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# Enzymatic asymmetric hydroxylation of unnatural substrates with soybean lipoxygenase

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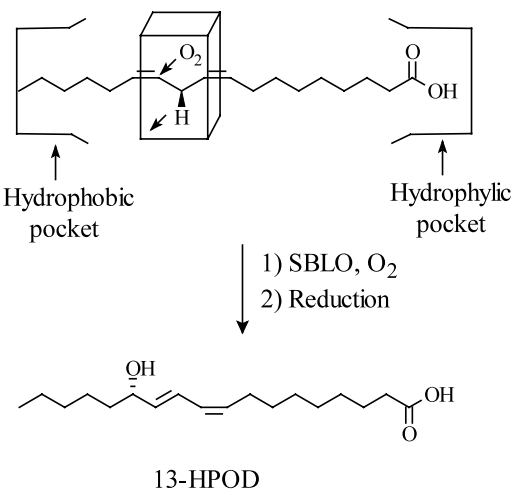
**Abstract**—Surrogate substrates mimicking the natural substrate (linoleic acid) bearing a spacing prosthetic group with a non-ionic hydroxyl terminus undergo asymmetric hydroxylation with soybean lipoxygenase. The prosthetic modifier supplies the missing structural features needed for enzymatic recognition and controls the regiochemical outcome of the reaction by its high hydrophobic content. The effect of pH on the regiochemistry clearly shows that all the substrates can arrange themselves at the active site of soybean lipoxygenase in only one orientation leading to formation of hydroperoxides by oxygenation at the ω-6 carbon. © 2001 Published by Elsevier Science Ltd.

## 1. Introduction

The lipoxygenases are a group of non-heme iron-containing dioxygenases which catalyze the addition of molecular oxygen to polyunsaturated fatty acids in a stereospecific and regiospecific way[1] (Scheme 1). They are involved in the biosynthesis of inflammatory mediators in cell differentiation and atherogenesis.[2] Previous studies of modified substrates with soybean lipoxygenase suggest that the essential structural requirements




**Scheme 1.** Substrate recognition by SBLO.

are a single ω-6-(*Z*,*Z*)-1,4-pentadienyl moiety and an appropriately spaced proximal carboxyl group as characterized by the natural substrate linoleic acid.[3] It had long been considered that the carboxyl group in the fatty acid was important for enzymatic recognition and binding.[4] However, Harris et al.[5] reported that glycerol esters of linoleic acid bearing an ionic phosphatidylcholine group are also substrates for the enzyme. Despite its high degree of stereo- and regioselectivity, soybean lipoxygenase has attracted little attention from a synthetic point of view.[6] As part of our continuing effort[7] to explore the synthetic utility of SBLOX, we describe herein an enzymatic method for the asymmetric hydroxylation of unnatural synthetic substrates **1a**–**1c** bearing a spacing prosthetic modifier, which contains a non-ionic hydroxyl terminus as shown below (Scheme 2). The primary purpose of the prosthetic modifier is to supply the missing structural features needed for enzymatic recognition and to influence the regiochemistry of the reaction by its high hydrophobic content.

## 2. Results and discussion

### 2.1. Synthesis

The substrates **1a**–**1c** were synthesised from a common precursor 3-butyn-1-ol, which was alkyated with $LiNH_2$ and bromopentane to give 3-nonyn-1-ol. Hydrogenation over Lindlar catalyst gives (*Z*)-3-nonen-1-ol in 90% yield, which upon treatment with iodine, triphenylphosphine and imidazole[8] gives the corre-

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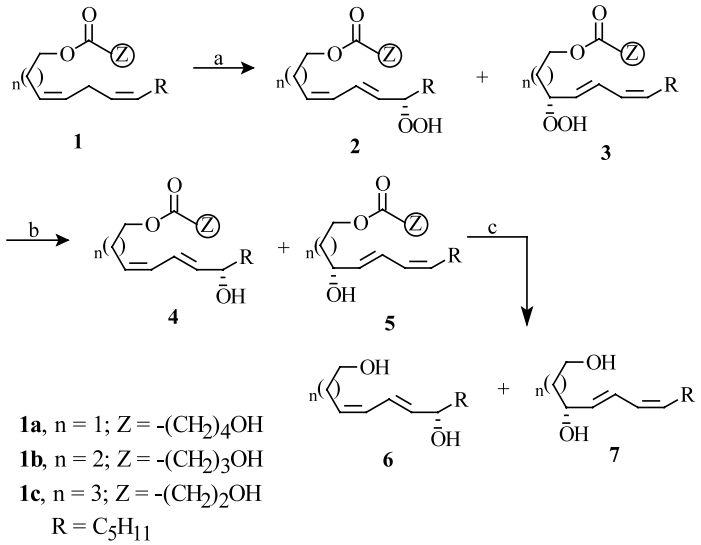



**Scheme 2.** Asymmetric hydroxylations of unnatural substrates **1a**–**1c** with soyben lipoxygenase. *Reagents and conditions*: (a) SBLO, $O_2$, 0.2 M sodium borate buffer (pH 9), 0°C, 1 h; (b) TPP, ether; (c) KOH, MeOH, rt, 12 h.

sponding iodo compound in 85% yield. The iodo compound when reacted with triphenylphosphine in refluxing acetonitrile yields the phosphonium salt (Scheme 3). The dienoic alcohols with (*Z*,*Z*) geometry were prepared by condensation of the ylide derived from the phosphonium iodide **8** with the appropriate aldehydic partner **9** and subsequent removal of the tetrahydropyranyl (THP) protecting groups with *p*-toluenesulphonic acid in methanol (Scheme 4). All the dienoic alcohol products have >95% (*Z*,*Z*) geometry, as determined by GC analysis.

