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# SYNTHESIS OF TWO ANALOGUES OF ARACHIDONIC ACID AND THEIR REACTIONS WITH 12-LIPOXYGENASE

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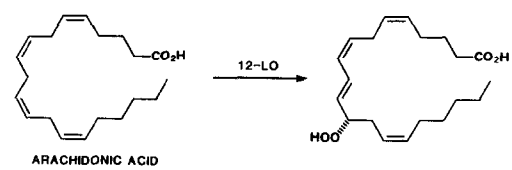
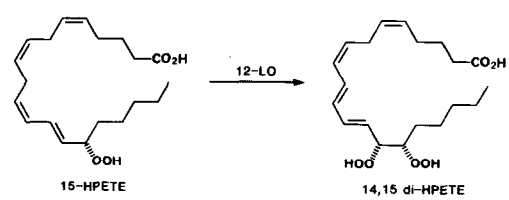
## ABSTRACT

Two analogues of arachidonic acid (AA) were synthesized and their reaction with purified porcine 12-lipoxygenase was investigated. The analogue (Z,Z,Z,E)-5,8,11,13 eicosatetraenoic acid **1** was found to be a substrate for the enzyme, being oxidized at one third the rate of AA. The structure of the lipoxygenation product, as well as its absolute stereochemistry, were determined by comparison of the enzymatic reaction product with a synthetic sample of known stereochemistry, prepared from L-arabinose. The oxygenation of **1** by 12-lipoxygenase occurred selectively at carbon 14 and yielded only the S-isomer. The second AA analogue (Z,Z,Z,E,E)-5,8,11,13,15 eicosapentaenoic acid **2** failed to give any detectable amount of product upon incubation with the enzyme. The results demonstrate that the 15-oxygenated function of 15-H(P)ETE is not a requirement for the stereoselective 14-oxygenation catalyzed by 12-lipoxygenase. Furthermore, these results support the proposal that AA binds in a horseshoe like conformation at the active site of lipoxygenase.

The lipoxygenases have attracted much attention since their discovery because of their key involvement in the arachidonic acid (AA) metabolic pathway.[1] An important aspect of the lipoxygenation reaction is the mechanism which allows for the stereoselective oxygen insertion at specific sites on the substrate molecule, since these enzymes are known to perform multiple oxygenation reactions.[2] The 12-lipoxygenase (12-LO) oxidation of AA involves the removal of one hydrogen from C-10, isomerisation of the 11,12-olefin and addition of dioxygen at C-12 (Scheme I). Aside of AA, 15-HPETE was also found[3] to be a substrate for 12-LO. It reacts to give an oxygenation product at C-14 (Scheme 2), which constitutes a 2 carbon shift in the position of oxygenation on the fatty acid chain. This reaction was of interest not only because it is likely to be involved in the formation of the lipoxins,[4] but also from a stereochemical point of view in terms of the conformation of the substrate in the active site of the enzyme. We therefore initiated a study of alternative substrates for the oxygenation reaction, by first preparing and testing an AA analogue in which no 15-oxygenated group was present (**1**), and also another substrate (**2**) in which the potential oxygenation position would be removed 2 more carbons towards the terminal methyl, namely at C-16. We herein describe the preparation of arachidonic acid analogues **1** and **2**, their oxydation by purified pig 12-LO and the stereochemical assignment of the enzymatic product of **1**.




SCHEME 1



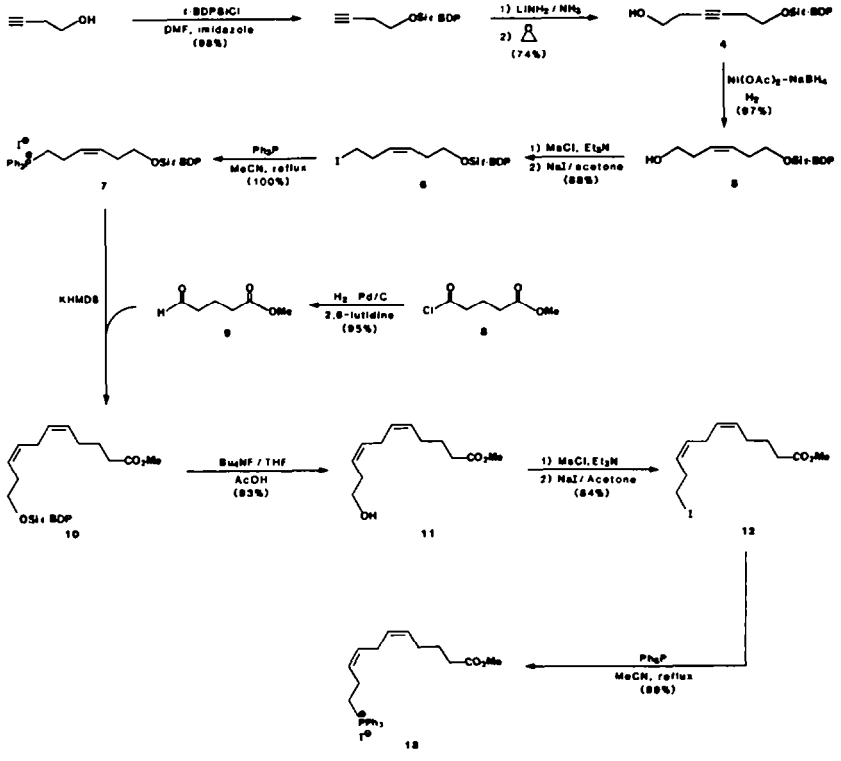

SCHEME 2

## RESULTS

The substrate 1 was synthesized as described in Scheme 3. Commercially available 3-butyn-1-ol was first protected with a t-butyldiphenylsilyl[5] group to give 3. The acetylenic proton was then removed with lithium amide in ammonia, and the anion was reacted with a large excess of ethylene oxide to generate 4. The use of the proper protecting group proved to be important in this sequence since a previous attempt at this 2 carbon homologation reaction using a tetrahydropyranyl protecting group gave much inferior yields and difficultly separable mixtures.

