3-Hydroxyleukotriene B_4 (3-OH-LTB_4): Total Synthesis and Stereochemical Assignment[1]

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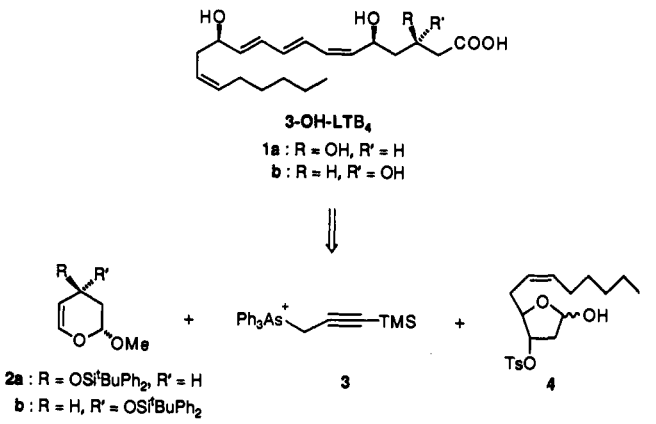
Abstract: The asymmetric total synthesis of 3-hydroxyleukotriene B_4 (3-OH-LTB_4), an ethanol-inducible proinflammatory autacoid, was achieved using a triply convergent strategy for the sequential union of propargylic arsonium salt **3** with pyranosides **2a,b** and furanose **4**. Both saccharide subunits were derived from commercial 2-deoxy-D-ribose. The key transformation involved palladium-mediated coupling of bromoacetylenide **9** with stannylglycal **6a,b**. Subsequent Rieke zinc hydrogenation of acetylene **10a,b** and controlled ionic reduction of the cross-conjugated cyclic enol ether using $NaBH_3CN$ at pH ~4–4.5 established the *cis*-$\Delta^{6,7}$-olefin and C(5)-hydroxyl stereochemistry, respectively, and led to **11a,b**. Methyllactol hydrolysis, PCC oxidation, methanolysis, and desilylation afforded 3(*R*)- and 3(*S*)-OH-LTB_4 methyl esters, respectively. On the basis of chromatographic and mass spectral comparisons, enzymatically derived 3-OH-LTB_4 is composed principally of the 3(*S*)-isomer (>95%).

Introduction

Leukotriene B_4 (LTB_4) is a dihydroxylated arachidonate metabolite of the 5-lipoxygenase pathway. As a consequence of its potent and unique biological properties, it is thought to play a major role in a broad range of inflammatory, allergic, and immunologic responses.[2] An important mechanism for the regulation of LTB_4 is its clearance from the circulation via hepatic uptake,[3] whereupon it is extensively modified.[4] The generally inactive or attenuated catabolites[5] are then eliminated by biliary excretion. Recently, however, Shirley and Murphy noted[6] that relatively low concentrations of ethanol interfere with the normal process of LTB_4 degradation by hepatocytes *in vitro*, with the consequent accumulation of a new metabolite identified by mass spectroscopy as 3-hydroxy-leukotriene B_4 (3-OH-LTB_4). This has significance when one realizes even moderate consumers of alcoholic beverages are exposed to such levels of ethanol. More importantly, 3-OH-LTB_4 substantially retains or even transcends[7] the pharmacologic characteristics of the parent autacoid and, thus, may represent the long-sought alcohol inducible chemotactic agent associated with fibrotic and cirrhotic liver degeneration.[8]

To expedite the physiologic evaluation of this unique, bioactive secondary metabolite[9] and to establish its absolute configuration, we describe herein the asymmetric total synthesis of 3(*R*)-and 3(*S*)-OH-LTB_4 (**1a** and **b**, respectively).[10] Our approach exploited a triply convergent strategy outlined retrosynthetically

Scheme 1



in Scheme 1 for the sequential union of propargylic arsonium salt **3** with pyranosides **2a,b** and furanose **4**, corresponding to subunits C(6)–C(8), C(1)–C(5), and C(9)–C(20), respectively.

Preparation of Stannylglycal 6a,b

Methyl β-2-deoxy-D-ribopyranoside (**5**), obtained from commercial 2-deoxy-D-ribose according to literature procedure,[11] was transformed to glycal **2a** by regioselective protection of the C(3)-alcohol via $AgNO_3$-promoted[12] silylation in THF/pyridine (70%) (Scheme 2). Minor amounts (~5–8%) of contaminatory C(4)-silyl ether were easily removed chromatographically, and the remaining free alcohol was dehydrated by way of the corresponding triflate using DBU (53%). Low-temperature metalation[13] with *t*-BuLi and stannylation of the resultant vinyl anion proceeded smoothly to give the versatile 1,5-dicarbonyl chiron **6a** (77%). Its C(3)-epimer, **6b**, was also conveniently acquired from **5**, albeit in modest yield, by double Mitsunobu inversion using excess

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(1) Presented in part at the 204th ACS National Meeting, Washington, DC, August 23–28, 1992.
(2) Reviews: Samuelsson, B. *Science* **1983**, *220*, 568–575. Marx, J. L. *Ibid.* **1982**, *215*, 1380–1383.
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(4) Harper, T. W.; Garrity, M. J.; Murphy, R. C. *J. Biol. Chem.* **1986**, *261*, 5414–5418.
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(7) Shirley, M. A.; Reldhead, C. T.; Murphy, R. C. *Biochem. Biophys. Res. Commun.* **1992**, *185*, 604–610.
(8) Lehmann, W. D.; Furstenberger, G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1027–1029.
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(10) An alternative total synthesis of 3-OH-LTB_4 has been described recently: Chauhan, K.; Bhatt, R. K.; Falck, J. R.; Capdevila; J. H. *Tetrahedron Lett.* **1994**, *35*, 1825–1828.
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(12) Kinzy, W.; Schmidt, R. R. *Tetrahedron Lett.* **1987**, *28*, 1981–1984.
(13) Tius, M. A.; Galeno, J. G.; Gu, X.; Zaidi, J. H. *J. Am. Chem. Soc.* **1991**, *113*, 5775–5783.

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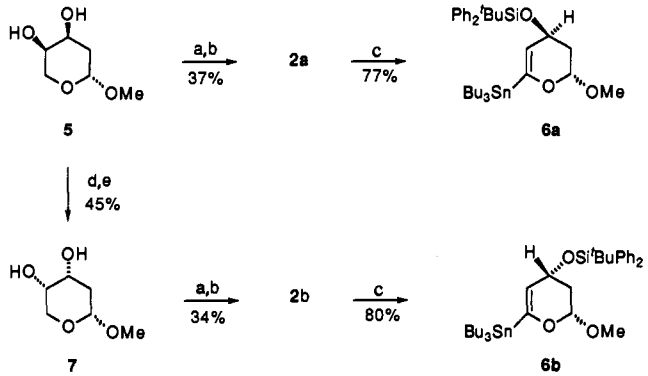
Scheme 2



[a] *tert*-$BuPh_2SiCl$, $AgNO_3$, THF/C_5H_5N (4:3), 23 °C, 3 h. [b] Tf_2O, CH_2Cl_2/C_5H_5N (6:1), −20 °C, 2 h; DBU (neat), 23 °C, 0.5 h. [c] *tert*-BuLi, THF, −45 °C, 1 h; *n*-Bu_3SnCl, 0 °C, 10 min. [d] DEAD, Ph_3P, $4\text{-}NO_2C_6H_4CO_2H$, $PhCH_3$, 50 °C, 4 h. [e] NaOMe/MeOH, 23 °C, 1 h.

4-nitrobenzoic acid (50%).[14] Saponification furnished diol **7** (90%), which was carried through to **6b** (27% overall) by the sequence described above. Interestingly, **7** behaved analogously to **5** during silylation, indicating the stereochemistry at the anomeric center has little influence on the differential reactivity of the *vic*-diols.

Convergence and Final Elaboration

The task of assembling the three subunits and concluding the functional group manipulations (Scheme 3) commenced with a Wittig condensation between the ylide of **3**[15] and the known furanose **4**,[16] which like the other chiral moiety, traces its origins to 2-deoxy-D-ribose. This step took advantage of the rapid, base-induced elimination of tosylate from the open-chain tautomer of **4** for the *in situ* generation of an *E*-enal.[16] Subsequent olefination by the excess ylide produced *E,E*-dienyne **8** (64%). A small amount of accompanying *E,Z*-dienyne was isomerized almost quantitatively to **8** by heating in cyclohexane with diphenyl disulfide for a few hours.[17] The somewhat labile **8** was converted uneventfully to bromide **9** in excellent yield (90% overall) by fluoride-mediated desilylation, protection of the hydroxyl, and treatment with $NBS/AgNO_3$.[18]

Palladium(0)[19]-catalyzed coupling[20] of the pivotal intermediate **9** with stannylglycal **6a** gave rise to adduct **10a** (71%) and completed the basic carbon framework.[21] The remaining stereocenters *en route* to **11a** were established by facile *cis*-hydrogenation[22] of the acetylene (90%) using Rieke zinc as described by White[23] and carefully controlled ionic reduction of the cross-conjugated cyclic enol ether with $NaBH_3CN$ at pH ~4–4.5.[13] The former reaction was completely stereoselective as judged by TLC and NMR. The latter reduction yielded **11a** (63%) and 5(*R*)-**11a** (16%) in an ca. 4:1 ratio, in agreement with kinetically controlled axial hydride delivery as observed by others[24] with analogous cyclic oxycarbenium ions. The configuration at C(5) was confirmed using lactone **12** (*vide infra*) by extensive spectral analysis including ^1H NMR data,[25] which were in generally close agreement with that of Evans[26] (Table 1). It is worth mentioning that reversing the order of reduction was unsuccessful; all attempts to reduce the enol ether without prior acetylene hydrogenation[27] returned starting material or, under forcing conditions, gave complex product mixtures.

