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Enantioselective reduction of γ-hydroperoxy-α,β-unsaturated carbonyl compounds catalyzed by lipid-coated peroxidase in organic solvents

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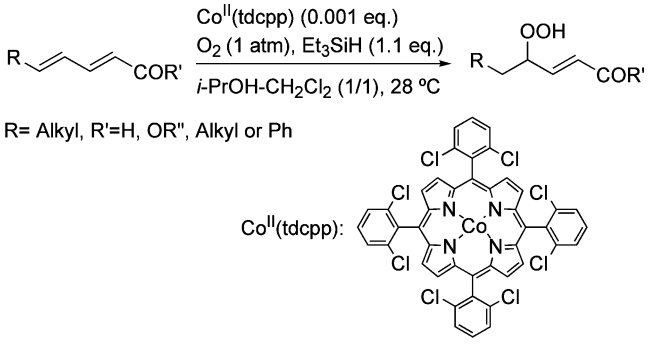
Abstract—The reduction of racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds in the presence of lipid-coated horseradish peroxidase as a homogenious catalyst in organic solvents such as toluene, benzene and chlororform containing a small amount of water enantioselectively afforded optical active (*R*)-alcohols and (*S*)-hydroperoxides, respectively.

1. Introduction

Naturally occurring compounds having a γ-hydroperoxy- or a γ-hydroxy-α,β-unsaturated carbonyl moiety are known, for example, 4-hydroperoxy-2-alkenal and 4-hydroxy-2-alkenal as cytotoxic products during lipid-peroxidation in the living body,[1–4] cyototoxic (*E*)-13-hydroxy-10-oxo-11-octadecenoic acid and its lactone from a water extract of corn,[5–8] and several macrocyclic antibiotics such as brefeldin A[9] and patulolide C.[10] We have reported novel and direct methods for conversion of α,β,γ,δ-unsaturated carbonyl compounds into the corresponding γ-hydroperoxy-α,β-unsaturated ones by cobalt(II) porphyrin-catalyzed reduction–oxygenation with oxygen and triethylsilane (Scheme 1) and its application for syntheses of several natural products.[11–15] Since racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds are easily synthesized by our method, we next investigated separating them into optically active γ-hydroperoxy and γ-hydroxy compounds. Several methods for the resolution of alkyl hydroperoxides by the use of chromatographic or enzymatic methods have been reported. The peracetalization of racemic alkyl hydroperoxides with chiral vinyl ether gave diastereomeric peracetals, which were separated by liquid chromatography followed by deprotection to afford each enantiomer.[16,17] Although this is an excellent and general method for the preparation of enantiomerically pure hydroperoxides, the chiral vinyl ether used therein is not easily available and both protection and deprotection steps are needed. Dussault et al. reported that lipoxygenase-catalyzed oxygenation of linoleic acid followed by protection of the hydroperoxy group and oxidative cleavage of the olefin moiety gave (4*S*)-peroxy-2-alkenal enantioselectively in high yield.[18–20] However, this chemoenzymatic method is enable to afford the corresponding (*R*)-enantiomer. The lipase-catalyzed resolution of racemic hydroperoxides in organic solvents was reported.[21,22] Since the hydroperoxides were enantioselectively acylated by the lipase and then disintegrated to ketone, the remaining hydroperoxides had optical activity with an excess of the (*S*)-enantiomer. Unfortunately, the corresponding (*R*)-hydroperoxides were not obtained by this enzymatic method. Recently, α-hydroperoxyalkylbenzenes and/or alkyl α-hydroperoxyalkanoates have been found to be enantioselectively



Scheme 1.

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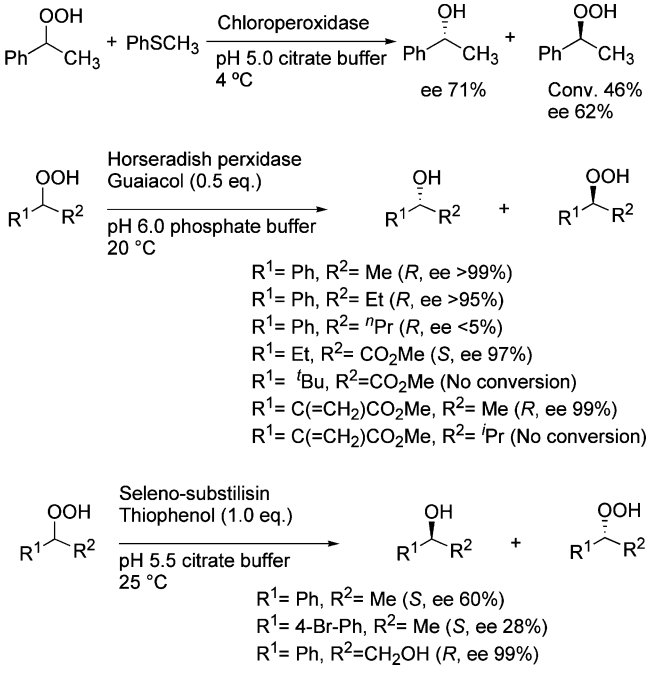
reduced with aryl methyl sulfide by chloroperoxidase,[23–25] with guaiacol by peroxidase,[26–30] and with thiophenol by seleno-subtilisin[31] in an aqueous medium, to give enantiomerically enriched hydroperoxides and alcohols, respectively, in moderate or good enantiomeric excess as shown in Scheme 2. We expected that the enantioselective reduction by peroxidase was applicable to the racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds. Since the racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds are slightly soluble in water, their reduction was considered to be favorable in an organic solvent. Recently, lipid-coating techniques to solubilize an enzyme in homogeneous organic solvents were reported by Okahata et al.[32–43] and Goto et al.[44–63] The lipid-coated enzymes prepared from several enzymes and amphiphilic lipids (surfactants) were found to show high catalytic activity in organic media. In particular, Goto et al. reported that lipid-coated horseradish peroxidase (lipid-HRP) acted as a homogeneous catalyst in anhydrous benzene for the oxidation of *o*-phenylenediamine using H_2O_2 or *t*-BuOOH as an oxidant.[60] Herein we report an enantioselective reduction of racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds in the presence of the lipid-HRP in organic media containing small amounts of water.

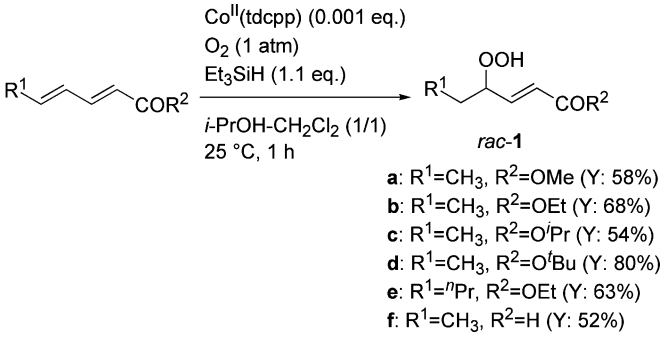


Scheme 2.

2. Results and discussion

2.1. Preparation of racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds and lipid-HRP

Racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds *rac*-**1a**–**f** used for substrates were prepared from α,β,γ,δ-unsaturated carbonyl compounds by the Co^{II}(tdcpp)-catalyzed reduction–oxygenation in moderate or good yield (Scheme 3).[15] The lipid-HRP was prepared according to the method of Goto et al. (Fig. 1).[44] The mixture of both buffer solution (pH 5.5–7.0) of HRP and benzene solution of dioleyl *N*-D-gluconoglutamate (DOGG) was emulsified at 16,000 rpm and then lyophilized, giving the lipid-HRP as light-brownish powder. The molar ratio of DOGG to HRP was changed between 330/1 and 2500/1.



Scheme 3.

