The Total Synthesis of 5-Oxo-12(*S*)-hydroxy-6(*E*),8(*Z*),10(*E*),14(*Z*)-eicosatetraenoic Acid and Its 8,9-*trans*-Isomer and Their Identification in Human Platelets

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Received July 8, 1998

The first total synthesis of 5-oxo-12(*S*)-hydroxy-6(*E*),8(*Z*),10(*E*), 14(*Z*)-eicosatetraenoic acid (5-oxo-12-HETE) **6** and its 8-*trans*-isomer **7** is reported. The synthetic 5-oxo-12-HETE **6** and its 8,9-*trans*-isomer **7** were used to identify their formation in mixtures of platelets and neutrophils by transcellular metabolism.

Introduction

We have recently been interested in oxoeicosanoids because these have been shown to possess significant biological activity, for example, 5-oxoeicosatetraenoic acid (5-oxo-ETE),[1] 12-oxo-ETE.[2] In addition, many of the oxoeicosanoids, for example, 12-oxo-ETE and 12-oxoleukotriene B_4 (12-oxo-LTB_4), have been shown to be intermediates in the formation of other bioactive molecules such as 12(*R*)-HETE,[2] 12-HETrE,[2−4] and c-LTB_3.[5] The strategy we have followed in every case involved the total synthesis of the target oxoeicosanoid[1,2,5−7] and its use as a standard for the positive identification of the biological products. The larger amount available through total synthesis has allowed the evaluation of the biological properties of these molecules.

The purpose of this report is to describe the total synthesis of 5-oxo-12-hydroxyeicosatetraenoic acid (5-oxo-12-HETE) (the abbreviation 5-oxo-12-HETE refers to the 5-oxo derivative of 5,12-diHETE) **6** and its *trans*-isomer **7**. These synthetic materials have been used to identify their formation by mixtures of platelets and neutrophils as a result of a transcellular metabolism between neutrophils and platelets. Neutrophils that contain 5-LO make 5-HPETE, 5-oxo-ETE, LTA_4, and LTB_4. Platelets do not contain 5-lipoxygenase (5-LO), but do contain 12-LO, the major product of which is 12-hydroperoxyeicosatetraenoic acid (12-HPETE). Coincubation of platelets and neutrophils results in the formation of two new oxo metabolites, **6** and **7**, in addition to the expected and known metabolites. Scheme 1 shows the two possible biochemical pathways by which **6** is produced by transcellular metabolism. Scheme 2 shows another possible mechanism for the formation of the 8-*trans*-isomer **7**.

Results and Discussion

The oxoeicosanoids of interest have in common a conjugated dienone or trienone functional group, which renders them unstable and difficult to handle. Any *cis*-double bonds present in the conjugated system are highly prone to isomerization. We have found that they can be stored for prolonged periods in solution at −20 °C. The purer the oxo-eicosanoid, the better and longer its chance for survival. We have been able to keep several of these oxoeicosanoids in acetonitrile or ethyl acetate for more than a year without noticeable deterioration. Except for a *cis*−*trans* isomerization, we are not certain of the decomposition products. We only observed a decline in concentration as judged by UV absorbance in 12-oxo-LTB_4. This indicates that the decomposition products have lost their conjugated polyenone chromophore. High-performance liquid chromatography (HPLC) shows no other significant product than the oxo-eicosanoid itself. This is reminiscent of the peptido leukotrienes LTC_4, LTD_4, and LTE_4, which can be kept intact when stored under carefully defined conditions, but will deteriorate rapidly, as judged by UV absorbance, with a drop in concentration, when in use, for example, in a biological experiment lasting a day or more.

For this reason we have, as illustrated in the total synthesis of 5-oxo-12-HETE **6** described here, designed a synthesis in which the carbonyl function is protected through the whole synthesis and deblocking is the last step in the synthesis. There is a price to pay for this strategy. In the deprotection of the carbonyl group at the end of the synthesis, it is impossible to prevent a partial *cis*−*trans* isomerization; however, we have been able to keep it to a minimum. As it turned out, however, this disadvantage has been turned to a benefit. In all the cases we studied, for example, 5-oxo-ETE,[1] 12-oxo-ETE,[2] 12-oxo-LTB_4[5], and the present case, the isolation of the *cis*-oxo-eicosanoid from biological sources invariably is accompanied by substantial amounts of the *trans*-isomers, and we needed the synthetic authentic material to identify them and study their biological properties.

