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A novel synthesis of 3(*R*)-HETE, 3(*R*)-HTDE and enzymatic synthesis of 3(*R*),15(*S*)-DiHETE

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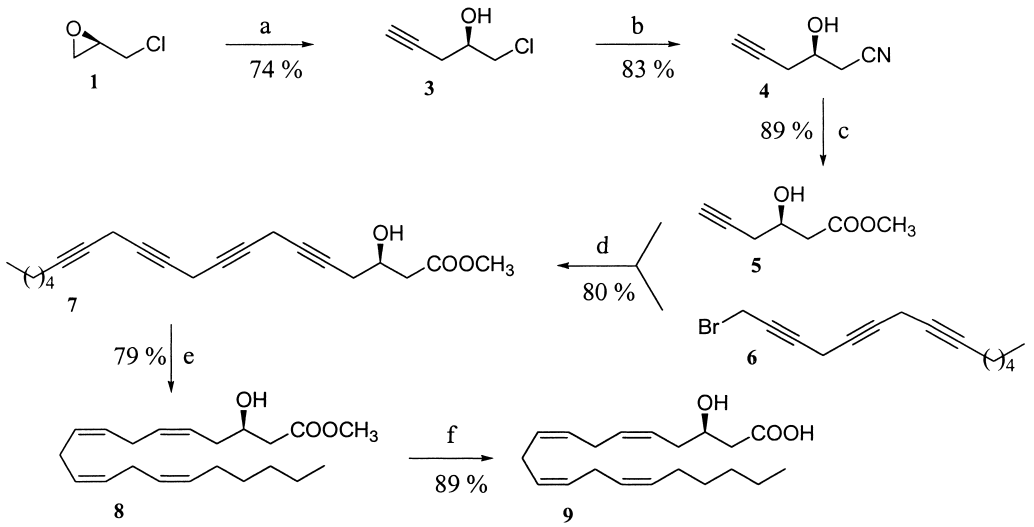
Abstract—3(*R*)-HETE and 3(*R*)-HTDE were prepared by cross-coupling of methyl 3(*R*)-hydroxyhex-5-ynoate either with 1-bromo-2,5,8-tetradecatriyne or 1-bromo-2-octyne followed by catalytic hydrogenation of the skipped triple bonds formed using Lindlar's catalyst. Enzymatic synthesis of 3(*R*),15(*S*)-DiHETE was accomplished by soybean LOX-1 using 3(*R*)-HETE as a substrate.

1. Introduction

While the oxygenated derivatives of arachidonic acid (AA) and related compounds produced in mammalian cells have been extensively studied during last 25 years, a little attention was paid to the respective compounds of fungal origin. 3(*R*)-Hydroxy fatty acid derivatives of both AA and the shorter chain fatty acids, a novel group of biologically active collectively termed 3(*R*)-hydroxy-oxylipins, were identified in some fungal species (*Dipodascopsis uninucleata* and *Mucor genevensis*)[1–3] when exogenous AA or linoleic acid were utilized as substrates. Recently, 3(*R*)-hydroxy-oxylipins have been shown to be potent microbial growth factors[4,5] and to play a crucial role in the morphogenesis of *Candida albicans*.[4] Inhibitors of 3(*R*)-hydroxy-oxylipins have been thus proposed as efficient therapeutic drugs against vulvovaginal candidosis.[6] Moreover, 3-HETE was found as a strong proinflammatory lipid



Scheme 1. (a) TMSC≡CH (**2**), *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78°C, 40 min; NH_4F, DMF/H_2O, rt; (b) NaCN, 18-crown-6, CH_3CN, rt, 4 h; (c) NaOH, 3 h, 100°C then CH_2N_2; (d) CuI, NaI, K_2CO_3, DMF, rt; (e) H_2/Lindlar's catalyst, quinoline, benzene, 10°C; (f) LiOH, MeOH, rt, 8 h.

