The Total Synthesis of Tritiated and Deuterated 5-Oxo-ETE, a Novel Inflammatory Mediator

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The total synthesis of 11,12,14,15-tetratritiated and deuterated 5-oxo-ETE is accomplished using a novel bisacetylene precursor **14**. The syntheses of these labeled derivatives are necessary in order to investigate further the role and biochemistry of the novel inflammatory mediator and eosinophilic chemotactic agent 5-oxo-ETE.

Introduction

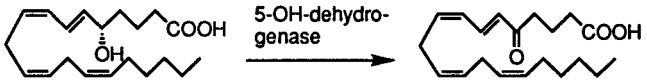
5-Oxo-ETE **2** is a novel arachidonate metabolite formed by a dehydrogenase present in microsomal fractions of human neutrophils (Scheme 1).[1] This enzyme catalyzes the oxidation of 5-HETE, a major product of the 5-LO pathway in these cells, to 5-oxo-ETE. This 5-hydroxyeicosanoid dehydrogenase is highly 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.[1] It also requires that the double bond in the 6-position of 5-hydroxyeicosanoids 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.

Monocytes, lymphocytes, and eosinophils also synthesize 5-oxo-ETE. Monocytes stimulated with the calcium ionophore A23187 and PMA synthesized the same or slightly greater amounts of 5-oxo-ETE than neutrophils, suggesting that these cells may also be a major site for the synthesis of this substance.[2]

5-Oxo-ETE is a potent chemoattractant for neutrophils[3] and eosinophils.[4] It also stimulates a variety of other responses in these cells, including calcium mobilization, adherence, integrin expression, actin polymerization, and degranulation.[5−7] There is substantial evidence that the effects of 5-oxo-ETE on granulocytes are mediated by interaction with a specific G protein-linked receptor.[8]

The availability of deuterium- and tritium-labeled analogues of 5-oxo-ETE is essential for further investigation of its biological role. Deuterium-labeled 5-oxo-ETE can be used as an internal standard for the mass spectrometric analysis of this substance, whereas a tritium-labeled analogue can be used in studies of its metabolism and binding to receptors. The first total synthesis of these labeled derivatives is presented here.

Scheme 1



5-(S)-HETE **1** 5-oxo-ETE **2**

Results and Discussion

We have recently reported the first total synthesis of 5-oxo-ETE.[9] The other preparation from 5-HETE[10−12] has also been reported.[3,13,14] Synthetic 5-oxo-ETE was used to unequivocally confirm the structure of the biologically derived mediator.[9] 5-Oxo-ETE and other oxoeicosanoids such as 12-oxo-LTB_4[15] and 12-oxo-ETEs[15−18] contain dienone or trienone systems that render these molecules reactive and unstable. For that reason, throughout the synthesis of 5-oxo-ETE we have carried the carbonyl function in a protected form as a dithiolane derivative.

In the synthesis of labeled 5-oxo-ETE described here we have retained this necessary feature. We also elected to use two acetylene functions as the precursors of the tritiated and deuterated molecules **16** and **17** (Scheme 2) and used the Lindlar catalyst for the synthesis of the radiolabeled and deuterated derivatives. The selection