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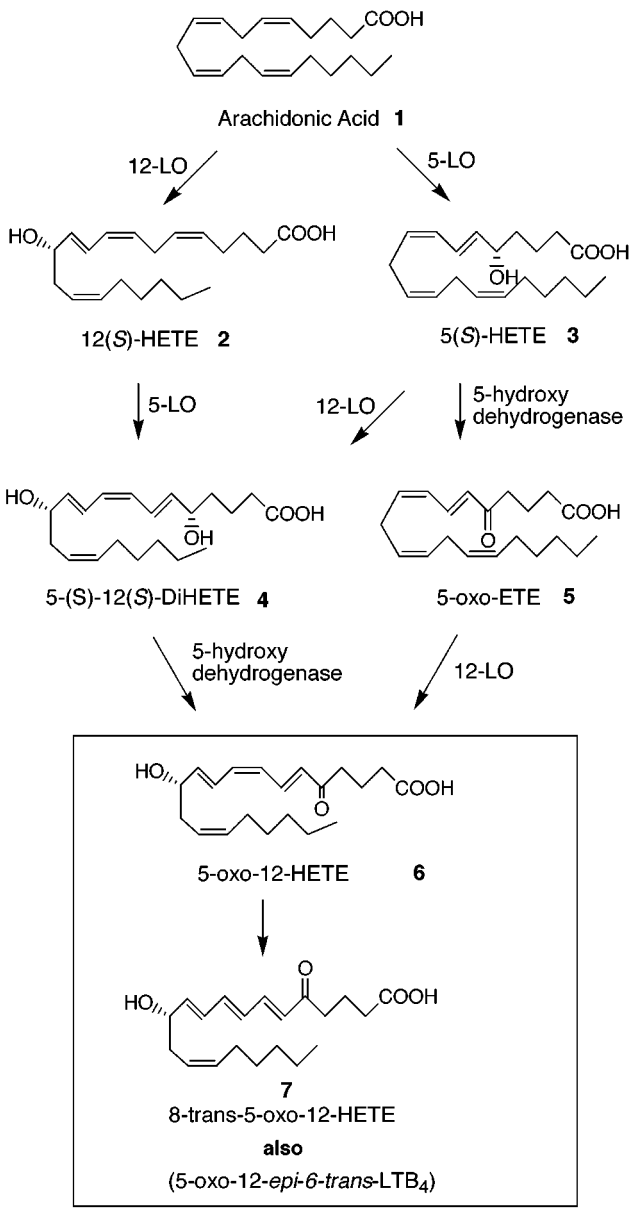
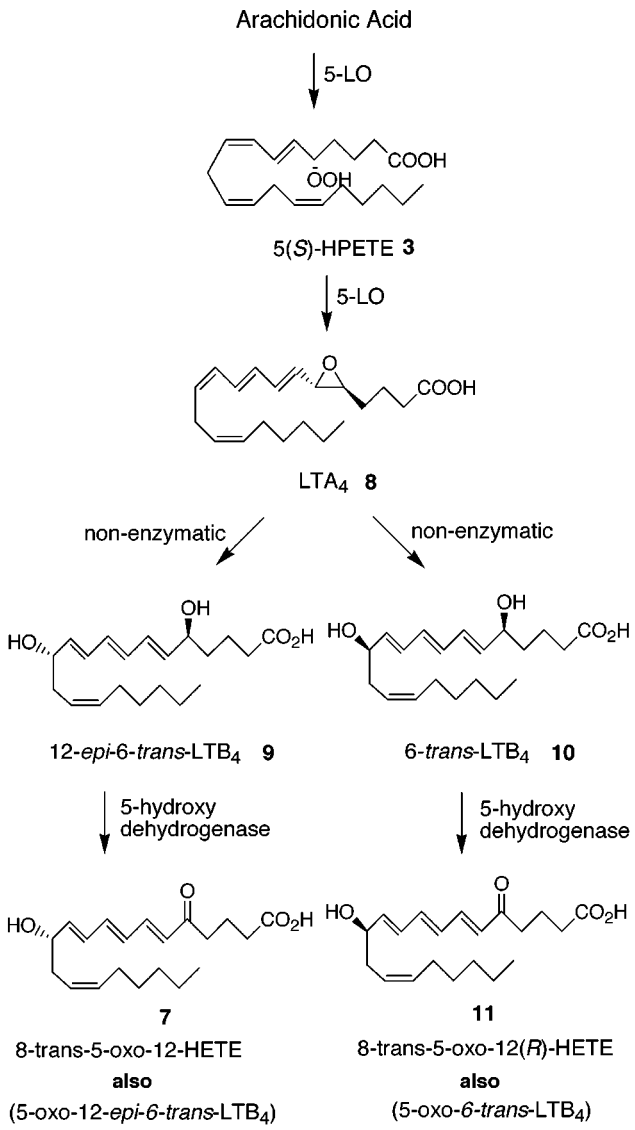
10.1021/jo9813282 CCC: $15.00
Published on Web 11/10/1998

Scheme 1



Scheme 2



Another related thought worth discussing here is the following. Do these isomerizations and, in the present case, the formation of the all-*trans* compound **7**, occur in vivo? We do not know whether any enzyme is performing such isomerization in vivo, and in view of the very facile acid-catalyzed isomerization, we cannot be sure whether the isomerization, if it is happening in vivo, is enzymatic or chemical. In addition, it is possible that some of the all-*trans*-compound formed is due to an artifact of the isolation procedure. In one instance, however, in 12-oxo-ETE and its 8,9-*trans*-isomer isolated from aplysia nervous tissue, we have shown by incubating the two synthetic isomers with aplysia neural homogenates that the formation of the *trans*-compound is not an artifact of the experiment.[2] These experiments not only suggest that the *trans*-isomer in this case is formed in vivo, but may be suggestive of the involvement of an enzyme.

The ease with which the chemical *cis*−*trans* isomerization occurs varies somewhat with the different structures, and the biochemical precedent described above for 8,9-*trans*-12-oxo-ETE may not be a general phenomenon. Independent verification of this isomerization, or the lack thereof in vivo, in 5-oxo-12-HETE **6** and 8-*trans*-5-oxo-12-HETE **7**, remains to be done. *Cis*−*trans* isomerization of oxoeicosanoids is likely to affect the biological activity of these compounds. For example, 8-*trans*-5-oxo-ETE is less potent than 5-oxo-ETE in activating neutrophils.[8]

The strategy we used to construct the 5-oxo-12-HETE is shown in Scheme 3. We elected to use two synthons **12** and **13** and construct the 8,9-*cis* double bond last. Synthon **12** was prepared as shown in Scheme 4 from the dithioacetonide derivative **15**, itself prepared from D-arabinose in four steps. Derivative **15**, which we used extensively in the past,[9,10] is the equivalent of a 1,4-dialdehyde **14** in which the two aldehyde poles can be used separately and independently. As shown in Scheme 4, we reworked the chemistry completely and to our advantage compared with that used in the synthesis of 12-*epi*-LTB$_4$.[9] The use of periodic acid, as we described recently,[11] saves steps and, more importantly, simplifies

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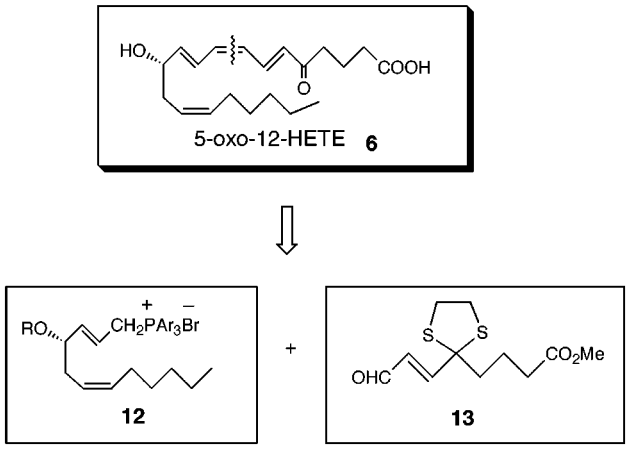
Scheme 3

