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Oxygenated *N*-Acyl Alanine Methyl Esters (NAMEs) from the Marine Bacterium *Roseovarius tolerans* EL-164

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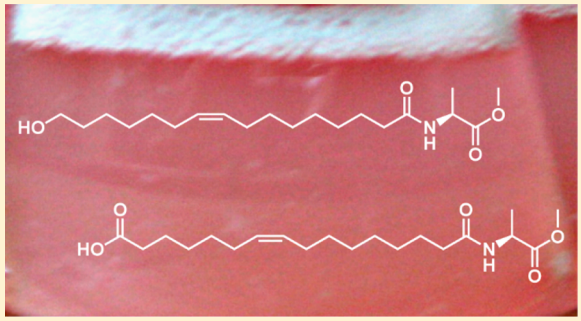
Supporting Information

ABSTRACT: The marine bacterium *Roseovarius tolerans* EL-164 (Rhodobacteraceae) can produce unique *N*-acylalanine methyl esters (NAMEs) besides strucutrally related *N*-acylhomoserine lactones (AHLs), bacterial signaling compounds widespread in the Rhodobacteraceae. The structures of two unprecedented NAMEs carrying a rare terminally oxidized acyl chain are reported here. The compounds (*Z*)-*N*-16-hydroxyhexadec-9-enoyl-L-alanine methyl ester (Z9-16-OH-C16:1-NAME, **3**) and (*Z*)-*N*-15-carboxypentadec-9-enoyl-L-alanine methyl ester (16COOH-C16:1-NAME, **4**) were isolated, and the structures were determined by NMR and MS experiments. Both compounds were synthesized to prove assignments and to test their biological activity. Finally, non-natural, structurally related Z9-3-OH-C16:1-NAME (**18**) was synthesized to investigate the mass spectroscopy of structurally related NAMEs. Compound **3** showed moderate antibacterial activity against microorganisms such as *Bacillus*, *Streptococcus*, *Micrococcus*, or *Mucor* strains. In contrast to AHLs, quorum-sensing or quorum-quenching activity was not observed.



Roseovarius tolerans EL-164 (Rhodobacteraceae), isolated from the hypersaline Ekho Lake (Antarctica),[1] is a bacterium of the Roseobacter group, an important group of marine bacteria. Roseobacters occur in many different habitats such as the open water column, algal or plankton surfaces, and algal blooms.[2−4] This group of bacteria produces various secondary metabolites such as the antibiotic tropodithietic acid (TDA),[5] antialgal roseobacticides,[6] antioxidants such as methyl 3-hydroxybutyrate oligomers,[7,8] or volatile compounds.[9] In addition, roseobacters can produce a wide range of *N*-acylhomoserine lactones (AHLs),[7,10−12] which are involved in different quorum-sensing regulated traits.[5,13]

Recently, we identified AHL-related compounds from *R. tolerans* EL-164, *N*-acylalanine methyl esters (NAMEs).[14] Despite their close structural similarity, differences occurred in the acyl chains of AHLs and NAMEs in this bacterium. The major compound was (*Z*)-*N*-9-hexadecenoyl-L-alanine methyl ester (**1**), accompanied by smaller amounts of saturated and unsaturated homologues.[14] AHLs were also produced; the major one was (*Z*)-*N*-7-tetradecenoyl-L-homoserine lactone (**2**). In contrast to AHLs, NAMEs did not show any activity in quorum-sensing assays,[14] but proved to have antialgal and antibacterial properties.[7] During the investigation of the extract of liquid cultures of this bacterium we observed new NAMEs that carry additional oxygen atoms in the side chain, while respective AHLs were absent. Here we report on the identification and synthesis of these new NAMEs found in culture medium extracts of *R. tolerans* EL-164.

RESULTS AND DISCUSSION

The secondary metabolites produced by liquid cultures of *R. tolerans* EL-164 were collected by extraction via Amberlite XAD-16 resin as described previously.[14] These extracts were investigated by HPLCMS (Figure 1). The known compounds Z9-C16:1-NAME (**1**) and Z7-C14:1-AHL (**2**) were readily identified by their ESI mass spectra with masses of *m*/*z* 340 and *m*/*z* 310 $[M + H]^+$ and typical ions at *m*/*z* 104 (alanine methyl ester unit) or *m*/*z* 102 (homoserine lactone unit), respectively. An HPLC/HR-MS^2 investigation in ESI positive mode (Figure 2) and comparison with a synthetic sample confirmed the assignment of **1**. The mass spectrum is characterized by the loss of H_2O (−18 Da), MeOH (−32 Da), and MeOH/CO (−60 Da) from $[M + H]^+$. The alanine methyl ester unit is indicated by the ions *m*/*z* 104 and *m*/*z* 237 $[M + H - 103]^+$, fragments occurring analogously at *m*/*z* 102 and $[M + H - 101]^+$ in AHLs.[15,16]

Two additional compounds, **A** and **B** in Figure 1, showed $[M + H]^+$ ions at *m*/*z* 356 and 370, respectively. The HR-MS data

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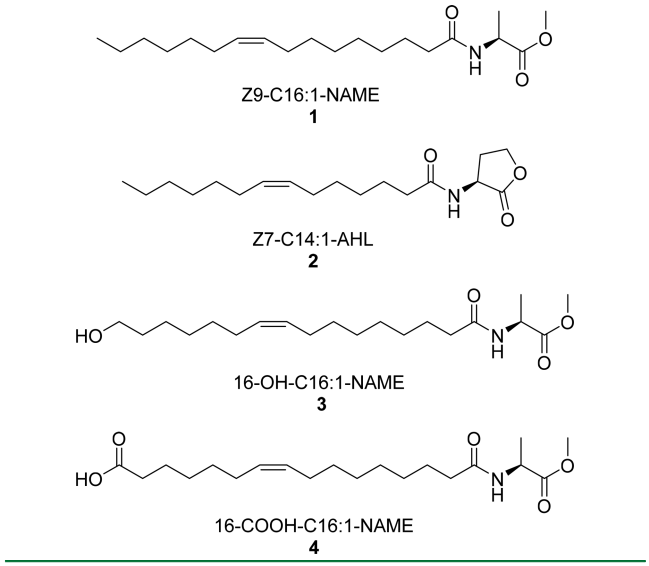

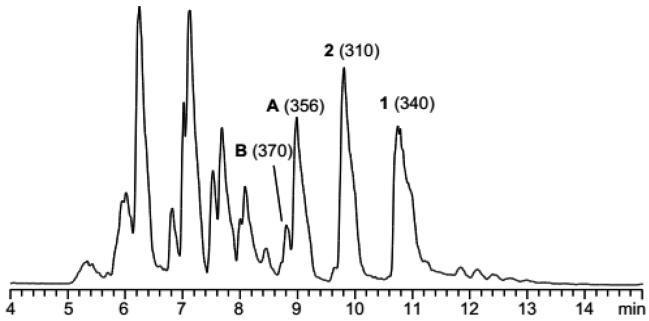

Figure 1. HPLCMS total ion chromatogram of an extract of *R. tolerans* EL-164 showing C16:1-NAME (**1**) and C14:1-AHL (**2**), as well as the unknown NAMEs **A** and **B**. The $[M + H]^+$ ion is shown in parentheses. Analysis was performed on an Agilent Eclipse Plus RP18 column (3.5 μm, 2.1 × 150 mm) with a gradient of H_2O, MeCN, and 2% formic acid in ESI positive mode.

of **A** and its MS^2 fragmentation pattern were similar to those of NAME **1** (Figure 2B) and indicated this compound also to be an acylated alanine methyl ester. The mass difference of 16 Da compared to **1** and an additional loss of H_2O in the mass spectrum, indicated by ions m/z 306 $[M + H - H_2O - CH_3COOH]^+$, m/z 253 $[M + H - 103]^+$, and m/z 235 $[M + H - 103 - H_2O]^+$, revealed an additional HO group or a keto or epoxide group instead of the double bond in the acyl side chain.

Compound **A** (0.8 mg) was then isolated by preparative HPLC. NMR analysis was performed with the help of ^1H, ^{13}C, DEPT, COSY, HSQC, HMBC, and NOESY experiments. As expected, the ^1H NMR spectrum showed signals of an alanine methyl ester moiety: an ester methyl group at δ 3.69 (s), a methine proton adjacent to a carbonyl and a methyl group (δ 4.54), the alanine methyl group (δ 1.33), and an amide proton (δ 5.97) were observed (Table 1).