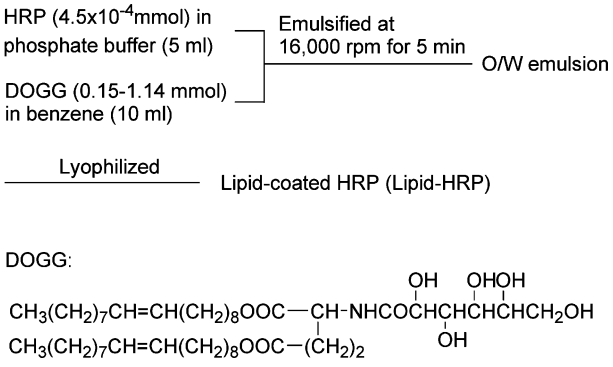
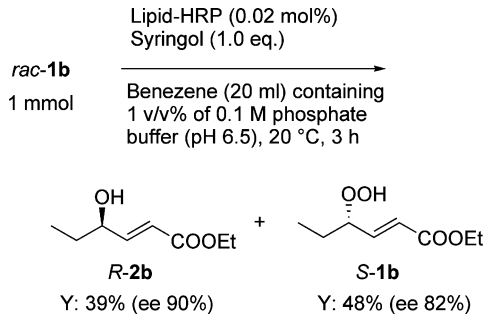


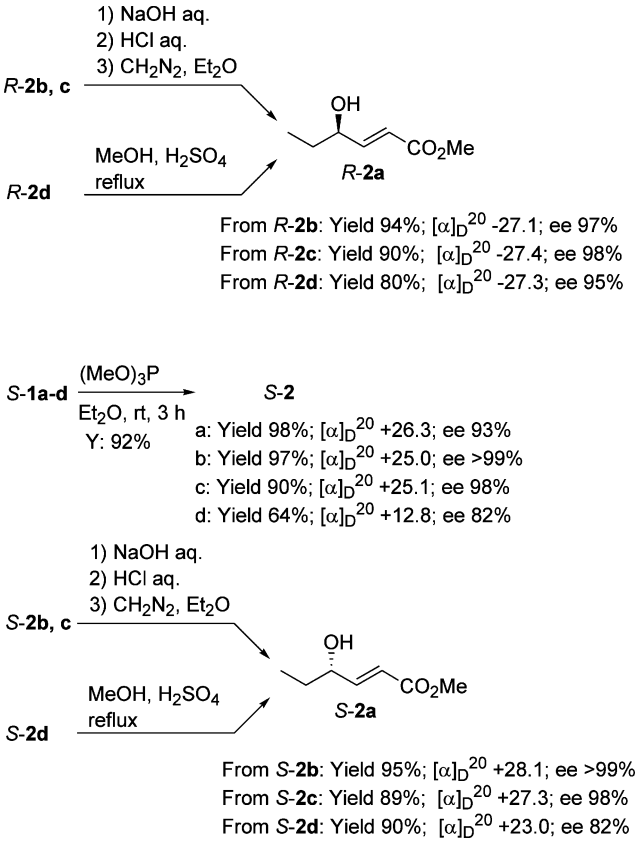
Figure 1. Preparation of lipid-coated horseradish peroxidase (lipid-HRP).

2.2. Determination of enantiomeric excesses of the products obtained from the lipid-HRP-catalyzed reduction of racemic ethyl 4-hydroperoxy-2(*E*)-hexenoate, *rac*-1b

The enzymatic reduction of 1.0 mmol of racemic ethyl 4-hydroperoxy-2(*E*)-hexenoate *rac*-**1b** with 3,5-dimethoxy-4-hydroxybenzaldehyde (syringol) (1.0 mmol) was first carried out in the presence of the lipid-HRP (2×10^{-4} mmol) in 20 ml of benzene containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) at 20°C for 3 h (Scheme 4). The solvent was evaporated in vacuo, and the residue was chromatographed on silica gel with hexane–EtOAc (9/1) as an eluent, to give the alcohol (*R*)-**2b** (Y: 39%) and the hydroperoxide (*S*)-**1b** (Y: 48%). After (*R*)-**2b** and (*S*)-**1b** were, respectively, converted into (*R*)- and (*S*)-enantiomers of methyl 4-hydroxy-2(*E*)-hexenoate by the procedure described in Scheme 5 of Section 2.5, the absolute configurations were determined by comparison of their specific rotation values with that of methyl (2*E*,4*S*)-4-hydroxy-2-hexenoate reported in the literature.[64] The enantiomeric



Scheme 4. The lipid-HRP was prepared from a mixture of HRP (20 mg, 4.5×10^{-4} mmol) in 5.0 ml of 0.1 M phosphate buffer (pH 6.5) and DOGG (338 mg, 0.4 mmol) in 10 ml of benzene.



Scheme 5.

excesses (ee) of both the alcohol (*R*)-**2b** and the hydroperoxide (*S*)-**1b** were determined based on the ratio of areas corresponding to the two enantiomers in the chromatogram analyzed by chiral HPLC on Daicel Chiralpak AS (Fig. 2).

2.3. Influences of molar ratio of DOGG to HRP for preparation of the lipid-HRP and pH of buffer on the lipid-HRP-catalyzed reduction of *rac*-1b

The catalytic activity of the lipid-HRP prepared under different conditions was evaluated on reduction of *rac*-**1b** with syringol in benzene containing 1 v/v% of 0.1 M

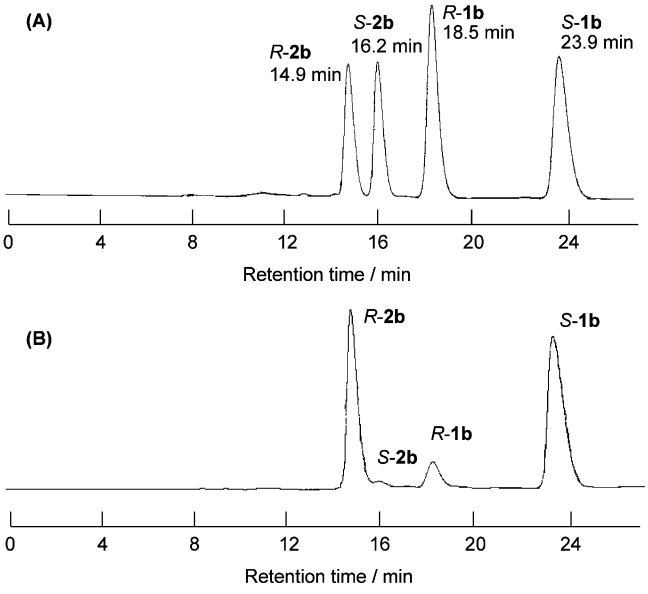
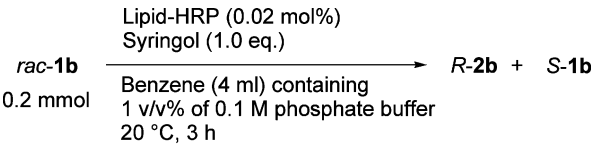


Figure 2. HPLC chromatograms of the products obtained from the lipid-HRP-catalyzed reduction of *rac*-**1b**. (**A**) Authentic racemic sample of the alcohol *rac*-**2b** and the hydroperoxide *rac*-**1b**. (**B**) The crude products obtained from the reduction of *rac*-**1b** for 3 h. Conditions: Shimadzu LC-9A with SPD-10A UV detector at 254 nm; column, Daicel Chiralpak AS (0.46×25 cm); eluent, 10% 2-propanol in hexane; flow rate, 0.5 ml/min; injected sample, 20 μl; column temp., rt.

phosphate buffer (pH 5.5–7.0) at 20°C for 3 h (Table 1). In the absence of the lipid, the yield of the (*R*)-alcohol (*R*)-**2b** was low (entry 1). The production of (*R*)-**2b** increased markedly with an increase in the molar ratio of DOGG to HRP, and 900/1 ratio afforded the best yield (entries 2–4). The molar ratio of DOGG to HRP slightly influenced on the ee of (*R*)-**2b** (entries 1–4). The pH of buffer affected to both the yield and the ee of (*R*)-**2b** (entries 3 and 5–7). The yield and the ee of (*R*)-**2b** were found to be highest at the range of pH 6.0–6.5.