Mild acidic hydrolysis of the pyranoside moiety in **11a** followed by pyridinium chlorochromate (PCC) oxidation formed **12a** (60% overall), from which **1a** methyl ester was obtained by conventional lactone methanolysis (100%) and desilylation (81%) with Bu_4NF in the presence of HOAc to modulate the basicity.[28] Repetition of the foregoing sequence beginning with **6b** proceeded analogously and with comparable yields to give **1b** methyl ester. Routine hydrolysis (LiOH, THF/H_2O (3:1), 0 °C, 2 h) provided **1a,b** (>95%) from the corresponding esters.

Stereochemical Assignment

Chromatographic (HPLC, GC) and mass spectral comparisons of **1a,b** methyl esters with material isolated from the incubation of LTB_4 with rat hepatocytes and esterified with CH_2N_2 demonstrated that the enzymatic product is principally composed of the 3(*S*)-isomer (>95%).[29] This is consistent with β-oxidation supported by mitochondrial or peroxisomal systems. The first step of β-oxidation in the peroxisome results in oxidation of LTB_4-CoA ester to a *trans*-α,β-enoyl-CoA ester which may be facilitated by the presence of ethanol.[30] Hydration of the intermediate *trans*-α,β-enoyl-CoA ester by enoyl-CoA hydratase (crotanase) forms the S_L-isomer of 3-hydroxyacyl-CoA ester. There have been several reports of the release of a 3-hydroxyacyl-CoA ester from the β-oxidation complex and the appearance of the 3-hydroxycarboxylic acid.[31] However, for LTB_4 metabolism in the presence of ethanol, the concentration of NAD^+ in the peroxisome is reduced by metabolism of ethanol in the cytosol. Since this cofactor is required for subsequent oxidation of the 3(*S*)-hydroxy-1-acyl-CoA ester into the 3-ketoacyl-CoA intermediate by 3-hydroxyacyl-CoA dehydrogenase, subsequent steps of β-oxidation are inhibited and the 3(*S*)-hydroxy metabolite of LTB_4 accumulates. Results concerning the physiologic significance of this process and the influence of stereochemistry on biological activity will be reported elsewhere.[29]

Experimental Section

Reagents and Methods. Unless otherwise stated, ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AC-250 spectrometer and

(14) Martin, S. F.; Dodge, J. A. *Tetrahedron Lett.* **1991**, *32*, 3017–3020. Coleman, R. S.; Fraser, J. R. *J. Org. Chem.* **1993**, *58*, 385–392.

(15) The less basic triphenylphosphonium analogue, in contrast, gave inferior yields (typically <20%) and/or complex product mixtures. Similar difficulties have been reported: Corey, E. J.; Ruden, R. A. *Tetrahedron Lett.* **1973**, 1495–1499.

(16) Lumin, S.; Falck, J. R.; Schwartzman, M. L. *Tetrahedron Lett.* **1991**, *32*, 2315–2318. On a preparative scale, the vinyl cuprate coupling was conducted in toluene at −10 °C for 14–15 h to avoid precipitation of the bis-tosylate, which is only partially soluble in Et_2O.

(17) Rokach, J.; Young, R. N.; Kakushima, M. *Tetrahedron Lett.* **1981**, *22*, 979–982.

(18) Magriotis, P. A.; Scott, M. E.; Kim, K. D. *Tetrahedron Lett.* **1991**, *32*, 6085–6088.

(19) Palladium(II) catalysts, *inter alia*, $(PPh_3)_2PdCl_2$, $(PPh_3)_2(PhCH_2)PdCl$, $PdCl_2(dppf)$, and $(CH_3CN)_2PdCl_2$, in a variety of solvents, were not satisfactory.

(20) Conceptually related couplings of stannylglycals: Dubois, E.; Beau, J.-M. *J. Chem. Soc., Chem. Commun.* **1990**, 1191. Friesen, R. W.; Sturino, C. F. *J. Org. Chem.* **1990**, *55*, 5808–5810.

(21) The more labile iodide version of **9** also participated in the coupling, but gave erratic yields of **10** (34–61%). Also, disappointing results were obtained by reversing the functionalities, i.e., having the tin on **9** in place of the bromide and a halogen on **6** instead of a stannyl group.

(22) Zinc copper–silver couple as recommended by Tropis et al. was somewhat sluggish and never reached completion: Tropis, M. A.; Pougny, J. R. *Tetrahedron Lett.* **1989**, *30*, 4951–4952. Catalytic hydrogenation using P2-Ni or Lindlar was complicated by incomplete reaction and/or overreduction.

(23) Chou, W.-N.; Clark, D. L.; White, J. B. *Tetrahedron Lett.* **1991**, *32*, 299–302.

(24) Dondoni, A.; Marra, A.; Scherrmann, M.-C. *Tetrahedron Lett.* **1993**, *34*, 7323–7326.

(25) It should be apparent that **12a** has the same relative stereochemical relationship to the Evans *syn*-lactone as 5(*R*)-**12a** does to the Evans *anti*-lactone. Since the absolute configuration at C(3) is known, the assignment at C(5) can be deduced. Likewise, **12b** relates to the *anti*-lactone as 5(*R*)-**12b** does to the *syn*-lactone.

(26) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem Soc.* **1988**, *110*, 3560–3578.

(27) Complexation of the acetylene with dicobalt octacarbonyl, followed by ionic reduction, returned mainly unreacted starting material or at higher temperatures (>0 °C) led to decomplexation without enol reduction.

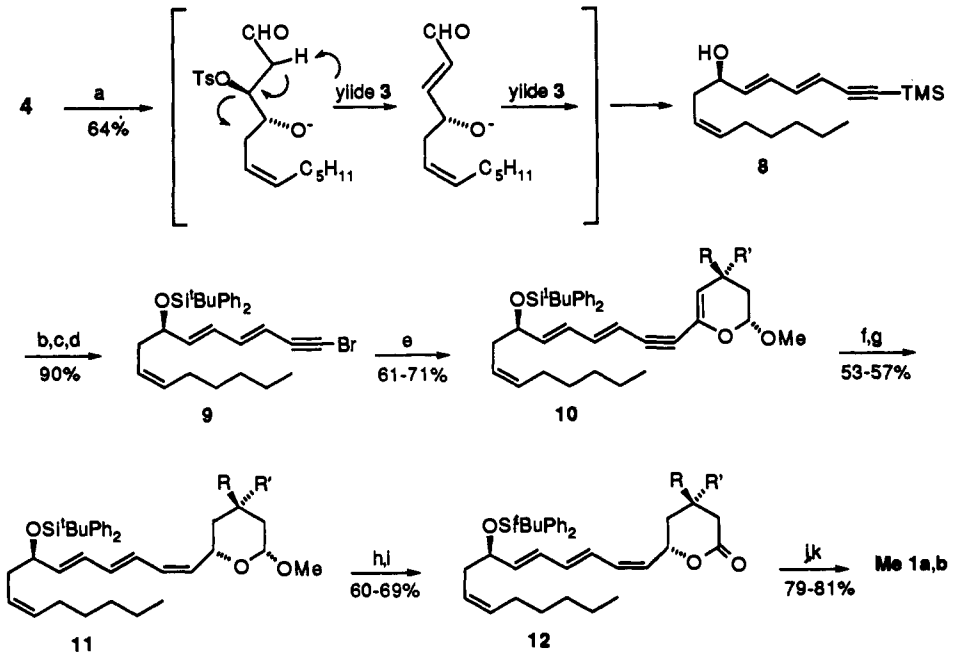
(28) Lee, T. J.; Holtz, W. J.; Smith, R. J. *J. Org. Chem.* **1982**, *47*, 4750–4757.

(29) For details of the stereochemical analysis as well as the results of recent biological testing consult, See: Wheelan, P.; Sala, A.; Folco, G.; Nicosia, S.; Falck, J. R.; Bhatt, R. K.; Murphy, R. C. Submitted for publication.

(30) Handler, J. A.; Thurman, R. G. *J. Biol. Chem.* **1990**, *265*, 1510–1515.

(31) Diczfalusy, U.; Alexson, S. E. H.; Sisfontes, L.; Olund, J.; Bjorkhem *Biochim. Biophys. Acta* **1990**, *1043*, 182–188. Tserng, K.-Y.; Jin, S.-J. *J. Biol. Chem.* **1990**, *266*, 11614–11620.

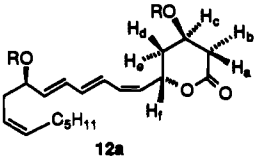
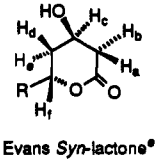
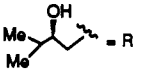
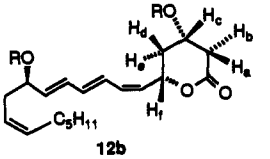
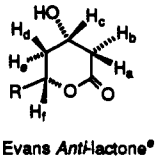
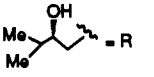
Scheme 3



a: R = OSi'BuPh$_2$, R' = H **b**: R = H, R' = OSi'BuPh$_2$

[a] **3**, NaN(SiMe$_3$)$_2$, THF/HMPA (5:1), −78° to −30 °C, 4 h. [b] *n*-Bu$_4$NF, THF, 24 °C, 1 h. [c] *tert*-BuPh$_2$SiCl/AgNO$_3$, C$_5$H$_5$N/CH$_2$Cl$_2$, 24 °C, 6 h. [d] NBS/AgNO$_3$, acetone, 24 °C, 1 h. [e] **6a,b**, (PPh$_3$)$_4$Pd(0), PhCH$_3$, 65 °C, 12 h. [f] Rieke Zn, THF/MeOH/H$_2$O (7:5:1), 65 °C, 3 h. [g] NaBH$_3$CN/6% HCl/MeOH (~pH 4–4.5), EtOH, 0° to 24 °C 5 h. [h] HOAc/H$_2$O/THF (2:1:1), 60 °C, 5 h. [i] PCC/Al$_2$O$_3$, CH$_2$Cl$_2$, 24 °C, 3 h. [j] MeOH/Et$_3$N, 24 °C, 1 h. [k] *n*-Bu$_4$NF/HOAc (2:1), THF, 45 °C, 14 h.