Synthetic Strategy

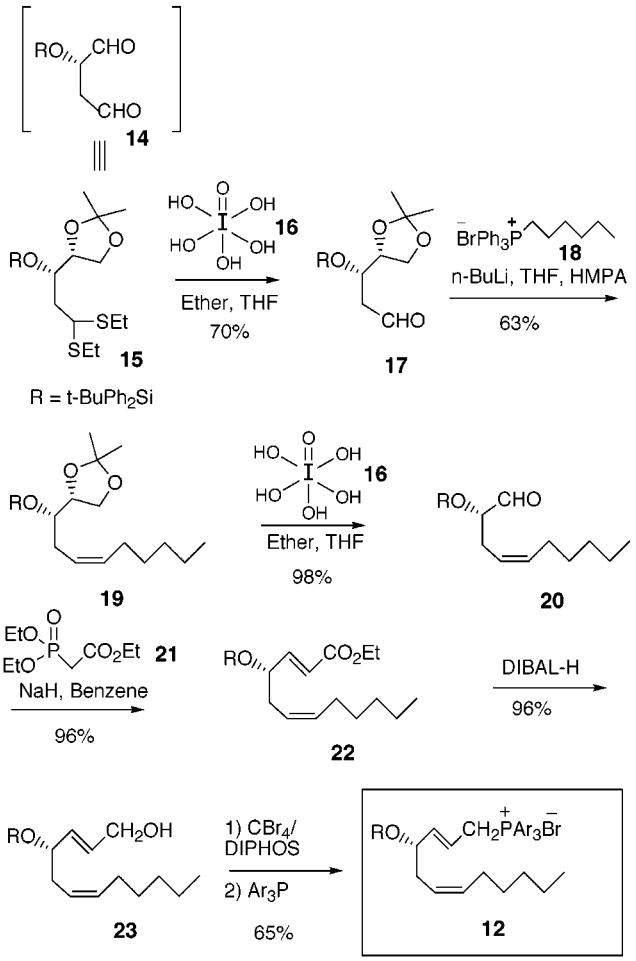


the workup of the reaction. Typically the substrate is dissolved in tetrahydrofuran (THF)/ether; the periodic acid, dissolved in THF, is added to the reaction, stirred for a short time, and filtered through Celite. The solvents are evaporated, and in many instances, the products are pure and can be used as such for the next step. As can be seen, we used the periodic acid twice in the preparation of synthon **12** to generate aldehydes **17** and **20**. We previously have performed transformation of **19** to **20** in two steps.[9] The first is the aqueous acid hydrolysis of the acetonide, and the second, the lead tetraacetate cleavage of the diol followed by chromatographic purification. Although the yields of the two steps were good, the one-step procedure described here is clearly superior because it saves time and avoids unnecessary chromatographic purifications.

Synthon **13**[1] was prepared from aldehyde **24** as shown. We previously used the stabilized Wittig reagent **25**.[9,12−15] For example, in our synthesis of LTA_4 we used either 1 or 2 equiv of **25** in benzene reflux to prepare a monoene aldehyde or diene aldehyde. Although the use of 2 equiv was convenient to prepare diene aldehyde, contamination with some triene aldehyde and monoene aldehyde occurred and the product was difficult to purify. One equivalent, however, can be used conveniently and efficiently for the formation of the monoadduct. In some cases yields were very good,[12,15] in others the yields were moderate,[9,14] but the starting material could be recovered and recycled. Because aldehyde **24** is more reactive than the α,β-unsaturated aldehyde **13**, we anticipated that the Wittig reaction of **24** and **25** could be performed selectively to give **13** with minimum diene aldehyde contamination.

The condensation of synthons **12** and **13** afforded the two Wittig products **26** and **27** as shown in Scheme 5. It is evident that there is an unusually high amount of the *trans*-product **27**. This is not unlike an observation we made in the synthesis of LTB_4[9,14] and is the subject of a separate investigation. The two triene products, **26** and

Scheme 4



27, do not separate at this stage, but are easily separated by column chromatography after fluoride deprotection of the silyl group to give pure **28** and **29**. After the ester hydrolysis of **28**, the thiolane deprotection of the *cis*-product **30** is effected as shown in Scheme 5, and the pure *cis*- and *trans*-products **6** and **7** are obtained in 41% and 5% yield, respectively. Separately, the *trans*-dithiolate derivative **31** was converted to **7** in 71% yield. The structural proof of the two end products **6** and **7** is based on proton NMR and mass spectral data. Electrospray MS analysis revealed an M-1 ion at *m*/*z* 333 for both 5-oxo-12-HETE **6** and 8-*trans*-5-oxo-12-HETE **7**.

The objective of the synthesis described herein was to determine whether platelets can synthesize and/or metabolize 5-oxoeicosanoids. Using synthetic **6** and **7** we have shown that these compounds are metabolites of endogenous arachidonic acid in mixtures of platelets and neutrophils.[16] 5-Oxo-12-HETE **6** is formed by the combined actions of 5-lipoxygenase (neutrophils), 12-lipoxygenase (platelets), and 5-hydroxyeicosanoid dehydrogenase (platelets and neutrophils) as shown in Scheme 1. 8-*trans*-5-Oxo-12-HETE **7** could be formed either by isomerization of the 8-*cis* double bond of **6** as described earlier or by a nonenzymatic degradation of LTA_4.

Examination of Scheme 2 reveals that there is a different biochemical pathway potentially leading to 8-*trans*-5-oxo-12-HETE (5-oxo-12-*epi*-6-*trans*-LTB_4) **7**. 5,12-

