Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemistry and Physics of Lipids 124 (2003) 63–67
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

The first total synthesis of the marine fatty acid (±)-9-methoxypentadecanoic acid: a synthetic route towards mid-chain methoxylated fatty acids

Néstor M. Carballeira*, Carlos Miranda

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, San Juan 00931-3346, Puerto Rico

Received 21 February 2003; accepted 12 March 2003

Abstract

The marine fatty acid (±)-9-methoxypentadecanoic acid was synthesized for the first time in seven steps (7.8% overall yield) starting from commercially available 9-decen-1-ol. The key step in the synthesis was the coupling of pentylmagnesium bromide with 1-benzyloxy-9,10-epoxydecane under 1,5-cyclooctadiene copper (I) chloride catalysis. Nuclear magnetic resonance data are provided for the first time for this type of methoxylated fatty acids and the synthetic approach utilized is of general applicability since it can be used in the synthesis of other mid-chain methoxylated fatty acids. This synthetic methodology should afford sufficient quantities of these fatty acids for biological evaluation. The spectral data obtained for the title compound will also be helpful in subsequent characterizations of other mid-chain methoxylated fatty acids using nuclear magnetic resonance spectroscopy.

Keywords: Algae; Fatty acids; 9-Methoxypentadecanoic acid; Synthesis

1. Introduction

Saturated mid-chain methoxylated fatty acids are rare in nature, but several interesting examples have been identified in bacteria and marine organisms. For example, the acid-producing bacterium *Thiobacillus* produces the fatty acids 10-methoxyoctadecanoic acid, 11-methoxyoctadecanoic acid, 12-methoxyeicosanoic acid, and 13-methoxyeicosanoic acid (Kerger et al., 1986). Some recent findings also reported the fatty acids 11-methoxyheptadecanoic acid and 11-methoxynonadecanoic acid in the cholesterol esters and triglycerides of the bacillus *Helicobacter pylori* (Inamoto et al., 1995). On the other hand, the four mid-chain methoxylated fatty acids 9-methoxypentadecanoic acid, 9-methoxyheptadecanoic acid, 13-methoxyheneicosanoic acid, and 15-methoxytricosanoic acid were identified in the red alga *Schizymenia dubyi* collected in Sicily, Italy (Barnathan et al., 1998). However, in all of these cases the absolute stereochemistry at the methoxylated carbon was never determined and since these compounds were only identified by gas chromatography-mass spectrometry, nuclear magnetic resonance spectral data for all of these fatty acids is still lacking in the lipid literature. In addition, there are no reported syntheses for these saturated mid-chain methoxylated fatty acids, but a couple of monounsaturated mid-chain methoxylated

* Corresponding author. Tel.: +1-787-764-0000x4791; fax: +1-787-756-8242.
E-mail address: ncarball@upracd.upr.clu.edu (N.M. Carballeira).

0009-3084/03/$ – see front matter
doi:10.1016/S0009-3084(03)00043-4

fatty acids have been synthesized, in particular those derived from *Lyngbya majuscula* (Müller et al., 1995; Mesguiche et al., 1999). Therefore, a general and practical synthetic methodology towards saturated mid-chain methoxylated fatty acids is needed if we are to fully characterize these fatty acids and explore their biological potential. In the present communication we report the first total synthesis of racemic 9-methoxypentadecanoic acid, since the absolute stereochemistry at the asymmetric carbon in the natural fatty acid is not known. This was achieved by a seven-step synthetic methodology that could very well be used in the synthesis of most of the known saturated mid-chain methoxylated fatty acids.

2. Materials and methods

2.1. Instrumentation

^{1}H NMR (300 and 500 MHz) and ^{13}C NMR (75 and 125 MHz) were either recorded on a Bruker DPX-300 spectrometer or a Bruker DRX-500 spectrometer. ^{1}H NMR chemical shifts are reported with respect to internal $(CH_3)_4Si$, ^{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 mm × 0.25 mm special performance capillary column (HP-5MS) of polymethyl siloxane cross-linked with 5% phenyl methylpolysiloxane. IR spectra were recorded on a Nicolet 600 FT-IR spectrophotometer.