Table 1. Influences of the molar ratio of DOGG to HRP for preparation of the lipid-HRP and pH of buffer on the reduction of *rac*-**1b**



Entry	Molar ratio	pH of buffer	Alcohol (*R*)-**2b**	
	DOGG/HRP		Yield (%)	Ee (%)
1	0/1	6.0	10	92
2	330/1	6.0	30	91
3	900/1	6.0	39	91
4	2500/1	6.0	27	90
5	900/1	5.5	14	85
6	900/1	6.5	40	94
7	900/1	7.0	30	70

The lipid-HRP-catalyzed reduction of *rac*-**1b** was followed by the measurement of consumption of the hydroperoxide **1b** and production of the alcohol **2b** by the chiral HPLC, and the time courses of the reduction are shown in Figure 3. The hydroperoxide **1b** was decreased in the reduction progress and its ee was gradually increased: The (*S*)-enantiomer of **1b** was on the increase. On the other hand, the ee of the alcohol **2b** was unchanged at ca. 90% during the reaction course and the (*R*)-enantiomer of **2b** was predominantly produced. Since the reduction of (*R*)-enantiomer of **1b** proceeds extremely fast compared with its (*S*)-enantiomer, the kinetic resolution of **1b** takes place to advantage on the reduction of *rac*-**1b** in benzene containing of a small amount of the buffer.

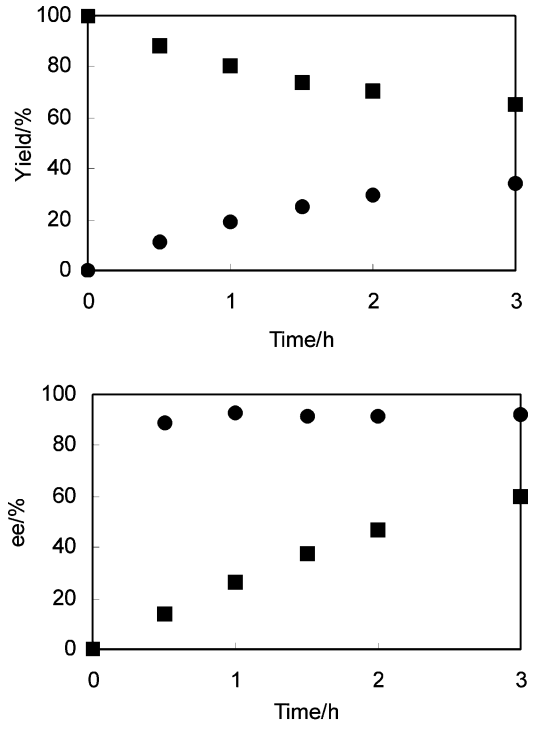
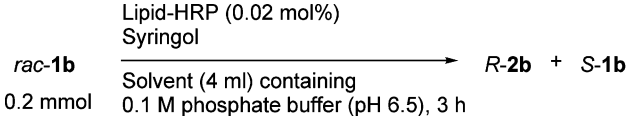


Figure 3. Time courses of the reduction of *rac*-**1b** with syringol (1.0 equiv.) in the presence of the lipid-HRP (0.02 mol%) in benzene containing 1 v/v% of 0.1 M phosphate buffer (pH 6.0) at 20°C: the alcohol **2b** (●), the hydroperoxide **1b** (■).

2.4. Effects of reaction conditions on the lipid-HRP-catalyzed reduction of *rac*-1b

In order to optimize the reaction conditions, effects of water content, the amount of syringol, solvent, and temperature were examined on the lipid-HRP-catalyzed reduction of *rac*-**1b** with syringol in solvent containing 0–1.0 v/v% of 0.1 M phosphate buffer (pH 6.5). Table 2 summarizes the effects of the reaction conditions. No reduction of *rac*-**1b** took place in anhydrous benzene (entry 1). The reduction was observed to proceed in the presence of a small amount of the buffer and the yield of the (*R*)-alcohol (*R*)-**2b** became constant at 41% in benzene containing 0.66 v/v% of buffer or above (entries 2–4). The water content was not influenced on the ee of (*R*)-**2b**. The effect of water content on the reactivity of enzymes in non-aqueous media has been a focus of recent research.[65–70] Although Goto et al. have noted that the lipid-coated HRP was active in anhydrous benzene for the oxidation of *o*-phenylenediamine using H_2O_2 or *t*-BuOOH,[60] we found that a small amount of water was required for the enhancement of the reactivity of the lipid-HRP on the reduction of the hydroperoxide **1b**. On the other hand, the molar ratio of syringol to *rac*-**1b** had no influence on the yield and the ee of (*R*)-**2b** (entries 4–6), and the use of 1.0 equiv. of syringol is sufficient for the reduction. We next tried to use chloroform, dichloromethane, or toluene instead

Table 2. Effects of buffer content, the amount of syringol, solvent, and temperature on the lipid-HRP-catalyzed reduction



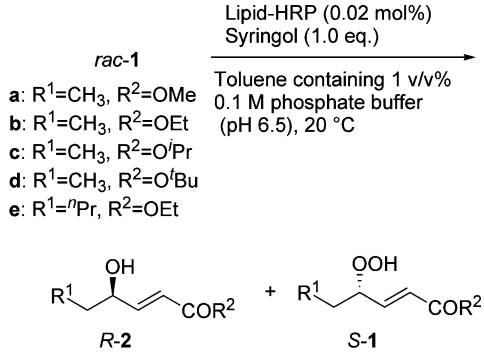
Entry	Buffer (v/v%)	Syringol (equiv.)	Solvent	Temp. (°C)	Alcohol (*R*)-**2b**	
					Yield (%)	Ee (%)
1	0	1.0	Benzene	20	No reaction	–
2	0.33	1.0	Benzene	20	21	94
3	0.66	1.0	Benzene	20	41	94
4	1.0	1.0	Benzene	20	41	94
5	1.0	2.0	Benzene	20	41	94
6	1.0	4.0	Benzene	20	40	94
7	1.0	1.0	CH_2Cl_2	20	27	92
8	1.0	1.0	$CHCl_3$	20	42	94
9	1.0	1.0	Toluene	20	48	95
10	1.0	1.0	Toluene	10	36	93
11	1.0	1.0	Toluene	15	43	94
12	1.0	1.0	Toluene	25	48	92
13	1.0	1.0	Toluene	30	46	92

of benzene on the reduction (entries 7–9): hexane, diethyl ether, ethyl acetate, and alcohols were not used because the lipid-HRP was insoluble in these solvents. When using dichloromethane instead of benzene, both the yield and the ee of (*R*)-**2b** were decreased. The reactivity of the lipid-HRP in chloroform was similar to that in benzene, and that in toluene was better than that in benzene. Benzene, chloroform and toluene showed almost the same enantioselectivity. Finally, the effect of the reaction temperature was examined (entries 9–13). The reaction at 10 or 15°C proceeded slower than that at 20°C. The ee of (*R*)-**2b** was somewhat decreased at 25°C and above. Thus, the reduction with 1.0 equiv. of syringol in toluene containing 0.1 M phosphate buffer (pH 6.5) at 20°C was found to be the most suitable.

2.5. The lipid-HRP-catalyzed reduction of racemic γ-hydroperoxy-α,β-unsaturated carbonyl compounds

To demonstrate the applicability of the lipid-HRP-catalyzed reduction, several alkyl 4-hydroperoxy-2-alkenoates were allowed to react with syringol (1.0 equiv.) in the presence of the lipid-HRP (0.02 mol%) in toluene containing 1.0 v/v% of 0.1 M phosphate buffer (pH 6.5) at 20°C (Table 3). The reaction was terminated at a conversion rate of about 50% which was detected by HPLC. The alcohol and the hydroperoxide were isolated by silica gel column chromatography, and then converted by the procedure shown in Scheme 5 into (*R*)- and (*S*)-enantiomers of methyl 4-hydroxy-2(*E*)-hexenoate, (*S*)-**2a** and (*R*)-**2a**, respectively. Their absolute configurations were determined by comparison of the specific rotation values of (*S*)-**2a** and (*R*)-**2a** with that of methyl (2*E*,4*S*)-4-hydroxy-2-hexenoate reported in the literature.[64] The reduction of racemic alkyl 4-hydroperoxy-2-alkenoates proceeded enantioselectively, to give the (*R*)-alcohols and the (*S*)-hydroperoxides in good yields except for *t*-butyl ester *rac*-**1d**. The reduction progress for *rac*-**1d** was very slow and ca. 55% of the hydroperoxide remained after 72 h, so that the ee of the hydroperoxide *S*-**1d** was somewhat low (ee 82%). The reactivity of alkyl 4-hydroxy-2-hexenoates was decreased with the increase in bulkyness of the ester. Adam et al. revealed that HRP catalyze efficiently the kinetic resolution of secondary hydroperoxides in buffer (Scheme 3) and the replacement of the alkyl side chain by a longer alkyl or a bulky branched one resulted in the decrease in the ee or the reactivity.[26–30] No conversion of racemic ethyl 4-hydroperoxy-2-octenoate *rac*-**1e** is presumably due to chain lengthening. The reactivity of the lipid-HRP for the hydroperoxides in toluene is similar to that of HRP in aqueous solution described by Adam et al.