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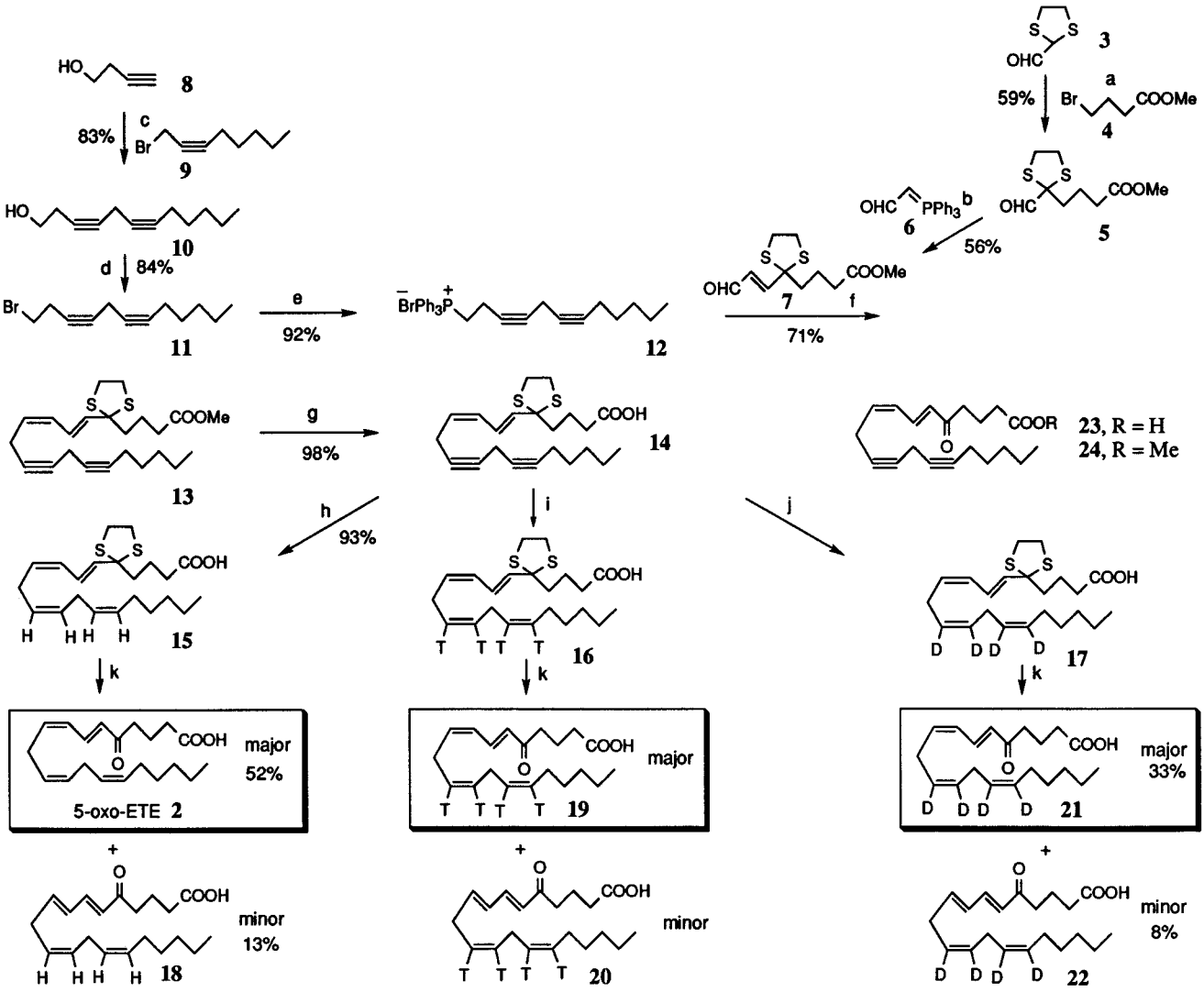
Scheme 2[a]



[a] Key: (a) NaH, room temperature; (b) reflux benzene; (c) ethylmagnesium bromide/CuBr; (d) CBr_4, PPh_3; (e) PPh_3, CH_3CN; (f) $LiN(SiMe_3)_2$; (g) LiOH; (h) H_2, Lindlar; (i) T_2, Lindlar; (j) D_2, Lindlar; (k) $PhI(OCOCF_3)_2$.

of two acetylenic functions in the precusor **14** permitted the incorporation of four tritium atoms in the molecule to give tritium-labeled 5-oxo-ETE with high specific activity. In addition, the bisacetylenic function is necessary for the incorporation of the 4-deuterium atoms, which is optimal for the development of a GC/MS or LC/MS assay. In our synthetic design, we took into account the desirability that the incorporation of tritium be performed as late in the synthesis as possible in order to carry out the minimum number of synthetic steps with a radioactive material. In this case, we introduced the tritium in the penultimate step, and in fact, we have performed the two last steps in a one-pot reaction. We also performed the partial hydrogenation of these acetylene groups using the Lindlar catalyst and obtained 5-oxo-ETE as shown in Scheme 2. This represents a new total synthesis for this important mediator, distinct from the one we recently reported.[9]

