

# 3-Hydroxyleukotriene B<sub>4</sub> (3-OH-LTB<sub>4</sub>): Total Synthesis and Stereochemical Assignment<sup>1</sup>

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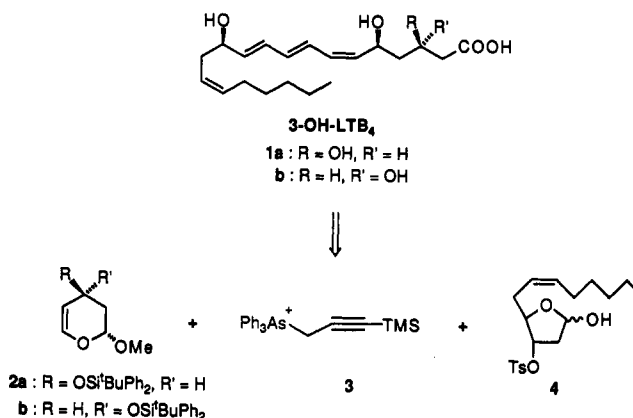
**Abstract:** The asymmetric total synthesis of 3-hydroxyleukotriene B<sub>4</sub> (3-OH-LTB<sub>4</sub>), an ethanol-inducible proinflammatory autacoid, was achieved using a triply convergent strategy for the sequential union of propargylic arsonium salt **3** with pyranosides **2a,b** and furanose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

## Introduction

Leukotriene B<sub>4</sub> (LTB<sub>4</sub>) 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.<sup>2</sup> An important mechanism for the regulation of LTB<sub>4</sub> is its clearance from the circulation via hepatic uptake,<sup>3</sup> whereupon it is extensively modified.<sup>4</sup> The generally inactive or attenuated catabolites<sup>5</sup> are then eliminated by biliary excretion. Recently, however, Shirley and Murphy noted<sup>6</sup> that relatively low concentrations of ethanol interfere with the normal process of LTB<sub>4</sub> degradation by hepatocytes *in vitro*, with the consequent accumulation of a new metabolite identified by mass spectroscopy as 3-hydroxy-leukotriene B<sub>4</sub> (3-OH-LTB<sub>4</sub>). This has significance when one realizes even moderate consumers of alcoholic beverages are exposed to such levels of ethanol. More importantly, 3-OH-LTB<sub>4</sub> substantially retains or even transcends<sup>7</sup> 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.<sup>8</sup>

To expedite the physiologic evaluation of this unique, bioactive secondary metabolite<sup>9</sup> and to establish its absolute configuration, we describe herein the asymmetric total synthesis of 3(*R*)- and 3(*S*)-OH-LTB<sub>4</sub> (**1a** and **b**, respectively).<sup>10</sup> 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 Stannylglycol **6a,b**

Methyl β-2-deoxy-D-ribosepyranoside (**5**), obtained from commercial 2-deoxy-D-ribose according to literature procedure,<sup>11</sup> was transformed to glycol **2a** by regioselective protection of the C(3)-alcohol via AgNO<sub>3</sub>-promoted<sup>12</sup> 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<sup>13</sup> 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.

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(8) Lehmann, W. D.; Furstenberger, G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1027–1029.

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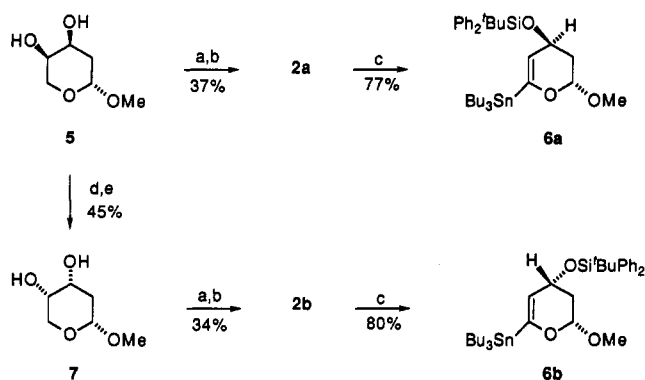
(10) An alternative total synthesis of 3-OH-LTB<sub>4</sub> has been described recently: Chauhan, K.; Bhatt, R. K.; Falck, J. R.; Capdevila, J. H. *Tetrahedron Lett.* **1994**, *35*, 1825–1828.

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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.