2.2. 1-Benzyloxy-9-decene

A solution of 9-decen-1-ol (0.5 g, 3.2 mmol) in dry tetrahydrofuran (THF), was added dropwise with stirring to a suspension of sodium hydride (0.23 g, 9.6 mmol) in 2 ml of THF at room temperature under nitrogen. Then, 0.6 ml of benzylchloride (4.15 mmol) was added dropwise and the resulting mixture was heated under reflux for 16 h. Subsequently, 10 ml of water was added, the solvent was evaporated and the mixture was extracted with diethyl ether (2 × 80 ml). The extracts were dried (Na_2SO_4), and the solvent was evaporated. The resulting residue was purified by distillation obtaining 0.65 g (83% yield) of the previously reported 1-benzyloxy-9-decene (Prugh et al., 1986). IR (neat) ν 3063, 3028, 2926, 2851, 1641, 1493, 1453, 1360, 1100, 909, 732, 695 cm^{-1}; ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 139.1 (d, C-2), 138.8 (s), 128.3 (d), 127.6 (d), 127.5 (d), 114.2 (t, C-1), 72.9 (t), 70.5 (t), 33.9 (t, C-3), 29.8 (t), 29.5 (t), 29.1 (t), 29.0 (t), 26.2 (t), 25.6 (t); GC-MS *m/z* (relative intensity) M^+ 246 (1), 161 (1), 155 (1), 147 (1), 137 (2), 118 (2), 108 (6), 107 (22), 106 (2), 105 (3), 104 (4), 95 (14), 92 (35), 91 (100), 83 (4), 81 (12), 79 (6), 77 (3), 69 (8), 67 (7), 65 (8), 55 (14).

2.3. 1-Benzyloxy-9,10-epoxydecane

Magnesium monoperoxyphthalate (MMPP) (6.12 g, 15.9 mmol) was added to a solution of 1-benzyloxy-9-decene (0.65 g, 2.65 mmol) dissolved in ethanol. The reaction mixture was stirred at room temperature for 48 h. A mixture of 120 ml of hexane and water (1:1) was added and the organic phase was separated, dried and evaporated. The residue was purified by silica gel chromatography using hexane–diethyl ether (8:2) as eluent, which afforded 0.51 g (73% yield) of 1-benzyloxy-9,10-epoxydecane. IR (neat) ν 3032, 2924, 2858, 1495, 1452, 1359, 1256, 1203, 1104, 911, 830, 735 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.24 (5H, m, C_6H_5), 4.50 (2H, s, CH_2-Ph), 3.46 (2H, t, J = 6.6 Hz, H-1), 2.90 (1H, m, H-9), 2.74 (1H, A part ABX system, H-10), 2.46 (1H, B part ABX system, J = 2.75 and 5.01 Hz, H-10), 1.61–1.26 (14H, m, CH_2); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 138.6 (s), 128.3 (d), 127.6 (d), 127.4 (d), 72.8 (t), 70.4 (t), 52.4 (d, C-2), 47.1 (t, C-1), 32.4 (t, C-3), 29.7 (t), 29.4 (t), 29.3 (t), 26.1 (t, C-8), 25.9 (t, C-4); GC-MS *m/z* (relative intensity) M^+ 262 (M^+, 0.1), 245 (0.1), 161 (0.1), 153 (1), 135 (1), 133 (1), 108 (10), 107 (53), 105 (5), 104 (6), 97 (2), 95 (5), 92 (24), 91 (100), 81 (10), 79 (9), 67 (10), 55 (13).

2.4. 1-Benzyloxy-9-pentadecanol

A solution of 0.3 ml (0.35 g, 2.34 mmol) of 1-bromopentane in 2 ml of THF was added dropwise under nitrogen to a suspension of 0.057 g (2.34 mmol) of magnesium turnings in 0.5 ml of refluxing THF. After completion of the addition the Grignard reagent was cooled to −20 °C and 0.51 g (1.95 mmol) of 1-benzyloxy-9,10-epoxydecane and