The synthesis starts with the bisacetylene derivative **10** prepared from butyn-1-ol (**8**).[11] Alkylation of butyn-1-ol (**8**) with bromooct-3-yne (**9**) gave bisacetylene alcohol **10** in 83% yield. Bromination of the alcohol **10** with CBr_4 and PPh_3 furnished bromide **11** in 84% yield. The bromide **11**, after purification, was immediately treated with Ph_3P to generate the phosphonium salt **12**, which was purified by flash column chromatography to give pure **12** in 92% yield. The aldehyde **7** was prepared using our recently reported procedure.[9] Wittig reaction of the aldehyde **7** with the phosphonium salt **12**, using LHMDS as base, produced selectively the *Z*-isomer **13** in 71% yield. The methyl ester **13** was treated with 1 M LiOH in THF/H_2O to give the dithio bisacetylene derivative of 5-oxo-ETE **14** in 98% yield, which was used as such in the next step. Hydrogenation of the bisacetylene compound **14** using Lindlar catalyst furnished **15** in 93% yield. A very pure sample of this dithio acid **15** was obtained by reversed-phase HPLC purification in 51% yield. Finally, deprotection of the HPLC-purified dithio acid **15** with [bis(trifluoroacetoxy)iodo]benzene[15,19] gave 5-oxo-ETE (**2**) in 52% yield. The 6,7-trans isomer **18** was also isolated in 13% yield. In anticipation of the radiolabeled preparation and in order to minimize the manipulations with the radioactive material, we have developed a two-step, one-pot operation in which the crude product, after hydrogenation, was used as such in the next and final step. The HPLC purification in

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the last step affords material as pure as the two-step procedure.

The deuteration of the bisacetylene compound **14** was performed using Lindlar catalyst as described for the hydrogenation procedure. In this case, too, the crude product **17** was used as such in the last deprotection step, and d_4-5-oxo-ETE (**21**) and its 8,9-trans-isomer **22** were isolated in 33% and 8% yields, respectively. The electrospray MS analysis of **21** as well as **22** revealed an M − 1 ion at *m*/*z* 321 for the corresponding d_4-5-oxo-ETE and d_4-8,9-*trans*-5-oxo-ETE.

The tritiation of bisacetylene derivative **14** was performed by a modification of the hydrogenation and deuteration experiment and is described in some detail in the Experimental Section. The crude tritiated dithio acid **16** was deblocked using [bis(trifluoroacetoxy)iodo]-benzene, and the pure T_4-5-oxo-ETE (**19**) (specific activity 63 Ci/mmol) and its 8,9-trans-isomer **20** (specific activity 57 Ci/mmol) were isolated by HPLC purification.

It is worth mentioning that during the hydrogenation, deuteration, or tritiation steps vigorous stirring of the reaction mixture is important. In addition, since we use large amounts of catalyst and because most of the product is adsorbed by the catalyst after the reaction is complete, stirring with methanol and then with methylene chloride/methanol (4:1) is necessary. The catalyst is also transferred to the column during filtration through Celite.

Before we settled for the convenient two-step, one-pot procedure for hydrogenation, deuteration, and/or tritiation reaction, we explored the possibility of performing tritiation as the last step in the synthesis. Dithio acid **14** was deblocked using the procedure mentioned above and the product purified by HPLC to give pure acid **23**. However, hydrogenation of **23** using Lindlar catalyst was complicated by competitive over-reduction of the 6,7- and 8,9-double bonds, and as a result we abandoned this approach. In addition, the use of ester derivative **24** as a model compound for the hydrogenation using Lindlar catalyst also resulted in partial reduction of the unsaturated dienone system.

Throughout the experimental procedures, we have used 4-hydroxy-TEMPO, which is a radical inhibitor, as a precautionary measure. We have previously observed during dithiane chemistry the easy isomerization of cis double bonds to trans double bonds due to the minute amount of sulfur radicals presumably present in the system.[15]

We are currently using tritium-labeled 5-oxo-ETE to investigate the metabolism of this substance in leukocytes. Preliminary studies indicate that both 5-oxo-ETE and its 8-trans isomer are converted to 6,7-dihydro metabolites by these cells. The availability of labeled substrates is critical for the investigation of this metabolic pathway, since the dihydro products do not absorb appreciably in the UV region above 200 nm. Deuterium-labeled 5-oxo-ETE is currently being used to set up a mass spectrometric assay for this compound.

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 Hz.

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 isolated and spectroscopically (^{1}H NMR) homogeneous materials. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light and/or 10% ethanolic phosphomolybdic acid and heat as developing agent. Flash column chromatography was carried out by using E. Merck 230−400 mesh silica gel. HPLC analyses and separations were carried out on a Waters instrument with a Waters 600E dual pump solvent delivery system controller, U6K injector, and Waters 994 programmable photodiode array detector.

