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Synthesis of docosahexaenoic acid derivatives designed as novel PPARγ agonists and antidiabetic agents

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Abstract—To discover novel peroxisome proliferator-activated receptor γ (PPARγ) agonists that could be used as antidiabetic agents, we designed docosahexaenoic acid (DHA) derivatives (**2** and **3**), which have a hydrophilic substituent at the C(4)-position, based on the crystal structure of the ligand-binding pocket of PPARγ. These compounds were synthesized via iodolactone as a key intermediate. We found that both DHA derivatives (**2** and **3**) showed PPARγ transactivation higher than, or comparable to, that of pioglitazone, which is a TZD derivative used as an antidiabetic agent. DHA derivatives related to these potent compounds **2** and **3** were also synthesized to study structure–activity relationships. Furthermore, 4-OH DHA **2**, which shows strong PPARγ transcriptional activity, was separated as an optically pure form.

1. Introduction

The incidence of type 2 diabetes has increased dramatically over the past two decades, and it is now becoming one of the biggest public health problems worldwide. Thiazolidinediones (TZDs), such as pioglitazone and rosiglitazone, are widely used for patients with type 2 diabetes to lower their plasma glucose level. However, these drugs have secondary effects such as obesity, edema, and hepatotoxicity.[1,2] Therefore, there is currently a need to develop innovative agents for treatment of type 2 diabetes without side effects.

It is well accepted that peroxisome proliferator-activated receptor γ (PPARγ) is the target of TZDs.[3,4] Peroxisome proliferator-activated receptors (PPARs) consist of three subtypes—α, γ, δ—and are members of the nuclear receptor superfamily of ligand-activated transcription factors. These three PPARs play an important role in lipid and glucose metabolism. PPARα promotes fatty acid catabolism in the liver and skeletal muscle, while PPARγ regulates fatty acid storage in adipose tissue. Mutations of PPARγ have been reported to cause type 2 diabetes, indicating the importance of PPARγ in glucose homeostasis.[5]

We are now developing novel PPARγ agonists as antidiabetic agents that do not cause side effects.[6] We considered that natural products and their derivatives, particularly biomolecules, would be appropriate for treatment of chronic diseases such as diabetes because humans have pathways for metabolism or excretion of biomolecules for protection against their undesirable effects. We therefore focused on docosahexaenoic acid (DHA, **1**) and its metabolites (Fig. 1). DHA is a long-chain polyunsaturated fatty acid present in large amounts in the adult mammalian brain and retina.[7] It is an essential fatty acid, a major constituent of nutrients rich in *n*-3 polyunsaturated fatty acids, and has beneficial effects on blood cholesterol levels and insulin sensitivity.[8,9] In addition, DHA and its metabolites would be expected not to have marked side effects because it has been used for some time as a functional food ingredient throughout the world. Here, we describe the synthesis of DHA derivatives, designed on the basis of the crystal structure of the ligand binding domain (LBD) of PPARγ, and their related compounds.

Keywords: PPARγ agonist; Docosahexaenoic acid; Antidiabetic agent; Iodolactonization.
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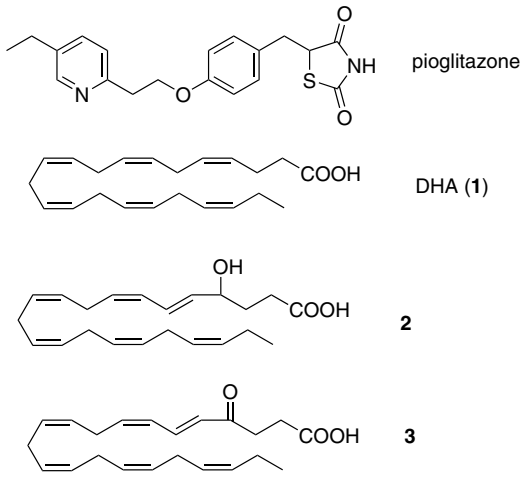


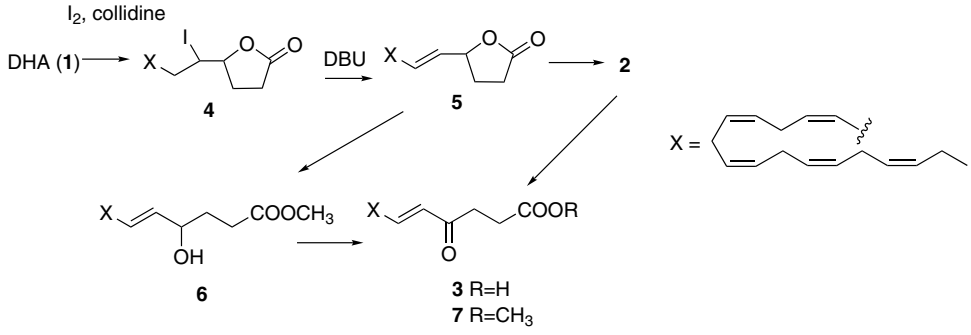
Figure 1.

Interestingly, our designed compounds are also putative metabolites of DHA (Fig. 1).

2. Results and discussion

The X-ray crystal structure of PPARγ-LBD docked with the TZD derivative, rosiglitazone, has been reported.[10] On the basis of the three-dimensional structure of the ligand-binding pocket, we designed 4-OH DHA (**2**) and 4-oxo-DHA (**3**) as agonists for PPARγ. Our docking analysis indicated that the 4-hydroxyl and 4-oxo groups of DHA derivatives could generate a new hydrogen bond with the hydroxyl group of Y327 lining the ligand-binding pocket of PPARγ.[6] Using DHA as a starting material, 4-OH DHA (**2**) and 4-oxo-DHA (**3**) were synthesized as shown in Scheme 1. Iodolactonization of DHA, which is the key reaction for introducing a functional group at C(4), was performed in dichloromethane in the presence of iodine together with γ-collidine. Treatment of iodolactone **4** with DBU afforded dehydrohalogenation product **5,** which was converted to 4-OH DHA **2** by basic hydrolysis. Oxo-compound **3** was synthesized in two different ways. Direct oxidation of 4-OH DHA **2** with Dess–Martin reagent[11,12] gave the desired product **3** in poor yield (25%). In the second method, methanolysis of lactone **5** gave the 4-hydroxyl methyl ester **6**, from which Swern or Dess–Martin oxidation gave the 4-oxo-compound **7**. Attempts to hydrolyze methyl ester **7** to carboxylic acid **3** under basic conditions resulted in production of a complex mixture by double bond isomerization. This reaction was improved (80% yield) by using lipase PS (Amano), which works under neutral conditions. Transactivation assay of PPARγ showed that all synthetic compounds (**2**–**7**) had higher activity than the parent compound DHA (**1**) (Fig. 2a).[6] It should be noted that 4-OH DHA **2** and 4-oxo-DHA **3**, designed with the aid of docking software and synthesized, showed activity that was comparable with, or higher than, that of pioglitazone.

As a next step, we evaluated the importance of the hydroxyl or oxo group at C(4) of DHA derivatives **2** and **3**. We synthesized the methyl ethers (**8** and **9**) and acetate (**10**) as compounds protecting the 4-hydroxyl group (Scheme 2). Ether **8** was obtained by treating **6** with CH_3I in the presence of Ag_2O. Transactivation assay showed that both methyl ethers, **8** and **9**, and the acetate **10** had significantly reduced activity (Fig. 2b), indicating the importance of the 4-hydroxyl group (Scheme 2). To confirm this hypothesis, we synthesized the 4-fluorinated compound (**11**) and compounds with no substituent at C(4) (**14** and **15**). The former (**11**) was designed as a molecule capable of forming a hydrogen bond between the F-substituent and the hydroxyl group of Y327, while the latter two (**14** and **15**) were designed as counterparts not capable of forming the corresponding hydrogen bond. A compound with a fluorine substituent (**11**) was successfully synthesized by treating **6** with DAST.[13] Attempts to reduce the 4-hydroxyl group to yield **14** and **15** failed to afford a complex mixture including isomerization products. We synthesized these by an alternative method (Scheme 3). Wittig reagent **12** was derived by (1) iodolactonization of eicosapentaenoic acid, (2) hydrolysis under basic conditions, (3) oxidative cleavage with sodium periodate followed by reduction with $NaBH_4$, and (4) direct bromination of alcohol in the presence of CBr_4 and Ph_3P, followed by treatment with Ph_3P in acetonitrile in a sealed tube. Aldehyde **13** was derived from δ-valerolactone by methanolysis followed by Swern oxidation and then the Wittig reaction with (triphenylphosphoranylidene)acetaldehyde. Target compound **15** was obtained by coupling of Wittig reagent **12** and aldehyde **13**. Fluorinated compound **11** showed potent activity, while compounds **14** and **15**, having no substituent at C(4), showed weak



Scheme 1.

