Bioorganic & Medicinal Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Construction of a series of intermediates in the β-oxidation pathway from THA to EPA via DHA in free acid form

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A B S T R A C T

β-Oxidation of most fatty acids occurs in the mitochondria. However, β-oxidation for ω-3 polyunsaturated fatty acids (PUFAs) is distinct from abundant fatty acids and occurs in the peroxisomes. Since little is known about peroxisomal β-oxidation, here we report the synthesis of proposed intermediates of ω-3 PUFA β-oxidation steps in free fatty acid form having a conjugated double bond, a β-hydroxyl group, a β-olefin and a β-carbonyl group. These fatty acids can serve as authentic samples for biological experiments.

1. Introduction

Eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are representative ω-3 polyunsaturated fatty acids (PUFAs) (Fig. 1).

These PUFAs and their oxidized metabolites are important research subjects in nutrition, metabolomics, molecular biology, cell biology, and medicine,[1–5] etc. The oxidation of EPA and DHA is catalyzed by lipoxygenase, cyclooxygenase[6] or cytochrome P450[7] for the synthesis of chemical mediators such as leukotrienes, prostaglandins, pro-resolvins mediators and neuroprotectins.[6–10] Additionally, β-oxidation is fundamental for life, maintaining homeostasis by producing energy. In mammals, β-oxidation takes place in mitochondria, with some exceptions.

β-Oxidation for ω-3 PUFAs occurs in peroxisomes for the synthesis of DHA and its retroconversion to EPA (Scheme 1).[11]

Although ω-3 PUFAs have recently been trending in lipid research, reports of peroxisomal β-oxidation are far fewer than those of mitochondrial β-oxidation. Scheme 1 shows the synthetic pathway for EPA and DHA by peroxisomal β-oxidation. EPA-CoA is converted to docosapentaenoyl-CoA (DPA-CoA, C22:5n-3) by elongase. Due to the lack of Δ4-desaturase in mammals,[12] there is an indirect route for the synthesis of DHA, the so called Sprecher's shunt.[13,14] In this route, DPA-CoA, C22:5n-3 is converted to tetracosahexaenoyl-CoA (THA-CoA, C24:6n-3) by elongase and Δ6-desaturase, and then acetyl-CoA is cleaved off by β-oxidation to afford DHA-CoA. EPA-CoA is then biosynthesized by β-oxidation of DHA-CoA. In the liver, there has been a report that the rate of retroconversion of DHA to EPA is 20% in a study using tritium labeled DHA.[15]

No β-oxidation intermediates have been reported via Sprecher's shunt or in the retroconversion of EPA in mammals. However, these intermediates can be proposed on the basis of two other pathways. Reaction mechanisms in the mitochondrial β-oxidation pathway are well known in other unsaturated fatty acids and the metabolic pathway of DHA to EPA in yeast is known.[11] It is thought that oxidation in mammals also passes through a similar pathway. The proposed pathway is shown in Scheme 2. THA-CoA is dehydrogenated to afford α,β-unsaturated-THA-CoA (**1**-CoA). The conjugated olefin is hydrated to afford β-hydroxy compound **2**-CoA. Next, the β-hydroxyl group of **2**-CoA is further oxidized to afford β-keto compound **3**-CoA, and the α-carbon of the β-keto ester is cleaved to generate DHA-CoA. The retroconversion pathway to EPA contains additional reactions because this pathway occurs via the highly conjugated compound **4**-CoA. DHA-CoA is dehydrogenated to afford **4**-CoA. The highly conjugated **4**-CoA is then reduced to **5**-CoA. The deconjugated **5**-CoA is converted to conjugated ester **6**-CoA and hydration of the conjugated olefin affords **7**-CoA. The β-hydroxyl group is further oxidized to afford **8**-CoA, in which the α -carbon of the β-keto ester is then cleaved to generate EPA-CoA.

Herein, we report the syntheses of seven PUFAs in their free fatty acid forms (Fig. 2), which are predicted to be peroxisomal β-oxidation intermediates from THA to EPA via DHA in mammals. In this paper, we

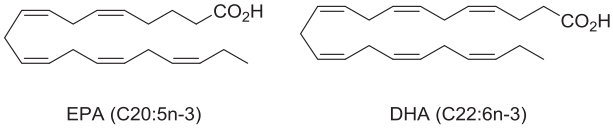

Fig. 1. Structures of representative ω-3 PUFAs.

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https://doi.org/10.1016/j.bmc.2018.07.004
Received 8 June 2018; Received in revised form 30 June 2018; Accepted 3 July 2018

show several synthetic approaches for obtaining the complete peroxisomal β-oxidation intermediates of ω-3 PUFAs.

2. Results and discussion

Since the synthesis of skipped *Z* olefins requires sensitive reactions and multiple steps, we planned to use EPA and DHA esters, which are abundant resources and easy to obtain.

2.1. Synthesis of β-olefinic acids

We synthesized isomers of DHA **5** and **6** and docosaheptaenoic acid **4** as follows. In our previous study, phosphorous ylide **9** was synthesized via iodolactonization and glycol cleavage using EPA as the starting material (Scheme 3).[16]

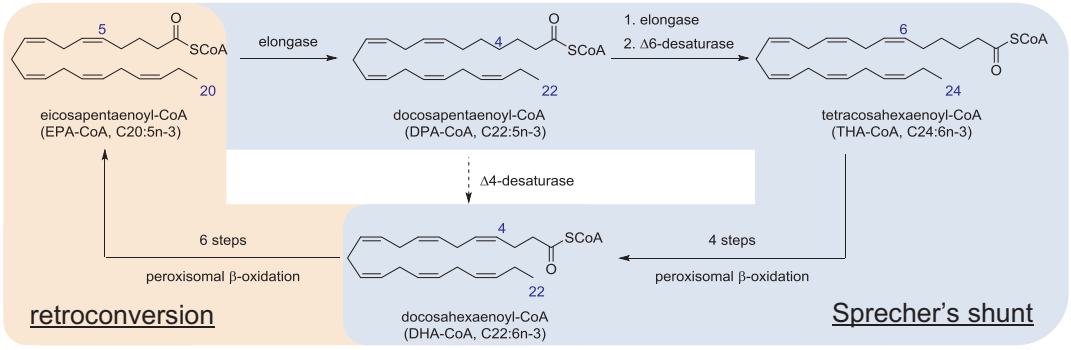
In order to synthesize **4**, we first synthesized phosphorous ylide **14** from DHA as the key intermediate. Iodolactonization of DHA afforded lactone **10**.[16] Lactone **10** was treated with KOH-MeOH aq. to afford glycol **11** and subsequent glycol cleavage was accomplished by $NaIO_4$ and $NaBH_4$ to give alcohol **12** in 33% yield for the 3 steps. After bromination of alcohol **12** (quant), followed by treatment with PPh_3, synthesis of ylide **14** was achieved in 43% yield over 2 steps (Scheme 4).

Using Dess-Martin periodinane, aldehydes **16** (97%) and **18** (83%) were prepared from alcohols **15** and **17**, respectively (Figs. S1 and S2).

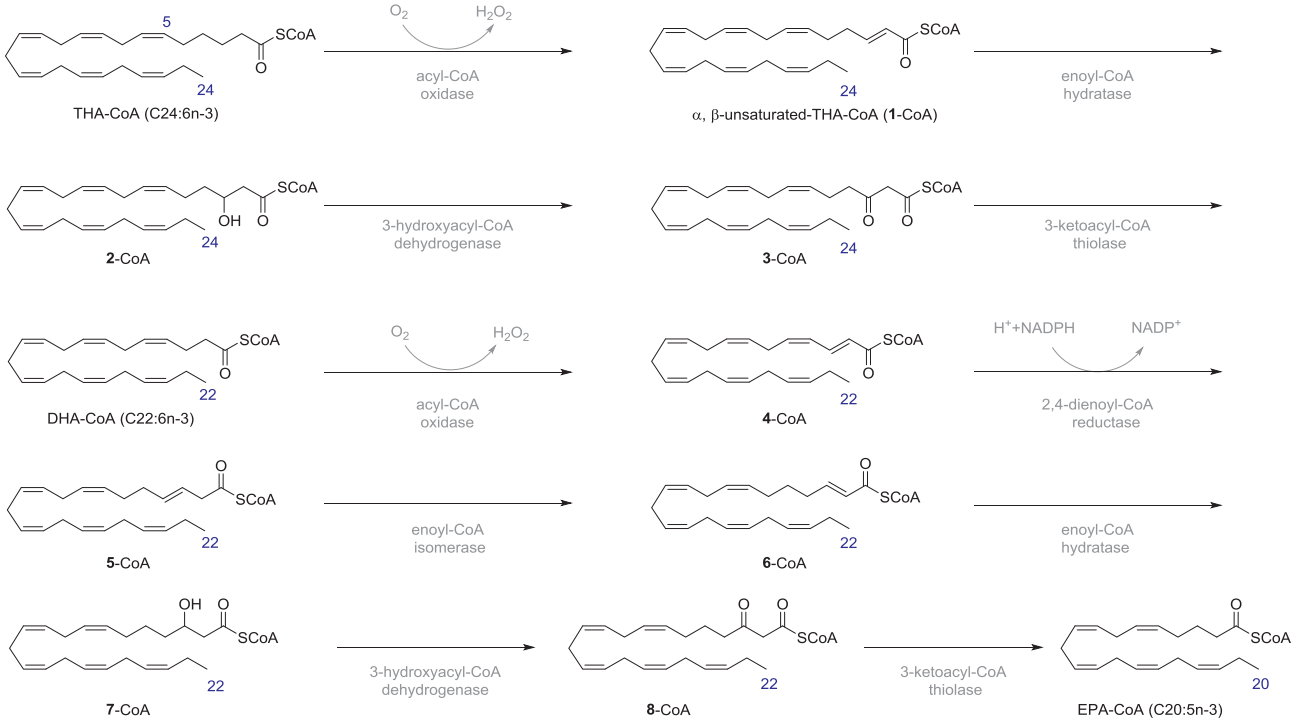
Wittig reactions were performed with ylide **9** using LHMDS as a base in THF with and without HMPA (Scheme 5). Ester **19** was obtained in 28% yield from **9** and aldehyde **16**. In the presence of HMPA, the yield was only slightly increased (30%). In the case of **20** from **9** and **18**, the yield of the Wittig reaction was 22% without HMPA, but 53% in the presence of HMPA. Esters **19** and **20** were treated with 5% KOH-^{i}PrOH/H_2O to give target DHA isomer **5** in 97% yield and **6** in quantitative yield, respectively.

Ester **21** was obtained by coupling of ylide **14** and (*E*)-ethyl-4-oxobut-2-enate (28% yield). In addition, since three Wittig reactions proceeded with multiple byproduct, we couldn't isolate *E* isomers for **19**, **20** and **21**.

