

282, 291 nm (ϵ 29 200, 35 300, 28 300). The vinyl protons showed the same pattern in the ^1H NMR spectrum as LTE_4 dimethyl ester. Anal. ($\text{C}_{25}\text{H}_{41}\text{NO}_5\text{S}$) C, H, N, S. (5*R*,6*R*) Isomer: $[\alpha]^{20}_{\text{D}} +7.9^\circ$ (c 0.47, dioxane). Anal. ($\text{C}_{25}\text{H}_{41}\text{NO}_5\text{S}$) C, H, N, S.

Desulfurization of the (5*S*,6*S*) material gave a lactone with a *positive* rotation.

Hydrolysis with potassium hydroxide in aqueous methanol and purification by reverse phase chromatography as before yielded the mono-potassium salt: UV max (methanol) 270, 279.5, 290 nm. Assuming an ϵ 280, 42 000, this material contained 85% LTE_4 equivalent.

(5*S*)-5-Hydroxyeicosanoic Acid Lactone (22). A solution of the dimethyl ester (5*R*,6*S* isomer, 1 g) in ethanol (30 mL) containing Raney nickel (5 mL, aqueous slurry) was stirred at room temperature for 2 h, filtered, and concentrated. The residue was dissolved in ethyl acetate (25 mL), dried (MgSO_4), stirred for 5 min with a palladium catalyst (5% on carbon, 500 mg), and filtered free of solids. The ethyl acetate solution was then treated with more of the same catalyst and hydrogenated at room temperature and pressure until the uptake of hydrogen stopped. The solids were then removed and the mixture was concentrated and the residue treated with base as in the case of **16**. The crude hydroxy acid was dissolved in dichloromethane (50 mL), treated with trifluoroacetic acid (0.1 mL), left at room temperature for 1 h, and then washed (Na_2CO_3 , brine), dried (MgSO_4), and concentrated. Purification of the residue by HPLC (4:1 hexane-ethyl acetate) gave the *total* lactone fraction (100 mg). This material was dissolved in heptane and subjected to flash chromatography to yield the pure lactone (98.3 mg). This second purification step is needed to remove impurities carried over by the reagent grade hexane used in the HPLC purification: mp 52–56°; $[\alpha]^{20}_{\text{D}} -26^\circ$ (c 2.07, dioxane). Anal. ($\text{C}_{20}\text{H}_{38}\text{O}_2$) C, H.

To estimate the optical purity of the lactone, the above sample was converted to the orthoester mixture as before. GLC indicated a 93:7 mixture of orthoesters.

Homo- LTE_4 Dimethyl Ester. The bromide **21** (14 g, 50 mmol) was converted into the salt as before and allowed to react with the aldehyde **7c** (14 g, 97 mmol, freshly distilled) as before to yield the pure *cis*-epoxide (2.4 g, 14%), a mixed fraction (1.5 g, mainly *trans*), and the pure *trans*-epoxide (4.9 g, 28%): UV max (hexane) 261, 273, 284 nm (ϵ 27 300, 34 100, 25 200); ^1H NMR (CCl_4) δ 6.48 (dd, 1, $J = 15$, 11 Hz, H-9), 6.32 (dd, 1, $J = 15$, 11 Hz, H-8), 5.54 (d, 1, $J = 15$ Hz, H-10), 5.42 (dd, 1, $J = 15$, 8 Hz, H-7), 3.62 (s, 3, OCH_3), 3.22 (m, 2, H-13), 2.99 (dd, $J = 8$, 2 Hz, H-6), 2.72 (m, 2, H-5). Anal. ($\text{C}_{22}\text{H}_{30}\text{O}_3$) C, H. Hydrogenation of this material (4.9 g, 14.3 mmol) in hexane (100 mL) followed by HPLC yielded the pure tetraene (1.7 g, 34%). The ^1H NMR (C_6D_6) showed the same features as in the case for racemic **2**. This epoxide (1.7 g, 4.9 mmol) was added to L-cysteine methyl ester (from the hydrochloride 1.7 g) in a mixture of methanol (8 mL) and water (2 mL) at pH 9 (triethylamine) and left at room temperature for 4 h. The solvents were then removed in vacuo and the residue was partitioned between ethyl acetate and water, and the crude cysteine derivatives were

then purified by HPLC (5% methanol in 1:1 hexane-ethyl acetate). (6*R*,7*S*) isomer (0.8 g): $[\alpha]^{20}_{\text{D}} -12.6^\circ$ (c 1.725, dioxane); UV max (ethanol) 272, 281, 290 nm (ϵ 26 500, 32 600, 26 400). Anal. ($\text{C}_{26}\text{H}_{43}\text{NO}_5\text{S}$) C, H, N, S. (6*S*,7*R*) Isomer (0.65 g): $[\alpha]^{20}_{\text{D}} +39.2^\circ$ (c 2.42, dioxane); UV max (ethanol) 271, 281, 290 nm (ϵ 29 100, 36 000, 29 100); ^1H NMR (CDCl_3) δ 6.54 (dd, 1, $J = 14$, 11 Hz, H-4), 6.28 (m, 1, $J = 14$ Hz, H-2), 6.20 (m, 1, $J = 14$ Hz, H-3), 6.03 (ddt, 1, $J = 11$, 2 Hz, H-5), 5.64 (dd, 1, $J = 14$, 10 Hz, H-1), 3.76 and 3.67 (2 s, 6, CO_2CH_3), 2.95 (m, 2, H-7), 2.84 (ddd, 2, $J = 12$, 8, 5 Hz, SCH_2). Anal. ($\text{C}_{26}\text{H}_{43}\text{NO}_5\text{S}$) C, H, N, S.

Nor- LTE_4 Dimethyl Ester. The bromide **21** (20 g, 71.7 mmol) was converted into the sulfonium salt and allowed to react with the aldehyde **7b** (25 g, 216 mmol, freshly distilled) as before. Purification by HPLC (2% triethylamine in 3:1 hexane-ether) gave the pure *cis*-epoxide (3.4 g, 15%) and the *trans*-epoxide (8.6 g, 37%). Crystallization of the *trans* isomer from hexane gave the analytical sample: mp 46–48°; UV max (hexane) 262, 272, 284 nm (ϵ 34 300, 44 000, 36 200); ^1H NMR (CDCl_3) δ 6.55 and 6.40 (2 dd, 2, $J = 14.5$, 11 Hz, H-2 and H-3), 5.61 (d, 1, $J = 14.5$ Hz, H-4), 5.45 (dd, 1, $J = 14.5$, 8 Hz, H-1), 3.69 (s, 3, CO_2CH_3), 3.29 (s, 2, H-7), 3.15 (dd, 1, $J = 8$, 2 Hz, H-3), 2.92 (dt, $J = 6$, 2 Hz, H-2). Anal. ($\text{C}_{20}\text{H}_{26}\text{O}_3$) C, H.

Hydrogenation of the *trans*-epoxide (8 g, 25.5 mmol) in hexane (150 mL) as before yielded the desired tetraene (3.5 g, 43%) after HPLC purification (2% triethylamine in 5% ethyl acetate-hexane): ^1H NMR (C_6D_6) δ 6.55 (dd, 1, $J = 15$, 11 Hz, H-4), 6.33 (dd, 1, $J = 15$, 11 Hz, H-2), 6.09 (dd, 1, $J = 15$, 11 Hz, H-3), 6.03 (t, 1, $J = 11$ Hz, H-5), 5.5 (m, 3, H-6, H-8, and H-9), 5.28 (dd, 1, $J = 15$, 8 Hz, H-1), 3.33 (s, 3, CO_2CH_3), 3.0 (m, 2, H-7), 2.96 (dd, 1, $J = 8$, 2 Hz, H-3), 2.7 (dt, 1, $J = 8$, 2 Hz, H-2). Anal. ($\text{C}_{20}\text{H}_{30}\text{O}_3$) C, H.

Cysteine methyl ester hydrochloride (2.4 g) was converted to the free amine in aqueous methanol as before and then allowed to react with the *trans*-epoxide (2.35 g, 7.4 mmol) for 1 h at room temperature. Workup as before and purification by HPLC (3% methanol in 1:1 hexane-ethyl acetate) yielded the pure (4*R*,5*S*) isomer (1 g) and the (4*S*,5*R*) isomer (1.25 g). The (4*S*,5*R*) diastereomer was contaminated by the γ -lactone which could be removed by repeated HPLC purification. (4*R*,5*S*) Isomer: $[\alpha]^{20}_{\text{D}} -19.5^\circ$ (c 3.07, dioxane). Anal. ($\text{C}_{24}\text{H}_{39}\text{NO}_5\text{S}$) C, H, N, S. (4*S*,5*R*) Isomer: $[\alpha]^{20}_{\text{D}} +27^\circ$ (c 3.14, dioxane). Anal. ($\text{C}_{24}\text{H}_{39}\text{NO}_5\text{S}$) C, H, N, S.

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Enantiospecific Syntheses of Leukotrienes C_4 , D_4 , and E_4 and $[14,15\text{-}^3\text{H}_2]$ Leukotriene E_4 Dimethyl Ester

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Abstract: A "chiral-pool" approach was employed to synthesize various leukotrienes (slow-reacting substance of anaphylaxis, SRS-A) enantiospecifically. The pivotal (*S,S*)-*trans*-epoxy alcohol **9** was prepared by efficient and facile routes starting from erythorbic acid (*D*-araboascorbic acid, **13**). This epoxide could also be produced starting from D-glucose. The epimeric (*S,R*)-*cis*-epoxide **38** was obtained utilizing L-tartaric acid as the chiral starting material. Elaboration of **9** into leukotriene A_4 methyl ester (**5**) and the potassium salts of leukotrienes C_4 (**4a**), D_4 (**4b**), and E_4 (**4c**) was accomplished by standard methods. These salts exhibited potent contractile activities in the in vitro guinea pig ileum assay. Reduction of 14,15-dehydroleukotriene A_4 methyl ester (**44**) with tritium gas gave $[14,15\text{-}^3\text{H}_2]$ -**5** and subsequently the dimethyl ester of $[14,15\text{-}^3\text{H}_2]$ leukotriene E_4 having a high specific activity of 40 Ci/mmol.

Slow-reacting substance of anaphylaxis (SRS, SRS-A) is, along with histamine and certain chemotactic peptides, an important

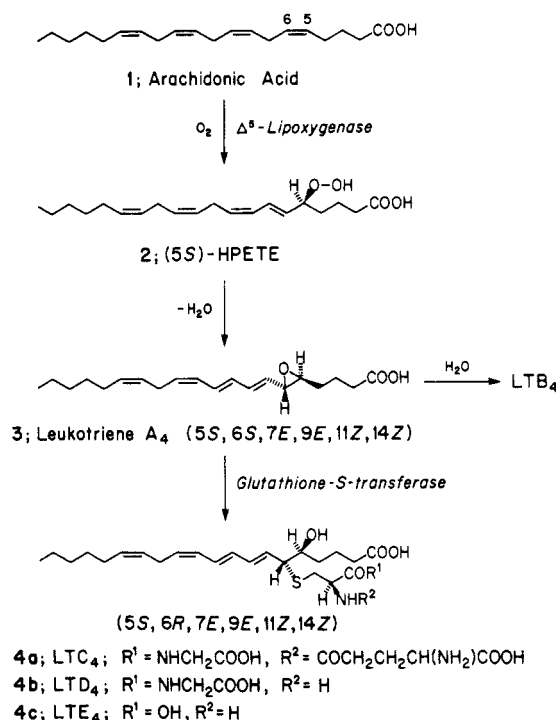
mediator of asthma and other conditions of hypersensitivity.¹ This factor has been isolated from a variety of mammalian cells upon

antigen (SRS-A) or chemical (SRS) challenge and is now known to be composed of a family of potent bronchoconstricting peptidolipids whose effects are slower in onset but stronger and longer in duration than those of histamine.

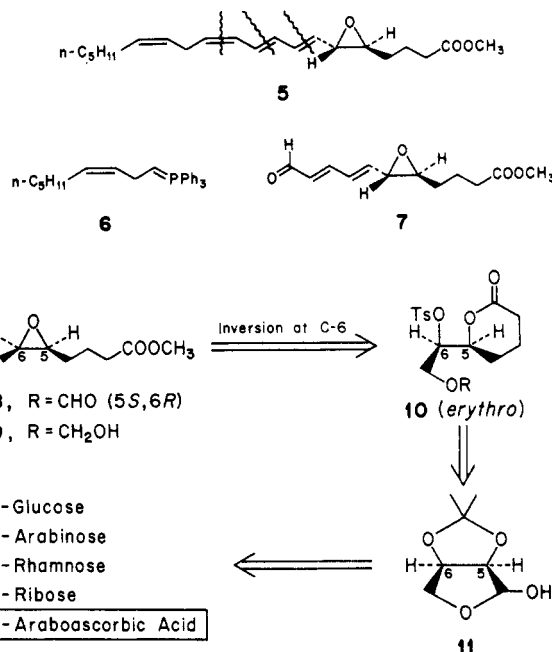
While the existence of SRS-A has been known for over 40 years, it was not until 1979 that the characterization of this extremely unstable spasmogenic factor as a family of arachidonic acid metabolites was achieved, largely through the efforts of Samuelsson and co-workers. Thus far, three compounds named leukotriene C₄ (LTC₄, **4a**), leukotriene D₄ (LTD₄, **4b**), and leukotriene E₄ (LTE₄, **4c**) have been fully characterized and shown to be the components of SRS-A.² This new and structurally intriguing class of substances thus joins the prostaglandins, thromboxanes, and prostacyclin as metabolites of arachidonic acid (eicosanoids) whose biochemical and physiological significance is immense. The structural elucidation of the leukotrienes has therefore led to an explosion in synthetic, biochemical, and pharmacological research¹⁻³ in both academic and industrial institutions, the ultimate goals of which are novel and improved treatments for serious afflictions such as bronchial asthma and related conditions of hypersensitivity.