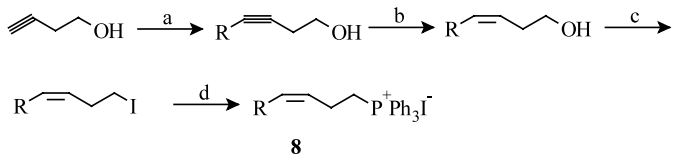
Coupling of the dienols with mono *p*-methoxybenzyl protected acids derived from the appropriate diols under DCC/DMP conditions afforded the coupled esters **11** in 80% yield. Removal of the 4-methoxybenzyl group with DDQ[9] furnished the lipoxygenase substrates **1a**–**1c** in 80% yield (Scheme 5). All the products were characterised by $^1H$ and $^{13}C$ NMR spectroscopy.

For the enzymatic oxidation reactions, solutions of substrates **1a**–**1c** in 0.2 M sodium borate buffer (pH 9) at 0°C were treated with lipoxygenase type-1 (activity = 127,000 units/mg of protein). The reaction mixture was then stirred under a stream of oxygen for 1 h. The reaction was stopped by addition of ethanol and water, extracted with ether and evaporated to give the crude hydroperoxide, which was then converted to alcohol by treatment with triphenylphosphine. No isomerization was observed in the reduction of the hydroperoxides to the corresponding alcohols **5**. The prosthetic group then was removed by hydrolysis with KOH/MeOH to give the chiral diols, which have comparable specific rotation values to those previously reported.[12]

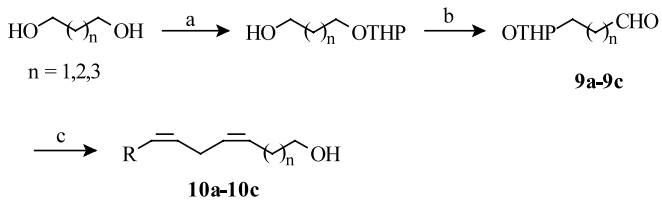
## 2.2. Regiochemistry

The present study shows that synthetically useful chiral diols can be obtained in high chemical yield and enantiomeric purity through lipoxygenase-catalyzed transformation of unnatural substrates. These chiral diols **6a**–**6c** can be efficiently employed for the total synthesis of some unsaturated hydroxy fatty acids (Scheme 6). Currently, we are actively engaged in the total synthesis of some hydroxy fatty acids and their analogs, which have tremendous biological importance.

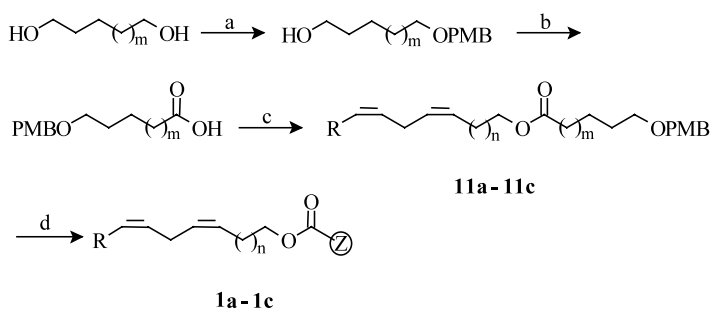
In all of the above cases oxygenation occurs at the appropriate olefinic site yielding the product **2**, whereas unwanted formation of **3** by attack of oxygen at the other olefinic site is minimal. This clearly indicates that substrates **1a**–**1c** preferentially arrange themselves at the active site of SBLO-1 in an orientation leading to



**Scheme 3.** *Reagents and conditions*: (a) $LiNH_2$; THF/HMPA (6:1), $C_5H_{11}Br$; (b) $H_2$, Lindlar catalyst; (c) $I_2$, TPP/imidazole; acetonitrile:ether (1:3); (d) TPP, acetonitrile, (reflux, 48 h).



**Scheme 4.** *Reagents and conditions*: (a) DHP/TsOH 1; (b) IBX/DMSO, rt 6 h; (c) **8**, *n*-BuLi, THF–HMPA (10:1), 0°C, 2 h, MeOH, TsOH, 80%.



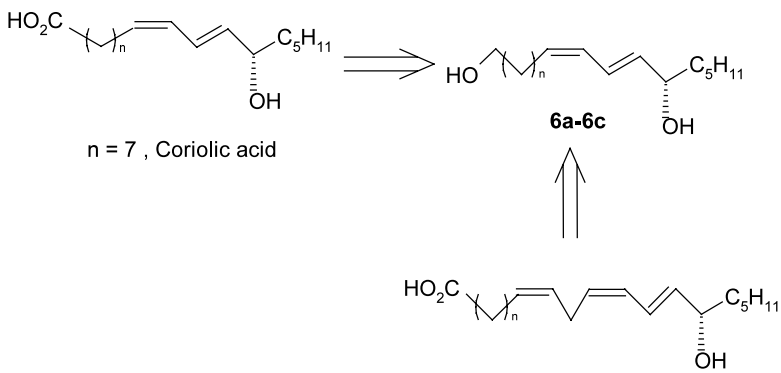

**Scheme 5.** *Reagents and conditions*: (a) 4-Methoxybenzylbromide, NaH, $n$-$Bu_4N^+I^-$; (b) $CrO_3$, $H_2SO_4$ (8N); (c) **10a**–**10c** DCC, DMAP; (d) DDQ, DCM–$H_2O$ (19:1), rt.

formation of **2** by oxygenation at the ω-6 carbon, whereas formation of **3** by attack of oxygen at the ω-10 carbon is possible only if the substrate arranges itself head to tail (i.e. in opposite orientation) at the active site of SBLO-1.