Additionally, an acyl chain with a *Z*-configured double bond was present. A Δ^9-double bond was evident from COSY correlations along the chain between C-14 and C-20. The *Z*-configuration was indicated by the chemical shift of the carbons next to the double bond, δ_C 27.1.[17] The connectivity of the chain carbons was elucidated by COSY, HSQC, and HMBC correlations (Figure 3). The CH_2 group at δ 3.57 and 63.0 in the ^{13}C NMR indicated an additional HO group at the terminal end of the chain, in accordance with the mass spectrometric data. The absolute configuration of alanine in the NAMEs has been previously shown to be L, and the same configuration was tentatively assigned for **A**.[14] The direction of the optical rotation of the natural compound was not clearly assignable due to the low specific rotation value. Based on these data, the structure of **A** was therefore (*Z*)-*N*-16-hydroxyhexadec-9-enoyl-L-alanine methyl ester (16OH-C16:1-NAME, **3**), a terminally oxidized analogue of NAME **1**.

Compound **B** could not be isolated in sufficient quality for NMR investigations. Nevertheless, the HRMS data of **B** (Figure 2C) showed the presence of a second additional oxygen compared to **1**, with the composition $C_{20}H_{26}NO_5$. The ion m/z 104, characteristic for NAMES, was observed, while instead of the ion $[M + H - 103]^+$, m/z 249 $[M + H - 103 - H_2O]^+$ occurs. This indicated, together with the acyl ion m/z 231 $[C_{16}H_{23}O]^+$, the presence of two double bonds and three O atoms in the acyl chain. Ions of the NAME- and AHL-typical loss of H_2O and/or MeOH (m/z 352, 338, 320), and MeOH, CO, and H_2O (m/z 292) were also present. We therefore concluded that this compound is likely (*Z*)-*N*-15-carboxypentadec-9-enoyl-L-alanine methyl ester (16COOH-C16:1-NAME, **4**), a further oxidized analogue of **1**. The earlier retention times on an RP-HPLC column are consistent with these assignments.

To verify the structures of both **A** and **B**, compounds **3** and **4** were synthesized (Scheme 1). 1,7-Heptanediol (**5**) was monoprotected with *tert*-butyldimethylsilyl chloride (TBDMSCl) and oxidized via Parikh–Doering reaction to give **6**. The aldehyde was coupled with the Wittig salt **8**, obtained from 9-bromononanol (**7**). Wittig reaction furnished monoprotected diol **9** in 76% yield with a *Z*-configured double bond at C-9. After oxidation with tetrapropylammonium perruthenate (TPAP) to the acid **10**, coupling with L-alanine methyl ester hydrochloride gave amide **11**. Deprotection with tetrabutylammonium fluoride (TBAF) furnished the desired compound **3**. Because direct oxidation proceeded only in poor yield, a two-step oxidation process via Parikh–Doering and subsequent Pinnick oxidations was performed, furnishing acid **4**.

The synthetic material proved to be identical in all aspects to the natural compounds **A** and **B**, e.g., in MS^2 and MS^3 experiments (Supporting Information). The absolute configuration of the alanine residue in NAMEs of *R. tolerans* EL-164 has been previously elucidated to be L.[14] The new compounds **3** and **4** are obviously terminally oxidized analogues of the already known NAME **1**. Such oxidized acyl chains have not been reported for the closely related AHLs that contain an additional oxygen usually at C-3.[10,18−20] Currently we are analyzing a whole range of bacteria for similar analogues. Therefore, it seemed plausible to also synthesize a 3-hydroxyacyl-NAME (**18**), although this compound is not known from nature. Knowledge of its mass spectrometric properties would help in identification. Therefore, 3OH-C16:1-NAME **18** was synthesized according to Scheme 2.

Ethyl-7-bromoheptanoate (**12**) was transformed into Wittig salt **13**. A Z-selective Wittig reaction with heptanal furnished ethyl (*Z*)-tetradec-7-enoate (**14**). The free acid **15**, obtained by alkaline hydrolysis, was used to acylate Meldrum's acid to furnish **16**. The crude product was directly coupled with L-alanine methyl ester hydrochloride, furnishing (*Z*)-*N*-3-oxohexadec-9-enoyl-L-alanine methyl ester (**17**). Final reduction with sodium borohydride delivered the target compound, (*Z*)-*N*-3-hydroxyhexadec-9-enoyl-L-alanine methyl ester (3OH-C16:1-NAME, **18**).

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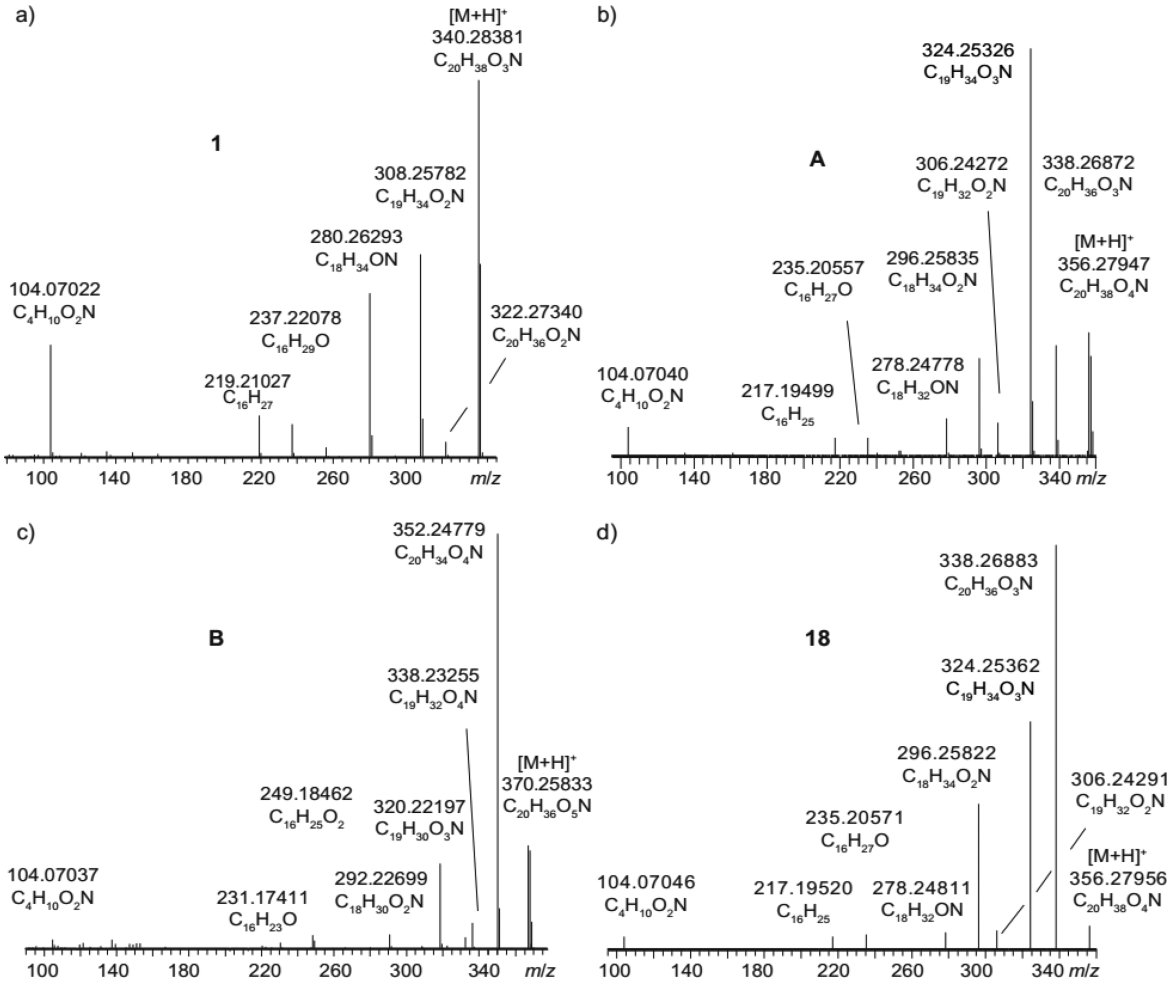

Figure 2. HR-MS/MS mass spectrum of C16:1-NAME (**1**) in ESI positive mode with m/z 340 as the precursor ion (a), of compound **A**, precursor m/z 356 (b), compound **B**, precursor m/z 370 (c), and 3-OH-C16:1-NAME (**18**), precursor m/z 356 (d).