Whereas the prostaglandins, thromboxanes, and prostacyclin originate by way of the cyclooxygenase pathway of arachidonic acid metabolism, the leukotrienes are formed via the lipoxygenase mode as shown in Scheme I.² Thus arachidonic acid (**1**) is released from phospholipids and undergoes addition of oxygen mediated by the enzyme Δ^5 -lipoxygenase affording the (5S)-hydroperoxide **2**. Dehydration of the latter intermediate (presumably enzymatically mediated and initiated by loss of a proton at C-10) produces the pivotal and extremely unstable epoxy acid **3** (LTA₄) having the absolute configuration and olefin geometry depicted. Enzymatic hydration of LTA₄ yields a (5S,12R,6Z,8E,10E,14Z) diol tetraene known as LTB₄ which is a potent chemotactic factor for leukocytes thought to be involved in inflammatory processes.^{2d} On the other hand, glutathione-S-transferase converts LTA₄ stereo- and regiospecifically to the conjugate **4a** (LTC₄). Further enzymatic processes transform **4a** into the Cys-Gly adduct **4b** (LTD₄) and the cysteine derivative **4c** (LTE₄). This sequence of events can occur, for example, in

Scheme I



Scheme II



the lung mast cell of a sensitized individual upon exposure to an antigen, and the leukotrienes (SRS-A) thus released (IgE-dependent) are thought to contribute significantly to constriction of the peripheral airways of the lung during an allergic asthmatic episode. In addition, the leukotrienes have been shown to induce increased vascular permeability and plasma leakage.

In view of the obvious importance of this class of compounds, we, as well as many other groups of workers,³ sought synthetic access to the leukotrienes. Our main goal was to provide a reliable supply of the pure, natural LT's. With these materials in hand, the establishment and maintenance of pharmacological assays designed to detect novel bronchopulmonary agents such as SRS-A antagonists and synthesis inhibitors would be facilitated. Hopefully, the synthetic methodology developed would be applicable to the preparation of isotopically labeled LT's for use in the development of radioimmunoassay procedures⁴ and for receptor

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16 readily afforded D-erythrionolactone (**15**) which gave the acetonide lactone **17** in standard fashion.¹⁹ After modification of this sequence to facilitate scale-up, we were able to obtain **17** in 30% overall yield.

A much more efficacious route to **17** involves oxidation of **13** with alkaline hydrogen peroxide in the manner that Isbell and Frush²⁰ obtained L-threonic acid from L-ascorbic acid. Their oxidation procedure was modified and, after acidification, a mixture of the desired lactone **15** and oxalic acid was produced. While the diol can be isolated at this stage if desired, direct exposure of the mixture to 2,2-dimethoxypropane-*p*-toluenesulfonic acid¹⁹ gave **17** in 60–70% overall yield. We believe this is the most expeditious route to **15** and **17** presently available. The peroxide-mediated extrusion of oxalic acid from **13** most likely involves the indicated fragmentation of intermediate **12**, derived from attack of peroxide anion on dehydroerythorbic acid (cf. **14**).

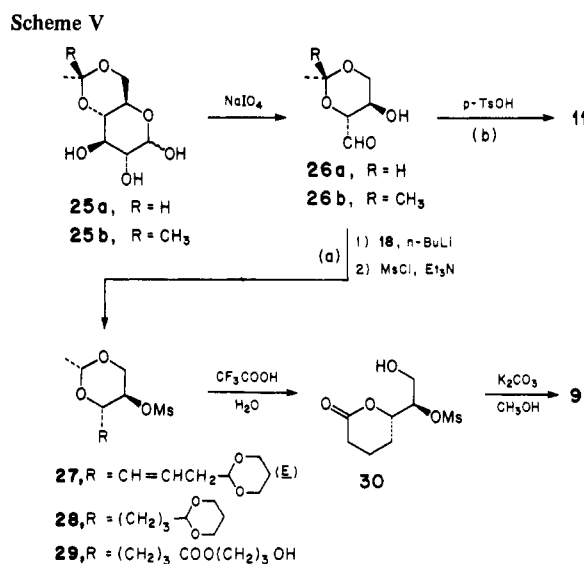
Our approach might be considered uneconomical in that we are eliminating as oxalic acid, two carbons of our six-carbon "chiral pool" starting material only to subsequently add three carbons (see below). The loss is minimal, however, since the carbon atoms discarded bear no chirality and are already in a highly oxidized and therefore essentially useless state.

Diisobutylaluminum hydride reduction of **17**,¹⁹ in dichloromethane at -78 °C, furnished, in 90% yield, the D-erythrose derivative **11**. By means of these processes, this lactol, our key chiral building block,²¹ was readily available in quantities of hundreds of grams.

We had initially hoped to introduce the required propionic acid moiety (C-1-C-3 of **9**) directly via a Wittig reaction of lactol **11** with the known²² (3-carboxypropyl)triphenylphosphonium bromide. Unfortunately, all attempts to effect this coupling were unsuccessful. In contrast, the phosphorane derived from acetal phosphonium salt **18**²³ (Scheme IV) smoothly condensed with **11** giving, after benzylation of the intermediate primary alcohol, the Z-unsaturated bis-acetal ester **19a** in excess of 70% yield. Catalytic hydrogenation then furnished **20a**. No epimerization was detected throughout this sequence.

We now addressed the problem of adjusting the oxidation state at C-1. While selective hydrolytic liberation of the protected aldehyde, in the presence of the isopropylidene moiety, and subsequent oxidation did not appear to be a viable approach, the studies of Deslongchamps²⁴ involving acetal oxidations with ozone provided us with an attractive alternative. Thus exposure of **20a** to ozone at -70°C smoothly gave the hydroxypropyl ester **21a**. In a crucial transformation, aqueous trifluoroacetic acid treatment of **21a** afforded the beautifully crystalline *erythro*-hydroxy lactone **22a**, in 70% yield. In this process, the hydroxyl functions at C-5 and C-6 are differentiated by lactone formation selectively liberating the C-6 OH for conversion into a leaving group.

Although the toluenesulfonate derivative of **22a** formed only sluggishly, the corresponding methanesulfonate **23** (cf. **10**) could be obtained in nearly quantitative yield under standard conditions.²⁵ Having our key intermediate in hand, we were now ready to test the epoxide synthesis. We were pleased to find that treatment of **23** with sodium methoxide or methanolic potassium carbonate readily provided the desired epoxide **9**. No isomeric epoxides could be detected in the crude reaction mixture. A sample of **9** prepared in this way was converted to the (*R*)-MTPA derivative. Examination of the ¹H NMR spectrum of this epoxy diester revealed no diastereomeric impurity, thus indicating that



our epoxy alcohol was essentially enantiomerically pure.²⁶

Subsequently, we found that protection of the primary alcohol throughout the preceding sequence as the benzoyl derivative was unnecessary, and the scheme could be shortened by the elimination of one step as follows. Tosylation of the initial product obtained by Wittig reaction of **11** and **18** gave the olefinic bis-acetal **19b** which, upon catalytic hydrogenation, afforded **20b**. This tosylate smoothly underwent ozone oxidation²⁴ yielding ester **21b**. In analogy with the benzoyl derivative, tosylate **21b** produced the desired *erythro*-hydroxy lactone **22b**, again a nicely crystalline intermediate, upon hydrolysis in aqueous trifluoroacetic acid. The initial product arising from methanolysis of **22b** was the terminal epoxide **24**^{3n,o} which could be isolated if desired; however, exposure to a catalytic amount of methoxide caused isomerization to the desired, thermodynamically preferred, disubstituted epoxide **9**.^{3n,o} It should be noted that conversion of **24** to **9** involves stereochemical inversion at C-6.

Synthesis of Epoxide 9 from D-Glucose

We also investigated the synthesis of the key C₇-epoxide **9** utilizing D-glucose as the "chiral pool" starting material (Scheme V). Following literature procedures,^{13c,27} 4,6-*O*-ethylidene-D-glucose (**25a**) was oxidatively degraded to the dimer²⁷ of 2,4-*O*-ethylidene-D-erythrose (**26a**). Freshly prepared monomeric **26a**²⁷ was then subjected to a sequence similar to those described above involving, initially, Wittig reaction with excess phosphorane derived from salt **18**. Mesylation of the crude product gave **27**. While not crucial to the synthetic plan, it is interesting to note that the olefinic linkage produced by this Wittig reaction possesses the *E* geometry, a result due to the effect of the β-alkoxy function being situated in a position which allows equilibration of the phosphobetaine intermediate. Catalytic hydrogenation of **27** gave bis-acetal **28** which was selectively oxidized to ester **29** with ozone at -78 °C.²⁴ Under these conditions, we found no evidence for oxidation of the trisubstituted dioxane ring.

Hydrolysis of **29** in aqueous trifluoroacetic acid gave the lactone **30** along with many other products. Alternative hydrolysis conditions failed to provide improved results. Treatment of crude **30** with methanolic potassium carbonate afforded, after chromatographic separation, **9** identical with the epoxide produced starting from erythorbic acid. Unfortunately, the overall yield of **9** produced by the sequence shown in Scheme V is unsatisfactory and we do not consider this scheme to be competitive with those described earlier.

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respectively, in the presence of triethylamine. Saponification of the adducts so obtained followed by reverse-phase chromatography afforded the purified salts which were found to be at least 95% isomerically pure by RPLC analysis.

Our synthetic leukotriene samples exhibited potent spasmogenic activity. Thus in the *in vitro* guinea pig ileum assay,⁴¹ EC₅₀ values of 1×10^{-10} , 3×10^{-10} , and 6×10^{-9} M were observed for **4a** dipotassium salt, **4b** dipotassium salt, and **4c** monopotassium salt, respectively. The contractions induced by these materials were blocked by the standard SRS-A antagonist FPL55712.⁴⁴

Synthesis of Tritium-Labeled Leukotrienes

For the purpose of establishing a radioimmunoassay for leukotrienes,⁴ we required materials labeled with tritium. Given the inherent instability of these substances, severe constraints were placed on our strategic planning. In particular, a minimum number of radiochemical steps and rapid purification methods were required. After several unsuccessful alternatives were explored, we selected 14,15-dehydro-LTA₄ methyl ester (**44**) (Scheme VII) as the substrate into which tritium would be introduced.⁴² This material was prepared by Wittig reaction of epoxy dienal **7** with acetylenic phosphonium salt **42d**. The latter intermediate was obtained in straightforward fashion starting from **42a**, via tosylate **42b** and iodide **42c**.

The reduction of **44** was carried out on a 0.18 mmol scale using 10 Ci of carrier-free tritium gas and Lindlar catalyst. The product ([14,15-³H₂]-**5**) was rapidly isolated and purified by HPLC prior to dilution with unlabeled **5** and conversion to [14,15-³H₂]-LTE₄ dimethyl ester by treatment with L-cysteine methyl ester-triethylamine. The adduct was again rapidly purified by reverse-phase HPLC, and the pure product (UV max 280 nm, 40 Ci/mmol) was stored in dilute methanol or aqueous methanol, at -80 °C, under argon. Under these conditions, [14,15-³H₂]-LTE₄ dimethyl ester shows minimal chemical or radiolytic decomposition after 2 months. Samples of the labeled diester, which is not biologically active, were saponified just prior to use.

Conclusions

The synthetic approaches to optically pure leukotriene synthons described above have proven effective for preparing a variety of important substances. In particular, the routes to epoxy alcohol **9** from erythorbic acid have been useful not only for the production of the leukotrienes themselves, but also for the synthesis of isotopically labeled materials and analogues possessing interesting biological activities.⁴³ These latter compounds will be the subjects of forthcoming reports.

Experimental Section

General. All reactions except hydrogenations were carried out under an atmosphere of argon. The "usual workup" involved three extractions with the specified solvent. Organic extracts were then combined, washed with saturated brine, dried over anhydrous MgSO₄, filtered, and concentrated under water aspirator pressure. Residues were dried to constant weight under high vacuum or water aspirator pressure in the case of volatile materials. Unless otherwise noted, column chromatography was performed using EM Silica Gel 60 (0.063–0.2 mm). Thin layer chromatography (TLC) was performed using EM Silica Gel 60F-254 precoated plates. Spots were detected with UV light and/or phosphomolybdic acid and ceric sulfate sprays followed by heating. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ¹H NMR spectra (100 or 200 MHz) were obtained in CDCl₃ solution unless otherwise noted. Chemical shifts are reported

relative to Me₄Si as an internal standard. Infrared spectra were obtained in CHCl₃ solution.