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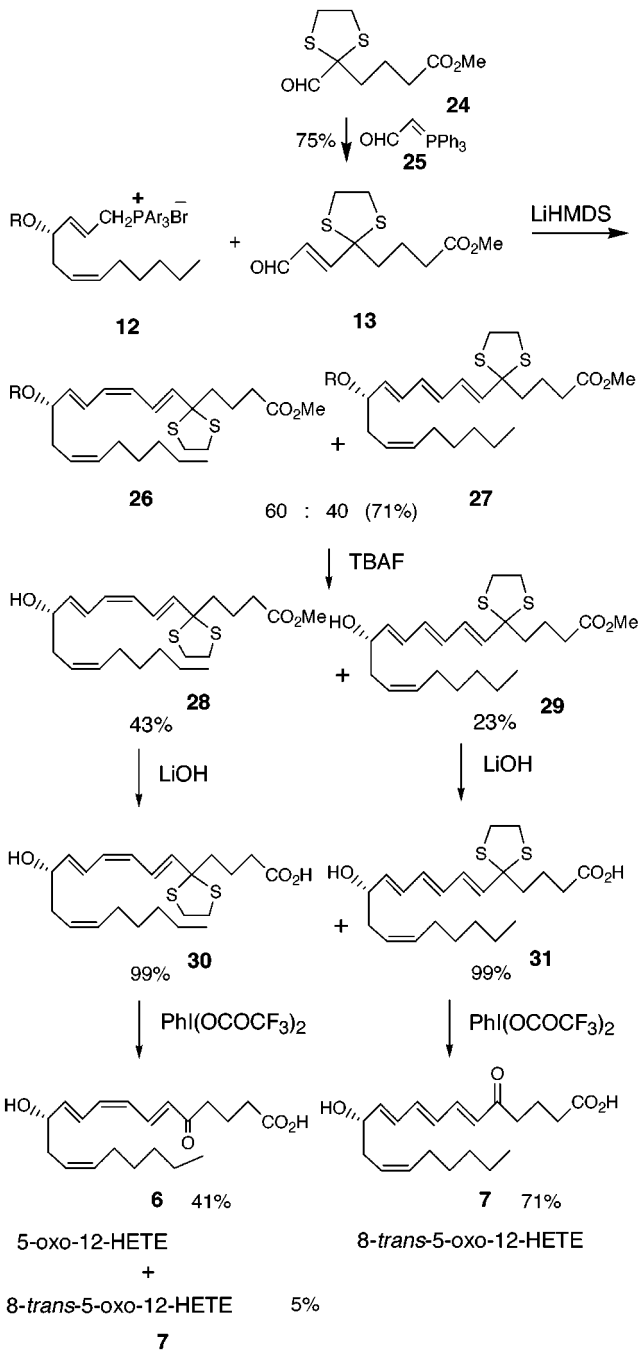
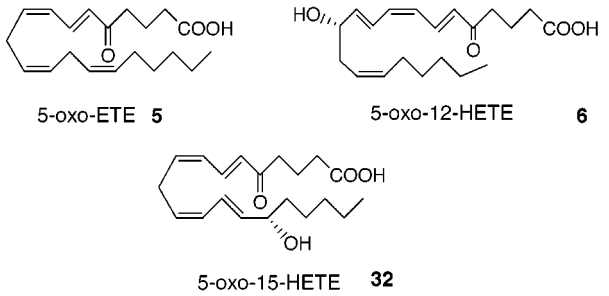
Scheme 5



Dihydroxy products **9** and **10** are found in cells as a result of LTA_4 hydrolysis and often have been used qualitatively and quantitatively as an index of LTA_4 production in cells, because LTA_4 is very unstable and cannot be measured intact in vivo. On oxidation by a dehydrogenase, these dihydroxy derivatives **9** and **10** will yield 5-oxo compounds **7** and **11**. Derivative **7** is identical with the *trans*-product isolated in vivo and shown in Scheme 1. In addition, **11** is enantiomeric with **7** and can only be assessed and identified by special studies. Hence it is not impossible that 5-oxo products **7** and **11**, derived from LTA_4 hydrolysis as shown in Scheme 2, contribute to the amount of **7** derived by the biosynthetic sequence shown in Scheme 1. We have not yet performed the special studies necessary to completely clarify the biochemical origin of the *trans*-oxo derivative **7**.

Scheme 6



Somewhat to our surprise, 5-oxo-12-HETE and its 8-*trans*-isomer have virtually no biological activity on neutrophils. In contrast, another 5-oxo-HETE, namely 5-oxo-15-HETE, displays substantial activity on both neutrophils[17] and eosinophils.[18] 5-Oxo-12-HETE was more than 10,000 times less active than 5-oxo-ETE in mobilizing calcium in neutrophils,[16] and preliminary experiments indicate that, in contrast to 5-oxo-ETE and 5-oxo-15-HETE, it does not induce neutrophil migration at concentrations as high as 10 μM.[19] Comparison of the structures of 5-oxo-ETE (**5**), 5-oxo-15-HETE (**32**), and 5-oxo-12-HETE (**6**) (Scheme 6) suggests that a saturated carbon at position 10 is essential for biological activity mediated by the activation of the 5-oxo-ETE receptor. The region from carbon 11 and beyond of 5-oxo-15-HETE is considerably different from 5-oxo-ETE, yet a substantial degree of biological activity is retained, presumably because of the presence of the methylene group at carbon 10. In contrast, the conjugated triene in **6**, which would be flat in conformation, confers rigidity at carbons 10 and 11, resulting in substantial distortion from the flexibility at carbon 10 of **5** and **32**, which is presumably required for interaction with the putative 5-oxo-ETE receptor.

Experimental Section

Reagents and Methods. Unless stated otherwise, all reagents and chemicals were obtained from commercial sources and used without further purification. ^{1}H NMR spectra were recorded on a 360-MHz spectrometer with tetramethylsilane as an internal standard *J* values are given in Hertz. All reactions were carried out under an inert (nitrogen or argon) atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise noted. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

Methyl-5,5-(dimethylenedithio)-6(*E*)-8-oxo-7-octenoate (13). A solution of aldehyde **24** (2.8 g, 11.95 mmol) and triphenylphosphoraniledene acetaldehyde **25** (3.7 g) in benzene (30 mL) was refluxed for 18 h. (The amount of the triphenylphosphoraniledene acetaldehyde was added in 3 stages: 1.3 g at the start of the reaction; 1.2 g after 2 h, and a further 1.2 g after another 2 h.) The reaction mixture was cooled to room temperature, filtered through Celite, and washed with ethyl acetate; the combined filtrate was concentrated under reduced pressure to afford the crude product, which was purified by flash column chromatography using 20% ethyl acetate in hexane. The less polar fractions gave 706 mg of starting material **24**, and from the more polar fractions pure product **13** (1.75 g, 56%) was obtained (yield based on consumed starting material 75%). ^{1}H NMR ($CDCl_3$) δ 1.80 (m, 2 H), 2.14

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(m, 2 H), 2.32 (t, J = 7.2 Hz, 2 H), 3.21 (m, 2 H), 3.32 (m, 2 H), 3.61 (s, 3 H), 6.25 (dd, J = 15.2 and 7.7 Hz, 1 H), 6.78 (d, J = 15.2, 1 H), 9.58 (d, J = 7.8, 1 H): ^{13}C NMR ($CDCl_3$) δ 22.3, 33.2, 39.0 (2 × C), 39.5, 51.2, 68.5, 128.5, 158.9, 172.8, 193.1; HREIMS calcd ($C_{11}H_{16}O_3S_2$, M^+) 260.0541, obsd 260.0545.