The effect of pH on the regiochemistry (Table 2) also proves this hypothesis. It was reported previously[3,10] that the difference in the hydrophobic content between the proximal and the distal unit significantly influences the regioselectivity of the oxygenation reaction. The current study also shows the relevance of hydrophobic interactions to the regioselectivity of oxygenation reactions with lipoxygenase-1. From Table 1 it is clear that all of the three substrates show excellent regiospecificity, since the hydrophobicity of the $C_5H_{11}$ (log $p=+2.8$) is much higher than that of the hydroxylated terminus[12] (log $p=-3.7$). The reactions of compounds **1a**–**1c** displayed similar regioselectivity to that of the natural substrate linoleic acid because the proximal units are of similar lengths (10.6 and 10.9 Å). It indicates that the orientation of the substrate is solely determined by the size of the binding pocket, whereby the enzyme preferentially binds the group that has proper pocket fit.

### 2.3. Effect of pH

The natural substrate, linoleic acid, undergoes SBLO-catalyzed oxygenation at pH 9.0, to afford a product ratio of (13*S*)-HPOD and (9*S*)-HPOD of 49:1. As the pH was decreased below 9, the proportion of (9*S*)-HPOD increased linearly until at pH 6.0 it constituted about 25% of the product mixture.[11] When substrates **1a**–**1c** are subjected to oxygenation with SBLO at different pH values (6–9), it was observed that the regioselectivity was unaffected by the change in pH (Table 2), suggesting that substrates **1a**–**1c** can arrange themselves at the active site of SBLO in only one orientation, when compared to linoleic acid, which can arrange itself in either (normal or reverse) orientation (Scheme 7) depending on the pH of the medium.




**Scheme 6.** Proposed synthetic plans for unsaturated hydroxy fatty acids from **6a**–**6c**.

**Table 1.** SBLO-catalyzed oxidation of modified substrates **1a**–**1c**

| | Regiospecificity[a] (**4**:**5**) | Yield of **6** (%) | $[\alpha]_D^{25}$ of **6**[b] (°), config. | (*S*:*R*)[c] |
|---|---|---|---|---|
| **1a** | 49:1 | 40 | +52, (*S*) | 49:1 |
| **1b** | 49:1 | 43 | +28, (*S*) | 97:3 |
| **1c** | 97:3 | 45 | +39, (*S*) | 49:1 |

[a] Values were determined by normal-phase HPLC at 235 nm.
[b] All rotations were measured as solutions in $CHCl_3$, $c=1$ at 25°C.
[c] (*S*:*R*) ratios of **6** were determined by chiral HPLC (Diacel, Chiral OD column, hexane:isopropanol, 9:1.

**Table 2.** Effect of pH on the regioisomeric ratio **2a**:**3a**

| pH | Buffer | Total hydroperoxide (%) | Regiospecificity (**2a**:**3a**) |
|---|---|---|---|
| 6.0 | Acetate | 42 | 9:1 |
| 6.5 | Acetate | 51 | 9:1 |
| 7.0 | Phosphate | 50 | 92:8 |
| 7.5 | Phosphate | 52 | 19:1 |
| 8.0 | Borate | 65 | 97:3 |
| 8.5 | Borate | 70 | 97:3 |
| 9.0 | Borate | 72 | 49:1 |

## 3. Conclusion

Our present study demonstrates a synthetically useful enzymatic method for asymmetric hydroxylation of unnatural alkenes. It also reveals two other important points, firstly the prosthetic group having a non-ionic hydroxy terminus can recognise the enzyme and secondly the regiochemical outcome appears to be strongly influenced by the high hydrophobic content of the proximal unit. These results lead us directly onto the design of new unnatural substrates and the study of their activity with lipoxygenase is currently under investigation.

## 4. Experimental

### 4.1. General

Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. THF was distilled from sodium benzophenone ketyl. HMPA was distilled from BaO and stored over 3 Å molecular sieves. Dichloromethane (DCM) was distilled from calciumhydride. Soybean lipoxygenase (type-I) was obtained from Sigma co. $^1$H and $^{13}$C NMR were obtained on a Varian VXR 200 (200 and 50 MHz). Chemical shifts are reported as ppm downfield of tetramethylsilane. Infrared spectra were recorded on a Perkin–Elmer 1420 spectrometer. Optical rotations were measured on a Jasco Dip 360 digital polarimeter. The abbreviation TF denotes thin film.

### 4.2. 3-Nonyl-1-ol

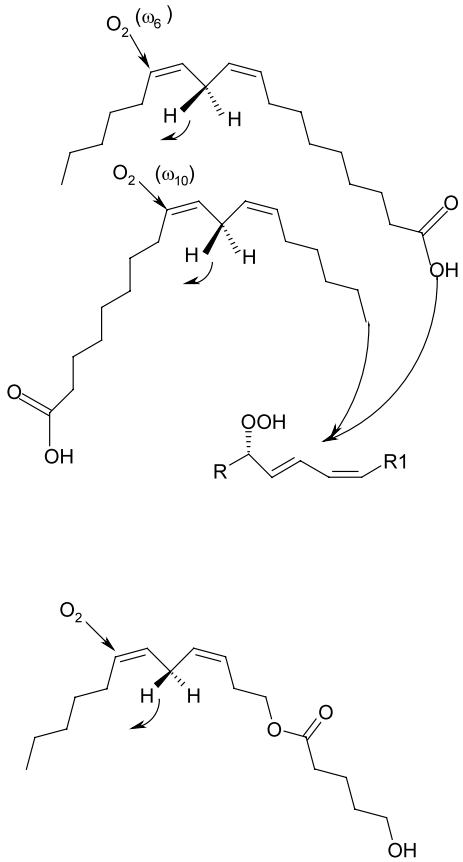
To a stirred suspension of lithium amide (prepared from 2.8 g, 0.4 g atom of Li) in liquid ammonia (300 mL) was added 3-butyn-1-ol (14 g, 200 mmol) over 30 min. The mixture was stirred for an additional 1 h. A solution of 1-bromopentane (30.2 g, 200 mmol) in THF/HMPA (60 mL, 6:1) was added and stirred for a further 6 h. The reaction was quenched with solid $NH_4Cl$ (20 g) and excess ammonia was then allowed to evaporate. The residue was dissolved in water and extracted with ether. The combined organic extract was washed with water, brine, and dried ($Na_2SO_4$). The crude product was purified by column chromatography to afford 3-nonyl-1-ol (20 g, 70%). IR (TF): 3400, 1090 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.3 (m, 4H), 1.5 (m, 2H), 2.1 (t, $J=7$ Hz, 2H), 2.4 (t, $J=7$ Hz, 2H), 3.6 (t, $J=7$ Hz, 2H). EIMS ($m/z$): 140 ($M^+$).