Table 1. Lactone Coupling Constants

 **12a** R = Si'BuPh$_2$	 Evans *Syn*-lactone[a]  = R	 **12b** R = Si'BuPh$_2$	 Evans *Anti*-lactone[a]  = R
J_{ab} = 17.2	J_{ab} = 17.7	J_{ab} = 17.2	J_{ab} = 17.0
J_{ac} = 3.9	J_{ac} = 3.6	J_{ac} = 5.8	J_{ac} = 5.8
J_{ad} = 1.5	J_{ad} = 1.4	J_{ad} = 1.3	J_{ad} = 1.3
J_{bc} = 5.2	J_{bc} = 4.7	J_{bc} = 7.9	J_{bc} = 7.6
J_{cd} = 3.6	J_{cd} = 3.6	J_{cd} = 4.5	J_{cd} = 4.3
J_{ce} = 3.2	J_{ce} = 3.1	J_{ce} = 9.1	J_{ce} = 9.1
J_{de} = 14.6	J_{de} = 14.5	J_{de} = 14.3	J_{de} = 13.8
J_{df} = 2.3	J_{df} = 2.4	J_{df} = 3.5	J_{df} = 3.1
J_{ef} = 11.7	J_{ef} = 11.6	J_{ef} = 11.7	J_{ef} = 11.7

[a] See ref 26.

are reported on the δ scale using tetramethylsilane as internal reference. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. Low-resolution mass spectra were obtained with a Finnigan SSQ 700 mass spectrometer, and high-resolution mass spectra were provided by the Midwest Center for Mass Spectrometry with partial support from NSF (DIR9017262). Elemental analyses were performed at Southern Methodist University, Dallas, TX.

All reactions were maintained under an argon atmosphere. Anhydrous solvents were freshly distilled from sodium benzophenone ketyl, except for CH$_2$Cl$_2$, toulene, and HMPA, which were distilled from CaH$_2$.

Tetrakis(triphenylphosphine)palladium(0) was freshly prepared as previously described.[32] Extracts were dried over anhydrous Na_2SO_4 and filtered prior to evaporation on a rotary evaporator under reduced pressure.

2(*R*)-Methoxy-4(*S*)-[(*tert*-butyldiphenylsilyl)oxy]-2,3-dihydro-4*H*-pyran (2a). To a stirring solution of methyl β-2-deoxy-D-ribopyranoside[11] (**5**) (6.4 g, 43 mmol) in THF (20 mL) was added pyridine (17 g, 0.215 mol) followed by $AgNO_3$ (8.83 g, 52 mmol). After 10 min in the dark, *tert*-butylchlorodiphenylsilane (15.4 g, 56 mmol) was added in one portion and the stirring continued for an additional 3 h. The reaction mixture was filtered through Celite 535, and the filter cake was washed with Et_2O (60 mL). The combined filtrate was washed with water (2 × 25 mL), saturated $CuSO_4$ solution (3 × 25 mL), and water (2 × 25 mL) and dried, and the solvent was removed *in vacuo*. Column chromatography (SiO_2) of the residue gave the C(3)-silyl ether of **5** (11.68 g, 70%) as a colorless syrup. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.43$. ^1H NMR: 7.60–7.68 (m, 4H), 7.32–7.50 (m, 6H), 4.68 (dd, J = 2.7, 3.2 Hz, 1H), 4.15 (ddd, J = 4.4, 6.5, 10.3 Hz, 1H), 3.70 (dd, J = 3.9, 12.7 Hz, 1H), 3.62 (dd, J = 1.7, 12.7 Hz, 1H), 3.52–3.58 (m, 1H), 3.20 (s, 3H), 2.52 (br s, 1H, D_2O exchangeable), 1.95–2.06 (m, 1H), 1.58–1.69 (m, 1H), 1.05 (s, 9H). ^{13}C NMR: 135.65, 135.58, 133.46, 133.32, 129.96, 129.91, 127.77, 127.70, 98.77, 68.07, 66.84, 61.64, 54.80, 33.52, 26.97, 19.14. MS (CI, CH_4) m/z (rel intensity): 387 ((M + H)$^+$, 0.5), 355 (8), 337 (18), 297 (83), 277 (92), 251 (100), 181 (77), 117 (69), 91 (24). HRMS: calcd for $C_{22}H_{30}O_4Si$ m/z 386.1913, found 386.1920.

To the above monosilyl ether (6.60 g, 17 mmol) in CH_2Cl_2 (25 mL) at −20 °C was added pyridine (4 g, 51 mmol) followed by neat trifluoromethanesulfonic anhydride (7.19 g, 26 mmol). The mixture was stirred at this temperature for 2 h, diluted with Et_2O (100 mL), washed with water (2 × 40 mL), saturated $CuSO_4$ solution (4 × 30 mL), and water (2 × 30 mL), dried, and concentrated under reduced pressure to give a labile, reddish syrup (8.60 g, 97%). This was immediately dissolved in neat DBU (10 mL). After 30 min, the reaction mixture was diluted with Et_2O (50 mL), washed with water (3 × 30 mL), dried, and concentrated *in vacuo*. Column chromatography (SiO_2, 2% EtOAc/hexane) furnished glycal **2a** (3.33 g, 53%) as a colorless oil. TLC (SiO_2): 10% EtOAc/hexane, $R_f \sim 0.47$. $[\alpha]^{24}D$: −158° (*c* 0.98, EtOH). ^1H NMR: 7.64–7.69 (m, 4H), 7.34–7.46 (m, 6H), 6.23 (dd, J = 0.7, 6.2 Hz, 1H), 4.94 (dd, J = 2.3, 8.1 Hz, 1H), 4.67 (ddd, J = 0.9, 4.3, 6.2 Hz, 1H), 4.28 (ddt, J = 0.7, 4.3, 5.1 Hz, 1H), 3.51 (s, 3H), 1.94–2.03 (m, 1H), 1.76 (ddd, J = 5.0, 8.0, 13.2 Hz, 1H), 1.06 (s, 9H). ^{13}C NMR: 142.33, 135.80, 135.73, 133.67, 133.49, 129.71, 129.63, 127.65, 127.58, 104.75, 98.58, 62.05, 56.27, 37.06, 26.98, 19.12. MS (CI, CH_4) m/z (rel intensity): 369 ((M + H)$^+$, 1), 337 (11), 311 (26), 252 (100), 233 (98), 213 (22), 113 (56), 81 (54). HRMS (FAB in 3-NBA): calcd for $C_{18}H_{19}O_3Si$ (M$^+$ − ^{t}Bu) m/z 311.1103, found 311.1108.

2(*R*)-Methoxy-4(*S*)-[(*tert*-butyldiphenylsily)oxy]-6-(tri-*n*-butylstannyl)-2,3-dihydro-4*H*-pyran (6a). A 1.6 M pentane solution of *t*-BuLi (217 mg, 3.39 mmol) was added dropwise to a −78 °C solution of glycal **2a** (0.50 g, 1.36 mmol) in THF (1.5 mL). After 1 h at −45 °C, the reaction was quenched by addition of tri-*n*-butyltin chloride (1.10 g, 3.40 mmol) and warmed to 0 °C. After 10 min, the reaction mixture was diluted with Et_2O (30 mL), washed with saturated NH_4Cl solution (20 mL), water (2 × 25 mL), and brine (25 mL), and dried, and the solvent was removed *in vacuo*. Flash column chromatography (SiO_2, 2% EtOAc/hexane) of the residue provided **6a** (687 mg, 77%) as a colorless oil. TLC (SiO_2): 20% EtOAc/hexane, $R_f \sim 0.63$. ^1H NMR: 7.50–7.70 (m, 4H), 7.18–7.45 (m, 6H), 4.84 (dd, J = 2.2, 7.9 Hz, 1H), 4.67 (dd, J = 1.0, 4.1 Hz, 1H), 4.22 (dt, J = 4.2, 4.5 Hz, 1H), 3.49 (s, 3H), 1.95–2.03 (m, 1H), 1.70–1.80 (m, 1H), 1.42–1.55 (m, 6H), 1.22–1.35 (m, 6H), 1.01 (s, 9H), 0.83–0.92 (m, 15H). ^{13}C NMR: 160.88, 135.81, 135.74, 134.60, 134.28, 129.73, 129.56, 127.69, 127.14, 115.98, 98.53, 62.73, 56.03, 37.31, 28.91, 27.84, 27.20, 26.98, 19.13, 13.68, 9.59. MS (CI, CH_4): m/z (rel intensity) 659 ((M + H)$^+$, 2), 657 (4), 601 (47), 403 (10), 335 (16), 309 (20), 213 (21), 199 (41), 57 (100). HRMS (FAB in 3-NBA) calcd for $C_{30}H_{45}O_3SnSi$ (M$^+$ − Bu) m/z 601.2160, found 601.2153.