It should be noted that 1,4-adducts were obtained when MeOH was used as the solvent in the hydrolysis of conjugated PUFA esters, thus ^{i}PrOH was used as the solvent.[17] Accordingly, highly conjugated ester **21** was treated with 5% KOH-^{i}PrOH/H_2O to provide docosaheptaenoic acid (**4**) in 35% yield. When the reaction was monitored by TLC, same products appeared, which were thought to contain 1,4- or 1,6-conjugate



Scheme 1. Biosynthetic pathway of EPA and DHA.



Scheme 2. β-Oxidation of THA to EPA via DHA.

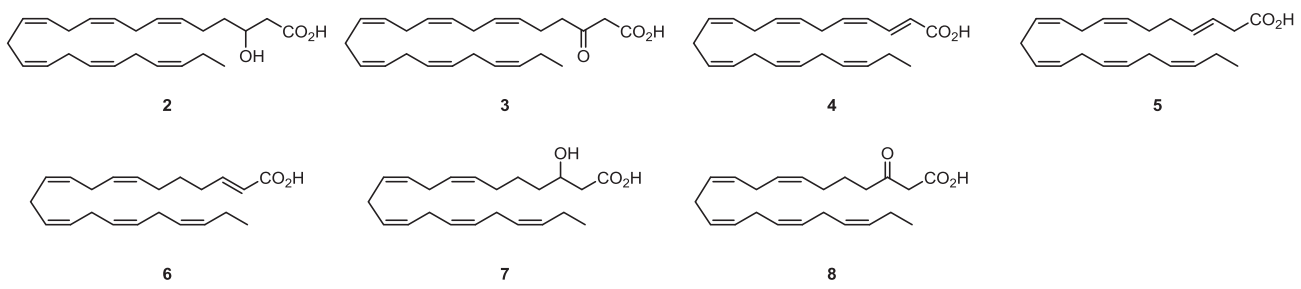
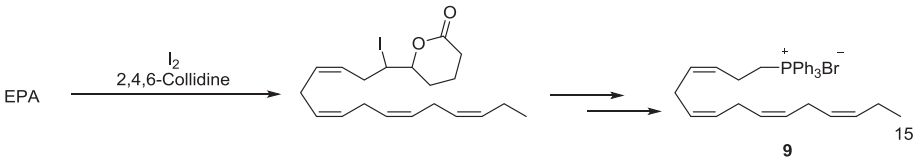
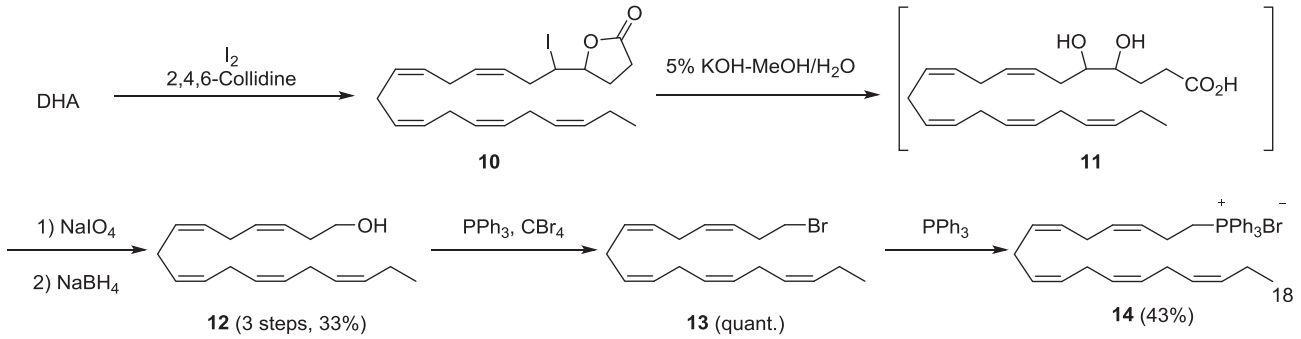


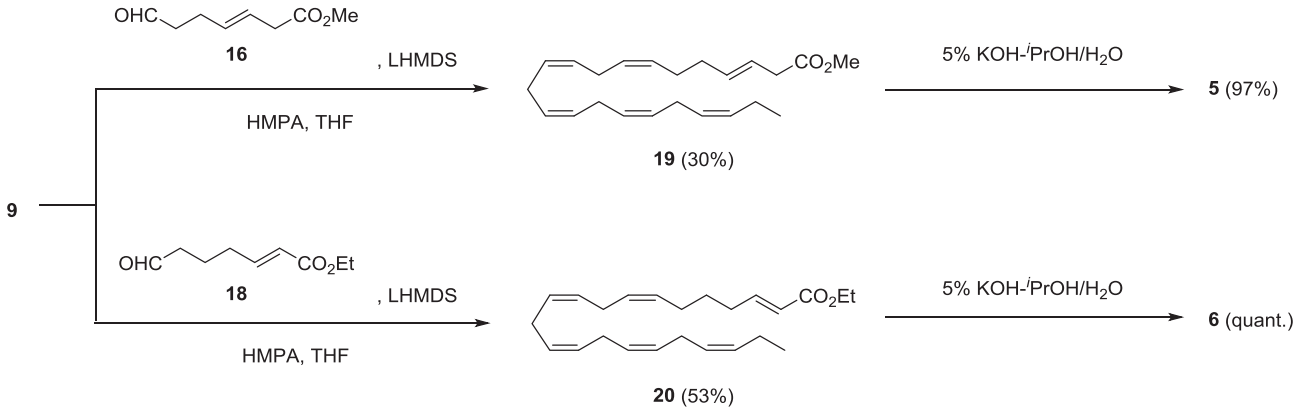
Fig. 2. Structures of the target compounds.



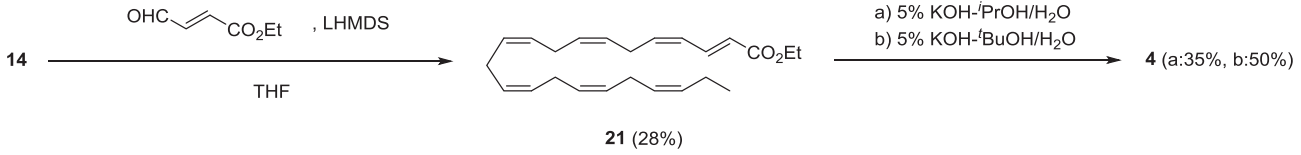
Scheme 3. Preparation of phosphorus ylide **9** via iodolactonization.



Scheme 4. Preparation of phosphorus ylide **14** via iodolactonization.



Scheme 5. Synthesis of DHA isomer **5** and **6**.

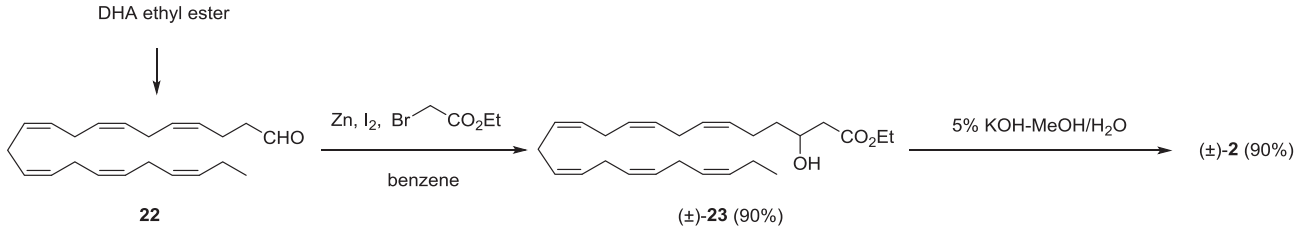


Scheme 6. Synthesis of docosaheptaenoic acid (**4**).

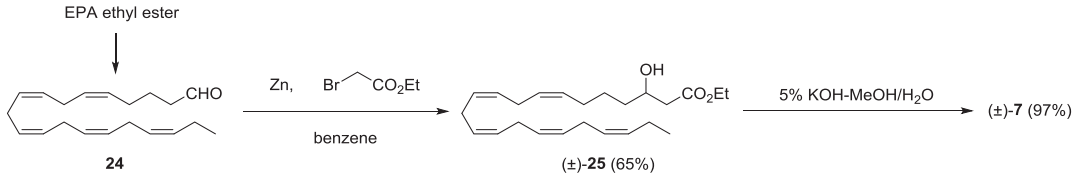
adducts. We thought that the conjugation reactions could be suppressed by using ^{t}BuOH, which is bulkier than ^{i}PrOH. As expected, using ^{t}BuOH as a solvent, we succeeded in improving the yield to 50% (Scheme 6).

2.2. Racemic synthesis of β-hydroxyl acids 2 and 7

We planned to synthesize β-hydroxyl acids **2** and **7** in racemic form in order to provide standard samples for the asymmetric synthesis and



Scheme 7. Synthesis of (±)-β-hydroxyl acid **2**.



Scheme 8. Synthesis of (±)-β-hydroxyl acid **7**.

for the synthesis of achiral β-keto acids. The β-hydroxyl group was constructed using the Reformatsky reaction,[18] as follows. Aldehyde **22**, prepared from DHA ethyl ester, was treated with Zn and ethyl bromoacetate to give (±)-**23** in 90% yield. Treatment of (±)-β-hydroxyl ester **23** with 5% KOH-MeOH/H_2O gave (±)-**2** in 90% yield (Scheme 7).

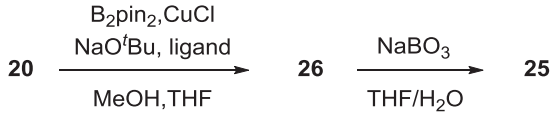
Likewise, β-hydroxyl ester (±)-**25** was produced from aldehyde **24**[19] derived from EPA ethyl ester (65% yield) and then treated with 5% KOH-MeOH/H_2O to obtain (±)-**7** in 96% yield (Scheme 8).

2.3. Asymmetric synthesis of β-hydroxyl acids 2 and 7

We planned to perform the asymmetric synthesis of **2** and **7**[20] as in the peroxisomal β-oxidation. However, the stereochemistry of the hydroxylation reaction in the metabolic pathway of DHA and THA has not yet been clarified. Furthermore, there are no examples to introduce a hydroxyl group stereoselectively to the β-position of a fatty acid. Therefore, the asymmetric synthesis was conducted with reference to the β-boration reaction, which is an asymmetric reaction applied to non-aromatic substrates.[21] The stereoselectivity was introduced with β-boration by 1,4-addition to the α,β-unsaturated ester **20**, and the desired compound was obtained by a two-step reaction in which the β-hydroxyl form was obtained by oxidation using Bpin with retention of configuration (Scheme 9).

Screening results of the ligands (Fig. S1) for this reaction are shown in Table 1. The reaction with josiphos catalyst was the best to achieve stereoselectivity in (*R*)-**25** at 84% *ee* (entry 1). Other ligands were moderate or poor in selectivity (entries 2–7). This result was sufficient to determine the absolute configuration of **7**. In order to confirm that the reaction occurred by contribution of this phosphine ligand, we

Table 1
Results of ligand screening for asymmetric β-boration.



