Total Synthesis of a Potent Proinflammatory 5-Oxo-ETE and Its 6,7-Dihydro Biotransformation Product

Subhash P. Khanapure, Xiao-Xin Shi, William S. Powell,† and Joshua Rokach*

Claude Pepper Institute and Department of Chemistry, Florida Institute of Technology, 150 West University Boulevard, Melbourne, Florida 32901 and Meakins-Christie Laboratories, McGill University, 3626 St-Urbain Street, Montreal, Quebec H2X 2P2, Canada

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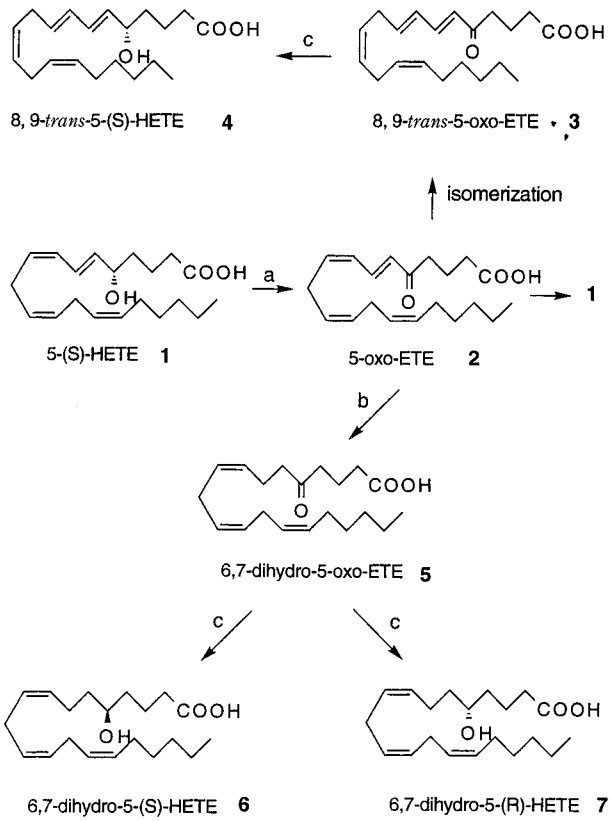
The first total synthesis of a potent inflammatory mediator 5-oxo-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic acid (5-oxo-ETE) **2** and its biotransformation product 6,7-dihydro-5-oxo-ETE **5** is reported. A convergent synthesis for the unstable title compounds is accomplished via two synthons, dithiolane aldehyde **13** and bisdienyl phosphonium bromide **19**. The synthetic 5-oxo-ETE **2** and its 8,9-trans isomer **3** were used to unequivocally confirm the structure of the biologically derived mediators. In addition, using synthetic 6,7-dihydro-5-oxo-ETE **5** we have been able to identify in neutrophils the formation of 6,7-dihydro-5-oxo-ETE **5**.

5-Oxo-ETE **2**, a novel arachidonate metabolite, is a potent chemotactic agent for human neutrophils. Although it is less potent than LTB_4 in this regard, it is about 100 times more potent than its precursor 5(*S*)-HETE **1**.[1] It has recently been shown that 5-oxo-ETE is also a potent chemotactic agent for human eosinophils which, unlike neutrophils, respond only weakly to LTB_4.[2,3] Of a variety of lipid mediators tested, 5-oxo-ETE was the most active in inducing chemotaxis of these cells (5-oxo-ETE > platelet-activating factor (PAF) ≈ 5-oxo-15-hydroxy-ETE ≫ LTB_4 ≈ LTD_4), with concentrations as low as 1 nM producing significant effects.[2] 5-Oxo-ETE also induces a variety of other responses in neutrophils and eosinophils including calcium mobilization,[1,4] degranulation,[4] superoxide production,[5] actin polymerization,[5,6] and the expression of cellular adhesion molecules.[6] There is substantial evidence that the stimulatory effects of 5-oxo-ETE on these cells are due to its interaction with its own specific G protein-linked receptor.[1,4,5,7]

Scheme 1 shows some of the proposed biochemical transformations which we plan to study. 5-Oxo-ETE is a product of the 5-LO pathway and is formed by a dehydrogenase present in microsomal fractions of human neutrophils (Scheme 1).[8] This enzyme catalyzes the oxidation of 5(*S*)-HETE **1**, a major product of the 5-LO pathway in these cells, to 5-oxo-ETE. This 5-hydroxy eicosanoid dehydrogenase is highly specific for the naturally occurring 5(*S*)-stereoisomer of 5-HETE **1**, since a variety of related positional and stereoisomers are not metabolized to a significant extent.[8] It also requires that the double bond in the 6-position of 5-hydroxy eicosanoids should be in the trans rather than the cis configuration. The high degree of specificity and the low K_m (200 nM) of this enzyme suggest that this reaction may be of physiological importance. On the basis of our experience in handling 5-oxo-ETE, the isomerization of 5-oxo-ETE **2** to the 8,9-*trans*-5-oxo-ETE **3** shown in the Scheme 1 most likely occurs in vivo and ex vivo by a simple chemical isomerization.

