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A Simple Method for the Preparation of (5*Z*,8*Z*,11*Z*,14*Z*)-16-Hydroxyeicosa-5,8,11,14-tetraenoic Acid Enantiomers and the Corresponding 14,15-Dehydro Analogues: Role of the 16-Hydroxy Group for the Lipoxygenase Reaction

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Abstract—(5*Z*,8*Z*,11*Z*,13*E*)-15-Hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) is not well oxygenated by arachidonate 15-lipoxygenases because of two structural reasons: (i) it contains a hydrophilic OH-group in close proximity to its methyl end and (ii) it lacks the bisallylic methylene at C_{13}. We synthesized racemic (5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxy-5,8,11,14-eicosatetraenoic acid (16-HETE) which still contains the bisallylic C_{13}, separated the enantiomers reaching an optical purity of >99% and tested them as substrates for 5- and 15-lipoxygenases. Our synthetic pathway, which is based on stereospecific hydrogenation of a polyacetylenic precursor, yielded substantial amounts (30%) of 14,15-dehydro-16-HETE in addition to 16-HETE. When 16-HETE was tested as lipoxygenase substrate, we found that it is well oxygenated by the soybean 15-lipoxygenase and by the recombinant human 5-lipoxygenase. Analysis of the reaction products suggested an arachidonic acid-like alignment at the active site of the two enzymes. In contrast, the product pattern of 16-HETE methyl ester oxygenation by the soybean lipoxygenase (5-lipoxygenation) may be explained by an inverse head to tail substrate orientation.

Introduction

Eicosanoids, such as prostaglandins[1,2] and leukotrienes,[2] are lipophilic mediators that have been implicated in a variety of physiological and pathophysiological processes. They are biosynthesized via cyclooxygenase (COX) or lipoxygenase (LOX) pathways and exhibit interesting biological activities. Originally, it was believed that lipoxygenases accept only naturally occurring free polyenoic fatty acids, but later it was shown that these enzymes exhibit a broader substrate specificity.[3–6] For instance, (15*S*,5*Z*,8*Z*,11*Z*,13*E*)-15-hydroperoxy-5,8,11,13-eicosatetraenoic acid [15(*S*)-HPETE] is oxygenated by the soybean lipoxygenase-1 to a mixture of (5*S*,15*S*,6*E*,8*Z*,11*Z*,13*E*)-5,15-dihydroperoxy-6,8,11,13-eicosatetraenoic acid [5(*S*),15(*S*)-DiHPETE] and (8*S*,15*S*,5*Z*,9*E*,11*Z*,13*E*)-8,15-dihydroperoxy-5,9,11,13-eicosatetraenoic acid [8(*S*),15(*S*)-DiHPETE] and this reaction was supposed to involve an inverse head to tail substrate orientation.[4] Compared with arachidonic acid, 15(*S*)-HPETE has an impaired binding affinity (lower K_M), and the oxygenation rate was lower by one order of magnitude.[4,6] Similar data have been reported for the oxygenation of 15(*S*)-HETE by the rabbit reticulocyte 15-LOX.[6] As mechanistic reasons for these unfavorable reaction kinetics two structural parameters have been discussed: (i) presence of a hydrophilic OH-group that may disturb the hydrophobic enzyme–substrate interaction, and (ii) lack of the n-8 bisallylic methylene that may sterically hinder initial hydrogen abstraction. In order to test, which of these structural features is more important we synthesized rac-(5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxyeicosa-5,8,11,14-tetraenoic acid (16-HETE), separated the enantiomers by chiral phase HPLC and assayed these compounds as substrate for the soybean 15-LOX and the recombinant

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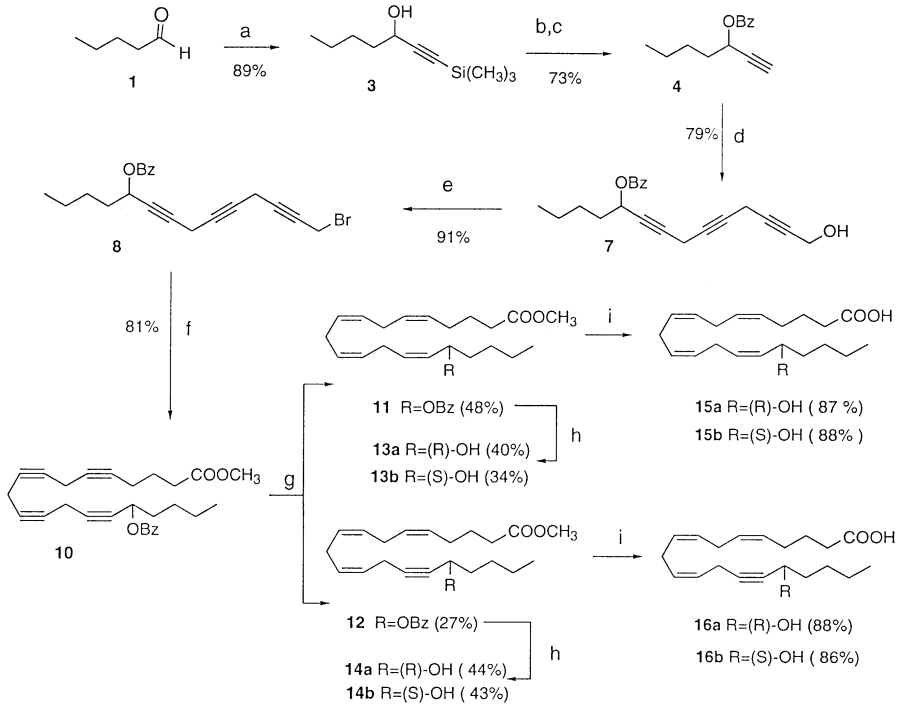
human 5-LOX. In biological systems 16(*R*)-HETE is formed from arachidonic acid via cytochrome P-450 monooxygenation.[7,8] In in vitro assays this metabolite selectively inhibits human polymorphonuclear leukocyte adherence and aggregation as well as leukotriene biosynthesis.[7] Here, we found that the soybean 15-LOX oxygenated both 16-HETE enantiomers with similar kinetics and introduced dioxygen at C-15. These data suggest that, in contrast to 15-HETE, 16-HETE may not be inversely aligned at the active site. Thus, the lack of the n-8 bisallylic methylene appears to be more important for the positional specificity than the presence of the hydrophilic OH-group.

Results and Discussion

Synthesis of rac-16-HETE and enantiomer separation

16(*R*)-HETE is naturally occurring arachidonic acid metabolite of the cytochrome P-450 pathway,[7,8] which constitutes a valuable tool for mechanistic studies on eicosanoid biosynthesis. To carry out such mechanistic investigations large amounts of 16(*R*)-HETE and its enantiomer are required, which can hardly be prepared from biological sources. Thus, a protocol for the enantiospecific synthesis of 16-HETE isomers has been worked out.[9] Unfortunately, this multi-step synthetic procedure did not exhibit an absolute enantioselectivity. We developed an alternative strategy for the preparation of 16(*S*)- and 16(*R*)-HETE, which is based on the synthesis of the racemic precursor and subsequent enantiomer separation by chiral phase HPLC. From Scheme 1, it can be seen that the tetraacetylenic precursor **10** can easily be prepared by cross-coupling of commercially available methyl 5-hexynoate (**9**), the bisfunctional C_7–C_{13} fragment—7-bromo-2,5-heptadiyne-1-ol (**6**) and a substituted alkynol (**4**). In detail, our synthesis procedure comprises the following steps: commercially available pentanal (**1**) was alkylated with lithium ethynyltrimethylsilane. Protection of the hydroxyl group and removal of the trimethylsilyl substituent afforded 3-benzoyloxy-1-heptyne (**4**). 7-bromo-2,5-heptadiyne-1-ol (**6**) was prepared from the corresponding 2,5-heptadiyne-1,7-diol (**5**).[10] Cross-coupling of **4** with bromoalcohol **6** under copper(I) catalysis[11] afforded skipped triacetylenic alcohol **7** in 79% yield. Subsequent displacement of hydroxyl group with bromine using PPh_3/CBr_4 as reactant followed by final cross-coupling of bromide **8** with methyl 5-hexynoate (**9**) resulted in methyl rac-16-(benzoyloxy)eicosa-5,8,11,14-tetraynoate (**10**). Stereospecific hydrogenation of the skipped-triple bonds of **10** with Lindlar's catalyst and quinoline in benzene, which was supposed to yield the skipped diene compound only, resulted in a mixture of two related products (**11** and **12**) in a ratio 3:1 as indicated by RP-HPLC (data not shown). A reasonable explanation for this product pattern was the assumption that catalytic hydrogenation was incomplete and that the minor side product may constitute the corresponding 14,15-dehydro derivative **12**. ^{1}H NMR studies of **12** revealed a characteristic triplet–triplet at $\delta_H = 5.55$ ppm, which is the result of decoupling of the C-16 proton on the neighboring 17-CH_2 hydrogens ($J = 6.4$ Hz) and the methylene protons at C-13 ($J = 1.7$ Hz). These data indicate the position a triple bond between C-14 and C-15. To elucidate the structures of the two hydrogenation products in more detail functional groups were