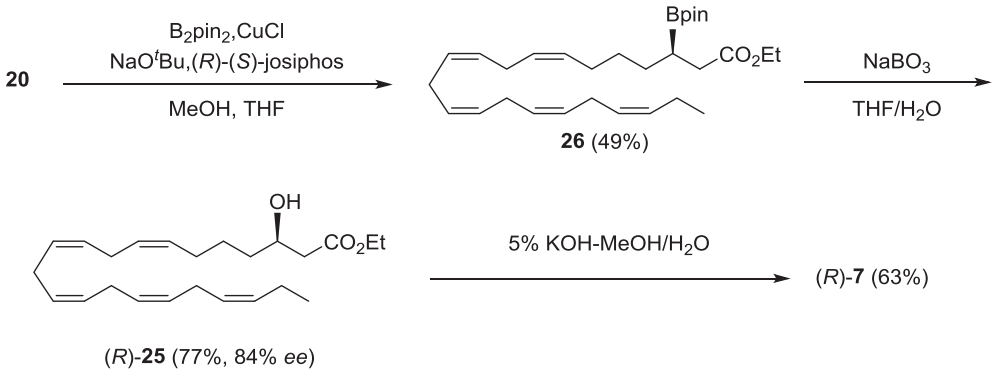
Entry	Ligand	mol%	2 steps yield (%)	*ee* of **25** (%)[a]
1	(*R*)-(*S*)-Josiphos	10	37	84
2	(*R*)-Segphos	10	60	56
3	(*R,R*)-Quinoxaline	10	7	16
4	(*R,R*)-Trost ligand	10	62	16
5	(*S*)-Bis(oxazoline)	10	21	34
6	MacMillan's cat	10	Trace	–
7	Cinchonidine	10	Trace	–
8	No	0	Trace	–
9	PPh_3	10	42	0

[a] Determined by chiral HPLC.

carried out two reactions with no ligand and with PPh_3 (entry 8, 9). It was found that the phosphine ligand was necessary for the reaction progress.

We determined the absolute configuration by applying the new Mosher method (Fig. 3, Fig. S3),[22] and the enantiomeric excess of **25** was measured by HPLC using a chiral column (Fig. S2). As expected from the literature, the *R* form was obtained.

Next, we attempted the asymmetric β-boration of α,β-unsaturated methyl ester **29**[23] (Scheme 10). Treatment of **29** with B_2pin_2, (*R*)-(*S*)-josiphos, NaO^{t}Bu and MeOH gave **30** in 64% yield, which was oxidized



Scheme 9. Enantioselective synthesis of (*R*)-β-hydroxyl acid **7** using asymmetric β-boration.

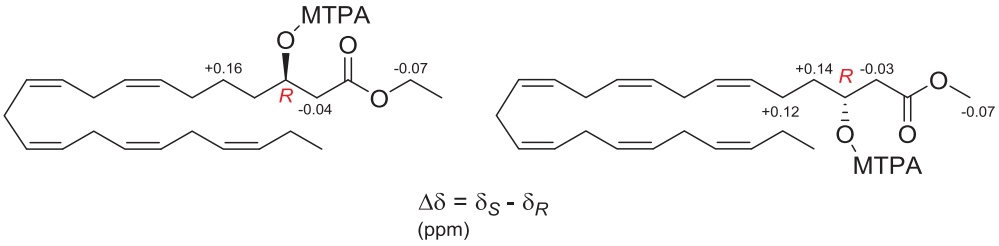
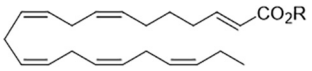
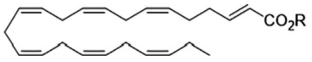


Fig. 3. Determination of absolute configuration.

to afford (*R*)-**31** in 95% yield and 83% *ee* as determined by ^{1}H NMR analysis (Fig. S3).

We investigated the effect of the esters on the resulting enantiomeric excess by comparing methyl and ethyl esters of **1** and **6** under the conditions of entry 1, Table 1. When the ethyl ester of **6** was used as a substrate (**20**), the optical yield was 84% *ee*. With the methyl ester as a substrate (**34**), the optical yield was 9% *ee* (Fig. S4, Table 2). In contrast, when the ethyl ester of **1** was used as a substrate (**38**), the optical yield was almost a racemic mixture (3% *ee*), but with methyl ester **29** as a substrate, the optical yield was 87% *ee*. (Fig. S5, Table 2). The reason for this selectivity is unclear as yet. This experiment is the first successful application of asymmetric synthesis using an unsaturated fatty acid as a substrate.

Table 2
Optical yield of product (% *ee*).

Substrate	R = Me	R = Et
	9[a)]	84[b)]
	83[a)]	3[a)]

[a)] Determined by ^{1}H NMR using diastereomer of MTPA ester.
[b)] Determined by chiral HPLC using **25.** It is because peaks of diastereomers of MTPA ester were overlapped in ^{1}H NMR chart.

2.4. Synthesis of β-keto acids *3* and *8*

We then attempted the synthesis of β-keto acids **3** and **8**. The first synthetic strategy for target β-keto acid **3** is shown in Scheme 11. The hydroxyl group of ester **23** was oxidized by Swern oxidation to obtain β-keto ester **42** in 47% yield. Hydrolysis of ester **42** under various conditions using LiOH or Lipase PS did not proceed.

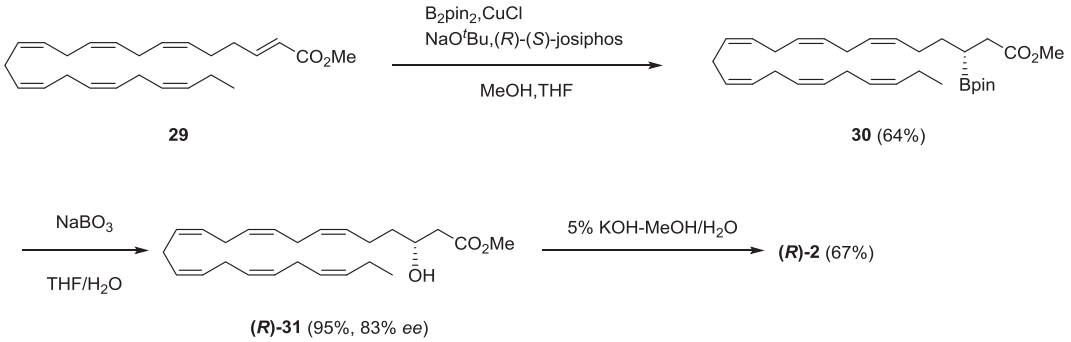
For the second approach, we hydrolyzed the β-hydroxyl ester to the acid and examined the oxidation reaction of the β-hydroxyl group. Compound **3** could not be obtained under any conditions (Dess–Martin, Jones, Parikh-Doering oxidations). It was considered that the β-keto carboxylic acid was unstable under these conditions. Therefore, we protected the β-keto group of **42** by an acetal to afford **43** in 50% yield (Scheme 12). Treatment of acetal-protected ester **43** with 5% KOH-MeOH/H_2O gave **44** (87% yield). Acetal **44** was deprotected under mild conditions using CBr_4 and PPh_3 to obtain **3** in 67% yield.[24]

In the synthesis of β-keto acid **8** (Scheme 13), **47** was prepared by the same synthetic route. However, the yield of **8** was only 32% in the deprotection step due to the difficulty of separating it from triphenylphosphine oxide. Thus, we attempted to use tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl), which is a water-soluble phosphine reagent used in molecular biology as a reducing agent. As a result, the phosphine oxide of TCEP was removed by a simple extraction step and the yield was improved (from 32% to 72%). In addition, we found that these β-keto acids were unstable and should be treated at less than 35 °C, otherwise a decarboxylated product was produced, which was observed by ^{1}H NMR.

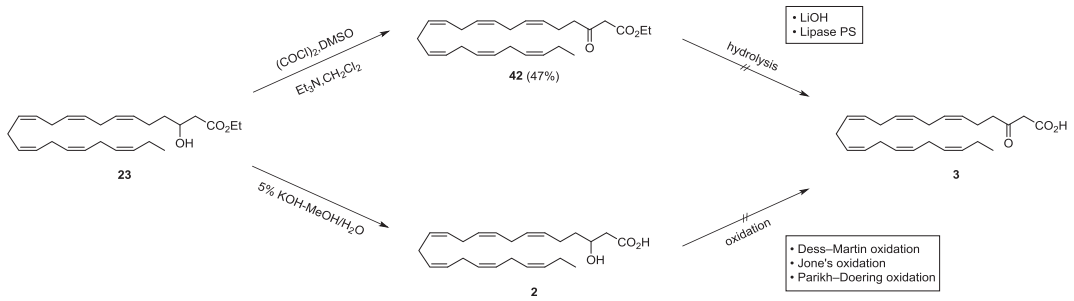
3. Conclusion

We achieved the construction of proposed intermediates in the β-oxidation pathway from THA to EPA via DHA. First, we synthesized three β-olefinic acids. At the hydrolysis step, we found that bulky ^{t}BuOH solvent suppressed byproducts, which resulted in increased yields. Second, asymmetric syntheses of β-hydroxyl acids were achieved by asymmetric β-boration-oxidation reactions. These syntheses are the first examples of the β-boration applied to unsaturated fatty acids. Third, we synthesized β-keto acids, which were found to be unstable. To solve this problem, the β-keto carboxyl group was protected, the ester moiety was hydrolyzed, and then the acetal group was deprotected with CBr_4 and PPh_3. Furthermore, the yield was improved using a water-soluble phosphine, TCEP.