Keywords: 3-hydroxy-oxylipins; DiHETE; total synthesis; coupling reactions; lipoxygenases.
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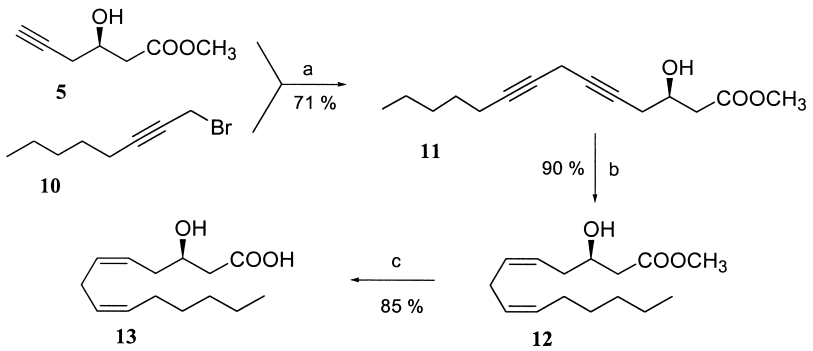


Scheme 2. (a) CuI, NaI, K_2CO_3, DMF, rt; (b) H_2/Lindlar's catalyst, quinoline, benzene, 10°C; (c) LiOH, MeOH, rt, 8 h.

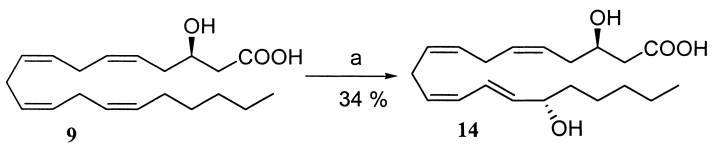
mediator, which modulates several human neutrophil functions, such as chemotaxis, phagocytosis, as well as secretion of IL-8 and leukotriene B_4.[7] Despite the progress in the research of 3(*R*)-hydroxy-oxylipins, a number of open issues on their role in intracellular signal transduction pathways still need to be clarified. Recovery of 3-hydroxyoxylipins by biotechnological methods from fungi is low, cumbersome and time consuming. For understanding their extensive biological role in mammalian cells, efficient synthetic methods are inevitably required. In the present study we describe novel methods for the synthesis of 3(*R*)-HETE, 3(*R*)-HTDE and 3(*R*),15(*S*)-DiHETE, which are simple and effective.

2. Results and discussion

Although chemical synthesis of 3(*R*)-HETE based on 5,6-epoxy-AA modification[8] has been reported previously, it turned out to be inappropriate both for preparation of tritiated analogues and for synthesis of fatty acids with shorter chain lengths. We therefore developed a new convergent procedure based on polyacetylenic approach for syntheses of (*R*,5*Z*,8*Z*,11*Z*,14*Z*)-3-hydroxyeicosa-5,8,11,14-tetraenoic acid (3(*R*)-HETE) (**9**) and (*R*,5*Z*,8*Z*)-3-hydroxytetradeca-5,8-dienoic acid (3(*R*)-HTDE) (**13**). Our synthetic strategy involves polyacetylenic precursors (Schemes 1 and 2), which could be prepared by cross-coupling of methyl 3(*R*)-hydroxyhex-5-ynoate (**5**) and bromides **6**, **10**, respectively. *R*-(−)-epichlorohydrin (**1**) was chosen as a starting material (Scheme 1). Oxirane ring opening using ethynyltrimethylsilyl borane as described by Yamaguchi and Hirao[9] followed by acetylene deprotection resulted in chloroalkynol **3**[10] with 74% yield. Substitution of chloride to cyano group using NaCN in the presence of 18-crown-6 in CH_3CN afforded organic cyanide **4** (83%). Saponification of the nitrile **4** by 15% aqueous solution of NaOH (3 h, 95–100°C) and the subsequent methylation of the acid formed yielded **5**. Methyl 3(*R*)-HETE (**8**) (Scheme 1) and 3(*R*)-HTDE (**12**) (Scheme 2) were synthesized by cross-coupling either of 1-bromo-2,5,8-tetradecatriyne (**6**) or of 1-bromo-2-octyne[11] (**10**) with **5** followed by catalytic hydrogenation of polyacetylenic esters formed on Lindlar's catalyst with quinoline in benzene. 1-Bromo-2,5,8-tetradecatriyne[12] (**6**) was prepared by cross-coupling of 1-heptyne with 7-bromo-2,5-heptadiyn-1-ol.[13] The corresponding free acids **9**, **13** as shown in Schemes 1 and 2 were prepared by saponification of respective methyl esters **8**, **12** using LiOH in MeOH–H_2O.