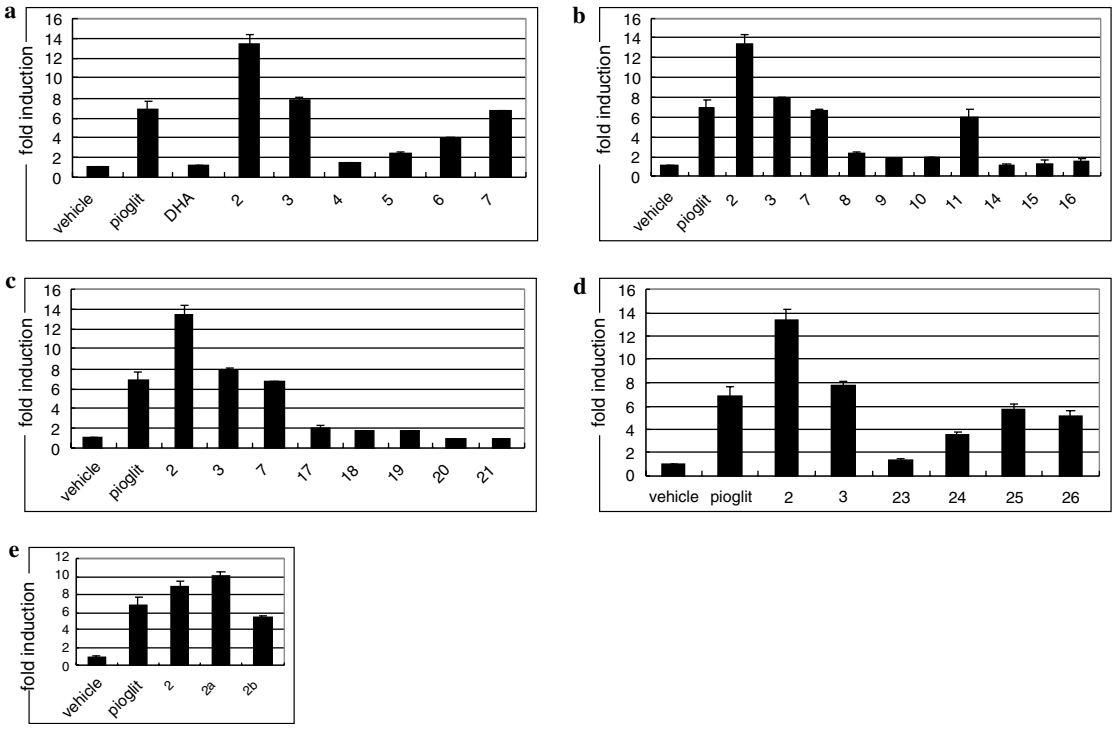
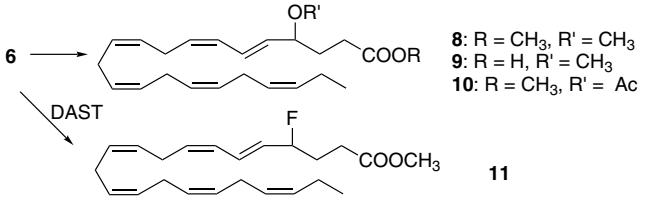


Figure 2. Activity of DHA derivatives on human PPARγ. All compounds were tested at 5 μM in the presence of 5% FBS using Cos7 cells by dual luciferase assay. Activities are presented by fold induction of PPARγ activation. Details were described in our previous paper.[6]



Scheme 2.

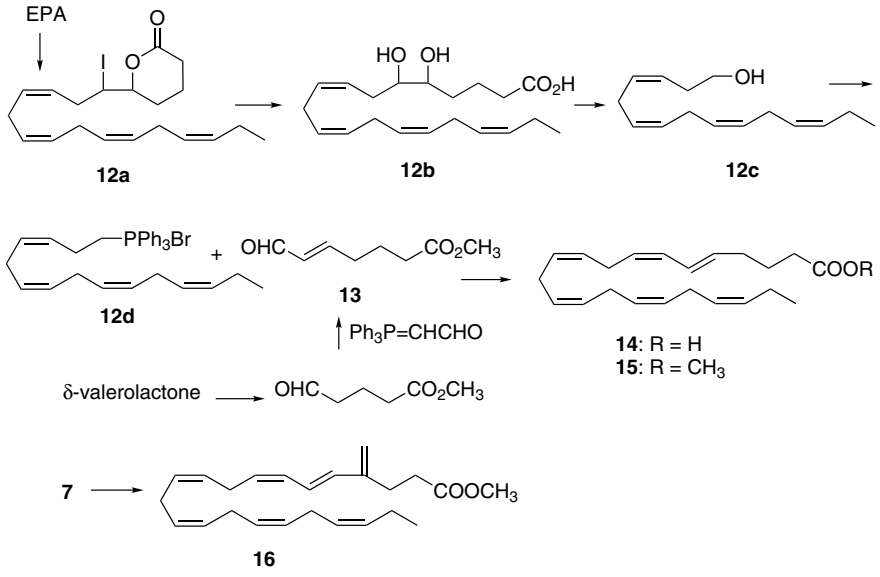
activity (Fig. 2b). These results demonstrate that the hydrophilic substituent at C(4) plays an important role in binding to PPARγ.

Compound **16** with a methylene substituent at C(4) was designed to test the importance of the 4-oxo group. This was synthesized by treating **7** with Tebbe reagent.[14] Methylene compound **16** showed significantly lower activity than 4-oxo-DHA **3**, indicating the importance of the 4-oxo group. Together, these results emphasize the indispensability of a hydrophilic substituent at C(4).

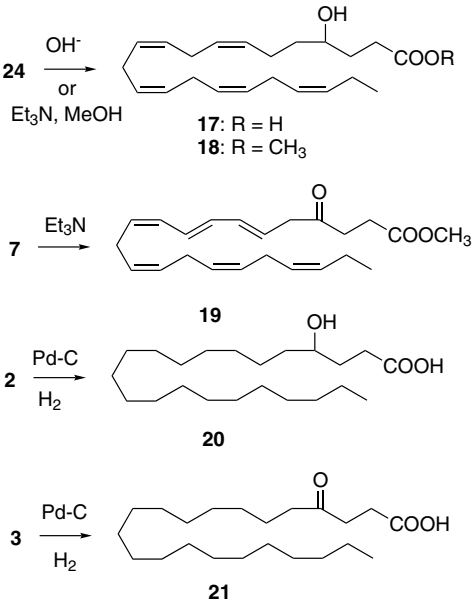
To examine the significance of the double bond at C(5), 5-dihydro derivatives **17** and **18**, and the deconjugated compound **19** were designed and synthesized (Scheme 4). In addition, fully saturated derivatives (**20** and **21**) were synthesized by catalytic hydrogenation. Saturation of the double bond at C(5) with hydrogen significantly lowered the potency, as confirmed by the poor activity of compound **17** (Fig. 2c). A similar result was obtained by isomerization of the double bond at C(5), as compound **19** showed poor activity (Fig. 2c). In addition, complete saturation eliminated the transactivation potency, as **20** and **21** showed no activity (Fig. 2c). Thus, it was clarified that both a hydrophilic group at C(4) and a 5*E*,7*Z*-conjugated diene structure are essential for producing highly potent DHA derivatives.

Since lactone **5** showed moderate PPARγ transactivation potency, we synthesized lactone derivatives (**23** and **24**) to investigate the structure–activity relationships of compounds having a lactone ring (Scheme 5). Iodolactone **4** was hydrolyzed under basic conditions to yield the 5-hydroxyl lactone **23**, which was produced via unstable but detectable 4-epoxy DHA **22**. Reduction of iodolactone **4** with Bu_3SnH afforded lactone **24** bearing no substituent at C(5). Compounds **23** and **24** showed poor and moderate activity, respectively (Fig. 2d). Lactol **25** and diol **26** were prepared as pro-drug candidates for both 4-OH DHA **2** and 4-oxo-DHA **3**. These two compounds were obtained by treating lactone **5** with DIBAL at low temperature. Interestingly, both compounds showed significant activity, but it is not known whether they would exhibit their activity after conversion to oxidative metabolites such as **2**.