Scheme 1[a]



[a] (a) 5-OH-dehydrogenase, (b) Δ^6 reductase; (c) 5-keto reductase.

(1) † McGill University.

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It is also possible that 5-oxo-ETE **2** could be further metabolized by neutrophils or other cells to the 6,7-dihydro metabolites **5**−**7** (Scheme 1). We have previously shown that neutrophils convert the 6-trans isomer of LTB_4 to similar dihydro products following initial oxidation of the 5-hydroxyl group.[8,9] In these studies the position of the reduced double bond was not clear, but recent mass spectrometric evidence suggests it is in the 6,7-position,[10] which we have confirmed by another approach. To test the hypothesis that neutrophils can also convert 5-oxo-ETE to 6,7-dihydro metabolites, 6,7-dihydro-5-oxo-ETE **5** has also been synthesized.

Our present interest in 5-oxo-ETE stems from our long-standing involvement in the area of oxoeicosanoids[11−15] as well as from recent evidence suggesting that the inhibitory effects of 5-lipoxygenase inhibitors on antigen-induced pulmonary eosinophil infiltration cannot be explained only on the basis of inhibition of leukotriene synthesis.[16,17] These finding raise the possibility that 5-oxo-ETE could be important in the physiological regulation of eosinophil accumulation in tissues such as the lung, and thus may be an important mediator in diseases such as asthma.

Because of the importance we attribute to 5-oxo-ETE, we started a synthetic program to provide larger amounts of this mediator in order to study its formation and transformation in biochemical systems and to evaluate in more detail its biological profile. Until now the source for this conjugated oxo-eicosanoid was the biologically derived material or the chemical oxidation of the very expensive 5(*S*)-HETE.

Results and Discussion

Leukotrienes which contain conjugated triene[18−29] and lipoxins[30−34] which contain conjugated tetraene are unstable compounds, and their syntheses require careful planning and execution. Oxoeicosanoids[11−14] present an additional degree of difficulty. For example, 5-oxo-ETE is unstable because of the presence of a conjugated dienone in the molecule. In addition, one of the double bonds in this system is a cis double bond which has a high propensity for trans isomerization. For these reasons, we elected early in the synthesis of 5-oxo-ETE to have the carbonyl function protected. This would reduce the instability of the conjugated diene system to an acceptable level. In addition, later in the synthesis we needed to hydrolyze the ester function to acid and that would have been impossible on a practical level without a protected carbonyl function.

The main focus of our synthetic plan was to prepare a masked carbonyl aldehyde synthon, such as **13**, and an intermediate, such as bisdienyl phosphonium salt **19**, and combine these components in a convergent and stereoselective manner, as shown in Scheme 2, to obtain the necessary precursor **20**. The bisdienyl phosphonium salt **19** was intended to serve not only as a precursor for 5-oxo-ETE **2**, but also to prepare the 6,7-dihydro-5-oxo-ETE **5**.

The key aldehyde synthon **13** was prepared in three steps from commercially available 2-carbethoxy-1,3-dithiolane **8**. DIBAL-H reduction of dithiolane **8** at −78 °C to −60 °C for 2 h gave the corresponding aldehyde **9**, which was purified by Kugelrohr distillation to yield pure 2-formyl-1,3-dithiolane **9** in 92% yield. The alkylation of dithiolane aldehyde **9** with methyl bromobutyrate **10** using NaH as a base in a mixture of DMSO/benzene (2:3) at room-temperature overnight gave aldehyde **11** in 59% yield after purification by column chromatography. The Wittig coupling of **11** with triphenylphosphoranylidene acetaldehyde **12** afforded the desired dithio aldehyde **13** in 56% yield (75% yield based on consumed starting material). The bis acetylene derivative **16** was prepared from butyn-1-ol **14**.[35] Alkylation of butyn-1-ol **14** with bromooct-3-yne **15** gave bis acetylene **16** in 83% yield. Hydrogenation of **16** using Lindlar catalyst afforded dienyl alcohol **17** in 60% yield. Bromination of the alcohol **17** with CBr_4 and triphenylphosphine provided the bromide **18** which, after purification, was immediately treated with Ph_3P to generate the phosphonium salt **19**, purified by flash column chromatography to give pure **19** in 92% yield. The three-step preparation of **19** from **16**, as just described, is a much improved synthesis over the related five-step one we reported a few years ago[35] for the chloride version of **19**. Wittig reaction of the aldehyde **13** with the phosphonium salt **19**, using LHMDS as base, produced **20** in 83% yield. The methyl ester **20** was treated with 1 M LiOH in THF/H_2O to give, after purification by column chromatography, the dithio

