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SYNTHESIS OF OXIDIZED FATTY ACID DERIVATIVES VIA AN IODOLACTONIZATION REACTION

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Abstract – To study structure-activity relationships of oxidized fatty acids for the activation of peroxisome proliferator-activated receptors (PPARs), which are ligand-dependent transcription factors, we synthesized a series of oxidized fatty acids via iodolactone as a key intermediate.

The peroxisome proliferator-activated receptors (PPARs) α, γ and δ are members of a nuclear receptor superfamily that play important roles in lipid homeostasis and glucose metabolism.[1] PPARs are known to play a role in chronic diseases such as obesity, diabetes, atherosclerosis and cancer.[2] They bind to most long-chain fatty acids and are activated by high micromolar concentrations of these acids.[1] Metabolic conversion of fatty acids in vivo and production of active metabolites could provide an additional level of hormonal regulation of PPARs and lead to the development of novel compounds for use in potential drugs for the treatment of PPAR-related diseases. We have been investigating the development of novel PPARγ ligands and have previously reported that several oxidized derivatives of docosahexaenoic acid (DHA) **1** potently induce gene transcription by binding to PPARγ.[2-4] We have recently succeeded in X-ray crystallographic analysis of the ligand-binding domain of PPARγ, which accommodates unsaturated fatty acids, including our synthetic oxidized DHA derivatives **4** and **5**.[5] The 4-hydroxy-DHA (**4**) is a natural metabolite and the 4-oxo-DHA (**5**) is a putative metabolite.[2] Herein we report the synthesis of a series of oxidized fatty acids, **6** and **7**, derived from eicosapentaenoic acid (EPA) **2**, and **8** and **9** derived from γ-linolenic acid (octadecatrienoic acid, OTE) **3** via the corresponding iodolactone as a common key intermediate.