To clarify how PPARγ distinguishes 4*S*-OH DHA (**2a**) and 4*R*-OH DHA (**2b**), we tried asymmetric iodolactonization as well as asymmetric reduction of 4-oxo-DHA **7**. However, the reactions yielded products only in poor enantiomeric excess. Therefore, we carried out optical resolution of racemic mixture **6**. We prepared the isocyanate **28** by reaction of dehydroabietylamine (**27**) with phosgene[15] and converted racemate **6** to a mixture of diasteromeric urethane derivatives, **29** and **30** (Scheme 6). We separated these diastereomers (**29** and **30**) by column chromatography on silica gel with an



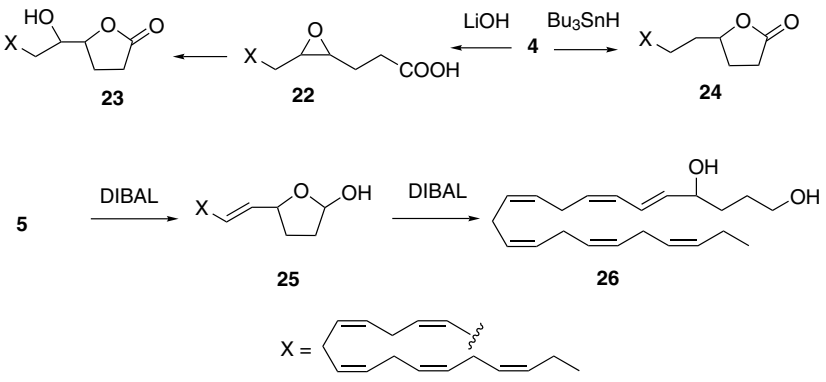
Scheme 3.



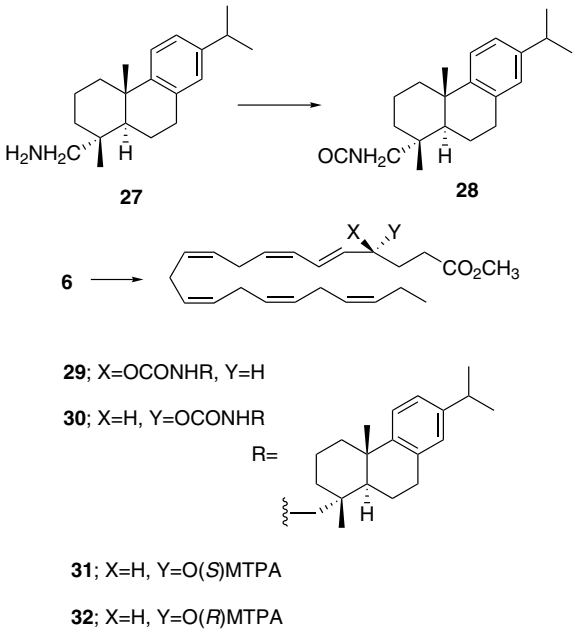
Scheme 4.

eluent of 5–8% AcOEt/hexane to obtain urethane derivatives of the optically pure forms of **6**. Both diastereomers (**29** and **30**) were treated with trichlorosilane in the presence of triethylamine to afford optically pure alcohols, (+)-**6a** and (−)-**6b**, respectively. These two compounds were hydrolyzed to afford (+)-**2a** and (−)-**2b**, respectively (Scheme 6).

The absolute configuration at C(4) was determined by the Kusumi–Mosher method.[16] Compound (−)-**6b** was converted to (*S*)-MTPA ester **31** and (*R*)-MTPA ester **32**. The difference in the chemical shifts of these two esters shown in Figure 3 indicates that (−)-**6b** and **2b** have the *R*-configuration, and (+)-**6a** and **2a** have the *S*-configuration. As shown in Figure 2e, compound (**2a**) with 4*S*-hydroxyl group showed higher activity than the racemate (**2**), while compound (**2b**) with 4*R*-hydroxyl group showed lower activity. However, it is clear that both 4*S*-OH DHA (**2a**) and 4*R*-OH DHA (**2b**) bind to PPARγ to induce luciferase activity. This result coincides with the docking analysis by which both 4*S*- and 4*R*-hydroxyl groups could form a hydrogen bond with the hydroxyl group of Tyr327 lining the ligand binding pocket of PPARγ (data not shown).



Scheme 5.



Scheme 6.

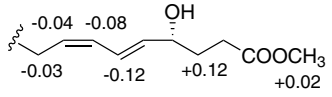


Figure 3. Determination of stereochemistry at C-4 of (−)-**6b** by ^{1}H NMR analysis (Kusumi–Mosher method). The difference of chemical shift values between *S*- and *R*-MTPA esters is shown in ppm.

We synthesized DHA derivatives modified at C(4), of which several showed PPARγ transactivation comparable to, or higher than, that of pioglitazone, which is a TZD derivative used as an antidiabetic agent. We have reported that one of them, the methyl ester of 4-oxo-DHA **7**, lowers blood glucose levels in diabetic animal models without producing undesirable effects such as obesity and hepatotoxicity.[6] Thus, DHA derivatives appear to have considerable promise for use as antidiabetic agents. We are now studying the detailed biological activities of DHA derivatives synthesized.

3. Experimental

3.1. General methods

NMR spectra were recorded using $CDCl_3$ as a solvent unless otherwise noted. ^{1}H NMR spectra were recorded at 400 MHz with TMS (0.00 ppm) as a reference. ^{13}C NMR spectra were recorded at 100 MHz with $CDCl_3$ (77.05 ppm) as a reference. ^{19}F NMR spectra were recorded at 376.5 MHz using trifluorotoluene (−63 ppm) as an external reference. Low- and high-resolution mass spectra were measured at an ionizing voltage of 70 eV. All air-sensitive reactions were run under argon or nitrogen atmosphere, and reagents were added through septa using oven-dried syringes. The phrase ''dried and evaporated'' indicates drying over $MgSO_4$ followed by evaporation of the solvents under house vacuum. Silica gel C200 (75–150 μm) was used for column chromatography, and precoated silica gel 60F254 plates (0.2 mm, Merck) were used for TLC.

3.2. 5-[(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-1-Iodo-3,6,9,12,15-octadecapentaenyl]dihydro-2(3*H*)-furanone (4)

To a solution of DHA (**1**) (425 mg, 1.30 mmol) and γ-collidine (686 μL, 5.19 mmol) in CH_3CN (43 mL) was added I_2 (660 mg, 2.60 mmol) at 0 °C. After being stirred at rt for 1 h, the mixture was quenched by addition of 5% aqueous $Na_2S_2O_3$ and extracted with ethyl acetate. The organic layer was washed with 10% aqueous HCl, water, and brine, dried, and evaporated. The residue was chromatographed on silica gel (20 g, 30% ethyl acetate–hexane) to give **4** (492 mg, 83%) as a yellow oil: ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.08 (3H, m, H-21, 3), 2.40 (1H, m, H-3), 2.56 (1H, m, H-2), 2.69 (1H, m, H-2), 2.80–2.88 (10H, m, H-6, 9, 12, 15, 18), 4.13 (1H, m, H-4), 4.25 (1H, m, H-5), 5.28–5.44 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.55 (1H, m, H-7); ^{13}C NMR δ 14.6, 20.8, 25.8, 25.9, 26.0, 26.1, 27.6, 28.8, 34.9, 38.0, 81.0, 127.0, 127.3, 127.6, 128.1, 128.2, 128.7, 128.9, 129.0, 131.8, 132.3, 176.4; MS *m*/*z* 454 (M^+, 5), 327 (22), 257 (15), 175 (60), 119 (48), 79 (100); HRMS Calcd for $C_{22}H_{31}IO_2$ (M^+) 454.1369, found 454.1358.

3.3. 5-[(1*E*,3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-1,3,6,9,12,15-Octadecahexaenyl]dihydro-2(3*H*)-furanone (5)

A solution of **4** (280 mg, 0.62 mmol) and DBU (111 μL, 0.740 mmol) in benzene (2.8 mL) was stirred at rt for 5 h. The reaction mixture was quenched by addition of 10% aqueous HCl and extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (15 g, 20% ethyl acetate–benzene) to give **5** (170 mg, 85%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.08 (3H, m, H-3, 21), 2.40 (1H, m, H-3), 2.58 (2H, m, H-2), 2.79–2.90 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.6 Hz, H-9), 5.0 (1H, q, J = 6.9 Hz, H-4), 5.34–5.43 (8H, m, H-10, 11, 13, 14, 16, 17, 19, 20), 5.52 (1H, m, H-8), 5.68 (1H, dd, J = 15.1, 6.9 Hz, H-5), 6.01 (1H, t, J = 11.0 Hz, H-7), 6.62 (1H, dd, J = 15.1, 11.0 Hz, H-6); ^{13}C NMR δ 14.6, 20.8, 25.9, 26.0 (2 carbons), 26.5, 28.9, 29.2, 53.3, 80.9, 127.3, 127.5 (2 carbons), 128.1, 128.4, 128.7, 128.9, 129.2, 130.3, 132.3, 132.8, 177.1; MS *m*/*z* 326 (M^+, 10), 257 (8), 246 (15), 131 (62), 79 (100); HRMS Calcd for $C_{22}H_{30}O_2$ (M^+) 326.2246, found 326.2234. UV (95% EtOH) λ_{max} 238 nm.

