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Preparation of the enantiomers of 19-epoxy docosahexaenoic acids and their 4-hydroxy derivatives

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Abstract

By the action of NBS in aq. DME, *dl*-19-bromo-20-hydroxy-DHA methyl ester **5** was prepared, which was effectively resolved by lipase PS and vinyl acetate in the presence of a thiacrown ether to give **7** and **8**, each being transformed into the corresponding epoxides, **1** and **2**, respectively. The absolute configuration of **8** was established by the Kusumi–Moscher method. For the purpose of biological evaluation, both epoxides were converted to the γ-lactones **3** and their 4-hydroxy derivatives **4**.

1. Introduction

In the course of our studies concerning the oxygenated fatty acids, we needed the preparation of both enantiomers of 19-epoxy docosahexaenoic acid (DHA) methyl ester **1** and **2** and its oxygenated derivatives **3** and **4** for the biological evaluation of the oxygenated DHA. DHA is found in its highest concentration in the brain, retina and testis of mammals and also in fish oil,[1] exhibiting various biological activities.[2] This paper deals with the preparation of both enantiomers and their 4-hydroxy derivatives.

2. Results and discussion

The preparation of both enantiomers **1** and **2** commenced from our recent findings, in which we revealed the terminal double bonds of ω-3 unsaturated fatty acids such as α-linolenic acid, DHA and EPA can be oxidized by the action of NBS in aqueous organic solvent with high regioselectivity.[3] When DHA methyl ester was submitted to the reaction with NBS (1.3 equiv.) in aqueous dimethoxyethane (DME) followed by purification with HPLC, racemic 19-bromo-20-hydroxy-DHA methyl ester **5** was

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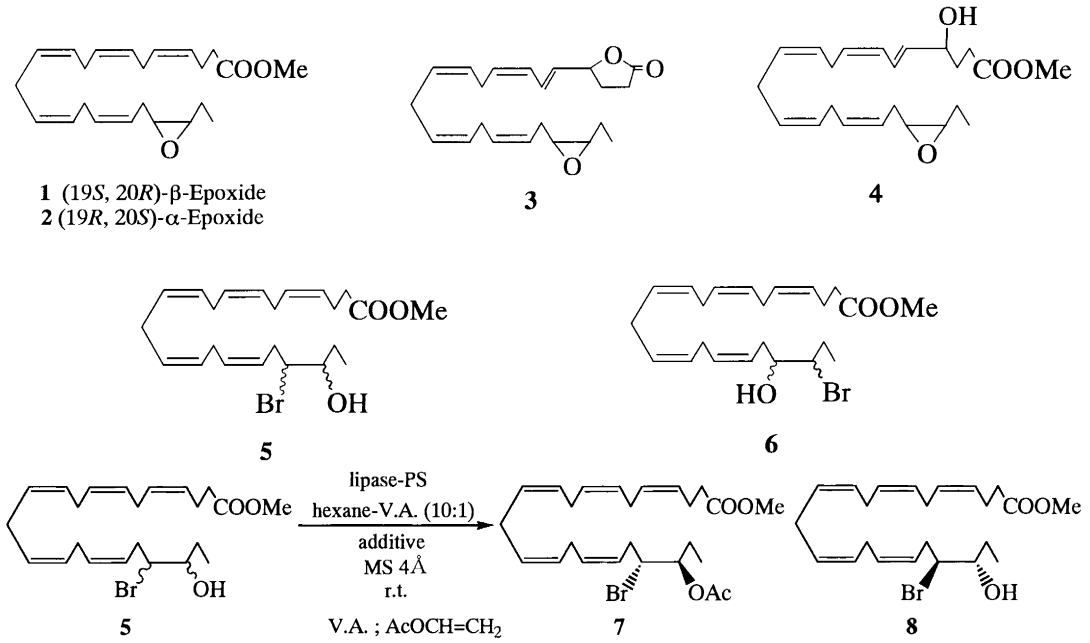
obtained in 37% conversion yield, accompanied by **6** as a major isomer. The bromohydrins **5** and **6** provided the same epoxide by base treatment. The isomer **6** was contaminated with other bromohydrins and could not be purified by the usual HPLC techniques.