2-Deoxy-3-*O*-(*tert*-butyldiphenylsilyl)-1,2-bis-*O*-(isopropylidene)-D-*erythro*-pentose (17). To a 0 °C cooled solution of dithioacetal **15** (10.4 g, 20 mmol) in anhydrous ethyl ether (200 mL) and THF (80 mL) was added a solution of periodic acid **16** (5.472 g, 24 mmol) in dry THF (20 mL) for 3 min under argon. The ice bath was removed, and the reaction mixture was stirred at room temperature for 5 min. A white solid was precipitated. Potassium carbonate (4 g) was added and stirred for 1 min. The reaction mixture was then diluted with ether (100 mL), filtered through Celite/florisil into a flask containing saturated aqueous Na_2SO_3 solution (60 mL), and washed with ether (50 mL). The combined filtrate was washed with aqueous Na_2SO_3 (2 × 60 mL), water (4 × 200 mL), and brine (100 mL); dried over anhydrous Na_2SO_4; and filtered and concentrated under reduced pressure to afford the crude product (8.4 g) which was purified by flash column chromatography with hexane/ethyl acetate (8:2) to afford the pure aldehyde **17**, 7.0 g in 84% yield. ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9 H), 1.28 (s, 3 H), 1.29 (s, 3 H), 2.58 (m, 2 H), 3.63 (m, 1 H), 3.95 (m, 1 H), 4.13 (m, 2 H), 7.44 (m, 6 H), 7.70 (m, 4 H), 9.6 (t, J = 2.3 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 19.3, 25.1, 26.2, 26.9, 48.0, 67.2, 70.1, 76.7, 78.6, 109.5, 127.7 (2 × C), 127.8 (2 × C), 130.0, 130.1, 133.0, 135.5, 135.8 (2 × C), 135.9, (2 × C).

3(*S*)-[(*tert*-Butyldiphenylsilyl)oxy]-1,2-bis-*O*-(isopropylidene)-5(*Z*)-undecene-1,2(*R*)-diol (19). To a stirred suspension of the hexyltriphenylphosphonium bromide **18** (23.485 g, 55 mmol) in THF (550 mL) was added dropwise sodium hexamethyldisilazide (1 M, 51 mL, 51 mmol) under argon. The reaction mixture turned orange, after being stirred for 30 min at room temperature. It was then cooled to −78 °C, and anhydrous hydroxymethylphosphoramide (HMPA) (115 mL) was added. The reaction mixture was stirred for 2 min, and then aldehyde **17** (7 g, 16.99 mmol) in THF (215 mL) was added dropwise at −78 °C to the resulting red solution. The reaction mixture was stirred for 2 h at −78 °C, and then allowed to warm slowly to 0 °C for 2 h. It was then quenched by the addition of aqueous saturated ammonium chloride solution (150 mL). The THF layer was separated, the solvent evaporated at reduced pressure, and the residue extracted with diethyl ether (3 × 150 mL). The combined extracts were washed with cold water (3 × 25 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product, which first was purified quickly by flash column chromatography using 5% ethyl acetate in hexane and then purified further by column chromatography using 1% ether in hexane to give pure product **19** (6.75 g, 83%). ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 7.1 Hz, 3 H), 1.11 (s, 9 H), 1.2−1.3 (m, 6 H), 1.35 (m, 3 H), 1.38 (m, 3 H), 1.85 (m, 2 H), 2.05−2.15 (m, 1 H), 2.23−2.35 (m, 1 H), 3.85 (t, J = 7.4 Hz, 1 H), 3.96 (m, 1 H), 4.12 (q, J = 5.5 Hz, 1 H), 5.39 (m, 2 H), 7.4 (m, 6 H), 7.75 (m, 4 H). ^{13}C NMR ($CDCl_3$) δ 14.0, 19.4, 22.5, 25.3, 26.5, 27.0, 27.2, 29.1, 31.5, 32.1, 66.0, 73.2, 77.83, 108.7, 124.1, 127.4 (2 × C), 127.5 (2 × C), 129.6, 129.7, 132.4, 133.7, 134.1, 136.0 (2 × C), 136.1 (2 × C).

2(*S*)-[(*tert*-Butyldiphenylsilyl)oxy]-4(*Z*)-decenal (20). To a solution of acetonide **19** (960 mg, 1.9 mmol) in anhydrous ethyl ether (20 mL), a solution of periodic acid (910 mg, 4 mmol) in dry THF (5 mL) was slowly added dropwise under argon. The reaction mixture was stirred overnight at room temperature. A white solid was precipitated. The reaction mixture was diluted with ether (50 mL), filtered through Celite, and washed with ether (50 mL). The combined filtrate was washed with aqueous Na_2SO_3 (40 mL), water (2 × 40 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product which was purified by flash column chromatography with hexane/ethyl acetate (19:1) to afford the pure aldehyde **20** (810 mg, 98%). ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 7.1 Hz, 3 H), 1.09 (s, 9 H), 1.26 (m, 6 H), 1.87 (m, 2 H), 2.3 (m, 1 H), 2.4 (m, 1 H), 4.03 (s, 1 H), 5.35 (m, 1 H), 5.5 (m, 1 H), 7.38 (m, 6 H), 7.65 (m, 4 H), 9.53 (d, J = 1.6 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 14.0, 14.5, 19.3, 22.5, 26.9, 27.3, 29.1, 31.1, 31.5,77.8, 122.6, 127.7 (2 × C), 127.8 (2 × C), 129.9 (2 × C), 130.0 (2 × C), 133.0, 133.1, 133.4, 135.8 (2 × C), 203.3.

Ethyl-4(*S*)-[(*tert*-butyldiphenylsilyl)oxy]-2(*E*),6(*Z*)-dodecadienoate (22). Sodium hydride (60% dispersion) (223 mg, 5.58 mmol) and dry hexane (3 mL) were placed in a 50-mL round-bottom flask under argon. After the mixture was stirred for 3 min, hexane was removed, and dry benzene (20 mL) was added. To this stirred suspension was added ethyl phosphonoacetate **21** (1.3 mL, 6.53 mmol), stirring continued at room temperature for 1 h, and then aldehyde **20** (810 mg, 1.98 mmol) in benzene (10 mL) was added at room temperature. The reaction mixture was stirred at room temperature for 3 h and then quenched with saturated aqueous ammonium chloride solution (20 mL), the benzene layer was separated, and the aqueous layer was extracted with ether (2 × 50 mL). The combined benzene and ether extracts were dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated at reduced pressure to give the crude product which was purified by column chromatography over silica gel using hexane/ethyl acetate (19:1) to yield the pure product **22**, 908 mg as a colorless oil in 96% yield. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 7.2 Hz, 3 H), 1.08 (s, 9 H), 1.2−1.3 (m, 6 H), 1.28 (t, J = 7.1 Hz, 3 H), 1.77 (m, 2 H), 2.1−2.3 (m, 2 H), 4.17 (q, J = 7.1 Hz, 2 H), 4.34 (m, 1 H), 5.21 (m, 1 H), 5.39 (m, 1 H), 5.94 (d, J = 15.6 Hz, 1 H), 7.72 (dd, J = 15.5, 4.9 Hz, 1 H), 7.3−7.4 (m, 6 H), 7.6−7.7 (m, 4 H). ^{13}C NMR ($CDCl_3$) δ 14.3, 14.5, 19.5, 22.7, 27.2, 27.4, 29.3, 31.6, 35.4, 60.4, 72.6, 120.5, 123.5 127.8 (4 × C), 129.9 (2 × C), 133.2, 133.6, 134.1, 136.0 (4 × C), 150.0, 166.7.