Methyl β-2-Deoxy-L-ribopyranoside (7). Diethyl azodicarboxylate (DEAD) (10.26 g, 59 mmol) was added dropwise to a 50 °C suspension of triphenylphosphine (15.4 g, 59 mmol), 4-nitrobenzoic acid (9.85 g, 59 mmol), and **5** (2.90 g, 19.6 mmol) in toluene (60 mL). The resultant homogeneous yellow solution was maintained for 4 h and then concentrated under reduced pressure. Column chromatography (SiO_2, 25% EtOAc/hexane) of the residue gave the bis(4-nitrobenzoate) of **5** (4.37 g, 50%) as a colorless syrup. TLC (SiO_2): 50% EtOAc/hexane, $R_f \sim 0.61$. ^1H NMR: 8.20–8.30 (m, 4H), 8.10–8.18 (m, 4H), 5.78 (dt, J = 5.3, 9.3 Hz, 1H), 5.39 (dt, J = 5.3, 8.7 Hz, 1H), 4.90 (br t, J = 2.0 Hz, 1H), 4.00 (dd, J = 5.5, 11 Hz, 1H), 3.78 (dd, J = 9.6, 11 Hz, 1H), 3.57 (s, 3H), 2.42 (ddd, J = 2.0, 5.2, 13 Hz, 1H), 2.05 (ddd, J = 2, 11, 13Hz, 1H). ^{13}C NMR: 163.85, 163.76, 150.77, 150.68, 134.89, 134.54, 130.89, 130.79, 123.62, 98.12, 71.16, 70.16, 59.24, 55.19, 34.82. MS (CI, CH_4): m/z (rel intensity) 447 ((M + H)$^+$, 0.5), 414 (4), 351 (2), 247 (7), 195 (17), 139 (100), 93 (87). HRMS (FAB in 3-NBA): calcd for $C_{20}HN_2O_{10}$ m/z 446.0961, found 446.0970.

To a 0 °C methanolic solution (15 mL) of the above dibenzoate (4.2 g, 9 mmol) was added a 25% methanolic NaOMe solution (0.2 mL). After 1 h, the volatiles were removed *in vacuo* and the residue was chromatographed (SiO_2: 10% MeOH/CH_2Cl_2) to yield **7** (1.25 g, 90%) as white micro-needles, mp 54–56 °C, $[\alpha]^{24}D$ −130.7° (*c* 1.21, $CHCl_3$). ^1H NMR: 4.79 (dd, J = 1.4, 3.5 Hz, 1H), 3.80–3.91 (m, 2H), 3.68 (dd, J = 4.0, 9.4 Hz, 1H), 3.37–3.49 (m, 2H), 3.36 (s, 3H), 2.12 (ddd, J = 1.4, 5.0, 13.0 Hz, 1H), 1.62 (ddd, J = 3.5, 11.3, 13.0 Hz, 1H). ^{13}C NMR: 98.83, 71.75, 69.24, 62.13, 54.79, 37.24. Anal. Calcd for $C_6H_{12}O_4$: C, 48.63; H, 8.16. Found: C, 48.58; H, 8.39.

2(*R*)-Methoxy-4(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-2,3-dihydro-4*H*-pyran (2b). Diol **7** (1.25 g, 8.4 mmol) was regioselectively protected as described for **2a** to give the corresponding C(3)-silyl ether (2.21 g, 68%) as a colorless oil. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.30$. ^1H NMR: 7.60–7.70 (m, 4H), 7.30–7.42 (m, 6H), 4.10 (dd, J = 2.5, 8.7 Hz, 1H), 4.08 (dd, J = 3.0, 12.7 Hz, 1H), 3.82 (ddd, J = 2.0, 3.4, 5.1 Hz, 1H), 3.58 (br s, 1H), 3.39 (s, 3H), 3.24–3.30 (m, 1H), 2.55 (t, J = 1.7 Hz, 1H, D_2O exchangeable), 1.65–1.95 (m, 2H). ^{13}C NMR: 134.30, 134.18, 133.18, 133.00, 128.96, 128.70, 127.12, 126.92, 98.18, 67.60, 66.14, 61.15, 54.36, 33.06, 26.43, 19.25. MS (CI, CH_4): m/z (rel intensity) 387 ((M + H)$^+$, 1), 355 (6), 337 (16), 297 (100), 277 (71), 251 (57), 199 (45), 181 (83), 163 (32), 57 (81). HRMS: calcd for $C_{22}H_{30}O_4Si$ m/z 386.1913, found 386.1944.

Dehydration of the above monoprotected diol (1.93 g, 5 mmol) as described for **2a** furnished **2b** (0.94 g, 51%) as a colorless oil, $[\alpha]_D^{24}$ −13.2° (*c* 2.0, EtOH). TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.70$. ^1H NMR (C_6D_6): 7.74–7.83 (m, 4H), 7.18–7.30 (m, 6H), 6.12 (dd, J = 0.83, 6.1 Hz, 1H), 4.74 (ddd, J = 0.9, 2.2, 6.2 Hz, 1H), 4.45 (ddt, J = 0.8, 2.9, 6.9 Hz, 1H), 4.36 (dd, J = 2.3, 8.0 Hz, 1H), 3.42 (s, 3H), 2.20 (ddd, J = 1.7, 6.9, 13.3 Hz, 1H), 1.94 (ddd, J = 2.3, 6.3, 13.2 Hz, 1H), 1.06 (s, 9H). ^{13}C NMR (C_6D_6): 141.99, 136.20, 136.13, 134.61, 134.54, 129.99, 129.96, 128.36, 127.99, 106.14, 99.74, 63.77, 55.78, 37.84, 27.08, 19.36. MS (CI, CH_4): m/z (rel intensity) 369 ((M + H)$^+$, 0.5), 367 (2), 311 (36), 253 (24), 233 (43), 207 (100), 199 (21), 113 (78), 81 (91). HRMS (FAB in 3-NBA) calcd for $C_{22}H_{28}O_3Si$ m/z 368.1808, found 368.1796.

Preparation of 2(*R*)-Methoxy-4-(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-6-(tri-*n*-butylstannyl)-2,3-dihydro-4*H*-pyran (6b). Stannylation of **2b** as described for **2a** provided **6b** (80%) as a colorless oil. TLC (SiO_2): 10% EtOAc/hexane, $R_f \sim 0.65$. ^1H NMR (C_6D_6): 7.70–7.84 (m, 4H), 7.12–7.24 (m, 6H), 5.08 (d, J = 3.7 Hz, 1H), 5.03 (dd, J = 2.3, 7.2 Hz, 1H), 4.52 (q, J = 5.0 Hz, 1H), 3.34 (s, 3H), 2.20 (ddd, J = 2.1, 5.2, 13.4 Hz, 1H), 2.00 (ddd, J = 2.1, 4.9, 13.4 Hz, 1H), 1.57–1.64 (m, 6H), 1.30–1.43 (m, 6H), 1.10 (s, 9H), 0.85–1.02 (m, 15H). ^{13}C NMR (C_6D_6): 161.49, 136.28, 136.09, 135.18, 134.49, 130.78, 129.93, 128.73, 128.44, 116.95, 99.17, 63.24, 55.75, 37.68, 29.37, 27.61, 27.23, 26.81, 19.38, 13.93, 9.90. MS (CI, CH_4): m/z (rel intensity) 659 ((M + H)$^+$, 4), 601 (51), 403 (20), 335 (17), 289 (23), 213 (22), 198 (37), 177 (21), 135 (38), 111 (31), 57 (100). HRMS (FAB in 3-NBA): calcd for $C_{30}H_{45}O_3SiSn$ (M$^+$ − Bu) m/z 601.2160, found 601.2180.

(3-Trimethylsilyl-2-propynyl)triphenylarsonium Bromide (3). A solution of 3-bromo-1-(trimethylsilyl)-1-propyne[33] (7.6 g, 40 mmol) and triphenylarsine (27.6 g, 90 mmol) in anhydrous CH_3CN (75 mL) was heated at 60 °C in a sealed tube. After 28 h, the solvent was evaporated and the residue was triturated with 5% CH_2Cl_2/benzene (2 × 25 mL). The precipitate was collected by filtration, washed with dry benzene (30 mL), and recrystallized (CH_2Cl_2/hexane 4:1) to give **3** (12.6 g, 64%), mp 196 °C. ^1H NMR: 7.80–7.89 (m, 6H), 7.65–7.80 (m, 9H), 5.21 (s, 2H), −0.02 (s, 9H). ^{13}C NMR: −0.59, 20.32, 93.99, 95.07, 120.99, 130.69, 133.21, 134.29. Anal. Calcd for $C_{24}H_{26}AsBrSi$: C, 57.95; H, 5.27. Found: C, 57.67; H, 5.28.

7(*R*)-Hydroxy-1-(trimethylsilyl)-3(*E*),5(*E*),9(*Z*)-pentadecatrien-1-yne (8). To a −78 °C suspension of arsonium salt **3** (1.02 g, 2.44 mmol) in THF/HMPA (18 mL, 5:1) was added a 1 M THF solution of sodium bis(trimethylsilyl)amide (422 mg, 2.3 mmol). The reaction mixture was

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warmed to −30 °C over 30 min, kept at this temperature for 1 h, and then recooled to −78 °C. To this was added a THF (0.5 mL) solution of lactol **4**[16] (282 mg, 0.76 mmol). The reaction mixture was warmed to −30 °C over 1 h, maintained for 3 h, and quenched with 25% NH_4OAc solution (5 mL). The mixture was extracted with EtOAc (3 × 15 mL), and the combined extracts were washed with water (2 × 10 mL) and brine (10 mL) and dried. All volatiles were removed *in vacuo*. PTLC (SiO_2, CH_2Cl_2/EtOAc/hexane 2:1:7) gave silyl *E,E*-dienyne **8** (128 mg, 58%) and *E,Z*-dienyne (13 mg, 6%): TLC (SiO_2), 30% EtOAc/hexane, $R_f \sim 0.66$ and 0.70, respectively. The latter was isomerized nearly quantitatively to **8** by refluxing in cylohexane for 4 h with diphenyl disulfide (1 equiv) for a combined total yield of 64%. ^1H NMR: 6.60 (dd, J = 11, 15.6 Hz, 1H), 6.25 (ddd, J = 1.3, 10.8, 15.2 Hz, 1H), 5.82 (ddt, J = 0.71, 5.9, 15.2 Hz, 1H) 5.61 (dd, J = 0.57, 15.6 Hz, 1H), 5.50–5.60 (m, 1H), 5.25–5.40 (m, 1H), 4.24–4.30 (m, 1H), 2.30 (br t, J = 7.2 Hz, 2H), 1.95–2.08 (m, 2H), 1.65 (d, J = 3.3 Hz, 1H, D_2O exchangeable), 1.22–1.40 (m, 6H), 0.87 (t, J = 6.9 Hz, 3H), 0.17 (s, 9H). ^{13}C NMR: 142.05, 138.46, 134.18, 129.33, 123.79, 111.29, 104.23, 97.30, 71.55, 35.25, 31.51, 29.28, 27.41, 22.56, 14.60, -0.08. MS (CI, CH_4): m/z (rel intensity) 291 ((M + H)$^+$, 6), 275 (14), 238 (3), 206 (10), 179 (100), 149 (29), 105 (12), 91 (51), 73 (65). HRMS (EI): calcd for $C_{18}H_{29}OSi$ (M − H)$^+$ 289.1988, found 289.1989.