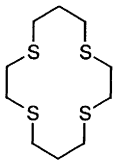
The resolution of *dl*-bromohydrin **5** was attempted by acylation with vinyl acetate in the presence of Lipase Amano PS. It was eventually found that the acylation took place, affording the acetate **7** and the resolved bromohydrin **8**. The reaction was quite slow and 1 week was not sufficient to complete the reaction, during which period some decomposition of the starting material occurred and hence the yields of **7** and **8** decreased seriously. Based on the recent demonstration[4] that addition of a crown ether leads to an increase of the reaction rate of lipase-catalyzed acylations, three different types of crown ether, 15-crown-5, thiacrown ether **I** and the Zn-complex of tropolonoid derivative **II**,[5] were examined. As shown in Table 1, the acylation was accelerated by addition of these crown ethers (entries 2–5), among which the thiacrown ether **I** was the most effective (entry 4) as reported by Takagi's group.[6] It is noteworthy that the acylation reaction depends largely on the kind of solvent employed. When diisopropyl ether was used as solvent, no reaction took place at all (entry 3). Table 2 shows the effect on the enantioselective acylation caused by the thiacrown ether, demonstrating that the reaction was complete after 1 day when 5 mol% of the thiacrown ether **I** was added. It was also shown that the thiacrown ether gave better results in the resolution in terms of the yield of the products in addition to the reaction rate. The position of the bromine atom and the hydroxyl group of the bromohydrin **5** was confirmed by the detailed inspection of the ^{1}H NMR spectra of **7** and **8**, wherein the chemical shifts and coupling modes of protons attached to the C19 to C22 are listed in Table 3. The downfield shift of the C20 proton of the acetate **7** indicates clearly that the hydroxyl group is located at the 20-position of the bromohydrin **5**. The *anti*-configuration of the Br atom with respect to the hydroxyl group is clear from the NBS–H_2O reaction.

The absolute configuration of the resolved bromohydrin **8** was elucidated by application of the Kusumi–Mosher method.[7] Thus, *dl*- and optically active bromohydrins **5** and **8** were submitted to the esterification with (*S*)- and (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) in the presence of DCC at room temperature, the results being summarized in Table 4. It was revealed that the reactivity of the bromohydrin depends largely on the absolute configuration of the MTPA employed. In the case of

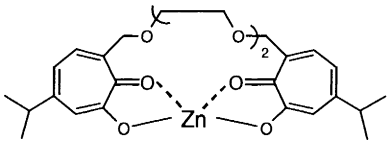
Table 1
Effect of the crown ethers on lipase-catalyzed acylation

Entry	Solvent	Additive	Time (days)	Result[a)]
1	hexane	none	7	+
2	hexane	15-crown-5	7	++
3	$(i\text{-}Pr)_2O$	thiacrown ether[b)]	7	-
4	hexane	thiacrown ether	7	+++
5	hexane	troponoid[c)]	7	++

a) The reactivity of the acylation was monitored by TLC wherein the results in entries 2~5 were based on that of entry 1.
b) 1, 4, 8, 11-Tetrathiacyclotetradecane **I** was used.
c) Zn complex of bis-tropolonyl diethyleneglycol derivative **II** was used.



I



II

Table 2
The effect of thiacrown ether **I** toward the acylation reaction of *dl*-bromohydrin **5**[a)]

Thiacrown ether	Time (days)	Specific rotation of **7** (yield)	Specific rotation of **8** (yield)
+ (5mol%)	1	$[\alpha]_D^{23}$ +19 (*c* 1.0, MeOH) (46%)[b)]	$[\alpha]_D^{24}$ -10 (*c* 1.0, MeOH) (50%)[b)]
–	6	$[\alpha]_D^{23}$ +18 (*c* 1.0, MeOH) (30%)	$[\alpha]_D^{23}$ -7.3 (*c* 1.0, MeOH) (50%)

a) The reaction was carried out with lipase PS, vinyl acetate and 4 Å molecular sieves in hexane in the presence or absence of thiacrown ether **I** at room temperature.
b) E.e % of **7** and **8**, obtained in the presence of the thiacrown ether , was ca. 97%.

Table 3
The chemical shifts and coupling modes[a)] of C19–C22 protons of the acylation products **7** and **8**

Compound	22-Me	21-CH_2	20-CHOR[b)]	19-CHBr
7	0.91 (t, 7.6 Hz)	1.76 (qd 7.6, 5.4 Hz)	4.93 (td, 5.4, 7.7 Hz)	4.05 (dt, 7.7, 3.4 Hz)
8	0.97 (t, 7.3 Hz)	1.61 (qd 7.3, 4.7 Hz)	3.40 (td, 4.7, 7.6 Hz)	4.08 (dt, 7.6, 3.0 Hz)

a) The NMR spectra were measured with 500 MHz in $CDCl_3$.
b) R = Ac and H in **7** and **8**, respectively.