## Scheme 2



<sup>a</sup> *tert*-BuPh<sub>2</sub>SiCl, AgNO<sub>3</sub>, THF/C<sub>5</sub>H<sub>5</sub>N (4:3), 23 °C, 3 h. <sup>b</sup> Tf<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>/C<sub>5</sub>H<sub>5</sub>N (6:1), -20 °C, 2 h; DBU (neat), 23 °C, 0.5 h. <sup>c</sup> *tert*-BuLi, THF, -45 °C, 1 h; *n*-Bu<sub>3</sub>SnCl, 0 °C, 10 min. <sup>d</sup> DEAD, Ph<sub>3</sub>P, 4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>CO<sub>2</sub>H, PhCH<sub>3</sub>, 50 °C, 4 h. <sup>e</sup> NaOMe/MeOH, 23 °C, 1 h.

4-nitrobenzoic acid (50%).<sup>14</sup> 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<sup>15</sup> and the known furanose 4,<sup>16</sup> 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.<sup>16</sup> 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.<sup>17</sup> 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<sub>3</sub>.<sup>18</sup>

Palladium(0)<sup>19</sup>-catalyzed coupling<sup>20</sup> of the pivotal intermediate 9 with stannylglycol 6a gave rise to adduct 10a (71%) and completed the basic carbon framework.<sup>21</sup> The remaining stereocenters *en route* to 11a were established by facile *cis*-hydrogenation<sup>22</sup> of the acetylene (90%) using Rieke zinc as

(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<sub>2</sub>O.

(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<sub>3</sub>)<sub>2</sub>PdCl<sub>2</sub>, (PPh<sub>3</sub>)<sub>2</sub>(PhCH<sub>2</sub>)PdCl, PdCl<sub>2</sub>(dppf), and (CH<sub>3</sub>CN)<sub>2</sub>PdCl<sub>2</sub>, in a variety of solvents, were not satisfactory.

(20) Conceptually related couplings of stannylglycols: 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.

described by White<sup>23</sup> and carefully controlled ionic reduction of the cross-conjugated cyclic enol ether with NaBH<sub>3</sub>CN at pH ~4–4.5.<sup>13</sup> 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<sup>24</sup> with analogous cyclic oxycarbenium ions. The configuration at C(5) was confirmed using lactone 12 (*vide infra*) by extensive spectral analysis including <sup>1</sup>H NMR data,<sup>25</sup> which were in generally close agreement with that of Evans<sup>26</sup> (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<sup>27</sup> 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<sub>4</sub>NF in the presence of HOAc to modulate the basicity.<sup>28</sup> Repetition of the foregoing sequence beginning with 6b proceeded analogously and with comparable yields to give 1b methyl ester. Routine hydrolysis (LiOH, THF/H<sub>2</sub>O (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<sub>4</sub> with rat hepatocytes and esterified with CH<sub>2</sub>N<sub>2</sub> demonstrated that the enzymatic product is principally composed of the 3(*S*)-isomer (>95%).<sup>29</sup> 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<sub>4</sub>-CoA ester to a *trans*-α,β-enoyl-CoA ester which may be facilitated by the presence of ethanol.<sup>30</sup> Hydration of the intermediate *trans*-α,β-enoyl-CoA ester by enoyl-CoA hydratase (crotonase) forms the *S*<sub>L</sub>-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.<sup>31</sup> However, for LTB<sub>4</sub> metabolism in the presence of ethanol, the concentration of NAD<sup>+</sup> 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<sub>4</sub> accumulates. Results concerning the physiologic significance of this process and the influence of stereochemistry on biological activity will be reported elsewhere.<sup>29</sup>

## Experimental Section

**Reagents and Methods.** Unless otherwise stated, <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> on a Bruker AC-250 spectrometer and

(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.