1-Bromo-7(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-3(*E*),5(*E*),9(*Z*)-pentadecatrien-1-yne (9). To a 0 °C THF solution (3 mL) of **8** (92 mg, 0.32 mmol) was added tetrabutylammonium fluoride (91 mg, 0.35 mmol) as a 1 M THF solution. After 1 h, the reaction mixture was diluted with water (10 mL) and extracted with Et_2O (2 × 15 mL), and the combined ethereal extracts were washed with 5% $NaHCO_3$ (2 × 10 mL), water (2 × 10 mL), and brine (10 mL), dried, and concentrated under reduced pressure to leave the *E,E*-dienyne (67.5 mg, 97%) as a colorless but somewhat labile oil, which was immediately used in the next step. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.61$. ^1H NMR: 6.60 (dd, J = 10.9, 15.6 Hz, 1H), 6.25 (dddd, J = 0.63, 1.3, 10.8, 15.2 Hz, 1H), 5.82 (dd, J = 6.0, 15.2 Hz, 1H), 5.61 (dd, J = 0.69, 15.6 Hz, 1H), 5.50–5.59 (m, 1H), 5.30–5.40 (m, 1H), 4.18–4.30 (m, 1H), 3.01 (d, J = 2.2 Hz, 1H), 2.32 (t, J = 7.3 Hz, 2H), 1.95–2.03 (m, 2H), 1.65 (d, J = 3.0 Hz, 1H, D_2O exchangeable), 1.25–1.30 (m, 6H), 0.88 (t, J = 6.7 Hz, 3H). ^{13}C NMR: 141.98, 138.45, 134.18, 129.29, 123.83, 111.35, 97.20, 94.38, 71.56, 35.42, 31.51, 29.29, 27.43, 22.58, 14.08.

To a solution of the above acetylenic alcohol (67 mg, 0.31 mmol) in anhydrous CH_2Cl_2 (3 mL) were added pyridine (123 mg, 1.55 mmol) and $AgNO_3$ (68 mg, 0.40 mmol) followed after 10 min by *tert*-butylchlorodiphenylsilane (111 mg, 0.40 mmol). After 6 h, the reaction mixture was filtered through a short bed of Celite 535, and the filtrate was diluted with Et_2O (25 mL), washed with saturated $CuSO_4$ solution (2 × 15 mL) and water (2 × 15 mL), and dried. The solvent was removed *in vacuo*. Chromatography of the residue gave the silyl ether (133 mg, 95%) as a colorless oil. TLC (SiO_2): 20% EtOAc/hexane, $R_f \sim 0.7$. ^1H NMR: 7.55–7.75 (m, 4H), 7.30–7.40 (m, 6H), 6.50 (dd, J = 10.6, 15.6 Hz, 1H), 5.85 (dd, J = 10.7, 15.3 Hz, 1H), 5.68 (dd, J = 6.2, 15.2 Hz, 1H), 5.35 (dd, J = 2.3, 15.7 Hz, 1H), 5.10–5.22 (m, 2H), 4.18–4.20 (m, 1H), 2.93 (d, J = 2.3 Hz, 1H), 2.08–2.22 (m, 2H), 1.70–1.92 (m, 2H), 1.10–1.30 (m, 6H), 1.03 (s, 9H), 0.80 (t, J = 6.4 Hz, 3H). ^{13}C NMR: 143.00, 139.95, 135.35, 135.00, 133.89, 133.61, 132.38, 132.18, 129.63, 128.75, 127.51, 127.46, 124.11, 109.44, 82.94, 79.19, 73.43, 35.83, 31.46, 29.18, 27.29, 27.00, 22.56, 19.33, 14.04. MS (CI, CH_4): m/z (rel intensity) 457 ((M + H)$^+$, 11), 399 (27), 379 (11), 345 (100), 199 (44), 135 (30). HRMS (EI): calcd for $C_{27}H_{31}OSi$ (M − Bu)$^+$ m/e 399.2144, found 399.2152.

To a solution of the above silyl ether (130 mg, 0.285 mmol) in acetone (4 mL) added $AgNO_3$ (5 mg, 0.028 mmol), followed by *N*-bromosuccinimide (NBS) (61 mg, 0.342 mmol). After 1 h, the solvent was evaporated under reduced pressure, and the residue was triturated with hexane. The precipitated succinimide was removed by filtration, and the filtrate was evaporated to give **9** (152 mg, 98%) as a pale yellow oil. TLC (SiO_2): 20% EtOAc/hexane, $R_f \sim 0.71$. ^1H NMR: 7.55–7.80 (m, 4H), 7.24–7.45 (m, 6H), 6.50 (dd, J = 10.6, 15.6 Hz, 1H), 5.95 (dd, J = 10.7, 15.3 Hz, 1H), 5.75 (dd, J = 6.3, 15.3 Hz, 1H), 5.40 (d, J = 15.3 Hz, 1H), 5.18–5.35 (m, 2H), 4.12–4.22 (m, 1H), 2.20–2.40 (m, 2H), 1.70–1.80 (m, 2H), 1.40–1.70 (m, 2H), 1.14-1.40 (m, 6H), 1.08 (s, 9H), 0.82 (t, J = 6.7 Hz, 3 H). ^{13}C NMR (C_6D_6): 143.10, 139.51, 136.28, 136.10, 135.10, 134.89, 132.53, 130.18, 130.00, 129.19, 128.78, 127.62, 124.60, 111.93, 110.55, 80.03, 73.99, 36.42, 31.78, 29.58, 27.68, 27.23, 22.95, 19.38, 14.28. MS (EI): m/z (rel intensity) 424 ((M − C_8H_{15})$^+$,16), 261 (17), 199 (100), 135 (24). HRMS: calcd for $C_{23}H_{24}{}^{79}BrOSi$ (M − C_8H_{15})$^+$ m/e 423.0780, found 423.0781.

Preparation of 2(*R*)-Methoxy-4(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-6-[7(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-3(*E*),5(*E*),9(*Z*)-pentadecatrien-1-ynyl]-2,3-dihydro-4*H*-pyran (10b). A solution of **6b** (237 mg, 0.36 mmol) in toluene (8 mL) was combined with a solution of **9** (161 mg, 0.30 mmol) and tetrakis(triphenylphosphine)palladium(0) (14 mg, 0.012 mmol) in toluene (2 mL). The initially homogeneous mixture was heated at 65 °C for 12 h and cooled to room temperature, and the solvent was removed *in vacuo*. Chromatography of the residue gave **10b** (176 mg, 71%) as a light yellow oil. TLC (SiO_2): 20% EtOAc/hexane, $R_f \sim 0.51$. ^1H NMR: 7.60–7.78 (m, 8H), 7.30–7.45 (m, 12H), 6.58 (dd, J = 10.8, 15.5 Hz, 1H), 6.00 (dd, J = 10.8, 15.2 Hz, 1H), 5.78 (dd, J = 6.3, 15.2 Hz, 1H), 5.58 (d, J = 15.6 Hz, 1H), 5.23–5.46 (m, 2H), 5.21 (d, J = 3.9 Hz, 1H), 5.02 (dd, J = 2.3, 6.8 Hz, 1H), 4.42 (q, J = 5.0 Hz, 1H), 4.20–4.30 (m, 1H), 3.55 (s, 3H), 2.18–2.36 (m, 2H), 1.90–2.10 (m, 1H), 1.75–1.89 (m, 3H), 1.20–1.35 (m, 6H), 1.10 (s, 18H), 0.90 (t, J = 6.7 Hz, 3H). ^{13}C NMR: 142.53, 139.48, 135.90, 135.71, 133.84, 132.41, 129.70, 129.63, 129.09, 127.68, 127.64, 127.50, 124.18, 111.00, 109.56, 99.26, 86.41, 73.82, 62.41, 56.45, 36.24, 35.91, 31.47, 29.19, 27.28, 26.99, 26.91, 22.56, 19.31, 19.19, 14.08. MS (CI, CH_4): m/z (rel intensity) 823 ((M + H)$^+$, 3), 794 (14), 766 (21), 652 (47), 623 (39), 577 (22), 565 (32), 504 (17), 429 (100). HRMS (FAB in 3-NBA): calcd for $C_{49}H_{57}O_4Si_2$ (M − Bu)$^+$ m/z 765.3795, found 765.3803.