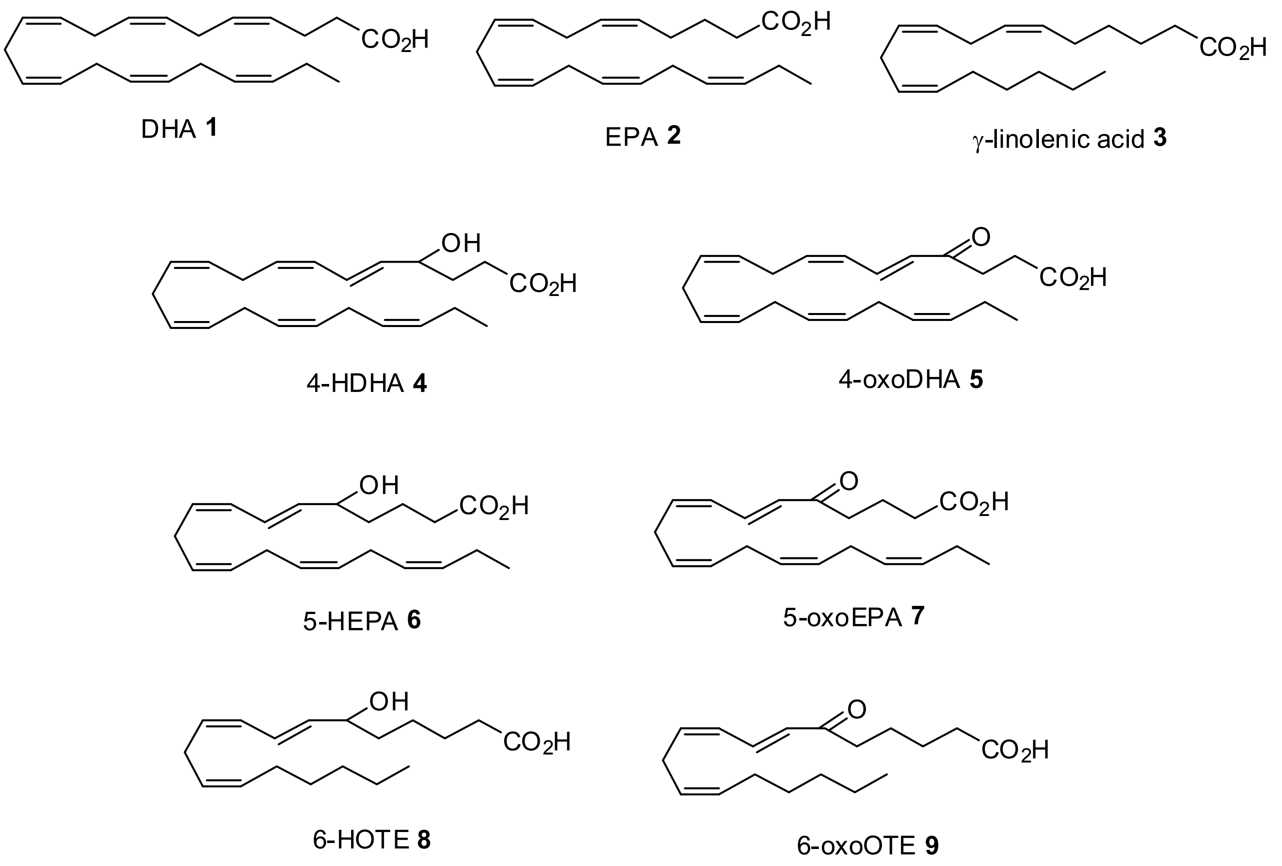
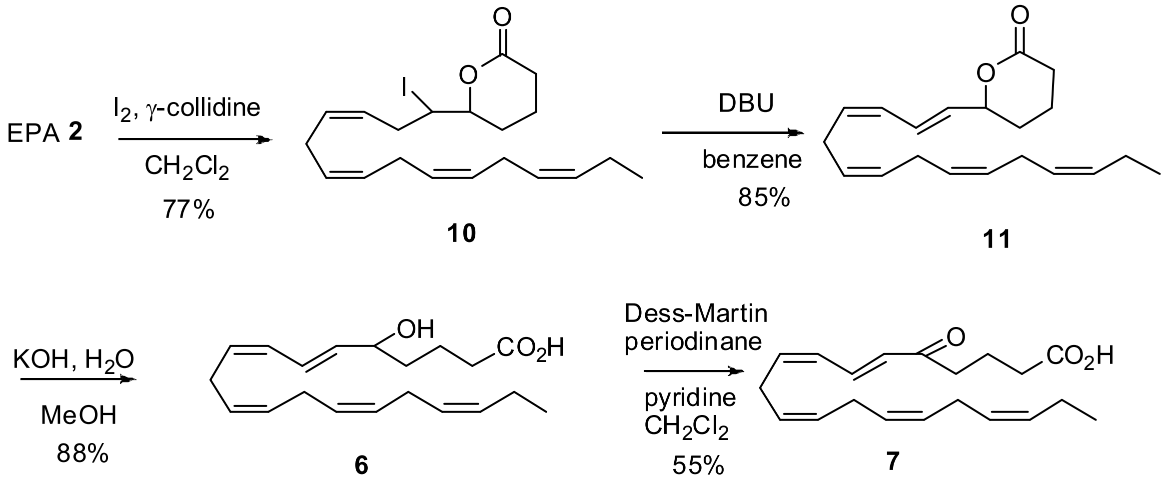


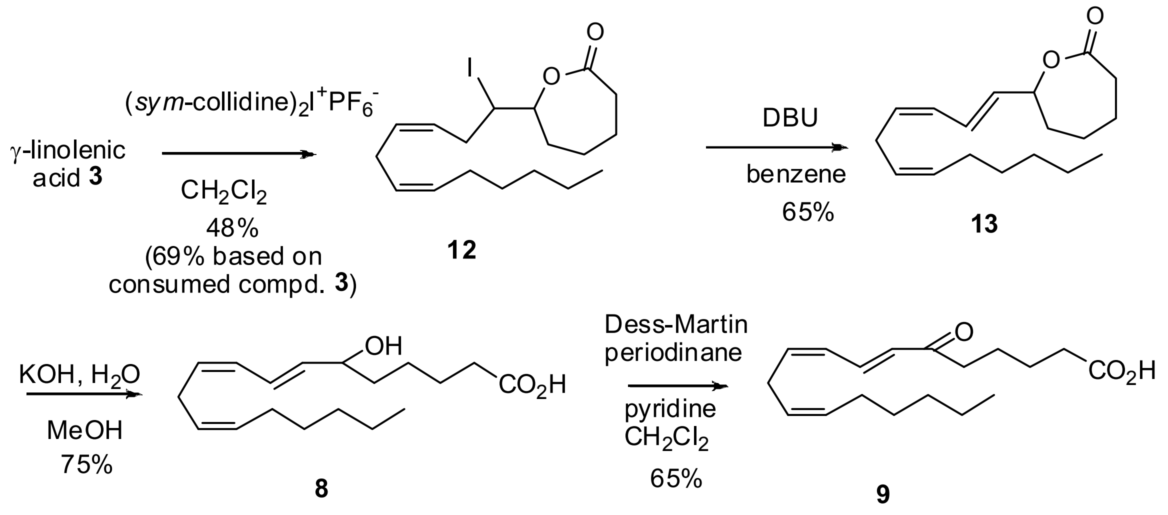
Figure 1 Structures of the fatty acids and the oxidized fatty acids.