As mentioned above, the current need for preparation of adequate amounts of 3(*R*)-hydroxy-oxylipins requires convenient methods for their synthesis. Among several methods available for the synthesis of 3(*R*),15(*S*)-DiHETE (**14**) with respect to stereo selectivity of the products, the enzymatic method appears to be the most suitable one (Scheme 3). Thus, soybean 15-LOX is known to oxygenate AA with a high stereo specificity (nearly exclusive formation of the product with *S*-configuration) to 15-HETE.[14] On the other hand, 3-HETE contains a *cis*-skipped tetraenoic system of AA rendering it to be an appropriate substrate for 15-LOX.

According to our protocol, 3-HETE was incubated with an aliquot of 15-LOX, and formation of conjugated dienes was monitored on UV-spectrophotometer. After standard work-up procedure the crude product mixture was purified successively by reverse phase (RP-) and normal phase (SP-) HPLC. Both RP- and SP-HPLC exhibited only conjugated diene formed (chromatograms are not shown, see Section 3). Chiral phase HPLC of corresponding methyl ester of **14** on a Chiral Pack AD column using hexane/EtOH (10%) as solvent system revealed a solitary peak with an elution time of 31.36 min. The optical purity was found to be more than 96%. The structure of the product was confirmed by GC–MS analysis of **14** as TMS-derivative. Thereby, prominent ions with *m*/*z* 175 [$^+$CH(OTMS)CH_2COOCH_3], 173 [$^+$CH(OTMS)$(CH_2)_4CH_3$] as well as molecular ion *m*/*z* 479 [M^+-15] were detected.



Scheme 3. (a) 15-LOX, $NaBH_4$.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded either on Brucker MSL 200 or Brucker MSL 300 spectrophotometer using $CDCl_3$ as a solvent. Chemical shifts are referenced to internal standard tetramethylsilane for ^{1}H NMR and to deuterium lock signal of $CDCl_3$ (δ ^{13}C=77.19 ppm) for ^{13}C NMR. IR spectra were recorded on Shimadzu IR-435. Optical rotation was measured by polarimeter DIP-360 (JASCO). HPLC analyses were carried out with the help of Shimadzu LC-10Avp liquid chromatograph connected to a SPD-10Advp UV-Detector. Analytical HPLC was performed on a Nucleosil C18-column; 250×4 mm, 5 μm particle size (Machery-Nagel, Düren, Germany) using: MeOH/H_2O/AcOH (85:15:0.1, by vol.) as solvent system at a flow rate of 1 mL/min. Preparative HPLC was carried out on a Lichrospher 100 RP18-column; 250×22.5 mm, 10 μm particle size (Knauer, Berlin, Germany) with MeOH/H_2O/AcOH (90:10, by vol.) as solvent system at a flow rate of 10 mL/min. Chiral HPLC analysis was carried out on Chiralpack AD column, 250×4.6 mm, (Dalcel Chemical Industries), using either hexane/MeOH (98:2, by vol.) or hexane/EtOH (9:1, by vol.) as solvent system at a flow rate of 1 mL/min. EIMS analysis was performed on a Shimadzu GC–MS QP-2000 system employing an ion source temperature of 180°C and electron energy of 70 eV. FAB-MS spectra were reordered on ZAB-HSQ VG Analytics mass-spectrometer (USA). Flash column chromatography was carried out on Silica Gel 60; particle size ranging from 70 to 230 mesh (Merck, Darmstadt, Germany). Thin-layer chromatography performed on Silica Gel 60 F_{254} sheets (Merck, Darmstadt, Germany). THF was freshly distilled from sodium/benzophenone ketyl. All other solvents and reagents used were of extra pure grade and purchased from Merck, Aldrich or Across (Germany). *n*-Butyllithium (Merck) was titrated as described by Watson and Eastman.[15] Prior to use all glassware and syringes were dried overnight at 140°C. All reactions were carried out under dry argon atmosphere.

