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# Facile Total Synthesis and Antimicrobial Activity of the Marine Fatty Acids (*Z*)-2-Methoxy-5-hexadecenoic Acid and (*Z*)-2-Methoxy-6-hexadecenoic Acid

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# Facile Total Synthesis and Antimicrobial Activity of the Marine Fatty Acids (Z)-2-Methoxy-5-hexadecenoic Acid and (Z)-2-Methoxy-6-hexadecenoic Acid

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The total synthesis of the naturally occurring (Z)-2-methoxy-5-hexadecenoic acid and (Z)-2-methoxy-6-hexadecenoic acid was accomplished using as a key step Mukaiyama's trimethylsilyl cyanide addition to 4- and 5-pentadecenal, respectively. These syntheses further confirm the structures of the natural marine fatty acids and corroborate their cis double-bond stereochemistry. The title compounds were antimicrobial against the Gram-positive bacteria *Staphylococcus aureus* (MIC 0.35 μmol/mL) and *Streptococcus faecalis* (MIC 0.35 μmol/mL).

Naturally occurring α-methoxy fatty acids are rare and have only been reported from the phospholipids of sponges.[1−3] Some examples include normal-chain saturated 2-methoxy fatty acids of between 16 and 24 carbons, and very long-chain monounsaturated fatty acids, such as (2*R*,21*Z*)-2-methoxy-21-octacosenoic acid, which was the first naturally occurring α-methoxy fatty acid reported from a phospholipid.[2] Some time ago, we identified (*Z*)-2-methoxy-5-hexadecenoic acid (**5**) and (*Z*)-2-methoxy-6-hexadecenoic acid (**9**) from the phospholipids of several Caribbean sponges, and recently, we identified the 2-methoxyhexadecanoic acid as a plausible biosynthetic precursor for these α-methoxylated hexadecenoic acids.[3,4] Despite most of these identification efforts, complete characterization of the α-methoxylated hexadecenoic acids **5** and **9** is still lacking. Moreover, these α-methoxylated fatty acids were identified, in trace amounts and in complex mixtures, by GC−MS, and no material is thus available for further biological screening.[3] Other middle-chain methoxy-branched fatty acids, such as (4*E*,7*S*)-7-methoxy-4-tetradecenoic acid, isolated from *Lyngbya majuscula*, display antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus*.[5] Therefore, it became of interest to us to develop an efficient synthesis for these α-methoxylated fatty acids and to test their antimicrobial activity. In this paper we describe the synthesis of (*Z*)-2-methoxy-5-hexadecenoic acid (**5**) and (*Z*)-2-methoxy-6-hexadecenoic acid (**9**), using as a key synthetic step Mukaiyama's trimethylsilyl cyanide addition to aldehydes under basic conditions.[6] We further confirm the cis double-bond stereochemistry of the naturally occurring compounds by coelution of the synthetic methyl esters with the methyl esters of the natural acids. We also report the antimicrobial activity of these α-methoxy fatty acids.

## Results and Discussion

The synthesis of (*Z*)-2-methoxy-5-hexadecenoic acid (**5**) first required the preparation of (*Z*)-4-pentadecenal.[7,8] This was accomplished starting with commercially available 1-dodecyne, which was coupled with 2-(2-bromoethyl)-1,3-dioxolane and *n*-BuLi in tetrahydrofuran-hexamethylphosphoramide, resulting in a 68% yield of 2-(3-tetradecyne)-1,3-dioxolane (Scheme 1). Subsequent catalytic hydrogenation, using Lindlar's catalyst, afforded 2-(3-tetradecenyl)-1,3-dioxolane in an 83% isolated yield. No double-bond isomerization was observed. The dioxolane was removed with 5% HCl in acetone−water (1:1), and the equilibrium favored the already-reported (*Z*)-4-pentadecenal.[7,8] Addition of trimethylsilyl cyanide to (*Z*)-4-pentadecenal, under triethylamine catalysis as described by Mukaiyama for other shorter-chain analogues, resulted in a 95% isolated yield of 2-trimethylsilyloxy-5-hexadecenonitrile.[6] Under basic conditions the trimethylsilyloxynitrile easily reverts to the original aldehyde. So, the trimethylsilyloxynitrile was first transformed into the corresponding α-hydroxy amide under concentrated acid conditions (HCl), and this was then hydrolyzed to the α-hydroxy acid with 50% NaOH. Under these conditions the intermediate (*Z*)-2-hydroxy-5-hexadecenoic acid was obtained in a 70% yield. Double methylation was then successfully accomplished with NaH and methyl iodide in DMSO resulting in the already-reported methyl (*Z*)-2-methoxy-5-hexadecenoate, which coeluted in capillary GC with an authentic sample from *Amphimedon compressa*.[4] This experiment confirmed the cis double-bond stereochemistry for this acid and verifies the structure by total synthesis. Final saponification with KOH in ethanol afforded the desired (*Z*)-2-methoxy-5-hexadecenoic acid (**5**). This is the first total synthesis for **5**; the overall yield was 12%.