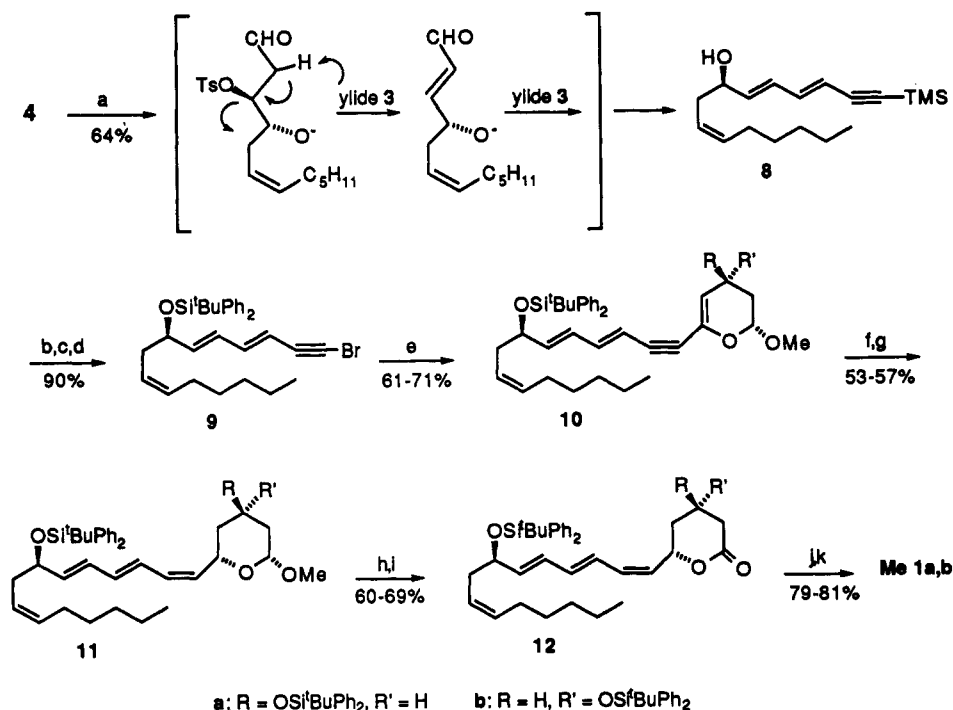
(28) Lee, T. J.; Holtz, W. J.; Smith, R. J. *J. Org. Chem.* **1982**, 47, 4750–4757.

(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) Diczfalussy, 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



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

Table 1. Lactone Coupling Constants

 12a R = Si <sup>t</sup> BuPh <sub>2</sub>	 Evans Syn-lactone <sup>a</sup> Me-CH(OH)-Me = R	 12b R = Si <sup>t</sup> BuPh <sub>2</sub>	 Evans Anti-lactone <sup>a</sup> Me-CH(OH)-Me = R
J <sub>ab</sub> = 17.2	J <sub>ab</sub> = 17.7	J <sub>ab</sub> = 17.2	J <sub>ab</sub> = 17.0
J <sub>bc</sub> = 3.9	J <sub>bc</sub> = 3.6	J <sub>bc</sub> = 5.8	J <sub>bc</sub> = 5.8
J <sub>cd</sub> = 1.5	J <sub>cd</sub> = 1.4	J <sub>cd</sub> = 1.3	J <sub>cd</sub> = 1.3
J <sub>de</sub> = 5.2	J <sub>de</sub> = 4.7	J <sub>de</sub> = 7.9	J <sub>de</sub> = 7.6
J <sub>ef</sub> = 3.6	J <sub>ef</sub> = 3.6	J <sub>ef</sub> = 4.5	J <sub>ef</sub> = 4.3
J <sub>fg</sub> = 3.2	J <sub>fg</sub> = 3.1	J <sub>fg</sub> = 9.1	J <sub>fg</sub> = 9.1
J <sub>gh</sub> = 14.6	J <sub>gh</sub> = 14.5	J <sub>gh</sub> = 14.3	J <sub>gh</sub> = 13.8
J <sub>af</sub> = 2.3	J <sub>af</sub> = 2.4	J <sub>af</sub> = 3.5	J <sub>af</sub> = 3.1
J <sub>ag</sub> = 11.7	J <sub>ag</sub> = 11.6	J <sub>ag</sub> = 11.7	J <sub>ag</sub> = 11.7

<sup>a</sup> See ref 26.

are reported on the  $\delta$  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<sub>2</sub>Cl<sub>2</sub>, toluene, and HMPA, which were distilled from CaH<sub>2</sub>.

Tetrakis(triphenylphosphine)palladium(0) was freshly prepared as previously described.<sup>32</sup> Extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and filtered prior to evaporation on a rotary evaporator under reduced pressure.

**2(R)-Methoxy-4(S)-[(*tert*-butyldiphenylsilyl)oxy]-2,3-dihydro-4H-pyran (2a).** To a stirring solution of methyl β-2-deoxy-D-ribofuranoside<sup>11</sup> (5) (6.4 g, 43 mmol) in THF (20 mL) was added pyridine (17 g, 0.215 mol) followed by AgNO<sub>3</sub> (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<sub>2</sub>O (60 mL). The combined filtrate was washed with water (2 × 25 mL), saturated CuSO<sub>4</sub> solution (3 × 25 mL), and water (2 × 25 mL) and dried, and the solvent was removed *in vacuo*. Column chromatography (SiO<sub>2</sub>) of the residue gave the C(3)-silyl ether of 5 (11.68 g, 70%) as a colorless syrup. TLC (SiO<sub>2</sub>): 30% EtOAc/hexane, *R<sub>f</sub>* ~ 0.43. <sup>1</sup>H 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<sub>2</sub>O exchangeable), 1.95–2.06 (m, 1H), 1.58–1.69 (m, 1H), 1.05 (s, 9H). <sup>13</sup>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<sub>4</sub>) *m/z* (rel intensity): 387 ((M + H)<sup>+</sup>, 0.5), 355 (8), 337 (18), 297 (83), 277 (92), 251 (100), 181 (77), 117 (69), 91 (24). HRMS: calcd for C<sub>22</sub>H<sub>30</sub>O<sub>4</sub>Si *m/z* 386.1913, found 386.1920.