(-)-(3R-cis)-Mono(tetrahydro-4-hydroxy-2-oxo-3-furanyl) Ester 2-(4-Methylphenyl)hydrazide of Ethanedioic Acid (16). In a 3-L, three-necked, round-bottomed flask equipped with an air-driven Teflon paddle stirrer, thermometer, and dropping funnel was placed 500 mL of deionized H₂O. With stirring, 53.5 mL (98.44 g, 0.96 mol) of reagent grade concentrated H₂SO₄ was added. The temperature rose to ca. 40 °C. A 53.5-g (0.5 mol) portion of ground *p*-toluidine was added with good stirring at this temperature, and within a few minutes a dark yellow solution resulted. The solution was then cooled in an ice bath and the sulfate precipitated. To the well-stirred, dense slurry, at 3 °C, was added a solution of 35 g (0.507 mol) of NaNO₂ in 63 mL of deionized H₂O dropwise, over a 25-min period, with the internal temperature kept at 3–8 °C. The resulting diazonium salt solution was stirred at 2–5 °C for 30 min whereupon a solution of 75.5 g (0.429 mol) of D-(-)-arabascorbic acid (**13**) in 500 mL of deionized H₂O was added dropwise over a 30-min period, at ca. 4–5 °C, with rapid stirring. An orange color formed along with gummy brown material which impeded stirring somewhat. After the addition was complete, the mixture was slowly warmed to room temperature with a water bath and then stirred for 3 h at room temperature during which time a yellow solid formed. The resulting yellow slurry was filtered with suction and washed with 700 mL of deionized H₂O in small portions. The solid was then washed with 200 mL of ice-cold 2B ethanol in small portions. The damp filter cake was refluxed in 1 L of 2B ethanol in a 3-L, one-necked, round-bottomed flask for 15 min. The slurry was allowed to cool to room temperature and kept at room temperature overnight. The slurry was filtered with suction and the solid was washed with 250 mL of ice-cold 2B ethanol in small portions. After the solid was dried at 45 °C using water aspirator pressure and then high vacuum, there was obtained 70.0 g (55.5%) of the hydrazide lactone **16** as an off-white solid, mp 182–184 °C dec, [α]_D²⁵ -59.3° (c 0.5, EtOH) (lit.¹⁷ mp 177 °C, [α]_D²⁰ -62.8° (c 0.53, EtOH)).

(-)-(3R-cis)-Dihydro-3,4-dihydroxy-2(3H)-furanone (D-Erythrone-lactone) (15). **A. From 16.** In a 2-L, one-necked, round-bottomed flask equipped with a reflux condenser, magnetic stirring bar, and heating mantle, a slurry of 69.9 g (0.238 mol) of the hydrazide **16** in 700 mL of deionized H₂O was heated to reflux under argon, with magnetic stirring, and refluxed for 50 min. The resulting yellow solution was cooled in an ice bath with stirring to produce a dense precipitate. At ca. 5 °C, the solid (oxalic acid tolyl hydrazide) was filtered with suction and washed with 250 mL of deionized H₂O in small portions. The combined filtrate and washings were extracted with 3 × 200 mL of ethyl acetate. The ethyl acetate layers were discarded. The aqueous layer was concentrated at 50 °C using water aspirator pressure, and the resulting oil was dried at 40 °C under high vacuum. The residue (which had crystallized on seeding) was taken up in 500 mL of ethyl acetate at reflux and filtered hot through Celite with suction to remove the small amount of insolubles present. The filter cake was washed with ca. 25 mL of hot ethyl acetate. The filtrate was allowed to cool to room temperature and refrigerated overnight at ca. 5 °C. The crystals were filtered with suction and washed with ca. 75 mL of cold ethyl acetate in small portions. After the crystals were dried under high vacuum at room temperature, there was obtained 20.7 g (73.7%) of the desired dihydroxy lactone **15** as colorless needles, mp 97.5–99 °C, [α]_D²⁵ -72.07° (c 0.487, H₂O) (lit.¹⁷ mp 104–105 °C, [α]_D²⁰ -73.2° (c 0.533, H₂O)).

B. By Alkaline Peroxide Oxidation of 13. A 2.72-g (15.45 mmol) sample of **13** was oxidized²⁰ with 8 mL (80 mmol) of 30% aqueous hydrogen peroxide and 4.24 g (40 mmol) of anhydrous sodium carbonate in 50 mL of water using the procedure described in detail below for the preparation of **17**. The dry solid residue obtained after Norit decomposition of excess peroxide, acidification (30 mL of 3 N HCl), and removal of water, containing **15**, oxalic acid, and sodium chloride, was treated with 25 mL of ethyl acetate, and the mixture was boiled on a steam bath for several minutes. The hot solution was decanted and the extraction was repeated with 10 mL of fresh ethyl acetate. The ethyl acetate extracts were combined and cooled to 0 °C. The precipitate was filtered with suction, washed with cold ethyl acetate, and dried under high vacuum. There was obtained 1.23 g (67.5%) of lactone diol **15** as colorless needles, [α]_D²⁵ -71.64° (c 0.53, H₂O).

(-)-(3aR-cis)-Dihydro-2,2-dimethylfuro[3,4-d]-1,3-dioxol-4(3aH)-one (2,3-O-Isopropylidene-D-erythrone-lactone) (17). **A. From Diol 15.** A mixture of 20.6 g (0.174 mol) of dihydroxy lactone **15**, 155 mL of reagent-grade acetone, 310 mL of 2,2-dimethoxypropane, and 0.35 g of *p*-toluenesulfonic acid monohydrate was stirred at room temperature for 6 h. The resulting pale yellow solution was treated with 3.5 mL of concentrated ammonium hydroxide solution. A slight precipitate formed. The mixture was concentrated under water aspirator pressure and the resulting solid was dissolved in 600 mL of ether. To the near solution was added 25 g of anhydrous magnesium sulfate and the slurry was

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stirred for 5 min. It was then filtered through Celite with suction, and the filter cake was washed with 200 mL of ether in small portions. Solvent removal afforded 29.8 g of a yellow solid. This material was dissolved in 200 mL of boiling ether to give a clear solution. At room temperature, 300 mL of hexane was added to produce an immediate precipitate. The slurry was refrigerated for 2 h, then filtered with suction, and the solid was washed with 200 mL of hexane in small portions. After drying under high vacuum at 35 °C, there was obtained 21.1 g (76.6%) of the desired acetone lactone **17** as an off-white solid, mp 66–68 °C, $[\alpha]_D^{25}$ –122.05° (c 1.03, H₂O) (lit.¹¹ mp 68–68.5 °C, $[\alpha]_D^{20}$ –112° (c 1.5, H₂O)).

B. Directly from 13. A solution of 35.2 g (0.20 mol) of **13** (D-isosorbic acid) in 500 mL of deionized H₂O was stirred with ice-bath cooling while 42.4 g (0.40 mol) of anhydrous, powdered sodium carbonate was added, cautiously, in small portions (CO₂ evolution). The resulting yellow solution was stirred with ice-bath cooling while 44 mL (0.45 mol) of 31.3% by weight aqueous hydrogen peroxide was added dropwise over a 10-min period. The internal temperature rose from 6 to 17 °C. The solution was stirred for 5 min with ice-bath cooling during which time the internal temperature continued to rise to 23 °C. The flask was now immersed in an oil bath pre-equilibrated to 40 °C and the solution was stirred for 30 min. An 8-g quantity of Norit A was added to decompose the excess peroxide and the mixture was heated on a steam bath with occasional swirling for 30 min, at which point gas evolution had essentially ceased and a negative starch-iodide test was observed. The hot mixture was filtered with suction on a Celite pad, and the filter cake was washed with a total of 100 mL of deionized H₂O in several small portions. The combined filtrate and washes were acidified to pH 1 by the cautious (CO₂ evolution!) addition of 150 mL (0.9 mol) of 6 N aqueous hydrochloric acid, in portions. The acidic solution was concentrated at 40–50 °C (aspirator pressure), using a rotary evaporator. The residue was dried at 40–50 °C (high vacuum) giving 84 g of a colorless, solid residue containing the dihydroxy lactone **15**, oxalic acid, and sodium chloride. To this material was added 175 mL of reagent grade acetone followed by 50 g of anhydrous, powdered magnesium sulfate. The mixture was stirred as 350 mL (2.85 mol) of 2,2-dimethoxypropane was added in one portion. To the mixture was added 0.42 g (2.2 mmol) of *p*-toluenesulfonic acid monohydrate at room temperature. The slurry was stirred at room temperature for 16 h. At the end of this time, 20 mL (0.3 mol) of concentrated ammonium hydroxide was added all at once. The mixture became quite dense and a mild exotherm was noted. After being stirred for a few minutes, the slurry was diluted with 500 mL of anhydrous ether and then filtered with suction. The solids were washed thoroughly with ether. After the combined filtrate and washes were concentrated on a rotary evaporator at aspirator pressure, and the residue was dried at 45 °C (high vacuum), there was obtained 32.8 g of a pale-yellow solid. This material was dissolved in 250 mL of boiling anhydrous ether. Some insoluble material was present. A 10-g portion of anhydrous, powdered magnesium sulfate was added and the mixture was filtered, with suction, through a pad of Celite. The filter cake was washed thoroughly with ether. The volume of the combined ether filtrate and washes was reduced to approximately 225 mL, and, when all of the solid had been redissolved by warming to reflux on a steam bath, 330 mL of hexane was added to the clear solution. An instantaneous, dense precipitate resulted. The mixture was stored at 0 °C for 2.5 h, then filtered with suction. The solid was washed with hexane and dried under high vacuum, at 40–50 °C, to constant weight. There was obtained 22.6 g (71.5%) of **17** as a colorless solid, mp 64–65 °C, $[\alpha]_D^{25}$ –120.15° (c 1, H₂O). This material was homogeneous on TLC analysis (1:3 hexane–ethyl acetate).

(–)-[3aR-(3α,6α)]-Tetrahydro-2,2-dimethylfuro[3,4-d]-1,3-dioxol-4-ol (2,3-O-Isopropylidene-D-erythrose) (11). **A. From Lactone 17.** A solution of 25 g (0.158 mol) of lactone **17** in 435 mL of dichloromethane was stirred rapidly at –72 °C (dry ice–acetone bath), while 125 mL (0.184 mol) of a 24.8% solution of diisobutylaluminum hydride in toluene was added dropwise over a 35-min period. The reaction mixture was stirred for 3.5 h at –72 °C, then cautiously decomposed (gas evolution) by the dropwise addition of 32 mL of methanol with continued cooling from the dry ice–acetone bath. The mixture was then slowly poured into a cold (ice bath), mechanically stirred mixture of 375 mL of water and 875 mL of ethyl acetate. To the resulting mixture was then added 350 mL of 1.5 N aqueous sulfuric acid while the pH was monitored with a pH meter. The final pH was 2.95. The mixture was transferred to a separatory funnel whereupon the layers were separated. The aqueous phase was extracted three times with ethyl acetate. The organic layers were combined and washed with 375 mL of saturated aqueous sodium bicarbonate solution. The bicarbonate wash was back-extracted once with ethyl acetate. Completion of the workup in the usual manner gave 22.8 g (90.2%) of lactol **11** as a colorless oil. $[\alpha]_D^{25}$ –77.03° (c 2.09, CHCl₃). This material crystallized on standing at 0 °C but was used

without further purification (lit.¹⁹ mp 30–32.5 °C, $[\alpha]_D^{25}$ –79.3° (c 0.925, CHCl₃)). TLC analysis (1:1 toluene–ethyl acetate) gave a single spot of slightly smaller *R_f* than the starting lactone.

B. From 4,6-O-Isopropylidene-D-glucose (25b). To a stirred solution of 6.7 g (31 mmol) of sodium metaperiodate in 60 mL of water cooled to 7 °C, was added a solution of 3.4 g (15.5 mmol) of **25b**^{28a} in 20 mL of water, over a 5-min period. The pH was maintained at ca. 4.0 by the addition of 3 N aqueous sodium hydroxide. After the addition was complete, the mixture was stirred for 15 min at 5–10 °C while continuing to maintain a pH of 4. The pH was then raised to 6.5 by the addition of 3 N NaOH and the slurry was concentrated at 50 °C (water aspirator pressure). The residue was dried under high vacuum and then stirred for 0.5 h with 200 mL of ethyl acetate after the addition of some anhydrous magnesium sulfate. The solids were filtered and the filtrate was concentrated. The solid residue (1.9 g) was chromatographed on 50 g of silica gel. Elution with 1:3 hexane–ethyl acetate gave 1.2 g (48.4%) of a colorless solid. The ¹H NMR spectrum of this material indicated it to be a mixture of dimeric forms of aldehyde **26b**. Only a trace of monomeric aldehyde was discernible in the IR and ¹H NMR spectra.

A solution of 394 mg (1.23 mmol) of this material and 50 mg of *p*-toluenesulfonic acid monohydrate, in 1 mL of anhydrous *N,N*-dimethylformamide, was stirred for 1 h and kept at room temperature for 5 days.^{29a} The solution was diluted with 15 mL of dichloromethane and treated with 0.5 g of anhydrous sodium bicarbonate. After stirring for 1 h, the solids were filtered and washed with dichloromethane. The filtrate and washes were combined and concentrated under reduced pressure. The oily residue was chromatographed on 50 g of silica gel. Elution with 2:1 hexane–ethyl acetate gave 111 mg (28.2%) of lactol **11** whose ¹H NMR spectrum and TLC mobility were identical with those of the lactol prepared as in part A above.