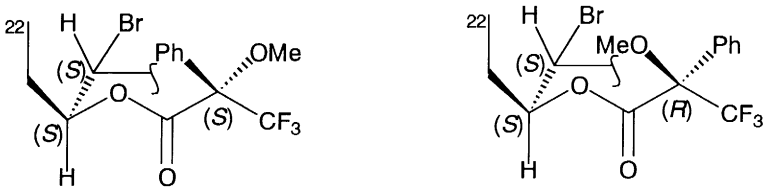
resolved bromohydrin **8**, 90% of the starting material **8** was recovered after 1 week by treatment with (*S*)-MTPA (entry 1) while 85% of the bromohydrin **8** was converted to the corresponding (*R*)-MTPA ester after 18 h when allowed to react with (*R*)-MTPA (entry 2). Alternatively, *dl*-bromohydrin **5** provided (*S*)-MTPA ester from (*S*)-MTPA in 64% yield after 94 h, accompanying the recovered bromohydrin possessing $[\alpha]_D$ −5.5 in 30% yield (entry 3). In the case of the reaction with (*R*)-MTPA, the recovered bromohydrin showed the opposite sign of the specific rotation (entry 4). It is evident that partial resolution of *dl*-bromohydrin **5** took place during MTPA esterification. These observations were explained by the concept of matched and mismatched relations[8] between the bromohydrins and the MTPA employed. In the NMR spectra of MTPA esters derived from *dl*-bromohydrin **5**, two kinds of methyl signals appeared at δ 0.95 and 0.84 in a 2:1 ratio. The upfield shift of the latter minor signal may be caused by the phenyl

group existing in a *syn* relation with the terminal 22-methyl group on the basis of the Kusumi–Mosher model as depicted in Fig. 1. This evidence leads to the conclusion that the resolved bromohydrin **8** has the 20*S* configuration. The non-shifted signal (0.95 ppm) observed in the NMR spectra of (*R*)-MTPA in entry 2, and also the major signals in entries 3 and 4, support this deduction. More than 97% enantiomeric excess (ee %) of the resolved bromohydrin **8** was estimated from the methyl signals (0.95 versus 0.84 ppm) of the (*R*)-MTPA ester prepared by complete esterification. Consequently, the (19*R*,20*R*) configuration is derivable for the bromoacetate **7**.

Table 4
MTPA esterification of bromohydrins **5** and **8**[a)]

Entry	Bromohydrin	Employed MTPA	Reaction time (h)	Product	
				MTPA ester	recovered bromohydrin
1	opt-active (**8**)	(*S*)	168	10%	90%
2	opt-active (**8**)	(*R*)	18	85% δ CH_3 0.95	15%
3	*dl* (**5**)	(*S*)	94	64% δ CH_3 0.95 : 0.84 = 2:1	30% $[\alpha]_D^{27}$ -5.5 (*c* 0.5, MeOH)
4	*dl* (**5**)	(*R*)	90	61% δ CH_3 0.95 : 0.84 = 2:1	25% $[\alpha]_D^{27}$ +4.9 (*c* 0.7, MeOH)

a) Bromohydrins **5** and **8** were treated with a mixture of (*R*) or (*S*)-MTPA (3 eq), DCC (3 eq) and DMAP (cat.) in CH_2Cl_2 at 25°C for the indicated time.

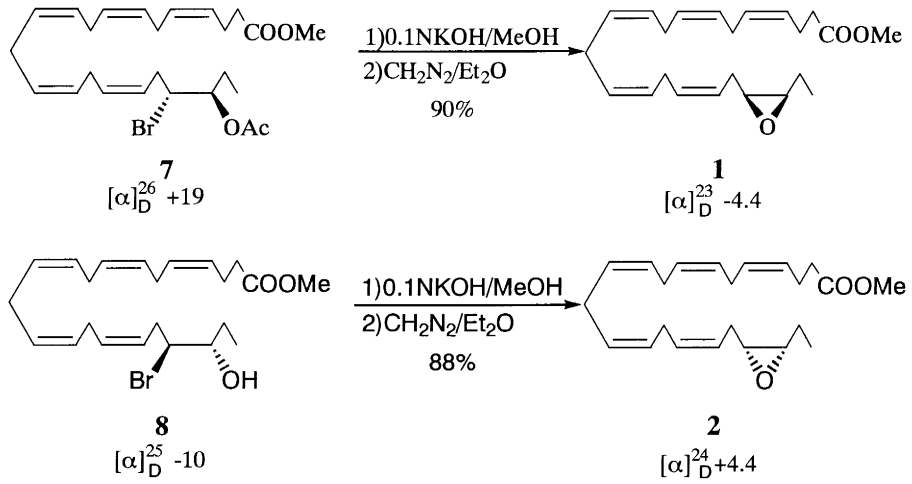


H-22 δ 0.84 H-22 δ 0.95

Fig. 1. Partial conformation of (*S*)- and (*R*)-MTPA esters of bromohydrin **8**

