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ORIGINAL ARTICLE

2-Methoxylated FA Display Unusual Antibacterial Activity Towards Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* (CIMRSA) and *Escherichia coli*

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Abstract The naturally occurring (6*Z*)-(±)-2-methoxy-6-hexadecenoic acid (**1**) and (6*Z*)-(±)-2-methoxy-6-octadecenoic acid (**2**) were synthesized in 7–8 steps with 38 and 13% overall yields, respectively, by using an acetylide coupling approach, which made it possible to obtain a 100% *cis*-stereochemistry for the double bonds. In a similar fashion, the acetylenic analogs (±)-2-methoxy-6-hexadecynoic acid (**3**) and (±)-2-methoxy-6-octadecynoic acid (**4**) were also synthesized in 6–7 steps with 48 and 16% overall yields, respectively. The antibacterial activity of acids **1**–**4** was determined against clinical isolates of methicillin-resistant *Staphylococcus aureus* (ClMRSA) and *Escherichia coli*. Among the series of compounds, acid **4** was the most active bactericide towards CIMRSA displaying IC_{50s} (half maximal inhibitory concentrations) between 17 and 37 μg/mL, in sharp contrast to the 6-octadecynoic acid, which was not bactericidal at all. On the other hand, acids **1** and **3** were the only acids that displayed antibacterial activity towards *E. coli*, but **1** stood out as the best candidate with an IC_{50} of 21 μg/mL. The critical micelle concentrations (CMCs) of acids **1**–**4** were also determined. The C18 acids **2** and **4** displayed a five-fold lower CMC (15–20 μg/mL) than the C16 analogs **1** and **3** (70–100 μg/mL), indicating that **4** exerts its antibacterial activity in a micellar state. None of the studied acids were inhibitory towards *S. aureus* DNA gyrase discounting this type of enzyme inhibition as a possible antibacterial mechanism. It was concluded that the combination of α-methoxylation and C-6 unsaturation increases the bactericidal activity of the C16 and C18 FA towards the studied bacterial strains. Acids **1** and **4** stand out as viable candidates to be used against *E. coli* and CIMRSA, respectively.

Keywords Antibacterial · *Escherichia coli* · Methicillin-resistant · Methoxylated fatty acids · *Staphylococcus aureus* · Synthesis

Abbreviations

ClMRSA	Clinical isolates of methicillin-resistant *Staphylococcus aureus*
CMC	Critical micelle concentration
DCM	Dichloromethane
DMI	1,3-Dimethyl-2-imidazolidinone
DMSO	Dimethyl sulfoxide
FA	Fatty acid(s)
GC/MS	Gas chromatography-mass spectrometry
IC_{50}	Half maximal inhibitory concentration
MIC	Minimum inhibitory concentration
PCC	Pyridinium chlorochromate
PTSA	*p*-Toluenesulfonic acid
Rel DNA	Relaxed deoxyribonucleic acid
SEM	Standard error of the mean
SC DNA	Super coiled deoxyribonucleic acid
TSI	Trypticase Soy Broth
THF	Tetrahydrofuran
TMSCN	Trimethylsilyl cyanide
UPLC-MS	Ultra high performance liquid chromatography-mass spectrometry

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Introduction

Despite the fact that the antibacterial activity of FA (fatty acids) has been amply studied [1], the bactericidal activity of the less ubiquitous α-methoxylated FA has been less documented in the literature. The α-methoxylated FA are a group of mainly marine FA, which have been isolated from many different species of Caribbean sponges [2]. These FA can range between 14 and 28 carbon atoms and can possess either the iso/anteiso methyl branching and/or double bond unsaturations at C-6 in the shorter chain analogs [2] or at C-5 and C-9 in the longer chain analogs, among other variants [3, 4]. The stereochemistry at the chiral center of the naturally occurring acids has been established as R [2].

The little research reported so far tends to indicate that the α-methoxy functionality imparts to a FA biophysical properties that increases their toxicity towards leukemia and neuroblastoma cell lines [5, 6], fungitoxicity towards *C. albicans* and *C. neoformans* [7, 8], and bactericidal activity towards Gram-positive bacteria (*Staphylococcus aureus* and *S. faecalis*) as well as toxicity towards mycobacteria (*Mycobacterium tuberculosis* $H_{37}Rv$) [9, 10]. The reasons behind these rather interesting differences seems to be speculative at this point, which could range from changes in the lipophilicity (logP) and pH of the acids to different rates of micelle formation. In order to get more insight into the properties behind the antibacterial potential of these rather peculiar compounds, we explored the antibacterial profile of the two naturally occurring (6*Z*)-(±)-2-methoxy-6-hexadecenoic acid (**1**) and (6*Z*)-(±)-2-methoxy-6-octadecenoic acid (**2**) together with their corresponding acetylenic analogs (±)-2-methoxy-6-hexadecynoic acid (**3**) and (±)-2-methoxy-6-octadecynoic acid (**4**). In this study we chose primarily antibacterial profiles against clinical isolates of methicillin-resistant *S. aureus* (ClMRSA). Compounds **1** and **2** are ideal to be compared to the corresponding non methoxylated analogs since it has been well established in the literature that 16:1 isomers, in particular the 6- and 9-hexadecenoic acids, are toxic towards *S. aureus* while the 18:1 isomers, such as the 6- and 9-octadecenoic acids, are relatively nontoxic to some *S. aureus* strains [11]. In this work we also present the first total synthesis for **2**, a natural product previously identified by us in the Caribbean sponge *Spheciospongia cuspidifera* [12] as well as a third generation synthesis for **1**, initially isolated from the same sponge.

Materials and Methods

Instrumentation

All compounds were analyzed by ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) using a Bruker DPX-300 spectrometer. The samples were diluted in 99.8% chloroform-d ($CDCl_3$) and the solvent signals at 7.26 (^{1}H) and 77.0 (^{13}C) ppm were used as internal standards for hydrogen and carbon, respectively. Mass spectral data were acquired on a GC–MS (Agilent 5977E MS ChemStation) equipped with a 30 × 0.25 mm (film 0.25 μm) special performance capillary column (HP-5MS) of polymethylsiloxane cross-linked with 5% phenyl methylpolysiloxane [4]. IR spectra were measured neat on a Bruker Tensor 27 FT-IR spectrometer. High-resolution mass spectral data were acquired using a quadrupole time-of-flight mass spectrometer (Q-TOF, Synapt, G2-S, Waters) with electrospray ionization in either negative or positive ion mode as previously described [4].

Synthesis

*5-Heptadecyn-1-ol (**6**)*

To a stirred solution of 0.34 g (1.9 mmol) of 1-tridecyne (**5**) in 5.0 mL of dry THF was added 0.70 mL (7.6 mmol) of *n*BuLi (2.5 M) in hexane at 0 °C under an argon atmosphere. The mixture was stirred at 0 °C for 45 min, and then 1.70 mL (10.3 mmol) of HMPA (Caution! HMPA is toxic and safety precautions should be taken handling this solvent) was added to the reaction mixture followed by the addition of 0.90 g (3.8 mmol) of 2-(4-bromobutoxy)tetrahydro-2*H*-pyran and the solution was stirred for 24 h. After that time, the reaction mixture was quenched with water, and the organic crude was washed with a brine solution (2 × 10 mL), diethyl ether (2 × 10 mL), dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Then, to the extracted product was added 20 mL of methanol followed by catalytic amounts (0.1 M) of PTSA and stirred for 12 h at 35 °C. The reaction mixture was then neutralized with a sodium bicarbonate solution (2 × 15 mL) and extracted with diethyl ether (2 × 15 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The crude product was purified using silica gel column chromatography while eluting with hexane/ether (9:1). The heptadec-5-yn-1-ol (**6**) [13] was obtained as a colorless oil 0.34 g (1.3 mmol) for a combined 71% yield for the two steps. IR (NaCl) ν_{max}: 3355 (O–H), 2928, 2852, 1462, 1377, 1330, 1065, 717 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 3.67 (2H, t, J = 6.3 Hz, H 1), 2.18-2.11 (4H, m), 1.67 (2H, m, H-2), 1.56–1.48 (4H, m), 1.27 (16H, br s, –C$\underline{H}_2$–), 0.87 (3H, t, J = 6.7 Hz, –C$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm) 80.7 (s), 79.7 (s), 62.5 (t, C-1), 31.9 (t), 31.8 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.3 (t), 29.2 (t), 29.1 (t), 28.9 (t), 25.3 (t, C-4), 22.7 (t), 18.7 (t), 18.5 (t), 14.1 (q, C-17); GC/MS (70 eV) *m/z* (relative intensity): 252 (M^+, 1), 234 (M^+–H_2O, 1), 231

(1), 217 (4), 215 (4), 203 (2), 189 (1), 179 (3), 173 (1), 165 (1), 161 (1), 151 (2), 149 (2), 139 (1), 137 (9), 135 (5), 123 (11), 121 (5), 111 (4), 109 (25), 107 (8), 105 (4), 97 (16), 96 (16), 95 (78), 93 (22), 91 (21), 83 (24), 81 (91), 79 (48), 77 (26), 71 (5), 69 (24), 67 (100), 65 (17), 57 (26), 55 (62).

