA (~70% purity, 285 mg, ~1.15 mmol) were added to a solution of crude **4** in EtOAc (4.6 mL) at room temperature. The reaction mixture was stirred at room temperature for 4 h. The resulting solution was directly purified by flash column chromatography on silica gel twice (20 g, hexane/EtOAc 3/1 to EtOAc/MeOH/AcOH 10/1/0.1; 20 g, hexane/EtOAc 1/1 to EtOAc/MeOH/AcOH 10/1/0.7) to afford akazaoxime (**1**, 22.2 mg, 85.3 mmol) as a colorless oil and the crude product (67.5 mg). The crude product was repurified by HPLC (Mightysil RP-18GPII, 10 mm × 250 mm) with a MeOH/0.1% HCO_2H solution (50/50) at a rate of 4 mL/min (t_R = 7.1 min). Collected fractions were extracted with EtOAc, and the organic layers were dried over Na_2SO_4, filtered, and concentrated to afford akazaoxime (**1**, 18.6 mg, 71.5 mmol) as a colorless oil. The combined yield was calculated as 33% over three steps: $[\alpha]^{28}_D$ −31 (*c* 0.95, MeOH); IR (ATR) ν_{max} 3270, 2958, 2931, 1708, 1657, 1537, 1462, 1255, 1200, 1200, 1016 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.47 (s, 1H), 4.24 (m, 1H), 3.68 (dd, 1H, *J* = 9.3, 1.5 Hz), 2.41 (dq, 1H, *J* = 9.3, 7.0 Hz), 1.65−1.56 (m, 2H), 1.37 (m, 1H), 1.17 (d, 3H, *J* = 7.0 Hz), 0.954 (d, 3H, *J* = 6.3 Hz), 0.947 (d, 3H, *J* = 6.3 Hz); $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD) δ 179.6, 164.9, 143.9, 75.9, 49.2, 45.4, 42.9, 26.1, 23.6, 22.8, 14.8; HRMS (ESI/TOF) *m*/*z* 283.1269 $[M + Na]^+$ (calcd for $C_{11}H_{20}N_2NaO_5$, 283.1264).

Analogue ***17***. TFA (0.12 mL) was added to a solution of methyl ester **14** (36.6 mg, 121 μmol) in CH_2Cl_2 (0.7 mL) at room temperature. The reaction mixture was stirred at room temperature for 12 h, and then toluene was added. The resulting solution was concentrated to afford crude amine **5**, which was used in the next reaction without further purification.

Et_3N (50 μL, 362 μmol), EDCI·HCl (61.7 mg, 322 μmol), *N*-Boc-DL-alanine (23.5 mg, 124 μmol), and DMAP (32.6 mg, 267 μmol) were added to a solution of crude **5** in CH_2Cl_2 (1.2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4 h, and then saturated aqueous NH_4Cl (1.0 mL) and H_2O (2.0 mL) were successively added. The resulting solution was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel twice (10 g, hexane/EtOAc 3/1 to 1/1; 10 g, hexane/EtOAc 2/1 to EtOAc) to afford crude amide **19**, which was used in the next reaction without further purification.

A NaOH solution (1 M in H_2O, 120 μL) was added to a solution of crude **19** (33.2 mg, 88.7 μmol) in THF (1.2 mL) at room temperature. The reaction mixture was stirred at room temperature for 11 h, and then a HCl solution (1 M in H_2O, 1 mL) was added. The resulting solution was extracted with EtOAc (5 × 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford crude carboxylic acid **20**, which was used in the next reaction without further purification.

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TFA (200 μL) was added to a solution of crude **20** in CH_2Cl_2 (0.9 mL) at room temperature for 2 h, and then toluene was added. The resulting solution was concentrated to afford crude amine **21**, which was used in the next reaction without further purification.

Na_2HPO_4 (68.4 mg, 482 μmol) and *m*-CPBA (~70% purity, 58.9 mg, ~239 μmol) were added to a solution of crude **21** in EtOAc (0.94 mL) at room temperature. The reaction mixture was stirred at room temperature for 11 h. The resulting solution was directly purified by flash column chromatography on silica gel (10 g, hexane/EtOAc 2/1 to 1/1 to EtOAc to EtOAc/MeOH 10/1) and PTLC (hexane/EtOAc/AcOH 4/1/0.1) to afford analogue **17** (11.6 mg, 42.3 μmol) in 35% yield over five steps as a colorless oil: $[\alpha]^{28}_D$ −9.34 (*c* 1.07, MeOH); IR (ATR) ν_{max} 3320, 2962, 1668, 1556, 1453, 1193, 1142, 1024, 801, 724 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.34 (br d, 1H, *J* = 10.1 Hz), 4.21 (m, 1H), 3.69 (br d, 1H, *J* = 9.5 Hz), 2.39 (m, 1H), 1.98 (s, 3H), 1.63−1.53 (m, 2H), 1.36 (m, 1H), 1.15 (d, 3H, *J* = 7.0 Hz), 0.95 (d, 3H, *J* = 6.6 Hz), 0.94 (d, 3H, *J* = 6.6 Hz); $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD) δ 179.8, 166.1, 151.2, 75.9, 49.2, 45.5, 43.3, 26.1, 23.6, 22.9, 14.8, 9.3; HRMS (ESI/TOF) *m*/*z* 273.1456 $[M - H]^-$ (calcd for $C_{12}H_{21}N_2O_5$, 273.1448).