The synthesis of (*Z*)-2-methoxy-6-hexadecenoic acid (**9**) first required the preparation of (*Z*)-5-pentadecenal as the key intermediate (Scheme 2). In this case, commercially available decyl aldehyde was coupled with 4-carboxybutyltriphenylphosphonium bromide, under Wittig conditions, resulting in a 10:1 mixture of the known (*Z*)- and (*E*)-5-pentadecenoic acids.[9,10] The acids were then reduced to the desired (*Z*)-5-pentadecenal via (*Z*)-5-pentadecen-1-ol, a known pheromone.[11−13] Addition of trimethylsilyl cyanide to (*Z*)-5-pentadecenal, also under triethylamine catalysis, yielded 2-trimethylsilyloxy-6-hexadecenonitrile in a 95% isolated yield. The trimethylsilyl cyanide was also transformed into the intermediate α-hydroxy amide with concentrated HCl, and then hydrolyzed to (*Z*)-2-hydroxy-6-hexadecenoic acid with 50% NaOH. Double methylation was also successfully accomplished with NaH and methyl iodide, in DMSO, resulting in methyl (*Z*)-2-methoxy-6-hexadecenoate, which has only been identified in the sponge *Spheciospongia cuspidifera*.[3] Final saponification with KOH in ethanol afforded the desired (*Z*)-2-methoxy-6-hexadecenoic acid (**9**) in an overall 3% yield. It is of interest to mention that a different synthesis for

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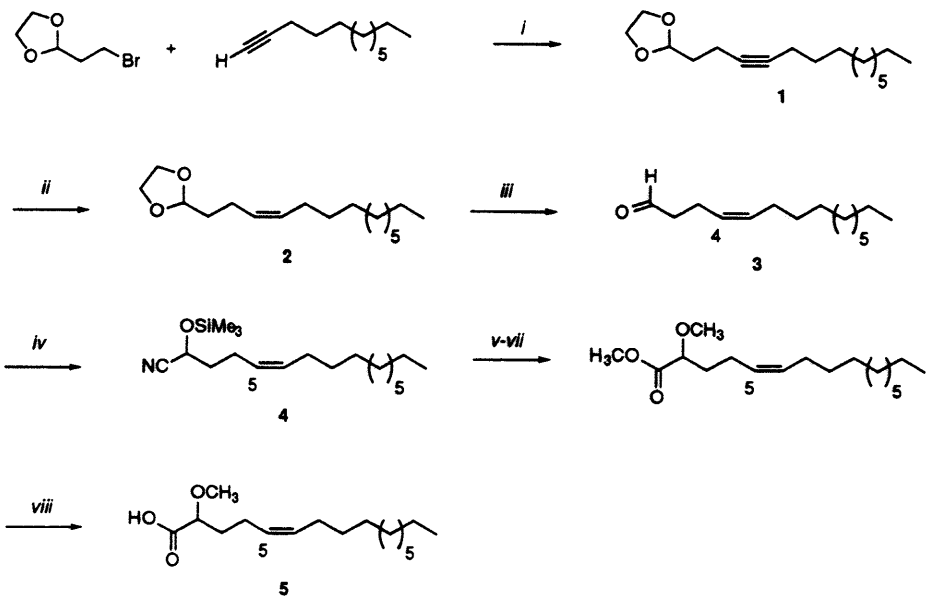
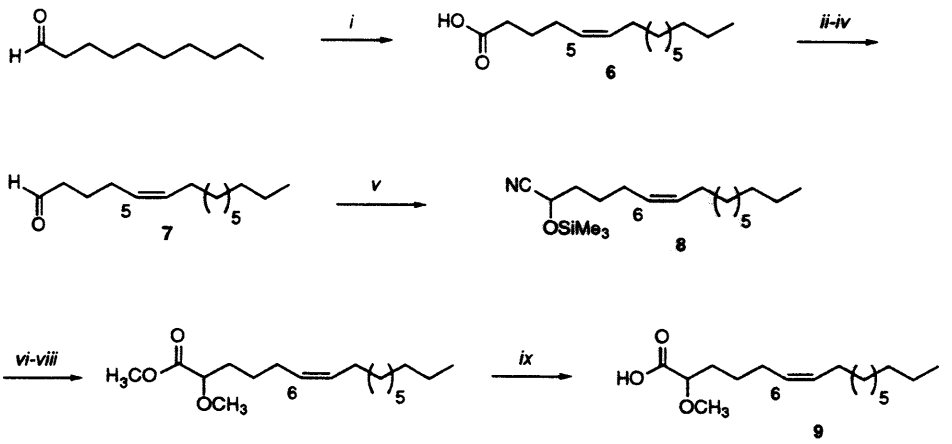
**Scheme 1**[a]