*5-Heptadecynal (**7**)*

To a stirred solution of 0.40 g (1.86 mmol) of pyridinium chlorochromate (PCC) and 20.0 mL of dry DCM was added 0.31 g (1.24 mmol) of **6** at rt under an argon atmosphere and the reaction was left stirring for 24 h. The product was obtained by fluorisil column chromatography purification eluting with diethyl ether. The 5-heptadecynal (**7**) was obtained as colorless oil (0.26 g, 1.02 mmol) for an 82% yield. IR (NaCl) ν_{max}: 2958, 2931, 2852 (C<u>H</u>O), 2213, 1718 (C=O), 1674, 1462, 722, 634 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 9.78 (1H, t, J = 1.2 Hz, –C<u>H</u>O), 2.56 (2H, dt, J = 7.3, 1.2 Hz, H-2), 2.22–2.12 (4H, m), 1.81 (2H, m), 1.45 (2H, m), 1.26 (16H, br s, –C<u>H</u>$_2$–), 0.87 (3H, t, J = 6.7 Hz, –C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 202.2 (d, <u>C</u>HO), 81.6 (s), 78.6 (s), 42.8 (t, C-2), 31.9 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.3 (t), 29.1 (t), 29.0 (t), 28.9 (t), 22.7 (t), 21.5 (t), 18.7 (t), 18.2 (t), 14.1 (q, C-17); GC/MS (70 eV) *m/z* (relative intensity): 250 (M^+, 1), 209 (1), 191 (1), 175 (1), 165 (1), 151 (6), 147 (3), 137 (10), 133 (7), 123 (14), 119 (16), 111 (16), 110 (29), 109 (20), 107 (12), 106 (10), 105 (11), 98 (6), 97 (15), 96 (16), 95 (50), 93 (31), 92 (47), 91 (36), 83 (40), 82 (68), 81 (52), 79 (77), 77 (29), 69 (26), 67 (85), 65 (24), 57 (28), 55 (100).

*2-[(Trimethylsilyl)oxy]-Octadec-6-Ynenitrile (**8**)*

To a stirred solution of **7** (0.26 g, 1.0 mmol) in dry DCM (10 mL) at 0 °C was added trimethylsilyl cyanide (TMSCN) (0.19 g, 1.9 mmol) followed by catalytic amounts (0.025 M) of trimethylamine under an argon atmosphere. The mixture was left stirring under argon for 3 h. Then, the solvent was removed *in vacuo* and the crude product was washed with water (2 × 10 mL), diethyl ether (2 × 10 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo* affording 0.29 g (0.84 mmol) of **8** as an oil in an 82% yield. The product was used as such for the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 4.43 (1H, t, J = 6.4 Hz, H-2), 2.20 (4H, m), 1.63 (2H, m), 1.56 (2H, m), 1.45 (2H, m), 1.25 (16 H, brs, –C<u>H</u>$_2$–), 0.87 (3H, t, J = 6.7 Hz, –C<u>H</u>$_3$), 0.18 (9H, s, –Si(C<u>H</u>$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 120.4 (s, –<u>C</u>N), 61.1 (d, C 2), 31.9 (t), 29.7 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.3 (t), 29.2 (t), 29.1 (t), 28.9 (t), 26.9 (t), 24.7 (t), 22.6 (t), 14.1 (q, –<u>C</u>H$_3$), 0.4 (q, –Si(<u>C</u>H$_3$)$_3$); GC/MS (70 eV) *m/z* (relative intensity): 349 (M^+, 1), 334 (M^+-CH_3, 4), 264 (1), 259 (1), 234 (1), 223 (2), 209 (9), 208 (12), 194 (9), 181 (3), 174 (3), 168 (7), 157 (2), 155 (7), 152 (3), 146 (5), 135 (4), 133 (10), 129 (11), 121 (7), 119 (32), 118 (13), 116 (18), 109 (8), 105 (10), 101 (11), 95 (20), 93 (20), 92 (43), 91 (26), 84 (27), 81 (24), 79 (41), 75 (61), 73 ($C_3H_9Si^+$, 100), 69 (14), 67 (36), 65 (10), 59 (15), 57 (28), 55 (47).

*Methyl (±)-2-Hydroxy-6-Octadecynoate (**9**)*

To a solution of 0.29 g (0.84 mmol) of **8** and 12.3 mL (152 mmol) of 2-methyltetrahydrofuran (2-MeTHF) was added 4.9 mL (159 mmol) of concentrated HCl and refluxed for 24 h. Then, the solvent was removed *in vacuo* and the crude product was washed with ether (2 × 10 mL), a brine solution (2 × 10 mL), and concentrated. The methyl ester was obtained by esterification of the crude product with HCl/MeOH while refluxing for 3 h. The ester was purified using silica gel column chromatography eluting with hexane/ether (7:3), affording **9** as colorless oil 0.11 g (0.36 mmol) for a 44% yield for the two steps. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm); 4.20 (1H, m, H-2), 3.78 (3H, s –OC<u>H</u>$_3$), 2.77 (1H, d, J = 5.3 Hz, –O<u>H</u>), 2.19–2.12 (4H, m), 1.88 (2H, m), 1.64 (2H, m), 1.45 (2H, m), 1.26 (16H, brs, –CH_2–), 0.87 (3H, t, J = 6.7 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 175.6 (s, C-1), 80.9 (s), 79.2 (s), 70.1 (d, C-2), 52.5 (q, –O<u>C</u>H$_3$), 33.5 (t), 31.9 (t, C-3), 29.6 (t), 29.0 (t), 29.5 (t), 29.3 (t), 29.2 (t), 29.1 (t), 28.9 (t), 24.4 (t), 22.7 (t, C-4), 18.7 (t), 18.5 (t), 14.1 (q, –<u>C</u>H$_3$); GC/MS (70 eV) *m/z* (relative intensity): 310 (M^+, 6), 251 (M^+–$C_2H_3O_2$, 6), 233 (M^+–$C_2H_3O_2$–H_2O, 6), 221 (4), 197 (4), 169 ($C_9H_{13}O_3^+$, 19), 156 (40), 151 (21), 135 (20), 128 (12), 123 (20), 121 (18), 111 (14), 109 (28), 105 (12), 103 (28), 97 (31), 95 (33), 90 ($C_3H_6O_3^+$, 65), 81 (55), 79 (57), 77 (28), 73 (39), 69 (47), 67 (70), 59 (33), 57 (100), 55 (99).

*(±)-2-Methoxy-6-Octadecynoic Acid (**4**)*

To a stirred solution of NaH (0.02 g, 0.61 mmol) in dry THF (3.0 mL) and under argon was added a solution of 0.05 g (0.17 mmol) of **9** in THF (2.0 mL). After 10 min at rt, the solution temperature was lowered to 0 °C and 0.04 mL (0.61 mmol) of methyl iodide was added dropwise. Then, HCl (conc) was added to the solution until the pH was acidic (pH = 1–2). The crude was extracted with diethyl ether (2 × 10 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified using silica gel column chromatography first eluting with hexane/ether (9:1) and then with diethyl ether affording the methyl (±)-2-methoxy-6-octadecynoate. To obtain **4**, a solution of KOH/MeOH (1.5 M) (8.0 mL) and the methyl ester was stirred for 2 h at 60 °C. After this time, the solvent was removed in vacuo and hexane (15.0 mL) and HCl (3.0 mL)

were added to the solution. The crude was washed with a brine solution (1 × 10 mL), diethyl ether (1 × 10 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The product was purified using fluorisil column chromatography eluting with diethyl ether affording **4** as a colorless oil 0.04 g (0.14 mmol) and in a 78% yield. IR (NaCl) ν_{max}: 3500–2500 (–OH), 3440, 2922, 2852, 2213, 1733 (C=O), 1459, 1380, 1262, 1171, 1124, 803, 717 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 3.83 (1H, m, H-2), 3.43 (3H, s, –OC$\underline{H}_3$), 2.18-2.11 (4H, m), 1.84 (2H, m, H-3), 1.61 (2H, m), 1.45 (2H, m), 1.24 (16H, br s, –C$\underline{H}_2$–), 0.86 (3H, t, J = 6.4 Hz, –C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 177.8 (s, C-1), 81.1 (s), 79.7 (d, C-2), 79.0 (s), 58.2 (q, –O$\underline{C}H_3$), 31.9 (t), 29.6 (t), 29.5 (t), 29.4 (t), 29.1 (t), 29.0 (t), 28.9 (t), 28.6 (t), 28.5 (t), 24.5 (t), 22.7 (t), 18.7 (t), 18.4 (t), 14.1 (q, –$\underline{C}H_3$); UPLC-HRMS (negative ion mode) Calcd for $C_{19}H_{33}O_3$ $[M–H]^+$ 309.2430, found 309.2439.

Methyl (±)-2-Methoxy-6-Octadecynoate

GC/MS (70 eV) *m/z* (relative intensity): 324 (M^+, 1), 292 (M^+-CH_3OH, 1), 281 (1), 265 (M^+-$C_2H_3O_2$, 1), 249 (1), 233 (M^+–$C_2H_3O_2$–H_2O, 17), 209 (4), 195 (5), 184 (9), 165 (7), 159 (1), 151 (28), 149 (11), 139 (14), 135 (38), 129 (34), 123 (27), 121 (50), 119 (19), 109 (50), 104 ($C_4H_8O_3^+$, 100), 97 (49), 95 (80), 91 (53), 81 (85), 79 (92), 71 (62), 69 (35), 67 (74), 59 (10), 57 (28), 55 (49).

