Synthesis of 10,11-Dihydro-12-oxo-LTB_4, a Key Biochemical Intermediate

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The first total synthesis of the 5(*S*)-hydroxy-10,11-dihydro-12-oxo-6(*Z*),8(*E*),14(*Z*)-eicosatrienoic acid (10,11-dihydro-12-oxo-LTB_4) (**3**) is reported. This compound is a key pivotal intermediate in the biotransformation of LTB_4 by the so-called "LTB_4 reductase pathway".

Leukotriene B_4 (LTB_4) is a metabolite of arachidonic acid which is synthesized by neutrophils and other inflammatory cells.[1] Several total syntheses of this key mediator have appeared over the years.[2] LTB_4 is a potent stimulator of neutrophils,[3] monocytes,[4] and lymphocytes.[5] It stimulates the expression of integrins[6] on neutrophils and promotes their adhesion to the vascular endothelium[7] and migration to tissues[8] in response to proinflammatory stimuli. Because of its potent biological effects, it is very important to understand the pathways responsible for the metabolism of LTB_4 as well as the biological activities of its metabolites. In common with other fatty acids, LTB_4 is metabolized by omega-oxidation[9,10] and β-oxidation.[11] ω-oxidation results in substantial loss of its biological activity,[12] whereas one of the initial products of β-oxidation, 3(*S*)-hydroxy-LTB_4, still retains substantial biological activity.[13]

We have identified an additional pathway for the metabolism of LTB_4 in neutrophils resulting in the formation of 10,11-dihydro metabolites[14] (Scheme 1). The 10,11-double bond of LTB_4 is not reduced directly, but first requires oxidation of the 12-hydroxyl group by a microsomal 12-hydroxyeicosanoid dehydrogenase to give 12-oxo-LTB_4[15] (Scheme 1). This is followed by reduction of the 10,11-double bond by a cytosolic olefin reductase to give 10,11-dihydro-12-oxo-LTB_4 (**3**) (Scheme 1), which is the initial stable product of this pathway isolated after incubation of intact neutrophils with LTB_4,[15] and is the main focus of this paper. The 12-oxo group of the dihydro-oxo compound can then be reduced to either 12-(*R*)- or 12(*S*)-10,11-dihydro-LTB_4.[16] Complete identity of the very unstable metabolite 12-oxo-LTB_4 has been established by comparison with an authentic synthetic sample.[17] In addition, using synthetic 6-*trans*-12-oxo-LTB_4, we have identified variable amounts of 6-*trans*-12-oxo-LTB_4 during metabolism in porcine leukocytes.[17] The 12-keto reductase appeared to be stereospecific during LTB_4 metabolism by subcellular fractions of porcine PMNL resulting in formation of 10,11-dihydro-LTB_4 (**4**) and not in the formation of 10,11-dihydro-12-*epi*-LTB_4 (**5**). The 10,11-dihydro-LTB_4 metabolite (**4**) was formed initially and the 12-*epi* isomer (**5**) was only detected following longer incubation times.[18] However, both dihydro epimers were found during LTB_4 metabolism by human monocytes,[19] mesangial cells,[20] and human lung.[21] In another study, both epimers of 10,11-dihydro-LTB_4 were detected following a 24-h incubation of LTB_4 with keratinocytes.[22] Additional time-course studies in keratinocytes are necessary to determine if both isomers are formed directly from 10,11-dihydro-12-oxo-LTB_4 (Scheme 1) or if only one isomer is initially formed, followed by the appearance of the 12-*epi*-isomer.

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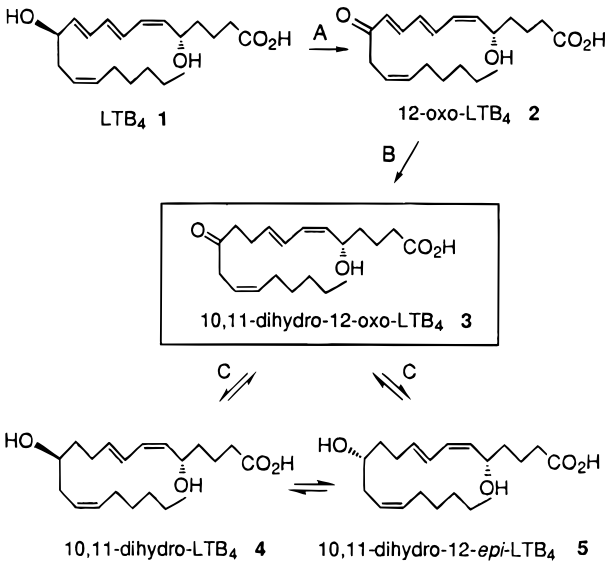
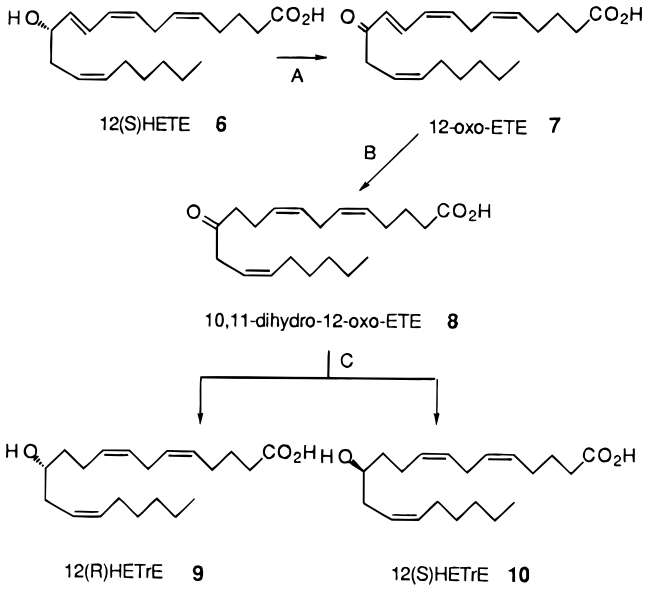
Scheme 1



A = 12-hydroxyeicosanoid dehydrogenase; B = Δ^{10}-reductase; C = 12-keto reductase.