[a] Key: (i) *n*-BuLi, THF–HMPA, −70 °C; (ii) $H_2$, Lindlar; (iii) 5% HCl, $Me_2CO$–$H_2O$, 60 °C; (iv) TMS–CN, $Et_3N$, $CH_2Cl_2$, −10 °C; (v) HCl concd room temperature; (vi) 50% NaOH, heat; (vii) NaH/DMSO, $CH_3I$; (viii) KOH–EtOH.

**Scheme 2**[a]



[a] Key: (i) $HO_2C(CH_2)_3CH_2PPh_3^+Br^-$, *n*-BuLi, THF/DMSO (1:1), −10 °C; (ii) 1 N HCl–MeOH, 3 h; (iii) $LiAlH_4$–THF, −78 °C; (iv) PCC (1.5 equiv), $CH_2Cl_2$, room temperature; (v) TMS–CN, $Et_3N$, $CH_2Cl_2$, −10 °C, 2 h; (vi) HCl concd, room temperature; (vii) 50% NaOH, heat; (viii) NaH/DMSO, $CH_3I$; (ix) KOH–EtOH.

**Table 1.** Antimicrobial Activity of **5** and **9**

| | MIC (μmol/mL) | |
|---|---|---|
| bacteria | **5** | **9** |
| *S. aureus* | 0.35 | 0.35 |
| *S. faecalis* | 0.35 | 0.35 |
| *P. aeruginosa*[a] | | |
| *E. coli*[a] | | |

[a] Not active (MIC > 200 μg/mL).

methyl (*Z*)-2-methoxy-6-hexadecenoate was recently reported, and the key step was the addition of tris(methylthio)methyllithium to (*Z*)-5-pentadecenal followed by methylation (NaH, MeI) and hydrolysis ($HgCl_2$, HgO, MeOH–$H_2O$).[14] However, acid **9** was not synthesized in the latter report.

Both (*Z*)-2-methoxy-5-hexadecenoic acid (**5**) and (*Z*)-2-methoxy-6-hexadecenoic acid (**9**) displayed moderate and similar antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* (MIC 0.35 μmol/mL) and *Streptococcus faecalis* (MIC 0.35 μmol/mL) (Table 1). They were not, however, active against the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. The 2-methoxyhexadecanoic acid was not antimicrobial against any of these four microorganisms (MIC >100 μg/mL).[4]

We have presented here a facile synthetic approach to α-methoxylated fatty acids. Isomers **5** and **9** showed similar antimicrobial activity against Gram-positive bacteria and no activity against Gram-negative bacteria.

## Experimental Section

**General Experimental Procedures.** IR spectra were recorded on a Nicolet 600 FT-IR spectrophotometer. $^1H$ and $^{13}C$ NMR were recorded on a General Electric QE-300 or Bruker DPX-300 spectrometers. $^1H$ NMR chemical shifts are reported with respect to internal $Me_4Si$, and $^{13}C$ NMR chemical shifts are reported in parts per million relative to $CDCl_3$ (77.0 ppm). GC–MS analyses were recorded at 70 eV using a Hewlett–Packard 5972A MS ChemStation equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS) of polymethyl siloxane cross-linked with 5% phenyl methylpolysiloxane. HRMS data was obtained in a VG AutoSpec high-resolution mass spectrometer.