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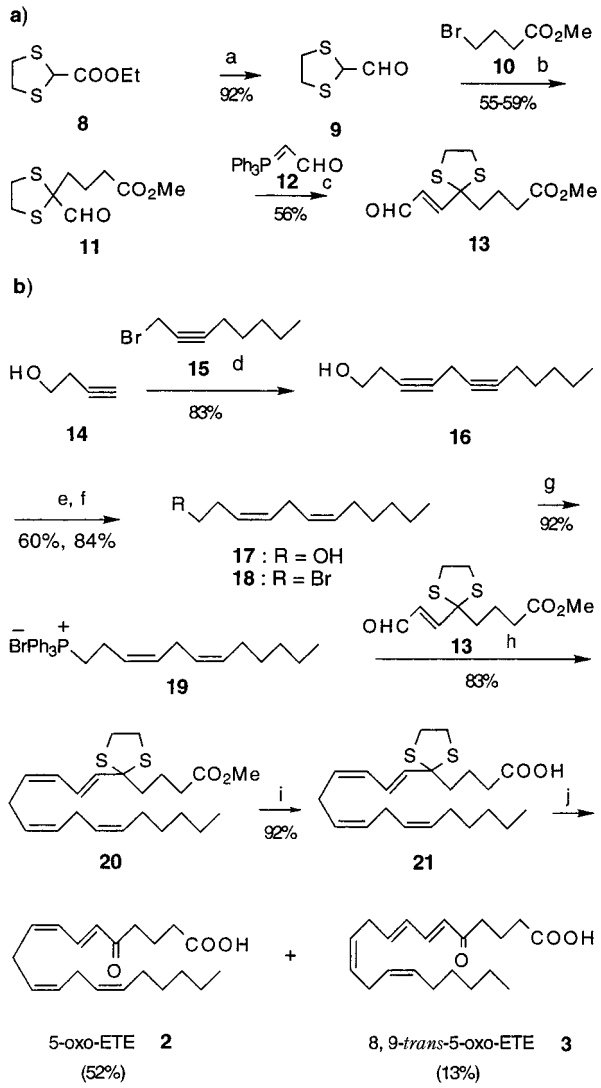
Scheme 2[a]

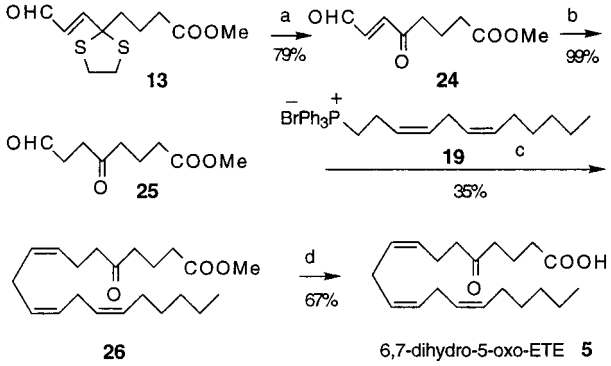


[a] (a) DIBAL-H, −78 to −60 °C, 2 h; (b) NaH, room temperature; (c) reflux in benzene; (d) ethylmagnesium bromide/CuBr; (e) H_2/Lindlar; (f) Ph_3P, CBr_4 0−5 °C 30 min; (g) bromide **18** in dry acetonitrile, Ph_3P, reflux 36 h; (h) $LiN(SiMe_3)_2$, HMPA, −98 to 0 °C; (i) 1 M LiOH, THF/H_2O, room temperature 24 h; (j) $PhI(OCOCF_3)_2$, −10 to −8 °C, 2 min.

derivative of 5-oxo-ETE **21** in 98% yield. Finally, deprotection of the dithio acid **21** with [bis(trifluoroacetoxy)-iodo]benzene[13,36] gave the 5-oxo-ETE **2** in 52% yield. The 8,9-trans isomer **3** was also isolated in 13% yield after purification by RP HPLC and then NP HPLC (for details, see Experimental Section). We tried our recently developed method for deblocking dithio derivatives to corresponding carbonyl compounds using periodic acid in anhydrous ether/THF.[37] This method works extremely well for most of the dithio derivatives under nonaqueous conditions, with ease of handling and simplicity of the workup procedure. Using this procedure to deblock **21**, we observed considerable double bond isomerization to give a mixture of **2** and **3** in the ratio of 55:45.