Scheme 2



A = 12-hydroxyeicosanoid dehydrogenase; B = Δ^{10}-reductase; C = 12-keto reductase.

We cannot completely eliminate the possibility that direct enzymatic epimerization at the 12-hydroxy position results in the production of the epimeric metabolite **5** (Scheme 1). 12(*S*)-HETE is metabolized in a similar fashion by neutrophils to give 12-oxo-ETE (**7**), 10,11-dihydro-12-oxo-ETE (i.e. 12-oxo-ETrE) (**8**), and the 12-(*R*) and 12(*S*) isomers of 10,11-dihydro-12-HETE (i.e. 12-HETrE) (**9** and **10**).[18,23,24]

Preliminary evidence suggested that 12-oxo-LTB_4[25] and the R and S isomers of 10,11-dihydro-LTB_4[26,27] all possess lower biological activities than LTB_4. 12(*R*)-HETrE (**9**), on the other hand, was found to be a potent proinflammatory agent.[28,29] As can be seen from Scheme 2, 12(*R*)-HETrE (**9**) is probably formed through the intermediacy of 12-oxo-ETE (**7**), and we have previously described the total synthesis of 12-oxo-ETE (**7**) and studied its preliminary biological and biochemical profile.[24,30] In order to study the biological activity of 10,11-dihydro-12-oxo-LTB_4 (**3**) and to investigate its metabolism to 10,11-dihydro-LTB_4 (**4**) and its 12-*epi* isomer (**5**), it was important to have the authentic, chemically synthesized compound.

This paper describes the first total synthesis of 10,11-dihydro-12-oxo-LTB_4 (**3**) along with its 6,7-*trans* isomer (**27**). Scheme 3 describes the steps involved. The synthesis starts with the octenyl dithiane **12** prepared from nonenol **11** in a two-step one-pot reaction.[17] Alkylation of octenyl dithiane **12** with 2-(2-bromoethyl)-1,3-dioxolane gave dioxolane **13** in 94% yield. Aldehyde **14** was obtained in 74% yield by the hydrolysis of the dioxolane **13**. Wittig reaction of the aldehyde **14** with commercial trimethyl phosphonoacetate, using LDA as base, produced a mixture of *E*- and *Z*-isomers **16** and **15** in 65% and 21% yields, respectively. The *Z*-isomer **15**, upon treatment with iodine, was converted to the *E*-isomer **16**. Thus the *E*-isomer **16** was obtained in effective 86% yield. We have also developed conditions which minimize the formation of the *Z*-isomer **15**. The treatment of trimethyl phosphonoacetate with sodium hydride at room temperature followed by addition of aldehyde **14** afforded *E*-isomer **16** in 73% yield with the *Z*-isomer **15** being formed in 2.5% yield. Both procedures are described in the Experimental Section. The reduction of the ester **16** with DIBAL-H was clean and gave the alcohol **17** in nearly quantitative yield. Bromination of the alcohol **17** with CBr_4 and DIPHOS furnished bromide **18**. Due to the high reactivity of the allyl bromide **18** and presence of sulfur in the molecule, the bromide **18** was unstable. We prepared the bromide **18** and immediately treated it with Ph_3P to generate the phosphonium salt **19** which was purified by flash column chromatography to give pure **19** in 72% yield. The benzoate aldehyde **20**, which contains the chiral component of the 12-oxo-10,11-dihydro-LTB_4 molecule, was prepared by two different routes, as described before. One uses 2-deoxy-D-ribose as starting material,[2c,d,17] the other starts with D-arabinose.[2g,17] The Wittig coupling of the phosphonium salt **19** with aldehyde **20** followed by purification gave an 87:17 *Z* and *E* mixture of **21** and **24** in 89.6% yield. The separation of the individual isomers was carried out by normal phase HPLC. Hydrolysis of the benzoate **21** with sodium methoxide in methanol gave the hydroxy ester **22** in 75% yield. Hydroxy ester **22** was treated with 1 M LiOH in THF/H_2O to give, after purification by column chromatography, the hydroxy acid **23** in 93% yield. Finally, deprotection of the dithio acid **23** with [bis(trifluoroacetoxy)iodo]benzene[31,17] gave the 10,11-dihydro-12-oxo-LTB_4 **3** in 79% yield. The 6,7-*trans* isomer **24**, isolated