3,6-Dodecadiyn-1-ol (10). 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 (**8**) (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 (**9**) (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 extracts were 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 **10** (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.

Dodecadiyn-1-yltrisphenylphosphonium Bromide (12). To a cooled (0−5 °C), stirred solution of alcohol **10** (3.64 g, 20 mmol) and triphenylphosphine (7.89 g, 3.0 mmol) in dry CH_2Cl_2 (50 mL) was slowly added dropwise a solution of carbon tetrabromide (9.55 g, 28.8 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 **11** was filtered through Celite. The filtrate was evaporated at reduced pressure, the residue was purified by flash column chromatography with 9:1 hexane/ethyl acetate to afford the pure bromide **11**, 4.1 g, in 84% yield: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 6.7 Hz, 3 H), 1.30 (m, 4 H), 1.46 (m, 2 H), 2.12 (t, J = 7.0 Hz, 2 H), 2.71 (t, J = 7.3 Hz, 2 H), 3.11 (s, 2 H), 3.40 (t, J = 7.4 Hz, 2 H). The bromide **11** was used immediately in the next step. The bromide **11** (4.1 g, 16.65 mmol) was dissolved in dry acetonitrile (85 mL), and triphenylphosphine (8.81 g, 33.3 mmol) was added. The reaction mixture was refluxed for 36 h under argon atmosphere and then concentrated *in vacuo*. The phosphonium salt was purified by flash column chromatography with 19:1 methylene chloride/methanol to afford the pure phosphonium salt **12** (7.82 g, 92%) as a colorless fluffy solid: ^{1}H NMR ($CDCl_3$) δ 0.83 (t, J = 6.9 Hz, 3 H), 1.25 (m, 4 H), 1.39 (m, 2 H), 2.03 (t, J = 7.1 Hz, 2 H), 2.61 (s, 2H), 2.73 (dt, J = 10.6, 7.0 Hz, 2 H), 3.98 (m, 2 H), 7.65 (m, 2 H), 7.7−7.85 (m, 9 H); ^{13}C NMR ($CDCl_3$) δ 9.27, 12.9, 13.76, 18.4, 21.9, 22.4, 28.17, 30.83, 72.75, 76.5 (d, J = 27 Hz), 79.44, 80.75, 117.79, (d, J = 342 Hz), 130.15 (d, J = 50 Hz), 133.8 (d, J = 40 Hz), 134.88 (d, J = 11 Hz).

Methyl 5,5-(Dimethylenedithio)-6(*E*),8(*Z*)-eicosadiene-11,14-diynoate (13). To a cooled (−78 °C), stirred solution of the phosphonium salt **13** (0.86 g, 1.36 mmol) in THF (16 mL) was added lithium hexamethyldisilazide (1 M, 1.23 mL, 1.23 mmol) dropwise under argon. After the mixture was stirred for 30 min at −78 to −70 °C, HMPA (3 mL) was added and reaction mixture stirred for 5 min, and then aldehyde **7** (320 mg, 1.23 mmol) in THF (4 mL) was added to the resulting red solution at −78 °C. The reaction mixture was stirred for 30 min at −78 °C and then allowed to warm slowly to 10 °C

over a period of 1 h. It was then quenched by the addition of aqueous saturated ammonium chloride solution (25 mL) and extracted with diethyl ether (3 × 75 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 10% ethyl acetate in hexane to give pure **13** (352 mg, 71%): $R_f = 0.36$ (10% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, $J = 7.1$ Hz, 3 H), 1.32 (m, 4 H), 1.44 (m, 2 H), 1.75 (m, 2 H), 2.15 (m, 4 H), 2.37 (t, $J = 7.3$ Hz, 2 H), 3.1 (m, 4 H), 3.25 (m, 4 H), 3.62 (s, 3 H), 5.45 (dt, $J = 10.5$, 7.0 Hz 1 H), 5.88 (d, $J = 14.8$ Hz, 1 H), 6.11 (t, $J = 10.9$ Hz, 1 H), 6.61 (dd, $J = 14.8$, 11.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 9.66, 13.89, 17.75, 18.58, 21.94, 22.90, 28.95, 30.96, 38.81, 38.89, 41.42, 51.47, 70.40, 73.95, 74.86, 77.85, 80.51, 123.48, 126.26, 128.28, 128.43, 138.71, 173.42.