(+)-(4R-cis)(Z)-2,2-Dimethyl-5-[3-(1,3-dioxan-2-yl)-1-propenyl]-1,3-dioxolane-4-methanol Benzoate (19a). To a stirred slurry of 129.9 g (0.284 mol) of phosphonium salt **18**²³ in 325 mL of dry tetrahydrofuran, at –25 °C (dry ice–acetone bath), was added, dropwise, 177.5 mL (0.284 mol) of 1.6 M *n*-butyllithium in hexane over a 15-min period. The addition funnel was rinsed with 25 mL of dry THF. The mixture turned yellow at first and then brown and was stirred for 20 min at ca. –25 °C. To the resulting phosphorane mixture there was now added a solution of 22.7 g (0.142 mol) of crude lactol **11** in 150 mL of dry THF, dropwise, over a 30-min period, at –25 °C. The addition funnel was rinsed with 25 mL of dry THF. The yellow-orange mixture was stirred at –25 °C for 30 min, then warmed to room temperature and stirred for an additional 1.5 h. After being cautiously treated dropwise with 21 mL of water, the tan mixture was transferred to a separatory funnel containing 560 mL of water and 700 mL of ether. The organic layer was separated and the aqueous phase was extracted three times with ether. Completion of the usual workup gave 68.7 g of an oily solid. This material was dissolved in 200 mL of dry pyridine and the solution was stirred with ice-bath cooling (5 °C) while 19.8 mL (24 g, 0.17 mol) of benzoyl chloride was added dropwise. The separation of a solid occurred. The mixture was stirred for 3 h at 5 °C, then poured into 1100 mL of saturated aqueous sodium bicarbonate solution and extracted four times with ether. The organic extracts were processed in the usual manner giving 94.0 g of an oily solid. This material was treated with a solution of 66 mL of toluene and 190 mL of hexane. The resulting slurry was suction filtered and the solid cake of triphenylphosphine oxide was washed with 30 mL of cold 2.9:1 hexane–toluene. The filtrate and wash were combined and concentrated under water aspirator pressure and then high vacuum giving 54.2 g of a red oil. This material was chromatographed in two approximately equal portions on columns of 500 g of silica gel packed in toluene. Elution with 19:1, 9:1, and 4:1 toluene–ethyl acetate gave a total of 40.8 g (79.3%) of pure (TLC, *R_f* 0.5, 1:1 toluene–ethyl acetate) ester **19a** as a viscous, yellow oil. The analytical specimen was obtained from a separate experiment and exhibited bp 135–140 °C (bath temperature) (0.03 mm), $[\alpha]_D^{25}$ +48.68° (c 1.03, CHCl₃); NMR 8.07 (m, 2, aromatic), 7.70–7.30 (m, 3, aromatic), 5.73, 5.65 (2 m, 2, *J*(cis) = 11 Hz, CH=CH), 5.08 (dd, 1, *J* = 6, 7 Hz, OCHO), 4.60–3.90 (m, 6, 2 × CH of CH₂O, OCHCHO, CH₂OCOC₆H₅), 3.19 (m, 2, 2 × CH of CH₂O), 2.43 (t, 2, *J* = 5.5 Hz, CH₂C=), 2.30–1.70, 1.29 (2 m, 2, 2 × CH of CH₂), 1.50, 1.39 ppm (2 s, 6, (CH₃)₂C).

Anal. Calcd for C₂₀H₂₆O₆: C, 66.28; H, 7.23. Found: C, 66.36; H, 7.11.

(+)-(4R-cis)-2,2-Dimethyl-5-[3-(1,3-dioxan-2-yl)propyl]-1,3-dioxolane-4-methanol Benzoate (20a). A mixture of 40.8 g (0.113 mol) of unsaturated acetal ester **19a**, 0.7 g (3.08 mmol) of platinum oxide, and 600 mL of ethyl acetate was stirred in an atmosphere of hydrogen, at room temperature, for 6 h at the end of which time gas uptake had ceased. A total of 2976 mL of hydrogen was taken up (2979 mL theory). The catalyst was removed by suction filtration of the reaction mixture through a pad of Celite. The filtrate was concentrated on a rotary

evaporator at water aspirator pressure, then high vacuum, to give 42.0 g (ca. 100%) of **20a** as a viscous, pale-yellow oil, containing a trace of solvent. This material was used without further purification. The analytical specimen obtained from a separate experiment exhibited bp 130–140 °C (bath temperature) (0.01 mm), $[\alpha]^{25}_D +15.18^\circ$ (*c* 1.05, CHCl_3); IR 1720 cm^{-1} (ester C=O).

Anal. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_6$: C, 65.92; H, 7.74. Found: C, 65.60; H, 7.79.

(+)-(4*S*-*cis*)-2,2-Dimethyl-5-[(benzoyloxy)methyl]-1,3-dioxolane-4-butanoic Acid 3-Hydroxypropyl Ester (21a). A solution of 17.5 g (0.048 mol) of acetal ester **20a** in 300 mL of ethyl acetate was stirred and cooled to –70 °C (dry ice–acetone bath), whereupon ozone gas in oxygen was passed in for 5 h (Welsbach Ozonator). The resulting solution was purged with argon, at –70 °C, until the blue color had disappeared. After being warmed to room temperature, the solution was washed with saturated aqueous sodium bicarbonate solution, and workup was completed in the usual manner giving 17.9 g of crude **21a** as a pale-yellow oil. This material was chromatographed on 400 g of silica gel packed in toluene. Elution with 9:1, 4:1, 2:1, 3:2, and 1:1 toluene–ethyl acetate afforded 12.7 g (69.4%) of pure **21a** (TLC R_f 0.4, 1:1 toluene–ethyl acetate) as a pale-yellow oil. The analytical specimen of **21a** obtained from a separate experiment exhibited $[\alpha]^{25}_D +11.48^\circ$ (*c* 1, CHCl_3); IR 3620, 3520 (OH), 1720 cm^{-1} (ester C=O); NMR 8.04 (m, 2, aromatic), 7.70–7.30 (m, 3, aromatic), 4.50–4.10 (m, 4, OCHCHO , $\text{CH}_2\text{OCOC}_6\text{H}_5$), 4.19 (t, 2, $J = 6$ Hz, $\text{CH}_2\text{OC=O}$), 3.65 (t, 2, $J = 5.5$ Hz, CH_2OH), 2.50–2.20 (m, 3, OH, $\text{CH}_2\text{C=O}$), 2.00–1.50 (m, 6, $3 \times \text{CH}_2$), 1.45, 1.35 ppm (2 s, 6, $(\text{CH}_3)_2\text{C}$).

Anal. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_7$: C, 63.14; H, 7.42. Found: C, 63.26; H, 7.69.

(+)-[*S*(*R)]-6-[2-[(Benzoyloxy)-1-hydroxy]ethyl]tetrahydro-2*H*-pyran-2-one (22a).** In a flask equipped with a magnetic stirring bar was placed 3.0 g (7.89 mmol) of diester **21a**. The flask was immersed in an ice bath, and, when the sample was well chilled, 30 mL of ice-cold 9:1 trifluoroacetic acid–water solution was added. The stirring bar became free to stir within a few seconds of the addition of the acid and the resulting solution was stirred at 0 °C for 3.5 h, then cautiously (foaming) added to a stirred, cold (0–5 °C) mixture of 750 mL of saturated aqueous sodium bicarbonate solution and 150 mL of ethyl acetate. The mixture was stirred for an additional 5 min, then transferred to a separatory funnel and worked up in the usual manner. There was obtained 2.7 g of an oil which crystallized to an off-white solid. This material was triturated with 40 mL of ether and the mixture refluxed for 40 min. The slurry was cooled to room temperature, refrigerated overnight (0 °C), and then kept at –20 °C for 3 h. The solid was filtered with suction, washed with some cold (–20 °C) ether, and dried under high vacuum giving 1.5 g (71.9%) of hydroxy lactone ester as a colorless solid, mp 89.5–91 °C (homogeneous on TLC analysis; R_f 0.25, 1:3 toluene–ethyl acetate). Lactone **22a** can be recrystallized by dissolution in one part of ethyl acetate, adding four parts of ether, seeding, and storing the mixture at –20 °C overnight. The analytical specimen obtained from a separate experiment exhibited mp 90.5–91.5 °C, $[\alpha]^{25}_D +34.78^\circ$ (*c* 1, CHCl_3); IR 3605, 3460 (OH), 1725 cm^{-1} (lactone, ester C=O); NMR 8.05 (m, 2, aromatic), 7.70–7.30 (m, 3, aromatic), 4.70–4.30 (m, 3, CHOC=O , $\text{CH}_2\text{OC=O}$), 4.10 (dt, 1, $J = 5.5$ Hz, CHOH), 3.30–2.80 (m, 1, OH), 2.80–2.20 (m, 2, $\text{CH}_2\text{C=O}$), 2.20–1.50 (m, 4, $(\text{CH}_2)_2$).

Anal. Calcd for $\text{C}_{14}\text{H}_{16}\text{O}_5$: C, 63.63; H, 6.10. Found: C, 63.94; H, 6.10.

(+)-(4*R*-*cis*)(*Z*)-2,2-Dimethyl-5-[3-(1,3-dioxan-2-yl)-1-propenyl]-1,3-dioxolane-4-methanol 4-Methylbenzenesulfonate (19b). A Wittig reaction between lactol **11** (4.0 g, 25 mmol) and phosphonium salt **18**²³ (22.9 g, 50 mmol) was carried out as described above for the preparation of **19a**. There was obtained 9.1 g of crude, oily product. A 3.2-g sample of this material was dissolved in 25 mL of dry pyridine, and the solution was stirred with ice-bath cooling while 2.3 g (12.1 mmol) of *p*-toluenesulfonyl chloride was added in one portion. The resulting mixture was stirred at 0–5 °C for 5 h, then poured into a mixture of 200 mL of saturated aqueous sodium bicarbonate solution and 200 mL of ether. After being stirred for 20 min, the mixture was worked up with ether in the usual manner, giving 5.7 g of crude product as a yellow oil. Chromatography of this material on 200 g of silica gel afforded 1.6 g (44.2%) of tosylate **19b** (eluted with 4:1 toluene–ethyl acetate) as a viscous, pale-yellow oil, $[\alpha]^{25}_D +62.37^\circ$ (*c* 1, CHCl_3); NMR 7.81 (d, 2, $J = 8$ Hz, aromatic), 7.35 (d, 2, $J = 8$ Hz, aromatic), 5.70 (dt, 1, $J = 7$, 11 Hz, $=\text{CHCH}_2$), 5.42 (dd, 1, $J = 8$, 11 Hz, $=\text{CHCH}$), 4.98 (dd, 1, $J = 7$, 8 Hz, $=\text{CCHO}$), 4.54 (t, 1, $J = 5$ Hz, OCHO), 4.25–3.50 (m, 7, $2 \times \text{CH}_2\text{O}$, CH_2OTs , CHO), 2.37 (dd, 2, $J = 4.5$, 7 Hz, $\text{CH}_2\text{CH=}$), 2.20–1.85 (m, 1, CH of CH_2), 1.40–1.10 (m, 1, CH of CH_2), 1.36, 1.32 ppm (2 s, 6, $(\text{CH}_3)_2\text{C}$).

Anal. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_7\text{S}$: C, 58.24; H, 6.84. Found: C, 58.60; H, 6.87.

(+)-(4*R*-*cis*)-2,2-Dimethyl-5-[3-(1,3-dioxan-2-yl)propyl]-1,3-dioxolane-4-methanol 4-Methylbenzenesulfonate (20b). A 1.55-g (3.76 mmol) sample of olefinic tosylate **19b** was hydrogenated over 50 mg of prerduced platinum oxide, in 30 mL of ethyl acetate, at room temperature and atmospheric pressure. A total of 90 mL of hydrogen was taken up over 1.5 h. The catalyst was filtered and the filtrate concentrated under reduced pressure. The residue (1.6 g) was chromatographed on 50 g of silica gel. Elution with 4:1 and 2:1 toluene–ethyl acetate gave 1.5 g (96.4%) of tosylate **20b** as a pale-yellow oil, $[\alpha]^{25}_D +27.30^\circ$ (*c* 0.86, CHCl_3).

Anal. Calcd for $\text{C}_{20}\text{H}_{30}\text{O}_7\text{S}$: C, 57.95; H, 7.30. Found: C, 58.27; H, 7.44.

(+)-(4*S*-*cis*)-2,2-Dimethyl-5-[[[(4-methylphenyl)sulfonyl]oxy]-methyl]-1,3-dioxolane-4-butanoic Acid 3-Hydroxypropyl Ester (21b). A 3.2-g (7.28 mmol) sample of crude bis-acetal **20b** was ozonized in 60 mL of ethyl acetate, for 2 h, at –70 °C, as described above for the preparation of **21a**. Chromatography of the crude product (3.4 g) on 100 g of silica gel afforded 2.1 g (67.1%) of ester **21b** (eluted with 1:1 toluene–ethyl acetate) as a pale-yellow oil, $[\alpha]^{25}_D +20.40^\circ$ (*c* 0.98, CHCl_3); IR 3630 (OH), 1727 (ester C=O), 1370, 1177 cm^{-1} (SO_2).

Anal. Calcd for $\text{C}_{20}\text{H}_{30}\text{O}_8\text{S}$: C, 55.80; H, 7.02. Found: C, 56.35; H, 6.93.