3.1.1. (*R*)-1-Chloropent-4-yn-2-ol (3). Into previously dried argon filled round bottom flask containing ethynyltrimethylsilane **2** (990 mg, 10 mmol) and THF (20 mL) was placed a solution of *n*-BuLi in hexane (6.25 mL, 1.6 M) at −78°C. After the reaction mixture was stirred for 10 min, a solution of boron trifluoride diethyl etherate (1.18 mL, 10 mmol) was added, and the resulting mixture was further stirred for 10 min at −78°C. Finally a solution of *R*-(−)-epichlorohydrin (**1**) (376 mg, 4.06 mmol) in THF (5 mL) was added, and the mixture was stirred for another 40 min at −78°C. The mixture was quenched with sat. aq. NH_4Cl (100 mL), and the products were extracted with Et_2O (2×70 mL). Combined ethereal extracts were dried over Na_2SO_4 and concentrated under reduced pressure. To 775 mg of the raw product with R_f=0.37 (Et_2O/hexane, 1:1) dissolved in DMF (40 mL), a solution of NH_4F (592 mg, 16 mmol) in H_2O (7 mL) was added. The so obtained homogenous mixture was kept stirring overnight at rt. Subsequently, H_2O (25 mL) was added and organic products were extracted with EtOAc (3×50 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on Silica Gel (Et_2O/hexane, 1:1) gave 356 mg of **3** in 74% yield. TLC: $R_{f=}$0.26 (Et_2O/hexane, 1:1). $[\alpha]_D^{21}$=−16.93 (*c* 3.0, acetone). IR (neat)/cm^{-1}: ν=3600–3300 (OH), 3280 (C≡CH), 770 (Cl), 635 (C≡CH). ^{1}H NMR (200 MHz, $CDCl_3$): δ=4.01 (m, 1H, 2-CH), 3.67 (m, 2H, 1-CH_2), 2.55 (m, 2H, 3-CH_2), 2.04 (t, 1H, *J*=1.8 Hz, 6-CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=79.45, 71.52, 69.86, 48.25, 24.51. Anal calcd for C_5H_7ClO: C, 50.65; H, 7.95. Found C, 50.38; H, 8.17.

3.1.2. (*R*)-1-Cyanopent-5-yn-3-ol (4). A solution of chloride **3** (245 mg, 2.07 mmol) in dry acetonitrile (10 mL) was added to a suspension of NaCN (325 mg, 6.63 mmol) in acetonitrile (10 mL). After adding 18-crown-6 (190 mg, 0.5 mmol) the resulting mixture was stirred for 4 h under reflux. The reaction was quenched with H_2O (100 mL), the products were extracted with Et_2O (3×50 mL), washed with sat. aq. NaCl (2×60 mL) and dried over Na_2SO_4. Purification was accomplished by flash chromatography on Silica Gel (Et_2O/hexane, 5:2), yielding 187 mg of **4** (83%). TLC: R_f=0.33 (Et_2O/hexane, 5:1). $[\alpha]_D^{21}$=−55.33 (*c* 0.3, acetone). IR (neat)/cm^{-1}: ν=3600–3200 (OH), 3280 (C≡CH), 2210 (C≡N), 635 (C≡CH). ^{1}H NMR (200 MHz, $CDCl_3$): δ=4.20 (m, 1H, 3-CH), 2.61 (m, 2H, 4-CH_2), 2.42 (m, 2H, 2-CH_2), 2.05 (t, 1H, *J*=1.8 Hz, 6-CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=118.62, 74.15, 73.30, 67.87, 28.23, 26.27. Anal calcd for C_6H_7NO: C, 66.04; H, 6.47. Found C, 65.95; H, 6.57.

3.1.3. Methyl (*R*)-3-hydroxyhex-5-ynoate (5). In a round bottom flask equipped with magnetic stirrer were placed **4** (118 mg, 1.08 mmol) and a solution of 15% NaOH (15 mL). The mixture was heated for 3 h at 95–100°C, and then acidified with H_2SO_4 (1 M) to pH 4.0. The organic products were extracted by EtOAc (5×40 mL), washed with sat. aq. NaCl (150 mL) and dried over Na_2SO_4. Following evaporation of EtOAc the residue was dissolved in Et_2O (20 mL) and subjected to bubbling of the excess of diazomethane (5 mmol) in argon stream. Et_2O was evaporated under reduced pressure and the product was purified by flash chromatography on Silica Gel (Et_2O/hexane, 5:2) giving rise to 136 mg of **5** (89%). TLC: R_f=0.42 (Et_2O/hexane, 5:1). $[\alpha]_D^{21}$=−18.44 (*c* 0.9, acetone). IR (neat)/cm^{-1}: ν=3600–3300 (OH), 3280 (C≡CH), 1740 (C=O), 635 (C≡CH). ^{1}H NMR (200 MHz, $CDCl_3$): δ=4.75 (m, 1H, 3-CH), 3.71 (s, 3H, OCH_3), 2.60 (m, 2H, 2-CH_2), 2.45 (m, 2H, 4-CH_2), 2.05 (t, 1H, *J*=1.8 Hz, 6-CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=172.77, 80.12, 71.29, 69.70, 51.92, 40.16, 26.60. Anal calcd for $C_7H_{10}O_3$: C, 59.14; H, 7.09. Found C, 58.98; H, 7.12.