The next step consisted in the reduction of the alkyne 4 to the alkene 5, which was to become the 8,9-olefin of the products 1 and 2. This reduction was best performed with the "nickel boride" catalyst[6] which was more reproducible and efficient than the standard Lindlar catalyst. The terminal unprotected alcohol of 5 was then converted to the iodide 6 through the mesylate, and the iodide was in turn transformed to the phosphonium iodide 7 by the reaction with triphenylphosphine. This fragment represents the $C_6$-$C_{11}$ section of the molecule.

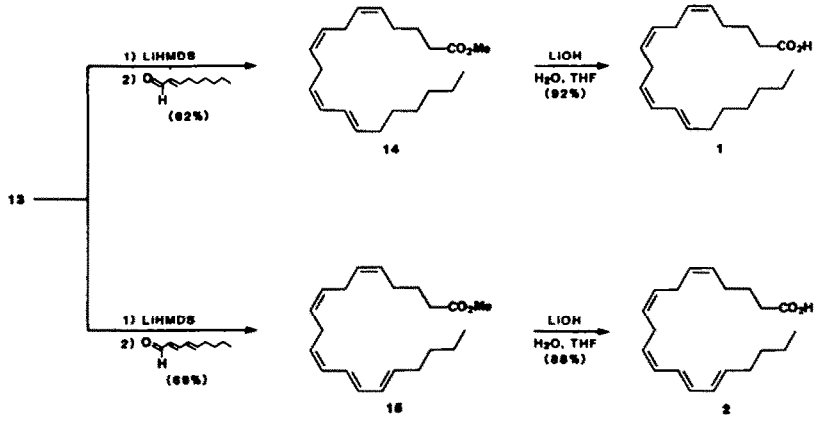
The $C_1$-$C_5$ fragment was prepared by Rosenmund reduction of the commercially available acid chloride 8. The resulting aldehyde 9 was then immediately coupled with the ylid derived from 7 to give 10, a synthon forming the head of the molecule and having a terminal protected hydroxyl for further elaboration of the tail section. The preparation for the junction of the top and bottom parts started with fluoride deprotection of 10 to the alcohol 11, followed by transformation to iodide 12 through the mesylate. Addition of triphenylphosphine then yielded the phosphonium salt 13 in near quantitative yield.



SCHEME 3

Both substrates 1 and 2 were prepared from the phosphonium salt 13 (Scheme 4). For the preparation of 1, the ylid from 13 was reacted with commercially available 2-nonenal to give a 62% yield of the diene ester 14. The ester was then hydrolyzed using lithium hydroxide in water-THF under nitrogen to give the desired 1 in 92% yield.

From the same ylid, the preparation of 2 involved a reaction with 2,4-nonadienal, affording a 69% yield the resulting ester triene 15 which was hydrolyzed with lithium hydroxide in water-THF, in the dark and under nitrogen, to give 88% yield of 2.




SCHEME 4

The stereochemistry of the isolated double bonds of 1 was Z, as evidenced by proton nmr. The diene was shown to be 11,12-Z, 13,14-E by n.O.e. measurements. This stereochemistry was expected from the synthetic steps used in the generation of the olefins.

The substrates 1 and 2 were incubated with immunoaffinity chromatography purified pig 12-LO and the reaction was followed by UV spectrophotometry. Incubation of 1 with 12-LO (Figure 1) resulted in a time dependent appearance of a new compound with UV absorption maxima at 264, 273.6 and 284 nm, analogous to those seen for conjugated triene compounds. The kinetics of the reaction monitored at 274 nm was characterized by a long lag phase at the start of the reaction, the optimal velocity being reached only after 70 sec. This lag phase was abolished by the addition of 2 μM (Z,E) 9,11-(13-hydroperoxy) octadecadienoic acid (13-HPOD) to the reaction mixture. The Km for substrate 1 was found to be 3.9 μM with a $V_{max}$ of 1.4 nmol/minute. The Km and $V_{max}$ obtained using AA as substrate under identical conditions were 27 μM and 4.8 nmol/minute, respectively. Incubation of 2 with 12-LO resulted in no detectable change in the UV spectrum of the reaction mixture.

In order to definitely establish the structure of the lipoxygenation product of 1 by 12-LO, we undertook the synthesis of 16, the reduced form (i.e. alcohol instead of peroxide) of the expected 14-lipoxygenation product of 1. Our strategy consisted in obtaining one enantiomer of 16 by total synthesis from a precursor of known stereochemistry, and to generate the other enantiomer through an oxidation-reduction racemization sequence.

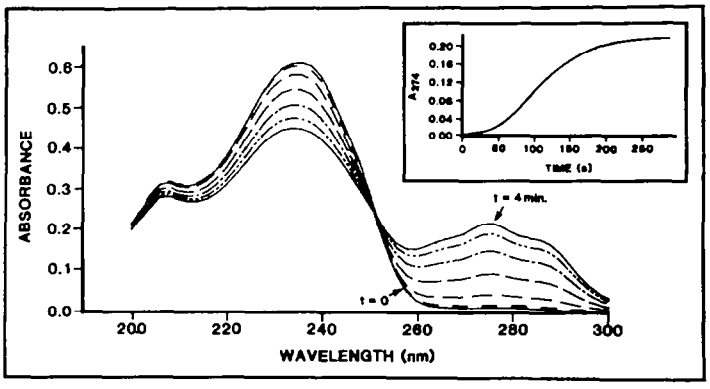
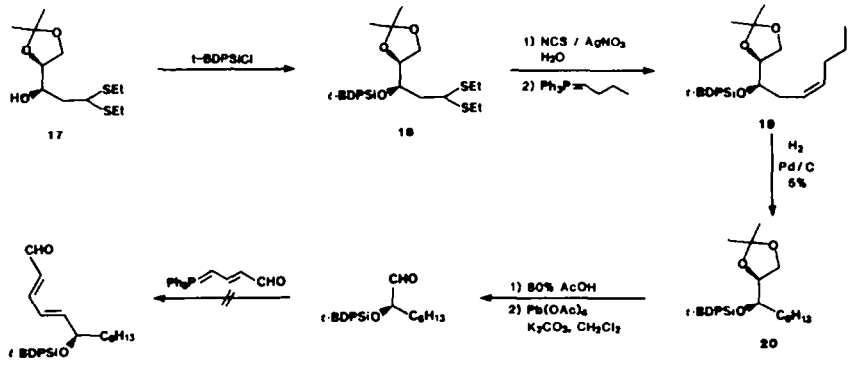



**Figure 1** Oxygenation of 1 by 12-LO. The UV spectrum of the reaction mixture containing 20 μM of 1 was monitored at time 0, 30, 60, 90, 120, 150 and 240 seconds after addition of the enzyme. The inset shows the absorbance of 274 nm as a function of time.