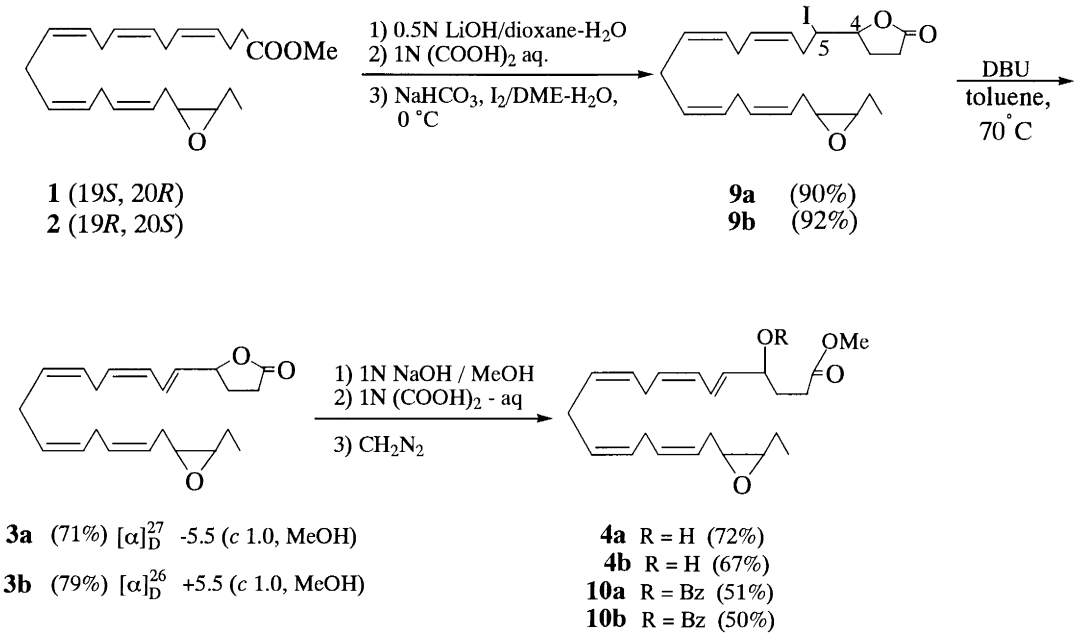
By treatment with methanolic KOH, bromoacetate **7** and the resolved bromohydrin **8** were transformed into the corresponding epoxides **1** and **2** in high yields, possessing specific rotations of −4.4 and +4.4, respectively (Scheme 1). More than 97% ee for **1** was derived from values of the specific rotations of each epoxide **1** and **2**, in which 97% ee for **2** was estimated from the original bromohydrin **8**.

The free acids obtained from the epoxy methyl esters **1** and **2** were submitted to the iodolactonization,[9] providing the corresponding iodolactones **9a** and **b** in high yields, wherein the formation of the γ-lactone ring was supported by observation of a sharp band at 1780 cm^{-1} in the IR spectra. The dehydroiodination with DBU afforded the corresponding pentaene-γ-lactones **3a** and **b**, in which the conjugated diene moiety possessing 5*E*,7*Z*-geometry in each compound was confirmed by comparison of the NMR spectra with those of the related fatty acids.[10] The pentaene-γ-lactones **3a** and **b** showed the same value for the specific rotation ($[\alpha]_D$) but with opposite signs. The alkaline hydrolysis of each lactone followed by careful acidification with aq. oxalic acid and then esterification with CH_2N_2 gave the 4-hydroxy esters **4a** and **b** (Scheme 2). The HPLC analysis of each benzoate derived therefrom with a chiral column showed that each of the benzoates **10a** and **b** is a 1:1 stereogenic mixture at the C4 position with respect



Scheme 1. Preparation and specific rotations of the epoxides **1** and **2** (each $[\alpha]_D$ measured at *c* 1.0 in MeOH)

to the asymmetric terminal epoxide ring. This evidence and the specific rotations of **3a** and **b** indicate no asymmetric induction occurred during iodolactonization of the epoxy DHA **1** and **2**.[11]



Scheme 2. Preparation of 4-hydroxy derivatives of each epoxide [**a** and **b** are (19*S*,20*R*) and (19*R*,20*S*) epoxy derivatives, respectively]

The biological activities of the oxygenated DHA derivatives prepared so far are now under investigation and the resolution of **4a** and **b** must be carried out if any positive biological activity is observed.

3. Experimental

3.1. General

Unless otherwise noted, ^{1}H NMR and ^{13}C NMR spectra were recorded on solutions in $CDCl_3$ with $SiMe_4$ as an internal standard with JNM-EX 270L (270 MHz) and GSX-500 (500 MHz) spectrometers. Chemical shifts are reported in δ_H and δ_C, and J values are in hertz. The following multiplicities were used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Infrared spectra were recorded on a Hitachi 270-30 spectrophotometer. The mass spectra were measured with a Hitachi M-80B spectrometer including EIMS (electron ionization, 70 eV) and HRMS mass spectrometries. Optical rotations were measured on a JASCO DIP-370 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Column chromatographic purification was carried out using Kiesel gel 60, Art 7734 (70–230 mesh). HPLC was performed with Waters Associates equipment and Guard-Pak Cartridge Prep Novapak HR (Waters Associates) and μ-porasil columns, respectively. Thin-layer chromatography was carried out on aluminium sheets coated with $60F_{254}$ silica. Plates were developed using a spray of 0.5% anisaldehyde in 2 M sulfuric acid. The starting material, DHA methyl ester, was purified using silica gel impregnated with 5% $AgNO_3$ column chromatography eluted with mixed solvents of hexane and AcOEt by changing the ratio from 60:1 to 30:1 and, finally, to 10:1. Solvents and commercially available reagents were dried and purified if necessary before use according to standard procedures. All the derivatives described in this paper are pale yellow oily substances and the purities were confirmed by ^{13}C NMR spectra. The usual work-up involved dilution of the reaction mixture with water, extraction with ether and evaporation after washing the organic extracts with water and brine, followed by drying over Na_2SO_4. The experiments concerning resolution of **5** to **7** and **8**, followed by epoxidation to **1** and **2**, were carried out five times and the average values of optical rotations were shown, the deviation of optical rotation being ± 0.2 in each experiment.