The synthesis of oxidized fatty acids via the six-membered iodolactone is shown in Scheme 1. Iodolactonization reaction of fish oil fatty acids containing EPA (**2**) together with DHA (**1**) has been reported, in which iodine was used in the presence of potassium iodide and potassium bicarbonate.[6] We have reported previously that iodolactonization reaction proceeded smoothly by treatment with iodine in the presence of pyridine or γ-collidine.[7] Treatment of EPA (**2**) in CH_2Cl_2 with iodine and γ-collidine led to an iodolactonization reaction that afforded the six-membered iodolactone **10** in 77% yield.[8] Treatment of the lactone **10** with DBU caused elimination of hydrogen iodide to afford the six-membered lactone **11**. This lactone **11** was hydrolyzed with KOH/H_2O/MeOH to afford the desired ring-opened product, 5-hydroxy-eicosapentaenoic acid (5-HEPA) **6**. Compound **6** was treated with the Dess-Martin oxidation reagent to afford 5-oxo-eicosapentaenoic acid (5-oxoEPA) **7**. Both 5-HEPA and 5-oxoEPA are known to be metabolites of EPA.[9,10] The chemical synthesis of 5-HEPA has been reported only in one patent,[11] and this is the first report of the chemical synthesis of 5-oxoEPA.

The same synthetic strategy described for Scheme 1 was applied to OTE (**3**) (Scheme 2). However, treatment of **3** in CH_2Cl_2 with iodine and γ-collidine afforded the seven-membered iodolactone **12** in poor yield (8%). However, we obtained **12** in moderate yield (48%) using bis(*sym*-collidine)iodine(I) hexafluorophosphate.[12] Elimination of hydrogen iodide from **12** afforded the lactone **13**, which was then hydrolyzed with an alkaline solution to give 6-hydroxyoctadecatrienoic acid (6-HOTE) **8**. Oxidation of **8** with the Dess-Martin reagent afforded 6-oxo-octadecatrienoic acid (6-oxoOTE) **9**. While compound **8** is known to be an in vitro metabolite of the parent fatty acid **3**,[13] 6-oxo compound **9** is not. This is the first report of the chemical synthesis of 6-HOTE and 6-oxoOTE.



Scheme 1 Synthesis of oxidized fatty acids via a six-membered iodolactone



Scheme 2 Synthesis of oxidized fatty acids via a seven-membered iodolactone

In conclusion, we synthesized a series of oxidized fatty acids from the natural unsaturated fatty acids **2** and **3** via iodolactones provided by the iodolactonization reaction to the double bond nearest to the terminal carboxylic acid group. Oxidation of the allylic alcohol to the corresponding ketone was achieved using the Dess-Martin reagent. In the future, we will evaluate the biological potency of these synthetic compounds (**6**-**9**) towards PPARα, γ, and δ.

EXPERIMENTAL

General

EPA was a gift from the Maruha Nichiro holdings (Tsukuba, Japan). All the other reagents were purchased from commercial sources and used without further purification. IR spectra were recorded on Simadzu FTIR-8400S spectrophotometer and data are given in cm^{-1}. NMR spectra were recorded on Bruker ARX 400 or AV 300 in $CDCl_3$ solution with TMS as an internal standard and the chemical shifts are given in δ values. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were recorded on JEOL MS700 spectrometer using NBA as positive-ion FAB matrix. UV spectra were recorded on Beckman DU750 spectrophotometer.