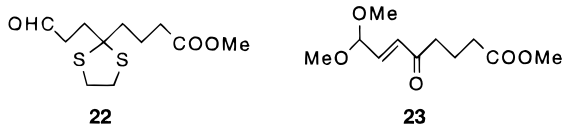
The synthesis of 6,7-dihydro-5-oxo-ETE **5** is outlined in Scheme 3. Our original intention, however, was to

Scheme 3[a]



[a] (a) $PhI(OCOCF_3)_2$, room temperature, 5 min; also periodic acid, THF/ether, room temperature 8 min (51%); (b) H_2/Pd, C; (c) $LiN(SiMe_3)_2$, HMPA, −78 °C to 0 °C; (d) 1 M LiOH, THF/H_2O, room temperature 24 h.

prepare **22** by hydrogenating **13** and continue the synthesis with the dithiolane protecting group and remove it later in the synthesis. All attempts at hydrogenation of **13** using different catalysts such as Pd/C, Ni-boride, tris(triphenylphosphone)chlororhodium, or Wilkinson's catalyst were unsuccessful, resulting in recovery of the starting material. Presumably the sulfur atoms in the molecule were responsible for poisoning the catalyst. We decided, therefore, to deblock the dithio group in **13** and then perform the hydrogenation. To our surprise, deprotection of dithio aldehyde **13** using [bis(trifluoroacetoxy)-



iodo]benzene in 9:1 MeOH:H_2O resulted mainly in the formation of the dimethyl acetal derivative **23** (yield 71%) of the desired **24**. This result is interesting in its own right since it can allow manipulation of the carbonyl function in the presence of an aldehyde. For the purpose of the synthesis at hand, we repeated the deprotection using [bis(trifluoroacetoxy)iodo]benzene in 9:1 CH_3CN:H_2O and obtained 79% of the desired keto aldehyde **24**. We also used the periodic acid procedure for the deprotection and obtained the keto aldehyde **24** in 51% yield after purification by column chromatography (Scheme 3). The hydrogenation of **24**, using Pd/C as catalyst, was clean and gave the reduced keto aldehyde **25** in nearly quantitative yield. We then performed the Wittig coupling of **25** and the bisdienyl phosphonium bromide **19**. The more reactive aldehyde reacted preferentially with the ylide in the presence of the less reactive ketone in a disappointing 35% yield. Hydrolysis of **26** gave 6,7-dihydro-5-oxo-ETE **5** in 67% yield.

It is worth mentioning that before we settled for the convenient preparation of aldehyde **11** in a two-step procedure from commercially available **8** as shown in Scheme 2, we attempted to alkylate the more popular dithiane carboxaldehyde **28** to prepare aldehyde **29** as shown in Scheme 4. In contrast to the dithiolane alkylation reaction, as shown in Scheme 2, the reaction between **28** and **10** is complicated and led to mixtures of products. The major product of this reaction was the O-alkylation product **31**, which during purification on silica gel was converted back to dithiane carboxaldehyde

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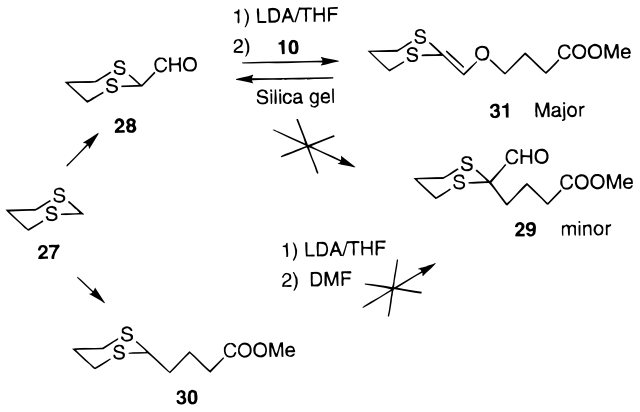
Scheme 4



28. It is not yet clear what causes a different course of reaction in the C-alkylation in the thiolane case versus the O-alkylation in the dithiane one.

The synthetic 5-oxo-ETE **2** and its 8,9-trans isomer **3** have been used to unequivocally confirm the structure of the biologically derived mediators. In addition, using synthetic 6,7-dihydro-5-oxo-ETE **5**, we have been able to identify in neutrophils the formation of 6,7-dihydro-5-oxo-ETE **5**, establishing the bioreduction at 6,7-double bond as a metabolic transformation in these cells. Preliminary studies suggest that the 6,7-dihydro derivative is approximately 100 times less potent than 5-oxo-ETE in stimulating cytosolic calcium mobilization in human neutrophils. It is therefore possible that reduction of this double bond contributes to the biological inactivation of 5-oxo-ETE.

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 and *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.

2-Formyl-1,3-dithiolane (9). To a cooled (−78 °C) stirred solution of dithiolane ester **8** (11.9 g, 66.7 mmol) in anhydrous dichloromethane (100 mL) was added DIBAL-H (1.0 M in toluene, 67 mL) (67 mmol) dropwise under argon. The reaction mixture was stirred at −78 °C to −60 °C for 1 h and then quenched with methanol (10 mL). 2 N HCl was added to adjust the pH to approximately 4, and the resulting mixture was extracted with dichloromethane (2 × 100 mL). The combined organic layers were washed with water (2 × 25 mL) and brine (1 × 25 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give the crude product **9**. It was purified by Kugelrohr distillation to give 8.2 g (92%) of the pure dithiolane aldehyde **9** as a colorless thick oil. ^{1}H NMR ($CDCl_3$) δ 3.26 (s, 4 H), 4.57 (d, J = 5.9 Hz, 1 H) 8.91 (d, J = 5.9 Hz, 1 H): ($CDCl_3$) δ 38.9, 55.5, 188.1.