*(Z)-5-Heptadecen-1-ol (**10**)*

Into a 50-mL two-necked round-bottomed flask containing 0.15 g (0.39 mmol) of palladium in activated carbon (Lindlar's catalyst) and 0.89 mL of quinoline were added a solution of **6** (0.28 g, 1.11 mmol) and 1.7 mL of dry hexane. One of the two necks was capped with rubber septa and the other was connected via tygon tubing to a 25 mL graduated pipet ending in a 150 mL beaker with distilled water. While stirring at rt a 20-mL syringe with needle was used to withdraw air from the system and draw water up into the graduated pipet to the 0.0 mL mark. Hydrogen was then introduced into the system using a balloon filled with hydrogen and attached to the hose barb-to-luer lock adapter with stopcock and a needle. The reaction mixture consumed 27.2 mL of hydrogen during 1 h. The mixture was filtered and the solvent removed *in vacuo*. The product was purified under vacuum distillation (Kugelrohr) by removing impurities and quinoline at 130 °C/3 mmHg. The (*Z*)-5-heptadec-en-1-ol (**10**) was obtained as a colorless oil 0.16 g (0.61 mmol) for a 55% yield. IR (NaCl) ν_{max}: 3384 (O–H), 3008 (=C–H), 2922, 2852, 1462, 1374, 1062, 717 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 5.63-5.32 (2H, m, –C$\underline{H}$=C$\underline{H}$–), 3.64 (2H, t, J = 6.5 Hz, H-1), 2.13-1.99 (4H, m), 1.58 (2H, m), 1.44 (2H, m, H-3), 1.25 (18 H, brs, –C$\underline{H}_2$–), 0.87 (3H, t, J = 6.7 Hz, –C$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm) 130.4 (d), 129.2 (d), 62.9 (t, C-1), 32.4 (t, C-3), 31.9 (t), 29.7 (t), 29.67 (t), 29.64 (t), 29.56 (t), 29.4 (t), 29.3 (t), 29.2 (t), 27.2 (t), 26.9 (t), 25.8 (t, C-4), 22.7 (t, C-17), 14.11 (q, –$\underline{C}H_3$); GC/MS (70 eV) *m/z* (relative intensity): 254 (M^+, 1), 236 (M^+-H_2O, 5), 208 (3), 179 (1), 165 (1), 152 (3), 151 (2), 1137 (7), 123 (12), 109 (24), 97 (29), 96 (24), 95 (58), 83 (44), 82 (100), 81 (65), 79 (17), 71 (18), 69 (38), 68 (65), 67 (81), 65 (4), 57 (36), 55 (56).

*(Z)-5-Heptadecenal (**11**)*

To a stirred solution of 0.2 g (0.92 mmol) of PCC and 15.0 mL of dry DCM under argon was added 0.16 g (0.61 mmol) of **10** at rt for 24 h. After that time the product was obtained by fluorisil column chromatography eluting with diethyl ether. The (*Z*)-5-heptadecenal (**11**) was obtained as a colorless oil 0.15 g (0.58 mmol) for a 95% yield. IR (NaCl) ν_{max}: 2960, 2928, 2852 (C$\underline{H}$O), 1712 (C=O), 1450, 729, 639 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 9.75 (1H, t, J = 1.7 Hz, C$\underline{H}$O), 5.36-5.31 (2H, m, –C$\underline{H}$=C$\underline{H}$–), 2.42 (2H, dt, J = 7.3, 1.7 Hz, H-2), 2.05 (4H, m), 1.68 (2H, m), 1.24 (18H, brs, –C$\underline{H}_2$–), 0.86 (3H, t, J = 6.7 Hz, –C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 202.7 (d, $\underline{C}$HO), 130.2 (d), 129.7 (d), 43.3 (t, C 2), 31.9 (t), 29.7 (t), 29.64 (t), 29.56 (t), 29.34 (t), 29.32 (t), 29.2 (t), 28.9 (t), 27.2 (t), 27.1 (t), 22.7 (t), 22.0 (t, C-4), 14.1 (t, –CH_3); GC/MS (70 eV) *m/z* (relative intensity): 252 (M^+, 1), 234 (2), 208 (6), 180 (2), 167 (1), 152 (3), 149 (1), 138 (4), 124 (7), 123 (5), 121 (3), 111 (13), 109 (12), 107 (3), 98 (67), 97 (26), 96 (40), 95 (24), 84 (24), 83 (35), 82 (47), 81 (43), 79 (29), 69 (37), 67 (81), 57 (38), 55 (100).

*(Z)-2-[(Trimethylsilyl)oxy]-Octadec-6-Enenitrile (**12**)*

To a stirred solution of **11** (0.14 g, 0.54 mmol) in dry DCM (12.0 mL) at 0 °C and under argon was added 0.11 mL (0.82 mmol) of TMSCN followed by catalytic amounts (0.025 M) of triethylamine. The mixture was stirred for 3 h under argon. Then, the solvent was removed *in vacuo* and the crude was washed with water (2 × 10 mL), diethyl ether (2 × 10 mL), dried over $MgSO_4$, filtered and evaporated in vacuo, affording **12** (0.12 g, 0.33 mmol) as a dark oil in a 61% yield. The product was used as such for the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm); 5.34 (2H, m, –C$\underline{H}$=C$\underline{H}$–), 4.38 (1H, t, J = 6.5 Hz, H-2), 2.12 (2H, m), 2.01 (4H, m), 1.46 (2H, m), 1.25 (18H, brs, –C$\underline{H}_2$–), 0.86 (3H, t, J = 6.7 Hz, –C$\underline{H}_3$), 0.19 (9H, s, –OSi(C$\underline{H}_3$)$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm); 130.1 (d), 129.4 (d), 119.9 (s, –$\underline{C}$N), 61.3 (d, C-2), 31.9 (t), 29.73 (t), 29.65 (t), 29.63 (t), 29.5 (t), 29.3 (t), 29.3 (t), 29.15 (t), 29.12 (t),

28.9 (t), 27.1 (t), 24.6 (t), 22.7 (t), 14.1 (q, –$\underline{C}H_3$), 0.45 (q, –OSi($\underline{C}H_3$)$_3$); GC/MS (70 eV) *m/z* (relative intensity): 351 (M^+, 3), 336 (M^+–CH_3, 3), 238 (2), 210 (4), 169 (6), 168 (7), 155 (6), 135 (16), 129 (16), 121 (10), 109 (6), 101 (17), 95 (19), 93 (14), 84 (28), 81 (25), 79 (31), 75 (53), 73 ($C_3H_9Si^+$, 100), 69 (26), 67 (51), 59 (14), 57 (40), 55 (83).

*Methyl (±)-2-Hydroxy-6Z-Octadecenoate (**13**)*

To a solution of 0.12 g (0.33 mmol) of **12** and 4.9 mL (60.4 mmol) of 2-MeTHF was added concentrated HCl (2.0 mL, 64.7 mmol) and the reaction was refluxed for 24 h. The solvent was then removed *in vacuo* and the crude product was washed with ether (2 × 10 mL), a brine solution (2 × 10 mL), and concentrated. Then, the product was obtained by esterification of the crude using HCl/methanol while refluxing for 3 h. The product was purified using silica gel column chromatography eluting with hexane/ether (7:3) affording **13** as a colorless oil 0.07 g (0.21 mmol) in a 64% yield for both steps. IR (NaCl) ν_{max}: 3364 (O–H), 2945, 2922, 2854, 1739 (C=O), 1462, 1374, 1124 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 5.34 (2H, m, –C$\underline{H}$=C$\underline{H}$–), 4.19 (1H, m, H-2), 3.78 (3H, s, –OC$\underline{H}_3$), 2.05 (2H, m), 1.78 (2H, m), 1.62 (2H, m), 1.43 (2H, m), 1.25 (18H, brs, –C$\underline{H}_2$–), 0.87 (3H, t, J = 6.7 Hz, –C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 175.8 (s, C-1), 130.7 (d), 128.8 (d), 70.3 (d, C-2), 52.5 (q, –O$\underline{C}H_3$), 31.9 (t), 29.73 (t), 29.69 (t), 29.67 (t), 29.63 (t), 29.55 (t), 29.34 (t), 29.32 (t), 27.2 (t), 26.9 (t), 24.8 (t, C-4), 22.7 (t, C-17), 14.1 (q, –$\underline{C}H_3$); GC/MS (70 eV) *m/z* (relative intensity): 312 (M^+, 7), 281 (M^+–CH_3O, 1), 263 (1), 253 (M^+–$C_2H_3O_2$, 44), 234 (4), 221 (1), 208 (6), 180 (2), 151 (4), 140 (6), 129 (4), 127 (12), 121 (11), 111 (19), 109 (22), 103 (22), 97 (33), 95 (43), 90 ($C_3H_6O_3^+$, 100), 83 (35), 81 (42), 69 (31), 67 (45), 57 (30), 55 (46).