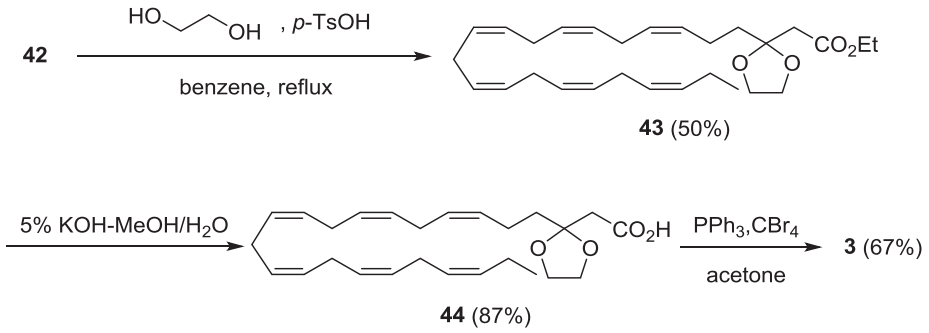
These intermediates are potential β-oxidized metabolites in peroxisomes. We expect these compounds to be valuable in leading to the elucidation of mechanisms *in vivo*. Moreover, information on diseases that cause abnormalities in the involved enzymes, such as peroxisome disease, may be obtained. Since these synthetic intermediates have



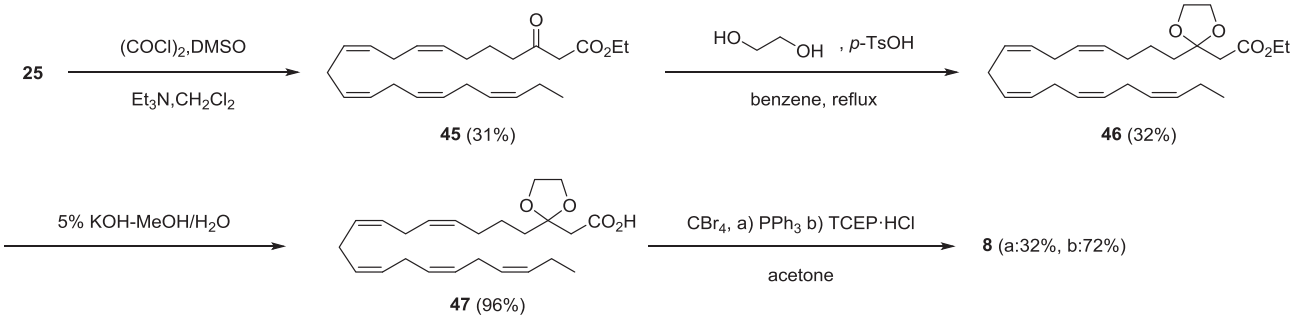
Scheme 10. Enantioselective synthesis of (*R*)-β-hydroxyl acid **2** using asymmetric β-boration.



Scheme 11. Attempted from **23** to **3**.



Scheme 12. Synthesis of β-keto acid **3**.



Scheme 13. Synthesis of β-keto acid **8**.

their own characteristics, e.g., conjugated double bonds, β-hydroxyl groups, β-olefins, β-carbonyls, their biological activities may be different.

4. Experimental procedures

4.1. General experimental procedures

All reagents were purchased from commercial sources and were used without further purification. Organic solvents were dried by standard methods. All air and moisture sensitive reactions were carried out under nitrogen atmosphere. Unless otherwise stated, NMR spectra were recorded at 300, 400 or 600 MHz for ^{1}H NMR and 75, 100 or 150 MHz for ^{13}C NMR in $CDCl_3$ solution with TMS as an internal standard and the chemical shifts are given in δ values. High resolution mass spectra were obtained using JEOL AccuTOF LC-plus JMS-T100LP spectrometer. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer or a JASCO FT/IR-420 spectrometer, and data are given in cm^{-1}. Optical rotations were measured on a JASCO P-2200 polarimeter, using a sodium lamp (589 nm).

4.2. Synthesis

4.2.1. (3Z,6Z,9Z,12Z,15Z)-Octadeca-3,6,9,12,15-pentaen-1-ol (12)

A solution of **10** (4.00 g, 8.81 mmol) in 5% KOH/MeOH–H_2O (19:1, 20 mL) was stirred at 60 °C for 3 h. The reaction mixture was acidified with 1 N aqueous HCl and then extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The crude product in THF/H_2O (2:1, 24 mL) was added $NaIO_4$ (4.67 g, 13.21 mmol) and the mixture was stirred at 0 °C for 1 h. The mixture was extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The residue was solved in MeOH (20 mL) and treated with $NaBH_4$ (1.65 g, 43.70 mmol) at 0 °C for 2 h. The reaction was quenched with water at 0 °C and then extracted with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (100 g, 8% ethyl acetate–hexane) to give **12** (1.27 g, 33%, 3 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.5 Hz, H-15), 2.08 (2H, q, J = 7.5 Hz, H-17), 2.36 (2H, q, J = 7.0 Hz, H-2), 2.80–2.88 (8H, m, H-5, 8, 11, 14), 3.65 (2H, m, H-1), 5.24–5.62 (10H, m, H-3, 4, 6, 7, 9, 10, 12, 13, 15, 16). ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3, 20.6, 25.6 (2 carbons), 25.7, 25.8, 30.8, 62.2, 125.7, 127.0, 127.9, 128.0 (2 carbons), 128.3 (2 carbons), 128.6, 131.1, 132.1. IR (neat) 3012, 2962, 2931, 1668, 1440, 1394, 1267, 1049.

4.2.2. ((3Z,6Z,9Z,12Z,15Z)-Octadeca-3,6,9,12,15-pentaen-1-yl) triphenylphosphonium bromide (14)

To a solution of **12** (101 mg, 0.387 mmol) in CH_2Cl_2 (3 mL) were added PPh_3 (305 mg, 1.16 mmol) and CBr_4 (385 mg, 1.16 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated. The residue was chromatographed on silica gel (100 g, 100% hexane) to give 1-brominated **13**. To a solution of the crude material (0.20 g, 0.63 mmol) in CH_3CN (3.0 mL) was added PPh_3 (0.49 g, 1.89 mmol) and the mixture was stirred at 80 °C for 24 h in a sealed tube. The reaction mixture was evaporated and the residue was chromatographed on silica gel (70 g, 10% MeOH–CH_2Cl_2) to give **14** (150 mg, 43% in 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (3H, t, J = 7.4 Hz, H-15), 2.17–2.80 (8H, m, H-5, 8, 11, 14), 3.91 (2H, m, H-1), 5.25–5.40 (10H, m, H-3, 4, 6, 7, 9, 10, 12, 13), 5.64 (1H, m, H-13), 7.70–7.90 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.4 (2 carbons), 20.5 (2 carbons), 22.6, 23.3, 25.5 (4 carbons), 117.8, 118.9, 126.6, 126.8, 126.9, 127.3, 127.7 (2 carbons), 128.4, 128.5, 128.7, 130.1, 130.3 (2 carbons), 130.5 (2 carbons), 132.1, 133.7 (2 carbons), 133.8 (2 carbons), 134.9 (2 carbons), 135.0 (2 carbons). HRMS (ESI$^+$) caled. for $C_{36}H_{43}BrP$ $[M+H]^+$ 587.22653, found 587.22743. IR (neat) 3564, 3521, 3502, 3461, 3440, 3421, 3404, 3373, 3305, 3286, 3247, 1699, 1436, 1101, 989, 744, 719, 688, 649, 617, 599 cm^{-1}.

4.2.3. Methyl (E)-7-oxohept-3-enoate (16)

To a solution of **15** (393 mg, 2.48 mmol) in CH_2Cl_2 (50 mL) was added Dess–Martin Periodinane (1.58 g, 3.72 mmol), $NaHCO_3$ (3.96 g, 47.12 mmol) at room temperature. The mixture was stirred at that temperature for 1 h and the reaction was quenched with sat $NaHCO_3$ and sat $Na_2S_2O_3$. The reaction mixture was extracted 3 times with CH_2Cl_2, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (15 g, 40% ethyl acetate–hexane) to give **16** (377 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.40 (2H, m), 2.55 (2H, m), 3.08 (2H, m), 3.68 (3H, s), 5.60 (2H, m), 9.77 (1H, t, J = 1.5 Hz).

4.2.4. Ethyl (E)-7-oxohept-2-enoate (18)

To a solution of **17** (1.02 g, 5.96 mmol) in CH_2Cl_2 (50 mL) was added Dess–Martin Periodinane (3.79 g, 8.94 mmol), $NaHCO_3$ (9.52 g, 113.2 mmol) at room temperature. The mixture was stirred at that temperature for 1 h and the reaction was quenched with sat $NaHCO_3$ and sat $Na_2S_2O_3$. The mixture was extracted 3 times with CH_2Cl_2, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (50 g, 30% ethyl acetate–hexane) to give **18** (849 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (3H, t, J = 7.1 Hz), 1.81 (2H, m), 2.24 (2H, m), 2.49 (2H, dt, J = 1.2, 7.2 Hz), 4.19 (2H, q, J = 7.1 Hz), 5.84 (1H, td, J = 1.5, 15.6 Hz), 6.86–6.97 (1H, td, J = 6.9, 15.6 Hz), 9.78 (1H, t, J = 1.3 Hz).

4.2.5. Methyl (3E,7Z,10Z,13Z,16Z,19Z)-docosa-3,7,10,13,16,19-hexaenoate (19)

To a solution of phosphonium bromide **9** (104.6 mg, 0.196 mmol) and HMPA (47.7 μL, 0.274 mmol) in THF (0.2 mL) at −78 °C under argon was added LHMDS (0.235 mL of a 1.0 M solution in hexane, 0.235 mmol). The mixture was added slowly to a solution of **18** (91.8 mg, 0.588 mmol) in THF (0.2 mL). The reaction mixture was allowed to warm to 0 °C for 1.5 h. The reaction mixture was quenched by addition of saturated aqueous NH_4Cl, and the mixture was diluted with ethyl acetate. The mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, 5% ethyl acetate–hexane) to give **19** (20 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.03–2.17 (6H, m, H-5, 6, 21), 2.85 (8H, m, H-9, 12, 15, 18), 3.03–3.11 (2H, m, H-2), 3.68 (3H, s, O-*Me*), 5.30 (1H, ttd, 1.4, 7.0, 10.7 Hz, H-7), 5.38 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.56 (2H, m, H-3, 4). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 25.5, 25.6 (3 carbons), 26.8, 32.4, 37.8, 51.7, 122.0, 127.0, 127.8, 128.0, 128.1, 128.2 (2 carbons), 128.3, 128.5, 129.2, 132.0, 134.0, 172.5. HRMS (ESI$^+$) caled. for $C_{23}H_{34}O_2Na$ $[M+Na]^+$ 365.24565, found 365.24733. IR (neat) 3012, 2962, 2929, 2376, 2312, 1743, 1542, 1508, 1433, 1259, 1193, 1166, 968, 705 cm^{-1}.

4.2.6. (3E,7Z,10Z,13Z,16Z,19Z)-Docosa-3,7,10,13,16,19-hexaenoic acid (5)

A solution of **19** (29 mg, 0.085 mmol) in 5% KOH/^{i}PrOH–H_2O (1:1, 0.8 mL) was stirred at 60 °C for 30 min. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate, dried over $MgSO_4$, and evaporated to give **5** (27.3 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.03–2.17 (6H, m, H-5, 6, 21), 2.84 (8H, m, H-9, 12, 15, 18), 3.07–3.15 (2H, m, H-2), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.56 (2H, m, H-3, 4). ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3, 20.6, 25.5, 25.6 (3 carbons), 25.7, 26.8, 29.7, 32.4, 37.5, 121.3, 127.0, 127.9, 128.1, 128.2, 128.3, 128.6, 129.2, 132.1, 134.7, 176.9. HRMS (ESI$^+$) calcd. for $C_{22}H_{32}NaO_2$ $[M+Na]^+$ 351.2300, found 351.23158. IR (neat) 3012, 2962, 2929, 2376, 2312, 1743, 1508, 1433, 1259, 1166, 968, 705 cm^{-1}.