3.4. (5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-Hydroxy-5,7,10,13,16,19-docosahexaenoic acid (2)

A solution of **5** (93 mg, 0.285 mmol) in 5% KOH/CH_3OH–H_2O (19:1, 2.9 mL) was stirred at rt for 5 h. The reaction mixture was neutralized with 5% aqueous HCl and then extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 40% ethyl acetate–hexane) to give **2** (91 mg, 93%): ^{1}H

NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.88 (2H, m, H-3), 2.08 (2H, m, H-21), 2.48 (2H, t, J = 7.3 Hz, H-2), 2.80–2.91 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.6 Hz, H-9), 4.25 (1H, dd, J = 12.6, 6.6 Hz, H-4), 5.32–5.43 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.68 (1H, dd, J = 15.1, 6.5 Hz, H-5), 6.01 (1H, t, J = 11.0 Hz, H-7), 6.54 (1H, dd, J = 15.1, 11.0 Hz, H-6) ; ^{13}C NMR δ 14.4, 18.2, 20.7, 25.7, 25.8, 26.3, 30.3, 31.9, 58.3, 71.8, 126.1, 127.1, 127.7, 128.01, 128.08, 128.12, 128.5, 128.76, 128.80, 130.8, 132.2, 135.4, 178.6; MS *m*/*z* 344 (M^+, 1), 327 (3), 326 (7), 297 (4), 246 (14), 187 (16), 117 (50), 108 (55), 79 (100); HRMS Calcd for $C_{22}H_{30}O_2$ (M^+-H_2O) 326.2246, found 326.2256. UV (95% EtOH) λ_{max} 237 nm (ε = 29,500).

3.5. Methyl(5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-hydroxy-5,7,10,13,16,19-docosahexaenoate (6)

A solution of **5** (170 mg, 0.52 mmol) and Et_3N (217 μL, 1.56 mmol) in CH_3OH (10 mL) was stirred at rt for 6 h. The reaction mixture was evaporated and the residue was chromatographed on silica gel (10 g, 10% ethyl acetate–benzene) to give **6** (113 mg, 61%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.88 (2H, m, H-3), 2.08 (2H, quint, J = 7.4 Hz, H-21), 2.45 (2H, t, J = 7.3 Hz, H-2), 2.80–2.91 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.6 Hz, H-9), 3.68 (3 H, s), 4.25 (1H, m, H-4), 5.32–5.43 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.68 (1H, dd, J = 15.1, 6.5 Hz, H-5), 6.01 (1H, t, J = 11.0 Hz, H-7), 6.54 (1H, dd, J = 15.1, 11.0 Hz, H-6) ; ^{13}C NMR δ 14.4, 18.4, 20.7, 25.7, 25.8, 26.3, 30.3, 32.2, 51.9, 58.3, 71.7, 125.9, 127.2, 127.8, 128.0, 128.2, 128.5, 128.8, 130.6, 132.2, 135.8, 174.7; MS *m*/*z* 358 (M^+, 5), 192 (24), 79 (100), 67 (53); HRMS Calcd for $C_{23}H_{32}O_2$ (M^+-H_2O) 340.2402, found 340.2380. UV (95% EtOH) λ_{max} 238 nm.

3.6. Methyl(5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-oxo-5,7,10,13,16,19-docosahexaenoate (7)

To a solution of $(COCl)_2$ (97 μL, 1.12 mmol) in CH_2Cl_2 (10 ml) was added a solution of DMSO (158 μL, 2.24 mmol) in CH_2Cl_2 (1 ml) at −78 °C and the mixture was stirred at −78 °C for 10 min. A solution of **6** (200 mg, 0.559 mmol) in CH_2Cl_2 (4 ml) was added and the mixture was stirred at that temperature for 10 min. Et_3N (640 μL, 4.60 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 with ethyl acetate. The organic layer was washed with water and brine, dried, and evaporated. The residue was chromatographed on silica gel (20 g, 10% ethyl acetate–hexane) to give **7** (152 mg, 76%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.07 (2H, quint, J = 7.5 Hz, H-21), 2.66 (2H, t, J = 6.7 Hz, H-2), 2.77–2.88 (6H, m, H-12, 15, 18), 2.92 (2H, t, J = 6.7 Hz, H-3), 3.10 (2H, t, J = 7.3 Hz, H-9), 3.69 (3H, s, CO_2Me), 5.28–5.49 (8H, m, H-10, 11, 13, 14, 16, 17, 19, 20), 5.87 (1H, m, H-8), 6.15 (1H, t, J = 11.4 Hz, H-7), 6.20 (1H, d, J = 15.5 Hz, H-5), 7.56 (1H, dd, J = 15.5, 11.4 Hz, H-6); ^{13}C NMR δ 14.3, 20.6, 25.6, 25.7, 25.8, 26.7, 28.0, 35.5, 51.9, 126.4, 127.0, 127.1, 127.7, 128.6, 128.7, 129.5, 129.7, 132.1, 137.1, 140.1, 173.4, 198.3; MS *m*/*z* 356 (M^+, 5), 276 (7), 189 (22), 167 (25), 137 (25), 115 (100), 79 (43); HRMS Calcd for $C_{23}H_{32}O_3$ (M^+) 356.2351, found 356.2335. UV (95% EtOH) λ_{max} 280 nm (ε = 24,000).

3.7. (5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-Oxo-5,7,10,13,16,19-docosahexaenoic acid (3)

To a solution of Dess–Martin Periodinane (370 mg, 0.872 mmol) and Et_3N (484 μL, 3.49 mmol) in CH_2Cl_2 (5.8 mL) was added **2** (200 mg, 0.581 mmol) in CH_2Cl_2 (5.8 mL) at ambient temperature. The mixture was stirred at that temperature for 30 min and the reaction was quenched with water. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (20 g, 10% ethyl acetate–hexane) to give **3** (51 mg, 26%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.79–1.93 (2H, m, H-3), 2.08 (2H, quint, J = 7.4 Hz, H-21), 2.45 (2H, t, J = 7.4 Hz, H-2), 2.80–2.88 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.6 Hz, H-9), 3.27 (3H, s, *Me*O), 3.64 (1H, q, J = 6.7 Hz, H-4), 5.28–5.46 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.50 (1H, dd, J = 15.2, 6.7 Hz, H-5), 6.01 (1H, t, J = 10.9 Hz, H-7), 6.50 (1H, dd, J = 15.2, 10.9 Hz, H-6); ^{13}C NMR δ 14.5, 20.8, 25.7, 25.8, 25.9, 26.3, 30.3, 30.5, 56.5, 81.3, 127.2, 127.9, 128.0, 128.1, 128.2, 128.8, 128.9, 130.9, 132.3, 133.2, 179.5; MS *m*/*z* 342 (M^+, 7), 324 (4), 313 (2), 288 (4), 262 (18), 255 (6), 241 (15), 207 (26), 189 (27), 153 (56), 79 (100); HRMS Calcd for $C_{22}H_{30}O_3$ (M^+) 342.2195, found 342.2201.

3.8. Methyl(5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-methoxy-5,7,10,13,16,19-docosahexaenoate (8)

To a solution of **6** (100 mg, 0.279 mmol) and CH_3I (140 μL, 2.24 mmol) in CH_3CN (280 μL) was added Ag_2O (129 mg, 0.558 mmol) at ambient temperature. After being stirred for 18 h, the mixture was diluted with ethyl acetate and washed with water. The organic layer was dried and evaporated. The residue was chromatographed on silica gel (10 g, 4 % ethyl acetate–hexane) to give **8** (61 mg, 59%): ^{1}H NMR δ 0.98 (3H, t, J = 7.5 Hz, H-22), 1.79–1.93 (2H, m, H-3), 2.08 (2H, quint, J = 7.4 Hz, H-21), 2.39 (2H, t, J = 7.5 Hz, H-2), 2.79–2.90 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.5 Hz, H-9), 3.26 (3H, s, *Me*O), 3.64 (1H, q, J = 6.7 Hz, H-4), 3.67 (3H, s, CO_2Me), 5.28–5.46 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.50 (1H, dd, J = 15.2, 6.7 Hz, H-5), 6.01 (1H, t, J = 10.9 Hz, H-7), 6.49 (1H, dd, J = 15.2, 10.9 Hz, H-6); ^{13}C NMR δ 14.5, 20.8, 25.8, 25.9, 26.3, 30.2, 30.8, 51.8, 56.5, 81.3, 127.2, 127.8, 128.0 (2 carbons), 128.2, 128.6, 128.8 (2 carbons), 128.9, 130.7, 132.3, 133.6, 174.2; MS *m*/*z* 372 (M^+, 1), 357 (1), 340 (11), 285 (8), 271 (9), 253 (8), 183 (40), 131 (100), 79 (75); HRMS Calcd for $C_{23}H_{32}O_2$ (M^+-CH_3OH) 340.2402, found 340.2394.