*(6Z)-(±)-2-Methoxy-6-Octadecenoic Acid (**2**)*

To a stirred solution of NaH (0.013 g, 0.54 mmol) in dry THF (2.0 mL), under argon, was added a solution of 0.05 g (0.16 mmol) of **13** in THF (3.0 mL). After 10 min at rt, the solution temperature was lowered to 0 °C and 0.04 mL (0.64 mmol) of methyl iodide was added dropwise. The reaction was left to stir for 3 h. After that, HCl (conc) was added to the solution until the pH was acidic (pH = 1–2). The crude was extracted with diethyl ether (2 × 10 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified using silica gel column chromatography first eluting with hexane/ether (9:1) and then with diethyl ether affording the methyl (6*Z*)-(±)-2-methoxy-6-octadecenoate. To obtain **2**, a solution of KOH/MeOH (1.5 M) (6.0 mL) and the methyl ester was stirred for 2 h at 60 °C. After this time, the solvent was removed *in vacuo* and hexane (12.0 mL) and HCl (2.0 mL) were added to the solution. The crude was washed with a brine solution (1 × 10 mL), diethyl ether (1 × 10 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The product was purified using a fluorisil column chromatography eluting with diethyl ether affording the (6*Z*)-(±)-2-methoxy-6-octadecenoic acid (**2**) as a colorless oil 0.045 g (0.14 mmol) in a 90% yield. IR (NaCl) ν_{max}: cm^{-1}; 3500–2500 (–OH), 3438, 2955, 2925, 2852, 1733 (C=O), 1459, 1377, 1286, 1121, 1074 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 5.34 (2H, m, –C$\underline{H}$=C$\underline{H}$–), 3.79 (1H, m, H-2), 3.43 (3H, s, –OC$\underline{H}_3$), 2.01 (4H, m), 1.75 (2H, m), 1.49 (2H, m), 1.25 (18H, brs, –C$\underline{H}_2$–), 0.86 (3H, t, J = 6.7 Hz, –C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 177.4 (s, C-1), 130.7 (d), 128.7 (d), 80.0 (d, C-2), 58.3 (q, –O$\underline{C}H_3$), 31.9 (t, C-3), 29.72 (t), 29.68 (t), 29.66 (t), 29.63 (t), 29.5 (t), 29.45 (t), 29.34 (t), 29.32 (t), 27.2 (t), 26.7 (t), 24.9 (t), 22.6 (t, C-17), 14.11 (q, –$\underline{C}H_3$); UPLC-HRMS (negative ion mode) Calcd for $C_{19}H_{35}O_3$ $[M\text{-}H]^+$ 311.2586, found 311.2595.

*2-(5-Pentadecyn-1-oxy)Tetrahydro-2H-Pyran (**14**)*

Into a 50-mL round-bottomed flask, was added (0.70 g, 3.8 mmol) of 2-(5-hexyn-1-yloxy) tetrahydro-2*H*-pyran, 6.0 mL of dry THF, and the mixture was cooled at 0 °C. To the cooled solution was added 2.9 mL (5.8 mmol) of *n*-BuLi (2 M in hexane) under argon, and the reaction was stirred for 15 min. To the stirring solution was added dropwise 3.0 mL of 1,3-dimethyl-2-imidazolidinone (DMI) and 1.1 mL (5.8 mmol) of 1-bromononane. After 20 min the cold bath was removed and the reaction was left stirring for 24 h. The reaction mixture was then washed with a brine solution (2 × 10 mL), extracted with diethyl ether (4 × 15 mL), dried over $MgSO_4$ and filtered. The solvent of the filtered solution was removed *in vacuo* and the crude product was purified using silica gel column chromatography eluting **14** with hexane/ether (9:1). The final product **14** (1.1 g, 3.4 mmol) was obtained as a colorless oil in a 90% yield. IR ν_{max}(neat): 2922, 2853, 1454, 1351, 1200, 1119, 1033, 905, 868, 813 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 4.57 (1H, t, J = 3.5 Hz), 3.89-3.36 (2H, m), 3.52-3.36 (2H, m), 2.21-2.09 (4H, m), 1.86-1.26 (25H, m), 0.87 (3H, t, J = 6.7 Hz, –CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm) 98.9 (d), 80.7 (s), 80.0 (s), 67.2 (t), 62.4 (t), 32.0 (t), 30.9 (t), 29.6 (t), 29.42 (t), 29.30 (t), 29.1 (t), 29.0 (t), 26.1 (t), 25.7 (t), 22.8 (t), 19.8 (t), 18.9 (t), 18.8 (t), 14.2 (q). GC/MS (70 eV) *m/z* (relative intensity): 308 (M^+, 0.01), 263 (1), 235 (8), 224 (1), 195 (1), 181 (6), 167 (1), 151 (1), 138 (2), 123 (2), 111 (6), 109 (5), 101 (6), 95 (19), 85 ($C_5H_9O^+$, 100), 67 (16), 55 (12); UPLC-HRMS (positive mode) Calcd for $C_{20}H_{37}O_2$ $[M + H]^+$ 309.2794, found 309.2817.

*5-Pentadecyn-1-ol (**15**)*

Into a 50-mL round-bottomed flask, was added (0.90 g, 2.9 mmol) of **14**, catalytic amounts of PTSA and 20 mL of MeOH. The stirring solution was refluxed at 65 °C for 12 h. After the reaction time, the solvent was removed *in vacuo*, the reaction crude was washed with a sodium bicarbonate ($NaHCO_3$) saturated aqueous solution (2 × 10 mL), extracted with diethyl ether (2 × 15 mL), dried over $MgSO_4$ and filtered. The solvent of the filtered solution was removed *in vacuo* and the crude product thus obtained was purified using silica gel column chromatography eluting **15** with hexane/ether (7:3). The pentadecynol (0.60 g, 2.7 mmol) was obtained as a colorless oil in a 93% yield. IR ν_{max} (neat): 3340 (–OH), 2922, 2853, 1456, 1377, 1332, 1057, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 3.68 (2H, t, J = 6.2 Hz, H-1), 2.21-2.09 (4H, m, H-4, H-7), 1.67–1.26 (19H, brs, –CH_2–), 0.87 (3H, t, J = 0.87 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 80.9 (s), 79.9 (s), 62.7 (t, C-1), 32.04 (t), 32.03 (t), 29.6 (t), 29.4 (t), 29.3 (t), 29.0 (t), 25.5 (t), 22.8 (t), 18.9 (t), 18.7 (t), 14.2 (q, C-15). GC/MS (70 eV) *m/z* (relative intensity): 224 (M^+, 0.01), 196 (M^+-H_2O, 0.01), 180 (3), 167 (1), 152 (3), 135 (4), 121 (6), 111 (29), 97 (54), 79 (100), 67 (41), 55 (36); UPLC-HRMS (positive mode) Calcd for $C_{15}H_{29}O$ $[M + H]^+$ 225.2218, found 225.2240.

*5-Pentadecynal (**16**)*

Into a 50-mL round-bottomed flask and under argon was placed 0.86 g (4.0 mmol) of PCC in 20 mL of dry DCM. To the stirring solution was added 0.60 g (2.7 mmol) of **15** and the reaction was left stirring for 12 h. After this time, the crude was filtered using silica gel and diethyl ether as eluent. The solvent of the filtered solution was removed *in vacuo* and the obtained yellowish liquid was purified using silica gel column chromatography eluting with hexane/ether (9:1). The 5-pentadecynal (0.53 g, 2.4 mmol) was obtained as a colorless oil in an 89% yield. IR ν_{max} (neat): 2953, 2922, 2853 (C<u>H</u>O), 2715 (C<u>H</u>O), 1710 (C=O), 1456, 1389, 1378, 722 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 9.80 (1H, t, J = 1.5 Hz, –C<u>H</u>O), 2.59-2.53 (2H, td, J = 7.5 and 1.5 Hz, H-2), 2.25-2.09 (4H, m, H-4, H-7), 1.85-1.76 (2H, m), 1.51–1.26 (16H, brs, –CH_2–), 0.87 (3H, t, J = 6.7 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 202.2 (d, C-1), 81.8 (s), 78.8 (s), 43.0 (t, C-2), 32.0 (t), 29.7 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.0 (t), 22.8 (t), 21.8 (t), 18.8 (t), 18.4 (t), 14.2 (q, C-15). GC/MS (70 eV) *m/z* (relative intensity): 222 (M^+, 1), 204 (1), 193 (M^+-CHO, 1), 178 (2), 161 (4), 151 (11), 123 (27), 137 (19), 119 (30), 110 (40), 95 (67), 79 (100), 67 (87), 55 (69).

*2-[(Trimethylsilyl)oxy]-6-Hexadecynenitrile (**17**)*

Into a 50-mL round bottomed flask and under argon was added 0.45 g (2.0 mmol) of and 8.1 mL of dry DCM. The stirring aldehyde solution was cooled to 0 °C, TMSCN (0.3 mL, 2.3 mmol) was added followed by the addition of catalytic amounts of triethylamine (0.03 mL, 2.2 mmol) and the mixture was stirred for 12 h. Then, the solvent was removed *in vacuo*, the reaction mixture was washed with a brine aqueous solution (2 × 10 mL), extracted with diethyl ether (3 × 15 mL), dried over $MgSO_4$, and filtered. The solvent was removed and the crude product was purified using silica gel column chromatography eluting the silyloxy nitrile **17** with hexane/ether (9:1), which was obtained as colorless oil (0.52 g, 1.8 mmol) in an 88% yield. IR ν_{max} (neat): 2954, 2924, 2854, 1256, 1254, 1110, 842, 752 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 4.45 (1H, t, J = 6.5 Hz, H-2), 2.25-2.10 (4H, m, H-5, H-8), 1.94–1.87 (2H, m), 1.69-1.27 (19H, brs, –CH_2–), 0.88 (3H, t, J = 6.5 Hz, –CH_3), 0.21 (9H, s, –Si(C<u>H</u>$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 120.0 (s, C-1), 81.6 (s), 78.8 (s), 61.3 (d), 35.4 (t), 32.0 (t), 29.7 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.1 (t), 24.2 (t), 22.8 (t), 18.9 (t), 18.3 (t), 14.8 (q, CH_3), 0. 24 (q, –Si(<u>C</u>H$_3$)$_3$). GC/MS (70 eV) m/z (relative intensity): 321 (M^+, 3), 306 (M^+–CH_3, 31), 293 (3), 278 (17), 264 (3), 250 (4), 236 (4), 232 (6), 222 (9), 208 (35), 194 (27), 181 (9), 168 (15), 155 (12), 133 (19), 129 (20), 119 (58), 107 (14), 92 (63), 73 ($C_3H_9Si^+$, 100), 67 (30), 55 (26); UPLC-HRMS (positive mode) Calcd for $C_{19}H_{36}NOSi$ $[M + H]^+$ 322.2566, found 322.2566.