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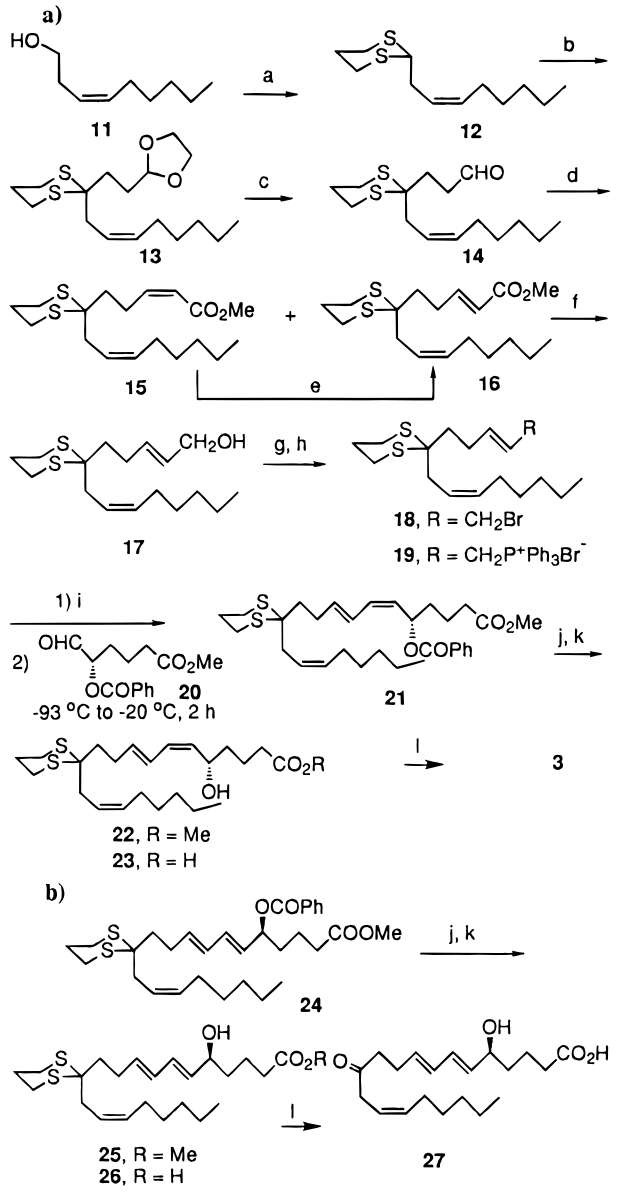
Scheme 3

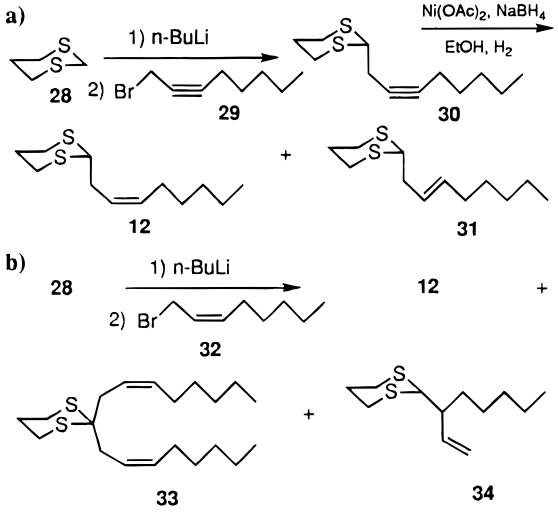


(a) 1) PCC/SiO_2 , 0 °C, 8 h, 2) 1,3-Propanedithiol, $BF_3{\cdot}Et_2O$, -30 °C to room temperature; (b) 1.2 eq BuLi -78 °C, at -20 °C 2 h; HMPA added at -78 °C, 10 min, then 2-(2-bromoethyl)-1,3-dioxolane, -78 °C to room temperature; (c) 2.0 M HCl/THF (2:3), reflux 6h; (d) Trimethyl phosphonoacetate, LDA -78 °C to -30 °C then aldehyde **14**, or NaH, room temperature then aldehyde **14**; (e) I_2; (f) DIBAL-H, -78 °C 1 h; (g) DIPHOS, CBr_4 0 °C 20 min; (h) bromide**18** in dry dichloromethane, Ph_3P room temperature overnight; (i) phosphonium salt **19** in THF, cooled to -93 °C, 1.05 eq. $NaN(SiMe_3)_2$,1 min; (j) NaOMe added at 0 °C, room temperature 10 min; (k) 1 M LiOH, THF/H_2O, room temperature 48 h; (l) $PhI(OCOCF_3)_2$, room temperature 3 min.

in the Wittig reaction, was converted to the 10,11-dihydro-6,7-*trans*-12-oxo-LTB$_4$ **27**, as shown in Scheme 3b.

Before we settled for the very convenient preparation of **12** in a two-step one-pot procedure, we developed conditions to alkylate dithiane **28** as shown in Scheme 4a,b. Alkylation of dithiane **28** with the allyl bromide **32** afforded the desired compound in 57% yield. Two byproducts **33** and **34** were isolated. The formation of byproduct **34** is reminiscent of the alkylation of formyl

Scheme 4



dithiane **35** with allyl halides (*vide infra*). In an attempt to improve on this reaction, we used the 3-nonynyl bromide **29** in the alkylation reaction (Scheme 4a).[24] This approach, which worked well, led also to the formation in varying degrees (2−5%) of the *trans* product. The purification of the desired dithiane **12** proved tedious, as the *trans* product **31** is very close to the *cis* on TLC. We attributed the formation of the *trans* product **31** to a free-radical-induced isomerization. The free radicals are presumably formed by hydrogenolysis of the sulfur−carbon bond during hydrogenation. The addition of a free-radical inhibitor such as 4-hydroxy-TEMPO reduces substantially the amount of *trans* product **31** formed, but does not eliminate it entirely.

It is worth mentioning that we had initially approached the synthesis of aldehyde **14** by another route shown in Scheme 5a. It is interesting to note that the main product of the reaction between **29** and **35** is the allene **42**, not the desired **36**. Intermediates **40** and **41** represent an attempt to rationalize the formation of the allene product **42**. This reaction is similar to the known alkylation of formyl dithiane **35** with allyl halides[32,33] in which the nucleophilic attack occurs at carbon 3 of the allyl halides. Although we were able to prepare **37** by formylation of **12**,[17] this approach was dropped because the reduction of **38** to **39** using hydride reducing agents did not work as well as some of our model compounds.