Table 1. NMR Data for Compound A in DMSO-d_6

position	δ_C, type	δ_H, mult. (J in Hz)
1	52.5, CH_3	3.69, s
2	173.8, C	
3	47.8, CH	4.54, quin (7.2)
4	18.6, CH_3	1.33, d (7.2)
5	172.7, C	
6	36.6, CH_2	2.14, t (7.7)
7	25.55, CH_2	1.59−1.54, m
8	29.01, CH_2	1.24−1.21, m
9	29.03, CH_2	1.30−1.25, m
10	29.20, CH_2	1.24−1.21, m
11	29.67, CH_2	1.30−1.25, m
12	27.1, CH_2	1.97−1.92, m
13	129.8, CH	5.30−5.25, m
14	129.9, CH	5.30−5.25, m
15	27.1, CH_2	1.97−1.92, m
16	29.61, CH_2	1.30−1.25, m
17	29.18, CH_2	1.24−1.21, m
18	25.61, CH_2	1.30−1.25, m
19	32.8, CH_2	1.53−1.48, m
20	63.0, CH_2	3.57, t (6.7)
NH		5.97, d (6.9)

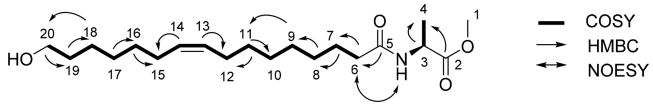

Figure 3. Important COSY, HMBC, and NOESY correlations of compound **A**.

The same ions were observed in the ESI mass spectra of **3** and **18**. The only obvious difference was the intensity of the ions m/z 324 and 338 in the HRESIMS^2 spectra (Figure 2). Low-resolution ESIMS^2 spectra (Figure S3, Supporting Information) also showed enhanced intensity of m/z 338, explainable by the easier loss of H_2O from C-3 compared to C-16. Although **3** did show more fragmentation in MS^3 compared to **18** (Figure S3), it seems questionable whether the differences observed are sufficient to differentiate these compounds in complex samples. Compounds **3** and **18** can also be analyzed by GCMS after trimethylsilylation. The respective mass spectra are clearly different (Figure S4). The derivative of **18** indicates the location of the OH substituent by the ion m/z 246. No related ion is observable in the spectrum of trimethylsilylated **3**.

Moderate antibacterial activity against TolC-deficient *E. coli* (minimum inhibitory concentration, MIC 8−16 μg/mL), *Micrococcus luteus* DSM-1790 (MIC 32−64 μg/mL), *Staphylococcus aureus* Newman (MIC 32−64 μg/mL), and *Mucor*

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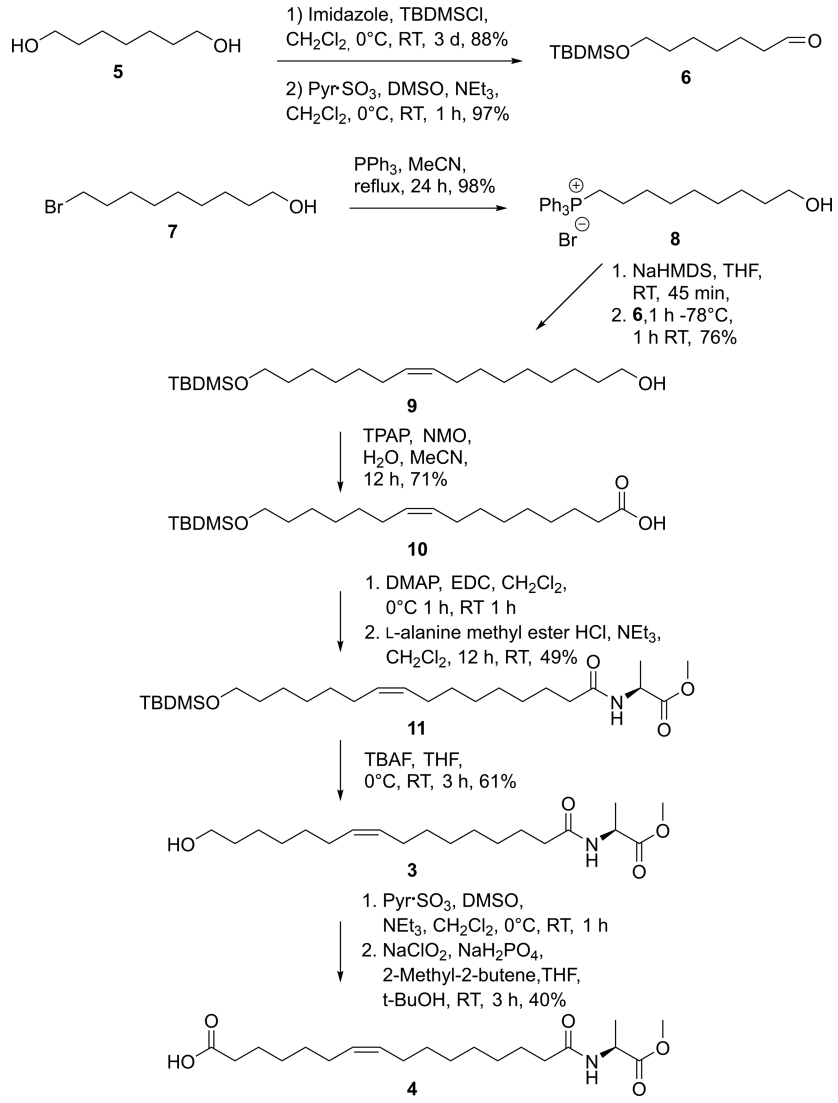
Scheme 1. Synthesis of 3 and 4 Starting from 1,7-Heptanediol 5 and Bromo Alcohol 7[a]



[a]TBDMSCl: *tert*-butyldimethylsilyl chloride, EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, TBAF: tetrabutylammonium fluoride.

hiemalis DSM-2656 (MIC 32−64 μg/mL) was observed for **3**. The synthetic compounds **17** and **18** also showed moderate antibacterial activity against *Bacillus subtilis* DSM10 (MIC 16 and 128 μg/mL), *Staphylococcus carnosus* DSM20501 (MIC 8 and 16 μg/mL), *Micrococcus luteus* DSM-1790 (MIC 16 and 8 μg/mL), and *E. coli* TolC (MIC 16 μg/mL for both). Similar to the nonoxidized parent compound **1**, NAME **3** did not induce or suppress quorum sensing signaling in reporter strains for long-chain and short-chain AHLs.[14] If the NAMEs indeed function as signaling compounds, it seems logical that no cross-activity with AHLs is observable. The function of NAMEs is currently being investigated by us. In summary, we identified unique NAMEs carrying terminally oxidized acyl chains and developed a straightforward approach to their synthesis.

The NAMEs **3** and **4** comprise new natural products. They resemble structurally not only AHLs, important signaling compounds, but also acylated amino acids (AAs) identified from various bacteria.[21−24] AAs are known from the amino acids tyrosine, tryptophan, arginine, or phenylalanine and carry in all cases nonoxidized saturated and unsaturated acyl chains. Acyl chains oxidized at positions other than C-3 are not known from either AHLs or AAs. Recently, AHLs have been detected that obviously carry additional O along the chain, but their exact structures were not determined.[25] Terminally oxidized fatty acids occur in plant materials, such as suberin[26] or cutin,[27] but are rare in microorganisms.[28]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measuired with an Anton-Paar MD 150 instrument at 20 °C with a 100 mm path length. UV spectra were obtained with a Varian Cary 100 Bio spectrometer. IR spectra were obtained with a Bruker Tensor 27 ATR spectrometer. NMR spectra were obtained with Bruker DRX-400, AV III-400, or AV II-600 spectrometers. The spectra were referenced to internal tetramethylsilane (δ_H 0.00 ppm) for ^{1}H NMR and to $CHCl_3$ (δ 77.01 ppm) for ^{13}C NMR. Chemical shifts are given in ppm; coupling constants *J*, in Hz.