2(*R*)-Methoxy-4(*S*)-[(*tert*-butyldiphenylsilyl)oxy]-6(*S*)-[7(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-1(*Z*),3(*E*),5(*E*),9(*Z*)-pentadecatetraen-1-yl]tetrahydropyran (11b). A methanolic solution (0.3 mL) of **10b** (75 mg, 0.091 mmol) was added dropwise to a suspension of Rieke zinc (60 mg, 10 equiv) in THF/MeOH/H_2O (3 mL, 7:5:1) heated to reflux. After 3 h, the reaction mixture was cooled to ambient temperature and filtered over Celite 535, and the clear filtrate was dried azeotropically using anhydrous benzene (5 mL). Chromatography of the residue afforded the *cis*-olefinic reduction product (68 mg, 90%) as a pale yellow oil. TLC (SiO_2): 10% EtOAc/hexane, $R_f \sim 0.51$. ^1H NMR: 7.65–7.80 (m, 8H), 7.32–7.55 (m, 12H), 7.15 (dd, J = 12.1, 13.6 Hz, 1H), 6.18 (dd, J = 10.7, 14.5 Hz, 1H), 6.10 (dd, J = 10.7, 13.6 Hz, 1H), 6.01 (t, J = 11.9 Hz, 1H), 5.78 (dd, J = 6.3, 14.2 Hz, 1H), 5.50 (d, J = 12 Hz, 1H), 5.35–5.43 (m, 2H), 5.10 (dd, J = 2.1, 7.9 Hz, 1H), 4.82 (d, J = 4.6 Hz, 1H), 4.45 (q, J = 4.5 Hz, 1H), 4.22–4.30 (m, 1H), 3.55 (s, 3H), 2.15–2.32 (m, 1H), 1.92–2.10 (m, 1H), 1.72–1.90 (m, 3H), 1.20–1.38 (m, 6H), 1.10 (s, 18H), 0.90 (t, J = 6.7 Hz, 3H). ^{13}C NMR (C_6D_6): 151.20, 137.18, 135.99, 135.91, 135.10, 132.61, 130.59, 130.42, 129.81, 129.74, 127.12, 127.01, 125.03, 123.92, 108.10, 99.01, 73.63, 62.78, 55.93, 36.77, 36.43, 31.57, 29.27, 27.40, 27.13, 27.03, 22.61, 19.48, 19.40, 14.32. MS (CI, CH_4): m/z (rel intensity) 825 ((M + H)$^+$, 3), 729 (6), 627 (15), 597 (16), 585 (33), 569 (100), 491 (37), 457 (83), 407 (43). HRMS (FAB in 3-NBA): calcd for $C_{52}H_{65}O_4Si_2$ (M − Me)$^+$ m/e 809.4421, found 809.4415.

Solutions of 6% methanolic HCl (0.1 mL) and 1 M ethanolic $NaBH_3CN$ (0.5 mL) were added simultaneously, but slowly over 2 h, to a 0 °C solution of the above reduction product (45 mg, 0.055 mmol) in absolute EtOH (1.5 mL). Upon complete addition, the reaction mixture was warmed to ambient temperature, where it was maintained for 3 h, quenched with saturated $NaHCO_3$ (2 mL), and extracted with Et_2O (3 × 10 mL). The combined ethereal extracts were washed with water (2 × 10 mL), dried, and evaporated *in vacuo*. Chromatography gave **11b** (28 mg, 63%) and 5(*R*)-**11b** (6.5 mg, 16%). TLC (SiO_2) of **11b** and 5(*R*)-**11b**: 15% EtOAc/hexane, $R_f \sim 0.42$ and 0.45, respectively. ^1H NMR of **11b**: 7.60–7.71 (m, 8H), 7.25–7.40 (m, 12H), 6.35 (dd, J = 11.6, 14.2 Hz, 1H), 6.18 (dd, J = 11, 14.9 Hz, 1H), 6.10 (dd, J = 10.7, 14.9 Hz, 1H), 6.00 (t, J = 11.4 Hz, 1H), 5.18–5.75 (m, 4H), 5.05–5.15 (m, 1H), 4.55–4.70 (m, 1H), 4.15–4.23 (m, 2H), 3.52 (s, 3H), 2.25–2.39 (m, 4H), 1.72-1.90 (m, 3H), 1.60–1.71 (m, 1H), 1.10–1.35 (m, 6H), 1.06 (s, 18H), 0.84 (t, J = 6.7 Hz, 3H). HRMS (FAB in 3-NBA): calcd for $C_{52}H_{67}O_4Si_2$ (M − Me)$^+$ m/z 811.4578, found 811.4539.

3(*S*),12(*R*)-Bis[(*tert*-butyldiphenylsilyl)oxy]-5(*S*)-hydroxy-6(*Z*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoic δ-lactone (12b). Methyllactol **11b** (28 mg, 0.034 mmol) was heated at 60 °C in a mixture of THF/H_2O/HOAc (1:1:2, 3 mL). After 5 h, the cooled reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with ice cold 5% $NaHCO_3$ solution (2 × 10 mL) and water (2 × 10 mL), dried, and concentrated *in vacuo* to give the lactol (19 mg, 69%) as a light yellow oil. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.45$. This was dissolved in CH_2Cl_2 (0.5 mL) and added to a suspension of pyridinium chlorochromate (11 mg, 0.05 mmol) and neutral Al_2O_3 (10 mg) in CH_2Cl_2 (2 mL). After 2 h, the reaction mixture was filtered, the filtrate concentrated *in vacuo*, and the residue chromatographed to yield **12b** (14 mg, 69% overall) as a colorless oil. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.60$. ^1H NMR: 7.60–7.86 (m, 8H),

7.38–7.59 (m, 12H), 6.32 (dd, J = 11.6, 14.2 Hz, 1H), 6.29 (dd, J = 10.7, 14.8 Hz, 1H), 6.20 (dd, J = 10.7, 14.8 Hz, 1H), 6.18 (t, J = 11.4 Hz, 1H), 5.84 (dd, J = 6.1, 15.1 Hz, 1H), 5.35–5.55 (m, 3H), 4.94–5.08 (m, 1H), 4.22–4.45 (m, 2H), 2.91 (ddd, J = 1.3, 5.8, 17.2 Hz, 1H), 2.61 (dd, J = 7.9, 17.2 Hz, 1H), 2.22–2.41 (m, 2H), 2.10–2.20 (m, 1H), 1.80–1.96 (m, 3H), 1.22–1.38 (m, 6H), 1.10 (s, 18H), 0.96 (t, J = 6.6 Hz, 3H). ^{13}C NMR: 170.08, 138.54, 135.90, 135.85, 135.66, 134.71, 134.30, 134.01, 133.03, 132.74, 132.33, 132.10, 130.13, 130.08, 129.62, 129.58, 129.10, 127.91, 127.86, 127.59, 127.52, 127.46, 126.10, 125.43, 124.16, 73.41, 73.05, 65.33, 39.82, 39.05, 35.94, 31.46, 29.18, 27.28, 27.06, 26.81, 26.42, 22.56, 19.74, 19.38, 14.06. HRMS (FAB in 3-NBA): calcd for $C_{48}H_{57}O_4Si_2$ (M – Bu)$^+$ m/z 753.3795, found 753.3801.

3(*S*)-Hydroxyleukotriene B_4 Methyl Ester (Me 1b). Methanolysis of **12b** (12 mg, 0.015 mmol) in dry MeOH (1 mL) containing Et_3N (0.1 mL) for 1 h and evaporation of all volatiles *in vacuo* generated the corresponding methyl ester (12 mg, 100%) as a colorless oil. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.51$. ^{1}H NMR: 7.62–7.85 (m, 8H), 7.25–7.42 (m, 12H), 6.18 (dd, J = 11.6, 14.2 Hz, 1H), 6.12 (dd, J = 10.7, 14.8 Hz, 1H), 6.07 (dd, J = 10.7, 14.8 Hz, 1H), 5.97 (t, J = 11.5 Hz, 1H), 5.72 (dd, J = 6.1, 15.1 Hz, 1H), 5.20–5.39 (m, 3H), 4.68–4.80 (m, 1H), 4.31–4.43 (m, 1H), 4.18–4.23 (m, 1H), 3.52 (s, 3H), 2.58 (dd, J = 1.4, 6.6 Hz, 2H), 2.12–2.33 (m, 2H), 1.96 (d, J = 3.0 Hz, 1H, D_2O exchangeable), 1.58–1.80 (m, 4H), 1.10–1.28 (m, 6H), 1.07 (s, 18H), 0.84 (t, J = 6.6 Hz, 3H). ^{13}C NMR: 171.56, 137.33, 135.89, 134.42, 134.17, 133.23, 132.67, 132.41, 132.19, 130.00, 129.90, 129.78, 129.54, 127.83, 127.71, 127.51, 127.48, 126.51, 124.34, 74.81, 68.53, 64.49, 51.48, 43.69, 41.95, 36.04, 31.47, 29.21, 27.29, 27.07, 26.93, 22.58, 19.34, 19.26, 14.08. MS (CI, CH_4): m/z (rel intensity) 843 ((M + H)$^+$, 6), 790 (63), 674 (75), 489 (90), 398 (100), 165 (74). HRMS (FAB in 3-NBA)/NaI): calcd for $C_{53}H_{70}O_5Si_2Na$ (M + Na)$^+$ m/z 865.4660, found 865.4684.