**2-(3-Tetradecynyl)-1,3-dioxolane (1).** Into a 250-mL two-necked round-bottom flask, equipped with a magnetic stirrer, was placed 1-dodecyne (4.6 g, 27 mmol) in 50 mL of anhydrous

THF. The reaction temperature was lowered to −78 °C, and 27 mL (54 mmol) of 2.0 M *n*-butyllithium in hexane was added. After 0.5 h HMPA (12.5 mL) was added at −50 °C, followed by the addition of 5.0 g (27.6 mmol) of 2-(2-bromoethyl)-1,3-dioxolane in THF. Stirring was continued for 8 h at this temperature, and the reaction mixture was finally quenched with a saturated $NH_4Cl$ solution (20 mL). Purification by Si gel column chromatography using hexane−ether 1:1 (v/v) as eluent, furnished 5.0 g (68% yield) of the tetradecynedioxolane: IR (neat) $\nu_{max}$ 2956, 2924, 2852, 1457, 1142, 1040 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 4.87 (1H, t, $J_{1,2}$ = 4.8 Hz, H-1), 3.84 (2H, AA'BB', $-OCH_2-$), 3.74 (2H, AA'BB', $-OCH_2-$), 2.18 (2H, tt, $J_{3,2}$ = 7.4 Hz and $J_{3,6}$ = 2.3 Hz, H-3), 2.02 (2H, tt, $J_{6,7}$ = 6.7 Hz and $J_{6,3}$ = 2.3 Hz, H-6), 1.73 (2H, dt, $J_{2,3}$ = 7.4 Hz and $J_{2,1}$ = 4.8 Hz, H-2), 1.18 (16H, br s, $CH_2$), 0.79 (3H, t, $J$ = 6.5 Hz, $-CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 103.2 (d, C-1), 80.2 (s, C-4), 78.7 (s, C-5), 64.6 (t, $-OCH_2-$), 33.3 (t, C-2), 31.7 (t, C-13), 29.4 (t), 29.1 (t), 29.0 (t), 28.9 (t), 28.7 (t), 22.5 (t), 18.5 (t, C-6), 13.8 (q, $CH_3$), 13.5 (t, C-3); GC−MS (70 eV) *m/z* 266 [$M^+$] (1), 238 (1), 237 (7), 195 (1), 167 (3), 153 (4), 140 (2), 139 (16), 125 (4), 113 (1), 99 (4), 97 (1), 95 (5), 93 (2), 92 (1), 91 (3), 87 (3), 86 (18), 81 (5), 80 (2), 79 (7), 77 (4), 74 (4), 73 [$C_3H_5O_2^+$] (100), 68 (2), 67 (9), 65 (4), 57 (3), 55 (10); HREIMS *m/z* 265.2168 [$M^+ - H$] (calcd for $C_{17}H_{29}O_2$, 265.2167).

**2-(3-Tetradecenyl)-1,3-dioxolane (2).** Into a 50-mL round-bottom flask, equipped with a magnetic stirrer and 40 mL of dry hexane were placed 2.7 g (10 mmol) of 2-(3-tetradecynyl)-1,3-dioxolane, 0.2 equivalents of quinoline, and 1.5 g of Lindlar's catalyst. After a couple of purging cycles, hydrogen was added until a volume equivalent to the initial amount of alkyne was consumed. After filtration and removal of the solvent in vacuo, 2.2 g (83% yield) of the alkene **2** was obtained: IR (neat) $\nu_{max}$ 3006, 2957, 2929, 2855, 1466, 1407, 1141, 1040, 724 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.37 (2H, m, H-4, H-5), 4.85 (1H, t, $J$ = 4.8 Hz, H-1), 3.97 (2H, AA'BB', $-OCH_2-$), 3.84 (2H, AA'BB', $-OCH_2-$), 2.15 (2H, m, H-3), 2.03 (2H, m, H-6), 1.70 (2H, m, H-2), 1.25 (16H, br s, $-CH_2-$), 0.87 (3H, t, $J$ = 6.8 Hz, $-CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 130.7 (d, C-4), 128.4 (d, C-5), 104.2 (d, C-1), 64.8 (t, $-OCH_2-$), 33.9 (t, C-2), 31.9 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.3 (t), 29.28 (t), 27.1 (t, C-6), 22.7 (t), 21.9 (t, C-3), 14.1 (q, C-15); GC−MS (70 eV) *m/z* 268 [$M^+$] (6), 239 (4), 225 (11), 211 (4), 206 (1), 197 (3), 183 (3), 169 (4), 156 (2), 155 (16), 141 (14), 127 (7), 113 (4), 100 (9), 99 [$C_5H_7O_2^+$] (96), 95 (3), 87 (4), 86 (25), 83 (7), 81 (5), 80 (6), 79 (4), 74 (3), 73 [$C_3H_5O_2^+$] (100), 69 (6), 67 (10), 57 (5), 55 (14); HREIMS *m/z* 268.2408 (calcd for $C_{17}H_{32}O_2$, 268.2402).