To the above monosilyl ether (6.60 g, 17 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>O (100 mL), washed with water (2 × 40 mL), saturated CuSO<sub>4</sub> 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<sub>2</sub>O (50 mL), washed with water (3 × 30 mL), dried, and concentrated *in vacuo*. Column chromatography (SiO<sub>2</sub>, 2% EtOAc/hexane) furnished glycol 2a (3.33 g, 53%) as a colorless oil. TLC (SiO<sub>2</sub>): 10% EtOAc/hexane, *R<sub>f</sub>* ~ 0.47. [α]<sub>D</sub><sup>25</sup>: –158° (c 0.98, EtOH). <sup>1</sup>H 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). <sup>13</sup>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<sub>4</sub>) *m/z* (rel intensity): 369 ((M + H)<sup>+</sup>, 1), 337 (11), 311 (26), 252 (100), 233 (98), 213 (22), 113 (56), 81 (54). HRMS (FAB in 3-NBA): calcd for C<sub>18</sub>H<sub>19</sub>O<sub>3</sub>Si (M<sup>+</sup> – <sup>1</sup>Bu) *m/z* 311.1103, found 311.1108.

**2(R)-Methoxy-4(S)-[(*tert*-butyldiphenylsilyl)oxy]-6-(*tri-n*-butylstannyl)-2,3-dihydro-4H-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 glycol 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<sub>2</sub>O (30 mL), washed with saturated NH<sub>4</sub>Cl solution (20 mL), water (2 × 25 mL), and brine (25 mL), and dried, and the solvent was removed *in vacuo*. Flash column chromatography (SiO<sub>2</sub>, 2% EtOAc/hexane) of the residue provided 6a (687 mg, 77%) as a colorless oil. TLC (SiO<sub>2</sub>): 20% EtOAc/hexane, *R<sub>f</sub>* ~ 0.63. <sup>1</sup>H 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). <sup>13</sup>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<sub>4</sub>) *m/z* (rel intensity): 659 ((M + H)<sup>+</sup>, 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<sub>30</sub>H<sub>45</sub>O<sub>3</sub>SnSi (M<sup>+</sup> – Bu) *m/z* 601.2160, found 601.2153.

**Methyl β-2-Deoxy-L-ribofuranoside (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<sub>2</sub>, 25% EtOAc/hexane) of the residue gave the bis(4-nitrobenzoate) of 5 (4.37 g, 50%) as a colorless syrup. TLC (SiO<sub>2</sub>): 50% EtOAc/hexane, *R<sub>f</sub>* ~ 0.61. <sup>1</sup>H

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, 13 Hz, 1H). <sup>13</sup>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<sub>4</sub>) *m/z* (rel intensity): 447 ((M + H)<sup>+</sup>, 0.5), 414 (4), 351 (2), 247 (7), 195 (17), 139 (100), 93 (87). HRMS (FAB in 3-NBA): calcd for C<sub>20</sub>H<sub>20</sub>O<sub>10</sub> *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<sub>2</sub>: 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to yield 7 (1.25 g, 90%) as white micro-needles, mp 54–56 °C, [α]<sub>D</sub><sup>24</sup> –130.7° (c 1.21, CHCl<sub>3</sub>). <sup>1</sup>H 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 (dd, *J* = 1.4, 5.0, 13.0 Hz, 1H), 1.62 (ddd, *J* = 3.5, 11.3, 13.0 Hz, 1H). <sup>13</sup>C NMR: 98.83, 71.75, 69.24, 62.13, 54.79, 37.24. Anal. Calcd for C<sub>6</sub>H<sub>12</sub>O<sub>4</sub>: C, 48.63; H, 8.16. Found: C, 48.58; H, 8.39.