We compared the effects of 10,11-dihydro-12-oxo-LTB$_4$ on calcium mobilization and chemotaxis in neutrophils to those of LTB$_4$ and a number of its metabolites.[34] Although 10,11-dihydro-12-oxo-LTB$_4$ stimulated both calcium mobilization (EC_{50}, 400 nM) and chemotaxis (EC_{50}, ca. 20 uM), it was considerably less active than LTB$_4$. Introduction of a 12-oxo group or reduction of the 10,11-double bond each reduced the biological activity of LTB$_4$ by about 100-fold.[34] When these two modifications were combined in 10,11-dihydro-12-oxo-LTB$_4$, there was a further 10- to 100-fold reduction in potency.[34] The 6,7-*trans* isomer of 10,11-dihydro-12-oxo-LTB$_4$ displayed substantially lower potency than the 6,7-*cis* compound,

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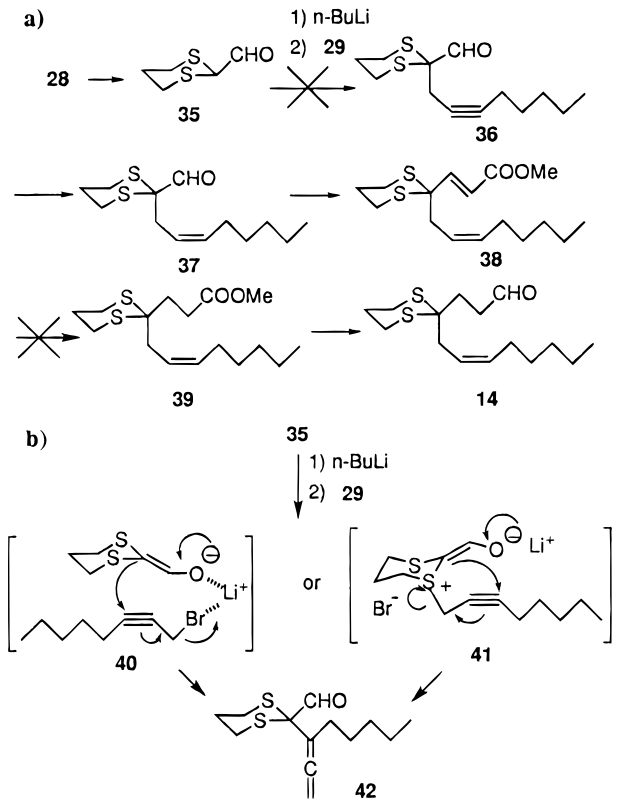
Scheme 5



as might be expected from the relatively low potencies of 6,7-*trans* isomers of LTB_4.[35,36]

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.

1-(2,4-Dioxolanyl)-4,4-(trimethylenedithio)dodec-6(*Z*)-enal (13). To a cooled (−78 °C) stirred solution of 2-octenyl dithiane **12** (2.3 g, 10 mmol) in THF (40 mL) was added n-BuLi (1.6 M, 7.5 mL, 12 mmol). The reaction mixture was stirred for 30 min at −78 °C and then slowly allowed to warm to −10 °C and stirred for 3 h at −10 °C. The reaction mixture was cooled to −78 °C, and HMPA (5 mL) was added dropwise. The resulting mixture was stirred further at −78 °C for 10 min, and then 2-(2-bromoethyl)-1,3-dioxolane (2.728 g, 15 mmol) in THF (4 mL) was added and allowed to warm to room temperature during 1 h. Workup consisted of the addition of saturated aqueous ammonium chloride solution (100 mL), extraction of the aqueous layer with diethyl ether (2 × 100 mL), washing the combined organic layers with brine (2 × 50 mL), drying over anhydrous Na_2SO_4, filtration, and concentration *in vacuo*. The crude product was obtained as a slightly yellow oil, which was purified by flash column chromatography using 10% ethyl acetate in hexane to give pure **13** (3.1 g, 94%). ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 6.9 Hz, 3 H), 1.27−1.40 (m, 6 H), 1.83 (m, 2 H), 1.87−2.0 (m, 2 H), 2.04 (m, 4 H), 2.57 (d, J = 6.7 Hz, 2 H), 2.71−2.80 (m, 2 H), 2.82−2.92 (m, 2 H), 3.84 (m, 2 H), 3.95 (m, 2 H), 4.95 (t, J = 4.5 Hz, 1 H), 5.42−5.60 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.22, 22.74, 25.39, 26.20 (2 × C), 27.89, 29.18, 29.36, 31.72, 32.01, 36.48, 52.98, 65.12 (2 × C), 104.37, 123.06, 133.62.

4,4-(Trimethylenedithio)dodec-6(*Z*)-enal (14). A solution of **13** (3.1 g, 9.1 mmol) in THF (40 mL) and 2 M HCl (30 mL) was refluxed for 6 h. The reaction mixture was cooled to room temperature, diluted with water (100 mL), neutralized with $NaHCO_3$ and extracted with diethyl ether (2 × 100 mL). The combined organic extract washed with water (2 × 50 mL) and brine (1 × 50 mL), dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated *in vacuo*. The crude product was obtained as a slightly yellow oil, which was purified by flash column chromatography using 5% ethyl acetate in hexane to give the aldehyde **14** (2.21 g, 85%). ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3 H), 1.21−1.40 (m, 6 H), 1.85−2.00 (m, 2 H), 2.05 (q, J = 7.2 Hz, 2 H), 2.27 (t, J = 7.2 Hz, 2 H), 2.57 (d, J = 6.9 Hz, 2 H), 2.64 (t, J = 7.1 Hz, 2 H), 2.70−2.78 (m, 2 H), 2.82−2.90 (m, 2 H), 5.41−5.50 (m, 1 H) 5.51−5.62 (m, 1 H), 9.82 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.26, 22.75, 25.15, 26.26 (2 × C), 27.92, 29.37, 30.44, 31.75, 36.92, 39.82, 52.62, 122.72, 133.98, 201.31.