**(*Z*)-4-Pentadecenal (3).** Into a 25-mL round-bottom flask, equipped with a magnetic stirrer, were placed 2.0 g (7.5 mmol) of 2-(3-tetradecenyl)-1,3-dioxolane in 15 mL of 5% HCl in 1:1 $Me_2CO-H_2O$. The reaction mixture was stirred overnight at 60 °C and extracted with ether (2 × 15 mL), dried over $MgSO_4$, filtered, and the ether removed in vacuo affording 1.8 g (90% yield) of the previously reported (*Z*)-4-pentadecenal.[7,8]

**2-Trimethylsilyloxy-5(*Z*)-hexadecenonitrile (4).** To an anhydrous $CH_2Cl_2$ solution (5 mL) of trimethylsilyl cyanide (0.2 g, 2 mmol) and 4-pentadecenal (0.45 g, 2 mmol), catalytic amounts of $Et_3N$ (10 mol %) were added at −10 °C. The reaction mixture was stirred for 2 h at this temperature, and the solvent removed in vacuo, affording 0.61 g (1.9 mmol) of product for a 95% yield: IR (neat) $\nu_{max}$ 3013, 2955, 2926, 2855, 1471, 1458, 1255, 1114, 846 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.30 (1H, m, H-5), 5.42 (1H, m, H-6), 4.38 (1H, t, $J$ = 6.5 Hz, H-2), 2.19 (2H, br q, $J$ = 7.0 Hz, H-4), 2.1 (2H, br q, $J$ = 6.5 Hz, H-7), 1.81 (2H, br q, $J$ = 7.0 Hz, H-3), 1.25 (16H, br s, $-CH_2-$), 0.86 (3H, t, $J$ = 7.0 Hz, $-CH_3$), 0.20 (9H, s, $-Si(Me_3)$; $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 132.1 (d, C-5), 126.7 (d, C-6), 119.9 (d, C-1), 60.8 (2H, d, C-2), 36.2 (t, C-4), 31.9 (t), 29.6 (t), 27.2 (t, C-7), 22.6 (t), 22.5 (t, C-3), 14.0 (q, $CH_3$), −0.45 (q, $-SiMe_3$); GC−MS (70 eV) *m/z* 323 [$M^+$] (9), 308 (16), 281 (3), 280 (3), 265 (1), 218 (4), 210 (4), 204 (7), 192 (4), 190 (7), 184 (4), 180 (4), 176 (9), 169 (13), 168 [$C_8H_{14}NOSi^+$] (43), 162 (10), 155 (11) 152 (7), 150 (6), 148 (11), 144 (10), 135 (11), 134 (14), 129 (17), 128 [$C_5H_{10}NOSi^+$] (16), 121 (11), 120 (16), 116 (13), 109 (8), 101 (36), 96(29), 84 (24), 81 (38), 75 (46), 73 [$C_3H_9Si^+$] (100), 69 (31), 67 (56), 57 (39), 55 (67); HREIMS *m/z* 323.2653 (calcd for $C_{19}H_{37}NOSi$, 323.2644).

**2-Hydroxy-5(*Z*)-hexadecenoic acid.** Into a 100-mL round-bottom flask equipped with a magnetic stirrer was placed trimethylsilyloxyhexadecenonitrile (0.59 g, 1.8 mmol) in 10 mL of THF. Concentrated HCl (5 mL) was added, and the reaction mixture was heated at 65 °C for 24 h. The reaction mixture was then cooled (ice bath) and made alkaline by the slow addition of 10 mL of 50% NaOH. The reaction mixture was steam-distilled until no more $NH_3$ passed into the distillate, $H_2O$ (25 mL) was then added, followed by extraction with ether (3 × 15 mL). After drying over $Na_2SO_4$, the ether was suction-filtered and evaporated in vacuo, affording 0.3 g (70% yield) of the acid, which was used for the next step without further purification: $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.37 (2H, m, H-5, H-6), 4.24 (1H, m, H-2), 2.35 (2H, m), 2.16 (2H, m), 2.02 (2H, br t, $J$ = 6.5 Hz, H-7), 1.26 (16H, br s, $-CH_2-$), 0.87 (3H, t, $J$ = 7.0 Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 178.7 (s, C-1), 131.6 (d), 127.7 (d), 70.2 (d, C-2), 34.1 (t, C-7), 31.9 (t), 29.62 (t), 29.56 (t), 29.53 (t), 29.3 (t), 29.2 (t), 27.2 (t), 26.7 (t), 24.9 (t), 22.6 (t), 14.0 (q, $CH_3$).