The preceding methyl ester (11 mg, 0.013 mmol) was dissolved in THF (1 mL) and added to a mixture of tetrabutylammonium fluoride trihydrate (21 mg, 0.065 mmol) and acetic acid (9 mg, 0.143 mmol) in THF (1 mL). After stirring at 45 °C for 14 h, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with ice cold 5% $NaHCO_3$ solution (2 × 10 mL) and water (2 × 10 mL), dried, and evaporated *in vacuo*. Chromatographic purification gave **1b** methyl ester (4 mg, 79%) as a colorless oil. TLC (SiO_2): 10% MeOH/CH_2Cl_2, $R_f \sim 0.44$. ^{1}H NMR: 6.50 (dd, J = 11.7, 14.1 Hz, 1H), 6.30 (dd, J = 10.6, 14.8 Hz, 1H), 6.19 (dd, J = 10.7, 14.7, 1H), 6.05 (t, J = 11.2 Hz, 1H), 5.80 (dd, J = 6.5, 14.4 Hz, 1H), 5.45–5.66 (m, 2H), 5.30–5.42 (m, 1H), 4.85–4.94 (m, 1H), 4.30–4.40 (m, 1H), 4.17–4.28 (m, 1H), 3.71 (s, 3H), 3.43 (d, J = 3.3, 1H, D_2O exchangeable), 2.32 (dd, J = 1.8, 7.1 Hz, 2H), 2.27 (d, J = 4.2 Hz, 1H, D_2O exchangeable), 2.24–2.40 (m, 2H), 1.95–2.16 (m, 2H), 1.62–1.80 (m, 2H), 1.08–1.20 (m, 6H), 0.84 (t, J = 6.6 Hz, 3H). ^{13}C NMR (C_6D_6): 172.93, 137.49, 134.78, 134.23, 133.00, 130.27, 129.99, 128.01, 125.63, 71.97, 65.42, 65.40, 51.17, 43.30, 41.50, 35.84, 31.79, 29.67, 27.75, 22.93, 14.26. HRMS: calcd for $C_{30}H_{58}O_5Si_3$ (tris-TMS ether of **1b** methyl ester) m/z 582.3592, found 582.3598.

Preparation of 2(*R*)-Methoxy-4(*S*)-[(*tert*-butyldiphenylsilyl)oxy]-6-[7(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-3(*E*),5(*E*),9(*Z*)-pentadecatrien-1-ynyl]-2,3-dihydro-4*H*-pyran (10a). Palladium-mediated coupling of **9** with **6a** as described for the preparation of **10b** gave **10a** (61%) as a pale yellow oil. TLC (SiO_2): 20% EtOAc/hexane, $R_f \sim 0.64$. ^{1}H NMR: 7.55–7.75 (m, 8H), 7.23–7.48 (m, 12H), 6.50 (dd, J = 10.6, 15.5 Hz, 1H), 5.98 (dd, J = 10.7, 15.2 Hz, 1H), 5.75 (dd, J = 6.3, 15.2 Hz, 1H), 5.50 (d, J = 15.6 Hz, 1H), 5.18–5.40 (m, 2H), 5.15 (d, J = 4.0 Hz, 1H), 5.00 (dd, J = 2.3, 6.8 Hz, 1H), 4.40 (q, J = 5.2 Hz, 1H), 4.18–4.25 (m, 1H), 3.50 (s, 3H), 2.15–2.40 (m, 2H), 1.88–2.04 (m, 1H), 1.71–1.82 (m, 3H), 1.15–1.35 (m, 6H), 1.07 (s, 18H), 0.86 (t, J = 6.7 Hz, 3H). ^{13}C NMR: 142.53, 139.49, 135.97, 135.83, 135.77, 134.06, 132.39, 129.75, 129.64, 129.13, 127.39, 127.53, 124.24, 111.51, 109.57, 99.36, 86.47, 73.66, 62.60, 56.35, 36.21, 35.98, 31.51, 29.22, 27.35, 27.08, 27.00, 22.58, 19.36, 19.16, 14.00. MS (CI, CH_4): m/z (rel intensity) 823 ((M + H)$^+$,4), 794 (16), 766 (20), 652 (44), 623 (37), 577 (21), 565 (33), 504 (18), 429 (100). HRMS (FAB in 3-NBA): calcd for $C_{49}H_{57}O_4Si_2$ (M – Bu)$^+$ m/z 765.3795, found 765.3814.

Preparation of 2(*R*)-Methoxy-4(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-6(*S*)-[7(*R*)-[(*tert*-butyldiphenylsilyl)oxy]-1(*Z*),3(*E*),5(*E*),9(*Z*)-pentadecatetraen-1-yl]tetrahydropyran (11a). Rieke zinc reduction of **10a** as described for **10b** afforded the corresponding *cis*-olefin (79%) as a light yellow oil. TLC (SiO_2): 10% EtOAc/hexane, $R_f \sim 0.52$. ^{1}H NMR: 7.60–7.72 (m, 8H), 7.27–7.47 (m, 12H), 7.08 (dd, J = 11.8, 13.8 Hz, 1H), 6.07 (dd, J = 10.7, 14.4 Hz, 1H), 6.04 (dd, J = 10.6, 13.9 Hz, 1H), 5.95 (t, J = 11.7 Hz, 1H), 5.68 (dd, J = 6.3, 14.4 Hz, 1H), 5.44 (d, J = 11.8 Hz, 1H), 5.15–5.34 (m, 2H), 5.05 (dd, J = 2.1, 8.0 Hz, 1H), 4.77 (d, J = 4.5 Hz, 1H), 4.40 (q, J = 4.5 Hz, 1H), 4.18–4.23 (m, 1H), 3.54 (s, 3H), 2.10–2.25 (m, 2H), 1.90–2.10 (m, 1H), 1.70–1.85 (m, 3H), 1.10–1.30 (m, 6H), 1.03 (s, 18H), 0.92 (t, J = 6.7 Hz, 3H). ^{13}C NMR: 151.80, 137.05, 135.98, 135.88, 134.56, 132.13, 130.57, 130.33, 129.59, 129.54, 127.63, 127.49, 124.55, 123.04, 107.19, 99.17, 73.82, 63.11, 56.58, 36.59, 36.18, 29.22, 27.35, 27.12, 27.05, 22.58, 19.39, 19.21, 14.00. MS (CI, CH_4): m/z (rel intensity) 825 ((M + H)$^+$, 4), 729 (7), 627 (15), 597 (17), 585 (32), 569 (100), 491 (38), 457 (84), 407 (43). HRMS (FAB in 3-NBA): calcd for $C_{52}H_{67}O_3Si_2$ (M – OCH_3)$^+$ m/z 795.4629, found 795.4608.

The above enol ether was reduced with $NaBH_3CN$ in the presence of methanolic HCl as described for the preparation of **11b** to give **11a** (67%) and 5(*R*)-**11a** as colorless syrups. TLC (SiO_2) of **11a** and 5(*R*)-**11a**: 10% EtOAc/hexane, $R_f \sim 0.39$ and 0.37, respectively. ^{1}H NMR of **11a**: 7.60–7.81 (m, 8H), 7.25–7.48 (m, 12H), 5.85–6.45 (m, 3H), 5.60–5.75 (m, 2H), 5.07–5.58 (m, 4H), 4.60–4.75 (m, 1H), 4.25–4.50 (m, 2H), 3.50 (s, 3H), 2.30–2.35 (m, 4H), 1.60–1.78 (m, 4H), 1.25–1.45 (m, 6H), 1.10 (s, 18H), 0.83 (t, J = 6.6 Hz, 3H).

Preparation of 3(*R*),12(*R*)-Bis[(*tert*-butyldiphenylsilyl)oxy]-5(*S*)-hydroxy-6(*Z*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoic δ-lactone (12a). Immediate hydrolysis and PCC oxidation of **11a** as described for the synthesis of **12b** furnished **12a** (60%) as a colorless oil. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.58$. ^{1}H NMR: 7.63–7.87 (m, 8H), 7.40–7.59 (m, 12H), 6.31 (dd, J = 11.6, 14.2 Hz, 1H), 6.27 (dd, J = 10.7, 14.8 Hz, 1H), 6.23 (dd, J = 10.7, 14.8 Hz, 1H), 6.16 (t, J = 11.4 Hz, 1H), 5.84 (dd, J = 6.2, 14.7 Hz, 1H), 5.25–5.58 (m, 3H), 4.89–4.97 (m, 1H), 4.20–4.48 (m, 2H), 2.83 (ddd, J = 1.5, 3.9, 17.2 Hz, 1H), 2.58 (dd, J = 5.2 , 17.2 Hz, 1H), 2.20–2.38 (m, 2H), 2.07–2.16 (m, 1H), 1.73–1.97 (m, 3H), 1.20–1.35 (m, 6H), 1.09 (s, 18H), 0.93 (t, J = 6.7 Hz, 3H). ^{13}C NMR: 170.17, 138.43, 135.80, 135.81, 135.68, 134.73, 134.40, 134.08, 133.00, 132.16, 132.33, 132.11, 130.18, 130.07, 129.63, 129.57, 129.11, 127.83, 127.71, 127.60, 127.50, 127.45, 126.08, 125.41, 124.20, 73.14, 72.97, 65.43, 39.81, 39.15, 35.95, 31.45, 29.32, 27.31, 27.16, 26.78, 26.43, 22.60, 19.78, 19.40, 14.16. MS (CI, CH_4): m/z (rel intensity) 811 (M$^+$ + 1, 0.5), 753 (6), 699 (100), 399 (3), 199 (18), 135 (31). HRMS (FAB in 3-NBA): calcd for $C_{52}H_{65}O_4Si_2$ (M – Me)$^+$ m/z 809.4421, found 809.4416.