5,5-(Dimethylenedithio)-6(*E*),8(*Z*)-eicosadien-11,14-diynoic Acid (Bisacetylenic Dithio acid) (14). A solution of the dithio compound **13** (36.9 mg) in THF (9 mL) and 1 M LiOH (4.5 mL) was stirred at room temperature for 20 h. To the reaction mixture was added water (8 mL), and THF was removed under a stream of argon and acidified by the addition of 5% aqueous KH_2PO_4 (60 mL) and extracted with ethyl acetate (3 × 30 mL). The combined ethyl acetate extracts were washed with cold water (4 × 25 mL) and brine (1 × 25 mL), dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated under reduced pressure to afford the bisacetylenic dithio acid **14** (35 mg) in nearly quantitative yield, which was used in the next step without further purification: $R_f =$ 0.43 (35% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 0.91 (t, $J = 7.1$ Hz, 3 H), 1.35 (m, 4 H), 1.50 (m, 2 H), 1.75−1.90 (m, 4 H), 2.15 (m, 4 H), 2.40 (t, $J = 7.4$ Hz, 2 H), 3.05−3.15 (m, 4 H), 3.2−3.4 (m, 4 H), 5.5 (dt, $J = 10.6$, 7.2 Hz, 1 H), 5.82 (d, $J = 14.6$ Hz, 1 H), 6.07 (t, $J = 11.0$ Hz, 1 H), 6.66 (dd, $J =$ 14.7, 11.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 9.67, 13.88, 17.75, 18.58, 22.1, 22.57, 28.32, 30.97, 33.64, 38.93, 41.31, 70.36, 74.88, 76.86, 123.53, 126.33, 127.65, 128.42, 138.65, 178.5.

5,5-(Dimethylenedithio)-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic Acid (15). The bisacetylenic dithio acid **14** (24 mg) was dissolved in 24 mL of 9:1 hexane/ethyl acetate. The hydrogenation was performed at 0 °C over a period of 2 h using Lindlar catalyst (5% Pd) (1.2 g). More catalyst (0.6 g) was added, and the hydrogenation was continued for 30 min more. Then hydrogen was removed by applying vacuum and purging with air. To the reaction mixture was added HPLC-grade methanol (12 mL). The mixture was stirred for 5 min, and then 20 mL of methanol/methylene chloride (20:80) was added and further stirred for 5 min. The mixture including the catalyst was loaded on a pad of Celite (1 g) (before loading the product on Celite, it was washed with 10 mL of methanol/methylene chloride (20:80) and the washings were discarded) and filtered and washed with 200 mL of methanol/methylene chloride (20:80). Concentration of the filtrate gave the dithio acid **15** (21.6 mg, 93%). The crude product **15** can be used as such in the next step. The pure sample was obtained by RP HPLC [Spherisorb C-18, 10 × 250 mm column, solvent system: 20% water in acetonitrile containing 0.05% AcOH; flow rate 2 mL/min] in 51% yield: ^{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; EIHRMS calcd for $C_{22}H_{34}O_2S_2$ 394.2000, observed 394.1994.