Scheme 1. (a) TMSC≡CH (**2**), *n*-BuLi, THF, −78 °C, 0.5 h; (b) BzCl, Py, benzene, rt, 10 h; (c) *n*-Bu_4NF, THF, rt, 2 h; (d) **6**, CuI, NaI, K_2CO_3, DMF, rt, 12 h; (e) CBr_4, PPh_3, CH_2Cl_2, rt, 1 h; (f) **9**, CuI, NaI, K_2CO_3, DMF, rt; (g) H_2/Lindlar's catalyst, quinoline, benzene, 10 °C then reverse-phase HPLC; (h) MeOH, rt then chiral phase HPLC; (i) LiOH, MeOH–H_2O, rt.

deprotected using aq NaOH, the free carboxylate was esterified with pentafluorobenzyl bromide (PFB)[12] and the hydroxy groups were silylated. Gas chromatography/mass spectrometry in the negative chemical ionization mode revealed single peak chromatograms for the derivatives of **15** and **16** and their mass spectra were characterized by ions of major intensity at *m/z* 391 [M−181], 301 [M−181–90] and 389 [M−181], 299 [M−181–90], respectively. Similar data (*m/z* 391 and 301) have been previously reported for 16-HETE isolated from biological sources.[8] The structure of **15** and **16** was further confirmed by the more informative electron ionisation mass spectra (Fig. 1). The mass spectral data as well as the results of ^{1}H- and ^{13}C NMR analysis (see Experimental) indicated the chemical structure of **15** as rac-(5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxyeicosa-5,8,11,14-tetraenoic acid. Compound **16**, the side product of catalytical hydrogenation, was identified as rac-(5*Z*,8*Z*,11*Z*)-16-hydroxyeicosa-5,8,11-trien-14-ynoic acid. It is of mechanistic interest that the bulky benzoate residue at C-16 hinders catalytic hydrogenation of the C-14–C-15 triple bond. This hindrance was stronger at lower catalyst concentrations. When the molar substrate/catalyst ratio was altered from 1:1 to 3:1 (decrease of catalyst concentration) the **11**:**12** was shifted from 3:1 to 1:1. It should be stressed that the chemical synthesis of rac-(5*Z*,8*Z*,11*Z*)-16-hydroxyeicosa-5,8,11-trien-14-ynoic acid has not been reported before and that our synthetic protocol provides a simple and economic route for the preparation of this compound.

In principle, chiral hydroxy fatty acids can be prepared by two alternative ways: (i) chiral total synthesis (using chiral building blocks)[9,13,14] or (ii) stereorandom synthesis followed by enatiomer separation.[15,16] The latter pathway may be preferred when enantiomer separation is straight forward (no major derivatization) and when it leads to a high degree of optical purity (<99%). In Figure 2, enantiomer separation of the methyl esters of racemic 16-HETE (**13**) and its 14,15-dehydro analogue **14** is shown. In both cases, the enantiomers are base-line resolved and their retention time are quite different. Upscaling of this chromatographic procedure enabled the preparation of optically pure 16-HETE isomers (>99%) in the multi-mg range. Measurements of the optical rotation and comparison with literature data identified **13a** as 16(*R*)-HETE methyl ester $[\alpha]_D^{21}$ +6 (*c* 0.7, acetone) and **13b** as corresponding 16(*S*)-enantiomer $[\alpha]_D^{21}$ −6 (*c* 0.7, acetone) (lit.[9] $[\alpha]_D^{23}$ −5.4). To determine the configuration of the chiral center in **14a** and **14b** both enantiomers were stereospecifically hydrogenated to the corresponding tetraenoates. Co-elution in chiral phase HPLC indicated that the early eluting product **14a** is methyl (16*R*,5*Z*,8*Z*,11*Z*)-16-hydroxy-5,8,11-eicosatrien-14-ynoate and the late eluting **14b** its corresponding 16(*S*)-enantiomer.

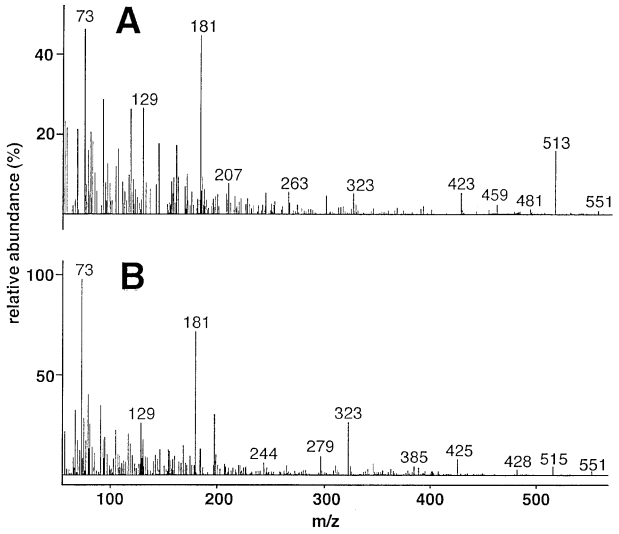


Figure 1. Mass spectra (electron ionization, 70 eV) of the pentafluorobenzyl-TMS-derivatives of rac-(5*Z*,8*Z*,11*Z*)-16-hydroxyeicosa-5,8,11-trien-14-ynoic acid (**16**) and rac-(5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxyeicosa-5,8,11,14-tetraenoic acid (**15**) (B) The analysis was performed as described in the Experimental.