### 4.3. (3*Z*)-3-Nonen-1-ol

3-Nonyl-1-ol (1 g, 7.14 mmol) in absolute ethanol (10 mL) was dissolved in a small round bottom flask. Lindlar catalyst (50 mg, Pd on $CaCO_3$) and few drops of quinoline were added and hydrogen was supplied from balloons. The reaction was monitored by TLC (visualization was carried out by dipping the plates in Eckerts reagent and heating it at 120°C), where the olefinic product moves a little faster than the starting material. After completion of the reaction the mixture was filtered to remove the catalyst. Ethanol was removed on a rotary evaporator. Dichloromethane was added to the residue and it was washed with 5% aq. HCl, brine, dried ($Na_2SO_4$) and evaporated. The resulting product was pure enough for the next step. IR (TF): 3390, 1630, 980 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.35 (brs, 6H), 2.1 (q, $J=7$ Hz, 2H), 2.35 (q, $J=7$ Hz, 2H), 3.6 (t, $J=7$ Hz, 2H), 5.25–5.4 (m, 2H); EIMS ($m/z$): 142 ($M^+$).

### 4.4. (3*Z*)-3-Nonenyliodide

(3*Z*)-Nonen-1-ol (2 g, 14 mmol) was dissolved in acetonitrile: ether (3:1, 75 mL). Imidazole (1.44 g, 21 mmol) was added at rt, followed by addition of $I_2$ (5.36 g, 21




**Scheme 7.** Orientation of natural substrate and synthetic substrates in the active site of SBLOX.

mmol) and triphenylphosphine (5.55 g, 21 mmol). The reaction mixture was stirred for 45 min at rt. The mixture was filtered and the filtrate was concentrated to give a semi-solid residue, which was purified through column chromatography to give the pure iodide (3 g, 85%). IR (TF): 2825–3000 $cm^{-1}$ (C=CH, alkane CH); $^1H$ NMR: 0.9 (t, $J=7$ Hz, 3H), 1.35 (brs, 6H), 2.1 (q, $J=7$ Hz, 2H), 2.65 (q, $J=7$ Hz, 2H), 3.1 (t, $J=7$ Hz, 2H), 5.26–5.4 (m, 2H); EIMS ($m/z$): 252 ($M^+$).

### 4.5. (3*Z*)-3-Nonenyl-1-triphenylphosphoniumiodide 8

(3*Z*)-3-Nonenyliodide (3 g, 12 mmol) was dissolved in dry acetonitrile (50 mL). Triphenylphosphine (3.11 g, 12 mmol) was added and the reaction mixture was heated at 60°C for 48 h under a nitrogen atmosphere. Acetonitrile was evaporated and dry ether (30 mL) was added to the semi-solid residue and shaken vigorously. Evaporation of the ether gave the phosphonium salt (6 g) as a white solid.

### 4.6. 3-Tetrahydropyranylpropanal 9a

3-Tetrahydropyranyl-1-propanol (1.5 g, 9.4 mmol) was dissolved in DMSO (10 mL). 2-Iodoxybenzoic acid (IBX, 5.25 g, 18.75 mmol) was added and the reaction mixture stirred for 4 h at rt. Water was added to the solution and 2-iodobenzoic acid was filtered off. The filtrate was extracted several times with ether, dried ($Na_2SO_4$) and concentrated. Purification of the residue by column chromatography gave 3-tetrahydropyranylpropanal (1.2 g, 80%). IR (TF): 2860, 1705 $cm^{-1}$; $^1H$ NMR: 1.45–1.88 (m, 6H), 2.5 (t, $J=6.8$ Hz, 2H), 3.5 (m, 2H), 3.82 (m, 2H), 4.63 (t, $J=3.9$ Hz, 1H), 9.6 (1H); EIMS ($m/z$): 158 ($M^+$).

### 4.7. (3*Z*,6*Z*)-3,6-Dodecadiene-1-ol 10a

A solution of **8** (1.5 g, 3 mmol) in THF: HMPA (6:1, 30 mL) was treated with a solution of *n*-BuLi (1.2 mL, 2.5 M in hexane) at 0°C, the resulting orange colored solution was stirred at the same temperature for 1 h. 3-Tetrahydropyranyl propanal **9a** (0.46 g, 3 mmol) in THF (5 mL) was added and the reaction mixture stirred for a further 2 h at the same temperature. The mixture was quenched with saturated $NH_4Cl$ solution and extracted with ether. The organic extract was washed with water, brine and dried ($Na_2SO_4$). The crude product was dissolved in methanol (15 mL) and catalytic PTSA was added. The mixture was stirred for 1 h at rt. Evaporation of methanol and purification by column chromatography afforded the (*Z*,*Z*) dienol in 60% yield. IR (TF): 3380, 1090 $cm^{-1}$; $^1H$ NMR: 0.9 (t, $J=7$ Hz, 3H), 1.25 (m, 6H), 2.1 (q, $J=7$ Hz, 2H), 2.2 (brs, 1H, -OH), 2.33 (q, $J=7$ Hz, 2H), 2.8 (t, $J=7$ Hz, 2H), 3.62 (t, $J=7$ Hz, 2H), 5.25–5.6 (m, 4H); $^{13}C$ NMR: 14.09, 22.62, 25.8, 27.3, 29.3, 30.9, 31.57, 62.3, 125.4, 127.5, 130.6, 131.5; EIMS ($m/z$): 182 ($M^+$).