6-[(3*Z*,6*Z*,9*Z*,12*Z*)-1-Iodopentadeca-3,6,9,12-tetraen-1-yl]tetrahydro-2*H*-pyran-2-one (10)

To a stirred solution of EPA (**2**) (920 mg, 3.05 mmol) and γ-collidine (1.68 mL, 12.2 mmol) in CH_2Cl_2 (35 mL) was added I_2 (1.54 g, 6.09 mmol) at 0 °C. After being stirred at rt for 1h, the mixture was quenched by addition of 5% aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with 10% aqueous HCl, water and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (40 g, 5-13% EtOAc–hexane) to give **10** (1.00 g, 77%). IR (neat) 3011, 2961, 1747, 1238 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J* = 6.8 Hz, 3H), 1.74-2.14 (m, 6H), 2.34-2.69 (m, 2H), 2.78-2.90 (m, 8H), 3.95 (m, 1H), 4.10 (m, 1H), 5.26-5.45 (m, 7H), 5.50-5.61 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 18.3, 20.6, 25.6, 25.7, 25.9, 28.0, 29.3, 29.6, 34.4, 36.9, 127.0, 127.1, 127.4, 127.8, 128.7, 128.8, 131.4, 132.1, 170.3; HRMS (FAB): Calcd for $C_{20}H_{30}IO_2$ $[M+H]^+$: 429.1290; found: 429.1289.

6-[(3*Z*,6*Z*,9*Z*,12*Z*)-Pentadeca-1,3,6,9,12-pentaen-1-yl]tetrahydro-2*H*-pyran-2-one (11)

A solution of **10** (950 mg, 22.22 mmol) and DBU (417 μL, 2.80 mmol) in benzene (11.1 mL) was stirred at rt for 3h. The reaction mixture was quenched by addition of 10% aqueous HCl and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (50 g, 5% EtOAc–benzene) to give **11** (467 mg, 85%). IR (neat) 3011, 2962, 1742, 1520, 1236 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, *J* = 7.5 Hz, 3H), 1.69 (m, 1H), 1.83-2.13 (m, 5H), 2.49 (m, 1H), 2.62 (m, 1H), 2.78-2.89 (m, 4H), 2.99 (t, *J* = 6.9 Hz, 2H), 4.87 (m, 1H), 5.27-5.57 (m, 7H), 5.70 (dd, *J* = 15.2, 6.2 Hz, 1H), 6.01 (t, *J* = 11.0 Hz, 1H), 6.63 (dd, *J* = 15.2, 11.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 18.3, 20.6, 22.6, 25.6, 25.7, 26.1, 28.4, 29.6, 127.0, 127.28, 127.32, 127.4, 127.7, 128.7, 128.9, 130.7, 132.0, 132.1, 171.1; HRMS (FAB): Calcd for $C_{20}H_{29}O_2$ $[M+H]^+$: 301.2168; found: 301.2177; UV (EtOH): λ_{max} 236 nm.

(8*Z*,11*Z*,14*Z*,17*Z*)-5-Hydroxyeicosa-6,8,11,14,17-pentaenoic Acid (6)

A solution of **11** (417 mg, 1.39 mmol) in 5% KOH/MeOH–H_2O (19:1, 5 mL) was stirred at rt for 2h. The reaction mixture was neutralized with 10% aqueous HCl and then extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (1 g, 50 % EtOAc–hexane) to give **6** (389 mg, 88%). IR (neat) 3012, 2961, 1709, 1535 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.5 Hz, 3H), 1.52-1.83 (m, 4H), 2.05 (quintet, *J* = 7.5 Hz, 2H), 2.39 (t, *J* = 7.3 Hz, 2H), 2.80-2.85 (m, 4H), 2.97 (t, *J* = 6.6 Hz, 2H), 4.20 (q, *J* = 6.3 Hz, 1H), 5.28-5.46 (m, 7H), 5.68 (dd, *J* = 15.1, 6.8 Hz, 1H), 5.99 (t, *J* = 10.9 Hz, 1H), 6.53 (dd, *J* = 15.1, 10.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 20.56, 20.58, 25.55, 25.65, 26.1, 33.7, 36.4, 72.4, 125.8, 127.0, 127.5, 127.8, 127.9, 128.66, 128.70, 128.9, 130.6, 132.1, 135.7, 179.2; HRMS (FAB): Calcd for $C_{20}H_{31}O_3$ $[M+H]^+$: 319.2273; found: 319.2265; UV (EtOH): λ_{max} 237 nm.