The alcohol 17 (Scheme 5) was readily obtained from L-arabinose by the Wong and Gray procedure.[7] It was then protected as the t-butyldiphenylsilyl ether 18. The thioacetal group was then hydrolyzed using the N-chlorosuccinimide-silver nitrate method,[8] and the unstable aldehyde was immediately reacted with the ylid derived from butyltriphenylphosphonium bromide to give the olefin 19. The alkene was reduced to the alkane 20 with $H_2$ and 5% Pd/C catalyst, and the course of this reaction could be easily monitored by TLC using silica gel plates impregnated with silver nitrate. This completed the preparation of the methyl tail end of the molecule.

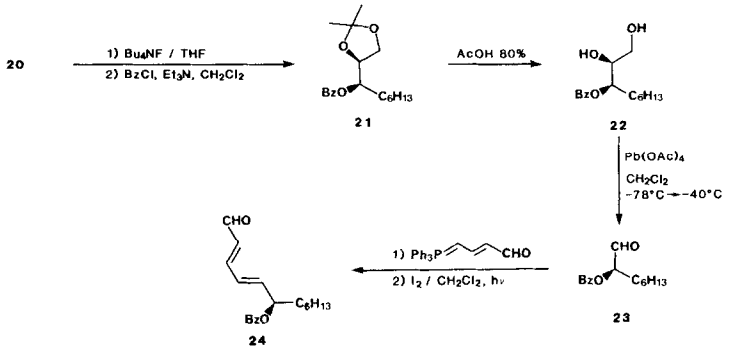



SCHEME 5

In order to assemble the middle section of the molecule, the acetonide group of 20 was removed by aqueous acid hydrolysis, and the resulting diol was cleaved with lead tetraacetate giving the aldehyde. All attempts to homologate this aldehyde (Scheme 5) had very limited success, typically <5% yield. Our past experience with this type of Wittig reaction suggested that the steric bulk of the silyl protecting group might have been the source of the problem, and we turned to different protecting group for the 14-hydroxyl group.

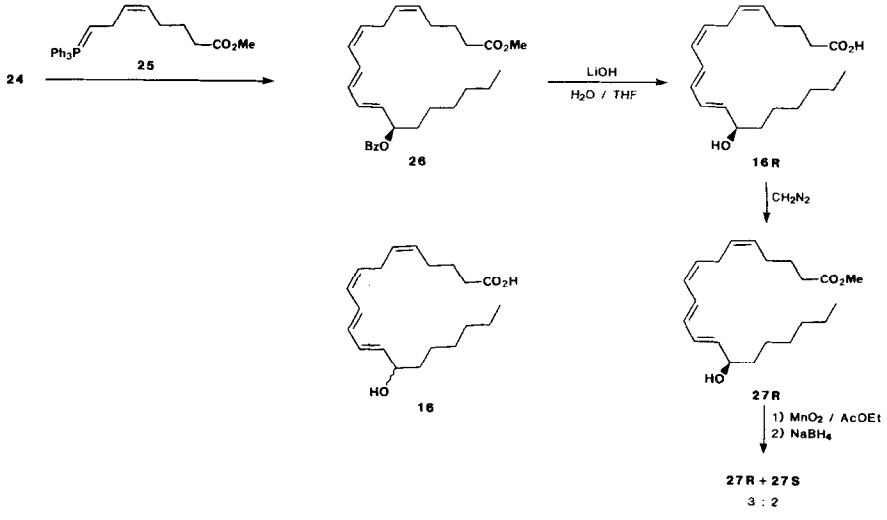
The silyl protecting group of 20 was therefore (Scheme 6) removed by fluoride treatment, and the resulting alcohol

was benzoylated to give **21**. Removal of the acetonide and diol cleavage as before smoothly afforded the aldehyde **23**. This aldehyde could then be homologated, with yields of 20-25%, to the dienal **24**, as a mixture of E and Z at the γ-olefin. The mixture could be isomerized to ca 90% E by neon light irradiation in the presence of a catalytic amount of iodine in dichloromethane.




SCHEME 6

The carboxylic acid head of the molecule (Scheme 7) was attached to **24** through a Wittig reaction using the known ylid **25**.[9] At this point, the two ester protecting groups were hydrolyzed to give the desired product **16R**. This product was compared with the enzymatic $NaBH_4$ treated product and was found to coelute in normal phase HPLC. As a further proof of their




SCHEME 7

identity, they were separately treated with diazomethane to give the corresponding methyl esters **27R** and **27E**, which again coeluted on normal phase HPLC.

The stereochemical assignment at C-14 was made as follows. The synthetic methyl ester **27R** was treated with $MnO_2$

until approximately half of the starting material had been converted to the trienone which eluted faster on normal phase HPLC. After a rapid work up, the mixture was treated with $NaBH_4$ in methanol, which reconverted the faster eluting trienone back to slower trienol. After workup, this mixture was injected on an ionic phenylglycine chiral HPLC column to resolve two peaks at 40.0 and 41.7 minutes, in 2:3 ratio (Figure 2b). The second peak coeluted with the methyl ester of original synthetic product 27R which was known to have the 14-(R) configuration as expected from its derivation from L-arabinose. This order of elution is in agreement with that reported for the many closely related HETE enantiomers.[10] The methyl ester of the $NaBH_4$ treated enzymatic product was then injected on the chiral column, and the chromatogram showed a single peak at 40.0 minutes (Figure 2a) indicating that the enzymatic reaction was totally stereoselective. Coinjection with the partly racemized mixture led to the enhancement of the first peak (Figure 2c), thereby assigning the 14-(S) stereochemistry to the enzymatic product.