4.2.7. Ethyl (2E,7Z,10Z,13Z,16Z,19Z)-docosa-2,7,10,13,16,19-hexaenoate (20)

To a solution of phosphonium bromide **9** (205.4 mg, 0.386 mmol) and HMPA (100 μL, 1.158 mmol) in THF (0.5 mL) at −78 °C under argon was added LHMDS (0.463 mL of a 1.0 M solution in hexane, 0.463 mmol). The mixture was added slowly a solution of **18** (131.4 mg, 0.772 mmol) in THF (0.7 mL). The reaction mixture was allowed to warm to 0 °C for 2 h. The reaction was quenched by addition of saturated aqueous NH_4Cl, and the mixture was diluted with ethyl acetate. The mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (60 g, 3% ethyl acetate–hexane) to give **20** (73.2 mg, 53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.29 (3H, t, J = 7.1 Hz, COO–CH_2–*CH_3*), 1.52 (2H, m, H-5), 2.10 (4H, m, H-6, 21), 2.68 (2H, m, H-4), 2.85 (8H, m, H-9, 12, 15, 18), 4.18 (2H, q, J = 7.1 Hz, COO–*CH_2*–CH_3), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.83 (1H, td, J = 1.5, 15.6 Hz, H-2), 6.90–7.02 (1H, td, J = 6.9, 15.6 Hz, H-3). ^{1}H NMR (300 MHz, benzene-d_6) δ 0.91 (3H, t, J = 7.5 Hz, H-22), 1.00 (3H, t, J = 7.1 Hz, COO–CH_2–*CH_3*), 1.21 (2H, m, H-5), 1.85 (4H, m, H-6, 4), 2.01 (2H, m, H-21), 2.73–2.95 (8H, m, H-9, 12, 15, 18), 4.06 (2H, q, J = 7.0 Hz, COO–*CH_2*–CH_3), 5.25 (1H, ttd, J = 1.5, 7.1, 10.7 Hz, H-7), 5.31–5.54 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.57 (1H, td, J = 1.5, 15.5 Hz, H-2), 7.04 (1H, td, J = 6.9, 15.6 Hz, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 25.5, 25.6 (3 carbons), 26.6, 27.9, 31.6, 60.1, 121.5, 127.0, 127.8, 128.0, 128.1, 128.2, 128.5 (2 carbons), 129.2 132.1, 133.2.0, 148.9, 166.6. HRMS (ESI$^+$) caled. for $C_{24}H_{36}O_2Na$ $[M+Na]^+$ 379.26130, found 379.26025. IR (neat) 3608, 3566, 3544, 3523, 3012, 2923, 2852, 23474, 1745, 1722, 1500, 1338, 1338, 1186, 1041, 979 cm^{-1}.

4.2.8. (2E,7Z,10Z,13Z,16Z,19Z)-Docosa-2,7,10,13,16,19-hexaenoic acid (6)

A solution of **20** (50.5 mg, 0.137 mmol) in 5% KOH/^{i}PrOH–H_2O (19:1, 3 mL) was stirred at room temperature for 17 h. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated, and evaporated to give **6** (49.9 mg, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.55 (2H, m, H-5), 2.09 (4H, m, H-21), 2.08 (4H, m, H-6, 21), 2.85 (8H, m, H-9, 12, 15, 18), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.77 (1H, td, J = 1.5, 15.5 Hz, H-2), 6.23 (1H, td, J = 6.9, 15.6 Hz, H-3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3, 20.6, 25.5, 25.6 (2 carbons), 25.7, 26.7, 27.8, 31.8, 120.8, 127.0, 127.9, 128.1, 128.2 (2 carbons), 128.3, 128.6, 128.7, 129.1, 132.0, 152.0, 171.8. HRMS (ESI$^+$) caled. for $C_{22}H_{32}O_2Na$ $[M+Na]^+$ 351.2300, found 351.22826. IR (neat)

3012, 2962, 2927, 2873, 2854, 1697, 1681, 1649, 1452, 1417, 1396, 1311, 1286, 1228, 979, 929, 705 cm^{-1}.

4.2.9. Ethyl (2E,4Z,7Z,10Z,13Z,16Z,19Z)-docosa-2,4,7,10,13,16,19-heptaenoate (21)

To a solution of phosphonium bromide **14** (1.29 g, 2.21 mol), (*E*)-Ethyl 4-oxobut-2-enoate (0.80 mL, 6.63 mmol) in THF (15 mL) was added LHMDS (1.0 M in THF, 6.63 mL, 6.63 mmol) at −78 °C. The reaction mixture was allowed to warm to 0 °C for 1 h, and the reaction was quenched with water. The mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (200 g, 3% ethyl acetate–hexane) to give **21** (222 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.30 (3H, t, J = 7.2 Hz, COO–CH_2–CH_3), 2.07 (2H, m, H-21), 2.71–3.13 (10H, m, H-9, 12, 15, 18), 4.18 (2H, q, J = 7.1 Hz, COO–CH_2–CH_3), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.79 (1H, tq, J = 0.7, 10.6 Hz, H-5), 5.89 (1H, d, J = 15.2 Hz, H-2), 6.14 (1H, t, J = 11.4 Hz, H-4), 7.58–7.67 (1H, ddd, J = 0.98, 11.6, 15.2 Hz, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 14.3, 20.5, 25.5, 25.6 (3 carbons), 26.5, 60.3, 121.8, 126.5, 126.6, 127.7, 127.8, 127.9, 128.3 (3 carbons), 128.5 (2 carbons), 129.4, 138.6, 138.95, 167.1 HRMS (ESI^+) caled. for $C_{24}H_{34}O_2Na$ $[M+Na]^+$ 377.24565, found 377.24167. IR (neat) 3012, 2964, 1714, 1433, 1365, 1265, 1163, 1118, 1039, 993, 869, 696 cm^{-1}.

4.2.10. (2E,4Z,7Z,10Z,13Z,16Z,19Z)-Docosa-2,4,7,10,13,16,19-heptaenoic acid (4)

A solution of **21** (16.3 mg, 0.046 mmol) in 5% KOH/^{t}BuOH–H_2O (1:1, 1 mL) was stirred at room temperature for 23 h. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate. The organic layer was washed with water and brine over $MgSO_4$, dried, and evaporated. The residue was chromatographed on silica gel (5 g, 30% ethyl acetate–hexane) to give **4** (7.5 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.07 (2H, m, H-21), 2.85 (8H, m, H-9, 12, 15, 18), 3.10 (2H, t, H-6), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.76–5.97 (2H, m, H-2, 5), 6.18 (1H, t, J = 11.0 Hz, H-4), 7.58–7.67 (1H, ddd, J = 1.0, 11.7, 15.3 Hz, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 25.5, 26.6 (3 carbons), 26.6, 120.7, 126.2, 126.4, 127.0, 127.6, 127.8, 127.9, 128.3, 128.5 (2 carbons), 129.6, 132.0, 140.0, 141.2, 171.9. HRMS (ESI^+) caled. for $C_{22}H_{30}NaO_2$ $[M+Na]^+$ 349.21435, found 349.21199. IR (neat) 3012, 2962, 2929, 2873, 2873, 2856, 2358, 2339, 1693, 1679, 1633, 1421, 1284, 1122, 995, 964, 873, 669 cm^{-1}.

4.2.11. Ethyl (6Z,9Z,12Z,15Z,18Z,21Z)-3-hydroxytetracosa-6,9,12,15,18,21-hexaenoate ((±)-23)

To a solution of aldehyde **22** (1.61 g, 5.16 mmol) in benzene (10 mL) was added Zn (0.6 g, 8.92 mmol), ethyl bromoacetate (0.66 mL, 6.192 mmol), and I_2 (224.3 mg, 1.54 mmol). The reaction was stirred at room temperature for 1 h. The mixture was diluted with ethyl acetate and filtered through Celite, evaporated. The residue was chromatographed on silica gel (30 g, 3% ethyl acetate–hexane) to give (±)-**23** (1.86 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.28 (3H, t, J = 7.1 Hz, COO–CH_2–CH_3), 1.54 (4H, m, H-4, 5), 2.08 (4H, m, H-23), 2.21 (2H, m, H-4), 2.35–2.54 (2H, m, H-2), 2.85 (10H, m, H-8, 11, 14, 17, 20), 4.00–4.11 (1H, br, H-3), 4.18 (2H, q, J = 7.1 Hz COO–CH_2–CH_3), 5.38 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.3, 23.2, 25.5, 25.6, 25.7 (3 carbons), 36.1, 41.2, 60.7, 67.4, 126.9, 127.8, 128.0, 128.2 (4 carbons), 128.3 (2 carbons), 128.6 (2 carbons), 129.3, 132.0, 173.0. HRMS (ESI) caled. for $C_{26}H_{40}O_3Na$ $[M+Na]^+$ 423.28751, found 423.28498. IR (neat) 3010, 2964, 2931, 1731, 1712, 1238, 1176, 1026, 723 cm^{-1}.

4.2.12. (6Z,9Z,12Z,15Z,18Z,21Z)-3-Hydroxytetracosa-6,9,12,15,18,21-hexaenoic acid ((±)-2)

A solution of (±)-**23** (1.86 g, 4.63 mmol) in 5% KOH/MeOH–H_2O (19:1, 47 mL) was stirred at 60 °C for 30 min. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (30 g, 40% ethyl acetate–hexane) to give (±)-**2** (1.56 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-24), 1.58 (2H, m, H-2), 2.08 (2H, m, H-23), 2.22 (2H, m, H-5), 2.44–2.62 (2H, m, H-4), 2.83 (10H, m, H-8, 11, 14, 17, 20), 4.05 (1H, m, H-3), 5.37 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 21.0, 23.2, 25.5, 25.6 (3 carbons), 36.2, 67.4, 127.0, 127.8, 128.0, 128.1 (3 carbons), 128.2 (3 carbons), 128.5, 128.7, 129.0, 132.0, 177.7. HRMS (ESI^+) caled. for $C_{22}H_{34}O_3Na$ $[M+Na]^+$ 369.24056, found 369.24117. IR (neat) 3346, 3012, 2964, 2931, 1712, 1402, 1217, 1068, 973, 757, cm^{-1}.