### 4.8. 5-(4-Methoxybenzyloxy)-1-pentanol

1,5-Pentanediol (4 g, 38 mmol) was dissolved in dry THF (80 mL) and NaH (60% dispersion in mineral oil, 1.54 g) was added to the solution portionwise at 0°C. The reaction mixture was stirred at 0°C for 1 h under a nitrogen atmosphere. Tetrabutylammonium iodide (catalytic) was added followed by the addition of 4-methoxybenzylbromide (9.2 g, 45.6 mmol) and the mixture was stirred for a further 2 h at rt. Water was added to the reaction mixture and extracted with EtOAc. The organic extract was washed with brine and dried ($Na_2SO_4$). Purification by means of column chromatography gave the product (6.8 g, 80%). IR (TF): 3400, 3000 $cm^{-1}$; $^1H$ NMR: 1.7 (m, 6H), 3.4 (t, $J=6.5$ Hz, 2H), 3.5 (t, $J=6.5$ Hz, 2H), 3.8 (s, 3H), 4.4 (s, 2H), 6.85 (d, $J=6$ Hz, 2H), 7.2 (d, $J=6$ Hz, 2H); EIMS ($m/z$): 224 ($M^+$).

### 4.9. 5-(4-Methoxybenzyloxy)pentanoic acid

5-(4-Methoxybenzyloxy)-1-pentanol (5 g, 22 mmol) was dissolved in distilled acetone (50 mL) at 0°C. Freshly prepared Jones reagent was added dropwise at the same temperature until the orange brown color persisted. The reaction mixture was allowed to warm to rt and stirred for a further 30 min, water was added and the mixture was extracted with EtOAc. Purification by chromatography gave the acid as a white solid (4 g, 75%). IR (KBr): 3000–2800, 1700 $cm^{-1}$; $^1H$ NMR: 1.7 (m, 4H), 2.35 (t, $J=6.5$ Hz, 2H), 3.5 (t, $J=6.5$ Hz, 2H), 3.8 (s, 3H), 4.4 (s, 2H), 6.85 (d, $J=6$ Hz, 2H), 7.2 (d, $J=6$ Hz, 2H). $^{13}C$ NMR: 21.5, 28.9, 33.6, 55.2, 69.4, 72.5, 114, 129, 130, 159.2, 180. EIMS ($m/z$): 238 ($M^+$).

### 4.10. (3*Z*,6*Z*)-3,6-Dodecadienyl (4-methoxybenzyloxy)-pentanoate 11a

5-(4-Methoxybenzyloxy)pentanoic acid (400 mg, 1.7 mmol) was dissolved in dichloromethane (4 mL). DCC (352 mg, 1.7 mmol) was added, followed by the addition of DMAP (10 mg) at 0°C. The reaction mixture was stirred for 5 min and a solution of (3*Z*,6*Z*)-3,6-dodecadiene-1-ol (367 mg, 2 mmol) in dichloromethane (2 mL) was added dropwise to the mixture. The reaction was stirred for a further 3 h at rt. Dicyclohexylurea was removed by filtration and the filtrate was washed with water, brine and dried ($Na_2SO_4$). After chromatographic separation **13a** was obtained (486 mg, 60%). IR (TF): 3030, 1740 $cm^{-1}$; PMR: 0.85 (t, $J=7$ Hz, 3H), 1.35 (brs, 6H), 1.7 (m, 4H), 2.1 (q, $J=7$ Hz, 2H), 4.4 (s, 2H), 5.25–5.6 (m, 4H), 6.85 (d, $J=6$ Hz, 2H), 7.2 (d, $J=6$ Hz, 2H). $^{13}C$NMR: 14, 21.5, 25.7, 27.3, 29.4, 30.9, 31.6, 60.2, 69.4, 72.5, 114, 125.5, 127.7, 129, 130, 130.8, 131.5, 159.2, 179. FABMS: 402 ($M^+$).

### 4.11. (3*Z*,6*Z*)-3,6-Dodecadienyl 5-hydroxypentanoate 1a

Compound **11a** (220 mg, 0.54 mmol) was dissolved in dichloromethane:water (19:1, 6 mL), DDQ (186 mg, 0.8 mmol) was added and the resulting solution stirred for 1 h at rt. The reaction mixture was filtered and the filtrate was washed with a 5% $NaHCO_3$ solution, brine and dried ($Na_2SO_4$). Purification by chromatography gave **1a** (125 mg, 80%). IR (TF): 3380, 1745 $cm^{-1}$; $^1H$ NMR: 0.9 (t, $J=7$ Hz, 3H), 1.3 (brs, 6H), 1.5–1.8 (m,

4H), 2.1 (q, $J=7$ Hz, 2H), 2.4 (m, 4H), 2.8 (t, $J=7$ Hz, 2H), 3.6 (t, $J=7$ Hz, 2H), 4.1 (t, $J=7$ Hz, 2H), 5.2–5.6 (m, 4H). $^{13}$C NMR: 13.9, 21, 22.4, 25.6, 26.8, 27.1, 29.2, 31.4, 32, 33.8, 62.1, 63.7, 124.6, 127, 130.5, 131, 172. EIMS ($m/z$): 282 ($M^+$).