3.2. 19-Bromo-20-hydroxy-DHA methyl ester *5*

Water (45 ml) was added to a dimethoxyethane (DME) (150 ml) solution of DHA methyl ester (3.98 g, 11.6 mmol) to obtain the saturated DHA methyl ester solution. A mass of NBS (200 mg) was added to the stirred saturated solution under ice cooling in the dark under an argon atmosphere. The reaction was monitored with KI–starch paper. After complete disappearance of HOBr in the solution, another mass of NBS (200 mg) was added; finally, a total amount of 2.69 g (15.1 mmol) of NBS was added. The reaction mixture was treated as usual and the crude reaction mixture was separated by SiO_2 (200 g) column chromatography eluted with hexane:AcOEt (40:1) to isolate first the recovered DHA methyl ester (2.02 g) and then a mixture of monobromohydrins (2.62 g). The mixture of monobromohydrins was submitted to HPLC purification with Guard-Pak Cartridge Prep Novapak HR column eluted with hexane:AcOEt (15:1) under the flow rate of 18 ml/min to obtain pure bromohydrins **5** (0.96 g, 37% conversion yield) and **6** (0.92 g), each as a pale yellow oil. The isomer **6** was contaminated with other bromohydrins and could not be purified by the usual HPLC technique. Compound **5**: δ_H (270 MHz, $CDCl_3$) 0.97 (3H, t, J=7.3 Hz), 1.61 (2H, m), 2.38 (4H, m), 2.80 (10H, m), 3.40 (1H, m), 3.67 (3H, s), 4.08 (1H, m) and 5.33–5.54 (10H, m). δ_C (67.8 MHz, $CDCl_3$) 173.6 (s), 131.0 (d), 129.3 (d), 128.5 (d), 128.2 (d) ×2, 128.1 (d) ×2, 127.9 (d), 127.7 (d), 126.1 (d), 74.2 (d), 62.9 (d), 51.6 (q), 34.0 (t), 33.8 (t), 29.0 (t), 25.9 (t), 25.7×2, 25.6 (t), 22.8 (t) and 10.0 (q). EIMS *m/e* (relative intensity) 438 (M^+, 10%), 409 (M^+-OMe, 9). HRMS calcd for $C_{23}H_{35}BrO_3$: 438.1770. Observed: 438.1748.

3.3. Resolution of dl-bromohydrin **5**

To a hexane (15 ml) solution of *dl*-bromohydrin **5** (714 mg) were added lipase PS (714 mg), 4 Å molecular sieves (ca 100 mg), 1,4,8,11-tetrathiacyclotetradecane (ca 20 mg) and vinyl acetate (1.5 ml) and the mixture was stirred for 24 h at room temperature under argon atmosphere. The insoluble materials were removed through a short silica gel column and the silica gel was washed with ether. After the combined organic layers were evaporated, the residue was passed through an SiO_2 (40 g) column eluted with hexane:AcOEt (30:1) to isolate the bromoacetate **7** (357 mg, 46%) and resolved bromohydrin **8** (360 mg, 50%) as a pale yellow oil, respectively. The experiments in Table 1 were carried out using the indicated crown ethers under similar conditions except for the reaction time.

Bromo acetate **7**: $[\alpha]_D^{24}$ +19 (*c* 1.0, MeOH). δ_H (270 MHz, $CDCl_3$) 0.9 (3H, t, *J*=7.3 Hz), 1.75 (2H, m), 2.12 (3H, s), 2.38 (4H, bs), 2.63 (2H, t, *J*=6.5 Hz), 2.84 (8H, m), 3.67 (3H, s), 4.04 (1H, m), 4.92 (1H, m) and 5.27–5.55 (10H, m). δ_C (67.8 MHz, $CDCl_3$) 173.5 (s, 1-C), 170.5 (s, Ac), 131.0 (d), 129.3 (d), 128.6 (d), 128.3 (d), 128.2 (d), 128.0 (d),127.9 (d), 127.6 (d), 125.8 (d),125.7 (d), 75.8 (d, 20-C), 55.9 (d, 19-C), 51.6 (q, Me ester), 34.0 (t), 33.0 (q), 25.9 (t), 25.7 (t) ×2, 25.6 (t), 25.4 (t), 22.7 (t), 21.0 (t) and 9.7 (q, 22-C). EIMS *m/e* (relative intensity) 480 (M^+, 2%), 451 [$(M-OMe)^+$, 2], 341 [$(M–Br–COMe–OMe)^+$, 38]. HRMS calcd for $C_{25}H_{37}BrO_4$: 480.1875. Found: 480.1876. Resolved Bromohydrin **8**: $[\alpha]_D^{24}$ −10 (*c* 1.0, MeOH). The NMR spectra (δ_H and δ_C) were identical to *dl*-bromohydrin **5**.