4.2.13. Ethyl (7Z,10Z,13Z,16Z,19Z)-3-hydroxydocosa-7,10,13,16,19-pentaenoate ((±)-25)

To a solution of aldehyde **24** (1.24 g, 4.36 mmol) in benzene (8.6 mL) was added Zn (2.56 g, 39.25 mmol), ethyl bromoacetate (4.37 mL, 26.2 mmol). The reaction was stirred at 110 °C for 30 min. The mixture was diluted with ethyl acetate and filtered through Celite, evaporated. The residue was chromatographed on silica gel (100 g, 5% ethyl acetate–hexane) to give (±)-**25** (1.07 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.28 (3H, t, J = 7.1 Hz, COO–CH_2–CH_3), 1.49 (4H, m, H-4, 5), 2.08 (4H, m, H-6, 21), 2.35–2.53 (2H, m, H-2) 2.85 (8H, m, H-9, 12, 15, 18), 4.00–4.11 (1H, br, H-3), 4.18 (2H, q, J = 7.1 Hz, COO–CH_2–CH_3), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.2, 20.5, 25.4, 25.5, 25.6 (3 carbons), 27.0, 36.0, 41.2, 60.6, 67.9, 127.0, 127.8, 128.0, 128.1 (2 carbons), 128.2, 128.3, 128.5, 129.7, 132.0, 173.0. HRMS (ESI^+) caled. for $C_{24}H_{38}O_3Na$ $[M+Na]^+$ 397.27186, found 397.27144. IR (neat) 3523, 3012, 2960, 2925, 2854, 2380, 2312, 2017, 1743, 1722, 1542, 1508, 1396, 1338, 1271, 1178, 1093, 1029 cm^{-1}.

4.2.14. (7Z,10Z,13Z,16Z,19Z)-3-Hydroxydocosa-7,10,13,16,19-pentaenoic acid ((±)-7)

A solution of (±)-**25** (80 mg, 0.213 mmol) in 5% KOH/MeOH–H_2O (19:1, 20 mL) was stirred at 60 °C for 20 min. The reaction mixture was acidified with 1 N aqueous HCl and then extracted with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and evaporated, and evaporated to give (±)-**7** (71.7 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.52 (4H, m, H-4, 5), 2.08 (4H, m, H-6, 21), 2.43–2.61 (2H, m, H-2), 2.85 (8H, m, H-9, 12, 15, 18), 4.00–4.11 (1H, br, H-3), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3, 20.6, 25.6 (2 carbons), 25.7 (2 carbons), 27.0, 36.0, 40.9, 67.8, 127.0, 127.9, 128.1 (2 carbons), 128.3 (4 carbons), 128.6, 129.6, 132.1, 177.0. HRMS (ESI^+) caled. for $C_{22}H_{34}O_3Na$ $[M+Na]^+$ 369.24056, found 369.24117. IR (neat) 3575, 3460, 3012, 2935, 2306, 1749, 1672, 1658, 1641, 1539, 1577, 1402, 1269, 1188, 1041, 719, 680, 649 cm^{-1}.

4.2.15. Ethyl (R,7Z,10Z,13Z,16Z,19Z)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)docosa-7,10,13,16,19-pentaenoate (26)

THF (0.50 mL) was added under nitrogen to CuCl (0.55 mg, 5.6 μmol, 2 mol%), NaO^{t}Bu (0.8 mg, 8.4 μmol), and (*R*)-(*S*)-josiphos ligand (12.8 mg,0.028 mmol). The reaction mixture was stirred for 30 min at 0 °C, then bis(pinacolato)diboron (78 mg, 0.38 mmol) in THF (0.20 mL) were added. The reaction mixture was stirred for 10 min. Then **20** (100 mg, 0.28 mmol) in THF (0.5 mL) and subseqently MeOH (22 μL, 0.56 mmol) were added. The reaction was stirred at 0 °C for 1 h. The mixture was diluted with ethyl acetate and filtered through Celite, evaporated. The residue was chromatographed on silica gel (4 g, 5% ethyl acetate–hexane) to give **26** (67.2 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.28 (15H, m, COO–CH_2–CH_3, B(pin)), 1.36 (5H, m, H-3, 4, 5), 2.08 (4H, m, H-6, 21), 2.85 (8H, m, H-

9, 12, 15, 18), 4.18 (2H, q, J = 7.2 Hz, COO–CH_2–CH_3), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 20.5, 24.6 (2 carbons), 24.8 (2 carbons), 25.5, 25.6 (3 carbons), 27.4, 28.8, 30.2, 35.8, 60.1, 83.1, 127.0, 127.7, 127.9 (3 carbons), 128.1 (4 carbons), 128.5 (2 carbons), 130.1, 132.0, 173.9. HRMS (ESI$^+$) caled. for $C_{30}H_{49}B_1O_4Na$ $[M+Na]^+$ 507.36216, found 507.36604. IR (neat) 3012, 2975, 2930, 1733, 1387, 1318, 1267, 1143, 1036, 968, 857, 205 cm^{-1}. $[\alpha]_D^{23}$ +7.1 (c 0.17, $CHCl_3$).

4.2.16. Ethyl (R,7Z,10Z,13Z,16Z,19Z)-3-Hydroxydocosa-7,10,13,16,19-pentaenoate ((R)-25)

A solution of **26** (40.3 mg, 0.08 mmol) in THF/H_2O (1:1, 1.6 mL) was added $NaBO_3{\cdot}4H_2O$ (104.8 mg, 0.68 mmol). The reaction was stirred at room temperature for 3 h. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, 5% ethyl acetate–hexane) to give (*R*)-**25** (25.5 mg, 77%, 84% *ee*). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.28 (3H, t, J = 7.1 Hz, COO–CH_2–CH_3), 1.49 (4H, m, H-4, 5), 2.08 (4H, m, H-6, 21), 2.35–2.53 (2H, m, H-2) 2.85 (8H, m, H-9, 12, 15, 18), 4.00–4.11 (1H, br, H-3), 4.18 (2H, q, J = 7.1 Hz, COO–CH_2–CH_3), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.2, 20.5, 25.4, 25.5, 25.6 (3 carbons), 27.0, 36.0, 41.2, 60.6, 67.9, 127.0, 127.8, 128.0, 128.1 (2 carbons), 128.2, 128.3, 128.5, 129.8, 132.0, 173.1. HRMS (ESI$^+$) caled. for $C_{24}H_{38}O_3Na$ $[M+Na]^+$ 397.27186, found 397.26758. IR (neat) 3011, 2963, 2931, 2865, 2362, 2325, 1734, 1716 cm^{-1}. $[\alpha]_D^{23}$ +4.5 (c 0.12, $CHCl_3$).

4.2.17. (R,7Z,10Z,13Z,16Z,19Z)-3-hydroxydocosa-7,10,13,16,19-pentaenoic acid (R)-7

A solution of (*R*)-**25** (49.7 mg, 0.124 mmol) in 5% KOH/CH_3OH–H_2O (19:1, 3 mL) was stirred at room temperature for 18 h. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and evaporated to give (*R*)-**7** (26.3 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.49 (4H, m, H-4, 5), 2.08 (4H, m, H-6, 21), 2.35–2.53 (2H, m, H-2), 2.85 (8H, m, H-9, 12, 15, 18), 4.00–4.11 (1H, br, H-3), 5.38 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 20.5, 23.3, 25.5 (2 carbons), 25.6 (2 carbons), 26.9, 36.0, 40.8, 67.7, 126.9, 127.8, 128.0 (2 carbons), 128.2 (3 carbons), 128.5, 129.5, 132.0, 176.9. HRMS (ESI$^+$) caled. for $C_{22}H_{34}O_3Na$ $[M+Na]^+$ 369.24056, found 369.24117. IR (neat) 3011, 2967, 2927, 2851, 1717 cm^{-1}. $[\alpha]_D^{24}$ +2.5 (c 0.11, $CHCl_3$).

4.2.18. Ethyl (R,7Z,10Z,13Z,16Z,19Z)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)docosa-7,10,13,16,19-pentaenoate (27)

To a solution of alcohol **25** (15.2 mg, 0.0379 mmol) in CH_2Cl_2 (0.38 mL) were added triethylamine (21 µL, 0.151 mmol), DMAP (23 mg, 0.189 mmol), and (*R*)-methoxy(trifluoromethyl)phenylacetyl chloride (14 µL, 0.075 mmol) at 0 °C. The solution was stirred at room temperature for 2 h. The reaction mixture was poured into ice and water, and extracted 3 times with ether. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (1 g, 5% ethyl acetate-hexane) to give (*S*)-MTPA ester **27** (17 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.19 (3H, t, J = 7.1 Hz, COO–CH_2–CH_3), 1.42 (2H, m, H-5), 1.72 (2H, m, H-4), 2.07 (4H, m, H-6, 21), 2.71 (2H, t, J = 6.8 Hz, H-4), 2.83 (8H, m, H-9, 12, 15, 18), 3.54 (3H, s, MTPA-OMe), 3.66 (1H, s, H-1), 5.22–5.44 (10H, m, H- 7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.49 (1H, m, H-3), 7.39 (3H, m, MTPA-Ph), 7.53 (2H, m, MTPA-Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.2, 20.5, 24.9, 25.5, 25.6 (3 carbons), 26.7, 33.2, 38.6, 55.3, 60.8, 73.3, 73.3, 127.0, 127.4 (3 carbons), 127.8, 128.0, 128.1 (2 carbons), 128.2, 128.3 (3 carbons), 128.5, 128.6, 129.1, 129.5, 132.0, 165.8, 169.7. HRMS (ESI$^+$) caled. for $C_{34}H_{45}F_3O_5Na$ $[M+Na]^+$ 613.30784, found 613.31168. IR (neat) 3012, 2935, 2360, 1748, 1453, 1268, 1169, 1122, 1019, 716 cm^{-1}.

4.2.19. Ethyl (R,7Z,10Z,13Z,16Z,19Z)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)docosa-7,10,13,16,19-pentaenoate (28)

To a solution of alcohol **25** (14.8 mg, 0.036 mmol) in CH_2Cl_2 (0.37 µL) were added triethylamine (20.5 µL, 0.147 mmol), DMAP (22.5 mg, 0.184 mmol), and (*S*)-methoxy(trifluoromethyl)phenylacetyl chloride (13.8 µL, 0.073 mmol) at 0 °C. The solution was stirred at room temperature for 1.5 h. The reaction mixture was poured into ice and water, and extracted 3 times with ether. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (6 g, 10% ethyl acetate-hexane) to give (*R*)-MTPA ester **28** (12 mg, 55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.09–1.29 (5H, m, H-4, COO–CH_2–CH_3), 1.56 (2H, m, H-5), 1.87–2.07 (4H, m, H-6, 21), 2.43–2.65 (2H, t, J = 6.8 Hz, H-2), 2.83 (8H, m, H-9, 12, 15, 18), 3.54 (3H, s, MTPA-OMe), 3.66 (1H, s, H-1), 5.22–5.44 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.49 (1H, m, H-3), 7.39 (3H, m, MTPA-Ph), 7.53 (2H, m, MTPA-Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.2, 20.5, 24.5, 25.5, 25.6 (4 carbons), 26.6, 29.6, 33.1, 38.8, 55.4, 60.9, 73.1, 127.0, 127.3 (2 carbons), 127.8, 128.0, 128.1, 128.2 (2 carbons), 128.3 (2 carbons), 128.5 (2 carbons), 129.1 (2 carbons), 129.5, 132.0, 165.8, 169.9. HRMS (ESI$^+$) caled. for $C_{34}H_{45}F_3O_5Na$ $[M+Na]^+$ 613.31168, found 613.30870. IR (neat) 3014, 2959, 2930, 2852, 2357, 1748, 1452, 1268, 1169, 1122, 1019, 716 cm^{-1}.