Oxygenation of 16-HETE (15) enantiomers by lipoxygenases

16-HETE has been identified as natural arachidonic metabolite of the cytochrome P-450 pathway[7,8] but its suitability to act as eicosanoid precursor has not been investigated so far. We first tested rac-16-HETE as substrate for the 15-LOXs and found that the soybean LOX-1 is capable of oxygenating this hydroxy fatty acid as indicated by spectrophotometric measurements (data not shown). More detailed kinetic studies revealed a K_M of 48.7 μM and a V_{max} of 2.2 s^{-1}. When compared with 15-HPETE oxygenation substrate affinity was higher whereas V_{max} was impaired (Table 1). Analysing the 16-HETE oxygenation products by RP-HPLC one

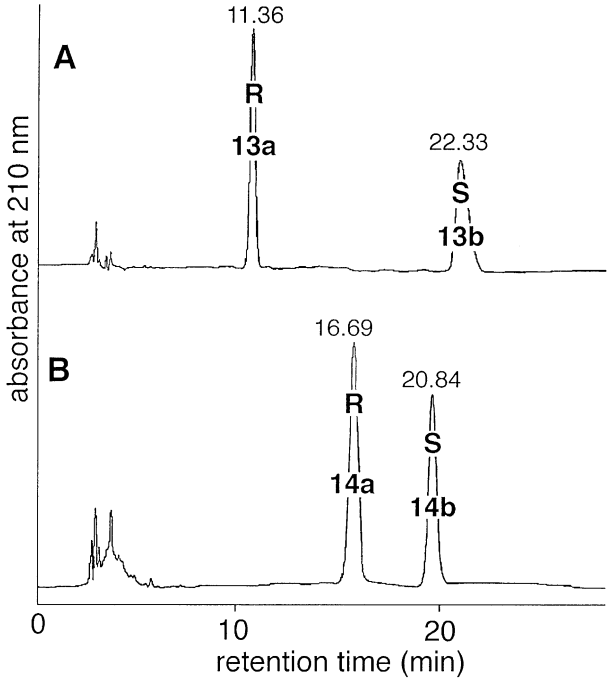


Figure 2. Enantiomer separation of (a) methyl rac-(5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxyeicosa-5,8,11,14-tetraenoate (**13**), and (b) methyl rac-(5*Z*,8*Z*,11*Z*)-16-hydroxyeicosa-5,8,11-trien-14-ynoate (**14**). For the analysis, a Chiralpack AD column (250×4.6 mm) from Daicel Chemical Industries was used. Solvent system: *n*-hexane/MeOH (98:2; by vol), flow rate 1 mL/min.

major compound absorbing at 235 nm was detected (Fig. 3A) and GC/MS data (see Experimental) indicated its chemical structure as (5*Z*,8*Z*,11*Z*,13*E*)-15,16-dihydroxy-5,8,11,13-eicosatetraenoate. These data, which suggest an arachidonic acid-like substrate alignment at the active site, were somewhat surprising since 15-HETE was oxygenated at C-8 and C-5 of the hydrocarbon backbone and this reaction involves an inverse substrate orientation.[4,6]

In order to find out whether configuration of the chiral center has any impact on the oxygenation process, the 16-HETE enantiomers were separately oxygenated by the soybean-LOX-1. Spectrophotometric measurements indicated that there was no major difference in the oxygenation kinetics of 16(*R*)- and 16(*S*)-HETE (data not shown). Thus, for the soybean LOX-1 the stereochemistry at C-16 does not influence C-15 lipoxygenation.

For several hydroxylated polyenoic fatty acids it has been reported that methylation of carboxylic group may force an inverse orientation at the active site of 15-LOXs.[6,17,18] For the methyl rac-16-HETE we made a similar observation. When this substrate was used for the soybean enzyme one major product was formed, which migrated more slowly in RP-HPLC (data not shown) and GC/MS data (see Experimental) indicated its chemical structure as (6E,8Z,11Z,14Z)-5,16-dihydroxy-6,8,11,14-eicosatetraenoate. This product pattern strongly suggests an inversely head to tail substrate orientation (Scheme 2). Comparison of the kinetic parameters revealed that 16-HETE methyl ester appears to be a better substrate than the free acid (Table 1).

Next, we studied the substrate behavior of 16-HETE for the human recombinant 5-LOX. 15(*S*)-HETE was shown to be a suitable substrate for the human 5-LOX and like arachidonic acid it is oxygenated at C-5.[19,20] When 16-HETE was used as substrate we also observed C-5 oxygenation (Fig. 3B). When we compared the product formation after a 15 min incubation period we found that 16(*R*)-HETE was more effectively oxygenated and its oxygenation rate was even higher than that of arachidonic acid. In contrast, 16(*S*)-HETE was oxygenated with a 5-fold lower rate (Table 2). These data indicate that the human 5-lipoxygenase oxygenates 16-HETE enantioselectively with a strong preference for the *R*-enantiomer.

Table 1. Kinetic parameters of hydroxy fatty acid oxygenation by the soybean LOX-1

Parameter	15-HPETE	16-HETE		14,15-dehydro-16-HETE	
	Free acid	Free acid **15**	Methyl ester **13**	Free acid **16**	Methyl ester **14**
K_M (μM)	440	48.7	4.1	No substrate	13.5
V_{max} (s^{-1})	25.0	2.2	7.1		8.2

Table 2. Reaction rate of fatty acid oxygenation by the recombinant human 5-lipoxygenase

Substrate	Relative reaction rate (%)
Arachidonic acid	100
16(*R*)-HETE	143
16(*S*)-HETE	29

Oxygenation of 14,15-dehydro-16-HETE (16) by soybean lipoxygenase-1

Acetylenic fatty acids are powerful inhibitors of the lipoxygenase reaction,[21–23] but the inhibition mechanism appears to be rather complex. 14,15-dehydro-16-HETE (**16**) was also supposed to act as LOX inhibitor because of its acetylenic structure. However, even at high concentrations (400 μM) we did not observe any significant inhibition of arachidonic acid oxygenation

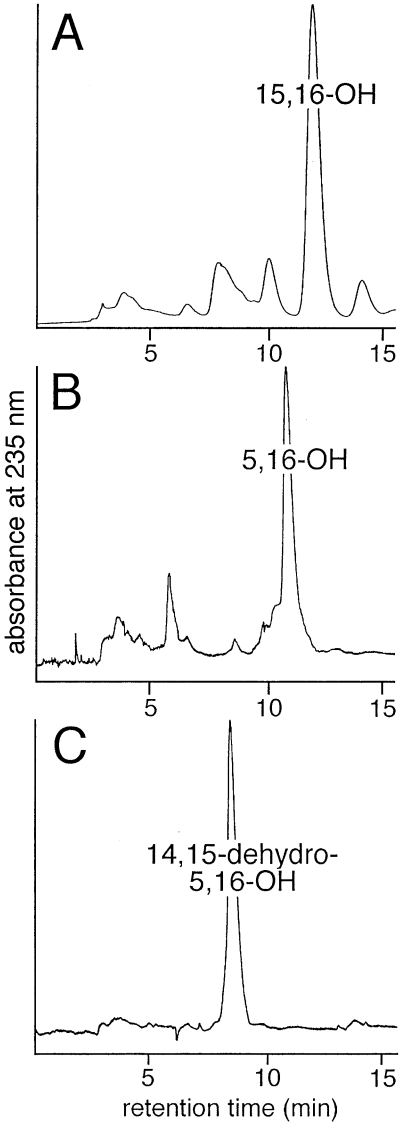
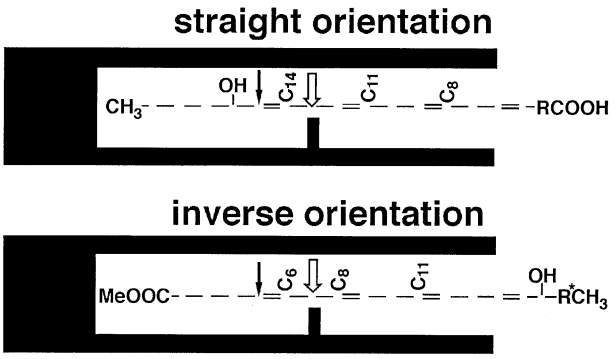


Figure 3. RP-HPLC analysis of the oxygenation products. The free acids of the lipoxygenase products were methylated with diazomethane and analyzed by RP-HPLC with a solvent system MeOH/H_2O/acetic acid (75:25:0.1, by vol) and a flow rate of 1 mL/min: (a) 16-HETE (**15**) oxygenation by the soybean LOX; (b) 16-HETE (**15**) oxygenation by the recombinant human 5-LOX; (c) oxygenation of methyl ester of 14,15-dehydro-16-HETE (**14**) by the soybean lipoxygenase-1.