3.1.4. Methyl (*R*)-3-hydroxyeicosa-5,8,11,14-tetraynoate (7). In a dry argon filled round bottom flask equipped with magnetic stirrer were suspended anhydrous K_2CO_3 (114 mg, 0.83 mmol), NaI (138 mg, 0.92 mmol) and CuI (131 mg, 0.69 mmol) in DMF (5 mL). After adding the solutions of methyl (*R*)-3-hydroxyhex-5-ynoate (**5**) (65 mg, 0.46 mmol) and the bromide **6** (146 mg, 0.55 mmol) each in DMF (2 mL), the resulting suspension was vigorously stirred overnight at rt. The mixture was then quenched with sat. aq. NH_4Cl (100 mL) and the lipophilic products were extracted with Et_2O (4×100 mL). Combined ethereal

extracts were washed with sat. aq. NH_4Cl (1×150 mL) and NaCl (2×150 mL) successively, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography on Silica Gel (Et_2O/hexane, 1:1) giving the pure **7** with an yield of 120 mg (80%). TLC: R_f=0.30 (Et_2O/hexane, 3:1). IR (neat)/cm^{-1}: ν=2240 (C≡C), 1740 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=4.79 (m, 1H, 3-CH), 3.65 (s, 3H, OCH_3), 3.19 (m, 6H, 7-, 10-, 13-CH_2) 2.45 (m, 2H, 2-CH_2), 2.22 (m, 2H, 4-CH_2), 2.14 (m, 2H, 16-CH_2), 1.25–1.45 (m, 6H, 17-, 18- and 19-CH_2), 0.89 (t, 3H, J=6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ=170.04, 81.72, 81.17, 79.77, 76.36, 75.57, 74.81, 73.88, 73.38, 68.98, 51.95, 40.34, 31.29, 28.65, 26.99, 22.40, 18.91, 14.10, 10.02 (3C). Anal calcd for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03. Found C, 77.48; H, 7.86.

3.1.5. Methyl (*R*,5*Z*,8*Z*,11*Z*,14*Z*)-3-hydroxyeicosa-5,8,11,14-tetraenoate (8). A suspension of Lindlar's catalyst (148 mg) in dry benzene (10 mL) was saturated with H_2 at rt and then cooled to 10°C. The ester **7** (84.8 mg, 0.26 mmol) in benzene (20 mL) and quinoline (0.15 mL) were added to the catalyst suspension under a stream of argon. The argon was subsequently exchanged with H_2 and the reaction mixture was stirred for 1 h at 10°C. H_2 uptake was measured with a gas burette. After the H_2 absorption was complete, the mixture was filtered and the filtrate was washed with HCl (2 M, 3×50 mL). Evaporation of the solvent yielded a crude residue, which was purified by a preparative RP-HPLC (MeOH/H_2O, 9:1) to give rise to 68.6 mg (79%) of pure **8**. TLC: R_f=0.46 (Et_2O/hexane, 3:1). RP-HPLC: RT=13.29 min (MeOH/H_2O/AcOH, 85:15:0.1). CP-HPLC: RT=9.34 min (hexane/MeOH, 98:2)). $[\alpha]_D^{20}$=−1.8 (*c* 0.67, acetone). ^1H NMR (200 MHz, $CDCl_3$): δ=5.21–5.41 (m, 8H, CH=CH), 3.98 (m, 1H, 3-CH), 3.65 (s, 3H, OCH_3), 2.75 (m, 6H, 7-, 10-, 13-CH_2), 2.42 (m, 2H, 2-CH_2), 2.21 (m, 2H, 4-CH_2), 1.92 (m, 2H, 16-CH_2), 1.15–1.25 (m, 6H, 17-, 18- and 19-CH_2), 0.85 (t, 3H, J=6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ=173.21, 131.24, 130.65, 128.81, 128.67 (2C), 127.93, 127.71, 125.06, 68.06, 51.74, 40.75, 34.72, 31.67, 29.47, 27.37, 25.93 (3C), 22.70, 14.14. EIMS *m*/*z*: 334 [M^+]. Anal calcd for $C_{21}H_{34}O_3$: C, 75.41; H, 10.25. Found C, 75.55; H, 9.97.