Methyl 5,5-(Dimethylenedithio)-6-oxohexanoate (11). To a 0 °C cooled and stirred suspension of sodium hydride (350 mg, 60%) in anhydrous DMSO (50 mL) and anhydrous benzene (100 mL) was added dropwise a solution of aldehyde **9** (1 g, 7.45 mmol) in DMSO (10 mL) under argon atmosphere. The reaction mixture was stirred at 0 °C for 1.5 h, and methyl bromobutyrate **10** (1.48 g, 8.2 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 5 h and then at room temperature for 20 h. The reaction mixture was then diluted with water (50 mL) and extracted with ether (3 × 150 mL). The combined extracts were washed with water (3 × 100 mL), dried over Na_2SO_4, and filtered, and the solvent was evaporated at reduced pressure to give the crude product, which was purified by flash column chromatography over silica gel using ethyl acetate/hexane (1:9) as an eluant to give the pure product **11** (1.02 g) in 55% yield. ^{1}H NMR ($CDCl_3$) δ 1.8 (m, 2 H), 1.97 (m, 2 H), 2.32 (t, J = 7.3 Hz, 2 H), 3.5 (m, 4 H), 3.61 (s, 3 H), 9.02 (s, 1 H). ^{13}C NMR ($CDCl_3$) δ 21.6, 33.5, 33.6, 39.7, 40.1, 71.8, 173.1, 188.0. HREIMS calcd ($C_8H_{13}O_2S_2$, M − CHO) 205.0354, obsd 205.0357.

Methyl 5,5-(Dimethylenedithio)-8-oxo-6(*E*)-octenoate (13). To a solution of aldehyde **11** (2.8 g, 11.95 mmol) in benzene (30 mL) was added (triphenylphosphoranylidene)acetaldehyde **12** (1.3 g, 4.6 mmol), and the solution was refluxed for 2 h; 1.2 g of (triphenylphosphoranylidene)acetaldehyde **12** was then added and refluxed for 2 h. Another 1.2 g of (triphenylphosphoranylidene)-acetaldehyde **12** was added and refluxed overnight. The reaction mixture was cooled to room temperature, filtered through Celite, and washed with ethyl acetate and the combined filtrate 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 **9**, 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 (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 x 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.

3,6-Dodecadiyn-1-ol (16). At room temperature 40 mL of ethylmagnesium bromide (3.0 M in ether, 120 mmol) was slowly added dropwise to a solution of butyn-1-ol **14** (3.85 g, 55 mmol) in dry THF (200 mL) under argon. The reaction mixture was refluxed for 1.5 h and then cooled to room temperature, CuBr (0.715 g) was added, and the mixture was stirred at room temperature for 15 min. To the milky white suspension obtained was added a solution of bromooct-3-yne **15** (9.45 g, 50 mmol) in THF (20 mL) dropwise and the reaction mixture was refluxed for 2 h. The reaction mixture was diluted with ether (50 mL), quenched with aqueous ammonium chloride solution (30 mL), and extracted with ether (2 × 100 mL) and the combined extract washed with water (4 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product. This was purified by flash column chromatography with 8:2 hexane/ethyl acetate to afford the pure bisacetylenic alcohol **16** (7.3 g, 83%). ^{1}H NMR ($CDCl_3$) δ 0.83 (t, J = 6.9 Hz, 3 H), 1.30 (m, 4 H), 1.45 (m, 2 H), 2.01 (m, 2 H), 2.40 (m, 2 H), 3.08 (m, 2 H), 3.64 (t, J = 6.4 Hz, 2 H). ^{13}C NMR ($CDCl_3$) δ 9.85, 14.01, 22.3, 23.2, 28.5, 31.2, 61.2, 74.1, 76.95, 76.97, 80.97.

3(*Z*),6(*Z*)-Dodecadien-1-ol (17). To a solution of **16** (7.1 g) in ethanol (100 mL) were added triethylamine (1

mL) and Lindlar catalyst (1 g) under argon at room temperature. Hydrogenation was performed at atmospheric pressure using a glass buret apparatus at 0 to 5 °C for 5 h and then at room temperature for 24 h. The solution was filtered to remove the catalyst, and the filtrate was then evaporated at reduced pressure to give bisdienyl alcohol **17** (7.1 g), which was purified by column chromatography to give 4.35 g of pure **17** in 60% yield. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.35 (m, 6 H), 2.05 (t, J = 6.9 Hz, 2 H), 2.35 (m, 2 H), 2.81 (t, J = 7.0 Hz, 2 H), 3.65 (m, 2 H), 5.25−5.56 (m, 4 H).