**Methyl 2-methoxy-5(*Z*)-hexadecenoate.** Into a two-necked round-bottom flask, equipped with a magnetic stirrer and under nitrogen, was placed (*Z*)-2-hydroxy-5-hexadecenoic acid (0.25 g, 0.9 mmol) dissolved in 5 mL of anhydrous DMSO. Two equivalents of NaH, dissolved in 1.0 mL anhydrous DMSO, were added, and the reaction mixture was stirred for 10 min. Subsequently, an excess of MeI (4 equivalents) was slowly added, and the reaction mixture was stirred for an additional 20 min. The reaction mixture was finally extracted with hexane (3 × 10 mL), dried over $MgSO_4$, and concentrated in vacuo, affording 0.15 g (54% yield) of the methoxylated methyl ester:[3] $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.35 (2H, m, H-5, H-6), 3.75 (1H, t, $J$ = 6.5 Hz, H-2), 3.74 (3H, s, $-CO_2CH_3$), 3.37 (3H, s, $-OCH_3$), 2.12 (2H, m), 2.0 (2H, m, H-7), 1.75 (2H, q, $J$ = 7 Hz), 1.25 (16H, br s, $-CH_2-$), 0.87 (3H, t, $J$ = 6.8 Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 173.3 (s, C-1), 131.4 (d), 127.8 (d), 79.8 (d, C-2), 58.1 (q, $-OCH_3$), 51.8 (q, $-CO_2CH_3$), 33.2 (t), 31.9 (t), 29.65 (t), 29.60 (t), 29.55 (t), 29.52 (t), 29.3 (t), 27.2 (t), 22.8 (t), 22.6, (t), 14.0 (q, C-16); GC−MS (70 eV) *m/z* 298 [$M^+$] (1), 266 (3), 239 (5), 207 (2), 192 (1), 180 (1), 150 (2), 140 (2), 136 (2), 125 (2), 111 (6), 109 (4), 105 (5), 104 (100), 97 (12), 96 (4), 95 (11), 93 (5), 89 (9), 87 (4), 83 (10), 81 (15), 79 (11), 75 (5), 69 (15), 67 (22), 57 (14), 55 (32).

**2-Methoxy-5(*Z*)-hexadecenoic acid (5).** Into a 25-mL round-bottom flask was placed methyl (*Z*)-2-methoxy-5-hexadecenoate (0.050 g, 0.17 mmol) in 10 mL of 1.0 M KOH in EtOH. The reaction mixture was refluxed for 1 h and then allowed to cool to room temperature. The EtOH was evaporaed in vacuo and the resulting salt washed with 5 mL of hexane to extract the nonsaponifiable matter, which was discarded. The salt was then dissolved in 15 mL of $H_2O$ and acidified with 6.0 M HCl followed by extraction (3 × 10 mL) with ethyl ether. The organic extracts were put together and the organic solvent evaporated in vacuo, obtaining 0.033 g of **5** for a 66% yield:[3] IR (neat) $\nu_{max}$ 3600−3100, 3009, 2961, 2931, 2855, 1714, 1457, 1206, 1124, 734 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.45−5.30 (2H, m, H-5,6), 3.78 (1H, br t, $J$ = 6.1 Hz, H-2), 3.43 (3H, s, $-OCH_3$), 2.36 (2H, m), 2.14 (2H, m), 2.0 (2H, m, H-7), 1.81 (2H, m, H-3), 1.25 (14H, br s, $-CH_2-$), 0.87 (3H, t, $J$ = 6.7 Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 175.8 (s, C-1), 131.9 (d), 127.9 (d), 79.8 (d, C-2), 58.6 (q, $-OCH_3$), 32.7 (t), 32.2 (t), 30.0 (t), 29.98 (t), 29.94 (t), 29.91 (t), 29.68 (t), 27.6 (t), 23.0 (t), 14.4 (q, $CH_3$); EIMS (70 eV) *m/z* 284 [$M^+$] (0.6), 266 [$M^+ - H_2O$] (0.9), 252 [$M^+ - CH_3OH$] (2.8), 239 (7.3), 207 (2.1), 180 (4.2), 129 (8.5), 111 (10), 109 (15.7), 91 (6.6), 90 (100), 71 (19), 69 (50.7), 68 (15.8), 67 (37.5), 57 (5), 55 (63); HREIMS *m/z* 284.2332 (calcd for $C_{17}H_{32}O_3$, 284.2351).