20 mg of 1,5-cyclooctadienecopper (I) chloride was added. After stirring the reaction mixture for 16 h at room temperature 3 ml of 1 M HCl was added and the THF was evaporated in vacuo. The residue was extracted with diethyl ether (2 × 80 ml) and the combined extracts were washed with 80 ml of 2 M $KHCO_3$, dried (Na_2SO_4), and the solvent evaporated. The resulting residue was chromatographed on silica gel (hexane–diethyl ether, 8:2) yielding 0.56 g (87% yield) of 1-benzyloxy-9-pentadecanol. IR (neat) ν 3450 (OH), 3024, 2921, 2845, 1453, 1365, 1273, 1104, 729 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.33–7.24 (5H, m, C_6H_5), 4.49 (2H, s, CH_2-Ph), 3.56 (1H, m, H-9), 3.45 (2H, t, $J = 6.6$ Hz, H-1), 1.60 (4H, m, H-8, H-10), 1.42–1.29 (20H, m, CH_2), 0.89 (3H, t, $J = 6.8$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 138.6 (s), 128.2 (d), 127.5 (d), 127.4 (d), 72.8 (t, CH_2OPh), 71.8 (d, C-9), 70.4 (t, C-1), 37.40 (t), 37.37 (t), 31.8 (t), 29.66 (t), 29.57 (t), 29.48 (t), 29.34 (t), 29.31 (t), 26.1 (t), 25.5 (t), 22.5 (t), 14.0 (q, –CH_3). GC-MS *m/z* (relative intensity) M^+ 334 (0.1), 316 (5), 249 (2), 225 (3), 207 (1), 188 (1), 161 (1), 157 (2), 151 (1), 137 (1), 125 (2), 111 (3), 107 (25), 105 (3), 104 (5), 97 (9), 95 (5), 92 (18), 91 (100), 83 (5), 81 (4), 79 (4), 69 (8), 65 (4), 57 (6), 55 (18).

2.5. 1-Benzyloxy-9-methoxypentadecane

Into a two-necked round-bottom flask and under a nitrogen atmosphere, was placed 0.56 g (1.68 mmol) of 1-benzyloxy-9-pentadecanol in 3 ml of dimethyl sulfoxide (DMSO). Separately, two equivalents of NaH (0.080 g, 3.33 mmol) were dissolved in 1 ml of DMSO and added dropwise to the reaction mixture followed by stirring at room temperature for 1 h. An excess of CH_3I was then added, and the reaction mixture was further stirred for 2 h. After this, the mixture was diluted with 30 ml of hexane–diethyl ether (1:1) and washed with H_2O (2 × 30 ml) to remove the DMSO. The organic phase was dried over Na_2SO_4, filtered, and evaporated in vacuo, affording 0.51 g (87% yield) of 1-benzyloxy-9-methoxypentadecane. IR (neat) ν 3024, 2927, 2839, 1437, 1278, 1114, 749 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.25 (5H, m, C_6H_5), 4.50 (2H, s, CH_2-Ph), 3.46 (2H, t, $J = 6.6$ Hz, H-1), 3.31 (3H, s, –OCH_3), 3.11 (1H, m, H-9), 1.73 (2H, m, H-2), 1.61 (4H, m, H-8, H-10), 1.35–1.22 (18H, m, CH_2), 0.88 (3H, t, $J = 6.8$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 138.7 (s), 128.3 (d), 127.6 (d), 127.4 (d), 81.0 (d, C-9), 72.8 (t, CH_2OPh), 70.5 (t, C-1), 56.3 (q, OCH_3), 34.6 (t), 33.4 (t), 29.8 (t), 29.7 (t), 29.5 (t), 29.42 (t), 29.39 (t), 26.9 (t), 26.2 (t), 25.2 (t), 22.63 (t), 22.62 (t), 14.1 (q, –CH_3). GC-MS *m/z* (relative intensity) M^+-32 316 (5), 263 (8), 225 (1), 207 (1), 171 (3), 157 (3), 142 (3), 130 (4), 129 (41), 117 (7), 108 (3), 107 (12), 97 (30), 92 (13), 91 (100), 83 (4), 79 (5), 71 (14), 67 (5), 55 (28).