3(*Z*),6(*Z*)-Dodecadien-1-yltriphenylphosphonium Bromide (19). To a cooled (0−5 °C), stirred solution of alcohol **17** (3.64 g, 20 mmol) and triphenylphosphine (7.89 g, 30 mmol) in dry CH_2Cl_2 (50 mL) was slowly added dropwise a solution of carbon tetrabromide (9.55 g, 30 mmol) in dry CH_2Cl_2 (10 mL) under argon. The reaction mixture was stirred for 30 min at 0−5 °C and then diluted with hexane/ethyl acetate (9:1) (400 mL), and the resulting solution of the bromide **18** was filtered through Celite. The filtrate was evaporated at reduced pressure and the residue purified by flash column chromatography with 9:1 hexane/ethyl acetate to afford the pure bromide **18** (4.1 g, 84%). The bromide **18** was immediately converted to the phosphonium salt **19**.

The bromide **18** (4.9 g, 20 mmol) was dissolved in dry acetonitrile (100 mL), and triphenylphosphine (10.59 g, 40 mmol) was added. The reaction mixture was refluxed for 36 h under argon atmosphere and then concentrated in vacuo. The phosphonium salt **19** was purified by flash column chromatography with 19:1 methylene chloride/methanol to afford the pure phosphonium salt **19** (9.35 g, 92%) as a colorless fluffy solid. ^{1}H NMR ($CDCl_3$) δ 0.7 (t, J = 7.2 Hz, 3 H), 1.07 (m, 6 H), 1.71 (q, J = 7.0 Hz, 2 H), 2.25−2.43 (m, 4 H), 3.66 (m, 2 H), 4.97−5.04 (m, 1 H), 5.1−5.3 (m, 2 H), 5.4−5.5 (m, 1 H), 7.55 (m, 6 H), 7.75 (m, 9 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 20.5, 22.6 (split), 23.4, 25.6, 25.61, 27.2, 29.1, 31.5, 117.7, 118.7, 126.6 (split), 130.7, 130.9, 133.7, 135.3.

Methyl 5,5-(Dimethylenedithio)-6(*E*),8(*Z*),11(*Z*),-14(*Z*)-eicosatetraenoate (20). To a cooled (−78 °C), stirred solution of the phosphonium salt **19** (2.03 g, 4 mmol) in THF (20 mL) was added lithium hexamethyldisilazide (1 M, 4 mL, 4 mmol) dropwise under argon. After stirring for 2 h at −78 °C, HMPA (2 mL) was added, the reaction mixture was stirred for 10 min, and then aldehyde **13** (521 mg, 2 mmol) in THF (4 mL) was added to the resulting red solution. The reaction mixture was stirred for 1 h at −78 °C and then allowed to warm slowly to 0 °C over a period of 1 h. It was then quenched by the addition of 2 N HCl 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 pure **20** (676 mg, 83%). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.23−1.38 (m, 6 H), 1.77−1.86 (m, 2 H), 2.03−2.16 (m,4 H), 2.35 (t, J = 7.3 Hz, 2 H), 2.84 (m, 2 H), 2.98 (m, 2 H), 3.2−3.34 (m, 4 H), 3.66 (s, 3 H), 5.4 (m, 5 H), 5.79 (d, J = 14.6 Hz, 1 H), 6.03 (t, J = 15.0 Hz, 1 H), 6.70 (dd, J = 14.7, 11.9 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 13.7, 22.5, 22.9, 25.5, 26.2, 27.1, 29.2, 31.4, 33.8, 38.9, 41.6, 51.4, 70.6, 124.2, 127.3, 127.4, 127.5, 128. 8, 130.4, 130.6, 137.6, 173.4. HREIMS calcd ($C_{23}H_{36}O_2S_2$, M^+) 408.2157, obsd 408.2148.

5,5-(Dimethylenedithio)-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic Acid (21). A solution of the dithio compound **20** (220 mg) in THF (50 mL) and 1 M LiOH (10 mL) was stirred at room temperature for 20 h. The reaction mixture was adjusted to pH 5 by the addition of 5% $KHSO_4$ (120 mL) and extracted with ethyl acetate (2 × 50 mL). The combined ethyl acetate extract was washed with cold water, dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated under reduced pressure to afford the dithio acid **21**, which was purified by flash column chromatography over silica gel using 1:19 MeOH/CH_2Cl_2 to give the pure dithio acid **21** (195 mg, 92%). ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 7.0 Hz, 3 H), 1.25−1.40 (m, 6 H), 1.78−1.88 (m, 2 H), 2.06 (q, J = 6.9 Hz, 2 H), 2.12−2.18 (m, 2 H), 2.41 (t, J = 7.3 Hz, 2 H), 2.83 (m, 2 H), 2.90 (m, 2 H), 3.31−3.37 (m, 4 H), 5.4 (m, 5 H), 5.80 (d, J = 14.7 Hz, 1 H), 6.04 (t, J = 10.9 Hz, 1 H), 6.71 (dd, J = 14.6, 11.2 Hz, 1 H). ^{13}C NMR (CD_3COCD_3) δ 14.8, 23.6, 24.1, 26.6, 27.2, 28.0, 30.4, 33.5, 34.3, 40.0 (2 × C), 42.59, 71.6, 125.0, 128.5, 128.7, 128.9, 129. 8, 131.1, 131.3, 139.3, 175.0.