5-Oxo-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic Acid (5-Oxo-ETE) (2). To a 0 °C cooled solution of dithio acid **15** (20.8 mg, 0.0527 mmol) in methanol/H_2O (9:1, 25 mL) was added a solution of 4-hydroxy-TEMPO (40 μg) in CH_2Cl_2 (40 μL) followed by a solution of [bis(trifluoroacetoxy)iodo]benzene (48 mg, 0.111 mmol) in methanol (0.4 mL) and the resulting mixture stirred at 0 °C for 2.5 min. The reaction mixture was quenched with cold water (20 mL) and extracted with ethyl acetate (2 × 50 mL). The combined ethyl acetate extracts were washed with cold water (5 × 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 first purified by reversed-phase HPLC. Approximately 20 injections were performed, and every time before loading the product on RP column, ethyl acetate was evaporated under a stream of argon and the residue dissolved in acetonitrile and then injected onto the Spherisorb C-18, 10 × 250 mm column. The mobile system CH_3CN/H_2O/AcOH (80:20:0.02%) at a flow rate of 3 mL/min was used to give a mixture of cis and trans isomers (retention time 13.5 min). The eluent containing the mixture of 5-oxo-ETE (**2**) and 8,9-*trans*-5-oxo-ETE (**18**) was neutralized with Et_3N before evaporation of solvents. The separation of individual isomers was carried out by NP HPLC [Spherisorb S10W, 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 were obtained in a ratio of 87:17, t_R 8,9-cis 16.49 min, t_R 8,9-trans 19.73 min. To the each of the eluents containing the pure 5-oxo-ETE (**2**) and 8,9-*trans*-5-oxo-ETE (**18**) was added an equal amount of ethyl acetate, and they were washed with water to remove the 2-propanol and acetic acid to give pure 5-oxo-ETE (**2**), 8.8 mg, in 52% yield: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, $J = 6.0$ Hz, 3 H), 1.25−1.32 (m, 6 H), 1.99 (m, 2 H), 2.03 (m, 2 H), 2.40 (t, $J = 6.1$ Hz, 2 H), 2.64 (m, 2 H), 2.79 (m, 2 H), 3.16 (m, 2 H), 5.34−5.4 (m, 4 H), 5.86 (m 2 H), 6.13 (dd, $J = 16.0$, 10.7 Hz, 1 H), 7.51 (dd, $J = 15.5$, 11.5 Hz, 1 H); ^{1}H NMR (CD_3COCD_3) δ 0.87 (t, $J = 7.0$ Hz, 3 H), 1.27−1.39 (m, 6 H), 1.86 (m, 2 H), 2.1 (m, 2 H, peak overlapped with solvent acetone peak), 2.34 (t, $J = 7.3$ Hz, 2 H), 2.72 (t, $J = 7.2$ Hz, 2 H), 2.9 (m, 2 H, peak overlapped with water peak), 3.16 (t, J = 6.6 Hz, 2 H), 5.35−5.5 (m, 4 H), 5.90 (m 2 H), 6.24 (dd, $J =$ 15.9, 10.7 Hz, 1 H), 7.63 (dd, $J = 15.3$, 11.6 Hz, 1 H); UV (EtOH) λ_{max} 278 ($\epsilon = 23\,500$). 8,9-*trans*-5-Oxo-ETE (**18**) was obtained, 2.2 mg, in 13% yield: ^{1}H NMR (CD_3COCD_3) δ 0.86 (t, $J = 7.0$ Hz, 3 H), 1.18 (m, 6 H), 1.84 (m, 2 H), 2.1 (m, 2 H, peak overlapped with solvent acetone peak), 2.33 (t, $J = 7.3$ Hz, 2 H), 2.66 (t, $J = 7.3$ Hz, 2 H), 2.9 (m, 2 H, peak overlapped with water peak), 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).

Deuteration Procedure and Deblocking of d_4-Dithio Acid. 5-Oxo-6(*E*),8(*Z*),11(*Z*),14(*Z*)-11,12,14,15-d_4-eicosatetraenoic Acid (d_4-5-Oxo-ETE) (21). In a 25 mL three-neck flask was placed a solution of dithio acid **14** (12 mg) in ethyl acetate/hexane 1:9 (12 mL), and 100 μg of 4-hydroxy-TEMPO in ethyl acetate (100 μL) was added. The solution was purged with argon and the flask connected to the deuteration apparatus. The argon was removed by applying vacuum and the flask purged with deuterium; the process was repeated three times. Then Lindlar catalyst (5% Pd) (0.6 g) was added quickly, and the reaction mixture was stirred well magnetically at 0−5 °C for 2 h. More catalyst (0.3 g) was added, and the deuteration was further continued for 30 min more. Then deuterium was removed by applying vacuum and purging with air. To the reaction mixture was added HPLC-grade methanol (5 mL), the mixture was stirred for 5 min, and then 10 mL of 20:80 methanol/methylene chloride was added and further stirred for 5 min. The mixture including the catalyst was loaded on a pad of Celite (1 g) (before loading the product on Celite, it was washed with 10 mL of 20:80 methanol/methylene chloride and the washings were discarded), filtered, and washed with 200 mL of methanol/methylene chloride (20:80). The filtrate on evaporation of solvent at reduced pressure gave 11 mg of the product **17**, $R_f = 0.55$ (35% ethyl acetate in hexane), which was used as such in the next step.