(+)-[*S*(*R)]-6-[2-[(4-Methylphenyl)sulfonyloxy]-1-hydroxyethyl]-tetrahydro-2*H*-pyran-2-one (22b).** A 2.0-g (4.65 mmol) sample of ester tosylate **21b** was hydrolyzed with 20 mL of ice-cold 9:1 trifluoroacetic acid–water as described above for the preparation of **22a**. The crude crystalline product (2.1 g) was chromatographed on 150 g of silica gel. Elution with 1:1 toluene–ethyl acetate gave 0.8 g (54.8%) of pure (TLC) lactone **22b** as a colorless solid. A sample of this material was recrystallized from ethyl acetate–ether affording colorless crystals, mp 95–96 °C, $[\alpha]^{25}_D +30.98^\circ$ (*c* 1.08, CHCl_3); IR 3615 (OH), 1737 (lactone C=O), 1177, 1365 cm^{-1} (SO_2); NMR 7.81 (d, 2, $J = 8.5$ Hz, aromatic), 7.37 (d, 2, $J = 8.5$ Hz, aromatic), 4.40–3.80 (m, 4, OCHCHO , CH_2O), 2.90–2.20 (m, 3, $\text{CH}_2\text{C=O}$, OH), 2.46 (s, 3, $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2$), 2.18–1.50 ppm (m, 4, $(\text{CH}_2)_2$).

Anal. Calcd for $\text{C}_{14}\text{H}_{18}\text{O}_6\text{S}$: C, 53.49; H, 5.77. Found: C, 53.71; H, 5.68.

(+)-(S,*R*)-6-Hydroxyoxiranepentanoic Acid Methyl Ester (24). A solution of 197 mg (0.627 mmol) of hydroxy tosylate lactone **22b** in 3 mL of dry methanol was stirred with ice-bath cooling while 113.7 mg (0.823 mmol) of anhydrous potassium carbonate was added. The slurry, which thickened as the reaction proceeded, was stirred at 0–5 °C for 4 h, then diluted with dichloromethane, treated with saturated brine, and worked up with dichloromethane in the usual manner. The oily residue (92.6 mg) was chromatographed on 10 g of silica gel. Elution with 1:3 toluene–ethyl acetate afforded 79.2 mg (72.6%) of epoxide **24** as a pale-yellow liquid, $[\alpha]^{25}_D +19.64^\circ$ (*c* 1, CHCl_3). TLC analysis indicated that this material was homogeneous and slightly less polar than the isomeric epoxide **9**: IR 3570 (OH), 1735 cm^{-1} (ester C=O); NMR 3.80 (m, 1, CHOH), 3.68 (s, 3, CO_2CH_3), 3.01 (q, 1, $J = 3$ Hz, CHO), 2.77 (m, 2, CH_2O), 2.40 (m, 2, $\text{CH}_2\text{C=O}$), 1.97 (d, 1, $J = 2$ Hz, OH), 1.95–1.50 ppm (m, 4, $(\text{CH}_2)_2$).

Anal. Calcd for $\text{C}_8\text{H}_{14}\text{O}_4$: C, 55.16; H, 8.10. Found: C, 55.13; H, 8.06.

(2*R*,4*S*,5*R*)(*E*)-4-[3-(1,3-Dioxan-2-yl)-1-propenyl]-2-methyl-1,3-dioxan-5-ol Methanesulfonate (27). To a stirred slurry of 11.2 g (24.5 mmol) of phosphonium salt **18** in 20 mL of anhydrous tetrahydrofuran at –30 °C was added 15.4 mL (24.6 mmol) of 1.6 *M* *n*-butyllithium in hexane, over a 3-min period. The yellow-orange mixture was stirred for 25 min at –30 °C, and then a solution of 1.6 g (10.96 mmol) of freshly prepared, monomeric **26a**²⁷ in 10 mL of tetrahydrofuran was added over 2 min. After being stirred at –30 °C for 20 min, the yellow mixture was allowed to warm to room temperature and stirred for 2.5 h. The mixture was decomposed with 1 mL of water, then poured into brine, and worked up with ether in the usual manner giving 4.7 g of a yellow semisolid. This material was dissolved in 65 mL of dichloromethane. The solution was stirred with ice-bath cooling while 3.2 mL (23 mmol) of triethylamine was added followed by 1.5 mL (19.4 mmol) of methanesulfonyl chloride. The mixture was stirred at 0–5 °C for 45 min, then poured into a stirred mixture of 150 mL of cold, saturated sodium bicarbonate solution and 75 mL of dichloromethane. After being stirred for 5 min, the mixture was worked up with dichloromethane in the usual manner giving 6.1 g of a semisolid. This material was chromatographed on 50 g of silica gel. Elution with 4:1 toluene–ethyl acetate afforded 2.8 g (79.3%) of mesylate acetal **27** as a pale-yellow oil: NMR 5.92 (dt, 1, $J = 6.5$, 15 Hz, $\text{CH}_2\text{CH=}$), 5.55 (dd, 1, $J = 6.5$, 15 Hz, CHCH=), 4.75 (q, 1, $J = 5$ Hz, CHCH_2), 4.62 (t, 1, $J = 5$ Hz, OCHO), 4.50–3.40 (m, 8, $3 \times \text{CH}_2$, $2 \times \text{CH}$), 2.99 (s, 3, SO_3CH_3), 2.42 (dd, 2, $J = 5$, 6.5 Hz, $\text{CH}_2\text{CH=}$), 2.04 (m, 1, CH_2), 1.33 (m, 1, CH_2), 1.34 ppm (d, 3, $J = 5$ Hz, CH_3CH).

(-)-(2*R*,4*S*,5*R*)-4-[3-(1,3-Dioxan-2-yl)propyl]-2-methyl-1,3-dioxan-5-ol Methanesulfonate (**28**). Hydrogenation of 2.8 g (8.7 mmol) of olefinic mesylate **27** over 100 mg of prerduced platinum oxide, in 25 mL of ethyl acetate was carried out as described above for the preparations of **20a,b**. There was obtained 2.8 g of **28** as an oil. Chromatography on 50 g of silica gel gave 2.15 g (76.3%) of pale-yellow, oily **28** (eluted with 2:1 toluene-ethyl acetate). The analytical specimen was obtained similarly from a separate experiment, $[\alpha]_D^{25} -41.22^\circ$ (*c* 1.04, CHCl₃).

Anal. Calcd for C₁₃H₂₄O₇S: C, 48.13; H, 7.46; S, 9.88. Found: C, 48.32; H, 7.50; S, 9.85.

(-)-(2*R*,4*S*,5*R*)-2-Methyl-5-[(methylsulfonyl)oxy]-1,3-dioxane-4-butanolic Acid 3-Hydroxypropyl Ester (**29**). A 2.1-g (6.48 mmol) sample of bis-acetal mesylate **28** was ozonized, at -70 °C, in 40 mL of ethyl acetate, for 3 h, as described above for the preparations of **21a,b**. The crude product (2.2 g) was purified by HPLC (1:3 toluene-ethyl acetate) giving 1.2 g (54.5%) of pure (TLC, *R_f* 0.3, 1:3 toluene-ethyl acetate) ester **29** as a pale-yellow oil, $[\alpha]_D^{25} -35.85^\circ$ (*c* 0.88, CHCl₃); IR 3630 (OH), 1727 (ester C=O), 1367, 1168 cm⁻¹ (SO₂).

Anal. Calcd for C₁₃H₂₄O₈S: C, 45.87; H, 7.11; S, 9.42. Found: C, 45.96; H, 6.87; S, 9.61.

(-)-(S,S-*trans*)-3-(Hydroxymethyl)oxiranebutanoic Acid Methyl Ester (**9**). A. From Lactone **22a**. A solution of 4.4 g (16.7 mmol) of hydroxy lactone ester **22a** in 100 mL of methylene chloride was cooled in an ice-salt bath, to ca. -5 °C with stirring, whereupon 3.7 mL (26.6 mmol) of triethylamine was added; then 1.7 mL (21.9 mmol) of methanesulfonyl chloride was added over a 1-min period at ca. -5 °C, and the reaction mixture was stirred for 45 min as the temperature was slowly allowed to rise to 0 °C. The reaction mixture was added to 75 mL of ice-cold 1 N aqueous sulfuric acid and the mixture worked up with dichloromethane in the usual manner (the combined organic extracts were additionally washed with saturated aqueous sodium bicarbonate solution). There was obtained 5.9 g of mesylate **23** as a pale-yellow gum (TLC, *R_f* 0.6, 1:3 toluene-ethyl acetate); NMR 3.09 ppm (s, 3, CH₃SO₂).

A solution of this mesylate in 60 mL of anhydrous methanol was cooled to 3 °C in an ice bath, with stirring, whereupon 2.6 g (18.8 mmol) of anhydrous potassium carbonate was added. The slurry, which thickened as the reaction proceeded, was stirred at ca. 3-5 °C for 7.5 h, at which point TLC indicated that reaction was complete. (The formation of epoxide **9** must be followed by TLC (*R_f* 0.25, 1:3 toluene-ethyl acetate) to ensure that the starting material (*R_f* 0.6) and an intermediate hydroxy mesylate (*R_f* 0.2) are absent. Because of the heterogeneity of the reaction mixture, the times required for complete epoxide formation have varied. Efficient stirring is important.) The mixture was diluted with dichloromethane and poured into saturated brine. Workup with dichloromethane in the usual manner gave 6.1 g of a yellow liquid consisting of epoxy alcohol **9** and methyl benzoate. This material was chromatographed on 50 g of silica gel packed in toluene. Elution with 1:3 toluene-ethyl acetate afforded 2.3 g (79.3%) of **9** as a pale-yellow liquid. A sample of this material was evaporatively distilled (essentially quantitative recovery), giving a colorless liquid, bp 85-95 °C (bath temperature) (0.1 mm), $[\alpha]_D^{25} -32.89^\circ$ (*c* 0.33, CHCl₃) (lit.^{3b} $[\alpha]_D^{25} -37.4^\circ$ (*c* 0.27, CHCl₃)). We have observed rotations in various runs in the range of -25 to -35° for this epoxide: NMR 3.66 (s, 3, CO₂CH₃), 3.59, 3.86 (2 m, 2, CH₂OH), 2.91 (m, 2, epoxide CH), 2.37 (t, 2, *J* = 6.5 Hz, CH₂CO₂CH₃), 2.19 (t, 1, *J* = 6 Hz, OH (exchangeable with D₂O)), 1.69 ppm (m, 4, (CH₂)₂); IR 3600 (OH), 1735 cm⁻¹ (ester C=O).

A 57.2-mg (0.33 mmol) sample of epoxide **9** ($[\alpha]_D^{25} -29.77^\circ$ (*c* 0.27, CHCl₃)) was esterified with 344.4 mg of (+)-MTPA chloride in 1.5 mL of dry pyridine, for 22 h at room temperature. Quenching with water and extractive workup (ether) gave 129 mg of diester as a yellow oil: NMR 4.55 (dd, 1, *J* = 12, 4 Hz, CH of CH₂OC=O), 4.22 ppm (dd, 1, *J* = 12, 6 Hz, CH of CH₂OC=O). In the spectrum of diester obtained from (+)-MTPA chloride and racemic **9** (spectrum provided by Drs. K. B. Sharpless and T. Katsuki²⁶), the doublet of doublets centered at 4.55 ppm is resolved into two doublets of doublets. No such resolution could be discerned in the spectra of the MTPA derivatives of our optically active epoxy alcohol samples, even those having as low an optical rotation as -24.92°. We conclude from these results that the rotation of **9** is very sensitive to minor chemical impurities and not a reliable indicator of enantiomeric purity.

B. From Lactone **22b**. To a stirred solution of 197.6 mg (0.629 mmol) of lactone **22b** in 2 mL of anhydrous methanol was added 0.47 mL (0.752 mmol) of 1.6 M methanolic sodium methoxide. The resulting solution was stirred at room temperature for 4 days. TLC analysis (1:3 toluene-ethyl acetate) revealed a rapid conversion to epoxide **24** (see above) followed by a slow isomerization to **9**. The solution was poured into ether and brine and worked up with ether in the usual manner. The crude product was chromatographed on 10 g of silica gel. Elution with 1:3 toluene-ethyl acetate gave 37 mg (33.8%) of epoxide **9** as a pale-yellow

liquid, $[\alpha]_D^{25} -34.65^\circ$ (*c* 0.32, CHCl₃). The ¹H NMR spectrum was identical with that of the material produced as in part A above.

C. From Mesylate Acetal **29**. A 712-mg (2.09 mmol) sample of mesylate acetal **29** was hydrolyzed in 9 mL of 9:1 trifluoroacetic acid-water, at 0-5 °C, for 5.5 h, as described above for the preparations of **22a,b**. There was obtained 622 mg of an oily, crude product containing the lactone **30**. This material was dissolved in 8 mL of anhydrous methanol, and the solution was stirred with ice-bath cooling as 457 mg (3.31 mmol) of anhydrous potassium carbonate was added. After being stirred at 0-5 °C for 5.5 h, the thick slurry was poured into a mixture of brine and ethyl acetate and worked up with ethyl acetate in the usual manner. The crude, oily product (238 mg) was chromatographed on 20 g of silica gel. Elution with 1:3 toluene-ethyl acetate gave 105 mg (29%) of epoxide **9** as a pale-yellow liquid. Evaporative distillation provided an essentially quantitative recovery of a colorless liquid, bp 125-135 °C (bath temperature) (0.45 mm), $[\alpha]_D^{25} -34.32^\circ$ (*c* 1.77, CHCl₃). The IR and ¹H NMR spectra of this material were identical with those of the epoxide produced as in parts A and B above.