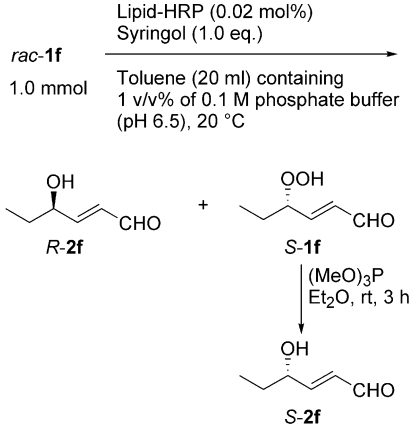
Table 3. The lipid-HRP-catalyzed reduction of racemic alkyl (*E*)-4-hydroperoxy-2-alkenoates *rac*-**1a**–**e**[a]



Entry	Hydroperoxide	Time (h)	Yield/% (ee/%)	
			(*R*)-**2**	(*S*)-**1**
1	*rac*-**1a**	1.5	39 (97)	44 (93)
2	*rac*-**1b**	3.0	45 (97)	40 (>99)
3	*rac*-**1c**	5.0	43 (98)	40 (98)
4	*rac*-**1d**	72	33 (95)	50 (82)
5	*rac*-**1e**	24	No reaction	–[b]

[a] Yield is isolated one, and ee was determined by chiral HPLC.
[b] Not determined.

The kinetic resolution of racemic (*E*)-4-hydroperoxyhexenal *rac*-**1f** by the lipid-HRP was next attempted (Scheme 6). After the reduction of *rac*-**1f** was carried out for 0.5 or 1.0 h, the alcohol (*R*)-**2f** and the hydroperoxide (*S*)-**1f** were isolated by silica gel column chromatography. Because the hydroperoxide *rac*-**1f** and the alcohol *rac*-**2f** were unseparable under the chiral HPLC conditions, the reaction time for 50% conversion of *rac*-**1f** could not determined. The hydroperoxide (*S*)-**6** was reduced with trimethylphosphite to the corresponding alcohol (*S*)-**2f**. The ee of the alcohols (*R*)-**2f** and (*S*)-**2f** were determined by chiral capillary GC on Ultra Alloy-CD (Fig. 4). The absolute configurations of the products were determined compatible with those of (*R*)- and (*S*)-enantiomers of **2f** derived from *R*-**2b** and *S*-**1b** according to the procedure described in Scheme 7. Table 4 shows the results of the reduction of *rac*-**1f**. When *rac*-**1f** was allowed to react for 0.5 h, (*R*)-**2f** and (*S*)-**1f** were obtained, respectively, in 35% yield and ee 93% and in 60% yield and ee 73%. On the other hand, the reduction of *rac*-**1f** for 1.0 h afforded (*R*)-**2f** (ee 72%) in 58% yield and (*S*)-**2f** (ee 97%) in 25% yield. Thus, we could prepare the (*S*)-hydroperoxide (*S*)-**1f** and both (*R*)- and (*S*)-enantiomers of the alcohol **2f** in high enantioselectivity. 4-hydroperoxy- and 4-hydroxy-



Scheme 6.

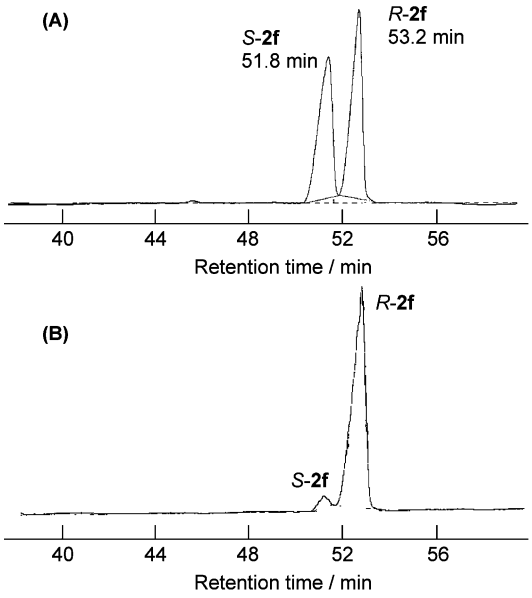


Figure 4. GC chromatograms of the products obtained from the lipid-HRP-catalyzed reduction of racemic (*E*)-4-hydroxy-2-hexenal *rac*-**1f**. (**A**) Authentic racemic sample of *rac*-**2f**. (**B**) The alcohol **2f** obtained from the reduction of *rac*-**1f** for 0.5 h. Conditions: column, Ultra Alloy-CD No. 2; flow gas, He; column temp., 90°C; detector, FID.

2-hexenal are important for the lipid-peroxidation products in fish,[3,4] but their syntheses in optically active forms have not been reported.

3. Conclusion

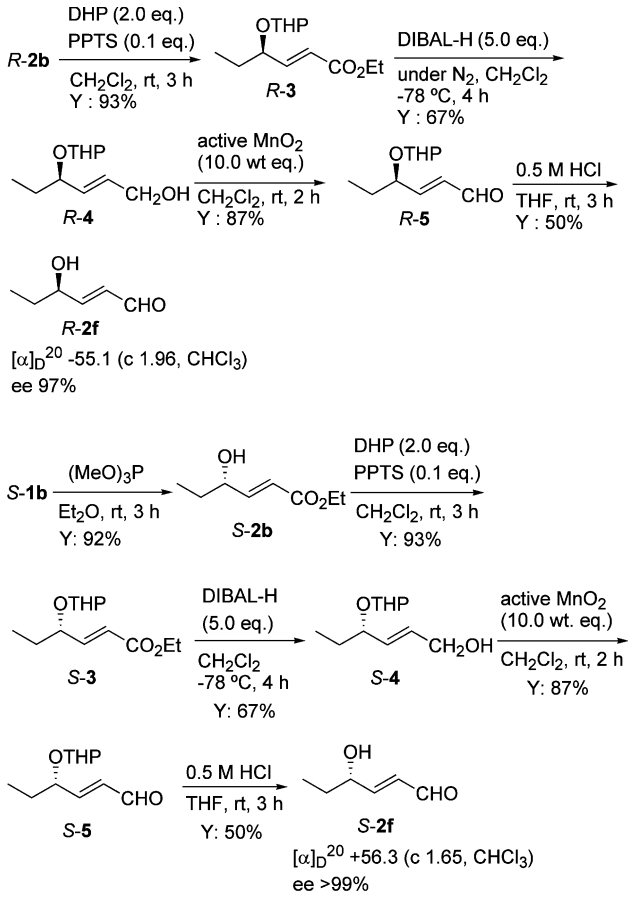
The lipid-HRP was found to catalyze the enantioselective reduction of γ-hydroperoxy-α,β-unsaturated carbonyl compounds in organic solvent containing a small amount of water, to afford the (*R*)-alcohol and (*S*)-hydroperoxide, respectively.

4. Experimental

4.1. General

Horseradish peroxidase (HRP) (Activity; 180 units/mg) was purchased from Wako Pure Chemicals Industries, Ltd. (Japan) and used without further purification. All commercial chemicals were analytical grade quality. All purchased solvents were of high purity and were redistilled before use. Column chromatography was executed with BW-300 purchased from Fuji Silysia Co., Ltd. (Japan).

^{1}H and ^{13}C NMR spectra were recorded on a AC-250P (Bruker, USA) in $CDCl_3$ solution with TMS ($\delta = 0.00$) as an internal standard. IR spectra were measured in $CHCl_3$ solution on a Hitachi IR 270-30 spectrometer (Japan). High resolution mass spectrometry (HRMS) was performed on a Hitachi M-2000AM mass spectrometer (Japan) in electron impact mode at 70 eV.



Scheme 7. The abbreviations used are as follows: DHP, 3,4-dihydro-2*H*-pyran, PPTS, pyridinium *p*-toluenesulfonate, DIBAL-H, diisobutyl aluminum hydride.