(8*Z*,11*Z*,14*Z*,17*Z*)-5-Oxoeicosa-6,8,11,14,17-pentaenoic Acid (7)

To a stirred solution of **6** (150 mg, 471 μmol) and pyridine (133 μL, 1.65 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise Dess–Martin periodinane (15 wt.% in CH_2Cl_2, 1.95 mL, 942 μmol) during 1h at -20°C. The solution was stirred for 1h and the reaction was quenched with hexane/EtOAc/AcOH (50 : 50 : 0.2, 5 mL). The mixture was warmed to rt, stirred for 10 min and passed through a 1:1 mixture of Celite/silica gel. The filtrate was evaporated and the residue was chromatographed on silica gel (4 g, 5-30% EtOAc–hexane) to give **9** (82 mg, 55%). IR (neat) 3013, 2964, 1704, 1274, 1259 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.5 Hz, 3H), 1.98 (quintet, *J* = 7.2 Hz, 2H), 2.08 (quintet, *J* = 7.5 Hz, 2H), 2.42 (t, *J* = 7.2 Hz, 2H), 2.68 (t, *J* = 7.2 Hz, 2H), 2.74 (t, *J* = 7.7 Hz, 2H), 2.86 (t, *J* = 7.7 Hz, 2H), 3.10 (t, *J* = 7.7 Hz, 2H), 5.47-5.53 (m, 6H), 5.88 (dt, *J* = 10.6, 7.7 Hz, 1H), 6.13 (t, *J* = 11.3 Hz, 1H), 6.19 (d, *J* = 15.3 Hz, 1H), 7.56 (dd, *J* = 15.3, 11.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 19.0, 20.6, 25.6, 25.7, 26.7, 33.0, 39.6, 126.3, 126.9, 127.0, 127.4, 128.9, 129.6, 129.7, 132.1, 137.0, 140.1, 178.9, 199.7; HRMS (FAB): Calcd for $C_{20}H_{29}O_3$ $[M+H]^+$: 317.2117; found: 317.2108; UV (EtOH): λ_{max} 283 nm.

7-[(3*Z*,6*Z*)-1-Iodo-3,6-dodecadienyl]-2-oxepanone (12)

To a stirred solution of γ-linolenic acid (**3**) (50 mg, 180 μmol) was added bis(2,4,6-trimethylpyridine)iodine(I) hexafluorophosphate (815 mg, 1.59 mmol) at rt. After being stirred at 40°C for 18h, the mixture was quenched by addition of 10% aqueous HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (3 g, 3-10% EtOAc–hexane) to give **12** (35 mg, 48%) and recovered **3** (15 mg, 30%). IR (neat) 3011, 2930, 1732, 1176 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3H), 1.29-1.33 (m, 6H), 1.56-2.10 (m, 8H), 2.54-2.88 (m, 6H), 4.07 (m, 2H), 5.25–5.58 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 23.0, 23.3, 26.3, 27.7, 28.4, 29.7, 31.9, 34.4, 35.0, 35.5, 37.1, 81.3, 127.1, 127.2, 131.4, 132.1, 174.3; HRMS (FAB): Calcd for $C_{18}H_{30}IO_2$ $[M+H]^+$: 405.1290; found: 405.1277.