**2(R)-Methoxy-4(R)-[(*tert*-butyldiphenylsilyl)oxy]-2,3-dihydro-4H-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<sub>2</sub>): 30% EtOAc/hexane, *R<sub>f</sub>* ~ 0.30. <sup>1</sup>H 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<sub>2</sub>O exchangeable), 1.65–1.95 (m, 2H). <sup>13</sup>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<sub>4</sub>) *m/z* (rel intensity): 387 ((M + H)<sup>+</sup>, 1), 355 (6), 337 (16), 297 (100), 277 (71), 251 (57), 199 (45), 181 (83), 163 (32), 57 (81). HRMS: calcd for C<sub>22</sub>H<sub>30</sub>O<sub>4</sub>Si *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, [α]<sub>D</sub><sup>24</sup> –13.2° (c 2.0, EtOH). TLC (SiO<sub>2</sub>): 30% EtOAc/hexane, *R<sub>f</sub>* ~ 0.70. <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>): 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). <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>): 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<sub>4</sub>) *m/z* (rel intensity): 369 ((M + H)<sup>+</sup>, 0.5), 367 (2), 311 (66), 253 (24), 233 (43), 207 (100), 199 (21), 113 (78), 81 (91). HRMS (FAB in 3-NBA) calcd for C<sub>22</sub>H<sub>28</sub>O<sub>3</sub>Si *m/z* 368.1808, found 368.1796.

**Preparation of 2(R)-Methoxy-4-(R)-[(*tert*-butyldiphenylsilyl)oxy]-6-(*tri-n*-butylstannyl)-2,3-dihydro-4H-pyran (6b).** Stannylation of 2b as described for 2a provided 6b (80%) as a colorless oil. TLC (SiO<sub>2</sub>): 10% EtOAc/hexane, *R<sub>f</sub>* ~ 0.65. <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>): 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). <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>): 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<sub>4</sub>) *m/z* (rel intensity): 659 ((M + H)<sup>+</sup>, 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<sub>30</sub>H<sub>45</sub>O<sub>3</sub>SiSn (M<sup>+</sup> – Bu) *m/z* 601.2160, found 601.2180.

**(3-Trimethylsilyl-2-propynyl)triphenylarsonium Bromide (3).** A solution of 3-bromo-1-(trimethylsilyl)-1-propyne<sup>33</sup> (7.6 g, 40 mmol) and triphenylarsine (27.6 g, 90 mmol) in anhydrous CH<sub>3</sub>CN (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<sub>2</sub>Cl<sub>2</sub>/benzene (2 × 25 mL). The precipitate was collected by filtration, washed with dry benzene (30 mL), and recrystallized (CH<sub>2</sub>Cl<sub>2</sub>/hexane 4:1) to give 3 (12.6 g, 64%), mp 196 °C. <sup>1</sup>H NMR: 7.80–7.89 (m, 6H), 7.65–7.80 (m, 9H), 5.21 (s, 2H), –0.02 (s, 9H). <sup>13</sup>C NMR: –0.59, 20.32, 93.99, 95.07, 120.99, 130.69, 133.21, 134.29. Anal. Calcd for C<sub>24</sub>H<sub>26</sub>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  $-30^{\circ}\text{C}$  over 30 min, kept at this temperature for 1 h, and then recooled to  $-78^{\circ}\text{C}$ . To this was added a THF (0.5 mL) solution of lactol **4**<sup>16</sup> (282 mg, 0.76 mmol). The reaction mixture was warmed to  $-30^{\circ}\text{C}$  over 1 h, maintained for 3 h, and quenched with 25%  $\text{NH}_4\text{OAc}$  solution (5 mL). The mixture was extracted with  $\text{EtOAc}$  ( $3 \times 15\text{ mL}$ ), and the combined extracts were washed with water ( $2 \times 10\text{ mL}$ ) and brine (10 mL) and dried. All volatiles were removed *in vacuo*. TLC ( $\text{SiO}_2$ ,  $\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{hexane}$  2:1:7) gave silyl *E,E*-dienyne **8** (128 mg, 58%) and *E,Z*-dienyne (13 mg, 6%); TLC ( $\text{SiO}_2$ ), 30%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.66$  and 0.70, respectively. The latter was isomerized nearly quantitatively to **8** by refluxing in cyclohexane for 4 h with diphenyl disulfide (1 equiv) for a combined total yield of 64%.  $^1\text{H}$  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,  $\text{D}_2\text{O}$  exchangeable), 1.22–1.40 (m, 6H), 0.87 (t,  $J = 6.9$  Hz, 3H), 0.17 (s, 9H).  $^{13}\text{C}$  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,  $\text{CH}_4$ ):  $m/z$  (rel intensity) 291 (( $\text{M} + \text{H}$ )<sup>+</sup>, 6), 275 (14), 238 (3), 206 (10), 179 (100), 149 (29), 105 (12), 91 (51), 73 (65). HRMS (EI): calcd for  $\text{C}_{18}\text{H}_{29}\text{OSi}$  ( $\text{M} - \text{H}$ )<sup>+</sup> 289.1988, found 289.1989.