### 4.12. 7-Hydroxy-(3*Z*,5*E*,7*S*)-3,5-dodecadienyl 5-hydroxypentanoate 4a

To a homogeneous solution of **1a** (100 mg, 0.35 mmol) in sodium borate buffer (0.2 M, pH 9, 20 mL) at 0°C was added Lipoxygenase type-1 (50 mg), while $O_2$ was bubbled through the solution at such a rate to produce the minimum of foaming. After 1 h the reaction mixture was acidified with citric acid to pH 5.0, and then extracted several times with either, dried ($NaSO_4$) and evaporated to afford the crude hydroperoxide.

The crude hydroperoxide (105 mg) was dissolved in diethyl ether (2 mL) and the solution cooled in an ice bath. Triphenylphosphine (TPP, 2 mmol) was added and the mixture was stirred overnight in an ice bath. Ether was evaporated to give the crude diol. Chromatographic purification afforded the diol (44 mg, 40%). IR (TF): 3400, 1740 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.3 (brs, 6H), 1.5–1.8 (m, 4H), 2.3 (t, $J=7$ Hz, 2H), 2.4 (t, $J=7$ Hz, 2H), 2.5 (brs, 2H, -H), 3.6 (t, $J=7$ Hz, 2H), 4.05 (t, $J=7$ Hz, 2H), 4.1 (q, $J=7$ Hz, 1H), 5.46 (dd, $J=7$, 10.4 Hz, 1H), 5.73 (dd, $J=7$, 15 Hz, 1H), 6.15 (dd, $J=10$, 10.4 Hz, 1H), 6.52 (dd, $J=10$, 15 Hz, 1H). $^{13}$C NMR: 14, 20.9, 22.5, 26, 26.9, 27.1, 29.4, 31.6, 34, 62.1, 64, 72, 124, 127.4, 128.5, 130.2, 173. EIMS ($m/z$): 298 ($M^+$), $[\alpha]_D^{25}=+11.0$ ($c=0.5$, $CHCl_3$).

### 4.13. (3*Z*,5*E*,7*S*)-3,5-Dodecadiene-1,7-diol 6a

Compound **4a** (44 mg, 0.147 mmol) was dissolved in methanol (2 mL) and the solution treated with KOH (1 g). The mixture was stirred for 12 h at rt and then diluted with water and extracted several times with EtOAc. Evaporation and purification by column chromatography gave the diol as a yellow oil (30 mg, 90%). IR (TF): 3400, 980 cm$^{-1}$; $^1$H NMR: 0.85 (t, $J=7$ Hz, 3H), 1.26 (m, 6H), 1.44 (m, 2H), 2.2 (brs, 2H, -OH), 2.4 (q, $J=7.0$ Hz, 2H), 3.69 (t, $J=7$ Hz, 2H), 4.1 (q $J=7$ Hz, 1H), 5.46 (dd, $J=7$, 10.4 Hz, 1H), 5.73 (dd, $J=7$, 15 Hz, 1H), 6.15 (dd, $J=10$, 10.4 Hz, 1H), 6.52 (dd, $J=10$, 15 Hz, 1H). $^{13}$C NMR: 13.9, 22.5, 26, 27, 29.5, 32, 62.5, 65, 124.3, 127.5, 128.6, 130.3. HRMS calcd for $C_{12}H_{22}O_2$ 198.1619 obsd. ($m/z$) 198.1592 ($M^+$).

### 4.14. (3*R*,4*E*,6*Z*)-4,6-Dodecadiene-1,3-diol 7a

IR (TF): 3400, 1740 cm$^{-1}$; $^1$H NMR: 0.85 (t, $J=7$ Hz, 3H), 1.25 (brs, 6H), 1.8 (m, 2H), 2.2 (q, $J=7$ Hz, 2H), 2.5 (brs, 2H, -OH), 3.8 (m, 2H), 4.4 (q, $J=7$ Hz, 1H), 5.48 (dd, $J=7$, 10.4 Hz, 1H), 5.65 (dd, $J=7$, 15 Hz, 1H), 5.98 (dd, $J=10$, 10.4 Hz, 1H), 6.52 (dd, $J=10$, 15 Hz, 1H).

### 4.15. (4*Z*,7*Z*)-4,7-Tridecadiene-1-ol 10b

IR (TF): 3400, 1090 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.25–1.45 (m, 8H), 2.1 (q, $J=7$ Hz, 4H), 2.2 (brs, 1H, -OH), 2.8 (t, $J=7$ Hz, 2H), 3.65 (t, $J=7$ Hz, 2H), 5.3–5.6 (m, 4H). $^{13}$C NMR: 14, 22.6, 24, 25.9, 27.2, 29.2, 31, 31.7, 63, 125.6, 128, 130.5, 131. EIMS ($m/z$): 196 ($M^+$).

### 4.16. (5*Z*,8*Z*)-5,8-Tetradecadiene-1-ol 10c

IR (TF): 3385, 1095 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.25 (m, 6H), 1.4–1.6 (m, 4H), 2.15 (q, $J=7$ Hz, 4H), 2.25 (brs, 1H, -OH), 2.75 (t, $J=7$ Hz, 2H), 3.68 (t, $J=7$ Hz, 2H), 5.28–5.6 (m, 4H). $^{13}$C NMR: 13.8, 22.7, 24.5, 25, 26.1, 27.2, 29, 31, 31.8, 62.5, 125.4, 128, 130.2, 131. EIMS ($m/z$): 210 ($M^+$).