7-[(3*Z*,6*Z*)-1,3,6-dodecatrienyl]-2-oxepanone (13)

A solution of **12** (10 mg, 23.7 μmol) and DBU (4 μL, 26 μmol) in benzene (119 μL) was stirred at rt for 3h. The reaction mixture was quenched by addition of 10% aqueous HCl and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (1 g, 5% EtOAc–benzene) to give **13** (4.5 mg, 65%). IR (neat) 3011, 2930, 2858, 1728, 1176 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.23-1.40 (m, 6H), 1.60-1.82 (m, 3H), 1.89-2.10 (m, 5H), 2.58-2.77 (m, 2 H), 2.93 (t, *J* = 7.3 Hz , 2H), 4.82 (dd, *J* = 8.5, 7.2 Hz , 1H), 5.32 (m, 1H) 5.38-5.45 (m, 2H), 5.71 (dd, *J* = 15.1, 6.4 Hz, 1H), 5.96 (t, *J* = 11.0 Hz, 1H), 6.60 (dd, *J* = 15.1, 11.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 23.0, 23.2. 26.5, 27.7, 28.6, 29.7, 31.9, 35.5, 35.8, 80.8, 126.9, 127.2, 127.7, 131.5, 131.7, 132.5, 175.3; HRMS (FAB): Calcd for $C_{18}H_{29}O_2$ $[M+H]^+$: 277.2168; found: 277.2156; UV (EtOH): λ_{max} 236 nm.

(9*Z*,12*Z*)-6-Hydroxy-7,9,12-octadecatrienoic Acid (8)

A solution of **13** (10 mg, 36.2 μmol) in 5% KOH/MeOH–H_2O (19:1, 0.5 mL) was stirred at rt for 2h. The reaction mixture was neutralized with 10% aqueous HCl and then extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (1 g, 50% EtOAc–hexane) to give **8** (7.2 mg, 75%). IR (neat) 3400 (br), 3011, 2930, 1713, 1409 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.0 Hz, 3H), 1.22-1.72 (m, 12H), 2.02-2.12 (m, 2 H). 2.35 (t, *J* = 7.5 Hz, 2H), 2.91 (t, *J* = 7.3 Hz, 2H), 4.17 (q, *J* = 6.4 Hz, 1H), 5.28-5.44 (m, 3H), 5.67 (dd, *J* = 15.2, 6.9 Hz, 1H), 5.97 (t, *J* = 11.0 Hz, 1H), 6.52 (dd, *J* = 15.2, 11.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 23.0, 25.0, 25.3, 26.5, 27.6, 29.7, 31.9, 34.2, 37.2, 73.0, 126.2, 127.4, 128.1, 131.3, 131.4, 136.3, 179.2; HRMS (FAB): Calcd for $C_{18}H_{31}O_3$ $[M+H]^+$: 295.2273; found: 295.2266; UV (EtOH): λ_{max} 237 nm.

(9*Z*,12*Z*)-6-Oxo-7,9,12-octadecatrienoic Acid (9)

To a stirring solution of **8** (62 mg, 211 μmol) and pyridine (52 μL, 633 μmol) in CH_2Cl_2 (1.5 mL) was added Dess–Martin periodinane (15 wt.% in CH_2Cl_2, 878 μL, 424 μmol) at -20°C. The solution was stirred for 30 min and the reaction was quenched with hexane/EtOAc/AcOH (50 : 50 : 0.2, 5 mL). The mixture was warmed to rt, stirred for 10 min and passed through a 1:1 mixture of Celite/silica gel. The fltrate was evaporated and the residue was chromatographed on silica gel (4 g, 5-30% EtOAc–hexane) to give **9** (40 mg, 65%). IR (neat) 2954, 2925, 1681, 1589, 1263 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3H), 1.22-1.40 (m, 6H), 1.64-1.74 (m, 4H), 2.07 (m, 2H), 2.38 (t, *J* = 6.8 Hz, 2H), 2.59 (t, *J* = 7.1 Hz, 2H), 3.05 (t, *J* = 7.4 Hz, 2H), 5.33 (m, 1H), 5.48 (m, 1H), 5.85 (dt, *J* = 10.9, 7.9 Hz, 1H), 6.12 (t, *J* = 10.9 Hz, 1H), 6.17 (d, *J* = 15.5 Hz, 1H), 7.53 (dd, *J* = 15.5, 10.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 23.5, 24.3, 26.7, 27.3, 29.2, 31.5, 33.7, 40.5, 125.7, 126.8, 129.6, 131.9, 137.0, 140.5, 178.5, 200.2; HRMS (FAB): Calcd for $C_{18}H_{29}O_3$ $[M+H]^+$: 293.2117; found: 293.2128; UV (EtOH): λ_{max} 283 nm.

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