Analogue ***18***. TFA (0.1 mL) was added to a solution of methyl ester **14** (24.9 mg, 82.1 μmol) in CH_2Cl_2 (0.7 mL) at room temperature. The reaction mixture was stirred at room temperature for 13 h, and then toluene was added. The resulting solution was concentrated to afford crude amine **5**, which was used in the next reaction without further purification.

Et_3N (35 μL, 246 μmol), EDCI·HCl (42.5 mg, 222 μmol), *N*-Boc-DL-phenylalanine (22.4 mg, 84.6 mmol), and DMAP (3.3 mg, 27 μmol) were added to a solution of crude **5** in CH_2Cl_2 (0.82 mL) at 0 °C. The reaction mixture was stirred for 5 h at room temperature, and then saturated aqueous NH_4Cl (1.0 mL) and H_2O (3.0 mL) were successively added. The resulting solution was extracted with CH_2Cl_2 (4 × 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel twice (10 g, hexane/EtOAc 5/1 to 1/1; 10 g, hexane/EtOAc 2/1 to 1/1) to afford crude amide **22**, which was used in the next reaction without further purification.

A NaOH solution (1 M in H_2O, 90 μL) was added to a solution of crude **22** (32.2 mg, 71.4 μmol) in THF (0.9 mL) at room temperature. The reaction mixture was stirred at room temperature for 11 h, and then a NaOH solution (1 M in H_2O, 90 μL) was added. The reaction mixture was stirred at room temperature for a further 11 h, and then a HCl solution (1 M in H_2O, 1 mL) was added. The resulting solution was extracted with EtOAc (5 × 5 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford crude carboxylic acid **23**, which was used in the next reaction without further purification.

TFA (150 μL) was added to a solution of crude **23** in CH_2Cl_2 (0.74 mL) at room temperature for 2 h, and then toluene was added. The resulting solution was concentrated to afford crude amine **24**, which was used in the next reaction without further purification.

Na_2HPO_4 (52.0 mg, 482 μmol) and *m*-CPBA (~70% purity, 48.5 mg, ~197 μmol) were added to a solution of crude **24** in EtOAc (0.71 mL) at room temperature. The reaction mixture was stirred at room temperature for 12.5 h. The resulting solution was directly purified by flash column chromatography on silica gel (10 g, hexane to hexane/EtOAc 2/1 to EtOAc to EtOAc/MeOH 10/1) and PTLC (hexane/EtOAc/AcOH 4/1/0.1) to afford analogue **18** (15.0 mg, 42.8 μmol) in 52% yield over five steps as a colorless oil: $[\alpha]^{28}_D$ −9.70 (*c* 1.03, MeOH); IR (ATR) ν_{max} 3384, 1662, 1459, 1198, 1143, 850, 801, 726 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.28−7.12 (m, 5H), 4.18 (m, 1H), 3.95 (d, 1H, *J* = 14.2 Hz), 3.91 (d, 1H, *J* = 14.2 Hz), 3.66 (dd, 1H, *J* = 9.7, 1.2 Hz), 2.31 (m, 1H) 1.58−1.45 (m, 2H), 1.31 (m, 1H), 1.08 (d, 3H, *J* = 7.0 Hz), 0.91 (d, 3H, *J* = 6.6 Hz), 0.89 (d, 3H, *J* = 6.4 Hz); $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 179.6, 165.7, 153.5, 138.1, 130.1, 129.4, 127.4, 75.9, 49.2, 45.5, 43.1, 30.1, 26.0, 23.6, 22.8, 14.6; HRMS (ESI/TOF) *m*/*z* 349.1767 $[M - H]^-$ (calcd for $C_{18}H_{26}N_2O_5$, 349.1769).

Bioassays. A cytotoxicity test and antibacterial assays were carried out according to the reported procedures,[15] with *K. rhizophila* ATCC9341, *S. aureus* FDA209P JC-1, *E. coli* NIHJ JC-2, *R. radiobacter* NBRC14554, *C. albicans* NBRC0197, *G. cingulata* NBRC5907, and *T. rubrum* NBRC5467.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00358.

UV and IR spectra of **1**, one- and two-dimensional NMR spectra of **1** and **2**, HRMS spectra of **1** and **2**, ^{13}C spectra of labeled **1** and **2**, NMR data of newly synthesized compounds, comparison of NMR spectra of natural and synthetic **1**, Cartesian coordinates of **2a** and **2b**, and crystallographic data of **11** (PDF)

Accession Codes

CCDC 2058723 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

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