*Methyl (±)-2-Hydroxy-6-Hexadecynoate (**18**)*

Into a 100-mL round-bottomed flask were placed 23 mL of MeOH and 9 mL of HCl 12.1 M. To the stirred solution was added 0.50 g (1.6 mmol) of the trimethylsilyl nitrile **17** and the reaction was refluxed at 60–65 °C for 12 h. The solvent was removed *in vacuo*, the reaction mixture was washed with a brine aqueous solution (2 × 10 mL), extracted with diethyl ether (3 × 15 mL), dried over $MgSO_4$, and filtered. The solvent was removed and the crude product was purified via silica gel column chromatography eluting with hexane/ether (9:1) affording the methyl ester **18** (0.4 g, 1.3 mmol), which was obtained as a colorless oil in an 87% yield. IR ν_{max} (neat): 3463 (–OH), 2953, 2923, 2853, 1736 (C=O), 1455, 1437, 1211, 1103, 996 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 4.24–4.18 (1H, m), 3.79 (3H, s, –OCH_3), 2.71 (1H, s), 2.23–2.10 (4H, m, H-5, H-8), 1.97–1.20 (19H, brs, –CH_2–), 0.88 (3H, t, J = 0.88 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 175.8 (s, C-1), 81.1 (s), 79.4 (s), 70.3 (d, C-2), 52.7 (q, –OCH_3), 33.7 (t), 32.0 (t), 29.7 (t), 29.4 (t), 29.32 (t), 29.27 (t), 29.0 (t), 24.6 (t), 22.8 (t), 18.9 (t), 18.6 (t), 14.2 (q, –CH_3). GC/MS (70 eV)

m/z (relative intensity): 282 (M^+, 0.1), 264 (M^+-H_2O, 0.1), 239 (2), 223 (M^+-$C_2H_3O_2$, 21), 205 (M^+-$C_2H_3O_2$ -H_2O, 5), 183 (3), 169 ($C_9H_{13}O_3^+$, 100), 156 (34), 151 (14), 141 (7), 124 (46), 113 (42), 109 (50), 97 (51), 90 ($C_3H_5O_3^+$, 35), 81 (52), 67 (54), 55 (48); UPLC-HRMS (positive mode) Calcd for $C_{17}H_{31}O_3$ $[M + H]^+$ 283.2273, found 283.2292.

*(±)-2-Methoxy-6-Hexadecynoic Acid (**3**)*

Into a 50-mL round-bottomed flask was added 0.09 g (3.8 mmol) of NaH, under argon, to 15 mL of dry THF together with 0.30 g (1.1 mmol) of **18**. After 15 min of stirring the suspension at rt the reaction was cooled to 0 °C and then 0.23 mL (3.7 mmol) of methyl iodide were added. The reaction was stirred for 4 h, and then washed with a sodium bicarbonate ($NaHCO_3$) saturated aqueous solution (1 × 15 mL), as well as with diethyl ether (2 × 15 mL). The aqueous solution was acidified with concentrated HCl, extracted with diethyl ether (3 × 15 mL), dried over $MgSO_4$, and filtered. The solvent was removed and the crude product was purified using silica gel column chromatography eluting **3** with hexane/ether (7:3), which was obtained as colorless oil (0.26 g, 0.9 mmol) in an 87% yield. IR ν_{max} (neat): 3500–2500 (–OH), 2921, 2853, 1719 (C=O), 1457, 1194, 1117, 940, 719 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 3.85–3.81 (1H, dd, J = 4.8 and 7.1 Hz, H-2), 3.44 (3H, s, $–OCH_3$), 2.20–2.10 (4H, m, H-5, H-8), 1.89–1.27 (21H, brs, $–CH_2–$), 0.88 (3H, t, J = 6.7 Hz, $–CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 176.9 (s), 81.2 (s), 79.9 (d), 79.2 (s), 58.4 (q, $–OCH_3$), 32.0 (t), 31.5 (t), 29.6 (t), 29.4 (t), 29.30 (t), 29.28 (t), 29.0 (t), 24.6 (t), 22.8 (t), 18.9 (t), 18.6 (t), 14.2 (q, $–CH_3$). HRMS (TOFMS) Calcd for $C_{17}H_{33}O_3$ $[M + H]^+$ 305.2093, found 305.2052.

Methyl (±)-2-Methoxy-6-Hexadecynoate

GC/MS (70 eV) *m/z* (relative intensity): 296 (M^+, 0.01), 264 (M^+-CH_3OH, 2), 237 (M^+-$C_2H_3O_2$, 56), 205 (23), 184 (10), 167 (5), 152 (24), 137 (25), 135 (34), 129 (30), 121 (52), 111 (55), 104 ($C_4H_8O_3^+$, 86), 93 (89), 81 (95), 79 (100), 77 (30), 71 (67), 67 (81), 55 (53).

*(6Z)-(±)-2-Methoxy-6-Hexadecenoic Acid (**1**)*

Into a 50-mL round-bottomed flask a 5–10% Lindlar catalyst/dry hexane solution was prepared as described for **10** above. To the stirring solution was added 0.60 g (2.1 mmol) of **18**. After 5 min of stirring the suspension, hydrogen was added. The reaction was stirred for 12 h, and then the reaction mixture was filtered and extracted with a 5% HCl aqueous solution (4 × 15 mL), washed with diethyl ether (3 × 15 mL), dried over $MgSO_4$, and filtered. The solvent was removed and the crude product was obtained. Into a 50-mL round-bottomed flask, under argon, was added 0.16 g (6.7 mmol) of sodium hydride (NaH), 15 mL of dry THF and 0.53 g (1.86 mmol) of the 2-hydroxy methyl ester. After 15 min of stirring the suspension it was cooled to 0 °C and then 0.41 mL (6.6 mmol) of methyl iodide was added. After stirring the reaction for 4 h, it was washed with a bicarbonate ($NaHCO_3$) saturated aqueous solution (1 × 15 mL) and with diethyl ether (2 × 15 mL). The aqueous solution was acidified using concentrated HCl and then extracted with diethyl ether (3 × 15 mL). The solvent was removed and the crude product was purified via silica gel column chromatography eluting acid **1** with hexane/ether (7:3). The α-methoxylated acid **1** (0.33 g, 1.2 mmol) was obtained as colorless oil in a 62% yield, with spectral data similar to the one previously reported [9].

(6Z)-6-Octadecenoic and 6-Octadecynoic Acids

The syntheses of these acids followed procedures previously reported [13]. The acetylenic coupling between commercially available 1-tridecyne and the 2-[(5-bromopentyl) oxy]tetrahydro-2*H*-pyran using *n*-BuLi in THF-HMPA at 0 °C followed by the deprotection of the pyranyl group using methanol and PTSA afforded the 6-octadecyn-1-ol in a 75% yield. The alkynol was hydrogenated under Lindlar's conditions, as described above, resulting in the (6*Z*)-6-octadecen-1-ol in a 70% yield. The oxidation of the alkenol with PDC in DMF resulted in the formation of the known (6*Z*)-6-octadecenoic acid in a 45% yield. The overall yield for the four-step synthesis was 24%.

The 6-octadecynoic acid was prepared from the 6-octadecyn-1-ol by means of PDC oxidation in DMF, which resulted in a 75% yield of the acid. The overall yield for the three-step synthesis was 56%. The purity of both acids was confirmed by ^{13}C-NMR spectroscopy.

(±)-2-Methoxyhexadecanoic Acid

Was synthesized from the catalytic hydrogenation of **3** in hexane with H_2 and 10% Pd/C and the purity of the final product was confirmed by ^{13}C-NMR spectroscopy as well as comparison of the MS spectral data with the one previously reported [12].

Antibacterial Activity

Microorganisms

Staphylococcus aureus (ATCC 29213), *Escherichia coli* (ATCC 25922), and Methicillin-resistant *S. aureus* (MRSA,

ATCC 43300) were obtained from the American Type Culture Collection (Manassas, VA). Clinical isolates of MRSA (CIMRSA) were kindly donated by a community hospital in San Juan, Puerto Rico. Stock cultures were kept on blood Trypticase Soy Agar (TSA with 5% sheep blood, Remel and Oxoid Microbiology Products, Lenexa, KS). Subcultures were incubated for 18-24 h on TSA at 37 °C. Suspension cultures were prepared by inoculation of single colonies in 5 mL Trypticase Soy Broth (TSB, BD Diagnostic Systems, Franklin Lakes, NJ). Prior to preparation of susceptibility assays, bacteria cells were re-suspended in TSB (not centrifuged) and visually standardized by using a 0.5 McFarland standard solution, which provided an equivalent concentration of 1.0×10^8 CFU/mL.