**1-Bromo-7(R)-[(*tert*-butyldiphenylsilyl)oxy]-3(E),5(E),9(Z)-pentadecatrien-1-yne (9).** To a  $0^{\circ}\text{C}$  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  $\text{Et}_2\text{O}$  ( $2 \times 15\text{ mL}$ ), and the combined ethereal extracts were washed with 5%  $\text{NaHCO}_3$  ( $2 \times 10\text{ mL}$ ), water ( $2 \times 10\text{ mL}$ ), 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 ( $\text{SiO}_2$ ): 30%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.61$ .  $^1\text{H}$  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,  $\text{D}_2\text{O}$  exchangeable), 1.25–1.30 (m, 6H), 0.88 (t,  $J = 6.7$  Hz, 3H).  $^{13}\text{C}$  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  $\text{CH}_2\text{Cl}_2$  (3 mL) were added pyridine (123 mg, 1.55 mmol) and  $\text{AgNO}_3$  (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  $\text{Et}_2\text{O}$  (25 mL), washed with saturated  $\text{CuSO}_4$  solution ( $2 \times 15\text{ mL}$ ) and water ( $2 \times 15\text{ mL}$ ), and dried. The solvent was removed *in vacuo*. Chromatography of the residue gave the silyl ether (133 mg, 95%) as a colorless oil. TLC ( $\text{SiO}_2$ ): 20%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.7$ .  $^1\text{H}$  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).  $^{13}\text{C}$  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,  $\text{CH}_4$ ):  $m/z$  (rel intensity) 457 (( $\text{M} + \text{H}$ )<sup>+</sup>, 11), 399 (27), 379 (11), 345 (100), 199 (44), 135 (30). HRMS (EI): calcd for  $\text{C}_{27}\text{H}_{31}\text{OSi}$  ( $\text{M} - \text{Bu}$ )<sup>+</sup>  $m/e$  399.2144, found 399.2152.

To a solution of the above silyl ether (130 mg, 0.285 mmol) in acetone (4 mL) added  $\text{AgNO}_3$  (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 ( $\text{SiO}_2$ ): 20%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.71$ .  $^1\text{H}$  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, 3H).  $^{13}\text{C}$  NMR ( $\text{C}_6\text{D}_6$ ): 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 (( $\text{M} - \text{C}_8\text{H}_{15}$ )<sup>+</sup>, 16), 261 (17), 199 (100), 135 (24). HRMS: calcd for  $\text{C}_{23}\text{H}_{24}^{79}\text{BrOSi}$  ( $\text{M} - \text{C}_8\text{H}_{15}$ )<sup>+</sup>  $m/e$  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-4H-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  $65^{\circ}\text{C}$  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 ( $\text{SiO}_2$ ): 20%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.51$ .  $^1\text{H}$  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).  $^{13}\text{C}$  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,  $\text{CH}_4$ ):  $m/z$  (rel intensity) 823 (( $\text{M} + \text{H}$ )<sup>+</sup>, 3), 794 (14), 766 (21), 652 (47), 623 (39), 577 (22), 565 (32), 504 (17), 429 (100). HRMS (FAB in 3-NBA): calcd for  $\text{C}_{49}\text{H}_{57}\text{O}_4\text{Si}_2$  ( $\text{M} - \text{Bu}$ )<sup>+</sup>  $m/z$  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/ $\text{H}_2\text{O}$  (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 ( $\text{SiO}_2$ ): 10%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.51$ .  $^1\text{H}$  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).  $^{13}\text{C}$  NMR ( $\text{C}_6\text{D}_6$ ): 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,  $\text{CH}_4$ ):  $m/z$  (rel intensity) 825 (( $\text{M} + \text{H}$ )<sup>+</sup>, 3), 729 (6), 627 (15), 597 (16), 585 (33), 569 (100), 491 (37), 457 (83), 407 (43). HRMS (FAB in 3-NBA): calcd for  $\text{C}_{52}\text{H}_{65}\text{O}_4\text{Si}_2$  ( $\text{M} - \text{Me}$ )<sup>+</sup>  $m/e$  809.4421, found 809.4415.