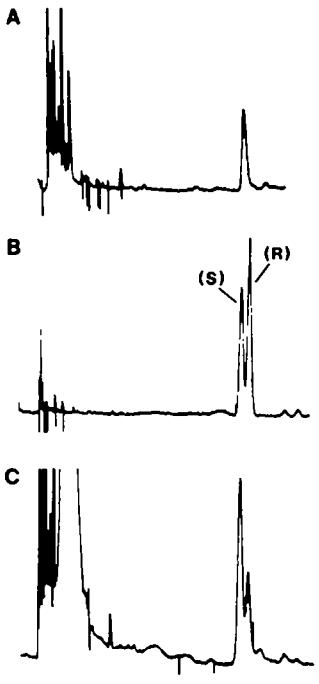



**Figure 2** Chiral phase HPLC of the $NaBH_4$ treated, methyl esterified enzymatic product (2a), of the partly racemized mixture of 27 (2b), and coinjection with the methyl ester derivative of the $NaBH_4$ treated enzymatic product (2c). The ionic phenyl glycine column was eluted with 100:0.5 hexane- isopropanol at a flow rate of 0.5 mL/minute.

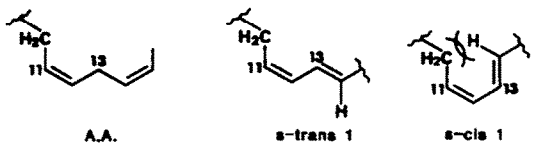
## DISCUSSION

The fact that 1 is a substrate for 12-LO and oxidized at a rate about one third that of arachidonic acid demonstrates the possibility of a direct 14-lipoxygenation in (Z,E)-11,13 dienes, such as 15-HPETE, 15-HETE and 5,15 di-HPETE. The 15-oxygenated function is therefore not an absolute requirement for these reactions, and the epoxide pathway in the 12-LO oxidation of 15-HPETE may very well be a minor one, as suggested by Maas and Brash.[3]

On the other hand, our stereochemical correlations clearly indicates the same stereochemistry (i.e. on the same face of the molecule) of lipoxygenation for 1, 15-HPETE and AA. One can therefore assume that these molecules adopt similar carbon skeleton conformations in the enzyme active site. As shown below, this determines the conformation of the 13-14 single bond of AA as being anti in the active site.Moreover, the conformation of the 12-13 bond can be deduced from our data. The radical or radicaloid species formed in the lipoxygenation reaction of 1 must have atoms 10 through 15 coplanar for optimal conjugation and minimum energy. Only two conformations therefore need to be considered, a s-cis and a s-trans. The s-cis can be readily ruled out as it has a very much higher energy than the s-transform, due to van der Waals radii penetration of the 14-H and the two 10-H. A MM2 calculation indeed indicates a 4.6 Kcal/mol preference for the s-trans form of the diene (a good model for the radical). This result indicates that 1 should be bound in the enzyme active site in the s-trans form, and would be in the horseshoe like conformation, at least in the C-10-C-16 region. This result nicely parallels the conformation of the C-4 through C-10 region of AA in the 5-LO active site, as demonstrated by Summers et al.[11]

The triene substrate 2, contrarily to 1 was found not to be oxidized by 12-LO. Our limit of detection for the tetraene product was such that the reactivity of 2 was at least 50-fold lower than that of 1. This was at first surprising since the formation of a radical or radicaloid from 2 is expected to be easier (less energetic) than that from 1. However, when the structure of 2 is

adjusted to fit in the horseshoe model, the 14,15 bond must lie in an s-cis conformation eclipsing two olefins, an energetically disfavored conformation. MM2 calculations indicates a cost of more than 2 Kcal/mol for this conformation. The lack of reaction of 2 with 12-LO may therefore be rationalized by the substrate's reluctance to adopt the proper conformation for binding.




## CONCLUSION

We have found that 12-LO can accept the modified arachidonic acid 1 as a substrate, and the products were found to be derived from a selective lipoxygenation reaction at C-14, from the same face of the molecule as for 15-HPETE, 15-HETE, 5,15 di-HPETE or AA substrate. From this result, we were able to determine the active site binding conformation of our substrate 1 and AA as having a W shape for the skip diene unit, in agreement with the published horseshoe conformation model.

## EXPERIMENTAL SECTION

NMR spectra were recorded as $CDCl_3$ solutions on a Bruker AM-250 instrument, and elemental analyses were performed by Galbraith. Immunoaffinity chromatography purified porcine 12-lipoxygenase[12] was obtained from T. Yoshimoto and S. Yamamoto, Tokushima University School of Medicine, Japan. UV spectra and kinetics were obtained on a Hewlett-Packard 8452A diode array spectrophotometer.

**1-(t-Butyldiphenylsilyloxy)-3-butyne 3.** A solution of 10 g (0.143 mol) of 3-butyn-1-ol in 100 mL of dry DMF was treated with 37.1 g (0.135 mol) of t-butyldiphenylsilyl chloride and 19.5 g (0.286 mol) of imidazole at 0°C. After the initial mixing, the ice bath was removed and the mixture stirred at room temperature for 8 h. The mixture was diluted with water and extracted with hexane, the organic phase was dried over $MgSO_4$ and evaporated to give 40.68 g (98%) of a colorless oil. nmr δ 7.7 (m, 4H), 7.4 (m, 6H), 3.78 (t, J = 7 Hz, 2H), 2.45 (dt, J = 2,7 Hz, 2H), 1.95 (d, J = 2 Hz, 1H), 1.06 (s, 9H). IR (neat) 3300, 3070, 1580 $cm^{-1}$. Anal. calcd for $C_{20}H_{24}OSi$: C, 77.87; H, 7.84; Si, 9.10. Found: C, 78.18; H, 7.76; Si, 9.44.

**6-(t-Butyldiphenylsilyloxy) 3-hexyn-1-ol 4.** In 300 mL of distilled ammonia under reflux were added 33 mL of a 1.6 M BuLi solution in hexane. After the addition was complete, 24 g (77 mmol) of alkyne 3 was added, followed by 38 mL (0.8 mol) of ethylene oxide. After 5 h, the mixture was canulated onto ice. This was then extracted with ether, the organic phase was washed with brine and dried over $Na_2SO_4$ to give a yellowish oil. This oil was purified by preparative medium pressure liquid chromatography using 10% AcOEt in hexane as the eluent to give 20.1 g (74%) of the desired alcohol 4. nmr δ 7.7 (m, 4H), 7.4 (m, 6H), 3.77 (br t, 2H), 3.66 (br q, 2H), 2.45 (br q, 4H), 2.0 (br s, 1H), 1.08 (s, 9H). IR (neat film) 3350 (br), 3070, 1580 $cm^{-1}$. Anal. calcd for $C_{22}H_{28}O_2Si$: C, 74.95; H, 8.01; Si, 7.97. Found: C, 75.00; H, 8.17; Si, 8.22.