3.9. (5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-Methoxy-5,7,10,13,16,19-docosahexaenoic acid (9)

According to the procedure described for **2**, the hydrolysis of the ester **8** was performed to give **9** in 87% yield: ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.79–1.93 (2H, m, H-3), 2.08 (2H, quint, J = 7.4 Hz, H-21), 2.45 (2H, t, J = 7.5 Hz, H-2), 2.80–2.88 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.6 Hz, H-9), 3.27 (3H, s, *Me*O), 3.64 (1H, q, J = 6.7 Hz, H-4), 5.28–5.46 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.50 (1H, dd, J = 15.2, 6.7 Hz, H-5), 6.01 (1H, t, J = 10.9 Hz, H-7), 6.50 (1H, dd, J = 15.2, 10.9 Hz, H-6); ^{13}C NMR δ 14.5, 20.8, 25.8, 25.9, 26.3, 30.3, 30.5, 56.5, 81.3, 127.2, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.6, 128.8, 128.9, 130.9, 132.3, 133.2, 179.5: MS *m*/*z* 358 (M^+, 3), 326 (11), 297 (5), 213 (10), 108 (100), 79 (95); HRMS Calcd for $C_{22}H_{30}O_2$ (M^+-CH_3OH) 326.2246, found 326.2234.

3.10. Methyl(5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-acetyloxy-5,7,10, 13,16,19-docosahexaenoate (10)

A solution of **6** (10 mg, 0.028 mmol) in pyridine (200 μL) and Ac_2O (50 μL) was stirred at 0 °C for 6 h. The mixture was neutralized with 10% aqueous HCl and extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (1 g, 10 % ethyl acetate–hexane) to give **10** (9 mg, 90%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.98 (2H, m, H-3), 2.05 (3H, s, CH_3CO), 2.08 (2H, m, H-21), 2.36 (2H, t, J = 7.5 Hz, H-2), 2.79–2.90 (6H, m, H-12, 15, 18), 2.96 (2H, t, J = 6.9 Hz, H-9), 3.67 (3H, s, *Me*O), 5.28–5.46 (10H, m, H-4, 8, 10, 11, 13, 14, 16, 17, 19, 20), 5.57 (1H, dd, J = 15.1, 7.3 Hz, H-5), 5.96 (1H, t, J = 11.0 Hz, H-7), 6.55 (1H, dd, J = 15.1, 11.0 Hz, H-6); MS *m*/*z* 400 (M^+, 1), 369 (4), 340 (66), 192 (73), 91 (100), 79 (90); HRMS Calcd for $C_{23}H_{32}O_2$ (M^+-AcOH) 340.2402, found 340.2383.

3.11. Methyl(5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-fluoro-5,7,10,13, 16,19-docosahexaenoate (11)

To a solution of diethylaminosulfur trifluoride (DAST) (54.8 μL, 0.418 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise **6** (100 mg, 0.279 mmol) in CH_2Cl_2 (0.5 mL) at −78 °C. The reaction mixture was stirred at that temperature for 10 min and then at 0 °C for 30 min. The reaction was quenched with brine and the mixture was extracted with ethyl acetate. The organic layer was washed with 10% aqueous HCl, saturated solution of aqueous $NaHCO_3$ and brine, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 3% ethyl acetate–hexane) to give **11** (42 mg, 42%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.07 (4H, m, H-3, 21), 2.44 (2H, m, H-2), 2.79–2.90 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.7 Hz, H-9), 3.68 (3H, s), 5.01 (1H, dq, J = 48.6, 6.3 Hz, H-4), 5.27–5.42 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.69 (1H, m, H-5), 6.00 (1H, t, J = 10.8 Hz, H-7), 6.60 (1H, m, H-6); ^{19}F NMR δ −175.55 (1F, ddt, J = 48.6, 21.1, 16.0 Hz); MS *m*/*z* 360 (M^+, 2), 340 (13), 271 (10), 260 (10), 197 (71), 131 (43), 79 (100); HRMS Calcd for $C_{23}H_{33}FO_2$ (M^+) 360.2465, found 360.2488.

3.12. 6-[(3*Z*,6*Z*,9*Z*,12*Z*)-1-Iodo-3,6,9,12-pentadecatetraenyl]tetrahydro-2*H*-pyran-2-one (12a)

Iodolactonization of eicosapentaenoic acid was performed by the same procedure described for **4** to give **12a** in 88% yield: ^{1}H NMR δ 0.98 (3H, t, J = 7.5 Hz, H-20), 3.96 (1H, m, H-5), 4.10 (1H, m, H-6), 5.36–5.39 (7H, m, H-9, 11, 12, 14, 15, 17, 18), 5.56 (1H, m, H-8).

3.13. (3*Z*,6*Z*,9*Z*,12*Z*)-3,6,9,12-Pentadecatetraen-1-ol (12c)

A solution of **12a** (2.17 g, 5.08 mmol) in 5% KOH/CH_3OH–H_2O (19:1, 20 mL) was stirred at 60 °C for 4 h. The reaction mixture was acidified with 5% aqueous HCl and then extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The crude product **12b** was obtained (1.56 g, 91%). A solution of **12b** (1.56 g, 4.63 mmol) and sodium periodate (1.46 g, 6.86 mmol) in THF/H_2O (2:1, 13.7 mL) 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 (13 mL) and treated with $NaBH_4$ (518 mg, 13.7 mmol) at 0 °C for 0.5 h. The reaction was quenched with water at 0 °C and then extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (40 g, 50% ethyl acetate–hexane) to give **12c** (758 mg, 75%): ^{1}H NMR δ 0.98 (3H, t, J = 7.5 Hz, H-15), 2.08 (2H, quint, J = 7.5 Hz, H-14), 2.36 (2H, q, J = 7.0 Hz, H-2), 2.80–2.88 (6H, m, H-5, 8, 11), 3.65 (2H, m, H-1), 5.24–5.62 (8H, m, H-3, 4, 6, 7, 9, 10, 12, 13); ^{13}C NMR δ 14.7, 21.0, 25.9, 26.0, 26.1, 31.2, 62.6, 126.0, 127.4, 128.2, 128.3, 128.8, 129.0, 131.6, 132.5.

3.14. Bromo[(3*Z*,6*Z*,9*Z*,12*Z*)-3,6,9,12-pentadecatetraenyl]triphenylphosphorane (12d)

To a solution of **12c** (450 mg, 2.05 mmol) in CH_2Cl_2 (20 mL) were added Ph_3P (1.61 g, 6.14 mmol) and CBr_4 (2.05 g, 6.14 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated. The residue was chromatographed on silica gel (15 g, 0–5% ethyl acetate–hexane) to give 1-brominated compound (510 mg, 88%). To a solution of 1-brominated compound (430 mg, 1.52 mmol) in CH_3CN (3.0 mL) was added Ph_3P (1.20 g, 4.57 mmol) and the mixture was stirred at 80 °C for 20 h in a sealed tube. The reaction mixture was evaporated and the residue was chromatographed on silica gel (26 g, 3–5% MeOH–CH_2Cl_2) to give **12d** (762 mg, 92%): ^{1}H NMR δ 0.96 (3H, t, J = 7.4 Hz, H-15), 2.04 (2H, quint, J = 7.4 Hz, H-14), 2.57 (2H, m, H-2), 2.58 (2H, t, J = 7.1 Hz, H-11), 2.69 (2H, t, J = 7.1 Hz, H-8), 2.75 (2H, t, J = 7.1 Hz, H-5), 3.91 (2H, m, H-1), 5.25–5.40 (7H, m, H-3, 4, 6, 7, 9, 10, 12), 5.64 (1H, m, H-13), 7.70–7.90 (15H, m, Ph).