4(*S*)-[(*tert*-Butyldiphenylsilyl)oxy]-2(*E*),6(*Z*)-dodecadienyl-1-ol (23). To a −60 °C cooled solution of silyl ester **22** (520 mg, 1.05 mmol) in anhydrous dichloromethane (12 mL) was slowly added dropwise a 1 M solution (in dichloromethane) of DIBAL-H (2.5 mL, 2.5 mmol) under argon. The reaction mixture was stirred for 15 min at −60 °C and then allowed to warm to −20 °C for 1 h. The reaction mixture was then quenched with water, neutralized with 10% HCl, and extracted with dichloromethane (2 × 25 mL). The combined extracts were washed with water (3 × 25 mL) and brine (1 × 25 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product which was purified by flash column chromatography with hexane/ethyl acetate (19:1) to give the pure alcohol **23** (438 mg, 96%). ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 7.3 Hz, 3 H), 1.04 (s, 9 H), 1.2−1.3 (m, 6 H), 1.85 (m, 2 H), 2.18 (m, 1 H), 2.27 (m, 1 H), 3.90 (d, J = 5.1 Hz, 2 H), 4.19 (s, 1 H), 5.29 (m, 1 H), 5.38 (m, 1 H), 5.45 (m, 1 H), 5.55 (dd, J = 14.5, 7.0 Hz, 1 H), 7.37 (m, 6 H), 7.65 (m, 4 H). ^{13}C NMR ($CDCl_3$) δ 14.3, 19.5, 22.8, 27.2, 27.5, 29.4, 31.7, 36.1, 63.2, 73.8, 124.8, 127.6 (2 × C), 127.7 (2 × C), 129.5, 129.7 (2 × C), 129.8 (2 × C), 132.3, 134.3, 134.4, 134.7, 136.2 (2 × C), 136.3 (2 × C).

4(*S*)-[(*tert*-Butyldiphenylsilyl)oxy]-2(*E*),6(*Z*)-dodecadien-1-yl-tris(3-methoxyphenyl)phosphonium bromide (12). To a cooled (0−5 °C), stirred solution of alcohol **23** (210 mg, 0.492 mmol) and 1,2-bis(diphenylphosphino)ethane (DIPHOS) (215 mg, 0.541 mmol) in dry CH_2Cl_2 (8 mL) carbon tetrabromide (244 mg, 0.738 mmol) in dry CH_2Cl_2 (8 mL) was slowly added under argon. The reaction mixture was stirred for 30 min at 0 °C and then diluted with CH_2Cl_2 (8 mL), and the resulting solution of the bromide was filtered through a small pad of silica/Celite and washed with CH_2Cl_2 (20 mL). The combined filtrate was dried over anhydrous Na_2SO_4 and filtered, and the solution was used as such in the next step. To the above-mentioned solution of labile bromide was added tris(3-methoxy)phenylphosphine (909 mg, 2.5 mmol), and the mixture was stirred at room temperature for 18 h under argon atmosphere. The solvent was evaporated in vacuo. The phosphonium salt was purified by flash column chromatography with methylene chloride/methanol (19:1) to afford the phosphonium salt **12** (248 mg, 65%) as a colorless fluffy solid. ^{1}H NMR ($CDCl_3$) δ 0.81 (t, J = 7.2 Hz, 3 H), 0.83 (s, 9 H), 1.1−1.2 (m, 6 H), 1.8−2.0 (m, 4 H), 3.86 (m, 9 H), 4.19 (s, 1

H), 4.71 (m, 1 H), 4.94 (m, 2 H), 5.15 (m, 1 H), 5.51 (m, 1 H), 6.24 (m, 1 H), 7.3−7.7 (m, 22 H).

Wittig Condensation of 12 and 13: Methyl-5,5-(dimethylenedithio)-12(*S*)-[(*tert*-butyldiphenylsilyl)oxy]-6(*E*),8(*Z*),10(*E*),14(*Z*)-icosatetraenoate (26) and Methyl-5,5-(dimethylenedithio)-12(*S*)-[(*tert*-butyldiphenylsilyl)oxy]-6(*E*),8(*E*),10(*E*),14(*Z*)-icosatetraenoate (27). To a cooled (−98 °C), stirred solution of the phosphonium salt **12** (248 mg, 0.291 mmol) in THF (4 mL) sodium hexamethyldisilazide (1 M, 233 μL, 0.233 mmol) was added dropwise under argon. After stirring for 15 min at −98 °C, HMPA (1.2 mL) was added, the reaction mixture was stirred for 2 min, and then aldehyde **13** (70 mg, 0.267 mmol) in THF (2 mL) was added dropwise at −98 °C to the resulting red solution. The reaction mixture was stirred for 15 min at −98 °C, and then allowed to warm slowly to 0 °C for 1 h. It was then quenched by the addition of aqueous saturated ammonium chloride solution (1 mL) and extracted with diethyl ether (3 × 50 mL). The combined extracts were washed with cold water (3 × 25 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product which was purified by flash column chromatography using 5% ethyl acetate in hexane to give a mixture of *cis*- and *trans*-isomers **26** and **27** (152 mg, 71%). This mixture was difficult to separate as such, so it was desilylated and the individual isomers were separated.