**(Z) 6-(t-Butyldiphenylsilyloxy) 3-hexen-1-ol 5.** The hydrogenation catalyst was prepared as reported,[6] using 7.5 g of $Ni(OAc)_2 \cdot 4H_2O$, 175 mL of 95% ethanol, 0.95 g of $NaBH_4$ and 2.5 mL of ethylene diamine. A solution of 10 g (28.4 mmol) of alkyne 4 in 75 mL of ethanol was then added, and an atmosphere of $H_2$ was set over the reaction mixture. After 15 minutes stirring, the mixture was diluted with 1 volume of 1:1 AcOEt-hexane and filtered through a plug of silica gel. The filtrate was evaporated and the residual gelly was diluted in 1:1 AcOEt-hexane, and again filtered through a plug of silica gel. Evaporation yields 9.77 g (97%) of a colorless oil. nmr δ 7.69 (m, 4H), 7.41 (m, 6H), 5.54 (m, 2H), 3.66 (t, J = 7 Hz, 2H), 3.59 (br q, 2H), 2.35 (q, J = 7 Hz, 2H), 2.27 (q, J = 7 Hz, 2H), 1.5 (br s, 1H), 1.05 (s, 9H). Anal. calcd for $C_{22}H_{30}O_2Si$: C, 74.53; H, 8.53; Si, 7.92. Found: C, 73.80; H, 8.78; Si, 8.07.

**(Z) 1-(t-Butyldiphenylsilyloxy) 6-iodo-3-butene 6.** To a solution of 9.6 g (27.1 mmol) of the alcohol 5 in 60 mL of $CH_2Cl_2$ was added, at -45°C, 6.05 mL (43 mmol) of $Et_3N$. A solution of 2.73 mL (35 mmol) of MsCl in 40 mL of $CH_2Cl_2$ was then added slowly, and the solution was allowed to reach room temperature slowly. After 1/2 hr at room temperature, the mixture was diluted with 3 volumes of $CHCl_3$, washed successively with water, 10% $CuSO_4$ and brine, and dried with $MgSO_4$. After evaporation, the residue was dissolved in 150 mL of acetone, and treated with 16.2 g (108 mmol) of NaI at reflux. After 2 h, the suspension was diluted with 3 volumes of hexane and filtered. The solid was triturated with hexane, and the combined organic phases were evaporated. The residue was redissolved in hexane, filtered and the filtrate evaporated. Purification by flash chromatography in 2% ether in hexane afforded 11.05 g (88%) of the iodide 6. nmr δ 7.66 (m, 4H), 7.40 (m, 6H), 5.47 (m, 2H), 3.67 (t, J = 7 Hz, 2H), 3.08 (t, J = 7 Hz, 2H), 2.56 (q, J = 7 Hz, 2H), 2.28 (q, J = 7 Hz, 2H), 1.05 (s, 9H). MS (CI, $NH_3$) calcd for $C_{22}H_{29}IOSi$: 465.11107. Found: 465.11055.

**6-(t-Butylsilyloxy) 3-buten-1-yl triphenylphosphonium iodide 7.** A solution of 10.85 g (23.4 mmol) of iodide **6** and 6.43 g (24.6 mmol) of triphenylphosphine in 47 mL of acetonitrile was refluxed for 48 hours. The solvent was then evaporated, and the thick oil was dissolved in 100 mL of benzene. With rapid stirring, 100 mL of hexane was added. A yellow oil separated which was dried under high vacuum, to give 17.41 g (100%) of desired compound. nmr δ 7.8-7.2 (m, 25H), 5.7 (m, 1H), 5.5 (m, 1H), 5.8-5.5 (m, 4H), 2.4 (m, 2H), 2.08 (br q, 2H), 0.95 (s, 9H). Anal. calcd for $C_{40}H_{44}IOPSi$: C, 66.11; H, 6.10; I, 17.46, P, 4.26; Si, 3.86. Found: C, 65.72; H, 6.14; I, 17.79; P, 4.77; Si, 4.60.

**(Z,Z)Methyl 11-(t-butyldiphenylsilyloxy) 5,8-undecadienoate 10.** To a solution of 2 g (2.75 mmol) of the phosphonium salt **7** in 15 mL of THF and 1.8 mL of HMPA, at -78°C was added 0.95 mL of a 1.6 M BuLi in hexane solution. The solution turned deep red. After 45 minutes, a solution of 488 mg (3.75 mmol) of aldehyde **9** in 3 mL of THF was added to the ylid solution, which discolored to pale yellow. This solution was allowed to warm up to room temperature, and was then dissolved with two volumes of hexane to separate an oil. The oil was placed on a plug of silica gel, which was rinsed with 1:9 AcOEt-hexane. After evaporation, the residue was purified by flash chromatography in 4:96 AcOEt-hexane to give 953 mg (93% based on BuLi) of a colorless oil. nmr δ 7.67 (m, 4H), 7.3 (m, 6H), 5.35 (m, 4H), 3.66 (t, J = 7 Hz, 5H) , 2.72 (br t, 2H), 2.32 (m, 4H), 2.08 (q, J = 7 Hz, 2H), 1.69 (quintet, J = 7 Hz, 5H), 1.05 (s, 9H). Anal. calcd for $C_{39}H_{52}O_2Si$: C, 74.62; H, 8.50; Si, 6.23. Found: C, 74.27; H, 8.52; Si, 6.84.