2.6. 9-Methoxypentadecan-1-ol

A solution of 0.51 g (1.46 mmol) of 1-benzyloxy-9-methoxypentadecane in 3 ml of methanol and 0.1 ml of acetic acid was stirred with 50 mg of Pd/C (10%) under H_2 (1 bar) for 12 h. The reaction mixture was filtered through Celite®, the solvent removed in vacuo, and the resulting residue was chromatographed on silica gel (hexane–ether, 8:2) resulting in 0.18 g (47% yield) of 9-methoxypentadecan-1-ol. IR (neat) ν 3383 (OH), 2931, 2861, 1460, 1375, 1281, 1196, 1097, 719 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.63 (2H, t, $J = 6.7$ Hz, H-1), 3.31 (3H, s, –OCH_3), 3.11 (1H, m, H-9), 1.65 (1H, brs, –O*H*), 1.55 (2H, m, H-2), 1.43 (4H, m, H-8, H-10), 1.39–1.22 (18H, m, CH_2), 0.89 (3H, t, $J = 6.9$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 81.0 (d, C-9), 63.1 (t, C-1), 56.3 (q, –OCH_3), 33.5 (t), 33.4 (t), 32.8 (t), 32.1 (t), 29.8 (t), 29.5 (t), 29.3 (t), 25.7 (t), 25.31 (t), 25.26 (t), 24.9 (t), 22.6 (t), 14.0 (q, –CH_3). GC-MS *m/z* (relative intensity) M^+-1 257 (0.1), 243 (1), 208 (1), 174 (6), 173 (52), 141 (26), 130 (9), 129 (100), 123 (8), 109 (2), 99 (3), 97 (50), 95 (8), 85 (6), 83 (5), 81 (34), 71 (45), 69 (17), 67 (21), 58 (9), 55 (60).

2.7. 9-Methoxypentadecanal

To a stirred solution of 9-methoxypentadecan-1-ol (0.18 g, 0.68 mmol) in 10 ml of CH_2Cl_2 was slowly added pyridinium chlorochromate (0.42 g, 1.98 mmol) at room temperature. After 12 h, the reaction mixture was filtered through Florisil and washed with diethyl ether (3 × 15 ml). Evaporation of the solvent afforded 0.15 g (90% yield) of 9-methoxypentadecanal. IR (neat) ν 2929, 2854, 1735, 1458, 1376, 1290, 1115, 1099, 948 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.7

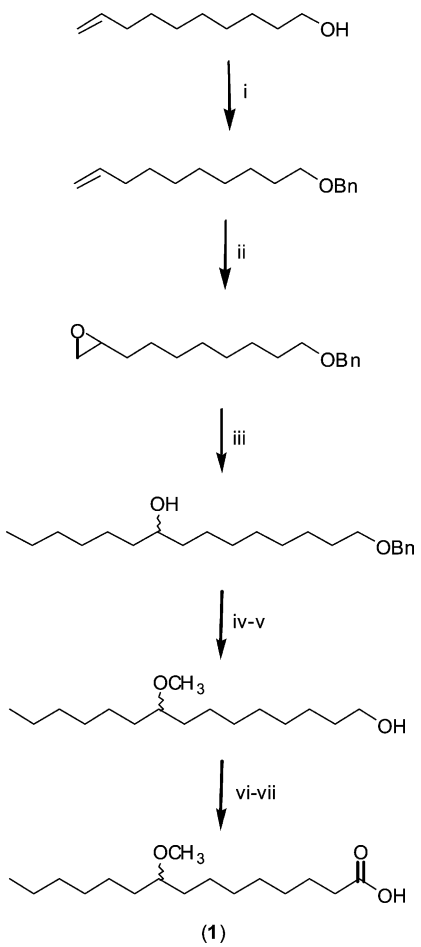
(1H, brt, $J = 1.7$ Hz, C*H*O), 3.30 (3H, s, –OCH_3), 3.10 (1H, m, H-9), 2.40 (2H, dt, $J = 7.4$ and 1.7 Hz, H-2), 1.61 (2H, m, H-3), 1.42 (4H, m, H-8, H-10), 1.35–1.25 (16H, m, CH_2), 0.86 (3H, t, $J = 6.6$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 200.6 (s, C-1), 81.0 (d, C-9), 56.3 (q, –OCH_3), 33.5 (t), 33.4 (t), 32.8 (t), 32.1 (t), 29.8 (t), 29.6 (t), 29.3 (t), 25.7 (t), 25.31 (t), 25.26 (t), 25.0 (t), 22.6 (t), 14.0 (q, –CH_3). GC-MS *m*/*z* (relative intensity) M^+-1 255 (0.1), 224 (1), 172 (10), 171 (90), 139 (8), 130 (8), 129 (100), 121 (16), 111 (3), 98 (6), 97 (61), 95 (21), 93 (11), 85 (3), 83 (6), 81 (10), 79 (9), 71 (24), 69 (26), 67 (16), 57 (11), 55 (65).