Methyl-5,5-(dimethylenedithio)-12(*S*)-hydroxy-6(*E*),8(*Z*),10(*E*),14(*Z*)-eicosa-tetraenoate (28) and Methyl-5,5-(dimethylenedithio)-12(*S*)-hydroxy-6(*E*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoate (29). A solution of the preceding mixture of **26** and **27** (37 mg, 0.05 mmol) in THF (4.5 mL) was treated with tetra-*n*-butylammonium fluoride (180 μL of 1 M solution in THF, 0.18 mmol), and the mixture was stirred at room temperature overnight. Then the reaction mixture was diluted with saturated aqueous ammonium chloride solution (2 mL) and extracted with ether (3 × 20 mL). The combined organic layers were washed with water (3 × 10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash chromatography (elution with ethyl acetate/hexane 1:9) to give the less polar product **28** (9 mg, 43%) as an oil. ^{1}H NMR (C_6D_6) δ 0.93 (t, J = 7.0 Hz, 3 H), 1.31 (m, 6 H), 1.9 (m, 2 H), 2.04 (m, 4 H), 2.13 (m, 2 H), 2.35 (m, 2 H), 2.78 (s, 4 H), 3.36 (s, 3 H), 4.14 (m, 1 H, C_{12}−H), 5.55 (m, 2 H, C_{14} and C_{15}−H), 5.76 (dd, J = 15.1, 5.8 Hz, 1 H, C_{11}−H), 5.98 (d, J = 14.7 Hz, 1 H, C_6−H), 6.09 (m, 2 H, C_8 and C_9−H), 7.10 (dd, J = 14.6, 10.1 Hz, 1 H, C_{10}−H), 7.28 (dd, J = 14.8, 10.2 Hz, 1 H, C_7−H). ^{13}C NMR (C_6D_6) δ 14.7, 23.3, 23.6, 28.1, 30.1, 32.2, 34.1, 36.3, 39.6, (2 × C), 42.3, 51.5, 71.4, 72.3, 125.0, 125.7, 125.8, 129.2, 130.2, 133.4, 138.4, 139.7, 173.5. Of the more polar *trans*-isomer **29**, 5.2 mg was obtained in 24% yield. ^{1}H NMR (C_6D_6) δ 0.94 (t, J = 7.0 Hz, 3 H), 1.44 (m, 8 H), 1.94 (m, 2 H), 2.10 (m, 6 H), 2.35 (m, 2 H), 2.80 (s, 4 H), 3.35 (s, 3 H), 5.57 (m, 2 H), 5.68 (dd, J = 14.5, 6.0 Hz, 2 H), 5.91 (d, J = 14.7 Hz, 1 H), 6.24 (m, 2 H), 6.29 (m, 1 H), 6.68 (m, 1 H). ^{13}C NMR (C_6D_6) δ 14.6, 23.3, 23.8, 28.1, 30.1, 30.5, 32.2, 34.2, 36.3, 39.5 (2 × C), 42.4, 51.3, 71.5, 72.4, 125.5, 129.9, 130.6, 132.4, 133.3, 133.5, 137.2, 139.0, 173.2.

5,5-(Dimethylenedithio)-12(*S*)-hydroxy-6(*E*),8(*Z*),10(*E*),14(*Z*)-eicosatetraenoic Acid (30). To a solution of the dithio compound **28** (29 mg, 0.0683 mmol) in THF (6 mL) and 1 M LiOH (1.6 mL) was added a solution of 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy, free radical (100 μg) in methanol (50 μL), and the solution was stirred at room temperature for 18 h. The solvent THF was removed by a stream of argon, then water (4 mL) and 5% KH_2PO_4 (80 mL) were added and extracted with ethyl acetate (2 × 40 mL). The combined ethyl acetate extracts were washed with cold water (4 × 20 mL) and brine (1 × 10 mL), dried over anhydrous Na_2SO_4 and filtered; the solvent evaporated under reduced pressure to afford 28 mg of the dithio acid **30** in nearly quantitative yield, which was pure enough to use as such in the next step. ^{1}H NMR (CD_3COCD_3) δ 0.88 (t, J = 6.8 Hz, 3 H), 1.34.40 (m, 8 H), 1.77 (m, 2 H), 2.16 (m, 2 H), 2.29 (m, 2 H), 2.36 (t, J = 7.3 Hz, 2 H), 3.32 (m, 4 H), 4.21 (dd, J = 12.2, 6.0 Hz 1 H), 5.46 (m, 2 H), 5.81 (dd, J = 15.1, 6.0 Hz, 1 H), 5.90 (d, J = 14.7 Hz, 1 H), 6.04 (m, 2 H), 6.78 (dd, J = 14.8, 10.2 Hz, 1 H), 6.9 (dd, J = 14.7, 10.2 Hz, 1 H). ^{13}C NMR (CD_3COCD_3) δ 14.8, 23.7, 24.4, 28.5, 30.5, 32.7, 34.5, 36.9, 40.3, (2 × C), 42.9, 71.8, 72.7, 125.4, 125.9, 126.9, 129.2, 130.9, 132.9, 139.9, 140.1, 174.9.

5,5-(Dimethylenedithio)-12(*S*)-hydroxy-6(*E*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoic Acid (31). To a solution of the dithio compound **29** (12.5 mg, 0.0295 mmol) in THF (3 mL) and 1 M LiOH (0.8 mL) was added a solution of 4-hydroxy-TEMPO (50 μg) in methanol (50 μL), and the reaction mixture was stirred at room temperature for 18 h. The solvent THF was removed by a stream of argon, then water (2 mL) and 5% KH_2PO_4 (40 mL) were added and extracted with ethyl acetate (3 × 25 mL). The combined ethyl acetate extracts were washed with cold water (4 × 20 mL) and brine (1 × 10 mL), dried over anhydrous Na_2SO_4, and filtered; the solvent was evaporated under reduced pressure to afford 12 mg of the dithio acid **31** in nearly quantitative yield, which was pure enough to use as such in the next step. ^{1}H NMR (CD_3COCD_3) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.29 (m, 8 H), 1.75 (m, 2 H), 2.14 (m, 2 H), 2.28 (m, 2 H), 2.35 (t, J = 7.4 Hz, 2 H), 3.30 (m, 4 H), 3.91 (br s, 1 H, OH), 4.15 (m, 1 H), 5.43 (m, 2 H), 5.77 (m, 1 H), 5.88 (d, J = 14.6 Hz, 1 H), 6.28 (m, 3 H), 6.44 (m, 1 H). ^{13}C NMR (CD_3COCD_3) δ 14.9, 23.8, 24.4, 28.6, 30.6, 32.8, 34.6, 37.0, 40.2 (2 × C), 42.9, 71.8, 72.8, 127.0, 130.4, 130.7, 132.6, 132.9, 134.3, 139.1, 139.3, 174.8.