**(Z,Z)Methyl 11-hydroxy 5,8-undecadienoate 11.** To 953 mg (2.32 mmol) of silyl ether **10** were added 1.4 mL of acetic acid, and then 9.3 mL of a 1 M solution of $Bu_4NF$ in THF. This solution was stirred overnight, and the conversion was completed by 3 h heating at 45°C. The reaction mixture was then diluted with 1 volume of water, extracted 3 times with 2 volumes of ether, and the organic phases were dried over $MgSO_4$. After evaporation, the residue was purified by flash chromatography to give 459 mg (93%) of a colorless oil. nmr δ 5.5-5.3 (m, 4H), 3.68 (s, 3H), 3.66 (t, J = 7 Hz, 2H), 2.81 (t, J = 6 Hz, 2H), 2.35 (m, 4H), 2.11 (m, 2H), 1.70 (quintet, J = 7 Hz, 2H), 1.63 (br s, 1H). IR (neat) 3400 (br), 2950, 1735 $cm^{-1}$

**(Z,Z)Methyl 11-iodo-5,8-undecadienoate 12.** A solution of 600 mg (2.83 mmol) of alcohol **11** and 630 µL (4.53 mmol) of $Et_3N$ in dry $CH_2Cl_2$ (6 mL) was cooled to -20°C and 283 µL (3.68 mmol) of methanesulfonyl chloride was added. After 5 minutes, the reaction was quenched by the addition of 5 mL water, and the mixture was allowed to reach room temperature. The mixture was diluted with chloroform, the organic layer was separated and dried over $MgSO_4$ before evaporation. This mesylate was immediately reacted with 1.7 g (11.3 mmol) of NaI in 15 mL of acetone, under gentle reflux for 90 minutes. The reaction mixture was then cooled to room temperature, diluted with 4 volumes of hexane, filtered and the filtrate was evaporated. Purification by flash chromatography afforded 765 mg (84%) of the title compound as a colorless oil. nmr δ 5.6-5.3 (m, 4H), 3.67 (s, 3H), 3.14 (t, J = 7 Hz, 2H), 2.76 (br t, 2H), 2.65 (br q, J = 7 Hz, 2H), 2.32 (t, J = 7 Hz, 2H), 2.10 (br q, J = 6 Hz, 2H), 1.70 (quintet, J = 7 Hz, 2H). IR (neat) 3000, 2950, 1735 $cm^{-1}$. Anal. calcd for $C_{12}H_{19}IO_2$: C, 44.74; H, 5.94; I, 39.39. Found: C, 44.58; H, 5.96; I, 41.77.

**((Z,Z)Methyl 5,8-undecadienoate-11-yl)triphenylphosphonium iodide 13.** The iodide **12** (225 mg, 0.70 mmol) was dissolved in 2.5 mL of acetonitrile, and 210 mg (0.80 mmol) of triphenylphosphine were added. The mixture was refluxed overnight, and then the solvent was allowed to evaporate at 80°C. The residual gum was suspended in 4 mL of benzene and 4 mL of hexane and the supernatant was discarded. After repeating this treatment once more, the residual colorless oil was dried under high vacuum at 100°C to give 407 mg (100%) of the expected product, used as such in the following step.

**(Z,Z,Z,E)Methyl 5,8,11,13 eicosatetraenoate 14.** At -78°C, 120 µL of a 2.63 M solution (0.316 mmol) of BuLi in hexane was added to a solution of 73 µL (0.347 mmol) of hexamethyldisilazane in 1 mL of THF and 0.25 mL of HMPA. The reaction temperature was raised to 0°C for 3 minutes, and cooled back to -78°C, where a solution of 203 mg (0.347 mmol) of the phosphonium salt **13** in 1.5 mL of THF was added. The reaction mixture turned orange and a solid appeared. After stirring at -78°C for 1/2 h, 79 µL (0.47 mmol) of (E) 2-nonenal were added, which turned the orange color to yellow. The cooling bath was then removed, and the reaction was stirred at room temperature for 50 minutes. Dilution of the reaction mixture with hexane formed a gummy phase. The liquid phase was separated and evaporated. Flash chromatography afforded 62 mg (62%) of a colorless oil. nmr δ 6.35 (dd, J = 11,14 Hz, 1H), 5.97 (br t, 1H), 5.69 (dt, J = 15,7 Hz, 1H), 5.5-5.3 (m, 4H), 5.25 (m, 1H), 3.67 (s, 3H), 2.93 (br t, 2H), 2.81 (br t, 2H), 2.32 (t, J = 7 Hz, 2H), 2.1 (m, 4H), 1.70 (quintet, 2H), 1.27 (br s, 8H), 0.88 (t, 3H). Anal. calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.31; H, 10.78.

**(Z,Z,Z,E,E)Methyl 5,8,11,13,15 eicosapentaenoate 15.** Proceeding as described for **14**, but using (E,E) 2,4-nonadienal as the aldehyde, 69 mg (69%) of **15** were obtained as a yellow oil. nmr δ 6.41 (dd, J = 12,14 Hz, 1H), 7.3-7 (m, 3H), 5.72 (dt, J = 15,7 Hz, 1H), 5.4 (m, 5H), 3.67 (s, 3H), 2.95 (br t, 2H), 2.81 (br t, 2H), 2.33 (t, J = 7 Hz, 2H), 2.11 (br q, 4H), 1.71 (quintet, J = 6 Hz, 2H), 1.4 (m, 4H), 0.90 (t, J = 7 Hz, 3H). Anal. calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 80.51; H, 10.48.

**(Z,Z,Z,E) 5,8,11,13 Eicosatetraenoic acid 1.** A solution of 50 mg (157 mmol) of the ester **14** in 1.5 mL of THF was treated with 930 µL of 0.33 M LiOH in water. After 14 h, the solution was heated to 50°C for 2 h to complete the reaction.

The mixture was then diluted with 1 mL of 5% citric acid and extracted with $CHCl_3$. After evaporation, the residue was prepurified by flash chromatography to give 44 mg of material. A small portion of this was purified by HPLC on a reversed phase C18 column, with an 85:15:1 methanol, water, acetic acid eluent. The peak at 23 minutes was collected, and 10 mM solution in ethanol was prepared for biochemical use.

**(Z,Z,Z,E,E) 5,8,11,13,15 Eicosapentaneoic acid 2.** As described for 1, but in the dark at all time, HPLC pure 2 was prepared.