Susceptibility Testing

Susceptibility testing was carried out as described by Sanabria-Ríos *et al*. [14]. Flat-bottomed microplate wells were previously inoculated with a 10 µL TSB solution containing 4–5 $\times$ 10^5 colony-forming units (CFU). Into these wells, dissolved in 100% DMSO, the FA were serially diluted with sterile TSB (stock solutions of 1.0×10^5, 1.0×10^4, and 1.0×10^3 µg/mL). For the spectrophotometrical analyses at 620 nm both a positive control well (containing the bacterial inoculated TSB but not the FA solution) and a negative control well (containing only TSB) were used. The minimum inhibitory concentration (MIC) was determined as the concentration at which the FA prevented turbidity in the well after incubation for 18–24 h at 37 °C. Turbidity was determined at 620 nm in a spectrophotometer at 25 °C for those samples with optical densities higher than 0.6–1.0. The IC_{50} values were calculated by plotting dose–response curves [15]. The dose–response curves were analyzed using the biostatistics software GraphPad Prism® (v 5.0, San Diego, CA). The IC_{50} was defined as the concentration in which the FA inhibited 50% of the bacterial growth.

S. aureus DNA Gyrase Inhibitory Tests

Staphylococcus aureus DNA gyrase inhibitory tests were performed as described Sanabria-Ríos *et al*. [15]. Briefly, the enzyme activity of DNA gyrase was assessed using DNA gyrase from *S. aureus* (1 unit will supercoil 0.05 µg of DNA in 60 min at 37 °C) and 0.20 µg of relax pHOT1 plasmid DNA. FA were dissolved in 50% DMSO obtaining a stock solution of 2.5 $\times$ 10^4 µg/mL and tested at different concentrations that ranged from 1.9 to 1000 µg/mL. Reactions (final volume 20 µL) were carried out for 60 min at 37 °C, after which 2 µL of 10% sodium dodecyl sulfate (SDS) was added. Bound protein was digested by incubation with proteinase K (0.05 mg/mL) for 30 min at 37 °C. Reaction was stopped by adding 4 µL of electrophoresis universal stop and loading buffer following electrophoresis in a 1% agarose gel (70 V/105 min). To visualize the reaction products, the gel was stained with ethidium bromide for 25 min and destained for 10–15 min in distilled water. DNA bands were detected and visualized in a Min BIS bioimaging system (model 241016P1, Israel).

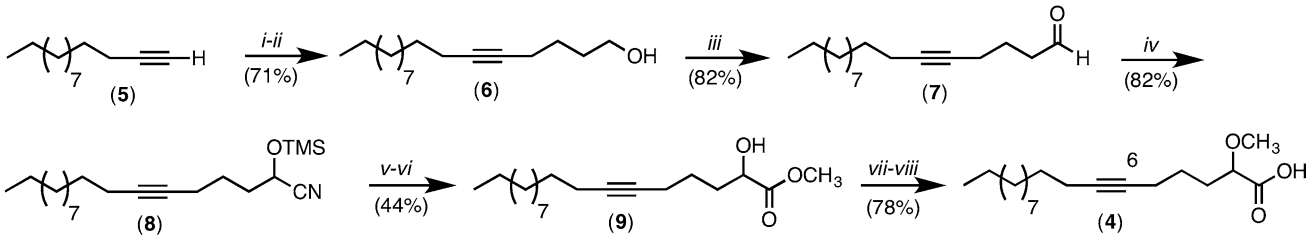
CMC Determination

Determination of CMC was performed as described by Sanabria-Ríos [14]. The FA, in either 95% ethanol or 100% DMSO, depending on the FA solubility, and starting from a stock solution of 1.0×10^5 µg/mL, were serially diluted (0.1–1000 µg/mL) in 1X phosphate-buffered saline (1X PBS, HyClon, Utah, USA) with Rhodamine 6G at a concentration of 2.5×10^{-6} M. The wavelength of maximum absorption (525 nm) for each dilution was determined in a Thermo Scientific Genesys 10S UV–Vis spectrophotometer (Cambridge, United Kingdom) and plotted as a function of FA concentration. The CMC value was described as the point at which the wavelength of maximum absorption first deviated from linearity.

Results

Synthesis of the α-methoxylated FA

The synthesis of the α-methoxylated acids **1**–**4** followed a synthetic procedure previously reported by us for other similar analogs [6]. The (±)-2-methoxy-6-octadecynoic acid (**4**) was prepared starting with the acetylenic coupling of commercially available 1-tridecyne with 2-(4-bromobutoxy)tetrahydro-2*H*-pyran using *n*-Buli in THF-HMPA at 0 °C, which after the deprotection of the pyranyl group using methanol and p-toluenesulfonic acid (PTSA) afforded the desired 5-heptadecyn-1-ol (**6**) in a 71% yield (Scheme 1). Alkynol **6** was then oxidized with Corey's pyridinium chlorochromate (PCC) in dichloromethane (DCM) affording the 5-heptadecynal (**7**) in an 82% yield. Then, aldehyde **7** was reacted with trimethylsilyl cyanide (TMSCN) in DCM and catalytic amounts of triethylamine (Et_3N) at 0 °C resulting in an 82% yield of nitrile **8**. Nitrile **8** was then transformed into the desired methyl ester **9** in two steps. First, the hydrolysis of **8** in 2-methyltetrahydrofuran (2-MeTHF) at 60 °C for 24 h afforded the (±)-2-hydroxy-6-octadecynoic acid, which was esterified in HCl/methanol resulting in the methyl ester **9** in a combined 44% yield for both steps. The methylation of **9** with sodium hydride and methyl iodide in tetrahydrofuran (THF) followed by an acidic workup afforded the (±)-2-methoxy-6-octadecynoic acid (**4**) in a 78% yield.



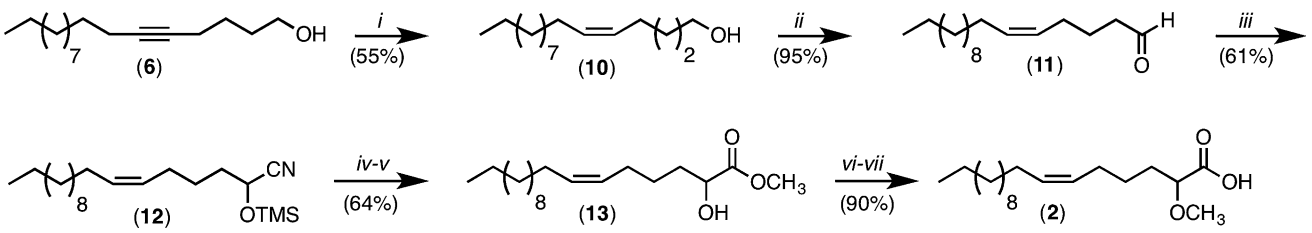
Scheme 1 Synthesis of (±)-2-methoxy-6-octadecynoic acid (**4**). (*i*) 2-(4-bromobutoxy)tetrahydro-2*H*-pyran, *n*-BuLi, THF-HMPA, 0 °C, 24 h; (*ii*) PTSA, MeOH, 35 °C, 12 h; (*iii*) PCC, CH_2Cl_2, 24 h, rt; (*iv*) TMSCN, Et_3N, CH_2Cl_2, 0 °C, 3 h; (*v*) HCl (conc.) 2-MeTHF, 60 °C, 24 h; (*vi*) HCl, MeOH, reflux, 3 h; (*vii*) NaH, THF, CH_3I, 0 °C, 3 h; (*viii*) KOH/MeOH (1.5 M), 60 °C, 2 h

The overall yield for the seven-step synthetic sequence was 16% and **4** is a novel FA.

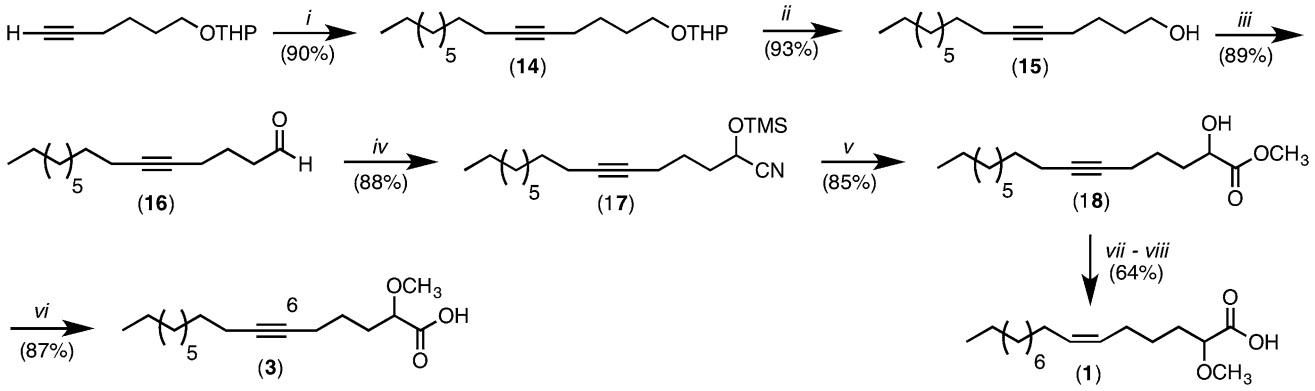
The (6*Z*)-(±)-2-methoxy-6-octadecenoic acid (**2**), a natural product whose synthesis has not been reported before, started from alkynol **6**, which was catalytically hydrogenated under Lindlar's conditions, resulting in a 55% yield of the (*Z*)-5-heptadecen-1-ol (**10**) as shown in Scheme 2. Characterization of the cis alkenol **10** by ^{13}C NMR confirmed the signals of the two sp^2 carbons that resonated at δ 130.4 and 129.2 ppm. The oxidation of the alkenol **10** using Corey's PCC in DCM resulted in a 95% yield of the (*Z*)-5-heptadecenal (**11**). Then aldehyde **11** was reacted with TMSCN in DCM and catalytic amounts of Et_3N at 0 °C that yielded the nitrile **12** in a 61% yield. As with the synthesis of **4** described above, nitrile **12** was transformed into the desired ester **13** in two steps. First, the acid hydrolysis of **12** in 2-MeTHF afforded the (6*Z*)-(±)-2-hydroxy-6-octadecenoic acid, and after esterification using HCl/MeOH the desired ester **13** was obtained in a combined 64% yield for the last two steps. The final acid **2** was obtained after methylation of **13** with sodium hydride and methyl iodide in THF, following an acidic workup, resulting in the (6*Z*)-(±)-2-methoxy-6-octadecenoic acid (**2**) in a 90% yield. The overall yield for this eight-step synthetic sequence (starting from **5**) was 13%, and this is the first reported total synthesis for **2** [12].