3.4. MTPA ester of bromohydrins

A mixture of the resolved bromohydrin **8** (16 mg, 0.034 mmol), DCC (15 mg, 0.073 mmol), DMAP (ca. 2 mg) and (*R*)-MTPA (13 mg, 0.073 mmol) in anhydrous CH_2Cl_2 (1 ml) was stirred at room temperature for 68 h. The reaction mixture was diluted with ether and the organic layer was successively washed with aq. $NaHCO_3$ solution, saturated aq. NH_4Cl solution and then brine and dried over Na_2SO_4. After evaporation of volatile materials, the residue was passed through an SiO_2 (2 g) column eluted with hexane:AcOEt (20:1) to remove urea. The (*R*)-MTPA ester of bromohydrin **8** was purified by HPLC using a μ-porasil column with hexane:AcOEt (20:1) as an elution solvent under the flow rate of 3 ml/min to obtain pure (*R*)-MTPA ester of **8** (24.7 mg, 98%). Similarly, the *dl*- and resolved bromohydrins were, respectively, treated with (*S*)- and (*R*)-MTPA under the conditions indicated in Table 4 to furnish the corresponding MTPA esters and recovered bromohydrin described in Table 4. (*R*)-MTPA ester of **8**: δ_H (270 MHz, $CDCl_3$) 0.95 (3H, t, *J*=7.6 Hz), 1.86 (2H, dq, *J*=5.6, 7.6 Hz), 2.38 (4H, bs), 2.47 (2H, m), 2.71–2.86 (8H, m), 3.58 (3H, s, OMe of MTPA), 3.67 (3H, s, 1-OMe), 4.03 (19-H, td, *J*=5.3, 8.9 Hz), 5.11 (20-H, td, *J*=5.6, 8.9 Hz), 5.29–5.48 (10H, m), 7.42 (3H, m) and 7.57 (2H, m).

3.5. (19S,20R)- and (19R,20S)-Epoxy-DHA **1** and **2**

After the bromoacetate **7** (282 mg, 0.59 mmol) in 0.1N KOH–MeOH (30 ml, 3 mmol) was stirred at room temperature for 20 h under an argon atmosphere, 0.5 N aq. oxalic acid solution was added to make the pH of the solution acidic (3–4). After the usual workup, the excess CH_2N_2 in ether solution was added to the crude reaction mixture and then the solvent was removed in vacuo. The crude methyl ester was purified first by SiO_2 (10 g) column chromatography eluted with hexane:AcOEt (20:1) to obtain the (19*S*,20*R*)-β-epoxide **1**: (185 mg, 88%) as a pale yellow oil. β-Epoxide **1**: $[\alpha]_D^{23}$ −4.4±0.2 (*c* 1.0, MeOH). δ_H (270 MHz, $CDCl_3$) 1.05 (3H, t, *J*=7.3 Hz), 1.57 (2H, m), 2.25 (1H, td, *J*=6.6, 5.6 Hz), 2.38 (5H, br m), 2.85 (10H, m), 3.67 (3H, s) and 5.38 (10H, m). δ_C (67.8 MHz, $CDCl_3$) 173.5 (s), 130.4 (d),

129.3 (d), 128.4 (d), 128.2 (d), 128.2 (d), 128.1 (d), 128.1 (d), 127.9 (d), 127.9 (d), 124.5 (d), 58.3 (d), 56.5 (d), 51.6 (q), 34.0 (t), 26.2 (t), 25.8 (t), 25.7 (t) ×2, 25.6 (t), 22.8 (t), 21.1 (t) and 10.7 (q). EIMS *m/e* (relative intensity) 358 (M^+, 4%), 327 [$(M-OMe)^+$, 3). HRMS calcd for $C_{23}H_{34}O_3$: 358.2508. Observed: 358.2500.

The resolved bromohydrin **8** (272 mg) was similarly treated with 0.1N KOH–MeOH for 20 h at room temperature under an argon atmosphere to obtain (19*R*,20*S*)-α-epoxide **2** (205 mg, 93%) as a pale yellow oil. $[\alpha]_D^{24}$ +4.4±0.2 (*c* 1.0, MeOH). δ_H (270 MHz, $CDCl_3$) and δ_C (67.8 MHz, $CDCl_3$) were identical to those of (19*S*,20*R*)-β-epoxide **1**. HRMS calcd for $C_{23}H_{34}O_3$: 358.2508. Observed: 358.2511.