### 4.17. (4*Z*,7*Z*)-4,7-Tridecadienyl (4-methoxybenzyloxy)-butanoate 11b

IR (TF): 1735 cm$^{-1}$; $^1$H NMR: 0.85 (t, $J=7$ Hz, 3H), 1.35 (brs, 6H), 1.6–1.75 (m, 4H), 2.12 (q, $J=7$ Hz, 4H), 2.4 (t, $J=7$ Hz, 2H), 2.75 (t, $J=7$ Hz, 2H), 3.5 (t, $J=7$ Hz, 2H), 3.8 (s, 3H), 4.1 (t, $J=7$ Hz, 2H), 4.5 (s, 2H), 5.2–5.45 (m, 4H), 6.9 (d, $J=6$ Hz, 2H), 7.2 (d, $J=6$ Hz, 2H). $^{13}$C NMR: 14, 21.3, 22.6, 25.5, 26.7, 27.2, 29.2, 31.5, 32, 34, 62.3m, 64.3, 69.5, 73, 115, 125.8, 128, 129.2, 131, 131.4, 159.2, 179. FABMS: 402 ($M^+$).

### 4.18. (5*Z*,8*Z*)-5,8-Tetradecadienyl (4-methoxybenzyl-oxy)propanoate 11c

IR (TF): 1740 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.35 (m, 6H), 1.65 (m, 4H), 2.1 (q, $J=7$ Hz, 4H), 2.5 (t, $J=7$ Hz, 2H), 2.78 (t, $J=7$ Hz, 2H), 3.6 (t, $J=7$ Hz, 2H), 3.8 (s, 3H), 4.1 (t, $J=7$ Hz, 2H), 4.5 (s, 2H), 5.25–5.5 (m, 4H), 6.9 (d, $J=6$ Hz, 2H), 7.2 (d, $J=6$ Hz, 2H). $^{13}$C NMR: 13.8, 21.4, 22.5, 25.7, 26.4, 27.4, 29.6, 31.5, 32, 33.8, 61.9, 64.7, 69.2, 70.8, 114.6, 125.65, 127.2, 128.5, 129.8, 131.1, 131.6, 160, 180.1. FABMS: 402 ($M^+$).

### 4.19. (4*Z*,7*Z*)-4,7-Tridecadienyl 4-hydroxybutanoate 1b

IR (TF): 3385, 1740 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.3 (brs, 6H), 1.6–1.8 (m, 4H), 2.1 (m, 4H), 2.4 (t, $J=7$ Hz, 2H), 2.78 (t, $J=7$ Hz, 2H), 3.65 (t, $J=7$ Hz, 2H), 4.09 (t, $J=7$ Hz, 2H), 5.2–5.45 (m, 4H). $^{13}$C NMR: 14, 21.1, 22.5, 25.6, 26.4, 27.1, 29.2, 31.4, 32, 33.8, 62.1, 64, 125, 127, 130.5, 131.2, 172. EIMS ($m/z$): 282 ($M^+$).

### 4.20. (5*Z*,8Z)-5,8-Tetradecadienyl 3-hydroxypropanoate 1c

IR (TF): 3400, 1740 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.2–1.8 (m, 10H), 2.0 (m, 4H), 2.5 (t, $J=7$ Hz, 2H), 2.75 (t, $J=7$ Hz, 2H), 3.8 (t, $J=7$ Hz, 2H), 4.15 (t, $J=7$ Hz, 2H), 5.2–5.5 (m, 4H). $^{13}$C NMR: 13.9, 21.2, 22.6, 25.5, 26, 27.1, 29.2, 31.4, 32, 34, 34, 62.2, 65.1, 125.1, 127, 131, 131.6, 173. EIMS ($m/z$): 282 ($M^+$).

### 4.21. 8-Hydroxy-(4*Z*,6*E*,8*S*)-4,6-tridecadienyl 4-hydroxybutanoate 4b

IR (TF): 3390, 1742 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.35 (brs, 6H), 1.6–1.8 (m, 4H), 2.35 (t, $J=7$ Hz,

2H), 2.4 (t, $J=7$ Hz, 2H), 3.65 (t, $J=7$ Hz, 2H), 4.1 (t, $J=7$ Hz, 2H), 4.15 (q, $J=7$ Hz, 1H), 5.48 (dd, $J=7$, 10.4 Hz, 1H), 5.75 (dd, $J=7$, 15 Hz, 1H), 6.15 (dd, $J=10$, 10.4 Hz, 1H), 6.54 (dd, $J=10$, 15 Hz, 1H). $^{13}$C NMR: 14.1, 21, 22.6, 25.9, 27, 27.2, 29.1, 31.5, 33.8, 62.1, 64.8, 72, 124.1, 127.6, 129, 130.2, 173. EIMS ($m/z$): 298 ($M^+$), $[\alpha]_D^{25}=+6.2$ ($c=1.1$, $CHCl_3$).

### 4.22. 9-Hydroxy-(5*Z*,7*E*,9*S*)-5,7-tetradecadienyl 3-hydroxypropanoate 4c

IR (TF): 3300, 1740 cm$^{-1}$; $^1$H NMR: 0.9 (t, $J=7$ Hz, 3H), 1.2–1.8 (m, 12H), 2.2 (t, $J=7$ Hz, 2H), 2.5 (t, $J=7$ Hz, 2H), 3.8 (t, $J=7$ Hz, 2H), 4.15 (t, $J=7$ Hz, 2H), 4.18 (q, $J=7$ Hz, 1H), 5.48 (dd, $J=7$, 10.4 Hz, 1H), 5.75 (dd, $J=7$, 15 Hz, 1H), 6.15 (dd, $J=10$, 10.4 Hz, 1H), 6.55 (dd, $J=10$, 15 Hz, 1H). $^{13}$C NMR: 14, 21.2, 22.3, 26, 26.8, 27.1, 29, 31.1, 33.7, 62.5, 65, 71, 124.2, 127.8, 128.5, 131, 173. EIMS ($m/z$): 298 ($M^+$), $[\alpha]_D^{25}=+9.4$ ($c=1.0$, $CHCl_3$).