5-Oxo-12(*S*)-hydroxy-6(*E*),8(*Z*),10(*E*),14(*Z*)-eicosatetraenoic Acid (5-Oxo-12-HETE) 6. To a solution of dithio acid **30** (14 mg, 0.0341 mmol) in methanol/H_2O (9:1, 5 mL) was added a solution of 4-hydroxy-TEMPO (100 μg) in methanol (50 μL) followed by [bis(trifluoroacetoxy)iodo]benzene (22 mg, 0.051 mmol) under argon, and the reaction mixture was stirred at room temperature for 2 min. The reaction mixture was quenched with cold water (45 mL) and extracted with ethyl acetate (2 × 25 mL). The combined ethyl acetate extracts were washed with cold water (8 × 25 mL) and brine (1 × 25 mL), dried over anhydrous Na_2SO_4, and filtered. 4-Hydroxy-TEMPO (50 μg) in ethyl acetate (50 μL) was added, and the product in ethyl acetate was kept at −20 °C. Analytical separation on Waters Nova-pak C-18, 3.9 × 150 mm column, using MeOH/H_2O/AcOH (70:30:0.1%) as the mobile phase and a flow rate of 0.5 mL/min, showed that 8,9-*cis*- and 8,9-*trans*-isomers **6** and **7** were obtained in the ratio of 87:13, t_r 8,9-*cis* 24.43 min, t_r 8,9-*trans* 18.62 min. The individual isomers were separated by reversed-phase HPLC (Sperisorb S10W, C-18, 10 × 250 mm column) using MeOH/H_2O/AcOH (70:30:0.1%) as the mobile phase and a flow rate of 6 mL/min to give pure *cis*- and *trans*-isomers. To the eluant (180 mL) containing the pure *cis*-isomer **6** was added triethylamine (600 μL) to neutralize the acetic acid. The mixture was stirred at room temperature for 1 min and then methanol was evaporated under high vacuum to concentrate the solution to approximately 60 mL. A 5% aqueous solution of KH_2PO_4 (80 mL) was added, extracted with ethyl acetate (2 × 40 mL), and washed with water (4 × 25 mL). The pure product (4.657 mg) was obtained in 40.5% yield. ^{1}H NMR (CD_3COCD_3) δ 0.866 (t, J = 6.8 Hz, 3 H), 1.29 (m, 8 H), 1.88 (t, J = 7.4 Hz, 2 H), 2.34 (m, 4 H), 2.72 (t, J = 7.2 Hz, 2 H), 4.27 (m, 1 H), 5.46 (m, 2 H), 6.03 (dd, J = 15.0, 6.6 Hz, 1 H), 6.16 (t, J = 11.3 Hz, 1 H), 6.22 (d, J = 15.4 Hz, 1 H), 6.44 (t, J = 11.2 Hz, 1 H), 6.97 (dd, J = 14.7, 11.9 Hz, 1 H), 7.36 (dd, J = 15.1, 11.9 Hz, 1 H). Electrospray MS calcd for $C_{20}H_{29}O_4$ (M-1) 333, obsd 333. Similarly, to the eluant (120 mL) containing the pure *trans*-isomer **7** was added triethylamine (400 μL) to neutralize the acetic acid. The mixture was stirred at room temperature for 1 min, and then methanol was evaporated under high vacuum to concentrate the solution to approximately 40 mL. A 5% aqueous solution of KH_2PO_4 (40 mL) was added, extracted with ethyl acetate (2 × 25 mL), and washed with water (4 × 10 mL). The pure product **7** (560 μg) was obtained in 5% yield. ^{1}H NMR (CD_3COCD_3) δ 0.93 (t, J = 7.4 Hz, 3 H), 1.34 (m, 8 H), 1.85 (m, 2 H), 2.29−2.36 (m, 4 H), 2.67 (t, J = 7.3 Hz, 2 H), 4.05 (br s, 1 H, OH), 4.21 (m, 1 H), 5.45 (m, 2 H), 6.03 (dd, J = 15.4, 5.7 Hz, 1 H), 6.20 (d, J = 15.5 Hz, 1 H), 6.42 (m, 2 H), 6.75 (dd, J = 14.7, 11.0 Hz, 1 H), 7.28 (dd, J = 14.4, 11.1 Hz, 1 H). Electrospray MS calcd for $C_{20}H_{29}O_4$ (M-1) 333, obsd 333.

5-Oxo-12(*S*)-hydroxy-6(*E*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoic acid (7): (8,9-*trans*-5-oxo-12-HETE) (7). To a solution of dithio acid **31** (8 mg, 0.0195 mmol) in methanol/H_2O (9:1, 3 mL) was added a solution of 4-hydroxy-TEMPO (50 μg) in methanol (50 μL) followed by [bis(trifluoroacetoxy)-iodo]benzene (12 mg, 0.028 mmol) under argon and the reaction mixture was stirred at room temperature for 3 min. The reaction mixture was quenched with cold water (25 mL) and extracted with ethyl acetate (2 × 30 mL). The combined ethyl acetate extracts were washed with cold water (8 × 10 mL) and brine (1 × 10 mL), dried over anhydrous Na_2SO_4, and filtered. 4-Hydroxy-TEMPO (50 μg) in ethyl acetate (50 μL) was added and the product in ethyl acetate was kept at −20 °C. The product was purified by reversed-phase HPLC (Sperisorb S10W, C-18, 10 × 250 mm column) using MeOH/H_2O/AcOH (70:30:0.1%) as the mobile phase and a flow rate of 6 mL/min to give a pure *trans*-isomer **7**. Triethylamine (500 μL) was added to the eluant (75 mL) containing the pure *trans*-isomer **7** to neutralize the acetic acid. The mixture was stirred at room temperature for 1 min and then methanol was evaporated under high vacuum to concentrate the solution to approximately 25 mL. A 5% aqueous solution of KH_2PO_4 (40 mL) was added, extracted with ethyl acetate (2 × 30 mL), and washed with water (4 × 15 mL). The pure product 4.639 mg was obtained in 71% yield. ^{1}H NMR (CD_3COCD_3) δ 0.93 (t, J = 7.4 Hz, 3 H), 1.34 (m, 8 H), 1.85 (m, 2 H), 2.29−2.36 (m, 4 H), 2.67 (t, J = 7.3 Hz, 2 H), 4.05 (br s, 1 H, OH), 4.21 (m, 1 H), 5.45 (m, 2 H), 6.03 (dd, J = 15.4, 5.7 Hz, 1 H), 6.20 (d, J = 15.5 Hz, 1 H), 6.42 (m, 2 H), 6.75 (dd, J = 14.7, 11.0 Hz, 1 H), 7.28 (dd, J = 14.4, 11.1 Hz, 1 H).

Acknowledgment. We acknowledge the National Institutes of Health for support under Grant DK-44730 (J. R.), the National Science Foundation for an AMX-360 NMR instrument (Grant CHE-9013145), the Medical Research Council of Canada for support under Grant MT-6254 (W. S. P.), and for support from the Quebec Heart and Stroke Foundation (W. S. P.).

Supporting Information Available: Copies of ^{1}H NMR and ^{13}C NMR spectral data for new compounds described herein (23 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9813282