Solutions of 6% methanolic HCl (0.1 mL) and 1 M ethanolic  $\text{NaBH}_3\text{CN}$  (0.5 mL) were added simultaneously, but slowly over 2 h, to a  $0^{\circ}\text{C}$  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  $\text{NaHCO}_3$  (2 mL), and extracted with  $\text{Et}_2\text{O}$  ( $3 \times 10\text{ mL}$ ). The combined ethereal extracts were washed with water ( $2 \times 10\text{ mL}$ ), dried, and evaporated *in vacuo*. Chromatography gave **11b** (28 mg, 63%) and **5(R)-11b** (6.5 mg, 16%). TLC ( $\text{SiO}_2$ ) of **11b** and **5(R)-11b**: 15%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.42$  and 0.45, respectively.  $^1\text{H}$  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  $\text{C}_{52}\text{H}_{67}\text{O}_4\text{Si}_2$  ( $\text{M} - \text{Me}$ )<sup>+</sup>  $m/z$  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  $\delta$ -lactone (12b).** Methyl lactol **11b** (28 mg, 0.034 mmol) was heated at  $60^{\circ}\text{C}$  in a mixture of THF/ $\text{H}_2\text{O}$ / $\text{HOAc}$  (1:1:2, 3 mL). After 5 h, the cooled reaction mixture was diluted with water (10 mL) and extracted with  $\text{EtOAc}$  ( $3 \times 10\text{ mL}$ ). The combined organic extracts were washed with ice cold 5%  $\text{NaHCO}_3$  solution ( $2 \times 10\text{ mL}$ ) and water ( $2 \times 10\text{ mL}$ ), dried, and concentrated *in vacuo* to give the lactol (19 mg, 69%) as a light yellow oil. TLC ( $\text{SiO}_2$ ): 30%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.45$ . This was dissolved in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) and added to a suspension of pyridinium chlorochromate (11 mg, 0.05 mmol) and neutral  $\text{Al}_2\text{O}_3$  (10 mg) in  $\text{CH}_2\text{Cl}_2$  (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 ( $\text{SiO}_2$ ): 30%  $\text{EtOAc}/\text{hexane}$ ,  $R_f \sim 0.60$ .  $^1\text{H}$  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). <sup>13</sup>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<sub>48</sub>H<sub>57</sub>O<sub>4</sub>Si<sub>2</sub> (M – Bu)<sup>+</sup>  $m/z$  753.3795, found 753.3801.

**3(S)-Hydroxyleukotriene B<sub>4</sub> Methyl Ester (Me 1b).** Methanolysis of **12b** (12 mg, 0.015 mmol) in dry MeOH (1 mL) containing Et<sub>3</sub>N (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<sub>2</sub>): 30% EtOAc/hexane,  $R_f \sim 0.51$ . <sup>1</sup>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<sub>2</sub>O 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). <sup>13</sup>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<sub>4</sub>):  $m/z$  (rel intensity) 843 ((M + H)<sup>+</sup>, 6), 790 (63), 674 (75), 489 (90), 398 (100), 165 (74). HRMS (FAB in 3-NBA/NaI): calcd for C<sub>33</sub>H<sub>70</sub>O<sub>5</sub>Si<sub>2</sub>Na (M + Na)<sup>+</sup>  $m/z$  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<sub>3</sub> 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<sub>2</sub>): 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f \sim 0.44$ . <sup>1</sup>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,  $J = 10.7, 14.7$  Hz, 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<sub>2</sub>O exchangeable), 2.32 (dd,  $J = 1.8, 7.1$  Hz, 2H), 2.27 (d,  $J = 4.2$  Hz, 1H, D<sub>2</sub>O 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). <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>): 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<sub>30</sub>H<sub>58</sub>O<sub>5</sub>Si<sub>3</sub> (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-4H-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<sub>2</sub>): 20% EtOAc/hexane,  $R_f \sim 0.64$ . <sup>1</sup>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). <sup>13</sup>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<sub>4</sub>):  $m/z$  (rel intensity) 823 ((M + H)<sup>+</sup>, 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<sub>49</sub>H<sub>57</sub>O<sub>4</sub>Si<sub>2</sub> (M – Bu)<sup>+</sup>  $m/z$  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)-pentadecatrien-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<sub>2</sub>): 10% EtOAc/hexane,  $R_f \sim 0.52$ . <sup>1</sup>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). <sup>13</sup>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<sub>4</sub>):  $m/z$  (rel intensity) 825 ((M + H)<sup>+</sup>, 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<sub>52</sub>H<sub>67</sub>O<sub>3</sub>Si<sub>2</sub> (M – OCH<sub>3</sub>)<sup>+</sup>  $m/z$  795.4629, found 795.4608.