In a separate experiment, the crude product (321 mg) from trifluoroacetic acid hydrolysis of 413 mg (1.21 mmol) of **29** (carried out as described above) was chromatographed on 50 g of silica gel. Elution with 1:3 toluene-ethyl acetate gave 56 mg (19.4%) of pure (TLC, *R_f* 0.2, 1:3 toluene-ethyl acetate) hydroxy mesyloxy lactone **30** as a colorless oil: IR 3620 (OH), 1738 (lactone C=O), 1178 cm⁻¹ (SO₂); NMR 4.90-4.40 (m, 2, OCHCHO), 3.97 (d, 2, *J* = 5 Hz, CH₂O), 3.17 (s, 3, SO₂CH₃), 2.90-2.25 (m, 3, CH₂, OH), 2.20-1.60 ppm (m, 4, (CH₂)₂).

(-)-(S,S)-2,2-Dimethyl-1,3-dioxolane-4,5-dimethanol Monobenzoate (**31**). A solution of 16.2 g (0.1 mol) of 2,3-O-isopropylidene-L-threitol²¹ in 32.5 mL of anhydrous pyridine was stirred with ice-bath cooling while 11.6 mL (14.04 g, 0.1 mol) of benzoyl chloride was added in a steady stream, from a pipet. A dense precipitate rapidly formed. The resulting slurry was stirred with ice-bath cooling for 15 min, then stored at 0 °C for 2 h. The mixture was diluted with 250 mL of ether and rapidly filtered with suction. The solids were washed rapidly several times with ether. The filtrate and washes were combined, washed with a solution of 10 mL of saturated aqueous potassium carbonate and 50 mL of brine, dried (anhydrous MgSO₄), filtered, and concentrated in vacuo. The oily residue (26.5 g) was chromatographed on 500 g of silica gel. Elution with 9:1 toluene-ethyl acetate gave 10.2 g (27.6%) of dibenzoate as a colorless solid. Elution with 4:1 and 1:1 toluene-ethyl acetate afforded 11.8 g (44.4%) of monoester **31** as a viscous, pale-yellow oil. Elution with ethyl acetate gave 3.3 g (20.4%) of recovered diol. The analytical specimen of **31** was obtained in a separate experiment by evaporative distillation, as a colorless oil, bp 123-127 °C (bath temperature) (0.05 mm), $[\alpha]_D^{25} -7.50^\circ$ (*c* 1.17, CHCl₃); IR 3600 (OH), 1720 cm⁻¹ (ester C=O); NMR 8.02 (m, 2, aromatic), 7.45 (m, 3, aromatic), 4.45 (m, 2, CH₂OCOPh), 4.24 (m, 1, CH₂OH), 4.02 (m, 1, CH₂OH), 3.78 (m, 2, OCHCHO), 2.24 (br s, 1, OH), 1.44 ppm (s, 6, (CH₃)₂C).

Anal. Calcd for C₁₄H₁₈O₅: C, 63.15; H, 6.81. Found: C, 63.30; H, 6.55.

(+)-(4*R-trans*)-5-[(Benzoyloxy)methyl]-2,2-dimethyl-1,3-dioxolane-4-carboxaldehyde (**32**). A solution of 0.33 mL (3.78 mmol) of oxaly chloride in 8.25 mL of dry dichloromethane was stirred and cooled to -60 °C while a solution of 0.56 mL (7.90 mmol) of dry dimethyl sulfoxide in 3.3 mL of dichloromethane was added dropwise. After the resulting mixture had been stirred for 2 min at -60 °C, a solution of 790 mg (2.97 mmol) of hydroxy ester **31** in 3.3 mL of dichloromethane was added dropwise. The mixture was stirred for 15 min at -55 °C; then 2.3 mL of triethylamine was added. The mixture was stirred at -60 °C for 5 min; then the cooling bath was removed. Stirring was continued for 45 min before the reaction was quenched by the addition of 25 mL of H₂O. Workup with dichloromethane in the usual manner gave 892 mg of a yellow, oily product which was chromatographed on 50 g of silica gel. Elution with 2:1 and 1:1 toluene-ethyl acetate afforded aldehyde **32** as an oil which was evaporatively distilled. There was obtained 508 mg (64.8%) of a colorless oil, bp 107-109 °C (bath temperature) (0.05 mm), $[\alpha]_D^{25} +17.1^\circ$ (*c* 2.03, CHCl₃); NMR 9.81 (d, 1, *J* = 1 Hz, HC=O), 1.50 (s, 3, CH₃), 1.44 ppm (s, 3, CH₃).

Anal. Calcd for C₁₄H₁₆O₅: C, 63.63; H, 6.10. Found: C, 63.93; H, 6.21.

(-)-(4*S-trans*)(Z)-2,2-Dimethyl-5-[3-(1,3-dioxan-2-yl)-1-propenyl]-1,3-dioxolane-4-methanol Benzoate (**33**). A Wittig reaction between aldehyde **32** (5.7 g, 21.6 mmol) and phosphonium salt **18**²³ (10.8 g, 23.6 mmol) was carried out as described above for the preparation of **19a** (23.7 mmol of *n*-butyllithium). The crude oily product obtained by extractive workup (ether) was treated with hexane which led to the precipitation of triphenylphosphine oxide. The mixture was filtered with suction and the solids were washed thoroughly with hexane. Concentration of the combined filtrate and washes in vacuo left 6.8 g of a yellow oil which was chromatographed on 300 g of silica gel. Elution with 4:1

toluene-ethyl acetate gave 3.67 g (46.9%) of pure (TLC, R_f 0.5, 1:1 toluene-ethyl acetate) bis-acetal ester **33** as a yellow oil. A sample was evaporatively distilled giving a colorless oil, bp 168–170 °C (bath temperature) (0.05 mm), $[\alpha]_D^{25} -32.84^\circ$ (c 0.95, CHCl_3): NMR 8.06 (m, 2, aromatic), 7.70–7.30 (m, 3, aromatic), 5.81 (dt, 1, $J = 7, 11$ Hz, $=\text{CHCH}_2$), 5.57 (m, 1, $=\text{CHCH}_2$), 4.74 (t, 1, $J = 8$ Hz, OCHO), 4.65–3.95 (m, 6, $2 \times \text{CH of CH}_2$, OCHCHO , $\text{CH}_2\text{OC=O}$), 3.68 (m, 2, $2 \times \text{CH of CH}_2$), 2.46 (m, 2, $\text{CH}_2\text{C=}$), 2.30–1.75 (m, 1, CH of CH_2), 1.45 (s, 6, $(\text{CH}_3)_2\text{C}$), 1.30–1.10 ppm (m, 1, CH of CH_2).

Anal. Calcd for $\text{C}_{20}\text{H}_{26}\text{O}_6$: C, 66.28; H, 7.23. Found: C, 66.22; H, 7.42.

(-)-(4*S*-*trans*)-2,2-Dimethyl-5-[3-(1,3-dioxan-2-yl)propyl]-1,3-dioxolane-4-methanol Benzoate (**34**). Hydrogenation of 337 mg (0.93 mmol) of **33** over 50 mg of prerduced platinum oxide, in 18 mL of ethyl acetate, was carried out as described above for the preparation of **20a,b**. The crude product was chromatographed on 17 g of silica gel. Elution with 9:1 and 4:1 toluene-ethyl acetate afforded 332 mg (98.2%) of bis-acetal ester **34** as a colorless oil, $[\alpha]_D^{25} -16.23^\circ$ (c 2.12, CHCl_3).

Anal. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_6$: C, 65.92; H, 7.74. Found: C, 65.89; H, 7.70.

(-)-(4*S*-*trans*)-5-[(Benzoyloxy)methyl]-2,2-dimethyl-1,3-dioxolane-4-butanolic Acid 3-Hydroxypropyl Ester (**35**). A 308-mg (0.846 mmol) sample of bis-acetal **34** was ozonized for 2.67 h, at -70 °C, in 10 mL of ethyl acetate, as described above for the preparation of **21a,b** and **29**. Chromatography of the crude product on 13 g of silica gel afforded 194 mg (60.3%) of diester **35** (eluted with 2:1 toluene-ethyl acetate) as a colorless oil, $[\alpha]_D^{25} -13.96^\circ$ (c 1.92, CHCl_3).

Anal. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_7$: C, 63.14; H, 7.42. Found: C, 63.36; H, 7.60.

(+)-[S(S*)]-6-[2-[(Benzoyloxy)-1-hydroxy]ethyl]tetrahydro-2H-pyran-2-one (**36**). A 1.16-g (3.05 mmol) sample of diester **35** was hydrolyzed in 10 mL of 9:1 trifluoroacetic acid-water, at 0–5 °C, for 2.5 h, and worked up as described above for the preparations of **22a,b**. The crude, crystalline product (753 mg) was chromatographed on 25 g of silica gel. Elution with 1:1 toluene-ethyl acetate gave 494 mg (61.3%) of pure (TLC) lactone **36** as a colorless solid. A sample was recrystallized from ethyl acetate-ether giving the analytical specimen as a colorless solid, mp 104.5–106 °C (mmp with **22a** 79–87 °C), $[\alpha]_D^{25} +30.68^\circ$ (c 1.00, CHCl_3): IR 3580 (OH), 1725 cm^{-1} (ester, lactone C=O); NMR 8.05 (m, 2, aromatic), 7.70–7.30 (m, 3, aromatic), 4.60–4.30 (m, 3, CHOC=O , $\text{CH}_2\text{OC=O}$), 4.01 (t, 1, $J = 4$ Hz, CHOH), 3.29 (d, 1, $J = 6$ Hz, OH), 2.80–2.25 (m, 2, $\text{CH}_2\text{C=O}$), 2.25–1.70 ppm (m, 4, $(\text{CH}_2)_2$).

Anal. Calcd for $\text{C}_{14}\text{H}_{16}\text{O}_5$: C, 63.63; H, 6.10. Found: C, 63.57; H, 6.26.

(+)-(S,R-*cis*)-3-(Hydroxymethyl)oxiranebutanoic Acid Methyl Ester (**38**). A 322-mg (1.22 mmol) sample of hydroxy lactone **36** was converted into the mesylate **37** as described above for the conversion of **22a** to **23**. The crude mesylate (406 mg), in 5.4 mL of dry methanol, was stirred with 177 mg (1.28 mmol) of anhydrous potassium carbonate, at 0–5 °C, for 2.5 h. The slurry was poured into brine and worked up with ether in the usual manner. The crude, oily product (313 mg) was chromatographed on 15 g of silica gel. Elution with ether followed by evaporative distillation afforded 120 mg (56.5%) of epoxide **38** as a colorless liquid, bp 95–97 °C (bath temperature) (0.05 mm), $[\alpha]_D^{25} +2.12^\circ$ (c 0.52, CHCl_3): IR 3615, 3515–3460 (OH), 1737 cm^{-1} (ester C=O); NMR 3.74 (m, 2, CH_2OH), 3.67 (s, 3, CO_2CH_3), 3.14, 3.00 (2 d of t, 2, $J(\text{vic}) = 4$ Hz, epoxide CH), 2.54 (m, 1, OH), 2.39 (t, 2, $J = 6.5$ Hz, $\text{CH}_2\text{CO}_2\text{CH}_3$), 1.70 ppm (m, 4, $(\text{CH}_2)_2$).

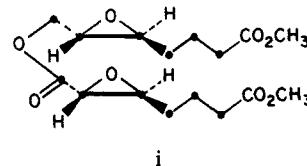
Anal. Calcd for $\text{C}_8\text{H}_{14}\text{O}_4$: C, 55.16; H, 8.10. Found: C, 54.93; H, 8.33.

On GC analysis (HP 5710A, 1 M \times 4 mm i.d., 5% SP 300 on Supelcoport 100/120, 125 °C isothermal, 30 mL/min), epoxide **38** exhibited a retention time of 62.3 min; epoxide **9**, 52.4 min.

(+)-(S,R-*trans*)-3-Formyloxiranebutanoic Acid Methyl Ester (**8**). A solution of 116 mL of pyridine in 4 L of reagent grade dichloromethane was stirred and cooled to 10–15 °C while 64 g (0.64 mol) of chromium trioxide (dried under high vacuum over P_2O_5) was added in portions, over 5–10 min. The ice bath was removed and the mixture was stirred for 1 h. The dark brown-orange suspension was again cooled to 15 °C and a solution of 19.3 g (0.11 mol) of epoxy alcohol **9** in 80 mL of dichloromethane was added dropwise over a 15–20-min period. After completion of the addition, the cooling bath was removed and the mixture was stirred for 1 h as the temperature rose from 15 °C to room temperature. The mixture was filtered through Celite and the residual chromium salts were washed with dichloromethane (2×250 mL). The combined filtrate and washes were concentrated in vacuo to a volume of ca. 200 mL and then diluted with 1 L of 1:1 hexane-ethyl acetate. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to a volume of ca. 50 mL, diluted with 250 mL of 1:1 hexane-ethyl acetate and again

filtered. Solvent removal left a pale-yellow liquid which was purified by HPLC (two silica gel columns; 2:1 hexane-ethyl acetate). There was obtained 13.3 g (69.7%) of epoxy aldehyde **8** as a pale-yellow liquid, $[\alpha]_D^{25} +74.9^\circ$ (c 0.27, CHCl_3) (lit.^{3b} $[\alpha]_D^{25} +68.6^\circ$ (c 0.31, CHCl_3); lit.^{3a} $[\alpha]_D^{25} +81^\circ$ (c 0.8, CDCl_3)): NMR 9.00 (d, 1, $J = 6.5$ Hz, HC=O), 3.14 ppm (dd, 1, $J = 2, 6.5$ Hz, OCHCHO).