3.15. Methyl(7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-5,7,10,13,16,19-docosahexaenoate (15)

To a solution of phosphonium bromide **12d** (30 mg, 55 μmol), aldehyde **13** (17.2 mg, 110 μmol), and HMPA

(58 μL, 333 μmoL) in THF (330 μL) was added LHMDS (1.0 M in THF, 66 μL, 66 μmol) at −78 °C. The reaction mixture was allowed to warm to 0 °C for 2 h, and the reaction was quenched with water. The mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, dried, and evaporated. The residue was chromatographed on silica gel (6 g, 1% ethyl acetate–hexane) to give **15** (6.0 mg, 33%): ^{1}H NMR δ 0.98 (3H, t, J = 7.5 Hz, H-22), 1.75 (2H, quint, J = 7.4 Hz, H-3), 2.08 (2H, quint, J = 7.5 Hz, H-21), 2.16 (2H, q, J = 7.4 Hz, H-4), 2.33 (2H, t, J = 7.4 Hz, H-2), 2.75–2.90 (6H, m, H-12, 15, 18), 2.94 (2H, m, H-9), 3.67 (3H, s, Me), 5.28–5.43 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.65 (1H, dt, J = 14.0, 7.4 Hz, H-5), 5.97 (1H, t, J = 11.0 Hz, H-7), 6.35 (1H, dd, J = 14.0, 11.0 Hz, H-6); ^{13}C NMR δ 14.7, 21.0, 24.9, 25.9, 26.1, 26.5, 32.6, 33.8, 51.9, 126.7, 127.4, 128.3, 128.4, 128.5, 128.7, 128.8, 128.9, 129.0, 129.1, 132.5, 134.1, 174.4; MS m/z 342 (M^+, 6), 313 (2), 288 (4), 273 (7), 241 (7), 227 (7), 145 (21), 131 (26), 119 (33), 108 (100), 91 (59), 79 (84); HRMS Calcd for $C_{23}H_{34}O_2$ (M^+) 342.2559, found 342.2529.

3.16. (7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-5,7,10,13,16,19-Docosahexaenoic acid (14)

Hydrolysis of ester **15** was performed, according to the procedure described for **2**, to give **14** in 91% yield: ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.76 (2H, quint, J = 7.4 Hz, H-3), 2.01 (2H, quint, J = 7.5 Hz, H-21), 2.17 (2H, q, J = 7.4 Hz, H-4), 2.37 (2H, t, J = 7.4 Hz, H-2), 2.75–2.88 (6H, m, H-12, 15, 18), 2.94 (2H, t, J = 5.9, H-9), 5.25–5.45 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.64 (1H, dt, J = 15.1, 7.4 Hz, H-5), 5.98 (1H, t, J = 11.5 Hz, H-7), 6.35 (1H, dd, J = 15.1, 11.5 Hz, H-6); ^{13}C NMR δ 14.7, 21.0, 24.6, 25.9, 26.5, 32.5, 33.6, 126.9, 127.4, 128.2, 128.3, 128.4, 128.5, 128.7, 128.8, 128.9, 129.0, 132.5, 133.9, 179.3; MS m/z 328 (M^+, 5), 299 (3), 274 (4), 241 (6), 219 (4), 145 (22), 131 (26), 108 (100), 91(68), 79 (91); HRMS Calcd for $C_{22}H_{32}O_2$ (M^+) 328.2402, found 328.2428.

3.17. Methyl(7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-methylene-5,7,10,13,16,19-docosahexaenoate (16)

To a solution of **7** (50 mg, 140 μmol) in THF (180 μL) was added 0.5 M Tebbe reagent in toluene (168 μmol, 336 μL) at −20 °C. After being stirred for 30 min at −20 °C, saturated solution of aqueous $NaHCO_3$ was added to the reaction mixture. The mixture was extracted with ethyl acetate, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (5 g, 3% ethyl acetate–hexane) to give **16** (7.1 mg, 14%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.06 (2H, quint, J = 7.5 Hz, H-21), 2.52–2.63 (4H, m, H-2, 3), 2.78–2.93 (6H, m, H-12, 15, 18), 3.00 (2H, t, J = 6.3 Hz, H-9), 3.69 (3H, s), 5.00 (1H, s), 5.06 (1H, s), 5.24–5.47 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 6.05 (1H, t, J = 11.1 Hz, H-7), 6.24 (1H, d, J = 15.5 Hz, H-5), 6.56 (1H, dd, J = 15.5, 11.1 Hz, H-6); ^{13}C NMR δ 14.7, 21.0, 25.9, 26.0, 26.1, 26.7, 27.5, 33.3, 52.0, 116.4, 124.1, 127.4, 128.1, 128.3, 128.4, 128.8, 129.0, 129.1, 129.3, 131.0, 132.4, 134.8, 145.0, 174.0; MS m/z 354 (M^+, 15), 285 (12), 267 (11), 213 (13), 145 (41), 131 (48), 108 (100), 91 (92), 79 (95); HRMS Calcd for $C_{24}H_{34}O_2$ (M^+) 354.2559, found 354.2564.

3.18. (7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-Hydroxy-7,10,13,16,19-docosapentaenic acid (17)

According to the procedure described for **2**, the hydrolysis of lactone **24** was performed to give **17** in 82% yield: ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.73 (2H, m, H-5), 1.84 (2H, m, H-3), 2.08 (2H, m, H-21), 2.20 (2H, m, H-6), 2.53 (2H, t, J = 7.2 Hz, H-2), 2.79–2.90 (8H, m, H-9, 12, 15, 18), 3.69 (1H, m, H-4), 5.29–5.45 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20); MS m/z 346 (M^+, 6), 328 (6), 259 (8), 175 (45), 119 (56), 79 (100); HRMS Calcd for $C_{22}H_{32}O_2$ (M^+-H_2O) 328.2402, found 328.2382.

3.19. Methyl(7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-hydroxy-7,10,13,16,19-docosapentaenoate (18)

According to the procedure described for **6**, methanolysis of lactone **24** was performed to give **18** in 33% yield: ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.53 (2H, m), 1.73, 1.82 (each 1H, m), 2.07 (2H, m, H-21), 2.19 (2H, m, H-6), 2.46 (2H, t, J = 7.4 Hz, H-2), 2.79–2.90 (8H, m, H-9, 12, 15, 18), 3.64 (1H, m, H-4), 3.68 (3H, s, H-Me), 5.29–5.44 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20); MS m/z 360 (M^+, 2), 342 (1), 322 (6), 259 (7), 175 (40), 119 (55), 79 (100); HRMS Calcd for $C_{23}H_{36}O_3$ (M^+) 360.2664, found 360.2636.

3.20. Methyl(6*E*,8*E*,10*Z*,13*Z*,16*Z*,19*Z*)-4-oxo-6,8,10,13,16,19-docosahexaenoate (19)

To a solution of **7** (100 mg, 279 μmol) in CH_2Cl_2 (10 mL) was added Et_3N (272 μL, 1.95 mmol) at 0 °C and the mixture was stirred at that temperature for 5 h. The reaction mixture was diluted with *n*-hexane, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (6 g, 8.5% ethyl acetate–hexane) to give **19** (65 mg, 65%): ^{1}H NMR δ 0.98 (3H, t, J = 7.5 Hz, H-22), 2.08 (2H, quint, J = 7.5 Hz, H-21), 2.59 (2H, t, J = 6.6 Hz, H-2), 2.77 (2H, t, J = 6.6 Hz, H-3), 2.80–2.88 (4H, m, H-15, 18), 2.99 (2H, t, J = 6.3 Hz, H-12), 3.27 (2H, d, J = 13.3 Hz, H-5), 3.67 (3H, s, Me), 5.30–5.46 (7H, m, H-11, 13, 14, 16, 17, 19, 20), 5.78 (1H, m, H-6), 6.03 (1H, t, J = 11.0 Hz), 6.20 (2H, m), 6.48 (1H, m); ^{13}C NMR δ 14.4, 20.7, 25.7, 25.8, 26.3, 27.8, 36.8, 46.9, 51.9, 125.4, 127.1, 127.7, 127.8, 127.9, 128.8 (3 carbons), 130.6, 132.2, 132.4, 134.6, 173.3, 206.7.

3.21. 4-Hydroxydocosanoic acid (20)

A mixture of **2** (3 mg, 8.7 μmol), 10% Pd–C (5 mg), and CH_3OH (1 mL) was stirred under a hydrogen atmosphere for 7 h. The reaction mixture was filtered and evaporated. The residue was chromatographed on silica gel (1 g, 10% MeOH–CH_2Cl_2) to give **20** (2.5 mg, 81%): ^{1}H NMR (CD_3OD) δ 0.90 (3H, t, J = 6.7 Hz, H-22), 1.19–1.50 (34H, m, H-5∼H-21), 1.54–1.84 (2H, m,

H-3), 2.39 (2H, m, H-2), 3.53 (1H, m, H-4); ^{13}C NMR δ 14.6, 23.9, 25.3, 27.0, 30.6, 30.9, (9 carbons), 31.6, 33.2, 33.6, 38.6, 50.9, 71.8, 178.0.