**(*Z*)-5-Pentadecenoic acid (6):** obtained as a 10:1 mixture (38% yield) of (*Z*)-5-pentadecenoic acid and (*E*)-5-pentadecenoic acid in the reaction of 4-carboxybutyltriphenylphosphonium bromide and decyl aldehyde with 2.0 M *n*-BuLi in dimethyl sulfoxide−tetrahydrofuran (1:1) as previously described.[9,10]

**Methyl (Z)-5-pentadecenoate:** obtained in a 95% yield from the reaction of (Z)-5-pentadecenoic acid and 1M HCl in MeOH following the standard literature procedure.[11]

**(Z)-5-Pentadecen-1-ol:** prepared in a 70% yield by reacting methyl (Z)-5-pentadecenoate with $LiAlH_4$ in dry THF as previously described.[12]

**(Z)-5-Pentadecenal (7):** obtained in a 60% yield by reacting (Z)-5-pentadecen-1-ol with pyridinium chlorochromate in dry $CH_2Cl_2$ using literature standard procedures.[13]

**2-Trimethylsilyloxy-6(Z)-hexadecenonitrile (8).** To an anhydrous $CH_2Cl_2$ solution (5 mL) of trimethylsilyl cyanide (0.2 g, 2 mmol) and 5-pentadecenal (0.45 g, 2 mmol), catalytic amounts of $Et_3N$ (10 mol %) were added at −10 °C. The reaction mixture was stirred for 2 h at the same temperature and the solvent removed in vacuo, affording 0.61 g (1.9 mmol) of product for a 94% yield: $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.39−5.26 (2H, m), 4.36 (1H, t, $J$ = 6.6 Hz, H-2), 2.20 (2H, m), 1.99 (2H, m), 1.69 (2H, m), 1.25 (16 H, br s, $-CH_2-$), 0.86 (3H, t, $J$ = 6.9 Hz, $-CH_3$), 0.20 (9H,s, $-SiMe_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 130.9 (d), 128.2 (d), 119.7 (d, C-1), 61.2 (2H, d, C-2), 35.6 (t), 31.6 (t), 29.5 (t), 29.2 (t), 26.5 (t), 22.5 (t), 13.9 (q, C-16), −0.58 (q, $-SiMe_3$); GC−MS (70 eV) *m/z* 323 [$M^+$] (8), 309 (7), 308 (28), 281 (7), 266 (4), 225 (5), 214 (3), 210 (11), 206 (6), 192 (6), 182 (5), 169 (9), 168 (9), 166 (5), 156 (6), 155 (9), 154 (5), 140 (7), 135 (20), 129 (34), 128 (6), 121 (11), 120 (28), 113 (9), 101 (14), 97 (8), 96 (13), 95 (17), 94 (8), 93 (14), 84 (22), 83 (15), 82 (19), 81 (24), 80 (10), 79 (21), 75 (48), 74 (11), 73 (100), 69 (26), 68 (20), 67 (39), 59 (17), 57 (26), 56 (15), 55 (56), 54 (33).

**2-Hydroxy-6(Z)-hexadecenoic acid.** Into a 100-mL round-bottom flask equipped with a magnetic stirrer was placed trimethylsilyloxyhexadecenonitrile (0.58 g, 1.8 mmol) in 10 mL of THF, 5 mL of concentrated HCl was added, and the reaction mixture was heated at 65 °C for 24 h. The reaction mixture was then cooled (ice bath) and made alkaline by the slow addition of 10 mL of 50% NaOH. The reaction mixture was steam-distilled until no more $NH_3$ passed into the distillate, $H_2O$ was then added (25 mL) followed by extraction with ether (3 × 15 mL). After drying over $Na_2SO_4$, the ether was suction filtered and evaporated in vacuo, affording 0.3 g (70% yield) of the acid, which was used for the next step without further purification: $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.38−5.28 (2H, m, H-5, H-6), 4.22 (1H, dd, $J$ = 7.2, 4.2 Hz, H-2), 2.20 (2H, m), 1.66 (2H, m), 1.45 (2H, m), 1.25 (16H, br s, $CH_2$), 0.85 (3H, t, $J$ = 6.5 Hz, $-CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz).$\delta$ 178.7 (s, C-1), 130.7 (d), 128.7 (d), 70.2 (d, C-2), 33.6 (t), 31.9 (t), 29.5 (t), 29.3 (t), 29.2 (t), 27.2 (t), 26.7 (t), 24.9 (t), 22.6 (t), 14.0 (q, $CH_3$).