**(4S)4-(Z)(1R)1-t-Butyldiphenylsilyloxy-3,3-dithioethyl propyl) 2,2-dimethyl-1,3-dioxolane.** The alcohol 17 was obtained from L-arabinose, using the published procedure.[7] A solution of 35.5 g (0.126 mol) of 17 in 150 mL $CH_2Cl_2$ was treated with 51.8 g (0.189 mol) t-butyldiphenylsilylchloride, 25.5 g (0.252 mol) of $Et_3N$ and 15.4 g (0.126 mol) of 4-dimethylaminopyridine. After 9 h of reflux, the mixture was poured into ice cold 0.5 N HCl and the organic phase was separated. Drying over $Na_2SO_4$ and evaporation yielded an oil which was purified by medium pressure liquid chromatography on silica gel using 2% AcOEt in hexane eluent to give 41.2 g (63%) of a colorless oil. nmr δ 7.75 (m, 4H), 7.4 (m, 6H), 4.1 (br t, 2H), 3.9 (m, 2H), 3.6 (br t, 1H), 2.4 (m, 4H), 2.0 (m, 2H), 1.55 (s, 3H), 1.3 (s, 3H), 1.27 (s, 3H), 1.12 (t, J = 7 Hz, 3H), 1.10 (t, J = 7 Hz, 3H), 1.04 (s, 6H). $[\alpha]_D$ -13.6 (c 3.3, $CHCl_3$). Anal. calcd for $C_{28}H_{42}O_3S_2Si$: C, 64.82; H, 8.16; Si, 5.41; S, 12.36. Found: C, 65.27; H, 8.55; Si, 7.15; S, 11.59.

**(4S)4-((Z)(1R)1-t-Butyldiphenylsilyloxy 3-heptenyl) 2,2-dimethyl 1,3-dioxolane 19.** The thioacetal 18 (4.8 g, 9.26 mmol) was dissolved in 30 mL of acetonitrile and this was added to a solution of 4.34 g of N-chlorosuccinimide and 5.34 g of $AgNO_3$ in 125 mL of 4:1 acetonitrile-water, at -10°C under $N_2$, over the course of 1/2 h. Ten minutes after the addition was complete, 2 mL of DMSO were added, and stirring was continued for 15 minutes. The reaction mixture was then neutralized by the addition of 80 mL of 1M, pH 7.21 phosphate buffer. Extraction of the mixture with $CH_2Cl_2$, followed by dilute pH 7.21 buffer wash, $Na_2SO_4$ drying and evaporation gave a residual oil. This oil was purified by filtration through 200 mL of silica gel, which was washed with 600 mL of 1:4 AcOEt-hexane. Evaporation gave the purified aldehyde which was immediately diluted with 20 mL of dry THF and used in the following reaction. In a different flask at -78°C under $N_2$, a suspension of 7.4 g of butyltriphenylphosphonium bromide in 70 mL of THF was treated with 10.6 mL of a 1.6 M solution of BuLi in hexane. The reaction was stirred at room temperature for 1 h, and cooled back to -78°C where the aldehyde solution was added over 1/2 h. The pale orange solution was allowed to reach room temperature, and was quenched 1/2 hr later with 1 mL of AcOH. Dilution with 3 volumes of hexane separated a gummy solid, and the supernatant was evaporated. Flash chromatography of the residue was 3:77:20 AcOEt-hexane-$CH_2Cl_2$ gave 901 mg (43%) of the title compound. nmr δ 7.71 (m, 4H), 7.39 (m, 6H), 5.35 (m, 2H), 4.06 (m, 1H), 3.92 (m, 2H), 3.80 (t, J = 8 Hz, 1H), 2.20 (m, 1H), 2.08 (m, 1H), 1.78 (q, J = 6 Hz, 2H), 1.33 (s, 3H), 1.30 (s, 3H), 1.25 (m, 2H), 1.06 (s, 9H), 0.81 (t, J = 7 Hz, 3H). Anal. Calcd for $C_{28}H_{40}O_3Si$: C, 74.29; H, 8.91; Si, 6.20. Found: C, 74.26; H, 8.87; Si, 6.28. $[\alpha]_D$ -36.4° (c 1.5, $CHCl_3$).

**(4S)4-((1R)1-benzoyloxyheptyl)2,2-dimethyl 1,3-dioxolane 21.** The alkene 19 (2.72 g) as an AcOEt solution was first reacted with $H_2$ at atmospheric pressure, with 10% Pd/c catalyst for 15 h. Filtration through celite and evaporation gave the reduced compound. A mixture of 1.5 g of this oil with 800 mL of AcOH was then treated with 33 mL of a 1 M solution of $Bu_4NF$ in THF. After 28 h at 50°C, the mixture was partitioned between water and ether, and the organic phase was dried with $MgSO_4$ and evaporated to dryness. This residue was taken into 1.1 mL of $CH_2Cl_2$ and 2.3 mL of $Et_3N$. After cooling to -10°C, 1.15 mL of benzoyl chloride were added, and the mixture was left at room temperature for 30 h. The solution was then diluted with $CH_2Cl_2$, washed with water, 15% citric acid and dried over $MgSO_4$. Flash chromatography with 15% ether in hexane eluent gave 1.09 g of a colorless oil. nmr δ 8.05 (dd, J = 1, 8 Hz, 2H), 7.7-7.3 (m, 3H), 5.26 (dt, J = 7,6 Hz, 1H), 4.26 (q, J = 6 Hz, 1H), 4.06 (dd, J = 7,8 Hz, 1H), 3.93 (dd, J = 7,8 Hz, 1H), 1.8 (m, 2H), 1.5-1.2 (m, 14H), 0.86 (br t, 3H). IR (neat) 1725 $cm^{-1}$. Anal. Calcd for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found: C, 71.66; H, 9.03. $[\alpha]_D$ -0.3 (c 1, $CHCl_3$); -0.1 (c 1, EtOH)

**(S,R) 3-benzoyloxy nonane-1,2-diol 22.** The acetonide 21 (330 mg, 1.03 mmol) was dissolved in 10 mL of 80% AcOH and heated at 45°C for 7 h. Evaporation to dryness yielded 288 mg of a colorless oil. An analytical sample was obtained by flash chromatography with 2:3 AcOEt-hexane eluent. nmr δ 8:05 (dd, J = 1,7 Hz, 2H), 7.60 (tt, J = 2,7 Hz, 1H), 7.46 (t, J = 7 Hz, 2H), 5.07 (m, 1H), 3.64 (m, 3H), 2.67 (br s, 1H), 2.56 (br s, 1H), 1.97 (m, 2H), 1.35 (m, 8H), 0.86 (br t, 3H). MS m/e calculated for $C_{16}H_{32}O_4$: 281.17528. Found: 281.17519. $[\alpha]_D$ +34.9 (c 2.1, $CHCl_3$)