To a 0 °C cooled solution of crude d_4-dithio acid **17** (11 mg, 0.0267 mmol) in methanol/H_2O (9:1, 5 mL) was added a solution of 4-hydroxy-TEMPO (40 μg) in CH_2Cl_2 (40 μL) followed by a solution of [bis(trifluoroacetoxy)iodo]-benzene (44 mg, 0.1 mmol) in methanol (1 mL) and the mixture stirred at 0 °C for 2.5 min. The reaction mixture was quenched with cold water (20 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 CH_2Cl_2 (50 μL) was added, and the solvent was evaporated under reduced pressure. The product was first purified by reversed-phase HPLC [Spherisorb C-18, 10 × 250 mm column; solvent system: CH_3CN/H_2O/AcOH (80:20:0.02%), flow rate 3 mL/min (retention time 13.5 min)] to give a mixture of cis and trans isomers. The eluent containing the mixture of d_4-5-oxo-ETE (**21**) and 8,9-trans isomer **22** was neutralized with Et_3N before evaporation of solvents. The separation of individual isomers was carried out by NP HPLC [Spherisorb S10W, 10 × 250 mm column, solvent system: 4.5% 2-propanol in hexane containing 0.05% AcOH; flow rate 4.5 mL/min]. To the each of the eluents containing the pure d_4-5-oxo-ETE (**21**) and d_4-8,9-*trans*-5-oxo-ETE (**22**) was added an equal amount of ethyl acetate, and they were washed with water to remove the 2-propanol and acetic acid to give pure d_4-5-oxo-ETE (**21**): 3.2 mg, in 33% yield; ^{1}H NMR (CD_3COCD_3) δ 0.87 (t, J = 7.0 Hz, 3 H), 1.25−1.37 (m, 6 H), 1.90 (m, 2 H), 2.1 (m, 2 H, peak overlapped with solvent acetone peak), 2.34 (d, J = 7.4 Hz, 2 H), 2.71 (t, J = 7.2 Hz, 2 H), 2.9 (m, 2 H, peak overlapped with water peak), 3.16 (d, J = 7.8 Hz, 2 H), 5.88 (m, 1 H), 6.21 (m 2 H), 7.63 (ddd, J = 15.4, 10.6, 1.0 Hz, 1 H); electrospray MS calcd for $C_{20}H_{25}D_4O_3$ (M − 1) 321, obsd 321. 8,9-*trans*-d_4-5-Oxo-ETE (**22**) was obtained, 0.8 mg, in 8% yield: ^{1}H NMR (CD_3COCD_3) δ 0.87 (t, J = 7.0 Hz, 3 H), 1.27−1.38 (m, 6 H), 1.86 (m, 2 H), 2.1 (m, 2 H, peak overlapped with solvent acetone peak), 2.33 (t, J = 7.3 Hz, 2 H), 2.66 (t, J = 7.3 Hz, 2 H), 2.9 (m, 2 H, peak overlapped with water peak), 3.0 (t, J = 3.9 Hz, 2 H), 6.13 (d, J = 15.6 Hz, 1 H), 6.27 (m, 2 H), 7.23 (dd, J = 15.7, 9.5 Hz, 1 H); electrospray MS calcd for $C_{20}H_{25}D_4O_3$ (M − 1) 321, obsd 321.

Tritiation Procedure. The tritiation reaction was performed using the procedure described above for the hydrogenation with slight modifications. To a solution of bisacetylenic dithio acid **14** (24 mg) in hexane/ethyl acetate (9:1) (24 mL) was added 200 μg of 4-hydroxy- TEMPO in 200 μL of ethyl acetate. Then 1.2 g of Lindlar catalyst was added under argon. Tritiation was performed at 0 °C (ice bath) for 2 h. The reaction was then stopped, more catalyst (0.6 g) was added, and tritiation was again carried out at 0−5 °C for another 30 min.

Workup Procedure. To the reaction mixture was added HPLC-grade methanol (12 mL). After the mixture was stirred at room temperature for 5 min, 25 mL of methylene chloride/methanol (80:20) was added and stirred well at room temperature for 5 min (this stirring is necessary because most of the product is adsorbed on the catalyst). Then 1 g of Celite was loaded into a small glass column, putting a small amount of sand at the bottom and on the top of the Celite for easier filtration. The Celite was washed with 25 mL of methylene chloride/methanol (80:20), and the washings were discarded. Then the reaction mixture including all the catalyst was loaded onto this column and the solution filtered by applying nitrogen, and then the Celite was washed with an additional 150 mL of methylene chloride/methanol (80:20). To the combined filtrate was added 200 μg of 4-hydroxy-TEMPO in 200 μL of ethyl acetate, and the material was stored under argon at −78 °C.