The above enol ether was reduced with NaBH<sub>3</sub>CN 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<sub>2</sub>) of **11a** and 5(R)-**11a**: 10% EtOAc/hexane,  $R_f \sim 0.39$  and 0.37, respectively. <sup>1</sup>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<sub>2</sub>): 30% EtOAc/hexane,  $R_f \sim 0.58$ . <sup>1</sup>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). <sup>13</sup>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<sub>4</sub>):  $m/z$  (rel intensity) 811 (M<sup>+</sup> + 1, 0.5), 753 (6), 699 (100), 399 (3), 199 (18), 135 (31). HRMS (FAB in 3-NBA): calcd for C<sub>52</sub>H<sub>65</sub>O<sub>4</sub>Si<sub>2</sub> (M – Me)<sup>+</sup>  $m/z$  809.4421, found 809.4416.

**3(R)-Hydroxyleukotriene B<sub>4</sub> 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<sub>2</sub>): 30% EtOAc/hexane,  $R_f \sim 0.53$ . <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>): 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). <sup>13</sup>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<sub>4</sub>):  $m/z$  (rel intensity) 843 ((M + H)<sup>+</sup>, 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<sub>53</sub>H<sub>70</sub>O<sub>5</sub>Si<sub>2</sub>Na (M + Na)<sup>+</sup>  $m/z$  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<sub>2</sub>): 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f \sim 0.45$ . <sup>1</sup>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<sub>2</sub>O exchangeable), 2.97 (br s, 1H, D<sub>2</sub>O 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<sub>2</sub>O exchangeable), 1.10–1.42 (m, 6H), 0.84 (t,  $J = 6.6$  Hz, 3H). <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>): 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<sub>30</sub>H<sub>58</sub>O<sub>5</sub>Si<sub>3</sub> (tris-TMS ether of **1a** methyl ester)  $m/z$  582.3592, found 582.3588.



**Isolation of Rat Hepatocytes and LTB<sub>4</sub> Incubation.** Hepatocytes were isolated as reported using a collagenase-perfusion procedure.<sup>34</sup> Cell viability was 80% as determined by trypan blue exclusion. Cells ( $7.5 \times 10^6$ /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<sub>4</sub>, 1.0 mM CaCl<sub>2</sub>, 3.0 mM Na<sub>2</sub>HPO<sub>4</sub>, 5.0 mM glucose). The P-450 inhibitor, ethoxycorufin (10  $\mu$ M), was added with ethanol (180 mM) and LTB<sub>4</sub> (24  $\mu$ M). 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 Ultramex column ( $4.6 \times 250$  mm, 5  $\mu$ m 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<sub>4</sub> 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  $\times$  0.25 mm DB-1 (J & W; Folsom, CA) column with 0.25- $\mu$ m film thickness. The injector temperature was maintained at 275 °C and the transfer line at 300 °C. Samples (1  $\mu$ L, 2–10 ng/ $\mu$ L acetonitrile for PFB/TMS derivatives and ECI analysis and 20–40 ng/ $\mu$ L 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  $\mu$ L) over 5% Rh/Al<sub>2</sub>O<sub>3</sub> (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  $\mu$ L) followed by the addition of a 10% solution (v/v) of pentafluorobenzyl bromide in acetonitrile (50  $\mu$ L). The sample was kept at room temperature for 30 min and evaporated under a N<sub>2</sub> stream. The dried sample was further derivatized with the addition of acetonitrile (50  $\mu$ L) and bis(trimethylsilyl)trifluoroacetamide (50  $\mu$ L) and kept at 60 °C for 5 min followed by evaporation again under a N<sub>2</sub> 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<sup>-</sup>) (*M* – 181) at *m/z* 567. Additional fragment ions were observed at *m/z* 495 [A<sup>-</sup> – (CH<sub>3</sub>)<sub>2</sub>SiCH<sub>2</sub>], *m/z* 477 [A<sup>-</sup> – TMSOH], *m/z* 405 [A<sup>-</sup> – TMSOTMS], *m/z* 387 [A<sup>-</sup> – 2(TMSOH)], *m/z* 361 (loss of CO<sub>2</sub> from *m/z* 405), *m/z* 315 [A<sup>-</sup> – TMSOTMS-TMSOH], *m/z* 297 [A<sup>-</sup> – 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<sup>-</sup> 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.<sup>4</sup> 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<sup>+</sup>, was observed at *m/z* 582 (0.15%), and additional odd electron ions were observed at *m/z* 492 (4.4%, M<sup>+</sup> – TMSOH) and at *m/z* 402 (2.6%, loss of 2(TMSOH) from M<sup>+</sup>). 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:** <sup>1</sup>H and <sup>13</sup>C spectra for all key intermediates and reverse-phase HPLC chromatograms of the LTB<sub>4</sub> 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.