For GC/MS an HP6890 GC system connected to an HP5973 MSD (Hewlett-Packard) fitted with a BPX-5 fused silica cap was used: column (25 m, 0.22 mm i.d., 0.25 mm film, SGE Inc.) or Zebron ZB-5 MSi column (30 m × 0.25 mm i.d., 0.25 μm film, Phenomenex) or HP-5 MS fused silica capillary column (30 m, 0.25 mm i.d., 0.22 mm film, Agilent); conditions: inlet pressure 97.0 kPa He, purge flow 45.5 mL/min; injection volume 1 μL; injector 250 °C, transfer line 300 °C,

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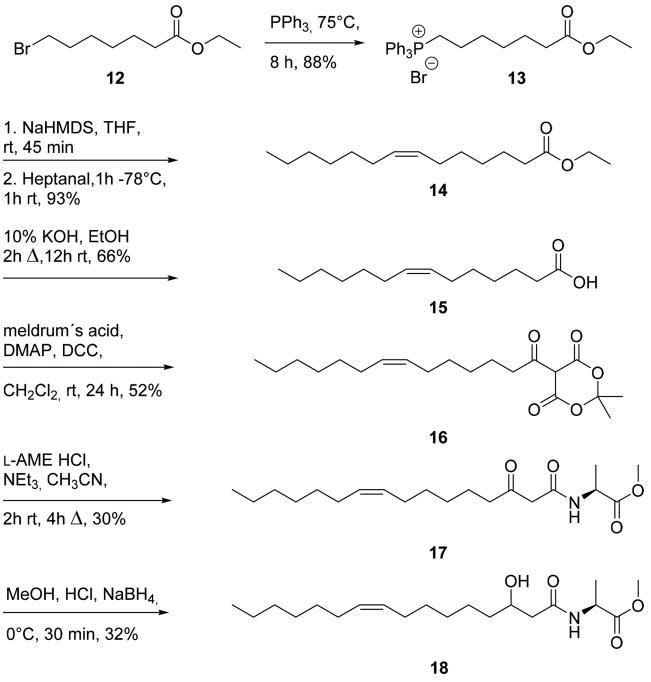
Scheme 2. Synthesis of 3-OH-C16:1-NAME 18[a]



[a]DMAP: 4-(*N*,*N*-dimethylamino)pyridine, DCC: dicyclohexylcarbodiimide, L-AME HCl: L-alanine methyl ester hydrochloride.

electron energy 70 eV. The gas chromatograph was programmed as follows: 50 °C (5 min isothermal), increasing at 10 °C/min to 320 °C, and operated in split mode (35:1), carrier gas (He) 1.2 mL/min. Alternatively, an Agilent GC 7890A system connected to a 5975C MSD (Agilent) fitted with an HP-5 MS fused silica capillary column (30 m, 0.25 mm i.d., 0.22 mm film, Agilent) was used. Conditions: inlet pressure 67.5 kPa He, purge flow 24.2 mL/min. The other data were identical to the first system. Gas chromatographic retention indices (*I*) were determined from a homologous series of *n*-alkanes (C_8−C_{33}). Acids and alcohols were derivatized with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) prior to injection. To a 60 μL sample in a glass vial dissolved in CH_2Cl_2 was added 20 μL of MSTFA, and the vial was closed and heated to 60 °C for 1 h. The solvent and excess MSTFA were evaporated in a stream of N_2, and the sample was taken up in CH_2Cl_2.

LC/MS data were acquired on a Thermo Fisher LTQ XL, equipped with an Accela pump, autosampler, and PDA detector. Only LC/MS grade eluents were used. Mass spectra were obtained in ESI positive mode. The temperature of the ion source was 40 °C, and the capillary temperature was 275 °C. The sheath and auxiliary gas flows were 15 and 10 mL/min, respectively. MS^2 and MS^3 analyses were carried out in CID mode with a normalized collision energy of 35%. The activation *Q* was 0.250 and activation time was 30 ms. Spectra were analyzed with Thermo Xcalibur 2.2 software. HRESIMS were performed with an LTQ Orbitrap Velos in MeOH acidified with 1% formic acid. The spray voltage was set to 1.8 to 2.3 kV, and the flow rate was 1 μL/min. HRMS/MS data were obtained via CID and a collision energy of 25−30.

For TLC, 0.20 mm Macherey-Nagel silica gel plates (Polygram SIL G/UV254) were used. Column chromatography (CC): Merck silica gel 60 (0.040−0.063 mm) using standard flash chromatographic methods.

Chemicals were purchased from Sigma-Aldrich Chemie GmbH or from Acros Organics and used without further purification. Solvents were purified by distillation and dried according to standard procedures. Moisture- and/or oxygen-sensitive reactions were carried out under N_2 in vacuum-heated flasks with dried solvents.

Strains, Culture Conditions, and Extraction. *Roseovarius tolerans* EL-164 was collected from hypersaline Ekho Lake in Antarctica.[1] This strain has been sequenced with the GenBank accession number LGVV00000000[29] and is in the strain collection of the Helmholtz Center for Infection Research, Braunschweig, Germany. Precultures were routinely grown in marine broth medium (MB, Carl Roth) in Erlenmeyer flasks at 28 °C on a rotary shaker at 150 rpm. Erlenmeyer flasks (500 mL) containing 100 mL of MB were inoculated with 2% preculture, and 2% of precleaned Amberlite XAD-16 (Sigma-Aldrich) was added, which was activated before by washing with 30 mL of MeOH and 3 × 30 mL of distilled H_2O. After growth of the culture for 3−5 days, the resin was filtered off and extracted with 3 × 50 mL of CH_2Cl_2/H_2O 3:1 (v/v). The two phases were separated, the organic phase was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The extract was concentrated at 60 °C under N_2 to a volume of ca. 500 μL. For HPLC analysis, 400 μL of the extract was evaporated to dryness and dissolved in 300 μL of MeCN. For isolation and purification of oxygenated NAMEs 4 L of culture was prepared as described above, which gave 91 mg of dry extract.

HPLC/MS of Extract. The dry extract was dissolved in MeCN/H_2O (1:1) and filtered. An Agilent Eclipse Plus C18 column (3.5 μm, 2.1 × 150 mm) was used with a flow of 250 μL/min and injection volume of 10 μL. Gradient elution was performed using 92.5% H_2O (B), 2.5% MeCN (C), and 5% MeCN containing 2% formic acid (D) from 0 to 1.5 min, 2.5% B, 92.5% C, and 5% D from 8 to 15 min, and reequilibration from 17 to 22 min to 92.5% B, 2.5% C, and 5% D.

Isolation and Purification. The extract was dissolved in H_2O and submitted to RP18 column chromatography with MeCN/H_2O (2:1, v/v) as eluents. Fractions were pooled after detection of compounds via HPLC/MS. Subsequent evaporation of MeCN under reduced pressure and lyophilization gave 1.5 mg of impure 16COOH-C16:1-NAME (**4**) and 0.8 mg of pure 16OH-C16:1-NAME (**3**). The purity was determined by LC/MS. Because the fraction of NAME **4** resisted further attempts of purification, only the fraction containing NAME **3** was submitted to NMR spectroscopic analysis.

Syntheses. *(9-Hydroxynonyl)triphenylphosphonium Bromide (**8**).* The reaction was performed under dry conditions. A solution of 9-bromononan-1-ol (**7**) (1.00 g, 4.48 mmol, 1.0 equiv) and Ph_3P (1.29 g, 4.93 mmol, 1.1 equiv) in MeCN (8 mL) was heated to reflux for 48 h.[32] After evaporation of MeCN under reduced pressure, the crude product was dissolved in CH_2Cl_2 and purified by flash column chromatography (SiO_2, CH_2Cl_2/MeOH, 15:1). Product **8** was obtained as a yellowish oil (2.13 g, 4.39 mmol, 98%): R_f (CH_2Cl_2/MeOH, 15:1) 0.16; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.85−7.80 (m, 9 arom. H), 7.75−7.71 (m, 6 arom. H), 3.68−3.62 (m, 2H, CH_2), 3.57 (t, 2H, *J* = 6.6 Hz, CH_2OH), 2.73 (s br, 1H, OH), 1.67−1.59 (m, 4H, 2 × CH_2), 1.49 (quin, 2H, *J* = 6.9 Hz, CH_2), 1.28−1.21 (m, 8H, 4 × CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 134.9 (d, *J* = 2.9, 3 Ar), 133.3 (d, *J* = 10.0 Hz, 6 Ar), 103.3 (d, *J* = 12.5 Hz, 6 Ar), 118.0 (d, *J* = 85.9 Hz, 3 Ar), 62.1 (CH_2OH), 32.3 (CH_2CH_2OH), 30.0 (d, *J* = 15.6 Hz, CH_2), 28.7 (d, *J* = 10.2 Hz, CH_2), 28.5 (CH_2), 25.3 (CH_2), 22.7 (CH_2), 22.3 (CH_2), 22.2 (CH_2); ^{31}P NMR ($CDCl_3$, 162 MHz) 24.66 (s, PPh_3).