3.22. 4-Oxodocosanoic acid (21)

A mixture of **7** (27 mg, 0.076 mmol), 10% Pd–C (27 mg), and CH_3OH (1 mL) was stirred under a hydrogen atmosphere for 7 h. The mixture was filtered and evaporated. The residue was chromatographed on silica gel (20 g, 2.5% ethyl acetate–hexane) to give methyl ester of **21** (27 mg, 97%): ^{1}H NMR δ 0.87 (3H, t, J = 6.8 Hz, H-22), 1.18–1.32 (30H, m, H-7∼H-21), 1.57 (2H, m, H-6), 2.43 (2H, t, J = 7.5 Hz, H-5), 2.58 (2H, t, J = 6.7 Hz, H-2), 2.71 (2H, t, J = 6.7 Hz, H-3), 3.67 (3H, s, MeO); ^{13}C NMR δ 14.6, 23.1, 24.3, 28.1, 29.6, 29.8, 29.9, 30.1 (10 carbons), 32.4, 37.4, 43.3, 53.2, 173.7, 209.6. According to the procedure described for **2**, the hydrolysis of the methyl ester was performed to give **21** in 76% yield: ^{1}H NMR δ 0.88 (3H, t, J = 6.8 Hz, H-22), 1.19–1.32 (30H, m, H-7∼H-21), 1.58 (2H, m, H-6), 2.44 (2H, t, J = 7.5 Hz, H-5), 2.63 (2H, m, H-2), 2.73 (2H, m, H-3); ^{13}C NMR δ 14.5, 23.1, 24.2, 27.9, 29.6, 29.8, 29.9, 30.0 (10 carbons), 32.3, 37.2, 43.2, 173.5, 209.9.

3.23. 5-[(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-1-Hydroxy-3,6,9,12,15-octadecapentaenyl]dihydro-2(3*H*)-furanone (23)

A solution of **4** (86 mg, 0.189 mmol) in 0.2 M LiOH/THF–H_2O (3:2, 5 mL) was stirred at 0 °C for 5 h. After the addition of 10% aqueous HCl, the mixture was extracted with ethyl acetate, washed with water, and evaporated. The residue was dissolved in chloroform (10 mL) and the solution was stored at rt for 24 h. The solution was evaporated and chromatographed on silica gel (5 g, 30 % ethyl acetate–hexane) to give **23** (54 mg, 83%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 2.08 (2H, m, H-21), 2.21 (2H, m, H-3), 2.40 (2H, m, H-2), 2.53 and 2.60 (each 1H, m, H-6), 2.79–2.90 (8H, m, H-9, 12, 15, 18), 3.63 (1H, m, H-5), 4.47 (1H, t, J = 7.2 Hz, H-4), 5.33–5.44 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20); ^{13}C NMR δ 14.5, 20.8, 24.3, 25.7 (2 carbons), 25.9, 26.0, 28.8, 31.6, 73.4, 82.2, 124.6, 127.3, 127.8, 128.1, 128.2 (2 carbons), 128.6, 128.8, 131.9, 132.3, 177.6; MS m/z 344 (M^+, 12), 288 (2), 276 (10), 175 (69), 119 (51), 79 (100); HRMS Calcd for $C_{22}H_{32}O_3$ (M^+) 344.2351, found 344.2365.

3.24. 5-[(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-3,6,9,12,15-Octadecapentaenyl]dihydro-2(3*H*)-furanone (24)

A solution of **4** (436 mg, 0.960 mmol) and Bu_3SnH (517 μL, 1.92 mmol) in THF (44 mL) was refluxed for 5 h. The mixture was evaporated and the residue was chromatographed on silica gel (20 g, 2.5% ethyl acetate–hexane) to give **24** (207 mg, 66%): ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, H-22), 1.66 (1H, m), 1.87 (2H, m), 2.08 (2H, m, H-21), 2.22 (2H, m), 2.33 (1H, m), 2.54 (2H, m, H-2), 2.79–2.90 (8H, m, H-9, 12, 15, 18), 4.50 (1H, m, H-4), 5.32–5.41 (10H, m, H-7, 8, 10, 11, 13, 14, 16, 17, 19, 20); MS m/z 328 (M^+, 14), 228 (10), 175 (63), 79 (100); HRMS Calcd for $C_{22}H_{32}O_2$ (M^+) 328.2402, found 328.2408.

3.25. 5-[(1*E*,3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-1,3,6,9,12,15-Octadecahexaenyl]tetrahydro-2-furanol (25)

To a solution of **5** (300 mg, 0.92 mmol) in THF (9.2 mL) was added dropwise 0.95 M diisobutylaluminum hydride in hexane (1.84 mmol, 1.94 mL) at −78 °C. After being stirred at −78 °C for 3 h, brine was added and the mixture was filtered through a Celite pad. The filtrate was extracted with ethyl acetate and the organic layer was dried and evaporated. The residue was chromatographed on silica gel (15 g, 10% ethyl acetate–benzene) to give **25** (146 mg, 48%): ^{1}H NMR δ 0.98 (3H, t, J = 7.5 Hz, H-22), 1.57–2.28 (6H, m, H-2, 3, 21), 2.78–2.89 (6H, m, H-12, 15, 18), 2.96 (2H, t, J = 6.6 Hz, H-9), 3.05 (1H, br s, OH), 4.50 and 4.73 (total 1H, each q, J = 7.9 Hz, H-4), 5.28–5.43 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.51 and 5.61 (total 1H, m, H-1), 5.62 and 5.77 (total 1H, dd, J=15.2, 7.8 Hz), 5.98 (1H, m), 6.53 (1H, m); MS m/z 328 (M^+, 1), 310 (4), 241 (3), 213 (8), 145 (21), 79 (100); HRMS Calcd for $C_{22}H_{30}O$ (M^+-H_2O) 310.2297, found 310.2303.

3.26. (5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-5,7,10,13,16,19-Docosahexaene-1,4-diol (26)

To a solution of **5** (300 mg, 0.92 mmol) in THF (9 mL) was added 0.95 M diisobutylaluminum hydride in hexane (2.76 mmol, 2.90 mL) at 0 °C and the mixture was stirred at 0 °C for 3 h. The reaction was worked up and purified, by the same procedure described for **25**, to give **26** (182 mg, 60%): ^{1}H NMR δ 0.98 (3H, t, J = 7.5 Hz, H-22), 1.54–1.73 (4H, m, H-2, 3), 2.07 (2H, m, H-21), 2.84 (6H, m, H-12, 15, 18), 2.97 (2H, t, J = 6.5 Hz, H-9), 3.68 (2H, m, H-1), 4.24 (1H, m, H-4), 5.28–5.45 (9H, m, H-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.72 (1H, dd, J = 15.2, 6.7 Hz, H-5), 6.00 (1H, t, J = 11.0 Hz, H-7), 6.52 (1H, dd, J = 15.2, 11.0 Hz, H-6) ; ^{13}C NMR δ 14.5, 20.8, 25.8, 25.9, 26.0, 26.3, 29.0, 34.5, 63.1, 72.8, 125.7, 127.2, 127.8, 128.1, 128.2, 128.3, 128.6, 128.8, 130.6 (2 carbons), 132.3, 136.4; MS m/z 330 (M^+, 1), 312 (6), 243 (8), 159 (15), 108 (70), 79 (100); HRMS Calcd for $C_{22}H_{32}O$ (M^+-H_2O) 312.2453, found 312.2471.

3.27. Methyl(4*S*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-{[({[(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydro-1-phenanthrenyl]methyl}amino)carbonyl]oxy}-5,7,10,13,16,19-docosahexaenoate (29) and methyl(4*R*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-{[({[(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydro-1-phenanthrenyl]methyl}amino)carbonyl]oxy}-5,7,10,13,16,19-docosahexaenoate (30)

A mixture of **6** (500 mg, 1.40 mmol), isocyanate **28** (1.31 g, 4.20 mmol), DMAP (342 mg, 2.80 mmol), and CH_2Cl_2 (4.2 mL) in a sealed tube was stirred at 50 °C for 20 h under nitrogen. Removal of solvent in vacuo and chromatography (10% AcOEt/hexane) on silica gel afforded urethane (**29**:**30** = 1:1) (850 mg, 91%) along with recovered **28** (451 mg). The diastereomeric carbamates were separated by chromatography on silica gel (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0% AcOEt/hexane) to afford **30** (261 mg, 28%) and **29** (243 mg, 26%) in this order. **30**:^{1}H NMR δ 0.92 (3H, s, Abieta-18), 0.97 (3H, t, J = 7.5 Hz, DHA-22),