A small polar impurity isolated during the HPLC separation (1.4 g, 7.4%) was the dimeric ester **i**: NMR 4.47 (dd, 1, $J = 2, 6$ Hz, CH of



CH_2O), 4.02 (dd, 1, $J = 3.5, 6$ Hz, CH of CH_2O), 3.68 (s, 6, $2 \times \text{CO}_2\text{CH}_3$), 3.28 (d, 1, $J = 1$ Hz, CHO), 3.15 (m, 1, CHO), 2.89 (m, 1, CHO), 2.37 (2t, 4, $2 \times \text{CH}_2\text{C=O}$), 2.00–1.40 ppm (m, 8, $4 \times \text{CH}_2$).

Anal. Calcd for $\text{C}_{16}\text{H}_{24}\text{O}_8$: C, 55.81; H, 7.03. Found: C, 55.51; H, 6.89.

(-)-(2*S*,3*S*,1*E*,3*E*)-3-(5-Oxo-1,3-pentadienyl)oxiranebutanoic Acid Methyl Ester (**7**). A. Using the Method of Corey et al.^{3b} A solution of 84.5 g (0.22 mol) of 1-tri(*n*-butylstannyl)-4-ethoxybutadiene³⁴ in 1 L of anhydrous tetrahydrofuran was stirred at -78 °C (dry ice-acetone bath) while 148 mL (0.236 mol) of 1.6 M *n*-butyllithium in hexane was added dropwise. After the mixture was stirred at -78 °C for 1 h, a solution of **33** (0.192 mol) of crude epoxy aldehyde **8** in 100 mL of tetrahydrofuran was slowly added (dropwise). Cooling to -78 °C was maintained while the mixture was stirred for an additional 1.75 h at which point the temperature was allowed to rise to -50 °C and 500 mL of saturated aqueous sodium bicarbonate was added. After being warmed to 0 °C, the mixture was worked up with ether in the usual manner giving a two-phase residue. The upper layer of tetrabutyltin was separated and discarded. The lower layer was dried under high vacuum, then dissolved in 300 mL of dichloromethane. With stirring and cooling to -40 to -50 °C, 27.3 g (0.27 mol) of triethylamine was added followed immediately by 26.3 g (0.23 mol) of methanesulfonyl chloride. The mixture was stirred at ca. -45 °C for 20 min, then treated with 500 mL of pH 7 buffer solution. After being warmed to 0 °C, the mixture was worked up with dichloromethane in the usual manner. The residual yellow oil was treated with ether and an insoluble yellow gum was removed by filtration of the ether solution through Celite. The filtrate was concentrated in vacuo and the residue was purified by HPLC (3:1 hexane-ethyl acetate), giving 2.12 g (4.9%) of epoxy dienal **7** as a yellow crystalline solid. A sample was recrystallized from ether-hexane, giving colorless crystals, mp 58–59 °C, $[\alpha]_D^{25} -35.34^\circ$ (c 2.04, CHCl_3): UV (EtOH) 276 nm (ϵ 35 000) (lit.^{3b} mp 36 °C, $[\alpha]_D^{25} -27.3^\circ$ (c 1.97, CHCl_3); UV (EtOH) 273 nm (ϵ 27 000); lit.^{3a} $[\alpha]_D^{25} -35^\circ$ (c 2.7, CHCl_3), lit.^{3r} mp 57.5–58.5 °C, $[\alpha]_D^{20} -31^\circ$ (c 1.98, CHCl_3); NMR 9.59 (d, 1, $J = 8$ Hz, HC=O), 7.11 (dd, 1, $J = 10, 15$ Hz, $\text{C}_3\text{-H}$), 6.64 (dd, 1, $J = 10, 15$ Hz, $\text{C}_2\text{-H}$), 6.16 (dd, 1, $J = 8, 15$ Hz, $\text{C}_4\text{-H}$), 5.98 (dd, 1, $J = 7.5, 15$ Hz, $\text{C}_1\text{-H}$), 3.68 (s, 3, CO_2CH_3), 3.24 (dd, 1, $J = 2, 7.5$ Hz, OCHCH=), 2.97 (dt, 1, $J = 2, 6$ Hz, OCHCH_2), 2.40 (d, 2, $J = 6$ Hz, $\text{CH}_2\text{C=O}$), 2.05–1.50 ppm (m, 4, $(\text{CH}_2)_2$).

Anal. Calcd for $\text{C}_{12}\text{H}_{16}\text{O}_4$: C, 64.27; H, 7.19. Found: C, 63.84; H, 7.09.

B. Using the Method of Ernest et al.^{3r} A solution of 10.6 g (32.1 mmol) of ylide **41**³⁶ (freshly prepared, recrystallized from CH_3CN) in 100 mL of dichloromethane, at room temperature, was treated with a solution of 4.6 g (26.7 mmol) of epoxide **8** in 40 mL of dichloromethane over ca. 15 min. The mixture was stirred at room temperature for 1 h. At the end of this time, 150 mL of 2:1 hexane-ethyl acetate was added and the mixture was filtered through a 50-mL plug of silica gel (deactivated by being packed in 1:1 hexane-ethyl acetate). The filtrate was concentrated in vacuo yielding a mixture of aldehyde isomers and triphenylphosphine oxide. This material was dissolved in 200 mL of dichloromethane containing 200 mg of iodine, and the resulting solution was kept at room temperature for 2.5 h. The isomerization can be followed by TLC (silica gel, 2:1 ether-hexane; the reported solvent system^{3r} failed to resolve the isomers in our hands). The solution was washed with aqueous sodium thiosulfate, then dried, and concentrated in vacuo. The solid residue was extracted by digestion-decantation with two 100-mL portions of 2:1 hexane-ethyl acetate, and the extracts were combined and concentrated in vacuo to give 5.5 g of crude **7**. Crystallization from ether-hexane afforded 4.05 g (67.7%) of the pure *E,E* dienal, mp 57–58 °C. The mother liquor residue was reexposed to iodine and purified by HPLC (deactivated silica gel, 2:1 hexane-ether) to yield an additional 0.47 g of pure **7** (total yield 75.5%) after crystallization.

(Z)-(3-Nonen-1-yl)triphenylphosphonium Iodide (43d). A mixture of 5.08 g (36.2 mmol) of 3-nonyn-1-ol (**42a**) (Farchan; redistilled, bp 63 °C (0.2 mm)), 160 mg of 5% Lindlar catalyst, 0.3 mL of synthetic quinoline, and 50 mL of hexane was stirred in an atmosphere of hydrogen for 75 min at which point gas uptake essentially ceased. The total uptake of hydrogen was 905 mL (905 mL theory). The mixture was suction filtered through Celite and the filter cake was washed thoroughly with hexane. The hexane filtrate and washes were combined, washed with 10 mL of 3 N aqueous HCl and 10 mL of saturated brine, then dried over anhydrous magnesium sulfate, filtered, and concentrated at aspirator pressure to give an oily residue. This material was evaporatively distilled affording 4.96 g (96.4%) of (Z)-3-nonen-1-ol (**43a**) as a colorless oil, bp 92–97 °C (bath temperature) (1 mm) (lit.³⁸ bp 107–109 °C (20 mm)). GC analysis (HP 402, 6-ft, 20% Carbowax column, 150 °C isothermal) revealed a purity of 99.7%. Under these conditions, the olefin product exhibits a retention time of 8.7 min; the starting acetylene, 13.1 min. To a stirred solution of 4.9 g (34.4 mmol) of this alcohol in 100 mL of anhydrous pyridine, at 0–5 °C (ice bath), there was added in one portion 13.24 g (69.4 mmol) of *p*-toluenesulfonyl chloride. The mixture was stirred at 0–5 °C for 1.5 h during which time separation of a solid occurred and then stored at 0 °C for 23 h. With stirring, the resulting mixture was slowly poured into 1.37 mL of cold 1.5 N aqueous HCl. The mixture was worked up with ether in the usual manner giving 9.9 g (97.1%) of (Z)-3-nonen-1-yl tosylate (**43b**) as an oil which was used without further purification. The following reactions were carried out in amber-colored flasks and worked up in a darkened room because of the light sensitivity of iodide **43c**. A mixture of this tosylate (33.4 mmol), 10 g (66.7 mmol) of sodium iodide, and 40 mL of acetone was stirred at room temperature for 67 h. The resulting brown mixture was poured into 500 mL of water and worked up with ether in the usual manner (the ether extracts were additionally washed with 12% aqueous sodium bisulfite solution). There was obtained 8.0 g (95%) of (Z)-1-iodo-3-nonene (**43c**) as a colorless oil which was used immediately. A solution of this iodide (31.7 mmol) and 8.3 g (31.6 mmol) of triphenylphosphine in 50 mL of benzene was stirred and heated in an 80 °C oil bath for 43 h. The reaction mixture was cooled to room temperature and the phosphonium salt separated as an oil. The benzene layer was removed using a pipet and discarded. To the residue was added 30 mL of ether and the mixture was shaken vigorously. Upon standing, two layers formed and the upper (ether) layer was removed and discarded. This washing process was repeated with 30 mL of fresh ether. The residue was dissolved in 75 mL of dichloromethane and the solution was concentrated under water aspirator pressure to yield a thick oil. This material was dried under high vacuum at 55 °C for 22 h giving 11.2 g of salt **43d** as a viscous oil which crystallized slowly. The overall yield from 3-nonyn-1-ol was 61.3%. This salt retains solvents tenaciously and should be thoroughly dried before use. A sample was recrystallized twice from methyl acetate, giving a colorless solid, mp 86.5 °C (lit.³⁴ mp 89–90 °C).

(3-Nonyn-1-yl)triphenylphosphonium Iodide (42d). A 2-g (14.3 mmol) sample of 3-nonyn-1-ol (**42a**) was converted into the iodide **42c** (1.9 g, 53.1%) via tosylate **42b**, essentially as described above for the preparation of **43c** from **43a**. A mixture of this iodide (7.6 mmol) and 2.2 g (8.4 mmol) of triphenylphosphine in 10 mL of benzene was stirred and heated at 80 °C for 2 days. The reaction mixture was concentrated and the residue purified by column chromatography on silica gel using a gradient elution of ethyl acetate to 5% methanol in ethyl acetate. There was obtained 2.5 g (64%) of salt **42d** as a colorless oil which could be recrystallized from tetrahydrofuran–ether, giving a solid, mp 97–100 °C.

Anal. Calcd for C₂₇H₃₀IP: C, 63.29; H, 5.90; P, 6.04; I, 24.77. Found: C, 63.47; H, 5.76; P, 6.18; I, 24.97.

(-)-(2S,3S,1E,3E,5Z,8Z)-3-(1,3,5,8-Tetradecatetraenyl)oxiranebutanoic Acid Methyl Ester (Leukotriene A₄ Methyl Ester) (5). The method of Corey et al.^{3b} was employed. A solution of 5.14 g (10 mmol) of phosphonium salt **43d** in 60 mL of anhydrous tetrahydrofuran was stirred at –78 °C (dry ice–acetone bath) as 6.25 mL (10 mmol) of 1.6 M *n*-butyllithium in hexane was added dropwise. Stirring at –78 °C was continued for 20 min whereupon 20.8 mL of hexamethylphosphoric triamide was added followed by 1.98 g (8.84 mmol) of epoxy diene **7**. After being stirred at –78 °C for 15 min, the mixture was allowed to warm to 0 °C and 100 mL of water was added. Rapid workup with ether in the usual manner gave a residue which was triturated in 300 mL of hexane. Removal of the solid triphenylphosphine oxide by suction filtration and concentration of the filtrate in vacuo afforded a yellow oil. Flash chromatography³⁷ of this material on silica gel, eluting with 3:1 hexane–ethyl acetate containing 2% triethylamine, gave 1.895 g (64.5%) of leukotriene A₄ methyl ester (**5**) as a nearly colorless solid. LC analysis revealed a purity of 96.1%. A sample was recrystallized from cold hexane giving crystals, mp 24–28 °C, [α]_D²⁵ –30.58° (c 0.28, cyclohexane); UV (MeOH) 270 (ε 34 550), 279 (49 170), 290 nm (36 520) (lit.^{3b} yellow oil; [α]_D²⁵ –21.9° (c 0.32, cyclohexane); UV (MeOH) 269, 278 (40 000), 289

nm); lit.^{3b} [α]_D –27° (c 0.8, cyclohexane); lit.^{3r} mp 28–32 °C; UV (MeOH) 270 (ε 43 900), 278 (56 700), 290 nm (43 100); NMR (C₆D₆) 7.06 (dd, 1, *J* = 12, 14 Hz, =CH), 6.35 (dd, 1, *J* = 12, 16 Hz, =CH), 6.20–5.95 (m, 2, 2 × =CH), 5.60–5.40 (m, 3, 3 × =CH), 5.31 (dd, 1, *J* = 8, 15 Hz, =CHCHO), 3.35 (s, 3, CO₂CH₃), 3.05–2.90 (m, 3, CHO and =CH₂C=), 2.56 (dt, 1, *J* = 2, 5.5 Hz, CHO), 2.08 (t, 2, *J* = 7.5 Hz, CH₂C=O), 2.05 (m, 2, CH₂C=), 1.61 (quintet, 2, *J* = 8 Hz, CH₂), 1.50–1.10 (m, 8, 4 × CH₂), 0.88 ppm (t, 3, *J* = 6 Hz, CH₃CH₂); mass spectrum *m/z* 332 (M⁺).