5-Oxo-6(*E*),8(*Z*),11(*Z*),14(*Z*)-11,12,14,15-T_4-eicosatetraenoic Acid (T_4-5-Oxo-ETE) (19). Tritiated dithio acid **16** (obtained from the above tritiation reaction of **14** on a 24 mg scale after evaporation of solvents) was dissolved in methanol/water (9:1) (10 mL), 4-hydroxy-TEMPO (200 μg) in ethyl acetate (100 μL) was added, and the solution was cooled to 0 °C (ice bath). Bis[(trifluoroacetoxy)iodo]benzene (47 mg) in methanol (1 mL) was added at 0 °C under argon, and the reaction mixture was stirred at 0 °C for 2.5 min. It was then diluted with cold water (70 mL) and extracted with ethyl acetate (2 × 70 mL). The extractions were performed by addition of solvent in the same reaction flask with mixing by magnetic stirring. The mixture was then transferred into a separatory funnel and the aqueous layer removed. Washings were also performed in the same manner as described for extraction. The combined ethyl acetate extracts were washed with cold water (8 × 50 mL), dried over Na_2SO_4, and filtered. 4-Hydroxy-TEMPO (100 μg) was added and the solvent evaporated at reduced pressure. The residue was dissolved in methanol (3 mL) and after addition of 4-hydroxy-TEMPO (50 μg) was kept in the freezer at −20 °C. The product was purified by reversed-phase HPLC on Spherisorb C18, 10 × 250 mm column using CH_3CN/H_2O/AcOH (80:20:0.02) as the mobile phase and a flow rate of 3 mL/min (retention time for cis and trans isomers was 13.5 min). Approximately thirty 150 μL injections were performed, and every time after collecting the eluate, 5 μg of 4-hydroxy-TEMPO was added and combined eluates stored at −20 °C. A mixture of cis and trans isomers (2.2 mg based on UV absorbance) was obtained in 90 mL of eluate. The acetic acid in the eluate was neutralized by the addition of 135 mL of triethylamine, followed by stirring at room temperature for 1 min. Acetonitrile was removed at reduced pressure, and 70 mL of 5% cold aqueous KH_2PO_4 (pH 4.3) was added to the aqueous solution and the product extracted with ethyl acetate (2 × 70 mL). The combined ethyl acetate extracts were washed with cold water (5 × 50 mL) as described above, dried over Na_2SO_4, and filtered, and 4-hydroxy-TEMPO (200 μg) was added. The solvent was concentrated at reduced pressure to approximately 1 mL, and after addition of 4-hydroxy-TEMPO (20 μg), the solution was kept in the freezer at −20 °C. The cis and trans isomers were separated by normal-phase HPLC on a Spherisorb S10W, 10 × 250 mm column using hexane/2-propanol/AcOH (95.5:4.5:0.05) as the mobile phase and a flow rate of 2 mL/min. (Approximately 10−12 injections of 80 μL were performed, and every time after the eluate was collected 5 μg of 4-hydroxy-TEMPO was added and the combined eluates were stored at −20 °C). The tritiated 5-oxo-ETE (**19**) and its trans isomer **20** were each obtained in volumes of 35 mL. To the each of the eluates was added ethyl acetate (35 mL), and then they were washed with cold water (5 × 50 mL) as described above. The organic phase was dried over Na_2SO_4 and filtered, 4-hydroxy-TEMPO (2 μg) was added, and the solvent was evaporated at reduced pressure. The pure products were dissolved in acetonitrile (10 mL), 4-hydroxy-TEMPO (2 μg) was added, and the materials were kept in the freezer at −80 °C. Pure tritium-labeled 5-oxo-ETE (**19**), 522 μg (based on UV absorbance), and its trans isomer **20**, 265 μg (based on UV absorbance), were obtained.

5-Oxo-6(*E*),8(*Z*)-eicosadien-11,14-diynoic Acid (23). Bisacetylenic acid (23) was prepared by deblocking **14** using bis[(trifluoroacetoxy)iodo]benzene and the product purified by reversed-phase and NP HPLC as described above.

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Supporting Information Available: Copies of HPLC data and ^{1}H NMR and ^{13}C NMR spectral data for new compounds described herein (27 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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