*3.6. Iodolactonization of β-epoxy DHA **1***

After a mixture of β-epoxy-DHA methyl ester **1** (33.8 mg, 0.094 mmol) and 0.5N LiOH (dioxane:H_2O 1:1) (1.9 ml, 0.94 mmol) was stirred at room temperature for 18 h under an argon atmosphere, the reaction mixture was condensed at 40°C under aspirator pressure after addition of water (5 ml). The aq. layer was made acidic (pH 3–4) by adding an aq. oxalic acid solution and then extracted with ether to obtain the hydrolyzed epoxy DHA. The free epoxy DHA was, without purification, taken up into DME (1 ml) and the DME solution was diluted with H_2O (1 ml). After $NaHCO_3$ (32 mg, 0.38 mmol) was added to the aq. DME solution and the mixture was stirred for 1 h at 0°C, iodine (24 mg, 0.094 mmol) dissolved in 2 ml of DME:H_2O (1:1) was added and then the mixture was kept stirring at 0°C for 15 h. The reaction mixture was quenched with an aq. $Na_2S_2O_3$ solution and the mixture was treated as usual after extraction with ether. The residue was purified by SiO_2 (7 g) chromatography eluted with hexane:AcOEt (8:1) to isolate the epoxy iodolactone **9a** (37 mg, 82%) as a pale yellow oil. The α-epoxy DHA **2** (102 mg) was similarly submitted to iodolactonization reaction to give iodolactone **9b** (118 mg, 90%). β-Epoxy iodolactone **9a**: δ_H (270 MHz, $CDCl_3$) 1.05 (3H, t, *J*=7.4 Hz), 1.59 (2H, dq, *J*=7.6, 7.3 Hz), 2.08 (1H, m), 2.25 (1H, m), 2.36–2.73 (4H, m), 2.76–2.99 (10H, m), 4.15 (1H, dt, *J*=3.0, 7.3 Hz), 4.27 (1H, dt, *J*=3.0, 7.6 Hz) and 5.31–5.61 (8H, m). δ_C (67.8 MHz, $CDCl_3$) 176.2 (s), 131.4 (d), 130.3 (d), 128.6 (d), 128.2 (d), 127.9 (d), 127.4 (d), 126.8 (d), 124.5 (d), 80.68 (d), 58.24 (d), 56.42 (q), 38.06 (d), 34.66 (t), 28.46 (t), 27.35 (t), 26.15 (t), 25.86 (t), 25.80 (t), 25.66 (t), 21.04 (t) and 10.64 (q). IR (neat) 1786 cm^{-1}.

*3.7. Dehydroiodination of iodolactones **9a** and **9b***

A mixture of β-epoxy iodolactone **9a** (210 mg, 0.447 mmol) and DBU (100 μl, 0.621 mmol) in anhydrous toluene (3 ml) was warmed at 70°C for 5 h under an argon atmosphere and then the reaction was quenched by adding an aq. NH_4Cl solution after being cooled to room temperature. The reaction mixture was treated as usual and the residue was purified by SiO_2 (35 g) column chromatography eluted with hexane:AcOEt (8:1) to obtain the β-epoxy pentaene lactone **3a** (135 mg, 88%) as a pale yellow oil. δ_H (270 MHz, $CDCl_3$) 1.05 (3H, t, *J*=7.6 Hz), 1.58 (2H, m), 2.01 (1H, m), 2.25 (1H, m), 2.36–2.59 (4H, m), 2.79–3.00 (8H, m), 5.01 (1H, q, *J*=6.9 Hz), 5.33–5.57 (7H, m), 5.69 (1H, dd, *J*=6.9, 15.2 Hz), 6.01 (1H, t, *J*=11.2 Hz) and 6.62 (1H, dd, *J*=11.2,15.2 Hz). δ_C (67.8 MHz, $CDCl_3$) 176.7 (s), 132.2 (d), 130.2 (d), 130.0 (d), 128.6 (d), 128.0 (d), 127.8 (d), 127.8 (d), 127.1 (d), 127.0 (d), 124.4 (d), 80.40 (d), 58.13 (d), 56.30 (q), 28.70 (t), 28.43 (t), 26.02 (t), 25.99 (t), 25.66 (t), 25.52 (t), 20.92 (t) and 10.48 (q).

By a similar treatment, α-epoxy pentaene lactone **3b** (166 mg, 82%) was obtained from α-epoxy iodolactone **9b** (278 mg, 0.591 mmol) as a pale yellow oil. HRMS of **3a** and **3b** calcd for $C_{22}H_{30}O_3$: 342.2195. Observed for **3a**: 342.2190; for **3b**: 342.2189.