Methyl 6,6-(Trimethylenedithio)-2(*E*),8(*Z*)-tetradecadienoate (16). Procedure a: Sodium hydride 60% dispersion (21 mg, 0.555 mmol) was placed in a flame-dried flask and washed with dry hexane (1 mL) under argon atmosphere. Anhydrous benzene (2 mL) was then added and, while stirring, trimethyl phosphonoacetate (134.7 mg, 0.185 mmol) was added dropwise under argon atmosphere. The white suspension formed was stirred at room temperature for 15 min, and then aldehyde **14** (53 mg, 0.185 mmol) in benzene (1 mL) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 1 h and then quenched with saturated aqueous ammonium chloride, diluted with benzene (5 mL), and acidified with 10% HCl. The benzene layer was separated and the aqueous layer extracted with benzene (10 mL). The combined extract was washed with water (3 × 5 mL) and dried over Na_2SO_4 and the solvent evaporated at reduced pressure to give the crude product, which was purified by flash column chromatography over silica gel using ethyl acetate/hexane (3:97) as an eluant. The less polar compound (1.6 mg), isolated in 2.5% yield, was characterized as the *Z*-isomer **15**. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 6.6 Hz, 3 H), 1.3−1.41 (m, 6 H), 1.90−2.12 (m, 6 H), 2.61 (d, J = 6.8 Hz, 2 H), 2.73−2.82 (m, 4 H), 2.83−2.92 (m, 4 H), 3.69 (s, 3 H), 5.45−5.60 (m, 2 H), 5.78 (d, J = 11.4 Hz, 1 H), 6.95 (dt, J = 11.5 and 3.7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.18, 22.79, 24.96, 25.42, 26.34 (2 × C), 27.96, 29.45, 31.79, 36.51, 37.36, 51.32, 53.18, 120.17, 123.16, 133.79, 149.31, 167.79. The more polar compound, obtained in 73% yield, was the desired ester **16**. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 6.7 Hz, 3 H), 1.22−1.35 (m, 6 H), 1.91−2.05 (m, 6 H), 2.34(q, J = 7.2 Hz, 2 H), 2.60 (d, J = 6.8 Hz, 2 H), 2.78−2.81 (m, 4 H), 3.70 (s, 3 H), 5.40−5.47 (m, 1 H), 5.51−5.61 (m, 1 H), 5.81(d, J = 15.7 Hz, 1 H), 6.95 (dt, J = 15.7 and 6.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.18, 22.71, 25.29, 26.26 (2 × C), 27.63, 27.92, 29.36, 31.72, 36.51 (2 × C), 51.57, 52.85, 121.41, 122.99, 133.75, 148.64, 167.09.

Procedure b: To a cooled (−78 °C), stirred solution of trimethyl phosphonoacetate (375 mg, 1.59 mmol) in THF (4 mL) was added LDA (1.5 M, 0.848 mL) (1.27 mmol) dropwise under argon. The reaction mixture was stirred at −78 °C for 15 min, and then a solution of aldehyde **14** (303 mg, 1.06 mmol) in THF (2 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 1 h, slowly allowed to warm to room temperature, and stirred at room temperature overnight. The resulting mixture was poured on saturated aqueous ammonium chloride solution (20 mL) and extracted with diethyl ether (2 × 50 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*. The product was purified by flash column chromatography using 5% EtOAc in hexane to give the less polar product **15** (75 mg, 21%) as a colorless thick oil. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 6.6 Hz, 3 H), 1.3−1.41 (m, 6 H), 1.90−2.12 (m, 6 H), 2.61 (d, J = 6.8 Hz, 2 H), 2.73−2.82 (m, 4 H), 2.83−2.92 (m, 4 H), 3.69 (s, 3 H), 5.45−5.60 (m, 2 H), 5.78 (d, J = 11.4 Hz, 1 H), 6.95

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(dt, J = 11.5 and 3.7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.18, 22.79, 24.96, 25.42, 26.34 (2 × C), 27.96, 29.45, 31.79, 36.51, 37.36, 51.32, 53.18, 120.17, 123.16, 133.79, 149.31, 167.79. The more polar product **16**, 236 mg, was isolated in 65% yield. The less polar *Z*-isomer **15** was dissolved in methylene chloride (5 mL), a catalytic amount of iodine was added, and the solution was stirred at room temperature for 2 days. It was converted to the (*E*)-ester **16**. Thus the (*E*)-ester **16** was obtained in total 86% yield. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 6.7 Hz, 3 H), 1.22−1.35 (m, 6 H), 1.91−2.05 (m, 6 H), 2.34 (q, J = 7.2 Hz, 2 H), 2.60 (d, J = 6.8 Hz, 2 H), 2.78−2.81 (m, 4 H), 3.70 (s, 3 H), 5.40−5.47 (m, 1 H), 5.51−5.61 (m, 1 H), 5.81(d, J = 15.7 Hz, 1 H), 6.95 (dt, J = 15.7 and 6.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.18, 22.71, 25.29, 26.26 (2 × C), 27.63, 27.92, 29.36, 31.72, 36.51 (2 × C), 51.57, 52.85, 121.41, 122.99, 133.75, 148.64, 167.09.