7-((tert-Butyldimethylsilyl)oxy)heptan-1-ol. 1,7-Heptanediol (**5**) (2.66 g, 20.10 mmol, 1 equiv) and imidazole (1.50 g, 22.11 mmol, 1.1 equiv) were dissolved in dry CH_2Cl_2 (140 mL), cooled to 0 °C, and stirred for 15 min at 0 °C under a nitrogen atmosphere. Slowly TBDMSCl (3.33 g, 22.11 mmol, 1.1 equiv) was added dropwise over a period of 10 min. The solution was allowed to warm to rt and was stirred for 3 days. H_2O (80 mL) was added, the phases were separated, and the H_2O phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure.[33,34] Column chromatography (SiO_2, pentane/EtOAc, 4:1) yielded the monoprotected alcohol (4.34 g, 17.61 mmol, 88%) as a yellowish liquid: R_f (SiO_2, pentane/EtOAc, 4:1) 0.29; GC (HP-5 MS) *I* 1572; IR (ATR) ν_{max} 3335 (br), 2929 (m), 2857 (m), 1468 (m), 1387 (w), 1361 (w), 1253 (m), 1096 (s), 1058 (m), 1006 (m), 833 (s), 773 (s), 771 (m), 661 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.58 (t, 2H, *J* = 6.2 Hz, $SiOCH_2$), 3.55 (t, 2H, *J* = 6.1 Hz, CH_2OH), 1.81 (s br, 1H, OH), 1.55−1.44 (m, 8H, 4 × CH_2), 1.35−1.26 (m, 2H,

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J. Nat. Prod. XXXX, XXX, XXX−XXX

CH_2), 0.85 (s, 9H, 3 × CH_3), 0.00 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 63.2 (CH_2OH), 62.9 ($SiOCH_2$), 32.8 (CH_2), 32.7 (CH_2), 29.2 (CH_2), 26.0 (3 × CH_3), 25.8 (CH_2), 25.7 (CH_2), 18.3 (C), −5.3 (2 × CH_3); EIMS m/z 246 $[M]^+$ (<1), 189 (5), 171 (6), 143 (13), 115 (23), 105 (48), 97 (65), 75 (98), 73 (39), 69 (29), 55 (100), 41 (32).

*7-((tert-Butyldimethylsilyl)oxy)heptanal (**6**).* The reaction was performed under a nitrogen atmosphere. 7-((*tert*-Butyldimethylsilyl)-oxy)heptan-1-ol (3.68 g, 14.81 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (120 mL), and NEt_3 (20.53 mL, 148.10 mmol, 10 equiv) was added. A solution of pyridine·SO_3 (7.07 g, 44.43 mmol, 3 equiv), which was dissolved in DMSO (45 mL) and stirred for 15 min at rt, was then added to the alcohol at 0 °C. The solution was stirred for 30 min at rt, and H_2O (100 mL) was added. The phases were separated, and the H_2O phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution and dried with $MgSO_4$.[35] After evaporation of the solvents under reduced pressure column chromatography (SiO_2, pentane/EtOAc, 17.1) gave aldehyde **6** (3.51 g, 14.35 mmol, 97%) as a yellowish liquid: R_f (SiO_2, pentane/EtOAc, 17:1) 0.36; GC (HP-5 MS) I 1506; UV/vis (CH_2Cl_2) λ_{max} (log ε) 227 (2.29), 222 (2.08) nm; IR (ATR) ν_{max} 2930 (s), 2857 (m), 1710 (s), 1467 (m), 1411 (w), 1389 (w), 1361 (w), 1253 (s), 1097 (s), 1005 (w), 937 (m), 833 (s), 774 (s), 661 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.72 (t, 1H, J = 1.8 Hz, HC═O), 3.55 (t, J = 6.5 Hz, 2H, $SiOCH_2$), 2.38 (dt, 2H, J = 7.4 Hz, 1.9 Hz, CH_2HC═O), 1.63−1.56 (m, 4H, 2 × CH_2), 1.51−1.44 (m, 2H, CH_2), 1.32−1.29 (m, 2H, CH_2), 0.85 (s, 9H, 3 × CH_3), 0.00 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.7 (HC═O), 63.0 (CH_2), 43.8 (CH_2), 32.6 (CH_2), 28.9 (CH_2), 25.9 (3 × CH_3), 25.6 (CH_2), 22.0 (CH_2), 18.3 (C), −5.3 (2 × CH_3); MS (70 eV, EI m/z 244 (<1, $[M]^+$), 187 (5), 173 (4), 157 (12), 131 (19), 115 (17), 105 (27), 101 (22), 95 (48), 75 (100), 73 (32), 67 (14), 59 (19), 57 (17), 44 (20), 41 (29).

*(Z)-16-((tert-Butyldimethylsilyl)oxy)hexadec-9-en-1-ol (**9**).* Wittig salt **8** (5.30 g, 10.91 mmol, 1.05 equiv) was dissolved in dry THF (120 mL), and NaHDMS was added dropwise (21.82 mL, 1 M in THF, 21.82 mmol, 2.1 equiv) at 0 °C under a nitrogen atmosphere. The solution was stirred for 45 min at rt, and a strong orange color evolved. The solution was cooled to −78 °C, and aldehyde **6** (2.54 g, 10.39 mmol, 1.0 equiv) was added slowly. After stirring for 1 h at −78 °C the temperature was allowed to rise to rt and ice cold pentane (300 mL) was added.[36,37] The formed Ph_3PO was filtered off, and after evaporation of two-thirds of the solvent SiO_2 was added to form a slurry. Column chromatography with gradient elution (SiO_2, pentane/EtOAc, 10:1, 5:1) furnished **9** (2.93 g, 7.90 mmol, 76%) as a colorless oil: R_f (pentane/EtOAc, 10:1) 0.4; GC (HP-5 MS) I 2463; IR (ATR) ν_{max} 3339 (br), 3005 (w), 2927 (s), 2855 (s), 1463 (m), 1438 (w), 1387 (w), 1361 (w), 1253 (m), 1185 (m), 1098 (s), 1006 (m), 938 (w), 834 (s), 774 (s), 722 (m), 695 (m), 662 (m), 541 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.34−5.25 (m, 2H, HC═CH), 3.59 (t, 2H, J = 6.7 Hz, $SiOCH_2$), 3.55 (t, 2H, J = 6.6 Hz, CH_2OH), 2.02−1.94 (m, 4H, CH_2HC═CHCH_2), 1.55−1.43 (m, 4H, 2 × CH_2), 1.33−1.23 (m, 16H, 8 × CH_2), 0.85 (s, 9H, 3 × CH_3), 0.00 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 129.9 (CH), 129.8 (CH), 63.3 (CH_2), 63.1 (CH_2), 32.9 (CH_2), 32.8 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 27.2 (CH_2), 26.0 (3 × CH_3), 25.7 (CH_2), 25.7 (CH_2), 18.4 (C), −5.3 (2 × CH_3); EIMS m/z 370 $[M]^+$ (<1), 313 (10), 295 (2), 221 (2), 151 (6), 137 (14), 123 (29), 109 (54), 95 (79), 83 (54), 81 (77), 75 (100), 69 (58), 67 (60), 55 (61), 41 (34).