For the synthesis of the (6*Z*)-(±)-2-methoxy-6-hexadecenoic acid (**1**) and (±)-2-methoxy-6-hexadecynoic acid (**3**) a different strategy was pursued in terms of hydrogenating the alkyne late in the synthetic sequence when aiming for **1** and thus saving several synthetic steps in between (Scheme 3). The synthesis of **3** started from commercially available 2-(5-hexyn-1-yloxy) tetrahydro-2*H*-pyran, which was coupled with 1-bromononane using *n*-BuLi in THF-DMI at 0 °C and after deprotection of the pyranyl group using methanol and PTSA, the desired 5-pentadecyn-1-ol (**15**) was obtained in a combined 84% yield for these two steps. The oxidation of alkynol **15** using Corey's PCC in DCM resulted in an 89% yield of the 5-pentadecynal (**16**). In an analogous fashion as the two syntheses above, aldehyde **16** was reacted with TMSCN in DCM and catalytic amounts of Et_3N resulting in an 88% yield of the nitrile **17**. Hydrolysis of **17** in HCl/MeOH at 60-65 °C for 12 h afforded the methyl ester **18** in an 85% yield. The methylation of **18** with sodium hydride and methyl iodide in THF followed by the acidic workup afforded the (±)-2-methoxy-6-hexadecynoic acid (**3**) in an 87% yield. The overall yield for this six-step synthetic sequence was 48% and acid **3** is a novel FA.

Our improved synthesis for the (6*Z*)-(±)-2-methoxy-6-hexadecenoic acid (**1**) took advantage of the synthetic route for **3** by starting with the methyl



Scheme 2 Synthesis of (6*Z*)-(±)-2-methoxy-6-octadecenoic acid (**2**). (*i*) H_2, Pd/C (10%), quinoline, hexane, rt; (*ii*) PCC, CH_2Cl_2, 24 h, rt; (*iii*) TMSCN, Et_3N, CH_2Cl_2, 0 °C, 3 h; (*iv*) HCl (conc.) 2-MeTHF, 60 °C, 24 h; (*v*) HCl, MeOH, reflux, 3 h; (*vi*) NaH, THF, CH_3I, 0 °C, 3 h; (*vii*) KOH/MeOH (1.5 M), 60 °C, 2 h



Scheme 3 Synthesis of (±)-2-methoxy-6-hexadecynoic acid (**3**) and the (6*Z*)-(±)-2-methoxy-6-hexadecenoic acid (**1**). (*i*) *n*-BuLi, THF-DMI, 1-bromononane, 0 °C, 24 h; (*ii*) MeOH, PTSA, 65 °C, 12 h; (*iii*) PCC, DCM, 12 h; (*iv*) TMS-CN, TEA, DCM, 0 °C; (*v*) HCl/MeOH, 60 °C, 12 h (*vi*) NaH/THF, MeI, 0 °C, 4 h, then HCl; (*vii*) H_2, Lindlar catalyst, hexane, 12 h; (*viii*) NaH/THF, MeI, 0 °C, 4 h, then HCl

(±)-2-hydroxy-6-hexadecynoate (**18**), which was catalytically hydrogenated under Lindlar's conditions, followed by methylation with sodium hydride/methyl iodide in THF, and after the acidic workup the (6*Z*)-(±)-2-methoxy-6-hexadecenoic acid (**1**) was obtained in a 64% yield for the last two steps. The overall yield for this seven-step synthetic sequence was 36%. The advantage of using this acetylide coupling approach over our previous methodology, which utilized a Wittig approach [9], is that a 100% cis stereochemistry was obtained for the C-6 double bond in **1**, while with the Wittig approach a 10:1 mixture of the *Z*/*E* isomers was obtained [9]. This is important for our aims since there is a considerable difference in the antibacterial activity of *cis* vs. *trans* FA [11]. For example, the *trans* 16:1 Δ9 acid has a considerably higher MIC than the cis 16:1 Δ9 acid towards *S. aureus* [11].

Antibacterial Studies

The antibacterial studies performed herein for the α-methoxylated acids **2** and **4** are presented in Table 1, while the corresponding studies for **1** and **3** are shown in Table 2. Assays were performed against clinical isolates of methicillin-resistant *S. aureus* (CIMRSA) as well as against one strain of *E. coli* (ATCC 25922) following the procedures described in Materials and Methods. As expected from previous studies with the 18:1Δ6 or 18:1Δ9 acids on *S. aureus* RN4220 [11], the 6-octadecenoic and 6-octadecynoic acids were ineffective towards all the studied strains of CIMRSA as well as against *E. coli*. However, the 6-octadecynoic acid did show some activity towards *S. aureus* (ATCC 29213) but with a very high IC_{50} of 274 µg/mL. The α-methoxylated octadecynoic acid **4** was the most bactericidal acid towards clinical isolates of methicillin-resistant

Table 1 Antibacterial activity of the C18 unsaturated FA against multi-drug resistant bacteria

Microorganisms	MIC/IC_{50} SEM, µg/mL Fatty acids			
	(±)-(**4**)	(±)-(**2**)	6-Octadecynoic acid	6-Octadecenoic acid
S. aureus (ATCC 29213)	125/67.7 ± 5.2	>1000	500/273.8 ± 11.2	>1000
E. coli (ATCC 25922)	>1000	>1000	>1000	>1000
ClMRSA I	62.5/32.2 ± 3.5	>1000	>1000	>1000
ClMRSA II	62.5/37.0 ± 1.6	>1000	>1000	>1000
MRSA (ATCC 43300)	31.3/17.7 ± 1.2	>1000	>1000	>1000
ClMRSA IX	31.3/21.3 ± 0.9	>1000	>1000	>1000

Experiments were performed in triplicate ($n = 3$). *E. coli* experiments were done in sextuplicate ($n = 6$)
MRSA methicillin-resistant *S. aureus*, *ClMRSA* clinical isolates of MRSA, *SEM* standard error of the mean

Table 2 Antibacterial activity of the C16 unsaturated FA against multi-drug resistant bacteria

Microorganisms	MIC/IC_{50} SEM, μg/mL Fatty acids			
	(±)-(**3**)	(±)-(**1**)	2-Methoxy-16:0[a]	6-Hexadecynoic acid
S. aureus (ATCC 29213)	62.5/37.9 ± 0.4	62.5/35.4 ± 0.3	250/92.9 ± 4.0	125/68.1 ± 4.5
E. coli (ATCC 25922)	250/140.8 ± 4.0	62.5/21.2 ± 0.6	>1000	>1000
ClMRSA I	250/151.3 ± 1.3	62.5/31.3 ± 0.5	250/155.5 ± 15.2	125/65.1 ± 3.4
ClMRSA II	250/121.5 ± 5.8	125/80.6 ± 4.3	ND	62.5/43.0 ± 0.7
MRSA (ATCC 43300)	62.5/38.2 ± 2.0	62.5/30.0 ± 0.9	250/146.1 ± 0.7	250/160.6 ± 1.7
ClMRSA IX	500/297.6 ± 1.0	125/77.5 ± 1.5	250/100.4 ± 4.3	125/58.0 ± 1.2

Experiments were performed in triplicate ($n = 3$). *E. coli* experiments were done in sextuplicate ($n = 6$)

MRSA methicillin-resistant *S. aureus*, *ClMRSA* clinical isolates of MRSA, *ND* not determined, *SEM* standard error of the mean

[a] Palmitic acid did not display any antibacterial activity against these strains (IC_{50} SEM >1000 μg/mL)

S. aureus (CIMRSA) displaying MIC between 31 and 63 μg/mL and $IC_{50's}$ between 17 and 37 μg/mL. In sharp contrast to the results obtained for **4**, the α-methoxylated octadecenoic acid **2** was not effective at all against all the studied strains, similar to what others have observed for the 6-octadecenoic acid against *S. aureus* [11] (Table 1). Therefore, among the studied C18 FA, acid **4** stands out as the best candidate against CIMRSA.

The antibacterial studies for the C16 α-methoxylated acids **1** and **3**, displayed in Table 2, presented a rather different scenario. Most of the studied acids displayed some sort of activity towards CIMRSA with MIC between 63 and 250 μg/mL and $IC_{50's}$ between 38 and 298 μg/mL. In this series both **1** and **3** were toxic to CIMRSA, but against CIMRSA I and IX the α-methoxylated hexadecenoic acid **1** displayed a 4-fold better toxicity profile than its hexadecynoic analog **3**. What was more surprising here is that both **1** and **3** also displayed toxicity towards *E. coli*, with MIC of 63 and 250 μg/mL, respectively, and $IC_{50's}$ of 21 and 141 μg/mL, respectively, with acid **1** displaying a 4-sevenfold better toxicity profile than **3** towards *E. coli*. Therefore, among the studied FA in the C16 series, acid **1** stands out as the best candidate against CIMRSA and *E. coli*. However, between the C16 acid **1** and the C18 acid **4**, the latter was more effective towards CIMRSA, while **1** was the most bactericidal towards *E. coli*. Important is the fact that both **1** and **4** contained the α-methoxy functionality in addition to a C-6 unsaturation in the acyl chain.