6,6-(Trimethylenedithio)-2(*E*),8(*Z*)-tetradecadien-1-ol (17). To a −78 °C cooled, stirred solution of ester (**16**) (830 mg, 2.42 mmol) in methylene chloride (80 mL) was slowly added DIBAL-H (5.3 mmol) dropwise under argon. The reaction mixture was stirred for 1 h at −78 °C and then quenched by the addition of methanol (1 mL), poured over cold water, acidified, and extracted with methylene chloride (2 × 100 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified by flash column chromatography (4:1 EtOAc/hexane) to afford the alcohol **17** (0.690 mg, 91%) as a colorless thick oil. ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 6.7 Hz, 3 H), 1.20−1.4 (m, 6 H), 1.95 (m, 4 H), 2.05 (m, 2 H), 2.2 (m, 2 H), 2.62 (d, J = 6.7 Hz, 2 H), 2.70 (m, 4 H), 4.13 (s, 2 H), 5.41−5.50 (m, 1 H), 5.51−5.59 (m, 1 H), 5.6−5.73 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.25, 22.77, 25.47, 26.31 (2 × C), 27.41, 27.97, 29.43, 31.78, 36.36, 37.88, 53.12, 63.88, 123.36, 129.70, 132.37, 133.56.

[6,6-(Trimethylenedithio)-2(*E*),8(*Z*)-tetradecadien-1-yl]-triphenylphosphonium Bromide (19). To a cooled (0−5 °C), stirred solution of alcohol **17** (640 mg, 2.03 mmol) and 1,2-bis(diphenylphosphino)ethane (800 mg, 2.03 mmol) in dry CH_2Cl_2 (5 mL) was slowly added carbon tetrabromide (1.01 g, 3.06 mmol) under argon. The reaction mixture was stirred for 10 min at 0−5 °C and then diluted with hexane (200 mL), and the resulting solution of the labile bromide **18** was quickly filtered through Celite. The filtrate was evaporated at reduced pressure, the residue was dissolved in dry acetonitrile (20 mL), and triphenylphosphine (800 mg, 3.06 mmol) was added. The reaction mixture was stirred for 24 h at room temperature 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 (930 mg, 72%) (overall yield from alcohol **17**) as a colorless fluffy solid. ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 6.7 Hz, 3 H), 1.2 (m, 6 H), 1.6−1.7 (m, 2 H),1.80−2.0 (m, 4 H), 2.0−2.1 (m, 2 H), 2.5 (d, J = 6.7 Hz, 2 H), 2.70 (m, 4 H), 4.6−4.7 (m, 2 H), 5.20−5.40 (m, 2 H), 5.40−5.50 (m, 1 H), 6.8−6.95 (m, 1 H), 7.6−7.7 (m, 6 H), 7.7−7.9 (m, 9 H); ^{13}C NMR ($CDCl_3$) δ 14.21, 22.66, 25.26, 26.21 (2 × C), 27.86, 28.25, 29.31, 31.65, 36.33, 52.83, 114.87, 117.78, 118.72, 123.20, 130.59, 133.47, 134.06, 134.17, 135.23, 147.87.

Methyl 5(*S*)-(Benzoyloxy)-12,12-(trimethylenedithio)-6(*Z*),8(*E*),14(*Z*)-icosatrienoate (21). To a cooled (−93 °C), stirred solution of the phosphonium salt **19** (384 mg, 0.6 mmol) in THF (16 mL) and HMPA (4 mL) was added lithium hexamethyldisilazide (1 M, 0.55 mL, 0.55 mmol) dropwise under argon. After stirring for 2 min, aldehyde **20** (131 mg, 0.5 mmol) in THF (2 mL) was added to the resulting red solution. The reaction mixture was stirred for 30 min at −93 °C, and at −78 °C for 2 h, and then allowed to warm slowly to 0 °C. It was then quenched by the addition of saturated aqueous ammonium chloride solution (50 mL) and extracted with diethyl ether (3 × 50 mL). The combined extract was 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 to give an 83:17 mixture of Z- and *E*-isomers (244 mg, 89.6%). The separation of the *Z*- and *E*-isomers was carried out by NP HPLC [μ-poracil silica, 7.8 × 300 mm; solvent system 8% EtOAc in hexane, flow rate 4 mL/min (retention time for *Z*-isomer 8.7 min, and retention time for *E*-isomer 10.85 min). The NP HPLC separation gave pure *Z*-isomer **21** (154 mg, 47%), ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.22−1.41 (m, 6 H), 1.67−2.03 (m, 8 H), 2.09 (q, J = 6.9 Hz, 2 H), 2.30 (m, 2 H), 2.37 (t J = 7.0 Hz, 2 H), 2.63 (d, J = 6.8 Hz, 2 H), 2.74− 2.92 (m, 4 H), 3.67 (s, 3 H), 5.34 (t, J = 10.1 Hz, 1 H), 5.46−5.63 (m, 2 H), 5.77 (dt, J = 15.0, 7.0 Hz, 1 H), 5.90 (m, 1 H), 6.11 (t, J = 11.0 Hz, 1 H), 6.52 (dd, J = 14.6, 11.5 Hz, 1 H), 7.43 (t, J = 7.4 Hz, 2 H), 7.55 (t, J = 7.4 Hz, 1 H), 8.04 (dd, J = 7.2 and 1.3 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.23, 20.79, 22.74, 25.43, 26.28 (2 × C), 27.93, 28.24, 29.40, 31.74, 33.90, 34.53, 36.46, 37.69, 51.70, 53.14, 70.89, 123.24, 125.78, 126.81, 128.78 (2 × C), 129.78 (2 × C), 130.73, 132.22, 133.00, 133.60, 137.27, 134.43, 166.04, 173.81; HREIMS calcd ($C_{31}H_{44}S_2O_4$, M^+) 544.2670, obsd 544.2693. Pure *E*-isomer **24**, 32 mg (9.8%), was obtained, ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.23−1.40 (m, 6 H), 1.69−1.88 (m, 4 H), 1.92−2.00 (m, 4 H), 2.06 (q, J = 7.1 Hz, 2 H), 2.2−2.28 (m, 2 H), 2.37 (t, J = 6.8 Hz, 2 H), 2.63 (d, J = 6.7 Hz, 2 H), 2.82 (m, 4 H), 3.68 (s, 3 H), 5.42−5.65 (m, 4 H), 5.76 (dt, J = 15.1, 6.8 Hz, 1 H), 6.04 (dd, J = 15.2, 10.6 Hz, 1 H), 6.30 (dd, J = 15.0, 10.5 Hz, 1 H), 7.45 (t, J = 7.4 Hz, 2 H), 7.56 (t, J = 7.4 Hz, 1 H), 8.05 (dd, J = 7.1 and 1.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.25, 20.87, 22.77, 25.45, 26.30 , 27.92 (2 × C), 29.42, 31.77, 33.89, 34.21, 36.41, 37.70, 51.74, 53.10, 74.83, 123.26, 128.53 (2 × C), 128.89, 129.80, 129.84, 130.75 (2 × C), 132.22, 133.07, 133.41, 137.61, 135.51, 166.02, 173.89