3.1.6. (*R*,5*Z*,8*Z*,11*Z*,14*Z*)-3-Hydroxyeicosa-5,8,11,14-tetraenoic acid (9). An aq. solution of LiOH (0.3 M, 7 mL) was added under argon to a solution of ester **8** (60.4 mg, 0.18 mmol) in MeOH (15 mL). The mixture was stirred for 8 h at rt, MeOH was removed by evaporation, and the pH was adjusted carefully to 5.0 using HCl (1 M). Lipophilic products were then extracted with Et_2O (3×40 mL), the ethereal extracts dried over Na_2SO_4 and concentrated under reduced pressure. The products were purified by flash chromatography (Et_2O/hexane, 1:3) to yield 51.2 mg (89%) of **9**. RP-HPLC: RT=10.24 min MeOH/H_2O/AcOH (85:15:0.1, by vol.). TLC: R_f=0.35 (Et_2O/hexane, 5:1 with 1% AcOH). $[\alpha]_D^{21}$=−1.8 (*c* 0.9, MeOH). ^1H NMR (200 MHz, $CDCl_3$): δ=5.20–5.40 (m, 8H, CH=CH), 3.95 (m, 1H, 3-CH), 2.74 (m, 6H, 7-, 10-, 13-CH_2), 2.42 (m, 2H, 2-CH_2), 2.21 (m, 2H, 4-CH_2), 1.96 (m, 2H, 16-CH_2), 1.15–1.25 (m, 6H, 17-, 18- and 19-CH_2), 0.85 (t, 3H, J=6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ=177.21, 131.10, 130.24, 128.80, 128.21 (2C), 127.63, 127.12, 125.10, 69.12, 40.70, 34.41, 31.40, 29.45, 27.30, 25.90 (3C), 22.60, 14.10. EIMS *m*/*z*: 320 [M^+], 302 [M^+-H_2O].

3.1.7. Methyl (*R*)-3-hydroxytetradeca-5,8-diynoate (11). Diynoate **11** was prepared by cross-coupling of methyl (*R*)-3-hydroxyhex-5-ynoate (**5**) (40 mg, 0.28 mmol) and the bromide **10** (56.7 mg, 0.30 mmol) as described for tetraynoate **7** affording pure **11** (50 mg, 71%). TLC: R_f=0.39 (Et_2O/hexane, 3:1)). $[\alpha]_D^{20}$=−26.3 (*c* 0.86, benzene). IR (neat)/cm^{-1}: ν=2240 (C≡C),1740 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=4.79 (m, 1H, 3-CH), 3.65 (s, 3H, OCH_3), 3.03 (m, 2H, 7-CH_2), 2.53 (m, 2H, 2-CH_2), 2.34 (m, 2H, 4-CH_2), 2.06 (m, 2H, 10-CH_2), 1.19–1.37 (m, 6H, 11-, 12- and 13-CH_2), 0.80 (t, 3H, J=6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ=172.86, 80.09, 78.01, 75.78 (2C), 67.01, 51.80, 40.28, 31.18, 28.54, 26.98, 22.26, 19.58, 18.78, 15.47. Anal calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found C, 72.01; H, 8.95.

3.1.8. Methyl (*R*,5*Z*,8*Z*)-3-hydroxytetradeca-5,8-dienoate (12). Compound **12** was prepared entirely analogous to the preparation of tetraenoate **8** from diynoate **11** (45 mg, 0.18 mmol) as described above. Yield of pure **12** was 41 mg (90%). TLC: R_f=0.44 (Et_2O/hexane, 3:1). RP-HPLC: RT=5.33 min (MeOH/H_2O/AcOH, 85:15:0.1). CP-HPLC: RT=9.27 min. $[\alpha]_D^{20}$=−2.5 (*c* 1.34, acetone).^1H NMR (300 MHz, $CDCl_3$): δ=5.25–5.51 (m, 4H, CH=CH), 4.02 (m, 1H, 3-CH), 3.67 (s, 3H, OCH_3), 2.81 (s, 1H, OH), 2.75 (t, 2H, J=7.10 Hz, 7-CH_2) 2.50 (dd, 1H, J=3.65, 16.27 Hz, 2-CH_2) and 2.41 (dd, 1H, J=8.70, 16.36 Hz, 2-CH_2), 2.27 (m, 2H, 4-CH_2), 2.01 (m, 2H, 16-CH_2), 1.18–1.35 (m, 6H, 17-, 18- and 19-CH_2), 0.85 (t, 3H, J=6.8 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ=173.38, 131.69, 130.83, 127.29, 124.62, 67.99, 51.88, 40.58, 34.49, 31.63, 29.41, 27.37, 25.90, 22.69, 14.19. FAB MS *m*/*z*: 255 [M+H]$^+$.