3(*R*)-Hydroxyleukotriene B_4 Methyl Ester (Me-1a). Methanolysis of **12a** as described for **12b** gave the corresponding methyl ester disilyl ether (100%) as a colorless oil. TLC (SiO_2): 30% EtOAc/hexane, $R_f \sim 0.53$. ^{1}H NMR (C_6D_6): 7.75–7.84 (m, 8H), 7.16–7.34 (m, 12H), 6.41 (dd, J = 11.6, 14.6 Hz, 1H), 6.28 (dd, J = 10.9, 15.2 Hz, 1H), 6.04 (dd, J = 10.8, 14.6, Hz, 1H), 5.86 (t, J = 11.3 Hz, 1H), 5.80 (dd, J = 6.5, 14.7 Hz, 1H), 5.40–5.58 (m, 2H), 5.31 (dd, J = 8.9, 10.5 Hz, 1H), 4.55-4.80 (m, 2H), 4.38–4.45 (m, 1H), 3.24 (s, 3H), 2.34–2.60 (m, 4H), 1.65–1.90 (m, 4H), 1.22–1.34 (m, 6H) 1.18 (s, 9H), 1.16 (s, 9H), 0.84 (t, J = 6.7 Hz, 3H). ^{13}C NMR: 171.68, 137.73, 136.31, 135.71, 135.38, 134.47, 134.16, 133.94, 132.60, 130.40, 130.18, 129.95, 128.12, 127.92, 127.81, 126.40, 124.71, 74.50, 69.18, 65.52, 51.89, 43.51, 42.36, 36.00, 31.88, 29.57, 27.71, 27.41, 27.28, 22.96, 19.69, 19.60, 14.49. MS (CI, CH_4): m/z (rel intensity) 843 ((M + H)$^+$, 0.3), 797 (36), 709 (38), 699 (44), 529 (43), 411 (83), 219 (62), 149 (100). HRMS (FAB in 3-NBA/NaI): calcd for $C_{53}H_{70}O_5Si_2Na$ (M + Na)$^+$ m/z 865.4660, found 865.4671.

Desilylation of the above methyl ester as described for the synthesis of **1b** methyl ester gave **1a** methyl ester (81%) as a colorless oil. TLC (SiO_2): 10% MeOH/CH_2Cl_2, $R_f \sim 0.45$. ^{1}H NMR: 6.58 (dd, J = 11.7, 13.6 Hz, 1H), 6.34 (ddd, J = 1.1, 6.0, 10.6 Hz, 1H), 6.22 (dd, J = 8.8, 10.6 Hz, 1H), 6.10 (t, J = 10.8 Hz, 1H), 5.80 (dd, J = 6.3, 14.6 Hz, 1H), 5.50–5.61 (m, 1H), 5.24–5.42 (m, 2H), 4.89 (ddd, J = 3.7, 8.8, 12.4 Hz, 1H), 4.18–4.37 (m, 2H), 3.76 (s, 3H), 3.65 (br s, 1H, D_2O exchangeable), 2.97 (br s, 1H, D_2O exchangeable), 2.52 (dd, J = 1.8, 7.1 Hz, 2H), 2.74–2.40 (m, 2H), 1.88–2.08 (m, 2H), 1.62–1.82 (m, 2H), 1.56 (br s, 1H, D_2O exchangeable), 1.10–1.42 (m, 6H), 0.84 (t, J = 6.6 Hz, 3H). ^{13}C NMR (C_6D_6): 172.34, 137.13, 134.71, 134.20, 133.18, 130.06, 129.81, 128.46, 125.60, 71.91, 65.48, 65.41, 51.25, 43.41, 41.58, 35.86, 31.82, 29.68, 27.71, 22.80, 14.02. HRMS: calcd for $C_{30}H_{58}O_5Si_3$ (tris-TMS ether of **1a** methyl ester) m/z 582.3592, found 582.3588.

Isolation of Rat Hepatocytes and LTB_4 Incubation. Hepatocytes were isolated as reported using a collagenase-perfusion procedure.[34] Cell viability was 80% as determined by trypan blue exclusion. Cells (7.5×10^6/mL) were incubated at 37 °C for 5 min in 10 mL of buffer (128.8 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 1.0 mM $CaCl_2$, 3.0 mM Na_2HPO_4, 5.0 mM glucose). The P-450 inhibitor, ethoxyresorufin (10 μM), was added with ethanol (180 mM) and LTB_4 (24 μM). Cells were incubated for 30 min at 37 °C followed by centrifugation. The supernatant was removed and passed through a C-18 solid-phase extraction column (Bond Elute), which was washed with water. The metabolites were then eluted with methanol (8 mL).

Reverse-Phase HPLC Analysis. The extracted supernatant from the rat hepatocyte incubation was evaporated under vacuum and reconstituted in the initial HPLC mobile phase. The sample was analyzed by RP HPLC on an Ultremex column (4.6 × 250 mm, 5 μm C-18; Phenomenex, Rancho Palos Verdes, CA). The mobile phase consisted of methanol/water, 0.05% acetic acid (pH adjusted to 5.75 with ammonium hydroxide) at an initial composition of 50% methanol followed by a linear gradient to 100% methanol over 40 min. UV absorbance was monitored (HP-1040A photodiode array detector, Hewlett-Packard, Palo Alto, CA) at 270 nm (see the supplementary material). Synthetic 3(*R*)- and 3(*S*)-LTB_4 methyl esters (**1a** and **1b** methyl esters, respectively) were mixed together in a 2:1 ratio and then analyzed by RP HPLC using the same conditions (see the supplementary material). Retention times for **1a** and **1b** were 27.8 and 28.4 min, respectively. A new, more polar product designated metabolite I was collected during HPLC of the incubation, taken to dryness, and methylated using ethereal diazomethane. The methyl ester of metabolite I had a retention time of 28.5 min (see the supplementary material). The methyl esters of **1a,b** were added to the methyl ester derivative of metabolite I and coinjected (see the supplementary material). Metabolite I coeluted with **1b** methyl ester, thus establishing the 3(*S*)-stereochemistry of the enzymatic product.

GC/MS Analysis. A Finnigan SSQ 70 (San Jose, CA) was employed for both ECI and EI analyses. ECI mass spectra (negative ions) were obtained in the chemical ionization mode with methane as the moderating gas, and EI mass spectra (positive ions) were obtained using an electron energy of 70 eV. The GC capillary column was a 10 m × 0.25 mm DB-1 (J&W; Folsom, CA) column with 0.25-μm film thickness. The injector temperature was maintained at 275 °C and the transfer line at 300 °C. Samples (1 μL, 2–10 ng/μL acetonitrile for PFB/TMS derivatives and ECI analysis and 20–40 ng/μL acetonitrile for methyl ester/TMS derivatives and EI analysis) were injected into the gas chromatograph using an initial column temperature of 150 °C followed by a linear program at 15 °C/min to 310 °C. Equivalent carbon values (EC values) for PFB derivatives were determined by comparison with standard PFB esters of straight chain fatty acids. Samples were hydrogenated in methanol (400 μL) over 5% Rh/Al_2O_3 (0.2–0.4 mg). Hydrogen gas was bubbled through the suspension for 2 min at room temperature. The methanol supernatant was removed from the catalyst after centrifugation and the catalyst washed with additional methanol. The combined methanol extracts were evaporated to dryness under a nitrogen stream, and the sample was derivatized for GC/MS analysis.

ECI GC/MS Analysis of Metabolite I. Lyophilized metabolite I was derivatized by the addition of a 10% solution (v/v) of *N,N*-diisopropylethylamine in acetonitrile (50 μL) followed by the addition of a 10% solution (v/v) of pentafluorobenzyl bromide in acetonitrile (50 μL). The sample was kept at room temperature for 30 min and evaporated under a N_2 stream. The dried sample was further derivatized with the addition of acetonitrile (50 μL) and bis(trimethylsilyl)trifluoroacetamide (50 μL) and kept at 60 °C for 5 min followed by evaporation again under a N_2 stream. The resultant PFB/TMS derivative of metabolite I was analyzed by ECI GC/MS (EC = 24.2). This revealed an abundant carboxylate anion (A^-) ($M-181$) at m/z 567. Additional fragment ions were observed at m/z 495 [$A^- - (CH_3)_2SiCH_2$], m/z 477 (A^- – TMSOH], m/z 405 (A^- – TMSOTMS), m/z 387 [A^- – 2(TMSOH)), m/z 361 (loss of CO_2 from m/z 405), m/z 315 (A^- – TMSOTMS-TMSOH], m/z 297 [A^- – 3(TMSOH)), and m/z 271 (loss of TMSOH from m/z 361). Analysis of the PFB/TMS derivative of hydrogenated metabolite I (EC = 24.4) confirmed the presence of four double bonds with A^- observed at m/z 575.

EI GC/MS Analysis of 1a and 1b Methyl Esters. TMS derivatives were prepared (see above) from hydrogenated **1a** and **1b** methyl esters. Analysis by EI GC/MS showed identical spectra and were identical to the published spectrum of the reduced TMS ether methyl ester derivative of the metabolite obtained from rat hepatocytes.[4] TMS derivatives of **1a** and **1b** methyl esters were also prepared. These derivatives displayed good GC behavior, and the positive ion mass spectra obtained were identical. The molecular ion, $M^{\bullet+}$, was observed at m/z 582 (0.15%), and additional odd electron ions were observed at m/z 492 (4.4%, $M^{\bullet+}$ – TMSOH) and at m/z 402 (2.6%, loss of 2(TMSOH) from $M^{\bullet+}$). A fragment ion indicative of the C-3 substituent was observed at m/z 175 (48.2%), and a fragment ion indicative of the C-5 substituent was observed at m/z 291 (58.0%). Fragment ions indicative of the C-12 substituent were observed at m/z 471 (4.0%) and at m/z 381 (45.7%, loss of TMSOH from m/z 471).

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Supplementary Material Available: ^1H and ^{13}C spectra for all key intermediates and reverse-phase HPLC chromatograms of the LTB_4 hepatocyte incubation extract, coinjection of **1a** and **1b** methyl esters, and coinjection of **1a/1b** methyl esters (1:1) with metabolite I methyl ester (33 pages). This information is contained in many 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.

(34) Berry, M. N.; Edwards, A. M.; Barritt, G. J. In *Isolated Hepatocytes: Preparation, Properties and Applications*; Elsevier: Amsterdam, 1991; pp 25–32.