Optical rotation was recorded on a Jasco DIP-100MU (Japan) with $CHCl_3$ as a solvent. The enantiomeric excesses (ee) of alkyl (*E*)-4-hydroperoxy-2-alkenoate and (*E*)-4-hydroxy-2-alkenoate were determined by chiral HPLC: Shimadzu LC-9A (Japan) with SPD-10A UV detector at 254 nm; column, Daicel Chiralpak AS column (0.46×25 cm) with Daicel Chiralpak AS precolumn (0.46×5 cm) (Japan); eluent, hexane-2-propanol (90/10); flow rate, 0.5 ml/min. The ee of (*E*)-4-hydroxy-2-hexenal was determined by chiral GC: Shimadzu GC-14B (Japan) with FID detector; column, Ultra Alloy-CD No. 2 (stationary phase, heptakis(2,3,6-tri-*O*-pentyl)-β-cyclodextrin; 0.25 mm×45 m; Frontier Lab, Japan); flow gas, He; flow rate, 1.61 ml/min; injection temp., 300°C; detector temp., 300°C; column temp., 90°C.

4.2. Preparation of lipid-coated horseradish peroxidase, lipid-HRP

A mixture of 5 ml of 0.1 M phosphate buffer (pH 6.5) solution of HRP (20 mg, 4.5×10^{-4} mmol) and 10 ml of benzene solution of dioleyl *N*-D-gluconoglutamate (DOGG) (338 mg, 0.41 mmol) was emulsified with Ultra Turrax T25 homogenizer (IKA Labortechick, German) at 16,000 rpm for 5 min to give W/O emulsions. The emulsions were lyophilized by use of FDU-

Table 4. The lipid-HRP-catalyzed reduction of racemic (*E*)-4-hydroperoxy-2-hexenal *rac*-**1f**[a]

Entry	Time (h)	Product			
		(*R*)-**2f**		(*S*)-**2f**	
		Yield/% (ee/%)	$[\alpha]_D^{20}$	Yield[b]/% (ee/%)	$[\alpha]_D^{20}$
1	0.5	35 (93)	–52.3	60 (70)	–[c]
2	1.0	58 (72)	–[c]	25 (97)	+55.0

[a] Yield was isolated one, ee was determined by chiral GC, and $[\alpha]_D^{20}$ was measured in $CHCl_3$.
[b] Based on the yield of the intermediate **1f**.
[c] Not determined.

506 freeze-dryer (EYELA, Japan) for 6 h to give lipid-HRP (406 mg) as light-brown powder.

4.3. Preparation of alkyl (*E*)-4-hydroperoxy-2-alkenoates and (*E*)-4-hydroperoxy-2-hexenal

Alkyl (2*E*,4*E*)-2,4-alkadienoate or (2*E*,4*E*)-2,4-hexadienal (40 mmol) and 5,10,15,20-tetrakis (2,6-dichlorophenyl)porphinatocobalt(II) (Co^{II}(tdcpp)) (37.3 mg, 0.04 mmol) were dissolved in 100 ml of 2-propanol–dichloromethane (1/1) in a 200 ml kjeldahl flask equipped with three-way stop-cock. The atmosphere in the flask was replaced with oxygen by bubbling for 5 min, and then an oxygen balloon was attached to the flask through the three-way stopcock. Triethylsilane (9.3 ml, 60 mmol) was added to the solution at 28°C. The reaction was terminated when the substrate was completely consumed by checking on TLC. After removing the solvent under reduced pressure, the residue was purified by silica gel column chromatography with hexane–ethyl acetate to afford alkyl (*E*)-4-hydroperoxy-2-alkenoate or (*E*)-4-hydroperoxy-2-hexenal.

4.3.1. Methyl (*E*)-4-hydroperoxy-2-hexenoate, *rac*-1a. Yield 58%; colorless oil; R_f (hexane–EtOAc, 4:1) 0.20; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3050, 3000, 2975, 2900, 1720, 1670, 1470, 1375, 1320, 1300, 1260, 1200, 1140, 1110, 1040, and 990; δ_H (250 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.4 Hz, CH_3), 1.53–1.76 (2H, m, CH_2), 3.73 (3H, s, OCH_3), 4.45 (1H, ddt, *J* 0.9, 6.8 and 6.9 Hz, C*H*OOH), 6.04 (1H, d, *J* 16.0 Hz, CH=C*H*-CO), 6.89 (1H, dd, *J* 6.8 and 16.0 Hz, C*H*=CH-CO), and 9.11 (1H, br s, OOH); δ_C (63 MHz, $CDCl_3$) 9.38, 26.17, 85.59 (C-OOH), 122.67, 146.86, and 166.88; *m*/*z* (HRMS) 161.1078 (MH^+; $C_7H_{13}O_4$ requires *MH*, 161.1112).

4.3.2. Ethyl (*E*)-4-hydroperoxy-2-hexenoate, *rac*-1b. Yield 68%; colorless oil; R_f (hexane–EtOAc 4:1) 0.19; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3050, 3000, 2975, 2900, 1720, 1670, 1470, 1375, 1320, 1300, 1260, 1200, 1140, 1110, 1040, and 990; δ_H (250 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.5 Hz, CH_3), 1.30 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 1.56–1.70 (2H, m, CH_2), 4.22 (2H, q, *J* 7.2 Hz, OCH_2), 4.45 (1H, dtd, *J* 0.9, 6.7 and 6.8 Hz C*H*OOH), 6.04 (1H, d, *J* 15.8 Hz, CH=C*H*-CO) and 6.89 (1H, dd, *J* 6.8 and 15.8 Hz, C*H*=CH-CO) 9.34 (1H, br s, OOH); δ_C (63 MHz, $CDCl_3$) 9.33, 14.02, 25.13, 60.69, 85.55 (C-OOH), 122.99, 146.60, and 175.37; *m*/*z* (HRMS) 175.0922 (MH^+; $C_8H_{15}O_4$ requires *MH*, 175.0970).

4.3.3. Isopropyl (*E*)-4-hydroperoxy-2-hexenoate, *rac*-1c. Yield 54%; colorless oil; R_f (hexane–EtOAc 4:1) 0.19; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3050, 3000, 2975, 2900, 1720, 1670, 1470, 1375, 1320, 1300, 1260, 1200, 1140, 1110, 1040, and 990; δ_H (250 MHz, $CDCl_3$) 0.96 (3H, t, *J* 7.6 Hz, CH_3), 1.26, 1.29 (6H, s, $OCHCH_3$), 1.53–1.76 (2H, m, CH_2), 4.45 (1H, dtd, *J* 0.9, 6.5 and 6.8 Hz, C*H*OOH), 5.06 (1H, m, OCH), 6.00 (1H, d, *J* 16.0 Hz, CH=C*H*-CO), 6.81 (1H, dd, *J* 6.8 and 16.0 Hz, C*H*=CH-CO), and 9.53 (1H, br s, OOH); δ_C (63 MHz, $CDCl_3$) 9.35, 21.65, 25.11, 68.17, 85.52 (C-OOH), 123.36, 146.41, and 166.11; *m*/*z* (HRMS) 189.1280 (MH^+; $C_9H_{17}O_4$ requires *MH*, 189.1325).

4.3.4. *t*-Butyl (*E*)-4-hydroperoxy-2-hexenoate, *rac*-1d. Yield 80%; colorless oil; R_f (hexane–EtOAc 4:1) 0.19; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3050, 3000, 2975, 2900, 1720, 1670, 1470, 1375, 1320, 1300, 1260, 1200, 1140, 1110, 1040, and 990; δ_H (250 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.4 Hz, CH_3), 1.49 (9H, s, $OCCH_3$), 1.55–1.72 (2H, m, CH_2), 4.41 (1H, dtd, *J* 0.9, 6.6, 6.9 Hz, C*H*OOH), 5.98 (1H, dd, *J* 0.9, 16.0 Hz, CH=C*H*-CO), 6.79 (1H, dd, *J* 6.9 and 16.0 Hz, C*H*=CH-CO) and 9.21 (1H, br s, OOH); δ_C (63 MHz, $CDCl_3$) 9.44, 25.17, 27.99, 80.95(C-OOH), 85.68, 124.91, 145.29, and 165.82; *m*/*z* (HRMS) 203.1205 (MH^+; $C_{10}H_{19}O_4$ requires *MH*, 203.1895).