Methyl 5(*S*)-Hydroxy-12,12-(trimethylenedithio)-6(*Z*),8(*E*),14(*Z*)-icosatrienoate (22). Sodium methoxide (1 mL, 25% wt in methanol) was added to a solution of benzoate ester **21** (36 mg, 0.066 mmol) in methanol (1.5 mL) at 0 °C and then the reaction mixture was stirred for 10 min at room temperature. After acidification with aqueous 5% KH_2PO_4 buffer (pH 4.3), the organic material was extracted with ethyl acetate (3 × 10 mL). The combined ethyl acetate extract was washed with cold water (1 × 10 mL) and brine (1 × 10 mL), dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated under reduced pressure to afford the crude hydroxy methyl ester product **22** which was purified by column chromatography using ethyl acetate hexane (20:80) to give pure **22** (27 mg, 93% yield). ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.9 Hz, 3 H), 1.25−1.43 (m, 6 H), 1.45−1.80 (m, 4H), 1.93−2.02 (m, 4 H), 2.08 (q, J = 6.9 Hz, 2 H), 2.22−2.33 (m, 2 H), 2.37 (t, J = 6.9 Hz, 2 H), 2.65 (d, J = 6.8 Hz, 2 H), 2.84 (m, 4 H), 3.68 (s, 3 H), 4.58 (m, 1 H), 5.30 (t, J = 10.1 Hz, 1 H), 5.45−5.53 (m, 1 H), 5.55−5.65 (m, 1 H), 5.75 (dt, J = 14.9, 6.9 Hz, 1 H), 6.03 (t, J = 10.1, 1 H), 6.35 (dd, J = 14.7, 10.4 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 14.03, 20.76, 22.53, 25.19, 26.06, 27.73, 27.90, 29.18, 31.53, 33.81, 33.16, 36.69, 37.56, 51.51, 52.85, 67.44, 123.04, 125.42, 130.47, 131.38, 133.39, 136.22, 173.95.

5(*S*)-Hydroxy-12,12-(trimethylenedithio)-6(*Z*),8(*E*),10(*E*),14(*Z*)-eicosatrienoic Acid (23). A solution of the dithio compound **22** (22 mg) in THF (2.3 mL) and 1 M LiOH (750 μL) was stirred at room temperature overnight. It was acidified with 1 M HCl and extracted with ethyl acetate (3 × 10 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 hydroxy acid, which was purified by flash column chromatography over silica gel using 1:9 MeOH/CH_2Cl_2 to give the pure hydroxy acid **23** (19 mg, 88%). ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3 H), 1.23−1.42 (m, 7 H), 1.50−1.70 (m, 3 H), 1.96 (m, 4 H), 2.08 (q, J = 7.1 Hz, 2 H), 2.41 (t, J = 7.1 Hz, 2 H), 2.65 (d, J = 6.8 Hz, 2 H), 2.84 (m, 4H), 4.60 (m, 1 H), 5.31 (t, J = 10.4 Hz, 1 H), 5.45−5.53 (m, 1 H), 5.54−5.63 (m, 1 H), 5.75 (dt, J = 14.3, 6.8 Hz, 1 H), 6.04 (t, J = 11.1 Hz, 1 H), 6.35 (dd, J = 14.5, 11.7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.00, 20.57, 22.30, 25.21, 26.09 (2 × C), 27.76, 27.92, 29.21, 31.56, 33.59, 36.15, 36.53, 37.53, 52.70, 67.52, 123.05, 125.39, 130.58, 133.38, 131.25, 133.44, 136.35, 178.93. Electrospray MS calcd [$C_{23}H_{38}S_2O_3$ + Na, (M + Na)$^+$] 449, obsd 449.