**(R) 2-Benzoyloxy octanal 23.** Under $N_2$, 30 mg of the diol 22 were dissolved in 800 mL of $CH_2Cl_2$, and 117 mg of finely milled $Na_2CO_3$ were added. The temperature was lowered to -78°C and under strong stirring, 66 mg of $Pb(OAc)_4$ were added. The bath was allowed to reach -40°C over 1 h, and the reaction was quenched by pouring it on a short silica gel column, which was eluted with 1:9:5 AcOEt-hexane-$CH_2Cl_2$. The eluate was evaporated to dryness to give 24.3 mg (85%) of a colorless oil, which is normally used immediately in the next reaction. nmr δ 9.63 (d, J = 1 Hz, 1H), 8.09 (m, 2H), 7.8-7.3 (m, 3H), 5.22 (dd, J = 5,8 Hz, 1H), 1.92 (m, 2H), 1.6-1.2 (m, 8H), 0.89 (t, J = 7 Hz, 3H). MS m/e calcd for $C_{15}H_{21}O_3$: 249.14907. Found: 249.14896. $[\alpha]_D$ +30.2° (c 1.2, $CHCl_3$)

**(E,E,R) 6-Benzoyloxy 2,4-dodecadienal 24.** A solution of 40 mg of fresh aldehyde 23 in 400 mL of DMSO was treated with 57 mg of 4-oxo butenylenetriphenylphosphorane. After 2 h at room temperature, the reaction mixture was diluted with 5 volumes of water and extracted twice with ether. After drying with $MgSO_4$, the solution was filtered quickly through a plug of silica gel, which was rinsed with ether. Evaporation of the ether below 0°C was immediately followed by dilution with a solution of 4 mg of $I_2$ in 5 mL of $CH_2Cl_2$. This solution was irradiated under a neon light for 5 minutes, then washed with $Na_2S_2O_3$ and dried with $MgSO_4$. Evaporation at sub-zero temperature immediately followed by dissolution in THF affords the desired aldehyde, ready for use in the next step. nmr δ 9.57 (d, J = 8 Hz, 1H), 8.07 (dd, J = 1,8 Hz, 2H), 7.7-7.3 (m, 3H), 7.09 (dd, J = 10,15 Hz, 1H), 6.54 (dd, J = 11, 15 Hz, 1H), 6.2 (m, 2H), 5.62 (q, J = 6 Hz, 1H), 1.84 (m, 2H), 1.32 (m, 8H), 0.88 (br t, 3H).

**(Z,Z,E,E,R) Methyl 14-benzoyloxy 5,8,10,12 eicosatetraenoate 26.** A solution of 51 µL of hexamethyldisilazane in 1 mL of THF was treated, at -78°C, with 140 µL of a 1.6 M solution of BuLi in hexane. The solution was then warmed up to 0°C for 15 minutes, and cooled back to -78°C where 130 mg of ((Z) methyl 5-octenoate-11-yl)triphenylphosphonium bromide (25) were added. After stirring for 20 minutes, the previously described aldehyde 24 solution in THF was added. This solution was protected from light and allowed to reach room temperature, where it was neutralized with pH 7.21 phosphate buffer. The resulting mixture was extracted with 1:1 ether-hexane, and the organic phase was dried with $MgSO_4$. Flash chromatography gave an oil which was then purified by HPLC, protecting the eluate from light and oxygen. nmr δ 8.05 (m, 2H), 7.6-7.4 (m, 3H), 6.53 (dd, J = 11,15 Hz, 1H), 6.40 (dd, J = 12,15 Hz, 1H), 6.18 (dd, J = 11,15 Hz, 1H), 6.01 (t, J = 11 Hz, 1H), 5.78 (m, 2H), 5.6-5.3 (m, 4H), 3.66 (s, 3H), 2.95 (m, 2H), 2.32 (t, J = 8 Hz, 2H), 2.12 (m, 2H), 1.9-1.5 (m, 4H), 1.5 (m, 4H), 1.5-1.2 (m, 10H), 0.88 (br t, 3H). $[\alpha]_D$ -123° (c 1.1, $CHCl_3$). UV $\lambda_{max}$ 273.7 nm, $\varepsilon_{max}$ $3.0 \times 10^4$ (EtOH)

**(Z,Z,E,E,R) 14-Hydroxy 5,8,10,12 eicosatetraenoic acid 16R.** The diester 26 (5 mg) was dissolved in MeOH (200 µL) in the dark, under $N_2$, and 305 µL of a 1 M LiOH solution were added. Stirring was continued at 40°C for 3 h. The mixture was then quenched with 15% citric acid and extracted with ether. After evaporation, the residue was purified by normal phase HPLC (15% AcOEt, 1% AcOH in hexane eluent), protecting the eluate from light and air. nmr δ 6.55 (dd, J = 11,14 Hz, 1H), 6.35 (dd, J = 10,14 Hz, 1H), 6.22 (dd, J = 10,14 Hz, 1H), 6.04 (t, J = 11 Hz, 1H), 5.83 (dd, J = 8, 14 Hz, 1H), 5.6-5.3 (m, 3H), 4.20 (m, 1H), 2.95 (br t, 2H), 2.39 (t, J = 8 Hz, 2H), 2.3-2.0 (m, 2H), 1.8-1.6 (m), 1.25 (br s, 10H), 0.88 (br t, 3H).

## SPECTROPHOTOMETRIC ASSAY OF 12-LIPOXYGENASE

Enzyme activity with the various substrates was monitored by UV spectrophotometry. Standard reaction mixtures contained 494 µL of 50 mM Tris-HCl, pH 7.4, 0.03% tween 20 and 5 µL of a concentrated fatty acid substrate solution in ethanol. The reaction was initiated by adding 1 µL of the enzyme solution (the protein concentration was 0.48 mg/mL) and the UV spectrum in the 200-350 nm range was recorded every 4 seconds. Product formation was calculated based on molar absorption coefficients of 30,000 $M^{-1}cm^{-1}$ at 274 nm for the triene product and 23,000 $M^{-1}cm^{-1}$ at 234 nm for the 12-HPETE 1. Enzyme activity was calculated from the optimal velocity attained after the initial lag phase.

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