1.21 (3H, s, Abieta-20), 1.22 (6H, d, $J = 7.5$ Hz, Abieta-16, 17), 1.25–1.89 (8H, m, Abieta-1, 2, 3, 6), 1.95 (2H, q, $J = 7.3$ Hz, DHA-3), 2.07 (2H, m, DHA-21), 2.29 (1H, d, $J = 12.9$ Hz, Abieta-5), 2.34 (2H, t, $J = 7.3$ Hz, DHA-2), 2.74–2.96 (11H, m, DHA-9, 12, 15, 18, Abieta-7, 15), 3.05, 3.10 (each 1H, dd, $J = 13.8$, 7.0 Hz, Abieta-19), 3.63 (3H, s, CO_2Me), 4.65 (1H, t, $J = 6.4$ Hz, NH), 5.20 (1H, q, $J = 7.3$ Hz, DHA-4), 5.29–5.44 (9H, m, DHA-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.56 (1H, dd, $J = 15.2$, 7.3 Hz, DHA-5), 5.95 (1H, t, $J = 10.8$ Hz, DHA-7), 6.52 (1H, dd, $J = 15.2$, 10.8 Hz, DHA-6), 6.89 (1H, s, Abieta-14), 6.99 (1H, d, $J = 8.0$ Hz, Abieta-12), 7.19 (1H, d, $J = 8.2$ Hz, Abieta-11); ^{13}C NMR δ 14.5, 18.7, 18.8, 19.1, 20.8, 24.1 (2 carbons), 25.5, 25.8, 25.9, 26.3, 30.0, 30.1, 30.4, 33.7, 36.2, 37.6, 37.6, 38.6, 45.5, 51.8, 51.9, 74.2, 124.1, 124.4, 127.1, 127.2, 128.0, 128.1, 128.2, 128.6, 128.8, 128.9, 131.4, 131.5, 132.3, 134.9, 145.9, 147.3, 156.3, 173.8. **29**: ^{1}H NMR δ 0.92 (3H, s, Abieta-18), 0.97 (3H, t, $J = 7.5$ Hz, DHA-22), 1.21 (3H, s, Abieta-20), 1.22 (6H, d, $J = 7.5$ Hz, Abieta-16, 17), 1.25–1.77 (8H, m, Abieta-1, 2, 3, 6), 1.95 (2H, dd, $J = 14.4$, 7.3 Hz, DHA-3), 2.08 (2H, m, DHA-21), 2.25 (1H, d, $J = 12.9$ Hz, Abieta-5), 2.35 (2H, dd, $J = 7.5$, 5.9 Hz, DHA-2), 2.77–2.95 (11H, m, DHA-9, 12, 15, 18, Abieta-7, 15), 3.00, 3.14 (each 1H, dd, $J = 13.8$, 6.5 Hz, Abieta-19), 3.64 (3H, s, CO_2Me), 4.66 (1H, t, $J = 6.4$ Hz, NH), 5.18 (1H, q, $J = 6.7$ Hz, DHA-4), 5.29–5.44 (9H, m, DHA-8, 10, 11, 13, 14, 16, 17, 19, 20), 5.56 (1H, dd, $J = 15.2$, 6.7 Hz, DHA-5), 5.93 (1H, t, $J = 10.8$ Hz, DHA-7), 6.54 (1H, dd, $J = 15.2$, 10.8 Hz, DHA-6), 6.87 (1H, s, Abieta-14), 6.95 (1H, d, $J = 8.0$ Hz, Abieta-12), 7.15 (1H, d, $J = 8.0$ Hz, Abieta-11); ^{13}C NMR δ 14.7, 19.0 (2 carbons), 19.2, 21.0, 24.4 (2 carbons), 25.7, 25.9, 26.0, 26.1, 26.5, 30.2, 30.3, 30.6, 33.8, 36.4, 37.8, 38.8, 45.5, 52.0, 52.1, 74.4, 124.2, 124.6, 127.3, 127.4, 128.0, 128.1, 128.2, 128.3 (2 carbons), 128.4, 128.8, 129.0, 129.1, 131.5,131.6, 132.5, 135.2, 146.0, 147.5, 156.5, 173.8.

(+)-**6a**. To a solution of carbamate **29** (115 mg, 172 μmol) and triethylamine (120 μL, 0.859 mmol) in benzene (1.7 mL) was added trichlorosilane (43 μL, 0.430 mmol) in benzene (0.86 mL) and the mixture was stirred for 2.5 h. The mixture was treated with a small amount of water (~0.2 mL) and subjected to rough chromatography on silica gel (2 g) with AcOEt followed by chromatography on silica gel (12–20% AcOEt/hexane) to afford isomer with (+) optical density of **6** (37.5 mg, 61%). $[\alpha]_D^{20}$ +7.0 (c 2, $CHCl_3$).

(−)-**6b**. Carbamate **30** (111 mg) was treated by the same procedure as described above to afford (−)-isomer **6** (36 mg). $[\alpha]_D^{20}$ −7.0 (c 2, $CHCl_3$).

3.28. Methyl(4*R*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-{[(2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropyl]oxy}-5,7,10,13,16,19-docosahexaenoate (31)

To a solution of alcohol (−)-**6b** (20 mg, 56 μmol) in dichloromethane (1.0 mL) were added triethylamine (38 μL, 273 μmol), DMAP (38 mg, 0.31 mmol), and (*R*)-methoxy(trifluoromethyl)phenylacetyl chloride (21 μL, 112 μmol) at 0 °C. The solution was stirred at room temperature for 20 min. The reaction mixture was poured into ice and water, and extracted with ether. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (4 g) with 20% AcOEt-hexane to give S-MTPA ester **31** (28.5 mg, 89 %). ^{1}H NMR δ 0.97 (3H, t, $J = 7.5$ Hz, H-22), 1.98–2.12 (4H, m, H-3, 21), 2.37 (2H, H-2), 2.78–2.87 (6H, m, H-12, 15, 18), 2.91 (2H, t, $J = 7.3$ Hz, H-9), 3.53 (3H, br s), 3.68 (3H, s, CO_2Me), 5.30–5.60 (11H, m, H-4, 5, 6, 10, 11, 13, 14, 16, 17, 19, 20), 5.94 (1H, t, $J = 11.0$ Hz, H-8), 6.59 (1H, dd, $J = 14.3$, 11.0 Hz, H-7), 7.34–7.43 (3H, m), 7.45–7.56 (2H, m).

3.29. Methyl(4*S*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4-{[(2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropyl]oxy}-5,7,10,13,16,19-docosahexaenoate (32)

Compound (−)-**6b** (10 mg, 28 μmol) was treated with (*S*)-methoxy(trifluoromethyl)phenylacetyl chloride according to the procedure described above to give *R*-MTPA ester **32** (13 mg, 81%). ^{1}H NMR δ 0.97 (3H, t, $J = 7.5$ Hz, H-22), 1.98–2.12 (4H, m, H-3, 21), 2.25 (2H, H-2), 2.78–2.87 (6H, m, H-12, 15, 18), 2.94 (2H, t, $J = 7.3$ Hz, H-9), 3.54 (3H, br s), 3.66 (3H, s, CO_2Me), 5.30–5.60 (11H, m, H-4, 5, 6, 10, 11, 13, 14, 16, 17, 19, 20), 5.98 (1H, t, $J = 11.3$ Hz, H-8), 6.67 (1H, dd, $J = 14.1$, 11.3 Hz, H-7), 7.34–7.43 (3H, m), 7.45–7.56 (2H, m).

(+)-(**4*S*)-2a** and (−)-(**4*R*)-2b**. Basic hydrolysis of (+)-(4*S*)-**6a** and (−)-(4*R*)-**6b** afforded (+)-(4*S*)-**2a** and (−)-(4*R*)-**2b**, respectively. (4*S*)-**2a**: $[\alpha]_D^{20}$ +5.9 (c 0.7, $CHCl_3$). (4*R*)-**2b**: $[\alpha]_D^{20}$ −5.9 (c 0.8, $CHCl_3$).

3.30. Transfection and transactivation assay

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS). Cells were seeded on 24-well plates at a density of 2×10^4 per well. After 24 h, the cells were transfected with a reporter plasmid containing four copies of MH100 GAL4 binding site (MH100 × 4-TK-Luc),[17] GAL4-hPPARγ chimera expression plasmid (pSG5-GAL-hPPARγ),[3] and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) by the lipofection method as described previously.[18] After 4 h-incubation, the medium was replaced with fresh DMEM containing 5% charcoal-treated FCS (HyClone, UT, USA). The next day, the cells were treated either with the ligand (final concentration, 5 μM) or ethanol vehicle and cultured for 24 h. Cells in each well were harvested with a cell lysis buffer, and the luciferase activity was measured with a luciferase assay kit (Toyo Ink, Inc., Japan). Transactivation measured by the luciferase activity was normalized with the internal control. All experiments were done in triplicate.

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