4.2.20. Methyl (R,6Z,9Z,12Z,15Z,18Z,21Z)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)tetracosa-6,9,12,15,18,21-hexaenoate (30)

THF (0.5 mL) was added under nitrogen to CuCl (0.53 mg, 5.43 µmol), NaO^{t}Bu (0.78 mg, 8.14 µmol), and (*R*)-(*S*)-josiphos ligand (17.3 mg, 0.027 mmol). The reaction mixture was stirred for 30 min at 0 °C, then bis(pinacolato)diboron (75 mg, 0.29 mmol) in THF (0.5 mL) were added. The reaction mixture was stirred for 10 min. Then, **29** (100.1 mg, 0.27 mmol) in THF (0.5 mL) and subsequently MeOH (0.54 mmol, 21 µL) were added. The reaction was stirred at 0 °C for 1.5 h. The reaction mixture is evaporated. The residue was chromatographed on silica gel (10 g, 5% ethyl acetate–hexane) to give **30** (86.6 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-24), 1.24 (12H, d, J = 2.6 Hz, B-pin), 1.37 (2H, m, H-2), 1.53 (1H, m, H-3), 2.08 (4H, m, H-5, 23), 2.44 (2H, m, H-4), 2.82 (10H, m, H-8, 11, 14, 17, 20), 3.65 (3H, s, H-1), 5.35 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 24.7 (3 carbons), 25.5, 25.6 (4 carbons), 26.4, 30.5, 35.4, 51.4, 83.1, 127.0, 127.8, 127.9 (4 carbons), 128.1 (4 carbons), 128.5 (3 carbons), 129.9, 132.0, 174.2. HRMS (ESI$^+$) caled. for $C_{31}H_{49}BNaO_4$ $[M+Na]^+$ 519.36216, found 519.36251. IR (neat) 3012, 2962, 2931, 2931, 2873, 1731, 1433, 1265, 1197, 1174, 1068, 918, 705 cm^{-1}. $[\alpha]_D^{24}$ +6.7 (c 0.11, $CHCl_3$).

4.2.21. Methyl (R,6Z,9Z,12Z,15Z,18Z,21Z)-3-hydroxytetracosa-6,9,12,15,18,21-hexaenoate ((R)-31)

A solution of **30** (66.9 mg, 0.13 mmol) in THF/H_2O (2:1, 2.6 mL) was added $NaBO_3{\cdot}4H_2O$ (246.9 mg, 1.60 mmol). The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, 15% ethyl acetate–hexane) to give (*R*)-**31** (49 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.5 Hz, H-24), 1.56 (2H, m, H-4), 2.08 (2H, m, H-23), 2.22 (2H, m, H-5), 2.47 (2H, m, H-2), 2.85 (10H, m, H-8, 11, 14, 17, 20), 3.72 (3H, s, COO–CH_3), 4.02 (1H, m, H-3), 5.37 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 23.3, 25.5, 25.6 (4 carbons), 36.2, 41.1, 51.7, 67.4, 127.0, 127.8, 128.1 (3 carbons), 128.2 (3 carbons), 128.5, 128.6, 129.2, 132.0, 173.3. HRMS (ESI$^+$) calcd. for $C_{25}H_{38}NaO_3$ 409.27186, found 409.27157. IR (neat) 3012, 2962, 2931, 2873, 1735, 1438, 1390, 1301, 1263, 1197, 1174, 1068, 927, 688 cm^{-1}. $[\alpha]_D^{24}$ +1.2 (c 0.19, $CHCl_3$).

4.2.22. (R,6Z,9Z,12Z,15Z,18Z,21Z)-3-Hydroxytetracosa-6,9,12,15,18,21-hexaenoic acid ((R)-2)

A solution of (**R**)-**31** (13.0 mg, 0.033 mmol) in 5% KOH/MeOH–H_2O (19:1, 0.5 mL) was stirred at room temperature for 1.5 h. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (3 g, 40% ethyl acetate–hexane) to give (*R*)-**2** (8.4 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-24), 1.58 (2H, m, H-2), 2.08 (2H, m, H-23), 2.22 (2H, m, H-5), 2.54 (2H, m, H-4), 2.83 (10H, m, H-8, 11, 14, 17, 20), 4.05 (1H, m, H-3), 5.37 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 20.5, 21.0, 23.2, 25.5, 25.6 (3 carbons), 36.1, 67.3, 126.9, 127.8, 128.0, 128.1 (3 carbons), 128.2 (3 carbons), 128.5, 128.8, 129.0, 132.0, 177.0. HRMS (ESI$^-$) caled. for $C_{24}H_{35}O_3$ $[M-H]^-$ 371.25862, found 371.25712. IR (neat) 3012, 2962, 2929, 2358, 2341, 1712, 1392, 1263, 684 cm^{-1}. $[\alpha]_D^{24}$ −6.7 (*c* 0.14, $CHCl_3$).

4.2.23. Methyl (R,6Z,9Z,12Z,15Z,18Z,21Z)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)tetracosa-6,9,12,15,18,21-hexaenoate (32)

To a solution of alcohol **31** (10.1 mg, 0.026 mol) in CH_2Cl_2 (0.26 mL) were added triethylamine (14 μL, 0.10 mmol), DMAP (15.8 mg, 0.13 mmol), and (*R*)-methoxy(trifluoromethyl)phenylacetyl chloride (9.7 μL, 0.052 mmol) at 0 °C. The solution was stirred at room temperature for 5 h. The reaction mixture was poured into ice and water, and extracted 3 times with ether. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, 10% ethyl acetate ethyl acetate-hexane) to give (*S*)-MTPA ester **32** (13.7 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-24), 1.72 (2H, m, H-2), 2.04 (4H, m, H-5, 23), 2.71 (2H, t, J = 6.8 Hz, H-4), 2.83 (10H, m, H-8, 11, 14, 17, 20), 3.54 (3H, s, MTPA-OMe), 3.66 (1H, s, H-1), 5.39 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22), 5.49 (1H, m, H-3), 7.39 (3H, m, MTPA-Ph), 7.53 (2H, m, MTPA-Ph). ^{13}C NMR (75 MHz, $CHCl_3$) δ 14.2, 20.8, 22.8, 25.7, 25.8 (2 carbons), 33.8, 38.7, 52.1, 55.6, 72.9, 127.0, 127.3, 127.8, 127.9, 128.0 (2 carbons), 128.3 (4 carbons), 128.5 (4 carbons), 128.6, 128.8, 129.1, 129.6, 132.0, 132.2, 165.9, 170.3. HRMS (ESI$^+$) calcd. for $C_{35}H_{45}F_3NaO_5$ 625.31168, found 625.31173. IR (neat) 3012, 2960, 2931, 2850, 1747, 1450, 1438, 1390, 1271, 1184, 1168, 1122, 1080, 1018, 715, 696 cm^{-1}.

4.2.24. Methyl (R,6Z,9Z,12Z,15Z,18Z,21Z)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)tetracosa-6,9,12,15,18,21-hexaenoate (33)

To a solution of alcohol **31** (9.8 mg, 0.025 mmol) in CH_2Cl_2 (0.25 mL) were added triethylamine (14.0 μL, 0.10 mmol), DMAP (15.4 mg, 0.12 mmol), and (*S*)-methoxy(trifluoromethyl)phenylacetyl chloride (37 μL, 0.20 mmol) at 0 °C. The solution was stirred at room temperature for 4 h. The reaction mixture was poured into ice and water, and extracted 3 times with ether. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, 5% ethyl acetate-hexane to give (*R*)-MTPA ester **33** (9.8 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-24), 1.80 (2H, m, H-2), 2.10 (4H, m, H-5, 23), 2.64 (2H, t, J = 6.8 Hz, H-4), 2.80 (10H, m, H-8, 11, 14, 17, 20), 3.53 (3H, s, MTPA-OMe), 3.59 (1H, s, H-1), 5.37 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22), 5.49 (1H, m, H-3), 7.40 (3H, m, MTPA-Ph), 7.53 (2H, m, MTPA-Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 22.9, 25.6 (5 carbons), 33.6, 38.3, 51.8, 55.3, 73.0, 127.0, 127.4 (2 carbons), 127.8 (2 carbons), 127.9, 128.3 (2 carbons), 128.0 (2 carbons), 128.3 (4 carbons), 128.5, 129.2, 129.5, 132.0, 165.8, 170.1. HRMS (ESI$^+$) calcd. for $C_{35}H_{45}F_3NaO_5$ 625.31168, found 625.31038. IR (neat) 3012, 2958, 2925, 2850, 1749, 1438, 1269, 1168, 1122, 1016, 717 cm^{-1}.

4.2.25. Ethyl (6Z,9Z,12Z,15Z,18Z,21Z)-3-oxotetracosa-6,9,12,15,18,21-hexaenoate (42)

To a solution of $(COCl)_2$ (2.0 M in CH_2Cl_2, 6.3 mL, 12.64 mmol) in CH_2Cl_2 (3 mL) was added a solution of DMSO (1.8 mL, 25.29 mmol) in CH_2Cl_2 (3 mL) at −78 °C and the mixture was stirred at −78 °C for 10 min. A solution of **23** (3.38 g, 8.43 mmol) in CH_2Cl_2 (4 mL) was added and the mixture was stirred at that temperature for 15 min. Et_3N (6.9 mL, 50.58 mmol) was added to the reaction mixture at −78 °C, and then the mixture was allowed to warm to 0 °C. The reaction was quenched with water and the mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (80 g, 5% ethyl acetate–hexane) to give **42** (1.58 g, 47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-24), 1.28 (3H, t, J = 7.2 Hz, COO–CH_2–*CH_3*), 2.08 (2H, m, H-23), 2.37 (2H, m, H-5), 2.61 (2H, t, J = 7.2 Hz, H-4), 2.83 (10H, m, H-8, 11, 14, 17, 20), 3.43 (2H, s, H-3), 4.20 (2H, q, J = 7.2 Hz, COO–*CH_2*–CH_3), 5.38 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22) ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.2, 20.5, 21.3, 25.5 (2 carbons), 25.6 (3 carbons), 42.7, 49.4, 61.3, 127.0, 127.8 (2 carbons), 128.0 (2 carbons), 128.1, 128.2 (3 carbons), 128.5, 129.3, 132.0, 167.1, 202.0. HRMS (FAB$^+$) caled. for $C_{26}H_{39}O_3$ $[M+H]^+$ 399.2899, found 399.2912. IR (neat) 3012, 2964, 2931, 1745, 1716, 1647, 1436, 1367, 1313, 1232, 1195, 1149, 1039, 927, 707 cm^{-1}.