Scheme 2. Two principal possibilities for substrate orientation at the active site of lipoxygenases. For straight orientation the substrate slips into the substrate binding pocket with its methyl end ahead (upper panel). In contrast, the carboxylate slides in first for inverse head to tail substrate alignment (lower panel). The black squares indicate the catalytic non-heme iron that is responsible for hydrogen abstraction. Thick open arrows: site of initial hydrogen abstraction; thin solid arrows: site of oxygen insertion.

(data not shown). Moreover, we found that 14,15-dehydro-16-HETE (**16**) was an inappropriate substrate for the soybean LOX-1 (Table 1). In contrast, its methyl ester **14** was well oxygenated by the enzyme and the kinetic constants (Table 1) did not reveal major differences to 16-HETE oxygenation. RP-HPLC of the oxygenation products (Fig. 3C) indicated a major conjugated diene that appeared to be homogenous in normal phase HPLC (hexane/2-propanol/acetic acid, 100:6:0.1). GC/MS analysis of the hydrogenated trimethylsilyl ethers (see Experimental) indicated the major product as methyl (6*E*,8*Z*,11*Z*)-5,16-dihydroxyeicosa-6,8,11-trien-14-ynoate, and this product pattern suggested an inverse substrate orientation at the active site.

General Conclusion

16-HETE containing a hydrophilic OH-group in the methyl ligand of the bisallylic C-13 is a suitable substrate for 5- and 15-lipoxygenases. Analysis of the product pattern suggested an arachidonic acid-like alignment at the active site. In contrast, the product composition of 16-HETE methyl ester oxygenation by the soybean lipoxygenase (5-lipoxygenation) may be explained with an inverse head to tail substrate orientation. In this case, the substrate slides into the hydrophobic substrate binding pocket with its carboxylate ahead. Thus, introduction of a hydrophilic substituent is not sufficient to force an inverse substrate orientation. This can only be achieved by simultaneous of the substrates carboxylate.

Experimental

General

^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker MSL 200 spectrophotometer in $CDCl_3$ as solvent. Chemical shifts are referenced to tetramethylsilane as an internal standard for ^{1}H NMR or to the deuterium lock signal of $CDCl_3$ ($\delta^{13}C = 77.19$ ppm). IR spectra were recorded on Shimadzu IR-435. UV spectra were recorded on Shimadzu UV-2100 spectrophotometer. HPLC analysis was carried out on a Shimadzu LC-10Avp liquid chromatograph connected to SPD-10Advp UV detector. RP-HPLC analysis was performed on a Nucleosil C18-column; 250×4 mm, 5 μm particle size (Machery-Nagel, Düren, Germany) with different solvent systems: MeOH/H_2O (95:5, by vol.) and a flow rate of 1 mL/min was used for the analysis of compounds **11** and **12**. In contrast, a solvent system of MeOH/H_2O (85:15, by vol) and a flow rate of 1 mL/min was used for analysing compounds 15 and 16. 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 (95:5, by vol) and a flow rate of 10 mL/min. Enantiomer separation of **13a**/**13b** and **14a**/**b** was performed either on Chiralpack AD column (250×4.6 mm, Daicel Chemical Industries) or on Chiralpack AD column (250×10 mm, Daicel Chemical Industries) using *n*-hexane/MeOH (98:2, by vol) as solvent system and a flow rate of 1 mL/min. The products of the lipoxygenase reaction were analyzed by RP-HPLC on a Nucleosil C18-column; 250×4 mm, 5 μm particle size (Machery-Nagel, Düren, Germany) with a solvent systems: MeOH/H_2O/acetic acid (75:25:0.1, by vol) and a flow rate of 1 mL/min. For SP-HPLC analysis of these products a Nucleosil 100-7 column 250×4 mm, 5 μm particle size (Machery-Nagel, Düren, Germany) was used and the analytes were eluted at a flow rate of 1 mL/min with the solvent systems *n*-hexane/2-propanol/acetic acid (94:6:0.1, by vol). GC/MS (EIMS) analyses were carried out on a Shimadzu GC-MS QP-2000 system equipped with a capillary column (30 m×0.32 mm, coating thickness 0.25 μm). An injector temperature of 280 °C, an ion source temperature of 180 °C and an electron energy of 70 eV were set up. The derivatized fatty acids were eluted with a following temperature program: isotermically at 180 °C for 2 min then from 180 to 290 °C at a rate 5 °C/min. For GC/MS analysis with negative ion detection a Shimadzu GC-MS-QP-5050A system equipped with a fused silica GC column DB-1 (30 m, film thickness 0.25 mm) from J&W Scientific was used. Samples were eluted with a flow of hellium 24 mL/min and following temperature program: isotermically at 150 °C for 3 min then from 150 to 190 °C at a rate 10 °C/min and from 190 to 310 °C at a rate 20 °C/min. HRMS was carried out on a MAT711 mass spectrometer (Finigan MAT). Column chromatography was carried out on silica Gel 60 (Merck, Darmstadt, Germany, particle size ranging from 70 to 230 mesh). For thin-layer chromatography we employed precasted Silica Gel 60 F254 sheets (Merck, Darmstadt, Germany). THF was freshly distilled from sodium/benzophenone ketyl, and HMPA was dried over CaH_2. All 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.[23] Prior to use, all glassware and syringes were dried at 140 °C overnight and all reactions were carried out under atmosphere of dry argon.

rac-1-(Trimethylsilyl)hept-1-yn-3-ol (3). A solution of acetylene **2** (3.01 g, 30.4 mmol) in THF (65 mL) was cooled to −78 °C. *n*-BuLi (18 mL, 1.6 M in hexane, 29.0 mmol) was added to the solution over 5 min followed by a solution of aldehyde **1** (0.83 g, 9.7 mmol) in THF (3 mL). After 0.5 h at −78 °C the reaction was quenched with satd aq NH_4Cl (100 mL) and the organic products were extracted with Et_2O (2×50 mL). The combined organic extracts were washed wih satd aq NaCl (1×50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The raw product was filtrated over silica gel (hexane/Et_2O, 5:1). Purified **3** was obtained in yield 1.58 g (89%). TLC: R_f = 0.49 (hexane/Et_2O, 1:1). IR (neat)/cm^{-1}: 3600–3200 (OH), 2240 (C≡C), 1273 (C–O), 1244, 840 ($Si(CH_3)_3$). ^1H NMR (200 MHz, $CDCl_3$) δ 4.33 (m, 1H, 3-CH), 1.71 (m, 2H, 4-CH_2), 1.35 (m, 4H, 5- and 6-CH_2), 0.91 (t, 3H, J = 6.8 Hz, CH_3), 0.15 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 89.06, 72.12, 62.86, 37.32, 27.98, 22.91, 14.09, 0.01. Anal. calcd for $C_{10}H_{20}OSi$: C, 65.15; H, 10.94. Found: C, 65.29; H, 10.71.