5-Oxo-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic Acid (5-oxo-ETE) (2). To a −10 °C cooled solution of dithio acid **21** (52 mg, 0.1318 mmol) in methanol/H_2O (9:1, 25 mL) was added 4-hydroxy-TEMPO (50 μg) in EtOAc (50 μL) followed by a solution of [bis(trifluoroacetoxy)iodo]-benzene (120 mg, 0.279 mmol) in methanol (1 mL) and stirred at −10 °C to −8 °C for 2 min. The reaction mixture was quenched with a pH 7.5 buffer solution (NaH_2PO_4/Na_2HPO_4) (20 mL) and extracted with ethyl acetate (150 mL). The ethyl acetate extract was washed with cold water (3 × 30 mL) and brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under reduced pressure to approximately 2 mL solution, and the product was purified by reverse phase HPLC [Sperisorb SW 10, novapack C-18, 10 × 250 mm column; solvent system: CH_3CN:H_2O:AcOH (80:20:0.02%); flow rate 2 mL/min to give a mixture of cis and trans isomers. The eluant containing the mixture of 5-oxo-ETE **2** and 8,9-*trans*-5-oxo-ETE **3** was neutralized with Et_3N before evaporation of the solvents. The separation of the individual isomers **2** and **3** was carried out by NP HPLC (μ-porasil, Sperisorb 10 × 250 mm column, solvent system: 4.5% 2-propanol in hexane containing 0.05% AcOH; flow rate 4.5 mL/min). Analytical separation showed that 8,9-cis and 8,9-trans isomers **2** and **3** were obtained in the ratio of 5:1, t_r 8,9-cis 16.49 min, t_r 8,9-trans 19.73 min. To each of the eluants containing the pure 5-oxo-ETE **2** and 8,9-*trans*-5-oxo-ETE **3** was added equal amounts of ethyl acetate, and they were washed with water to remove the 2-propanol and acetic acid to give pure 5-oxo-ETE **2** 22 mg, in 52% yield. ^{1}H NMR (CD_3COCD_3) δ 0.87 (t, J = 7.0 Hz, 3 H), 1.27−1.39 (m, 10 H), 1.86 (m, 2 H), 2.34 (t, J = 7.3 Hz, 2 H), 2.72 (t, J = 7.2 Hz, 2 H), 3.16 (t, J = 6.6 Hz, 2 H), 5.35−5.5 (m, 4 H), 5.90 (m, 1 H), 6.24 (m, 2 H), 7.63 (dd, J = 15.3, 11.6 Hz, 1 H). 8,9-*trans*-5-Oxo-ETE **3** was obtained 5.5 mg in 13% yield. ^{1}H NMR (CD_3COCD_3) δ 0.86 (t, J = 7.0 Hz, 3 H), 1.18 (m, 10 H), 1.84 (m, 2 H), 2.33 (t, J = 7.3 Hz, 2 H), 2.66 (t, J = 7.3 Hz, 2 H), 3.0 (t, J = 5.8 Hz, 2 H), 5.3−5.51 (m, 4 H), 6.13 (d, J = 15.6 Hz, 1 H), 6.28 (m, 2 H), 7.23 (dd, J = 15.7, 9.7 Hz, 1 H).

Methyl-5,8-Dioxo-6(*E*)-octenoate (24). **Procedure a:** To a solution of dithio aldehyde **13** (170 mg, 0.653

mmol) in acetonitrile/H_2O (9:1, 7 mL) was added [bis-(trifluoroacetoxy)iodo]benzene (1.13 g, 2.61 mmol) and the reaction mixture stirred at room temperature for 5 min. The reaction mixture was quenched with water (50 mL) and extracted with ethyl acetate (2 × 75 mL). The combined ethyl acetate extract was washed with cold water (3 × 30 mL) and brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under reduced pressure, and the product was purified by flash column chromatography over silica using 20% ethyl acetate in hexane to give the pure product **24** 95 mgs, in 79% yield as a thick oil (solidified on cooling). ^{1}H NMR ($CDCl_3$) δ 1.98 (m, 2 H), 2.39 (t, J = 7.1 Hz, 2 H), 2.79 (t, J = 7.1 Hz, 2 H), 3.81 (s, 3 H), 6.67 (dd, J = 16.2 and 7.2 Hz, 1 H), 6.79 (d, J = 16.2, 1 H), 9.77 (d, J = 7.2, 1 H). ^{13}C NMR ($CDCl_3$) δ 18.5, 32.5, 39.8, 51.4, 137.2, 144.4, 173.1, 193.1, 198.9.