4.3.5. Ethyl (*E*)-4-hydroperoxy-2-octenoate, *rac*-1e. Yield 63%; colorless oil; R_f (hexane–EtOAc 4:1) 0.25; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3030–2860, 1720, 1660, 1475 and 1375; δ_H (250 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.2 Hz, CH_3), 1.30 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 1.30–1.68 (6H, m, 3×CH_2), 4.19 (2H, q, *J* 7.3 Hz, OCH_2), 4.52 (1H, dt, *J* 6.7 and 6.8, C*H*OOH), 6.04 (1H, d, *J* 16.0 Hz, CH=C*H*-CO), 6.90 (1H, dd, *J* 6.8 and 16.0 Hz, C*H*=CH-CO), and 8.84 (1H, s, OOH); δ_C (63 MHz, $CDCl_3$) 13.87, 14.19, 22.56, 27.23, 31.84, 60.79, 84.64, 123.14, 146.88, and 166.53; *m*/*z* (HRMS) 203.1280 (MH^+; $C_{10}H_{19}O_4$ requires *MH*, 203.1283).

4.3.6. (*E*)-4-Hydroperoxy-2-hexenal, *rac*-1f. Yield 52%; colorless oil; R_f (hexane–EtOAc 4:1) 0.14; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3030–2830, 1700, 1640, 1465, and 1390; δ_H (250 MHz, $CDCl_3$) 0.99 (3H, t, *J* 7.5 Hz, CH_3), 1.59–1.78 (2H, m, CH_2), 4.60 (1H, dt, *J* 6.4 and 6.6 Hz, C*H*OOH), 6.31 (1H, dd, *J* 7.8 and 16.0 Hz, CH=C*H*-CHO), 6.83 (1H, dd, *J* 6.4 and 16.0 Hz, C*H*=CH-CHO), 8.90 (1H, br s, OOH), and 9.78 (1H, d,

J 7.8 Hz, CHO); δ_C (63 MHz, $CDCl_3$) 9.50, 25.23, 85.52, 133.09, 155.45, and 193.91; *m*/*z* (HRMS) 131.0702 (MH^+; $C_6H_{11}O_3$ requires *MH*, 131.0708).

4.4. The lipid-HRP-catalyzed reduction of ethyl (*E*)-4-hydroperoxy-2-hexenoate *rac*-1b in benzene

To a mixture of racemic ethyl (*E*)-4-hydroperoxy-2-hexenoate *rac*-**1b** (175 mg, 1.0 mmol) and syringol (154 mg, 1.0 mmol) in benzene (20 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) was added the lipid-HRP (200 mg, 2.0×10^{-4} mmol), and the reaction mixture was stirred at 20°C for 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 10/1–6/1) to give ethyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2b** (62 mg, ee 90%) in 39% yield and ethyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate (*S*)-**1b** (84 mg, ee 82%) in 48% yield. The ee of (*R*)-**2b** and (*S*)-**1b** were determined by the chiral HPLC under the conditions described above.

4.5. Measurement of the reaction progress on the lipid-HRP-catalyzed reduction of *rac*-1b

To a mixture of *rac*-**1b** (35 mg, 0.2 mmol) and syringol (32 mg, 0.2 mmol) in benzene (4.0 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.0) was added the lipid-HRP (41 mg, 4.0×10^{-5} mmol), and the reaction mixture was stirred at 20°C. At hourly intervals, 20 μl of the reaction mixture was withdrawn and analyzed by the chiral HPLC under the conditions described above: The consumption of the hydroperoxide **1b**, the production of the alcohol **2b**, and the ee of **1b** and **2b** were determined at the same time.

4.6. The lipid-HRP-catalyzed reduction of alkyl (*E*)-4-hydroperoxy-2-alkenoates

4.6.1. The reduction of methyl (*E*)-4-hydroperoxy-2-hexenoate *rac*-1a. To a mixture of methyl (*E*)-4-hydroperoxy-2-hexenoate *rac*-**1a** (206 mg, 1.25 mmol) and syringol (193 mg, 1.25 mmol) in toluene (25 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) was added the lipid-HRP (250 mg, 2.5×10^{-4} mmol), and the reaction mixture was stirred at 20°C for 1.5 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 10/1–6/1) to give methyl (2*E*,4*R*)-4-hydroxy-2-hexenoate, *R*-**2a** (70 mg, ee 97%) in 39% yield and methyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate, (*S*)-**1a** (90 mg, ee 93%) in 44% yield.

Methyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate (*S*)-**1a** (90 mg) was dissolved in ether (5.0 ml) and subsequently trimethyl phosphite (0.2 ml) was added to the solution. The reaction mixture was stirred at 0°C for 1 h and then at rt for 24 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 8/1–6/1) to give methyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2a** (80 mg, ee 93%) in 98% yield.

Methyl (2*E*,4*R*)-4-hydroxy-2-hexenoate, (*R*)-2a: colorless oil; $[\alpha]_D^{20}=-27.8$ (*c* 2.10 in $CHCl_3$); R_f (hexane–EtOAc, 4:1) 0.18; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3050, 3000, 2975, 2900, 1720, 1670, 1470, 1375, 1320, 1300, 1260, 1200, 1140, 1110, 1040, and 990; δ_H (250 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.5 Hz, CH_3), 1.56–1.70 (2H, m, CH_2), 3.73 (3H, s, OCH_3), 2.63 (1H, br s, OH), 4.45 (1H, dt, *J* 6.7 and 6.8 Hz, C*H*OH), 6.04 (1H, d, *J* 15.8 Hz, CH=C*H*-CO) and 6.89 (1H, dd, *J* 6.7 and 15.8 Hz, C*H*=CH-CO); δ_C (63 MHz, $CDCl_3$) 9.29, 29.30, 51.43, 71.96 (C-OH), 119.55, 150.52, and 167.08; *m*/*z* (HRMS) 144.0568 (MH^+; $C_7H_{13}O_3$ requires *MH*, 144.0897).

Methyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate, (*S*)-1a: colorless oil; $[\alpha]_D^{20}=-15.1$ (*c* 3.00 in $CHCl_3$); IR, NMR and mass spectral data were coincided with those of racemic one.

Methyl (2*E*,4*S*)-4-hydroxy-2-hexenoate, (*S*)-2a: colorless oil; $[\alpha]_D^{20}=+26.3$ (*c* 2.81 in $CHCl_3$); lit.[64] $[\alpha]_D^{20}=+24$ (*c* 3.1 in $CHCl_3$ for ee 95%).

4.6.2. The reduction of ethyl (*E*)-4-hydroperoxy-2-hexenoate *rac*-1b. To a mixture of *rac*-**1b** (700 mg, 4.0 mmol) and syringol (615 mg, 4.0 mmol) in toluene (80 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) was added the lipid-HRP (800 mg, 8.0×10^{-4} mmol), and the reaction mixture was stirred at 20°C for 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 10/1–6/1) to give ethyl (2*E*,4*R*)-4-hydroxy-2-hexenoate, (*R*)-**2b** (317 mg, ee 97%) in 45% yield and ethyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate, (*S*)-**1b** (252 mg, ee >99%) in 40% yield.

Ethyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate (*S*)-**1b** (252 mg) were dissolved in ether (5.0 ml) and subsequently trimethyl phosphite (0.3 ml) was added to the solution. The reaction mixture was stirred at 0°C for 1 h and then at rt for 24 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 8/1–6/1) to give ethyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2b** (222 mg, ee >99%) in 97% yield.

Ethyl (2*E*,4*R*)-4-hydroxy-2-hexenoate, (*R*)-2b: colorless oil; $[\alpha]_D^{20}=-24.2$ (*c* 2.01 in $CHCl_3$); R_f (hexane–EtOAc, 4:1) 0.17; ν_{max} ($CHCl_3$)/cm^{-1} 3630, 3500, 3030, 3000, 2950, 2900, 1720, 1660, 1460, 1380, 1300, 1280, 1240, 1180, 1140, 1100, and 1040; δ_H (250 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.0 Hz, CH_3), 1.29 (3H, t, *J* 7.0 Hz, OCH_2CH_3), 1.52–1.75 (2H, m, CH_2), 2.04 (1H, br s, OH), 4.21 (2H, q, *J* 7.0 Hz, OCH_2), 4.22 (1H, m, C*H*OH), 6.02 (1H, dd, *J* 1.1 and 15.8 Hz, CH=C*H*-CO) and 6.96 (1H, dd, *J* 5.3 and 15.8 Hz, C*H*=CH-CO); δ_C (63 MHz, $CDCl_3$) 9.33, 14.02, 25.13, 60.69, 72.55 (C-OH), 122.99, 146.60, and 175.37; *m*/*z* (HRMS) 159.0863 (MH^+; $C_8H_{15}O_3$ requires *MH*, 159.0065).

Ethyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate, (*S*)-1b: colorless oil; $[\alpha]_D^{20}=-20.3$ (*c* 1.00 in $CHCl_3$); IR, NMR and mass spectral data were coincided with those of racemic one.

Ethyl (2*E*,4*S*)-4-hydroxy-2-hexenoate, (*S*)-2b: colorless oil; $[\alpha]_D^{20}=+25.0$ (*c* 1.83 in $CHCl_3$).

4.6.3. The reduction of isopropyl (*E*)-4-hydroperoxy-2-hexenoate, *rac*-1c. To a mixture of isopropyl (*E*)-4-hydroperoxy-2-hexenoate *rac*-**1c** (590 mg, 3.1 mmol) and syringol (477 mg, 3.1 mmol) in toluene (50 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) was added lipid-HRP (600 mg, 6.0×10^{-4} mmol). The reaction mixture was stirred at 20°C for 4 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 10/1–6/1) to give isopropyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2c** (234 mg, ee 98%) in 43% yield and isopropyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate (*S*)-**1c** (222 mg, ee 98%) in 40% yield.

Isopropyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate (*S*)-**1c** (222 mg) were dissolved in ether (5.0 ml) and subsequently trimethyl phosphite (0.3 ml) was added to the solution. The reaction mixture was stirred at 0°C for 1 h and then at rt for 24 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 8/1–6/1) to give isopropyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2c** (202 mg, ee 98%) in 97% yield.

Isopropyl (2*E*,4*R*)-4-hydroxy-2-hexenoate, (*R*)-2c: colorless oil; $[\alpha]_D^{20}=-25.2$ (*c* 1.00 in $CHCl_3$); R_f (hexane–EtOAc 4:1) 0.17; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3050, 3000, 2975, 2900, 1720, 1670, 1470, 1375, 1320, 1300, 1260, 1200, 1140, 1110, 1040, and 990; δ_H (250 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.4 Hz, CH_3), 1.26 (6H, t, *J* 6.3 Hz, $OCH(CH_3)_2$), 1.56–1.69 (2H, m, CH_2), 2.46 (1H, br s, OH), 4.22 (1H, dt, *J* 5.7 and 5.8 Hz, C*H*OH), 5.06 (1H, m, OCH), 6.00 (1H, dd, *J* 1.5 and 15.8 Hz, CH=C*H*-CO), 6.91 (1H, dd, *J* 5.7 and 15.8 Hz, C*H*=CH-CO), and; δ_C (63 MHz, $CDCl_3$) 9.42, 21.75, 29.46, 67.75, 72.23 (C-OH), 120.73, 149.69, and 166.14; *m*/*z* (HRMS) 172.1103 (MH^+; $C_9H_{17}O_3$ requires *MH*, 172.1634).

Isopropyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate, (*S*)-1c: colorless oil; $[\alpha]_D^{20}=-19.8$ (*c* 1.00 in $CHCl_3$); IR, NMR and mass spectral data were coincided with those of racemic one.

Isopropyl (2*E*,4*S*)-4-hydroxy-2-hexenoate, (*S*)-2c: colorless oil; $[\alpha]_D^{20}=+25.1$ (*c* 1.30 in $CHCl_3$).

4.6.4. The reduction of *t*-butyl (*E*)-4-hydroperoxy-2-hexenoate, *rac*-1d. To a mixture of *t*-butyl (*E*)-4-hydroperoxy-2-hexenoate *rac*-**1d** (202 mg, 1.0 mmol) and syringol (155 mg, 1.0 mmol) in toluene (20 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) was added the lipid-HRP (200 mg, 2.0×10^{-4} mmol). The reaction mixture was stirred at 20°C for 72 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 12/1–6/1) to give *t*-butyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2d** (60 mg, ee 95%) in 33% yield and *t*-butyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate (*S*)-**1d** (101 mg, ee 82%) in 50% yield.

t-Butyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate (*S*)-**1d** (101 mg) were dissolved in ether (5.0 ml) and subsequently trimethylphosphite (0.3 ml) was added to the solution. The reaction mixture was stirred at 0°C for 1 h and then at rt for 24 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 8/1–6/1) to give *t*-butyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2d** (60 mg, ee 82%) in 64% yield.

***t*-Butyl (2*E*,4*R*)-4-hydroxy-2-hexenoate, (*R*)-2d**: colorless oil; $[\alpha]_D^{20}=-15.0$ (*c* 1.00 in $CHCl_3$); R_f (hexane–EtOAc, 4:1) 0.17; ν_{max} ($CHCl_3$)/cm^{-1} 3550, 3400, 3050, 3000, 2975, 2900, 1720, 1670, 1470, 1375, 1320, 1300, 1260, 1200, 1140, 1110, 1040, and 990; δ_H (250 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.4 Hz, CH_3), 1.30 (9 H, s, $OCCH_3$), 1.55–1.67 (2H, m, CH_2), 2.62 (1H, br s, OH), 4.19 (1H, dt, *J* 5.3 and 5.4 Hz, C*H*OH), 5.94 (1H, dd, *J* 1.5 and 15.7 Hz, CH=C*H*-CO), and 6.82 (1H, dd, *J* 5.3 and 15.7 Hz, C*H*=CH-CO); δ_C (63 MHz, $CDCl_3$) 9.46, 28.02, 29.49, 72.22(C-OH), 80.40, 121.99, 148.86, and 165.97; *m*/*z* (HRMS) 186.1356 (MH^+; $C_{10}H_{19}O_3$ requires *MH*, 186.2122).

***t*-Butyl (2*E*,4*S*)-4-hydroperoxy-2-hexenoate, (*S*)-1d**: colorless oil; $[\alpha]_D^{20}=-15.7$ (*c* 2.00 in $CHCl_3$); IR, NMR and mass spectral data were coincided with those of racemic one.

***t*-Butyl (2*E*,4*S*)-4-hydroxy-2-hexenoate, (*S*)-2d**: colorless oil; $[\alpha]_D^{20}=+12.8$ (*c* 2.01 in $CHCl_3$).

4.7. Conversion of alkyl (2*E*,4*R*)- and (2*E*,4*S*)-4-hydroxy-2-hexenoate to the corresponding methyl esters

4.7.1. Conversion of ethyl (2*E*,4*R*)- and (2*E*,4*S*)-4-hydroxy-2-hexenoate. A mixture of ethyl (2*E*,4*R*)-4-hydroxy-2-hexenoate *R*-**2b** (100 mg) and 2 M NaOH (5.0 ml) was stirred at room temperature for 2 h. 2 M HCl (8.0 ml) was added to the reaction mixture and the mixture was extracted with diethyl ether (3×10 ml). After the organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure. The residue was dissolved in diethyl ether (10 ml) and subsequently an excess of diazomethane in diethyl ether was added to the solution at 0°C. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 12/1) to give methyl (2*E*,4*R*)-4-hydroxy-2-hexenoate, (*R*)-**2a** (86 mg, ee 97%) in 94% yield; colorless oil; $[\alpha]_D^{20}=-27.1$ (*c* 1.00 in $CHCl_3$).

Ethyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2b** (100 mg) was similarly converted into methyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2a** (87 mg, ee >99%) in 95% yield; colorless oil; $[\alpha]_D^{20}=+28.1$ (*c* 1.00 in $CHCl_3$).

4.7.2. Conversion of isopropyl (2*E*,4*R*)- and (2*E*,4*S*)-4-hydroxy-2-hexenoate. A mixture of isopropyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2c** (100 mg) and 2 M NaOH (5.0 ml) was stirred at room temperature for 2 h. 2 M HCl (8.0 ml) was added to the reaction mixture and the mixture was extracted with diethyl ether (3×10

ml). After the organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure. The residue was dissolved in diethyl ether (10 ml) and subsequently an excess of diazomethane in diethyl ether was added to the solution at 0°C. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 12/1) to give methyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2a** (79 mg, ee 98%) in 90% yield; colorless oil; $[\alpha]_D^{20} = -27.4$ (*c* 1.00 in $CHCl_3$).

Isopropyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2c** (100 mg) was similarly converted into methyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2a** (77 mg, ee 98%) in 89% yield; colorless oil; $[\alpha]_D^{20} = +27.3$ (*c* 1.00 in $CHCl_3$).

4.7.3. Conversion of *t*-butyl (2*E*,4*R*)- and (2*E*,4*S*)-4-hydroxy-2-hexenoate. To methanol solution (5 ml) of *t*-butyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2d** 60 mg was added one drop of sulfuric acid, and the reaction mixture was stirred at 60°C for 20 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 12/1) to give methyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2a** (53 mg, ee 95%) in 80% yield; colorless oil; $[\alpha]_D^{20} = -27.3$ (*c* 1.12 in $CHCl_3$).

t-Butyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2d** (60 mg) was similarly converted into methyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2a** (56 mg, ee 82%) in 90% yield; colorless oil; $[\alpha]_D^{20} = +23.0$ (*c* 1.19 in $CHCl_3$).

4.8. The lipid-HRP-catalyzed reduction of (*E*)-4-hydroperoxy-2-hexenal *rac*-1f

4.8.1. Preparation of enantiomerically enriched (2*E*,4*S*)-4-hydroperoxy- and (2*E*,4*S*)-4-hydroxy-2-hexenal. To a mixture of (*E*)-4-hydroperoxy-2-hexenal *rac*-**1f** (131 mg, 1.0 mmol) and syringol (154 mg, 1.0 mmol) in toluene (20 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) was added the lipid-HRP (200 mg, 2.0×10^{-4} mmol). The reaction mixture was stirred at 20°C for 1 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 12/1–6/1) to give (2*E*,4*R*)-4-hydroperoxy-2-hexenal (*R*)-**2f** (56 mg, ee 72%) in 58% yield and (2*E*,4*S*)-4-hydroperoxy-2-hexenal (*S*)-**1f** (32 mg) in 25% yield.

(2*E*,4*S*)-4-hydroperoxy-2-hexenal (*S*)-**1f** (32 mg) were dissolved in ether (5.0 ml) and subsequently trimethylphosphite (0.1 ml) was added to the solution. The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 24 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 10/1–6/1) to give (2*E*,4*S*)-4-hydroxy-2-hexenal (*S*)-**2f** (23 mg, ee 97%) in 82% yield.

(2*E*,4*S*)-4-Hydroperoxy-2-hexenal, (*S*)-1f: colorless oil; $[\alpha]_D^{20} = -24.4$ (*c* 1.56 in $CHCl_3$); IR, NMR and mass spectral data coincided with those of racemic one.

(2*E*,4*S*)-4-Hydroxy-2-hexenal, (*S*)-2f: colorless oil; $[\alpha]_D^{20} = +55.0$ (*c* 0.73 in $CHCl_3$); R_f (hexane–EtOAc, 4:1) 0.12; ν_{max} ($CHCl_3$)/cm^{-1} 3620, 3500, 3050, 3010, 2970, 2920, 2860, 2780, 1700, 1640, 1480, 1400, 1230, 1130, 1060, 1020, and 980; δ_H (250 MHz, $CDCl_3$) 0.98 (3H, t, *J* 7.4 Hz, CH_3), 1.59–1.75 (2H, m, CH_2), 2.63 (1H, br s, OH), 4.38 (1H, ddt, *J* 1.5, 4.7, and 6.7 Hz, C*H*OH), 6.31 (1H, ddd, *J* 1.5, 7.9 and 15.7 Hz, CH=C*H*-CHO), 6.85 (1H, dd, *J* 4.7 and 15.7 Hz, C*H*=CH-CHO), and 9.57 (1H, d, *J* 7.9 Hz, CHO); δ_C (63 MHz, $CDCl_3$) 9.50, 29.45, 72.22 (C-OH), 130.82, 159.27, and 193.90; *m*/*z* (HRMS) 114.0621 (MH^+; $C_6H_{11}O_2$ requires *MH*, 114.0329).

4.8.2. Preparation of enantiomerically enriched (2*E*,4*R*)-4-hydroxy-2-hexenal (*R*)-2f. To a mixture of (*E*)-4-hydroperoxy-2-hexenal *rac*-**1f** (131 mg, 1.0 mmol) and syringol (154 mg, 1.0 mmol) in toluene (20 ml) containing 1 v/v% of 0.1 M phosphate buffer (pH 6.5) was added the lipid-HRP (200 mg, 2.0×10^{-4} mmol), and the reaction mixture was stirred at 20°C for 0.5 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 12/1–6/1) to give (2*E*,4*R*)-4-hydroperoxy-2-hexenal, (*R*)-**2f** (40 mg, ee 93%) in 35% yield, and (2*E*,4*S*)-4-hydroperoxy-2-hexenal (*S*)-**1f** (95 mg, ee 73%) in 60% yield.

(2*E*,4*R*)-4-Hydroxy-2-hexenal, (*R*)-2f: colorless oil; $[\alpha]_D^{20} = -52.3$ (*c* 1.21 in $CHCl_3$).

4.9. Synthesis of (2*E*,4*R*)- and (2*E*,4*S*)-4-hydroxy-2-hexenal from ethyl (2*E*,4*R*)- and (2*E*,4*S*)-4-hydroxy-2-hexenoate

Ethyl (2*E*,4*R*)-4-hydroxy-2-hexenoate (*R*)-**2b** (270 mg, 1.7 mmol, ee 97%) and 3,4-dihydro-2*H*-pyran (286 mg, 3.4 mmol) were dissolved in dichloromethane (5.0 ml). To the solution was added pyridinium *p*-toluenesulphonate (43 mg, 0.17 mmol), and the reaction mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate, 12/1) to give ethyl (2*E*,4*R*)-4-tetrahydropyranyloxy-2-hexenoate (*R*)-**3** (380 mg, 93%). After 5.0 ml of 1.0 M diisobutyl aluminium hydride in hexane was added to dichloromethane solution of (*R*)-**3** (380 mg) at −78°C, the reaction mixture was stirred for 3 h. To the reaction mixture was added 1.8 ml of water, and the solution was stirred at room temperature for 15 min. The reaction mixture was dried over Na_2SO_4 and then the solvent was removed under reduced pressure. The residue was purified by column chromatography (hexane–ethyl acetate, 5/1) to give (2*E*,4*R*)-4-tetrahydropyranyloxy-2-hexenol (*R*)-**4** (209 mg, 67%). A mixture of (*R*)-**4** (209 mg, 1.0 mmol) and manganese dioxide (2.1 g, 10 wt. equiv.) in dichloromethane (5.0 ml) was stirred at room temperature for 2 h. The active manganese dioxide was removed from the reaction mixture by filtration and the solvent was removed under reduced pressure. The residue was purified by column chromatography (hexane–ethyl acetate, 8/1) to give (2*E*,4*R*)-4-tetrahydropyranyloxy-2-hexenal (*R*)-**5**

(181 mg, 87%). To a THF (5.0 ml) solution of (2*E*,4*R*)-4-tetrahydropyranyloxy-2-hexenal (*R*)-**5** (181 mg, 0.9 mmol) was added 0.5 M HCl (2.5 ml), and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with 1 M K_2CO_3 (2.5 ml) and then extracted with dichloromethane (3×10 ml). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (hexane–ethyl acetate, 8/1) to give (2*E*,4*R*)-4-hydroxy-2-hexenal (*R*)-**2f** (57 mg, ee 97%) in 48% yield; colorless oil; $[\alpha]_D^{20} = -55.1$ (*c* 1.96 in $CHCl_3$).

Ethyl (2*E*,4*S*)-4-hydroxy-2-hexenoate (*S*)-**2b** (240 mg, 1.5 mmol, ee >99%) was similarly converted into (2*E*,4*S*)-4-hydroxy-2-hexenal (*S*)-**2f** (39 mg, ee >99%) in 39% yield from (*S*)-**2b**; colorless oil; $[\alpha]_D^{20} = +56.3$ (*c* 1.65 in $CHCl_3$).

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