rac-3-(Benzoyloxy)hept-1-yn (4). A solution of BzCl (1.54 g, 10.95 mmol) in benzene (20 mL) was added to a solution of **3** (1.55 g, 8.42 mmol) in benzene (30 mL) and pyridine (25 mL). The reaction mixture was stirred for 10 h at rt, then acidified with H_2SO_4 (1 M, 50 mL). Reaction products were extracted as described above (Et_2O, 2×50 mL) and the extracts were concentrated under reduced pressure. The residue was reconstituted in *n*-hexane and filtrated through a silica gel column (*n*-hexane/Et_2O, 4:1). The product which showed an R_f = 0.59 in silica gel thin layer chromatography (*n*-hexan/Et_2O, 1:1) was dissolved in THF (50 mL) and the silyl group was removed by addition of *n*-Bu_4NF (3.12 g, 11.95 mmol) at rt for 2 h. The mixture was quenched with H_2O (100 mL), organic layer was separated and lipophilic products were extracted from the water phase with Et_2O (2×40 mL). The combined organic layers were washed with satd aq NaCl (70 mL), dried over Na_2SO_4 and concentrated under vacuum. Silica gel column chromatography using gradient elution with hexane/Et_2O (from 1 to 10% Et_2O) gave pure **4** as a colorless oil in a yield 1.34 g (73.5%). TLC: R_f = 0.55 (hexane/Et_2O, 1:1). IR (neat)/cm^{-1}: 3274 (C≡C), 1725 (C=O), 1115 (C–O), 709 (Ph). ^1H NMR (200 MHz, $CDCl_3$) δ 8.05 (m, 2H, *o*-Bz), 7.49 (m, 3H, (*m*+*p*)-Bz), 5.55 (dt, 1H, J = 2.1 and 6.8 Hz, 3-CH), 2.47 (t, 1H, J = 2.1 Hz, 1-CH), 1.91 (m, 2H, 4-CH_2), 1.45 (m, 4H, 5- and 6-CH_2), 0.89 (t, 3H, J = 6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 165.55, 133.17, 132.69, 129.79 (2C), 128.50 (2C), 81.60, 73.70, 64.55, 34.67, 27.25, 22.36, 13.19. Anal. calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46. Found: C, 77.46; H, 7.21.

7-Bromo-2,5-heptadiyn-1-ol (6). A solution of PPh_3 (6.81 g, 26 mmol) in dry CH_2Cl_2 (50 mL) was added (dropwise for 0.5 h) to a stirred solution of diol **5** (3.13 g, 25.21 mmol) and CBr_4 (8.63 g, 26 mmol) in CH_2Cl_2 (70 mL) and DMF (10 mL) at 0 °C. The reaction mixture was stirred for 0.5 h at 0–10 °C. After the solvent was evaporated under reduced pressure, the residue was purified by chromatography on silica gel using the solvent system (*n*-hexane/Et_2O, 1:1). The eluent fractions containing the reaction product (R_f = 0.32 in TLC with hexane/Et_2O, 1:2) were combined and concentrated under vacuum. Yield of **6**: 2.59 g (72%). IR (neat)/cm^{-1}: 3600–3400 (OH), 2240, 2170 (C≡C), 600 (CH_2Br). ^1H NMR (200 MHz, $CDCl_3$): δ 4.21 (m, 2H, 1-CH_2), 3.87 (m, 2H, 7-CH_2), 3.27 (m, 2H, 4-CH_2). ^{13}C NMR (50 MHz, $CDCl_3$) δ 81.60, 79.98, 79.50, 78.47, 51.46, 15.22, 10.77. Anal. calcd for C_7H_7BrO: C, 44.95; H, 3.77. Found: C, 45.07; H, 4.05.

rac-10-(Benzoyloxy)tetradeca-2,5,8-triyn-1-ol (7). To a suspension of previously dried salts CuI (2.29 g, 12.04 mmol), NaI (1.81 g, 12.04 mmol) and K_2CO_3 (1.25 g, 9.03 mmol) in DMF (35 mL) were added the bromide **6** (1.12 g, 6.02 mmol) and acetylene **4** (1.32 g, 6.11 mmol) under argon atmosphere. After stirring for 12 h at rt, the mixture was quenched with satd aq NH_4Cl (100 mL) and the product was extracted with Et_2O (4×100 mL). The combined organic extracts were washed with satd aq NaCl (2×50 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexane/Et_2O, 1:2) to afford 1.53 g, (79%) of **7** as a colorless oil. TLC: R_f = 0.30 (hexane/Et_2O, 1:2). IR (neat)/cm^{-1}: 3600–3200 (OH), 2241 (C≡C), 1725 (C=O), 1115 (C–O), 710 (Ph). ^1H NMR (200 MHz, $CDCl_3$) δ 8.04 (m, 2H, *o*-Bz), 7.45 (m, 3H, (*m*+*p*)-Bz), 5.55 (m, 1H, 10-CH), 4.20 (m, 2H, 1-CH_2), 3.20 (m, 4H, 4- and 7-CH_2), 1.85 (m, 2H, 11-CH_2), 1.21–1.40 (m, 4H, 12- and 13-CH_2), 0.88 (t, 3H, J = 6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 165.69, 133.09, 130.30, 129.86 (2C), 128.43 (2C), 79.84, 79.14, 78.33, 74.77, 74.55, 74.40, 64.96, 51.14, 34.82, 27.32, 22.32, 13.94, 9.94 (2C). Anal. calcd for $C_{21}H_{22}O_3$: C, 78.23; H, 6.88. Found: C, 78.01; H, 7.18.

rac-5-(Benzoyloxy)-14-bromotetradeca-6,9,12-triyne (8). To a solution of alcohol **7** (1.49 g, 4.62 mmol) and CBr_4 (2.30 g, 6.93 mmol) in CH_2Cl_2 (30 mL) a solution of PPh_3 (1.82 g, 6.93 mmol) in CH_2Cl_2 (10 mL) was added at 10 ° C. The reaction mixture was stirred for 1 h at rt, quenched with MeOH (2 mL) and volatile components were removed under vacuum. Column chromatography on silica gel (hexane/Et_2O, 1:1) afforded pure **8** in a yield 1.62 g (91%). TLC: R_f = 0.51 (hexane/Et_2O, 1:1). IR (neat)/cm^{-1}: 2240, 2170 (C≡C), 1725 (C=O), 1115 (C–O), 710 (Ph), 600 (C-Br). ^1H NMR (200 MHz, $CDCl_3$) δ 8.05 (m, 2H, *o*-Bz), 7.49 (m, 3H, (*m*+*p*)-Bz), 5.55 (m, 1H, 5-CH), 3.85 (m, 2H, 14-CH_2), 3.15 (m, 4H, 8- and 11-CH_2), 1.85 (m, 2H, 4-CH_2), 1.20–1.41 (m, 4H, 2- and 3-CH_2), 0.85 (t, 3H, J = 6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 165.55, 133.06, 130.37, 129.86 (2C), 128.43 (2C), 81.31, 79.73, 78.44, 75.83, 74.69, 73.96, 64.88, 34.81, 27.32, 22.36, 14.49, 13.94, 10.16, 9.97. Anal. calcd for $C_{21}H_{21}BrO_2$: C, 65.46; H, 5.49. Found: C, 65.61; H, 5.66.

Methyl rac-16-(Benzoyloxy)eicosa-5,8,11,14-tetraynoate (10). In a dried round-bottomed flask equipped with magnetic stirrer anhydrous K_2CO_3 (0.77 g, 5.52 mmol), NaI (1.10 g, 7.36 mmol) and CuI (1.41 g, 7.36 mmol) were suspended in DMF (25 mL) under argon atmo-

sphere. Methyl 5-hexynoate (**9**) (0.46 g, 3.68 mmol) was added at once to the suspension followed by bromide **8** (1.42 g, 3.68 mmol). The reaction mixture was vigorously stirred overnight at rt, quenched with satd aq NH_4Cl (200 mL). The lipophilic products were extracted with Et_2O (4×100 mL). The combined organic extracts were washed with satd aq NaCl (2×150 mL). After drying over Na_2SO_4 the ethereal solution was concentrated in vacuum. The crude residue was purified by silica gel flash chromatography (hexane/Et_2O, 3:1) under argon atmosphere to give pure **10** as yellow oil; yield 1.28 g (81%). TLC: R_f=0.39 (hexane/Et_2O, 1:1). IR (neat)/cm^{-1}: 2240 (C≡C), 1740, 1725 (C=O), 1115 (C–O), 710 (Ph). ^1H NMR (200 MHz, $CDCl_3$) δ 8.01 (m, 2H, *o*-Bz), 7.49 (m, 3H, (*m*+*p*)-Bz), 5.55 (tt, 1H, *J*=1.5 and 6.5 Hz, 16-CH), 3.61 (s, 3H, OCH_3), 3.11 (m, 6H, 7-, 10- and 13-CH_2), 2.40 (t, 2H, *J*=7.0 Hz, 2-CH_2), 2.19 (m, 2H, 4-CH_2), 1.75 (m, 4H, 3- and 17-CH_2), 1.20–1.40 (m, 4H, 18- and 19-CH_2), 0.85 (t, 3H, *J*=6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.56, 165.66, 133.09, 130.39, 129.89 (2C), 128.46 (2C), 79.99, 79.66, 78.37, 75.23, 74.92 (2C), 74.29, 74.18, 64.96, 51.50, 34.89, 33.05, 27.39, 24.12, 22.39, 18.38, 13.98, 10.01, 9.86 (2C). Anal. calcd for $C_{28}H_{30}O_4$: C, 78.11; H, 7.02. Found: C, 78.18; H, 7.29.