5(*S*)-Hydroxy-12,12-(trimethylenedithio)-6(*Z*),8(*E*),14(*Z*)-eicosatrienoic Acid (10,11-dihydro-12-oxo-LTB$_4$) (3). A solution of dithio acid **23** (5.8 mg, 0.0136 mmol) in methanol/H_2O (9:1, 1.5 mL) and [bis(trifluoroacetoxy)iodo] benzene (15

mg, 0.0348 mmol) was stirred at room temperature for 2 min. The reaction mixture was diluted with water (20 mL) and extracted with ethyl ether (3 × 20 mL). The combined extract was washed with cold water (7 × 10 mL) and brine (1 × 10 mL), dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated under reduced pressure to afford the crude product **3** which was purified by flash column chromatography over silica gel using 1:19 MeOH/CH_2Cl_2 to give the pure 10,-11-dihydro-12-oxo-LTB$_4$ (**3**) (3.6 mg, 79%). ^{1}H NMR (C_6D_6) δ 0.95 (t, J = 7.2 Hz, 3 H), 1.25−1.75 (m, 10 H), 1.98 (q, J = 7.0 Hz, 2 H), 2.15 (m, 4 H), 2.35 (q, J = 7.2 Hz, 2 H), 2.92 (d, J = 6.8 Hz, 2 H), 4.69 (q, J = 7.1 Hz, 1 H), 5.31 (t, J = 10.1 Hz, 1 H), 5.55−5.75 (m, 3 H), 5.98 (t, J = 11.1 Hz, 1 H), 6.42 (dd, J = 11.9, 4.0 Hz, 1 H). Electrospray MS calcd [($C_{20}H_{32}O_4$ + Na) − H_2O, (M + Na)$^+$ − H_2O] 341, obsd 341.

Methyl 5(*S*)-Hydroxy-12,12-(trimethylenedithio)-6(*E*), 8(*E*),14(*Z*)-eicosatrienoate (25). 5(*S*)-(Benzoyloxy)-12,12-(trimethylenedithio)-6(*E*),8(*E*),14(*Z*)-eicosatrienoate (**24**) was hydrolyzed to **25** in nearly quantitative yield, as described for the preparation of **22**. ^{1}H NMR (C_6D_6) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.23−1.40 (m, 6 H), 1.50−1.60 (m, 2 H), 1.62−1.75 (m, 2 H), 1.92−2.00 (m, 4 H), 2.06 (q, J = 7.0 Hz, 2 H), 2.24 (m, 2 H), 2.35 (t, J = 7.4 Hz, 2 H), 2.64 (d, J = 5.7 Hz, 2 H), 2.83 (m, 4 H), 3.67 (s, 3 H), 4.12 (m, 1 H), 5.42−5.50 (m, 1 H), 5.55−5.62 (m, 2 H), 5.69 (dt, J = 14.2, 6.8 Hz, 1 H), 6.05 (dd, J = 15.0, 10.4 Hz, 1 H), 6.17 (dd, J = 15.1, 10.6 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 14.03, 20.79, 22.53, 25.21, 26.05 (2 × C), 27.06, 27.73, 29.19, 31.77, 36.09, 36.49, 37.56, 51.50, 52.85, 72.22 123.04, 129.84, 130.88, 133.36, 133.60, 134.25, 174.00.

5(*S*)-Hydroxy-12,12-(trimethylenedithio)-6(*E*),8(*E*), 10(*E*),14(*Z*)-eicosatrienoic Acid (26). Methyl-5(*S*)-hydroxy-12,12-(trimethylenedithio)-6(*E*),8(*E*),14(*Z*)-eicosatrienoate (**25**) was hydrolyzed to the hydroxy acid **26** in 94% yield as described for the preparation of **23.** ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.7 Hz, 3 H), 1.23−1.43 (m, 6 H), 1.51−1.80 (m, 4 H), 2.07 (m, 2 H), 2.25 (m, 2 H), 2.38 (t, J = 7.1 Hz, 2 H), 2.64 (d, J = 6.5 Hz, 2 H), 2.83 (m, 4 H), 4.15 (m, 1 H), 5.42−5.52 (m, 1 H), 5.52−5.63 (m, 2 H), 5.70 (dt, J = 14.3, 6.5 Hz, 1 H), 6.05 (dd, J = 14.8, 10.5 Hz, 1 H), 6.18 (dd, J = 14.9, 10.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.04, 20.56, 22.54, 25.21, 26.05 (2 × C), 27.73, 29.19, 31.54, 33.64, 36.10, 36.30, 37.54, 52.86, 72.31, 123.04, 129.80, 131.03, 133.38, 133.39, 134.39, 178.90.

5(*S*)-Hydroxy-12-oxo-6(*E*),8(*E*),14(*Z*)-eicosatrienoic Acid (10,11-dihydro-6,7-*trans*-12-oxo-LTB$_4$) (27). Deprotection of the dithio group in **26**, as described for the preparation of **3**, produced **27** in 77% yield. ^{1}H NMR (C_6D_6) δ 0.89 (t, J = 6.9 Hz, 3 H), 1.15−1.70 (m, 12 H), 1.95 (q, J = 7.0 Hz, 2 H), 2.10 (m, 4 H), 2.30 (q, J = 7.1 Hz, 2 H), 2.86 (d, J = 7.0 Hz, 2 H), 3.84 (m, 1 H), 5.4−5.70 (m, 4 H), 5.98 (dd, J = 14.6, 10.4 Hz, 1 H), 6.08 (dd, J = 14.4, 10.5 Hz, 1 H).

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectral data for new compounds described herein (25 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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