In a subsequent experiment carried out on a 20-mmol scale, the Wittig reaction was allowed to proceed for only 5 min at –70 °C; the reaction mixture was then immediately treated with 4:1 hexane–ethyl acetate. The mixture was washed five times with water, once with brine, dried (Na₂SO₄), filtered, and concentrated in vacuo. The residue was digested with 100 mL of hexane and the hexane solution was concentrated in vacuo to yield 6.6 g of crude **5**. This material was purified by HPLC (deactivated silica gel,^{3c} 95:5 hexane–ethyl acetate containing 1% triethylamine) giving the major component (4.65 g) as a solid. Recrystallization from hexane provided pure **5** (4.5 g, 67%), mp 34–35 °C, [α]_D²⁵ –38.6° (c 3.66, dioxane). The ¹H NMR spectrum of this material (200 MHz) was virtually identical with that of the racemic form.^{3c} Epoxide **5** should be stored at –80 °C, in the dark, under argon.

(2S,3S,1E,3E,5Z)-3-(1,3,5-Tetradecatrien-8-ynyl)oxiranebutanoic Acid Methyl Ester (14,15-Dehydroleukotriene A₄ Methyl Ester) (44). A solution of 384 mg (0.75 mmol) of phosphonium salt **42d** in 20 mL of tetrahydrofuran was treated with 0.35 mL (0.84 mmol) of *n*-BuLi (2.4 M in hexane), at –78 °C. After being stirred for 20 min, the mixture was treated with 1 mL of hexamethylphosphoric triamide. Stirring was continued for another 5 min whereupon 168 mg (0.75 mmol) of diene **7** was added. After 20 min at –78 °C, the reaction mixture was allowed to warm to 0 °C in 1.0 h and then quenched with H₂O. The solution was saturated with sodium chloride. The product was isolated rapidly by the usual extractive workup (ether) and chromatographed on a Lobar EM prepacked silica gel column (size B, predeactivated with methanol) with hexane–ethyl acetate–triethylamine (3:1:0.2). The yield of crude **44** was 106 mg as a yellow oil. A sample of this material was further purified by HPLC on a Waters μ-Porasil analytical column eluting with hexane–ethyl acetate–triethylamine (100:0.7:0.7) at a rate of 1 mL/min. The retention time of **44** was 15 min. Pure **44** was obtained as a solid, mp 32 °C; UV (CH₃OH) 266, 276 (ε 59 000), 287 nm; NMR 6.45 (m, 2, 2 × CH=), 6.21, 6.05 (2 m, 2, 2 × CH=), 5.46, 5.41 (2 m, 2, 2 × CH=), 3.68 (s, 3, CO₂CH₃), 3.15 (m, 1, =CHCHO), 3.06 (m, 2, C≡CCH₂CH=), 2.86 (m, 1, CH₂CHO), 2.38 (t, 2, *J* = 3.5 Hz, CH₂C=O), 2.14 (m, 2, CH₂C=), 1.79, 1.65, 1.47, 1.34 (4m, 10, 5 × CH₂), 0.89 ppm (t, 3, *J* = 3 Hz, CH₃CH₂).

[8,9-³H₂]-2S,3S,1E,3E,5Z,8Z)-3-(1,3,5,8-Tetradecatetraenyl)oxiranebutanoic Acid Methyl Ester ([14,15-³H₂]-Leukotriene A₄ Methyl Ester). A solution of 60 mg (0.18 mmol) of **44** in 15 mL of hexane was reduced under vacuum with 10 Ci (0.167 mol) of tritium gas over 60 mg of Lindlar catalyst (pretreated with 3% lead acetate). The mixture was stirred at room temperature for 1 h, and then unreacted tritium gas was removed from the system. The catalyst was filtered off and volatile components were removed by high-vacuum transfer at –180 °C. The residue was dissolved in 2 mL of hexane–ethyl acetate–triethylamine (9:1:0.2) and purified rapidly on a Lobar prepacked EM silica gel column (size A, predeactivated with methanol). The column was eluted with the same solvent mixture. Combination of the appropriate fractions gave crude product which was purified again by HPLC on a Waters μ-Porasil analytical column using hexane–ethyl acetate–triethylamine (100:0.7:0.7), at 1 mL/min. A total of 13 mg (0.04 mmol, 2.8 Ci) of [14,15-³H₂]-LTA₄ methyl ester was isolated with a retention time of 12 min. The yield was 24%.

[14,15-³H₂]-Leukotriene E₄ Dimethyl Ester. L-Cysteine methyl ester hydrochloride (100 mg, 0.74 mmol) was dissolved in 1 mL of a water–methanol mixture (30:5), and the solution was adjusted to pH 8.5 with triethylamine. This solution was then added to a mixture of 13 mg (0.04 mmol) of [14,15-³H₂]-LTA₄ methyl ester and 6 mg (0.018 mmol) of nonlabeled LTA₄ methyl ester. The mixture was stirred at room temperature overnight. The solvents were removed in vacuo, 25 mL of water was added to the residue, and the product was extracted with ether (3 × 25 mL). The combined ether extracts were dried over MgSO₄ and freed of solvent to give 2.8 Ci of crude [14,15-³H₂]-LTE₄ dimethyl ester. A sample of 100 mCi was purified rapidly by HPLC, and the rest was stored in 20% water/methanol at –80 °C to prevent decomposition. On a Waters μ-Bondapak reversed-phase analytical column, [14,15-³H₂]-LTE₄ dimethyl ester was isolated at a retention time of 15 min. The column was eluted with 20% water in methanol at 1 mL/min. The best fraction was concentrated to cloudiness and then extracted with ether. The combined ether extracts were dried over MgSO₄ and evaporated to give 18.7 mCi of pure product. Other fractions were discarded because

of fast decomposition. TLC analysis of the product on a silica gel plate (pretreated with hexane-ethyl acetate-triethylamine (9:1:0.2) and eluted with ethyl acetate) indicated the product to be 99% pure: UV (MeOH) 270, 280 (40 000), 290 nm. The specific activity (determination of mass by UV) was 39.6 Ci/mmol.

(5S,6R,7E,9E,11Z,14Z)-6-(S-L-Cysteinylglycyl)-5-hydroxy-7,9,11,14-eicosatetraenoic Acid Dipotassium Salt (Leukotriene D₄ (4b) Dipotassium Salt). Cysteinylglycine⁴⁰ (1 g, ca. 4.5 mmol; this material contained 1 equiv of lithium chloride) was dissolved in 10 mL of 6:1 methanol-water containing 1.5 mL of triethylamine. To the resulting solution was added 1 g (3 mmol) of LTA₄ methyl ester (5). After being stirred at room temperature for 18 h, the solution was diluted with 50 mL of water and most of the methanol was removed in vacuo, at 45 °C. The aqueous residue was freeze-dried. The residue was dissolved in water (30 mL), containing hydroquinone (10 mg), at 10 °C, and the solution was treated with aqueous KOH solution (600 mg in 10 mL). After 5 min at room temperature, water (50 mL) was added, the pH was adjusted to 9 with 0.5 N phosphoric acid, and the mixture was freeze-dried. The residue was dissolved in water and applied to a C₁₈-silica gel reverse-phase chromatography column (30 × 2 cm) packed in water. Elution with water removed the potassium phosphate while 7:3 methanol-water eluted the desired material (monitored by UV). Removal of methanol from the appropriate fractions in vacuo and freeze-drying of the aqueous residue gave 700 mg (40%) of LTD₄ dipotassium salt as a solid: UV (CH₃OH) 268 (ε 39 400), 280 (48 700), 292 nm (36 800). RPLC analysis (30 cm × 4 mm i.d. Waters C₁₈-silica gel; 65% CH₃OH, 35% pH 5.6 NH₄OAc; 280 nm) of this material revealed a purity of at least 94%. This salt was stored at -80 °C under argon, in the dark.

(5S,6R,7E,9E,11Z,14Z)-6-(S-Glutathionyl)-5-hydroxy-7,9,11,14-eicosatetraenoic Acid Dipotassium Salt (Leukotriene C₄ (4a) Dipotassium Salt). Glutathione (2 g, 6.5 mmol) was dissolved in 30 mL of 9:1 methanol-water, and the pH of the solution was adjusted to 8 by the addition of triethylamine. LTA₄ methyl ester (5, 1.5 g, 4.5 mmol) was added and the resulting mixture was stirred for 2 h at room temperature, then concentrated in vacuo. Flash chromatography³⁷ of the residue on silica gel (150 mL; CH₃OH-CH₂Cl₂-conc. NH₄OH 10:10:1) yielded 1.02 g of the adduct after concentration in vacuo and freeze-drying of the appropriate fractions (monitored by UV). This material was dissolved in 30 mL of water; the solution was treated with aqueous KOH solution (1 g in 10 mL) and allowed to stand at room temperature for 15 min. The pH of the solution was brought to 9 by the addition of 0.5 N phosphoric acid. The mixture was freeze-dried and the residue was digested with methanol. The insoluble solids were removed by filtration and the filtrate was evaporated in vacuo. The residue was desalted as described in the preceding experiment (30 × 2 cm C₁₈-silica gel RP column) giving 700 mg (21.2%) of LTC₄ dipotassium salt as a colorless solid: UV (CH₃OH) 268 (ε 39 000), 280 (49 400), 291 nm (40 000). Analysis of this material by RPLC (30-cm Waters C₁₈-silica gel; 65% CH₃OH, 35% pH 5.6 NH₄OAc; 280 nm) revealed a purity of at least

95%. This salt was stored at -80 °C, in the dark, under argon.

Anal. Calcd for C₃₀H₄₄K₂N₂O₆S₄H₂O: C, 46.60; H, 6.78; N, 5.43; K, 10.09. Found: C, 46.82; H, 6.51; N, 5.67; K, 10.14.

(5S,6R,7E,9E,11Z,14Z)-6-(S-L-Cysteinyl)-5-hydroxy-7,9,11,14-eicosatetraenoic Acid Monopotassium Salt (Leukotriene E₄ (4c) Monopotassium Salt). L-Cysteine methyl ester hydrochloride (2 g, 11.6 mmol) was dissolved in 20 mL of 6:1 methanol-water, and the solution was treated with triethylamine until pH 9 was reached. To this solution was added 2 g (6.0 mmol) of LTA₄ methyl ester (5), and after 1 h at room temperature the methanol was evaporated in vacuo. The residue was worked up with ethyl acetate in the usual manner, giving crude LTE₄ dimethyl ester. This material was purified by HPLC (deactivated silica gel,³⁶ one column; 1:1 hexane-ethyl acetate containing 3% CH₃OH), giving 2.65 g (94%) of pure diester. The spectral characteristics of this material were identical with those of material prepared by an alternative route.³⁶ A solution of this diester (5.57 mmol) in 20 mL of methanol was cooled to 5 °C, treated with KOH solution (2.5 g in 15 mL of H₂O), and stirred at room temperature for 15 min. Water (50 mL) was added and the solution was adjusted to pH 9 by the addition of 0.5 N phosphoric acid. *n*-Butyl alcohol (20 mL) was added and the organic solvents were evaporated in vacuo to yield a clear aqueous solution which was freeze-dried. The residue was digested with methanol (3 × 20 mL) and the methanol extracts were combined and concentrated in vacuo. The residue was dissolved in water and applied to a 30 × 2 cm RP column of C₁₈-silica gel. Desalting was carried out as described above. Elution with 1:4 CH₃OH-H₂O and 4:1 CH₃OH-H₂O gave two fractions (0.4 g and 1.8 g, respectively) after freeze-drying, both of which exhibited the characteristic leukotriene UV chromophore. The major fraction (less polar material—monopotassium salt) was at least 96% pure as determined by RPLC (same conditions as for LTC₄ and LTD₄): UV (CH₃-OH) 270 (ε 40 000), 280 (49 400), 291 nm (40 000). In several runs, microanalytical data indicated that the more polar material was the dipotassium salt and the less polar material was the monopotassium salt bis-hydrate. The 1.8 g obtained corresponds to a 62% yield based on a molecular weight of 513 for the latter species. This salt was stored at -80 °C, in the dark, under argon.

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Structure of the Sodium and Potassium Ion Activated Adenosinetriphosphatase Inhibitor L-681,110

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Abstract: Structure proposals for the major component A₁ of the Na⁺,K⁺-ATPase (EC 3.6.1.3; from porcine cerebral cortex or dog kidney) inhibitor L-681,110 and two minor components A₂ and B₁ (see Figure 4) are presented on the basis of spectroscopic evidence. They represent a new class of 16-membered macrocyclic lactones and are of considerable biological interest in that component A₁ also has comparable activity to the potent anthelmintic avermectins in stimulating GABA release from rat brain synaptosomes. Stereochemical assignments around the tetrahydropyran ring were deduced on the basis of spin-spin coupling constants.

The Na⁺,K⁺-ATPase [EC 3.6.1.3] inhibitor L-681,110 is produced by *Streptomyces* species MA-5038 and was isolated by

solvent extraction of the mycelia.¹ Besides inhibiting ATPase from dog kidney or porcine cerebral cortex, the major component