Methyl rac-(5Z,8Z,11Z,14Z)-16-(benzoyloxy)eicosa-5,8,11,14-tetraenoate (11). Into a 250-mL Erlenmeyer flask containing Lindlar's catalyst (1.60 g) dry benzene (30 mL) was added. The mixture was saturated with H_2 at rt and cooled to 10 °C. Then quinoline (1.6 mL) and a solution of **10** (1.01 g, 2.35 mmol) in benzene (70 mL) were added to the catalyst suspension and the reaction mixture was stirred for 1 h at 10 °C. The mixture was filtered, washed with 2 M HCl (2×30 mL) and the solvent was evaporated. RP-HPLC of the crude residue revealed two products, which were separated by preparative RP-HPLC (solvent system MeOH/H_2O, 95:5) to yield 0.48 g (48.6%) of the pure tetraenoate **11** and 0.28 g (27.4%) of the corresponding monoacetylenide **12**. Analytical data for **11**: TLC: R_f=0.54 (hexane/Et_2O, 1:1). RP-HPLC: RT=7.17 min. ^1H NMR (200 MHz, $CDCl_3$) δ 8.05 (m, 2H, *o*-Bz), 7.44 (m, 3H, (*m*+*p*)-Bz), 5.79 (m, 1H, 16-CH), 5.25–5.45 (m, 8H, CH=CH), 3.44 (s, 3H, OCH_3), 3.01 (m, 2H, 13-CH_2), 2.77 (m, 4H, 7- and 10-CH_2), 2.29 (t, 2H, *J*=7.0 Hz, 2-CH_2), 2.09 (m, 2H, 4-CH_2), 1.75 (m, 4H, 3- and 17-CH_2), 1.25–1.40 (m, 4H, 18- and 19-CH_2), 0.90 (t, 3H, *J*=6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.04, 166.03, 132.80, 132.10, 131.15, 129.93, 129.75 (2C), 129.09 (2C), 128.98 (2C), 128.43 (2C), 128.24, 127.77, 71.20, 51.50, 34.78, 33.64, 27.47, 26.81, 26.58, 25.85 (2C), 25.00, 22.73, 14.09. EIMS *m*/*z* (%): 316 (3.33) [M^+−BzCOO], 287 (0.37) [M^+−BzCOO–CH_3O], 259 (1.48)) [M^+−BzCOO–C_4H_9]. Anal. calcd for $C_{28}H_{38}O_4$: C, 76.68; H, 8.73. Found: C, 76.97; H, 8.85. Analytical data for 12: TLC: R_f=0.54 (hexane/Et_2O, 1:1). RP-HPLC: RT=5.91 min. ^1H NMR (200 MHz, $CDCl_3$) δ:=8.05 (m, 2H, o-Bz), 7.42 (m, 3H, (*m*+*p*)-Bz), 5.55 (tt, 1H, *J*=6.4 and 1.7 Hz, 16-CH), 5.25–5.45 (m, 6H, CH=CH), 3.61 (s, 3H, OCH_3), 2.93 (m, 2H, 13-CH_2), 2.74 (m, 4H, 7- and 10-CH_2), 2.27 (t, 2H, *J*=7.0 Hz, 2-CH_2), 2.05 (m, 2H, 4-CH_2), 1.83 (m, 2H, 17-CH_2), 1.65 (m, 2H, 3-CH_2), 1.25–1.45 (m, 4H, 18- and 19-CH_2), 0.88 (t, 3H, *J*=6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=174.97, 165.73, 133.02, 130.67, 130.05, 129.94 (2C), 129.24, 128.91, 128.47 (2C), 127.62, 124.57 (2C), 84.36, 78.04, 65.29, 51.43, 35.15, 33.64, 27.47, 26.81, 25.77 (2C), 25.00, 22.43, 17.43, 14.01. EIMS *m*/*z* (%):314 (1.48) [M^+−BzCOO], 285 (0.27) [M^+−BzCOO–CH_3O], 257 (0.59) [M^+−BzCOO–C_4H_9). Anal. calcd for $C_{28}H_{36}O_4$: C, 77.03; H, 8.31. Found: C, 77.12; H, 8.21.

Methyl (16R,5Z,8Z,11Z,14Z)-(13a) and methyl (16S,5Z,8Z,11Z,14Z)-16-hydroxyeicosa-5,8,11,14-tetraenoate (13b). A methanol solution of NaOMe (0.1 M, 8 mL) was added to a solution of racemic **11** (70.1 mg, 0.16 mmol) in dry methanol (10 mL) under argon atmosphere. The resulting mixture was stirred at rt for 18 h. After the reaction was completed, methanol was partly removed by evaporation under reduced pressure, the residue was acidified to pH 4 using 1 M HCl and the lipophilic products were extracted with Et_2O (3×30 mL). Combined organic extracts were dried over Na_2SO_4, concentrated under vacuum and the crude residue was purified by silical gel column chromatography (hexane/Et_2O, 1:1) to give pure racemic **13** (39.5 mg, 74%). Enantiomers were separated by chiral HPLC (solvent system *n*-hexane/MeOH, 98:2) to yield 20.7 mg of the pure **13a** and 17.8 mg of **13b**.

Analitical data for **13a**: $[\alpha]_D^{21}$ +6 (*c* 0.23, acetone). CP-HPLC: RT=11.36 min. RP-HPLC: RT=8.45 min. TLC: R_f=0.49 (hexane/Et_2O, 1:1). ^1H NMR (200 MHz, $CDCl_3$) δ 5.25–5.52 (m, 8H, CH=CH), 4.45 (m, 2H, 16-CH), 3.61 (s, 3H, OCH_3), 2.80 (m, 6H, 7-, 10- and 13-CH_2), 2.29 (t, 2H, *J*=7.0 Hz, 2-CH_2), 2.08 (m, 2H, 4-CH_2), 1.65 (m, 2H, 3-CH_2), 1.25–1.35 (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 174.71, 133.12, 130.41, 129.09, 128.79, 128.55 (2C), 128.13, 127.94, 68.04, 51.52, 37.37, 33.45, 27.67, 26.72, 26.32, 25.90 (2C), 24.82, 22.79, 14.08. EIMS *m*/*z* (%): 316 (2.1) [M^+−H_2O], 245 (1.6) [M^+−MeOH–C_4H_9]. HRMS calcd for $C_{21}H_{34}O_3$ [M^+]: 334.2508. Found 334.1312. Analytical data for **13b**: $[\alpha]_D^{21}$ −6 (*c* 0.23, acetone). CP-HPLC: RT=22.33 min. Other analytical data are identical to those obtained for **13a**.

Methyl (16*R*,5*Z*,8*Z*,11*Z*)-(14a) and methyl (16*S*,5*Z*,8*Z*,11*Z*)-16-hydroxyeicosa-5,8,11-trien-14-ynoate (14b). Enantiomers of **14** (**14a** and **14b**) were prepared from methyl (5*Z*,8*Z*,11*Z*)-rac-16-(benzoyloxy)eicosa-5,8,11-trien-14-ynoate (**12**) (47.9 mg, 0.11 mmmol) by an analogues procedure as described for **13a** and **13b** and yielded 16.2 mg of pure **14a** and 15.9 mg of **14b**.