*(Z)-16-((tert-Butyldimethylsilyl)oxy)hexadec-9-enoic Acid (**10**).* To a solution of alcohol **9** (2.93 g, 7.90 mmol, 1.0 equiv), *N*-methylmorpholin-*N*-oxide (NMO, 9.23 g, 79.0 mmol, 10.0 equiv), and H_2O (1.44 mL, 79.0 mmol, 10 equiv) in MeCN (100 mL) was added TPAP (0.28 g, 0.79 mmol, 0.1 equiv). The solution was stirred overnight at rt, and the solvent was evaporated under reduced pressure.[38] Acid **10** (2.16 g, 5.61 mmol, 71%) was obtained after column chromatography (SiO_2, pentane/EtOAc + 1% AcOH, 10:1) as a colorless liquid: R_f (pentane/EtOAc + 1% AcOH, 10:1) 0.36; IR (ATR) ν_{max} 3005 (w), 2927 (s), 2855 (s), 1710 (s), 1463 (m), 1412 (w), 1361 (w), 1252 (m), 1099 (s),1005 (w), 937 (w), 834 (s), 775 (s), 723 (w), 662 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.33−5.25 (m, 2H, HC═CH), 3.55 (t, 2H, J = 6.6 Hz, $SiOCH_2$), 2.29 (t, 2H, J = 7.5 Hz, CH_2COOH), 2.00−1.94 (m, 4H, CH_2HC═CHCH_2), 1.58 (quin, 2H, J = 7.4 Hz, CH_2CH_2COOH), 1.50−1.43 (m, 2H, CH_2), 1.33−1.24 (m, 14H, 7 × CH_2), 0.84 (s, 9H, 3 × CH_3), 0.00 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 80.0 (C), 130.0 (HC═CH), 129.8 (HC═CH), 63.3 ($SiOCH_2$), 34.1 (CH_2), 32.8 (2 × CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.1 (3 × CH_2), 29.0 (CH_2), 27.1 (2 × CH_2), 26.0 (3 × CH_3), 24.7 (CH_2), 18.4 (C), −5.3 (2 × CH_3).

*(Z)-(16-((tert-Butyldimethylsilyl)oxy)hexadec-9-enoyl)-L-alanine Methyl Ester (**11**).* Acid **10** (0.20 g, 0.52 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (10 mL) under a nitrogen atmosphere. After addition of 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 0.06 g, 0.52 mmol, 1 equiv), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.1 g, 0.68 mmol, 1.3 equiv) was added at 0 °C, and the solution was stirred for 1 h at 0 °C, allowed to warm to rt, and stirred for an additional 1 h. A solution of L-alanine methyl ester·HCl (0.09 g, 0.68 mmol, 1.3 equiv) in dry CH_2Cl_2 (10 mL) was prepared, and NEt_3 (0.07 g, 0.68 mmol, 1.3 equiv) was added. This solution was added dropwise to the solution containing **10** and was stirred overnight at rt. The solution was washed with 1 M HCl (10 mL), $NaHCO_3$ (10 mL), and H_2O (10 mL), the phases were separated, and the organic phase was dried with $MgSO_4$.[39−41] Product **11** (0.12 g, 0.26 mmol, 49%) was obtained by column chromatography (SiO_2, pentane/EtOAc, 4:1) as a colorless liquid: R_f (pentane/EtOAc, 4:1) 0.4; GC (HP-5 MS) I 3087; $[\alpha]_D^{20}$ −0.98 ± 0.05 (c 0.0215, CH_2Cl_2); IR (ATR) ν_{max} 3292 (br), 3002 (w), 2928 (s), 2855 (s), 1748 (m), 1649 (s), 1539 (m), 1459 (m), 1382 (w), 1360 (w), 1253 (m), 1207 (m), 1162 (m), 1098 (s), 1058 (m), 1005 (w), 990(w), 937 (w), 919 (w), 835 (s), 775 (s),732 (m), 661 (m), 539 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.97 (d, 1H, J = 6.8 Hz, NH), 5.31−5.28 (m, 2H, HC═CH), 4.56 (quin, 1H, J = 7.2 Hz, $CHCH_3$), 3.71 (s, 3H, OCH_3), 3.55 (t, 2H, J = 6.6 Hz, $SiOCH_2$), 2.16 (t, 2H, J = 7.6 Hz, CH_2C═O), 2.00−1.93 (m, 4H, CH_2HC═CHCH_2), 1.62−1.55 (m, 2H, CH_2), 1.51−1.43 (m, 2H, CH_2), 1.36 (d, 3H, J = 7.2 Hz, $CHCH_3$), 1.31−1.24 (m, 14H, 7 × CH_2), 0.85 (s, 9H, 3 × CH_3), 0.00 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7 (C), 172.6 (C), 129.9 (HC═CH), 129.8 (HC═CH), 63.3 ($SiOCH_2$), 52.4 (OCH_3), 47.8 ($CHCH_3$), 36.5 (CH_2), 32.8 (CH_2), 29.73 (CH_2), 29.68 (CH_2), 29.23 (CH_2), 29.18 (CH_2), 29.11 (CH_2), 29.09 (CH_2), 27.2 (CH_2HC═CHCH_2), 26.0 (3 × CH_3), 25.7 (CH_2), 25.5 (CH_2), 18.6 (C), 18.4 (CH_3), −5.3 (2 × CH_3); EIMS m/z 469 $[M]^+$ (6), 454 (4), 413 (37), 412 (100), 352 (6), 308 (5), 272 (2), 158 (2), 145 (4), 104 (14), 75 (21), 55 (7), 44 (17).

*(Z)-(16-Hydroxyhexadec-9-enoyl)-L-alanine Methyl Ester, 16OH-C16:1-NAME (**3**).* The educt **11** (0.17 g, 0.35 mmol, 1 equiv) was dissolved in dry THF (20 mL) and cooled to 0 °C. TBAF was added slowly (1 M in THF, 0.53 mL, 1.5 equiv), and the solution was stirred for 3 h at rt. Saturated NH_4Cl solution (20 mL) was added, the phases were separated, and the H_2O phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with saturated NaCl solution and dried with $MgSO_4$, and the solvent was evaporated under reduced pressure.[42] Column chromatography (RP18, MeCN/H_2O, 3:1) afforded product **3** (0.07 g, 0.21 mmol, 61%) as a colorless liquid: R_f (MeCN/H_2O, 3:1) 0.36; $[\alpha]_D^{20}$ −1.15 ± 0.39 (c 0.0026, CH_2Cl_2); IR (ATR) ν_{max} 3282 (br), 3002 (w), 2927 (s), 2855 (s), 1746 (s), 1651 (s), 1545 (m), 1456 (m), 1379 (w), 1263 (m), 1209 (m), 1163 (m), 1057 (m), 724 (w), 553 (w), 532 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.07 (d, 1H, J = 5.3 Hz, NH), 5.35−5.33 (m, 2H, HC═CH), 4.61 (quin, 1H, J = 7.2 Hz, $CHCH_3$), 3.75 (s, 3H, OCH_3), 3.64 (t, 2H, J = 6.7 Hz, CH_2OH), 2.21 (t, 2H, J = 7.6 Hz, CH_2C═O), 2.04−1.99 (m, 4H, CH_2HC═CHCH_2), 1.66−1.61 (m, 2H, CH_2), 1.60−1.55 (m, 2H, CH_2), 1.40 (d, 3H, J = 7.2 Hz, $CHCH_3$), 1.38−1.29 (m, 14H, 7 × CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8 (C═O), 172.7 (C═O), 129.9 (HC═CH), 129.8 (HC═CH), 63.0 (CH_2OH), 52.5 (OCH_3), 47.8 ($CHCH_3$), 36.5 (CH_2), 32.8 (CH_2), 29.64 (CH_2), 29.59 (CH_2), 29.18 (CH_2), 29.16 (CH_2), 29.01 (CH_2), 28.99 (CH_2), 27.1 (CH_2HC═CHCH_2), 25.6 (CH_2), 25.5 (CH_2), 18.6 (CH_3); HRESIMS m/z 356.27967 (calcd for $C_{20}H_{38}NO_4$, 356.27954).