### 4.23. (4*Z*,6*E*,8*S*)-4,6-Tridecadiene-1,8-diol 6b

IR (TF): 3400, 1000 cm$^{-1}$; $^1$H NMR: 0.88 (t, $J=7$ Hz, 3H), 1.32–1.75 (m, 10H), 2.2 (q, $J=7$ Hz, 2H), 3.65 (t, $J=7$ Hz, 2H). 4.15 (q, $J=7$ Hz, 1H), 5.50 (dd, $J=7$, 10.4 Hz, 1H), 5.73 (dd, $J=7$, 15 Hz, 1H), 6.18 (dd, $J=10$, 10.4 Hz, 1H), 6.52 (dd, $J=10$, 15 Hz, 1H). $^{13}$C NMR: 14, 22.6, 24, 26.3, 29, 31.2, 63, 65.6, 124.5, 128, 128.6, 130.6. HRMS calcd for $C_{13}H_{24}O_2$ 212.1777 obsd. ($m/z$) 212.1762 ($M^+$).

### 4.24. (5*Z*,7*E*,9*S*)-5,7-Tetradecadiene-1,9-diol 6c

IR (TF): 3395, 990 cm$^{-1}$; $^1$H NMR: 0.89 (t, $J=7$ Hz, 3H), 1.3–1.6 (m, 12H), 2.4 (q, $J=7$ Hz, 2H), 3.7 (t, $J=7$ Hz, 2H), 4.1 (q, $J=7$ Hz, 1H), 5.48 (dd, $J=7$, 10.4 Hz, 1H), 5.75 (dd, $J=7$, 15 Hz, 1H), 6.15 (dd, $J=10$, 10.4 Hz, 1H), 6.55 (dd, $J=10$, 15 Hz, 1H). $^{13}$C NMR: 13.9, 22.5, 24.3, 25, 26.2, 27.3, 29.2, 31, 62.3, 65.2, 124.3, 128.2, 128.5, 131. HRMS calcd for $C_{14}H_{26}O_2$ 226.1934 obsd. ($m/z$) 226.1927 ($M^+$).

## References


1. (a) Veldink, G. A.; Vilegenthart, J. F. G. In *Studies in Natural Product Chemistry*, *Vol. 9*, *Structure and Chemistry (part-B)*. Substrates and products of lipoxygenase catalysis. Amsterdam; Elsevier Science publishers BV, 1991; pp. 559–589; (b) Harmut, K.; Schewe, T.; Repoport, S. M. *Adv. Enzymol*. **1986**, *58*, 273–311; (c) Gardner, H. W. *Biochim. Biophys. Acta* **1991**, *1084*, 221; (d) Funk, C. D. *Biochim. Biophys. Acta* **1996**, *1304*, 65.
2. Brash, A. R. *J. Biol. Chem*. **1999**, *274*, 23679.
3. (a) Gunstone, F. D. In *Comprehensive Organic Chemistry*; Barton, D. H. R.; Oills, W. B.; Haslam, E., Eds.; Pregamon Press: New York, 1979; pp. 587–632; (b) Datcheva, V. K.; Kiss, K.; Solomon, L.; Kyler, K. S. *J. Am. Chem. Soc*. **1991**, *113*, 270.
4. Veldink, G. A.; Vliegenthart, J. F. G.; Boldingh, J. *Prog. Chem. Fats Other Lipids* **1975**, *15*, 131.
5. (a) Brash, A. R.; Ingram, C. D.; Harris, T. M. *Biochemistry* **1987**, *26*, 5465; (b) Eskola, J.; Laasko, S. *Biochim. Biophys. Acta* **1983**, *751*, 305.
6. (a) Dussault, P.; Lee, Q. *J. Org. Chem*. **1995**, *60*, 218; (b) Martini, D.; Buono, G.; lacazio, G. *J. Org. Chem*. **1996**, *61*, 9062; (c) Martini, D.; Buono, G.; Montillet, J.; Iacazio, G. *Tetrahedron: Asymmetry* **1996**, *7*, 1489; (d) Corey, E. J.; Su, W.-G.; Cleaver, M. B. *Tetrahedron Lett*. **1989**, *32*, 4181; (e) Maguire, N. M.; Mahon, M. F.; Molloy, K. C.; Read, G.; Roberts, S. M.; Sik, V. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2054; (f) Drouet, P.; Thomas, D.; Legoy, M. D. *Tetrahedron Lett*. **1994**, *23*, 3926; (g) Matsushita, Y.; Sugamato, K.; Nakama, T.; Matsui, T.; Hayashi, Y.; Uenakai, K. *Tetrahedron Lett*. **1997**, *38*, 6055; (h) Clapp, C. H.; Senchak, S. E.; Stover, T. J.; Potter, T. C.; Findies, P. M.; Novak, M. J. *J. Am. Chem. Soc*. **2001**, *123*, 747.
7. (a) Yadav, J. S.; Nanda, S.; Rao, A. B. *Synlett* **2001**, 787; (b) Yadav, J. S.; Nanda, S.; Rao, A. B. *Tetrahedron: Asymmetry* **2001**, *12*, 53.
8. Garegg, P. J.; Samuelson, B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2866.
9. Horita, K.; Yoshioka, T.; Tanaka, T.; Olikawa, Y.; Yonemitsu, O. *Tetrahedron* **1986**, *42*, 3021.
10. Gardner, H. W. *Biochim. Biophys. Acta* **1989**, *1001*, 274.
11. Zhang, P.; Kyler, K. S. *J. Am. Chem. Soc*. **1989**, *111*, 9241.
12. Rekker, R. F. *The Hydrophobic Fragmental Constant*; Elsevier: Amsterdam, 1977.