Analytical data for **14a**: $[\alpha]_D^{21}$ +12 (*c* 0.32, acetone). CP-HPLC: RT=16.69 min. RP-HPLC: RT=7.05 min. TLC: R_f=0.49 (hexane/Et_2O, 1:1). ^1H NMR (200 MHz, $CDCl_3$) δ 5.25–5.45 (m, 6H, CH=CH), 4.31 (tt, 1H, *J*=1.7 and 7.4 Hz, 16-CH_3), 3.61 (s, 3H, OCH_3), 2.95 (m, 2H, 13-CH_2), 2.74 (m, 4H, 7- and 10-CH_2), 2.33 (t, 2H, *J*=7.0 Hz, 2-CH_2), 2.09 (m, 2H, 4-CH_2), 1.65 (m, 4H, 3- and 17-CH_2), 1.25–1.40 (m, 4H, 18- and 19-CH_2), 0.88 (t, 3H, *J*=6.8 Hz, CH_3). ^{13}C NMR (50 MHz,

$CDCl_3$) δ 174.89, 129.97, 129.08 (2C), 128.87, 127.64, 124.75, 83.50, 81.53, 62.91, 51.35, 37.97, 33.49, 27.54, 26.69, 25.84, 25.78, 24.75, 22.54, 17.35, 14.05. EIMS *m/z* (%): 332 (0.01) [M^+], 271 (0.8) [$M^+-MeOH-C_2H_5$], 243 (0.99) [$M^+-MeOH-C_4H_9$]. HRMS calcd for $C_{21}H_{32}O_3$ [M^+]: 332.2351. Found 332.2376. Analytical data for **14b**: $[\alpha]_D^{21}$ −12 (*c* 0.32, acetone). CP-HPLC: RT = 22.33 min. Other analytical data are identical to those obtained for **14a**.

(16*R*,5*Z*,8*Z*,11*Z*,14*Z*)-16-Hydroxyeicosa-5,8,11,14-tetraenoic acid (15a). An aqueous solution (7 mL) of LiOH (10.0 mg, 0.41 mmol) was added to a solution of **11** (16.7 mg, 0.05 mmol) in methanol (10 mL) under argon atmosphere. The resulting mixture was stirred at rt overnight. After the reaction was completed, methanol was removed by evaporation under reduced pressure, the residue was acidified to pH 4 using 1 M HCl and the lipophilic products were extracted with Et_2O (3×15 mL). Combined organic extracts were dried over Na_2SO_4, concentrated in vacuum and the crude residue was purified by silical gel column chromatography (hexane/Et_2O, 1:3) to give pure **15a** (13.9 mg, 87%). TLC: R_f = 0.31 (hexane/Et_2O, 1:3). RP-HPLC: RT = 6.08 min. ^1H NMR (200 MHz, $CDCl_3$) δ 5.25–5.52 (m, 8H, CH=CH), 4.45 (m, 2H, 16-CH), 2.80 (m, 6H, 7-, 10- and 13-CH_2), 2.29 (t, 2H, *J* = 7.0 Hz, 2-CH_2), 2.08(m, 2H, 4-CH_2), 1.65 (m, 2H, 3-CH_2), 1.25–1.35 (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 178.08, 133.13, 130.41, 129.16, 128.79, 128.57 (2C), 128.13, 127.94, 68.04, 37.37, 33.45, 27.68, 26.72, 26.32, 25.92 (2C), 24.82, 22.79, 14.08. EIMS *m/z* (%): 302 (0.82) [M^+-H_2O], 245 (1.07) [$M^+-H_2O-C_4H_9$]. HRMS calcd for $C_{20}H_{30}O_2$ [M^+-H_2O]: 302.2246. Found 302.2264.

(16*S*,5*Z*,8*Z*,11*Z*,14*Z*)-16-Hydroxyeicosa-5,8,11,14-tetraenoic acid (15b). The free acid **15b** was prepared from the corresponding methyl ester (**13b**) (16.7 mg, mmol) as described for its corresponding (*R*)-enantiomer **13a** affording pure **15b** (14.1 mg, 88%). All spectral data and HPLC characteristica are identical to those of **15a**.

(16*R*,5*Z*,8*Z*,11*Z*)-16-Hydroxyeicosa-5,8,11-trien-14-ynoic acid (16a). The free acid **16a** was prepared from the corresponding methyl ester (**14a**) (13.3 mg, 0.04 mmmol) by an analogues procedure as described for **15a**. Yield of pure **16a** (11.2 mg, 88%). TLC: R_f = 0.30 (hexane/Et_2O, 1:3). RP-HPLC: RT = 5.42 min. ^1H NMR (200 MHz, $CDCl_3$) δ 5.25–5.45 (m, 6H, CH=CH), 4.31 (tt, 1H, *J* = 1.7 and 6.4 Hz, 16-CH_3), 2.95 (m, 2H, 13-CH_2), 2.74 (m, 4H, 7- and 10-CH_2), 2.33 (t, 2H, *J* = 7.0 Hz, 2-CH_2), 2.09 (m, 2H, 4-CH_2), 1.65 (m, 4H, 3- and 17-CH_2), 1.25–1.40 (m, 4H, 18- and 19-CH_2), 0.88 (t, 3H, *J* = 6.8 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.92, 129.97, 129.08 (2C), 128.83, 127.65, 124.75, 83.47, 81.53, 62.93, 37.97, 33.49, 27.54, 26.69, 25.84, 25.77, 24.74, 22.54, 17.35, 14.05. EIMS *m/z* (%): 318 (0.05) [M^+], 300 (0.24) [M^+-H_2O], 289 (0.31) [$M^+-C_2H_5$], 271 (0.55) [$M^+-H_2O-C_2H_5$], 261 (0.72) [$M^+-C_4H_9$], 243 (0.04) [$M^+-H_2O-C_4H_9$]. HRMS calcd for $C_{20}H_{30}O_3$ [M^+]: 318.2194. Found 318.2176.

(16*S*,5*Z*,8*Z*,11*Z*)-16-Hydroxyeicosa-5,8,11-trien-14-ynoic acid (16b). The free acid **16b** was prepared from its methyl ester (**14b**) (13.3 mg, 0.04 mmol) as described for **15a** affording pure **16b** (10.9 mg, 86%). All spectral data and HPLC characteristics were identical to those of **16a**.

Preparation and analysis of oxygenation products of compounds 13, 14, 15 by the soybean 15-lipoxygenase-1. An ethanolic solution of the fatty acid (10 μL, 50 mM) was added to sodium borate buffer pH 9.0 (5 mL, 0.1 M). After the mixture was sonicated for 30 s to achive homogenous substrate dispersion soybean 15-lipoxygenase-1 (40 μg) was added. After 25 min incubation at rt the hydroperoxy fatty acids formed were reduced by the addition of a saturated ethanolic solution of $NaBH_4$ (50 μL). The mixture was acidified to pH 3 with 0.1 M acetic acid and the lipophylic products were extracted with with ethyl acetate (2×3 mL). The extracts were combined and the solvent evaporated. After methylation of the carboxylate group with diazomethane the products were purified by RP-HPLC using the solvent system MeOH/H_2O/AcOH (75:25:0.1, by vol).

Analytical data for the products formed from methyl (5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxyeicosa-6,8,11,14-tetraenoate (**13**): UV: λ_{max} 234 nm. RP-HPLC: RT = 10.44 min (MeOH/H_2O/AcOH (75:25:0.1, by vol). SP-HPLC: RT = 5.31 min (*n*-hexane/2-propanol/AcOH (94:6:0.1, by vol). GC–MS (EIMS for TMS-derivative) *m/z* (%): 479 (0.17) [M^+-15], 255 (90), 203 (75).