2.8. 9-Methoxypentadecanoic acid

To a solution of 9-methoxypentadecanal (0.15 g, 0.61 mmol) in *t*-butyl alcohol was added a solution of $NaClO_2$ (0.22 g, 2.5 mmol) and NaH_2PO_4 (0.35 g, 3 mmol) as buffer in 3 ml of water over a period of 10 min. The reaction mixture was stirred at room temperature for 12 h. The mixture was diluted with water and extracted with diethyl ether (3×20 ml). The combined extracts were dried over Na_2SO_4 and concentrated. The resulting crude product was purified by phase transfer with an aqueous solution of $NaHCO_3$ and ether. Acidification of the aqueous phase and extraction with diethyl ether afforded 0.66 g (40% yield) of the previously reported 9-methoxypentadecanoic acid (Barnathan et al., 1998). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.31 (3H, s, –OCH_3), 3.12 (1H, m, H-9), 2.35 (2H, t, $J = 7.5$ Hz, H-2), 1.63 (2H, m, H-3), 1.43 (4H, m, H-8, H-10), 1.40–1.25 (16H, m, CH_2), 0.89 (3H, t, $J = 6.6$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 177.0 (s, C-1), 81.0 (d, C-9), 56.3 (q, –O*C*H_3), 33.5 (t), 33.4 (t), 32.8 (t), 32.1 (t), 29.8 (t), 29.6 (t), 29.2 (t), 25.7 (t), 25.31 (t), 25.26 (t), 24.9 (t), 22.7 (t), 14.1 (q, –*C*H_3). GC-MS *m*/*z* (relative intensity) M^+-1 271 (0.1), 240 (1), 188 (6), 187 (53), 169 (9), 155 (62), 137 (14), 130 (9), 129 (100), 119 (5), 113 (2), 109 (16), 97 (54), 95 (18), 93 (4), 71 (35), 69 (19), 67 (14), 60 (12), 57 (7), 55 (66).

3. Results and discussion

The synthesis of (±)-9-methoxypentadecanoic acid (**1**) was based on the opening of 1-benzyloxy-9,10-epoxydecane with pentyl magnesium bromide to generate the mid-chain hydroxylated 1-benzyloxy-9-pentadecanol (Scheme 1). This approach seems to be general, inasmuch as it can be used to generate other biosynthetically related mid-chain methoxylated fatty acids, such as the 11-methoxyheptadecanoic acid (Inamoto et al., 1995) by just chaining the terminal epoxide and Grignard reagent chain-lengths. Therefore, our synthesis started with 9-decen-1-ol, which was converted to the already reported 1-benzyloxy-9-decene through the reaction of the alcohol with benzyl chloride. The double bond was effectively epoxidized



Scheme 1. Synthesis of racemic 9-methoxypentadecanoic acid. (i) NaH, THF, BzCl; (ii) MMPP, EtOH, 48 h; (iii) C_5H_{11}MgBr, THF, 1,5-cyclooctadienecopper (I) chloride, −20 °C; (iv) NaH, CH_3I, DMSO, 2 h; (v) Pd/C (10%), H_2, CH_3OH-CH_3CO_2H; (vi) PCC, CH_2Cl_2, 12 h; and (vii) $NaClO_2$, NaH_2PO_4, H_2O, 12 h.