3.1.9. (*R*,5*Z*,8*Z*)-3-Hydroxytetradeca-5,8-dienoic acid (13). Free acid **13** was prepared by entirely analogous procedure as described for **9** from methyl (*R*,5*Z*,8*Z*)-3-hydroxytetradeca-5,8-dienoate (**12**) (35 mg, 0.14 mmol). Yield of pure **13** was 28 mg (85%). TLC: R_f=0.27 (Et_2O/hexane, 5:1 with 1% AcOH). Analytical RP-HPLC: RT=4.27 min. $[\alpha]_D^{20}$=−1.7 (*c* 1.0, acetone). ^1H NMR (300 MHz, $CDCl_3$): δ=5.25–5.50 (m, 4H, CH=CH), 4.01 (m, 1H, 3-CH), 2.76 (t, 2H, J=7.10 Hz, 7-CH_2), 2.55 (dd, 1H, J=3.65, 16.27 Hz, 2-CH_2) and 2.41 (dd, 1H, J=8.70, 16.36 Hz, 2-CH_2), 2.27 (m, 2H, 4-CH_2), 2.01 (m, 2H, 16-CH_2), 1.20–1.35 (m, 6H, 17-, 18- and 19-CH_2), 0.85 (t, 3H, J=6.8 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ=177.74, 132.07, 130.96, 127.21, 124.33, 67.99, 40.62, 34.44, 31.66, 29.43, 27.40, 25.93, 22.72, 14.22. FAB MS *m*/*z*: 263 [M+Na]$^+$.

3.1.10. (3*R*,15*S*,5*Z*,8*Z*,11*Z*,13*E*)-3,15-Dihydroxyeicosa-5,8,11,13-tetraenoic acid (14). An ethanolic solution of the fatty acid **9** (600 μL, 50 mM) was added to sodium borate buffer pH 9.0 (200 mL, 0.1 M). After the mixture was sonicated for 30 s to achieve homogeneous substrate dispersion, pure soybean 15-LOX-1 (2 mg) was added. After incubation for 25 min at rt hydroperoxy fatty acids formed were reduced by the addition of a saturated ethanolic solution of $NaBH_4$ (1 mL). The mixture was acidified to pH 3.0 with acetic acid (0.1 M), and the lipophilic products were extracted with EtOAc (3×40 mL). The organic

extracts were combined and the solvent was evaporated. The so obtained product was purified by RP-HPLC using the solvent system MeOH/H_2O/AcOH (85:15:0.1, by vol.). Yield of **14** was 3.42 mg (34%). RP-HPLC: RT=4.53 min MeOH/H_2O/AcOH (85:15:0.1, by vol.). UV: λ_{max}=234 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.56 (m, 1H, 13-CH), 5.97 (m, 1H, 14-CH), 5.69 (dd, 1H, J=15.21, 6.06 Hz, 12-CH), 5.55 (m, 1H, 11-CH), 5.35–5.45 (m, 4H, 5, 6, 8 and 9-CH), 4.21 (m, 1H, 15-CH), 4.03 (m, 1H, 3-CH) 2.84 (m, 4H, 7- and 10-CH_2), 2.59 (dd, 1H, J=4.12, 16.37 Hz, 2-CH_2) and 2.46 (dd, 1H, J=7.48, 16.40 Hz, 2-CH_2), 2.31 (m, 2H, 4-CH_2), 1.52 (m, 2H, 16-CH_2), 1.35 (m, 6H, 17-, 18-, and 19-CH_2), 0.87 (t, 3H, J=6.81 Hz, CH_3). FAB MS *m/z*: 335 $[M-H]^-$.

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