Critical Micelle Concentration (CMC) Studies

The question as to why the α-methoxylated acids **1** and **4** are more effective towards the studied cell lines prompted us to first study the critical micelle concentrations (CMC) of the α-methoxylated acids. Previous studies relate the biological activity of acids to their CMC as was demonstrated for the lipoteichoic acids [16] and the 2-alkynoic FA [14]. We have also shown that α-methoxylation lowers the CMC of the 6-icosynoic acid from 500 μM to 20–30 μM [6]. However, with just one single study, there is no clear general picture as to what extend α-methoxylation affects the CMC of a FA, and how this is related to the FA chain length [6]. Therefore, the CMC of the acids **1**–**4** were determined as previously described in a solution of rhodamine 6G and 1X PBS buffer and the obtained values are shown in Table 3. As expected from their longer chain lengths, the C18 acids **2** and **4** displayed a five-fold lower CMC (15–20 μg/mL) than the C16 analogs **1** and **3** (70–100 μg/mL). These results indicate that **4** mainly exerts its antibacterial activity in a micellar state. When the CMC value of acid **4** was compared to the CMC of the 6-octadecynoic acid (20–30 μg/mL), it was observed that it was slightly lower than the CMC of the 6-octadecynoic analog, but not as dramatic

Table 3 Critical micelle concentration (CMC) of the 2-methoxylated FA

	CMC (μg/mL)
Fatty acids	
(±)-(**4**)	15–18
(±)-(**2**)	15–20
6-Octadecynoic acid	20–30
(±)-(**3**)	90–100
(±)-(**1**)	70–90
2-Methoxy-16:0	10–50

The CMC determinations were done in triplicate ($n = 3$)

a difference as the one obtained for the C20 series previously reported by us [6]. In any instance, it is evident that α-methoxylation decreases the CMC of the parent FA, at least in the C_{18} and C_{20} series.

S. aureus DNA Gyrase Inhibitory Studies

Given that the *S. aureus* DNA gyrase is the target of several antibacterial agents, such as the quinolone-based antibacterials [17], it was of interest to study if the α-methoxylated FA **1** and **3** were inhibitory towards this topoisomerase II enzyme. Our results show that both **1** and **3** were not inhibitory towards the *S. aureus* DNA gyrase at concentrations as high as 1000 μg/mL (Fig. 1). Therefore, the mechanism of toxicity of these α-methoxylated FA towards *S. aureus* is different from the mechanisms displayed by the more classic antibacterials such as the quinolones or fluoroquinolones [17].

Discussion

FA can exert their antibacterial activities by a myriad of mechanisms that might include, among others, disruption of the electron transport chain [18], interference with oxidative phosphorylation [19], cell lysis with the concomitant leakage of cell metabolites [20], formation of peroxidation or auto-oxidation products [21], and inhibition of FA biosynthesis by inhibiting the FabI enzyme [22]. However, in the case of *S. aureus* RN4220 it was reported that the C16 FA are toxic by a rapid membrane depolarization mechanism coupled to the disruption of macromolecular biosynthesis as well as the release of solutes and low-molecular weight proteins from the cells [11]. We found herein that the α-methoxylated acid **4** was the most effective compound among those tested against CIMRSA. It is evident that the combination of α-methoxylation and a C-6 triple bond in the C18 structure augmented the antibacterial effect as evidenced by the lack of toxicity of **2**, where the only difference between **2** and **4** is a double bond versus a triple bond. The observed behavior for acid **2** is similar to what is known for an 18:1Δ6 FA, which is basically nontoxic to *S. aureus* RN4220 since it is an acid that is readily used for phospholipid biosynthesis given the fact that it is easily recognized by the biosynthetic machinery of *S. aureus* [11]. Acid **4** seems to be sufficiently foreign to the bacterium that it is not readily recognized by the *S. aureus* acyltransferase system [11]. It is very likely for **4** to work by disruption of the cytoplasmic membrane thereby releasing solutes and low-molecular weight proteins from the cells in a sort of a lysis mechanism of action. We should also mention here that oleic acid has been reported to be marginally toxic (MIC = 0.4 mM) towards other strains of *S. aureus*, such as *S. aureus* 285 or *S. aureus* 503, presumably by a mechanism involving inhibition of the enoyl-acyl carrier protein reductase (FabI), which is present in *S. aureus* and is essential for the bacterial FA biosynthetic machinery [22]. However, our bacterial strains were susceptible towards the studied FA in a similar fashion as it was reported for *S. aureus* RN4220 [11]. It is also important to mention that acid **4** had a low CMC at 15–18 μg/mL, while its antibacterial activity towards CIMRSA displayed $IC_{50's}$ between 17 and 37 μg/mL (Table 3). This implies that **4** might be interacting with the *S. aureus* membrane in a micellar state and α-methoxylation is helping in achieving lower CMCs since the corresponding nonmethoxylated analog has a slightly higher CMC (Table 3). This interaction with the bacterial membrane can result in an increase of the membrane fluidity resulting in a distortion of the respiratory chain and alteration of several membrane processes [23]. Since acid **4** is also expected to be more acidic than 6-octadecynoic acid (pKa 3.6 vs. pKa 4.7), it is also very likely that **4** might be acting like a better protonophore once inside the cell. This can only lead to the inability of the cell to control its internal pH that can ultimately result in the disruption of many enzymatic processes within *S. aureus* [23].

In the case of the tested C16 methoxylated acids, the α-methoxylated hexadecenoic acid **1** displayed the best overall antimicrobial profile. Presumably, acid **1** is also

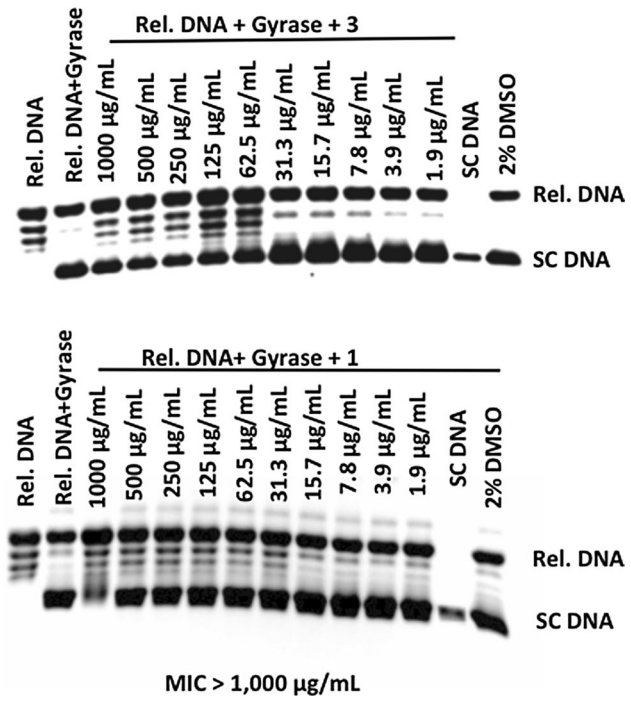


Fig. 1 Inhibitory effect of **1** and **3** on the supercoiling activity of *S. aureus* DNA gyrase. *Rel. DNA* relaxed DNA, *SC DNA* supercoiled DNA

poorly metabolized by *S. aureus*, i.e., it is a poor substrate for phospholipid biosynthesis and it accumulates for a longer time in the medium, similar to the 16:1Δ6 acid, since FA are normally not degraded by this bacterium [11]. Probably the unexpected result for **1** and **3** was their ability to be bactericidal towards *E. coli* (ATCC 25922), while almost all of the studied FA, including the 6-hexadecynoic acid, were not active at all towards this Gram-negative bacterium. The combination of an unsaturation at C-6 and the α-methoxylation was important for the observed activity since the acid 2-methoxy-16:0, which contains the α-methoxylation but no double bond, was not effective towards *E. coli*. Apparently, acids **1** and **3** can find their way more easily into the *E. coli* membrane, i.e., they have the right functional groups (α-methoxylation and C-6 unsaturation) so as to traverse the outer membrane more efficiently via a FadL porin or a similar transport system and once inside the cell create havoc [24]. Palmitic acid has a slightly better FA binding affinity than oleic acid towards FadL, while the affinity of myristic acid is nearly an order of magnitude less towards FadL [25]. The α-methoxylated acids **1** and **3** might interact more strongly with the amino acids (such as arginine and lysine) known to be present at the outside of the hydrophobic binding pockets of FadL which are important for binding long-chain FA to the protein [26]. It is also interesting to point out that **1** (the best of the two α-methoxylated acids against *E. coli*) displayed a high CMC of 70–90 μg/mL, while its antibacterial activity towards *E. coli* displayed an IC_{50} of 21 μg/mL (Table 3). In contrast to **4**, acid **1** is clearly interacting with the *E. coli* membrane in a monomeric state. In any instance, acid **1** could be used in conjunction with known antibiotics by serving as a debilitating agent that can disrupt the outer membrane of *E. coli*, thus allowing the antibiotic to definitely kill the Gram-negative bacteria.

This work identified **1** and **4** as viable candidates against *E. coli* and *S. aureus*, but other analogs will have to be synthesized coupled to further mechanistic work that could define the utility and scope of the α-methoxylated FA in fighting Gram-positive and Gram-negative bacteria. Such a work continues in our laboratories.

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