with MMPP in ethanol in a 73% yield. MMPP turned out to be more efficient than the classical *m*-chloroperoxybenzoic acid (*m*-CPBA) in epoxidizing these long chain alkenes, since the latter reagent only afforded moderate to low yields at long reaction times. In the next step towards the total synthesis of (**1**) the 1-benzyloxy-9,10-epoxydecane was opened with 1-pentylmagnesium bromide assisted by catalytic amounts of 1,5-cyclooctadiene copper (I) chloride (Cook et al., 1969; Küchler et al., 1991), which afforded the desired 1-benzyloxy-9-pentadecanol in an 87% yield. The use of the catalyst turned out to be essential since in the absence of the copper catalyst no coupling was observed. The 1-benzyloxy-9-pentadecanol was readily methylated with NaH and CH_3I in DMSO, which afforded in an 87% yield, the 1-benzyloxy-9-methoxypentadecane. Deprotection of the primary alcohol was achieved using hydrogen and catalytic amounts of 10% Pd/C for 12 h resulting in a 47% yield of 9-methoxypentadecan-1-ol (Scheme 1). Final oxidation to the acid was accomplished by first oxidizing the alcohol to the aldehyde with pyridinium chlorochromate (90% yield) and then oxidizing the aldehyde to the desired 9-methoxypentadecanoic acid (**1**) with $NaClO_2$ in *t*-butyl alcohol and NaH_2PO_4 as buffer. The overall yield for the seven steps of this synthesis was 7.8%.

The most significant absorption in the NMR spectrum of the (±)-9-methoxypentadecanoic acid (**1**) was observed for the carbons and hydrogens bearing the methoxy functionality. For example, the methoxy protons resonated at δ 3.31 ppm and the methoxy carbon was observed at δ 56.3 ppm, while the methine hydrogen resonated at δ 3.12 ppm and the methine carbon at δ 81.0 ppm. These ^{1}H and ^{13}C NMR displacements seem to be characteristic for saturated mid-chain methoxylated fatty acids and useful as a future reference for other similar analogs.

In summary, a general and practical synthetic methodology towards saturated mid-chain fatty acids is reported, the total synthesis of the marine fatty acid (±)-9-methoxypentadecanoic acid (**1**) was achieved in a 7.8% overall yield, and nuclear magnetic resonance spectral data for (**1**) is reported for the first time in the literature.

Acknowledgements

This work was supported by the SCORE program of the National Institutes of Health (NIH) under Grant No. SO6GM08102. C. M. thanks the Puerto Rico EPSCoR (NSF) program for a doctoral fellowship. The technical assistance of Marlene Cotto, an undergraduate student sponsored by the UPR NIH-RISE program, is appreciated.

References

Barnathan, G., Bourgougnon, N., Kornprobst, J.-M., 1998. Methoxy fatty acids isolated from the red alga, *Schizymenia dubyi*. Phytochemistry 47, 761–765.

Cook, B.W., Miller, R.G.J., Todd, P.F., 1969. A new route to olefin complexes of copper (I) compounds. J. Organometal. Chem. 19, 421–430.

Inamoto, Y., Hamanaka, S., Hamanaka, Y., Nagate, T., Kondo, I., Takemoto, T., Okita, K., 1995. Lipid composition and fatty acid analysis of *Helicobacter pylori*. J. Gastroenterol. 30, 315–318.

Kerger, B.D., Nichols, P.D., Antworth, C.P., Sand, W., Bock, E., Cox, J.J., Langworthy, T.A., White, D.C., 1986. FEMS Microbiol. Ecol. 38, 67.

Küchler, B., Voβ, G., Gerlach, H., 1991. Synthese der markierungssubstanz der kirschenfruchtfliege Rhagoletis cerassi L. Liebigs Ann. Chem. Pp. 545–552.

Mesguiche, V., Valls, R., Piovetti, L., Peiffer, G., 1999. Characterization and synthesis of (−)-7-methoxydodec-4(*E*)-enoic acid, a novel fatty acid isolated from *Lyngbya majuscula*. Tetrahedron Lett. 40, 7473–7476.

Müller, C., Voss, G., Gerlach, H., 1995. Synthesis of (4*E*,7*S*)-(−)-7-methoxy-4-tetradecenoic acid, a major constituent of the marine cyanophyte *Lyngbya majuscula*. Liebigs Ann. Chem. 673–676.

Prugh, J.D., Rooney, C.S., Deana, A.A., Ramjit, H.G., 1986. Synthesis and utilization of the chiral synthon methyl 3-*O*-benzyl-2,4,6-trideoxy-6-iodo-α-D-erythrohexopyranoside in the synthesis of a potent HMG-CoA reductase inhibitor. J. Org. Chem. 51, 648–657.