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J. Nat. Prod. XXXX, XXX, XXX−XXX

*(Z)-(15-Carboxypentadec-9-enoyl)-L-alanine Methyl Ester, 16COOH-C16:1-NAME (**4**).* The oxidation of the alcohol **3** (17 mg, 48 mmol, 1 equiv) to the aldehyde was performed as described for **6**. The aldehyde (16 mg, 51 mmol, 1 equiv) obtained was directly used in the following reaction. The aldehyde was dissolved in a *t*-BuOH/THF mixture (1.2 mL/1 mL), and 2-methyl-2-butene was added. A solution of $NaClO_2$ (32 mg, 356 mmol, 7 equiv) and NaH_2PO_4 (63 mg, 458 mmol, 9 equiv) in H_2O (0.4 mL) was added dropwise, resulting in a yellowish color of the solution. After stirring at rt for 3 h, a saturated NaCl solution was added and the phases were separated. The H_2O phase was extracted with CH_2Cl_2 (3 × 2 mL), and the combined organic layers were washed with a saturated NaCl solution. After drying with $MgSO_4$, the solvent was evaporated. A saturated $NaHCO_3$ solution was added to adjust the pH to 9, and the solution was extracted with diethyl ether (2 × 10 mL). After phase separation, 2 N HCl was added to lower the pH to 2 and the mixture extracted with diethyl ether (4 × 6 mL) again.[43−45] The organic phase was dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. Product **4** (7.2 mg, 19 mmol) was obtained in 40% yield: $[\alpha]_D^{20}$ −4.843 ± 0.32 (*c* 0.0031, CH_2Cl_2); IR (ATR) ν_{max} 3290 (br), 3002 (w), 2928 (s), 2856 (s), 1735 (s), 1645 (s), 1545 (m), 1457 (m), 1351 (w), 1213 (m), 1162 (m), 1060 (w), 985 (w), 729 (w), 566 (w), 542 (w) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.34 (d, 1H, *J* = 7.1 Hz, NH), 5.40−5.28 (m, 2H, HC═CH), 4.64 (quin, 1H, *J* = 7.2 Hz, $CHCH_3$), 3.76 (s, 3H, OCH_3), 2.34 (t, 2H, *J* = 7.3 Hz, CH_2COOH), 2.22 (t, 2H, *J* = 7.7 Hz, CH_2C═O), 2.07−1.97 (m, 4H, CH_2HC═$CHCH_2$), 1.69−1.58 (m, 6H, 3 × CH_2), 1.34 (d, 3H, *J* = 7.2 Hz, $CHCH_3$), 1.31−1.12 (m, 12H, 6 × CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.9 (COOH), 174.1 (C═O), 173.3 (C═O), 130.1 (HC═CH), 129.6 (HC═CH), 52.5 (OCH_3), 47.9 ($CHCH_3$), 36.4 (CH_2), 33.9 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.5 (CH_2), 27.0 (CH_2HC═$CHCH_2$), 26.8 (CH_2HC═$CHCH_2$), 25.6 (CH_2), 24.6 (CH_2), 18.5 (CH_3); HRESIMS *m*/*z* 370.25921 (calcd for $C_{20}H_{36}NO_5$, 370.25880).

*(6-Carboethoxyheptyl)triphenylphosphonium Bromide (**13**).* The Wittig salt was prepared as described for **8** from ethyl 7-bromoheptanoate (2.47 mL, 12.65 mmol, 1.05 equiv) and PPh_3 (3.15 g, 12.01 mmol, 1.05 equiv). The product **13** (5.56 g, 11.13 mmol, 88%) was obtained as a colorless oil: R_f (CH_2Cl_2/MeOH, 25:1) 0.56; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.84−763 (m, 15H, Ar), 4.00 (q, 2H, *J* = 7.1 Hz, CH_2CH_3), 3.72−3.58 (m, 2H, PCH_2), 2.18 (t, 2H, *J* = 7.2 Hz, CH_2COOEt), 1.71−1.54 (m, 4H, 2 × CH_2), 1.52−1.41 (m, 2H, CH_2), 1.35−1.22 (m, 2H, CH_2), 1.15 (t, 3H, *J* = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.6 (C═O), 134.2 (d, 3C, *J* = 2.9 Hz, 3 × CH Ar), 132.7 (d, 6C, *J* = 9.9 Hz, 6 × CH Ar), 129.7 (d, 6C, *J* = 12.5 Hz, 6 × CH Ar), 117.2 (d, 3C, *J* = 85.9 Hz, 3 × C Ar), 59.2 (OCH_2CH_3), 33.1 (CH_2), 29.2 (CH_2), 28.9 (CH_2), 27.5 (CH_2), 23.4 (CH_2), 22.2 (CH_2), 13.1 (OCH_2CH_3).

*Ethyl (Z)-Tetradec-7-enoate (**14**).* Ester **14** was obtained as described for the synthesis of **9** from Wittig salt **13** (6.00 g, 12.01 mmol, 1.05 equiv), NaHDMS (12.58 mL, 1 M in THF, 12.58 mmol, 1.1 equiv), and freshly distilled heptanal (1.6 mL, 1.30 g, 11.44 mmol, 1.00 equiv). Column chromatography (SiO_2, pentane/EtOAc, 20:1) furnished pure **14** (2.71 g, 10.64 mmol, 93%) as a yellowish oil: R_f (pentane/EtOAc, 20:1) 0.40, GC (ZB-5 MSi) *I* 1800; IR (ATR) ν_{max} 2926 (m), 2856 (m), 1738 (s), 1462 (w), 1373 (w), 1249 (w), 1179 (m), 1033 (w), 726 (w) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.23 (m, 2H, HC═CH), 3.99 (q, 2H, *J* = 7.7 Hz, CH_2CH_3), 2.17 (t, 2H, *J* = 7.7 Hz, CH_2COOEt), 1.86−1.93 (m, 4H, H_2CHC═$CHCH_2$), 1.47−1.56 (m, 2H, CH_2CH_3), 1.15−1.29 (m, 12H, 6 × CH_2), 1.13 (t, 3H, *J* = 7.2 Hz, CH_3), 0.76 (t, 3H, *J* = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.7 (C═O), 129.8 (HC═CH), 60.1 (CH_2CH_3), 34.3 (CH_2COOEt), 31.7 (CH_2), 29.5 (2 × CH_2), 28.9 (2 × CH_2), 27.1 (H_2CHC═$CHCH_2$), 24.8 (CH_2), 22.6 (CH_2CH_3), 14.1 (2 × CH_3); EIMS *m*/*z* 254 $[M]^+$ (37), 225 (4), 208 (74), 191 (16), 166 $[M^+ - C_4H_8O_2]$ (88), 151 (35), 124 (71), 109 (62), 88 $[C_4H_8O_2]^+$ (94), 70 (56), 55 (100), 39 (28).

*(Z)-Tetradec-7-enoic Acid (**15**).* The ester **14** (1.68 g, 6.60 mmol, 1 equiv) was dissolved in EtOH (25 mL), and KOH (0.57 g, 10.21 mmol, 1.55 equiv) was added. The solution was stirred overnight at rt. After acidification with 2 N HCl to pH 2.0, the solution was extracted with EtOAc (3 × 50 mL). The organic layers were combined, dried with $MgSO_4$, and concentrated in vacuo.[46] Column chromatography (SiO_2, pentane/EtOAc, 10:1) gave the product **15** (0.99 g, 4.36 mmol, 66%) as a yellowish oil: R_f (SiO_2, pentane/EtOAc, 10:1) 0.20; GC (ZB-5 MSi, MSTFA derivative) *I* 1857; IR (ATR) ν_{max} 3005 (m), 2924 (s), 2855 (s), 2671 (w), 1707 (s), 1460 (m), 1413 (m), 1278 (m), 1227 (m), 935 (m), 725 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.19 (s, 1H, OH), 5.22−5.33 (m, 2H, HC═CH), 2.28 (t, 2H, *J* = 7.7 Hz, CH_2COOH), 1.92−1.99 (m, 4H, H_2CHC═$CHCH_2$), 1.54−1.61 (m, 2H, CH_2CH_3), 1.13−1.37 (m, 12H, 6 × CH_2), 0.81 (t, 3H, *J* = 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3 (C═O), 129.8 (HC═CH), 34.1 (CH_2COOH), 31.8 (CH_2), 29.5 (2 × CH_2), 28.9 (2 × CH_2), 27.1 (H_2CHC═$CHCH_2$), 24.6 (CH_2), 22.6 (CH_2CH_3), 14.1 (CH_3); EIMS *m*/*z* (MSTFA derivative) 298 $[M]^+$ (21), 283 $[M^+-CH_3]$ (96), 255 (5), 227 (5), 199 (29), 183 (11), 166 (48), 145 (54), 117 (100), 96 (44), 73 (93), 55 (58), 39 (10).