4.2.26. Ethyl 2-(2-((3Z,6Z,9Z,12Z,15Z,18Z)-henicosa-3,6,9,12,15,18-hexaen-1-yl)-1,3-dioxolan-2-yl)acetate (43)

To a solution of **42** (104.0 mg, 0.26 mmol) in benzene (10 mL) was added ethylene glycol (278 μL, 2.6 mmol) and *p*-TsOH·H_2O (4.4 mg, 0.013 mmol). The reaction was stirred at 110 °C for 24 h. The reaction was quenched with water and the mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (20 g, 40% ethyl acetate–hexane) to give **43** (57.6 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-24), 1.27 (3H, t, J = 7.2 Hz, COO–CH_2–*CH_3*), 1.88 (2H, m, H-4), 2.08 (2H, m, H-23), 2.19 (2H, m, H-5), 2.66 (2H, s, H-2), 2.83 (10H, m, H-8, 11, 14, 17, 20), 3.98 (4H, m, acetal-CH_2), 4.15 (1H, m, COO–*CH_2*–CH_3), 5.37 (12H, m, H-6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.2, 20.5, 21.4, 25.5 (2 carbons), 25.6 (3 carbons), 37.4, 42.7, 60.5, 65.1 (2 carbons), 109.1, 127.0, 127.8, 128.0 (2 carbons), 128.1 (2 carbons), 128.2 (2 carbons), 128.3, 128.5, 129.3, 132.0, 169.4. HRMS (FAB$^+$) caled. for $C_{28}H_{43}O_4$ $[M+H]^+$ 443.3161, found 443.3154. IR (neat) 3012, 2964, 2931, 2894, 1735, 1442, 1390, 1369, 1265, 1218, 1110, 1095, 1039, 948, 711 cm^{-1}.

4.2.27. 2-(2-((3Z,6Z,9Z,12Z,15Z,18Z)-Henicosa-3,6,9,12,15,18-hexaen-1-yl)-1,3-dioxolan-2-yl)acetic acid (44)

A solution of **43** (57.6 mg, 1.36 mmol) in 5% KOH/MeOH–H_2O (19:1, 1.36 mL) was stirred at 60 °C for 1 h. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (6 g, 50% ethyl acetate–hexane) to give **44** (48.8 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.87 (2H, t, J = 5.0 Hz, H-2), 2.08 (2H, m, H-21), 2.19 (2H, m, H-3), 2.72 (2H, s, H-1), 2.83 (10H, m, H-6, 9, 12, 15, 18), 4.04 (4H, m, acetal-CH_2), 5.37 (12H, m, H-4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 21.4, 25.5 (2 carbons), 25.6 (4 carbons), 37.2, 42.3, 65.1 (2 carbons), 108.9, 127.0, 127.8, 128.1, 128.2 (4carbons), 128.3, 128.5, 129.0, 132.0, 173.2. HRMS (ESI$^+$) calcd. for $C_{26}H_{38}NaO_4$ $[M+Na]^+$ 437.26678, found 437.26869. IR (neat) 3012, 2964, 2931, 2360, 2341, 1712, 1436, 1394, 1267, 1222, 1118, 1047, 948, 684 cm^{-1}.

*4.2.28. (6Z,9Z,12Z,15Z,18Z,21Z)-3-Oxotetracosa-6,9,12,15,18,21-hexaenoic acid (**3**)*

A solution of **44** (56 mg, 0.135 mmol) in acetone (1 mL) added PPh_3 (71.2 mg, 0.27 mmol), CBr_4 (89.7 mg, 0.27 mmol). The mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine over $MgSO_4$, dried, and evaporated. The residue was chromatographed on silica gel (20 g, 50% ethyl acetate–hexane) to give **3** (33.7 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.5 Hz, H-23), 2.08 (2H, m, H-22), 2.40 (2H, m, H-4), 2.65 (2H, t, J = 7.4 Hz, H-5), 2.85 (10H, m, H-7, 10, 13, 16, 19), 3.54 (2H, s, H-3), 5.37 (12H, m, H-5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.5, 21.2, 21.6, 25.5 (2 carbons), 25.6 (3 carbons), 43.4, 29.9, 127.0, 127.3, 127.8, 128.0 (2 carbons), 128.2, 128.3, 128.5, 129.0, 129.7, 132.0, 208.3. HRMS (ESI$^-$) calcd. for $C_{24}H_{33}O_3$ $[M-H]^-$ 369.24297, found 369.24544. IR (neat) 3012, 2964, 2931, 1716, 1433, 1357, 1265, 1159, 1068, 927, 711 cm^{-1}.

*4.2.29. Ethyl (7Z,10Z,13Z,16Z,19Z)-3-oxodocosa-7,10,13,16,19-pentaenoate (**45**)*

To a solution of $(COCl)_2$ (2.0 M in CH_2Cl_2 1.25 mL, 2.50 mmol) in CH_2Cl_2 (5 mL) was added a solution of DMSO (0.43 mL, 5.0 mmol) in CH_2Cl_2 (2 mL) at −78 °C and the mixture was stirred at −78 °C for 10 min. A solution of **25** (781 mg, 2.08 mmol) in CH_2Cl_2 (4 mL) was added and the mixture was stirred at that temperature for 10 min. Et_3N (1.45 mL, 10.4 mmol) was added to the reaction mixture at −78 °C, and then the mixture was allowed to warm to 0 °C. The reaction was quenched with water and the mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (100 g, 5% ethyl acetate–hexane) to give **45** (291 mg, 31%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.28 (3H, t, J = 7.2 Hz, COO–CH_2–*CH_3*), 2.08 (4H, m, H-6, 21), 2.61 (2H, t, J = 7.2 Hz, H-4), 2.83 (8H, m, H-9, 12, 15, 18), 3.43 (2H, s, H-3), 4.20 (2H, q, J = 7.1 Hz, COO–*CH_2*–CH_3), 5.37 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (2 carbons), 20.5, 23.2, 25.5, 25.6 (3 carbons), 26.3, 42.2, 49.3, 61.3, 127.0, 127.8, 128.0, 128.1 (2 carbons), 128.2, 128.5, 1 28.9 (2 carbons), 132.0, 167.2, 202.6. HRMS (ESI$^+$) caled. for $C_{24}H_{36}O_3Na$ $[M+Na]^+$ 395.25621, found 395.25447. IR (neat) 3010, 2923, 2852, 2378, 2312, 1743, 1714, 1527, 1508, 1488, 1423, 1338, 1232, 1176, 1031 cm^{-1}.

*4.2.30. Ethyl 2-(2-((4Z,7Z,10Z,13Z,16Z)-nonadeca-4,7,10,13,16-pentaen-1-yl)-1,3-dioxolan-2-yl)acetate (**46**)*

To a solution of **45** (197 mg, 0.53 mmol) in benzene (15 mL) was added ethylene glycol (658 μL, 10.6 mmol) and *p*-TsOH·H_2O (45.6 mg, 0.26 mmol). The reaction was stirred at 110 °C for 13 h. The reaction was quenched with water and the mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (50 g, 5% ethyl acetate–hexane) to give **46** (72 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.27 (3H, t, J = 7.2 Hz, COO–CH_2–*CH_3*), 1.49 (2H, m, H-5) 1.83 (2H, m, H-4), 2.08 (4H, m, H-6, 21), 2.66 (2H, s, H-3), 2.83 (8H, m, H-9, 12, 15, 18), 3.98 (4H, m, acetal-CH_2), 4.15 (1H, q, J = 7.1, 7.2 Hz, COO–*CH_2*–CH_3), 5.37 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.2, 14.3, 20.6, 23.5, 25.5, 25.6 (3 carbons), 27.2, 37.3, 42.7, 60.5, 65.1, 65.2, 109.4, 127.0, 127.9, 128.0, 128.1, 128.2 (2 carbons), 128.4, 128.6, 129.9, 132.0, 169.5. HRMS (ESI$^+$) caled. for $C_{26}H_{40}O_4Na$ $[M+Na]^+$ 439.28243, found 439.28553. IR (neat) 3724, 3624, 2875, 2349, 2306, 1737, 1731, 1714, 1645, 1622, 1608, 1195, 1039, 948, 680, 649 cm^{-1}.

*4.2.31. 2-(2-((4Z,7Z,10Z,13Z,16Z)-Nonadeca-4,7,10,13,16-pentaen-1-yl)-1,3-dioxolan-2-yl)acetic acid (**47**)*

A solution of **46** (48.8 mg, 0.11 mmol) in 5% KOH/MeOH–H_2O (19:1, 2.2 mL) was stirred at 60 °C for 1 h. The reaction mixture was acidified with 1 N aqueous HCl and then extracted 3 times with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and evaporated to give **47** (43.7 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.49 (2H, m, H-5) 1.83 (2H, m, H-4), 2.08 (4H, m, H-6, 21), 2.70 (2H, s, H-3), 2.83 (8H, m, H-9, 12, 15, 18), 4.04 (4H, m, acetal-CH_2), 5.37 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3, 20.6, 23.5, 25.6 (2 carbons), 25.7 (2 carbons), 37.1, 42.2, 65.2 (2 carbons), 109.2, 127.0, 127.9, 128.2, 128.3 (4 carbons), 128.4, 128.6, 129.6, 132.1, 173.3. HRMS (ESI$^+$) caled. for $C_{24}H_{36}NaO_4$ $[M+Na]^+$ 411.25113, found 411.25184. IR (neat) 3012, 2960, 2923, 2358, 2341, 1737, 1714, 1699, 1224, 1041, 948, 719, 680, 649 cm^{-1}.

*4.2.32. (7Z,10Z,13Z,16Z,19Z)-3-Oxodocosa-7,10,13,16,19-pentaenoic acid (**8**)*

A solution of **47** (16 mg, 0.41 mmol) in acetone (0.41 mL) was added CBr_4 (27 mg, 0.082 mmol), TCEP· HCl (23.5 mg, 0.082 mmol). The reaction mixture was stirred for 12 days at room temperature. The reaction was quenched with water and the mixture was extracted 3 times with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, 40% ethyl acetate–hexane) to give **8** (10.3 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.08 (4H, m, H-6, 21), 1.71 (2H, m, H-5), 2.37 (2H, m, H-5), 2.57 (2H, t, J = 7.3 Hz, H-4), 2.83 (8H, m, H-9, 12, 15, 18), 3.52 (2H, s, H-2), 5.37 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20). ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3, 20.6, 23.6, 25.6 (3 carbons), 26.5, 29.7, 43.0, 127.0, 127.9, 128.1 (2 carbons), 128.2, 128.3, 128.6, 128.7, 129.2, 132.1, 132.2, 167.7, 208.9. HRMS (ESI$^-$) caled. for $C_{22}H_{31}O_3$ $[M-H]^-$ 343.22732, found 343.22701. IR (neat) 2923, 2852, 2374, 2318, 1745, 1710, 1687, 1639, 1542, 1500, 1396, 1338 cm^{-1}.

Declaration of interest

Conflicts of interest: none.

Acknowledgements

This work was financially supported by the Platform for Drug Discovery, Informatics, and Structural Life Science from the Japan Agency for Medical Research and Development (AMED), and was also supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (2013–2017).

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.07.004.

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