**Methyl 2-methoxy-6(Z)-hexadecenoate.** Into a two-necked round-bottom flask, equipped with a magnetic stirrer and under nitrogen, was placed (Z)-2-hydroxy-6-hexadecenoic acid (0.30 g, 1.1 mmol) dissolved in 5 mL of anhydrous DMSO. Two equivalents of NaH, in 1.0 mL anhydrous DMSO, was added, and the reaction mixture was stirred for 10 min. Subsequently, an excess of MeI (4 equivalents) was slowly added, and the reaction mixture was stirred for an additional 20 min. The reaction mixture was finally extracted with hexane (3 × 10 mL), dried over $MgSO_4$, and concentrated in vacuo affording 0.17 g (51% yield) of the already reported methoxylated methyl ester:[3,14] IR (neat) $\nu_{max}$ 3000, 2920, 2850, 1740, 1650, 1465, 1355, 1260, 1195, 1140, 720 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.39−5.25 (2H, m, H-5, H-6), 3.74 (1H, t, $J$ = 6.5 Hz, H-2), 3.73 (3H, s, $-CO_2CH_3$), 3.36 (3H, s, $-OCH_3$), 2.28 (4H, m, H-5, H-8), 1.99 (2H, m, H-3), 1.69 (2H, m, H-4), 1.25 (14 H, br s, $-CH_2-$), 0.87 (3H, t, $J$ = 6.5 Hz, $-CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz), $\delta$ 173.2 (s, C-1), 130.6 (d), 128.8 (d), 80.5 (d, C-2), 58.0 (q, $-OCH_3$), 51.8 (q, $-CO_2CH_3$), 32.4 (t, C-3), 31.9 (t, C-14), 29.6 (t), 29.57 (t), 29.54 (t), 29.3 (t), 29.2 (t), 27.2 (t, C-8), 26.8 (t, C-5), 25.2 (t), 22.6 (t, C-15), 14.0 (q, $CH_3$); GC−MS (70 eV) *m/z* 298 [$M^+$] (1), 266 (2), 239 (5), 207 (4), 206 (4), 180 (5), 150 (6), 136 (6), 127 (6), 124 (5), 123 (8), 121 (8), 117 (6), 111 (16), 110 (9), 109 (22), 108 (5), 104 (100), 98 (6), 97 (23), 96 (23), 95 (68), 94 (15), 93 (19), 89 (5), 87 (16), 85 (11), 84 (13), 83 (33), 82 (30), 81 (66), 80 (19), 79 (39), 77 (11), 75 (14), 71 (77), 70 (11), 69 (54), 68 (38), 67 (94), 59 (29), 58 (24), 57 (49), 56 (19), 55 (100), 54 (49). Anal. C, 72.21%; H, 11.49%; calcd for $C_{18}H_{34}O_3$: C, 72.44; H, 11.48.

**2-Methoxy-6(Z)-hexadecenoic acid (9).** Into a 25-mL round-bottom flask was placed methyl (Z)-2-methoxy-6-hexadecenoate (0.040 g, 0.13 mmol) in 10 mL of 1.0 M KOH in EtOH. The reaction mixture was refluxed for 1 h and then allowed to cool to room temperature. The EtOH was evaporated in vacuo, and the resulting salt was washed with 5 mL of hexane to extract the nonsaponifiable matter, which was discarded. The salt was then dissolved in 15 mL of $H_2O$ and acidified with 6.0 M HCl followed by extraction (3 × 10 mL) with ethyl ether. The organic extracts were put together and the solvent evaporated in vacuo, obtaining 0.025 g of **9** for a 63% yield:[3] $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.43−5.27 (2H, m, H-5, H-6), 3.8 (1H, dd, $J$ = 6.8 and 5.1 Hz, H-2), 3.43 (3H, s, $-OCH_3$), 2.02 (4H, m), 1.75 (2H, m, H-3), 1.49 (2H, m, H-4), 1.26 (14H, br s, $CH_2$), 0.87 (3H, t, $J$ = 6.5 Hz, $-CH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 179.9 (s, C-1), 130.8 (d), 128.7 (d), 80.1 (d, C-2), 58.2 (q, $-OCH_3$), 31.9 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.3 (t), 26.7 (t), 24.9 (t), 22.7 (t), 14.0 (q, $CH_3$).

**Antibacterial Activity.** Antibacterial activity against *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *S. faecalis*−group D (ATCC 29212) was determined following National Committee of Clinical Laboratory Standards (NCCLS).[15] A 200-$\mu$L solution of the α-methoxylated fatty acid in Mueller−Hinton broth was inoculated with $10^5$ colony-forming units in a 96-well plate. The minimal inhibitory concentration (MIC) was determined after an overnight incubation of the α-methoxylated acid and the microorganisms at 37 °C. The MIC was determined by observing the highest dilution of the fatty acid that inhibited growth when compared to an uninoculated chemical-control well. The generated data were taken from at least three separate experiments in duplicate.

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