*(Z)-2,2-Dimethyl-5-(tetradec-7-enoyl)-1,3-dioxan-4,6-dione (**16**).* The acid **15** (0.69 g, 3.05 mmol, 1.00 equiv), DCC (3.35 mL, 1 M in CH_2Cl_2, 0.69 mg, 3.53 mmol, 1.1 equiv), Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione, 0.44 g, 3.05 mmol, 1.0 equiv), and DMAP (0.39 g, 3.20 mmol, 1.05 equiv) were dissolved in 30 mL of dry CH_2Cl_2 and stirred at rt overnight in a nitrogen atmosphere. The mixture was filtered, the solvent was evaporated under reduced pressure, and the residue was redissolved in EtOAc (30 mL). After shaking with 2 N HCl (5 × 15 mL) the organic phase was dried with $MgSO_4$ and concentrated in vacuo.[47] The product **16** (0.56 g, 1.59 mmol, 52%) was obtained as a dark yellowish oil and used in the next reaction without further purification: R_f (SiO_2, pentane/EtOAc, 1:1) 0.46.

*(Z)-N-(3-Oxohexadec-9-enoyl)alanine Methyl Ester, 3-Oxo-C16:1-NAME (**17**).* The ester **16** (0.56 g, 1.56 mmol, 1.00 equiv), triethylamine (0.35 mL, 2.54 mmol, 1.60 equiv), and L-alanine methyl ester·HCl (0.22 g, 1.59 mmol, 1.0 equiv) were dissolved in 30 mL of MeCN. The solution was stirred for 2 h at rt and then heated to reflux for 4 h. The formed precipitate was filtered off, the solvent evaporated under reduced pressure, and the residue redissolved in EtOAc (30 mL).[47] After washing with 2 M HCl (3 × 10 mL) the organic phase was dried with $MgSO_4$ and dried in vacuo. Column chromatography (RP18, MeCN/H_2O, 5:1) gave 3-Oxo-C16:1-NAME **17** (0.16 g, 0.47 mmol, 30%) as a yellowish oil: R_f (RP18, MeCN/H_2O, 5:1) 0.32; $[\alpha]_D^{20}$ −0.02 ± 0.07 (*c* 0.0153, CH_2Cl_2); IR (ATR) ν_{max} 3309 (w), 3003 (w), 2926 (m), 2855 (m), 1748 (m), 1721 (m), 1648 (m), 1540 (m), 1455 (m), 1376 (w), 3151 (w), 1211 (m), 1163 (m), 1057 (w), 987 (w),852 (w), 724 (w), 567 (w) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.43 (d, 1H, *J* = 5.7 Hz, NH), 5.39−5.29 (m, 2H, HC═CH), 4.59 (quin, 1H, *J* = 7.2 Hz, $CHCH_3$), 3.75 (s, 3H, OCH_3), 3.41 (s, 2H, O═CCH_2C═O), 2.53 (t, 2H, CH_2C═O), 1.97−2.05 (m, 4H, H_2CHC═$CHCH_2$), 1.54−1.65 (m, 2H, CH_2), 1.43 (d, 3H, *J* = 7.3 Hz, $CHCH_3$), 1.39−1.22 (m, 12H, 6 × CH_2), 0.88 (t, 3H, *J* = 6.8 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.4 (C═O), 173.0 (C═O), 165.2 (C═O), 129.8 (HC═CH), 52.5 (OCH_3), 48.6 (O═CCH_2C═O), 48.1 ($CHCH_3$), 43.8 (CH_2C═O), 31.8 (CH_2), 29.6 (2 × CH_2), 28.8 (2 × CH_2), 27.1 (H_2CHC═$CHCH_2$), 22.9 (2 × CH_2), 18.2 ($CHCH_3$), 14.1 (CH_3).

*(Z)-N-(3-Hydroxyhexadec-9-enoyl)alanine Methyl Ester, 3-OH-C16:1-NAME (**18**).* NAME **17** (92 mg, 0.26 mmol, 1.00 equiv) was dissolved in MeOH (3.1 mL). The ice-cooled solution was adjusted with 2 M HCl to pH 3, and subsequently $NaBH_4$ (13.2 mg, 0.35 mmol, 1.4 equiv) was added in portions. After adding 2 M HCl to pH 3 the solution was stirred for 30 min at rt.[47] The solvent was evaporated under reduced pressure, and column chromatography (RP18, MeCN/H_2O, 8:1) afforded the diastereomeric product **18** (ratio 49:51) (29 mg, 0.08 mmol, 32%) as a bright yellowish oil: R_f (RP18, MeCN/H_2O, 8:1) 0.32; GC (HP-5 MS, MSTFA derivative) *I* 2604, 2611; $[\alpha]^{20}{}_D$ −2.72 ± 0.10 (*c* 0.0104, CH_2Cl_2); IR (ATR) ν_{max} 3312 (m, br), 3082 (w), 3003 (m), 2925 (s), 2854 (m), 1747 (m), 1730 (m), 1641 (s), 1540 (m), 1455 (m), 1374 (w), 1345 (w), 1212 (m), 1161 (m), 1091 (w), 1059 (w), 986 (w), 871 (w), 851 (w), 671 (m), 536 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.40 (d, 1H, *J* =

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J. Nat. Prod. XXXX, XXX, XXX−XXX

6.6 Hz, NH), 5.39−5.30 (m, 2H, HC═CH), 4.60 (dquin, 1H, J = 7.2 Hz, 2.3 Hz, $CHCH_3$), 4.01−3.94 (m, 1H, $CHOH$), 3.76 (s, 3H, OCH_3), 2.44−2.25 (m, 2H, CH_2C═O), 2.04−1.99 (m, 4H, CH_2HC═$CHCH_2$), 1.73−145 (m, 4H, 2 × CH_2), 1.42 (dd, 3H, J = 7.2 Hz, 1.0 Hz, $CHCH_3$), 1.38−1.26 (m, 12H, 6 × CH_2), 0.88 (t, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 173.5 (C═O), 172.2, 171.9 (C═O), 130.0 (HC═CH), 129.6 (HC═CH), 68.7, 68.5 (CHOH), 52.6, 52.5 (OCH_3), 48.0, 47.9 ($CHCH_3$), 42.9, 42.4 (CH_2C═O), 36.9, 36.7 (CH_2CHOH), 31.7 (CH_2), 29.70 (CH_2), 29.66 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 27.2 (CH_2HC═$CHCH_2$), 27.1 (CH_2HC═$CHCH_2$), 25.40, 25.39 (CH_2), 22.6 (CH_2), 18.3, 18.1 ($CHCH_3$), 14.1 (CH_3); EIMS m/z 355 $[M]^+$ (8), 296 (9), 187 (6), 174 (21), 145 (25), 114 (11), 104 (100), 102 (19), 81 (12), 67 (10), 55 (16), 44 (60); HRESIMS m/z 356.27949 (calcd for $C_{20}H_{38}NO_4$, 356.27954).

Bioassays. All compounds were dissolved in DMSO and were tested in standard microbroth dilution assays as described earlier.[30] Minimum inhibitory concentrations (MICs) were determined in standard microdilution assays. Cultures of *Bacillus subtilis* DSM-10, *Staphylococcus carnosus* DSM-20501, *Micrococcus luteus* DSM-1790, TolC-deficient *Escherichia coli*, *Staphylococcus aureus* Newman, and *Mucor hiemalis* DSM-2656 in mid log phase were diluted to achieve a final inoculum of ca. 5 × 10^5 to 5 × 10^6 cfu/mL in Mueller-Hinton broth (1.75% casein hydrolysate, 0.2% beef infusion, 0.15% starch; pH 7.4; used for all bacteria) or Myc medium (1% phytone peptone, 1% glucose, 50 mM HEPES, pH 7.0; *Mucor hiemalis*). Serial dilutions of samples were prepared from THF stock solutions (2 mg/mL) in sterile 96-well plates, the cell suspension was added, and microorganisms were grown for 16−20 h at their optimal growth temperature at either 30 or 37 °C. Given MIC values are the lowest concentration of antibiotic at which there was no visible growth. Quorum quenching and quorum sensing assays were performed as described previously.[14] Briefly, the sensor strain *Pseudomonas putida* F117 (pKRC12)[31] was grown overnight in Luria−Bertani medium with addition of gentamycin (20 μg/mL) at 30 °C with shaking (160 rpm), and compound **3** and **4** were added at different concentrations. The full assay is described in detail by Bruns et al.[14]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00757.

HPLC/MS TIC, mass spectra, and NMR spectra of isolated and synthesized products **A**, **B**, **3**, **4**, **17**, and **18** (PDF)

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Notes

The authors declare no competing financial interest.

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