Analytical data for the products formed from methyl (5*Z*,8*Z*,11*Z*)-16-hydroxyeicosa-6,8,11-trien-14-ynoate (**14**): UV: λ_{max} 234 nm. RP-HPLC: RT = 8.19 min (MeOH/H_2O/AcOH (75:25:0.1, by vol). SP-HPLC: RT = 6.87 min (*n*-hexane/2-propanol/AcOH (94:6:0.1, by vol). GC–MS (EIMS for TMS-derivative) *m/z* (%): 477 (0.10) [M^+-15], 255 (40), 203 (55).

Analytical data for the products formed from (5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxyeicosa-5,8,11,14-tetraenoic acid (**15**): UV: λ_{max} 234 nm. RP-HPLC: RT = 12.01 min (MeOH/H_2O/AcOH (75:25:0.1, by vol). SP-HPLC: RT = 5.99 min (*n*-hexane/2-propanol/AcOH (94:6:0.1, by vol). GC–MS (EIMS for TMS-derivative) *m/z* (%): 479 (0.12) [M^+-15], 335 (3.91), 159 (100).

Preparation and analysis of oxygenation products formed from 16(*R*)-(14a) and 16(*S*)-HETE (14b) by the recombinant human 5-lipoxygenase. The recombinant human 5-lipoxygenase (40 μL, 1.45 mg/mL) was preincubated in Tris–HCl buffer (960 μL, 0.1 M, pH 7.25) containing ATP (10 μL, 2.15 mM), $CaCl_2$ (4 μL, 100 mM), EDTA (0.2 μL, 0.5 M) and dipalmitoyl phosphatidyl choline (1.3 μg/mL) for 2 min at rt. Then an ethanolic solution of the substrate **14** (2 μL, 50 mM) was added and the enzyme was incubated for 15 min at rt. The hydroperoxy fatty acids formed were reduced by addition of a saturated ethanolic solution of $NaBH_4$ (50 μL). The mixture was acidified to pH 3 with acetic acid (0.1 M) and the lipids were extracted with ethyl acetate (2×3 mL). The extracts were combined and the solvent evaporated under vacuum. After methylation of the

carboxylic group with diazomethane, the products were purified by RP-HPLC using solvent system MeOH/H_2O/AcOH (75:25:0.1, by vol).

Catalytic hydrogenation and GC/MS analysis of hydrogenated lipoxygenase products

To get more informative mass spectra, the methylated lipoxygenase products formed from **13**–**15** were hydrogenated using 10% Pd/$CaCO_3$ as catalyst. For this purpose fatty acid derivatives (20 μg) were dissolved in MeOH (1 mL). Then the Pd-catalyst (3 mg) was added and hydrogen was bubbled through the mixture for 5 min at rt. The solution was filtered to remove catalyst, the solvent was evaporated, the residue was silylated with BSTFA and analysed by GC/MS as described in the Experimental.

Data for the oxygenation product formed from methyl (5*Z*,8*Z*,11*Z*,14*Z*)-16-hydroxyeicosa-6,8,11,14-tetraenoate (**13**) by soybean lipoxygenase-1. GC/MS (EIMS) *m*/*z* (%): 487 (8.7) [M^+-15], 445 (10.8), 203 (84), 159 (82).

Data for the the oxygenation product formed from methyl (5*Z*,8*Z*,11*Z*)-16-hydroxyeicosa-6,8,11-trien-14-ynoate (**14**) by the soybean lipoxygenase-1. GC/MS (EIMS) *m*/*z* (%): 487 (9.7) [M^+-15], 445 (12), 203 (40), 159 (41).

Data for the oxygenation product formed from (5*Z*,8*Z*, 11*Z*,14*Z*)-16-hydroxyeicosa-5,8,11,14-tetraenoic acid (**15**) by the soybean lipoxygenase-1. GC/MS (EIMS) *m*/*z* (%): 487 (1.16) [M^+-15], 416 (1.02), 343 (80), 159 (90).

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References and Notes

1. McGettigan, P.; Henry, D. *Curr. Pharm. Des* **2000**, *6*, 1693.
2. Bulger, E. M.; Maier, R. V. *Crit. Care Med.* **2000**, *28*, 27.
3. Brash, A. R.; Ingram, C. D.; Harris, T. M. *Biochemistry* **1987**, *26*, 5465.
4. van Os, C. P.; Rijke-Schilder, G. P.; van Halbeek, H.; Verhagen, J.; Vliegenthart, J. F. *Biochim. Biophys. Acta* **1981**, *663*, 177.
5. Kuhn, H.; Eggert, L.; Zabolotsky, O. A.; Myagkova, G. I.; Schewe, T. *Biochemistry* **1991**, *30*, 10269.
6. Schwarz, K.; Borngraber, S.; Anton, M.; Kuhn, H. *Biochemistry* **1998**, *37*, 15327.
7. Bednar, M. M.; Gross, C. E.; Russel, S. R.; Fuller, S. P.; Ahern, T. P.; Howard, D. B.; Falck, J. R.; Reddy, K. M.; Balazy, M. *Neurosurgery* **2000**, *47*, 1410.
8. Bednar, M. M.; Gross, C. E.; Balazy, M. K.; Belosludsev, Yu.; Colella, D. T.; Falck, J. R.; Balazy, M. *Biochem. Pharmacol.* **2000**, *60*, 447.
9. Heckmann, B.; Mioskowski, Ch.; Lumin, S.; Falck, J. R.; Wei, Sh.; Capdevila, J. H. *Tetrahedron Lett.* **1996**, *37*, 1425.
10. Ivanov, I. V.; Groza, N. V.; Romanov, S. G.; Kuhn, H.; Myagkova, G. I. *Synthesis* **2000**, 691.
11. Lapitskaya, M. A.; Vasiljeva, L. L.; Pivnitsky, K. K. *Synthesis* **1993**, 65.
12. Balazy, M. *J. Biol. Chem.* **1991**, *266*, 23561.
13. Takano, S.; Sugihara, T.; Ogasawara, K. *Heterocycles* **1990**, *31*, 1721.
14. Parker, K. A.; Ledeboer, M. W. *J. Org. Chem.* **1996**, *61*, 3214.
15. Wiesner, R.; Kühn, H. Handbook of Chromatography. Analysis of Lipid; Mukherjee, K. D.; Weber, N., Eds.; In *High Performance Liquid Chromatography of Oxygenated Fatty Acids Including Enantiomer Separation*; CRC: Boca Raton, 1993; p 89.
16. Schneider, C.; Boeglin, W. E.; Brash, A. R. *Anal. Biochem.* **2000**, *287*, 186.
17. Ivanov, I.; Schwarz, K.; Holzhutter, H. G.; Myagkova, G.; Kuhn, H. *Biochem. J.* **1998**, *336*, 345.
18. Walther, M.; Ivanov, I.; Myagkova, G.; Kuhn, H. *Chem. Biol.* **2001**, *8*, 779.
19. Petrich, K.; Ludwig, P.; Kuhn, H.; Schewe, T. *Biochem. J.* **1996**, *314*, 911.
20. Salari, H.; Braquet, P.; Borgeat, P. *Prostagl. Leukotr. Med.* **1984**, *13*, 53.
21. Richards, K. M.; Marnett, L. J. *Biochemistry* **1997**, *36*, 6692.
22. Nieuwenhuizen, W. F.; van der Kerk van Hoof, A.; van Lenthe, J. H.; van Schaik, R. C.; Versluis, K.; Veldink, G. A.; Vliegenthart, J. F. *Biochemistry* **1997**, *36*, 4480.
23. Watson, C. S.; Eastman, J. F. *Organomet. Chem.* **1967**, *9*, 165.