3.8. 19,20-β-Epoxy-4-hydroxy-DHA methyl ester **4a** and **4b**

After the β-epoxy pentaene lactone **3a** (26 mg, 0.075 mmol) in 0.7N NaOH–MeOH (7 ml) was kept at room temperature for 19 h, the mixture was diluted with water (5 ml) and then extracted with ether after an aq. 0.5N oxalic acid solution was added to make the solution acidic (pH 3–4). An excess of CH_2N_2 ether solution was added to the condensed residue at 0°C. Evaporation of the volatile materials and then SiO_2 (8 g) column chromatography eluted with hexane:AcOEt (10:1) gave the β-epoxy-4-hydroxy-DHA methyl ester **4a** (21 mg, 72%) as a pale yellow oil. δ_H (500 MHz, $CDCl_3$) 1.05 (3H, t, *J*=7.6 Hz), 1.57 (2H, m), 1.89 (2H, m), 2.22 (1H, m), 2.43 (1H, m), 2.45 (2H, bs), 2.84–2.98 (8H, m), 3.68 (3H, s), 4.24 (1H, dd, *J*=12.4, 6.3 Hz), 5.37–5.53 (7H, m), 5.69 (1H, dd, *J*=6.3, 15.3 Hz), 6.00 (1H, t, *J*=10.9 Hz), 6.54 (1H, dd, *J*=15.3, 11.2 Hz). δ_C (80 MHz, $CDCl_3$) 174.27 (s), 135.59 (d), 130.38 (d) ×2, 128.43 (d), 128.19 (d), 127.86 (d), 127.84 (d), 127.60 (d), 125.59 (d), 124.37 (d), 71.54 (d), 58.38 (d), 56.53 (d), 51.63 (q), 31.92 (t), 29.99 (t), 26.12 (t), 26.06 (t), 25.79 (t), 25.65 (t), 20.98 (t) and 10.56 (q).

Similarly, the α-epoxy pentaene lactone **3b** (31 mg) afforded the corresponding α-epoxy-4-hydroxy-DHA methyl ester **4b** (23 mg) in 67% yield. HRMS of **4a** and **4b** calcd for $C_{23}H_{34}O_4$: 374.2457. Observed for **4a**: 374.2450; for **4b**: 374.2449.

3.9. Benzoates **10a** and **10b** of epoxy-4-hydroxy-DHA methyl esters

A mixture of β-epoxy-4-hydroxy-DHA methyl ester **4a** (23 mg, 0.06 mmol), triethylamine (9 μl, 0.6 mmol), benzoic anhydride (41 mg, 0.18 mmol) and DMAP (ca. 4 mg) in CH_2Cl_2 (3 ml) was stirred at room temperature for 38 h under an argon atmosphere and then MeOH and an aq. NH_4Cl solution was added to the mixture to quench the benzoylation. The reaction mixture was treated as usual and the residue was purified with SiO_2 (8 g) column chromatography eluted with hexane:AcOEt (20:1) to obtain the β-epoxy benzoate **10a** (15 mg, 51%) after further purification with HPLC with μ-porasil column as a pale yellow oil. δ_H (270 MHz, $CDCl_3$) 1.05 (3H, t, *J*=7.6 Hz), 1.58 (2H, m), 2.13 (2H, m), 2.23 (1H, m), 2.38 (1H, m), 2.43 (2H, m), 2.83 (2H, bs), 2.86–2.99 (6H, m), 3.63 (3H, s), 5.35–5.52 (7H, m), 5.66 (1H, dd, *J*=7.3, 14.8 Hz), 5.99 (1H, t, *J*=10.9 Hz), 6.65 (1H, dd, *J*=10.9, 14.8 Hz), 7.44 (2H, m), 7.54 (1H, m) and 8.05 (2H, m). δ_C (67.8 MHz, $CDCl_3$) 170.30 (s), 165.66 (s), 132.97 (d), 131.59 (d), 130.46 (d),130.33 (d), 130.23 (d), 130.06 (s), 129.58 (d), 128.59 (d), 128.34 (d), 128.21 (d), 128.18 (d), 128.01 (d), 127.94 (d), 127.57 (d), 127.44 (d), 124.51 (d), 74.13 (d), 58.29 (d), 56.50 (d), 51.68 (q), 29.90 (t), 29.78 (t), 29.69 (t), 26.13 (t), 25.77 (t), 25.61 (t), 21.04 (t) and 10.60 (q).

By a similar reaction, α-epoxy-4-hydroxy-DHA methyl ester **4b** (23 mg, 0.06 mmol) was converted into the α-epoxy benzoate **10b** (13 mg) in 50% yield. The formation conditions of benzoates **10a** and **b** were not optimized. HRMS of **10a** and **10b** calcd for $C_{30}H_{38}O_5$: 478.2719. Observed for **10a**: 478.2715; for **4b**: 478.2724. The benzoates **10a** and **b** were, respectively, analyzed with the Daicel CHIRALCEL OD column eluted with hexane:*i*PrOH (9:1), showing two peaks at 7.0 and 7.7 min with the same intensities.

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