Procedure b: To a 0 °C cooled solution of aldehyde **13** (190 mg, 0.73 mmol) in anhydrous ethyl ether (1 mL) was slowly added dropwise a solution of periodic acid (333 mg, 1.46 mmol) in dry THF (1 mL) under argon. The ice bath was removed, and the reaction mixture was stirred for 8 min at room temperature. A white solid was precipitated. The reaction mixture was diluted with ether (5 mL), filtered through Celite/florisil, and washed with ether (30 mL), and the combined filtrate was washed with aqueous Na_2SO_3 (10 mL) and water (2 × 10 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 8:2 hexane/ethyl acetate to afford the pure aldehyde **24** (68 mg, 51%). The spectral data was identical as described above.

Methyl-5,8-Dioxooctanoate (25). To a solution of **24** (27 mg) in ethyl acetate:hexane (1:1) (15 mL) was added 10% Pd on carbon (25 mg) under argon at room temperature. Hydrogenation was performed at atmospheric pressure using a glass buret apparatus at room temperature for 10 h. The solution was filtered to remove the catalyst, and the filtrate was then evaporated at reduced pressure to give aldehyde **25** (27 mgs, 99% yield) which was used as such in the next step. ^{1}H NMR ($CDCl_3$) δ 1.90 (m, 4 H), 2.34 (t, J = 7.2 Hz, 2 H), 2.44−2.58 (m, 2 H), 2.66−2.78 (m, 2 H), 3.65 (s, 3 H), 9.79 (s, 1 H). ^{13}C NMR ($CDCl_3$) δ 18.8, 32.8, 34.5, 37.3, 41.3, 51.4, 173.4, 200.3, 207.7.

Methyl 5-Oxo-8(*Z*),11(*Z*),14(*Z*)-eicosatrienoate (26). To a cooled (−78 °C), stirred solution of the phosphonium salt **19** (165 mg, 0.329 mmol) in THF (3 mL) was added lithium hexamethyldisilazide (1 M, 0.25 mL, 0.25 mmol) dropwise under argon. After stirring for 1 h at −78 °C to −60 °C, it was cooled to −98 °C (MeOH/liq N_2), HMPA (0.4 mL) was added, and the reaction mixture was stirred for 2 min. Then aldehyde **25** (21 mg, 0.112 mmol) in THF (0.5 mL) was added to the resulting red solution. The reaction mixture was stirred for 20 min at −78 °C and allowed to warm slowly to 0 °C over a period of 1.5 h. It was then quenched by the addition of 1:1 THF water and extracted with ethyl acetate (3 × 25 mL). The combined extracts were washed with cold water (3 × 20 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 10% ethyl acetate in hexane to give pure **26** (13.1 mg, 35%). ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3 H), 1.23−1.40 (m, 6 H), 1.91 (m, 2 H), 2.05 (m, 2 H), 2.36 (m, 4 H), 2.36 (m, 4 H), 2.50 (m, 4 H), 2.82 (m, 2 H), 3.67 (s, 3 H), 5.30−5.44 (m, 6 H). ^{13}C NMR ($CDCl_3$) δ 14.0, 18.9, 21.6, 22.6, 25.5, 25.6, 27.2, 29.3, 31.5, 33.1, 41.6, 42.5, 51.5, 127.5, 127.8, 128.2, 128.6, 129.1, 130.5, 173.6, 209.5.

5-Oxo-8(*Z*),11(*Z*),14(*Z*)-eicosatrienoic Acid (6,7-dihydro-5-oxo-ETE) (5). A solution of ester **26** (7 mg) in THF (2 mL) and 1 M LiOH (0.52 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water (2 mL) and extracted with hexane (10 mL). The aqueous layer was separated, and the organic layer was extracted with water (3 × 10 mL). The combined aqueous extracts were acidified to pH 5 with 5% aqueous $KHSO_4$ and extracted with ethyl acetate (2 × 25 mL). The combined ethyl acetate extract was washed with cold water (2 × 10 mL), dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated under reduced pressure to afford the 6,7-dihydro-5-oxo-ETE **5**, which was purified by flash column chromatography over silica gel using 1:19 MeOH/CH_2Cl_2 to give the pure 6,7-dihydro-5-oxo-ETE **5** (4.5 mg, 67%). ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3 H), 1.23−1.40 (m, 8 H), 2.12 (m, 2 H), 2.17 (m, 2 H), 2.31−2.47 (m, 4 H), 2.48−2.56 (m, 4 H), 2.83 (m, 4 H), 5.37 (m, 6 H).

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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.

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