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*An efficient total synthesis of leukotriene B<sub>4</sub>*

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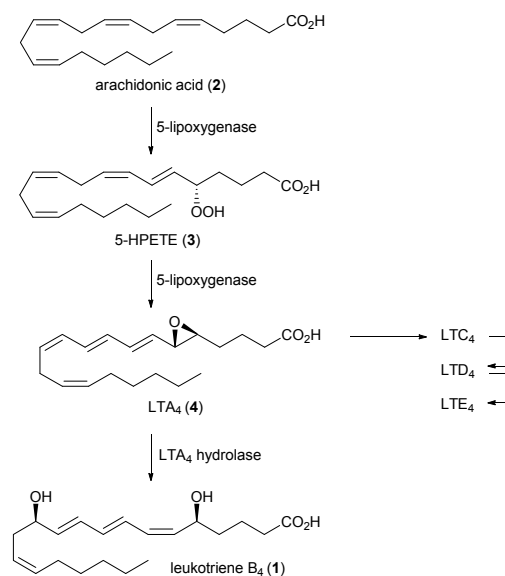
**Abstract**

Lipid mediators have attracted a great interest from scientist within the chemical, medicinal, and pharmaceutical research community. One such example is leukotriene B<sub>4</sub> which has been the subject of many pharmacological studies. Herein, we report a convergent and stereoselective synthesis of this potent lipid mediator in 5% yield over 10 steps in the longest linear sequence from commercial starting materials. The key steps were a stereocontrolled acetate-aldol reaction with Nagao's chiral auxiliary and a Z-selective Boland reduction. All spectroscopic data were in agreement with those previously reported.

**Introduction**

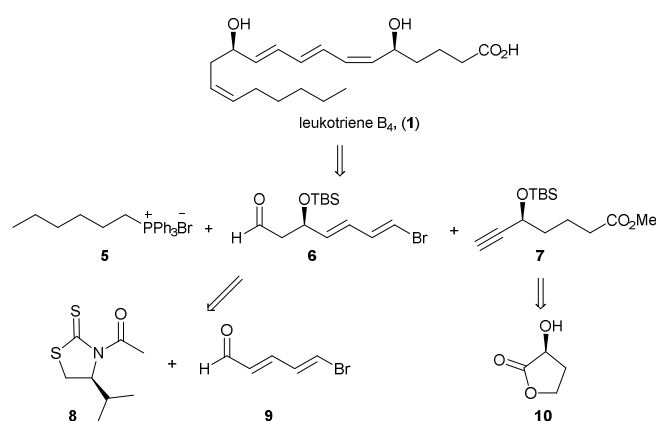
Leukotriene B<sub>4</sub> (LTB<sub>4</sub>, **1**) is a highly potent pro-inflammatory lipid mediator that promotes accumulation and activation of leukocytes at sites of inflammation.<sup>1</sup> In the first phase of acute inflammation, this dihydroxylated polyunsaturated fatty acid plays an important role, allowing leukocytes to cross from the bloodstream and into the tissue at the site of injury or infection.

LTB<sub>4</sub> (**1**) mediates its biological effects through two G protein-coupled receptors, BLT1 and BLT2.<sup>2</sup> BLT1 appears to mediate the major activities of LTB<sub>4</sub> on leukocytes and is important in inflammation, whereas BLT2 may be involved in various aspects of cancer progression.<sup>3</sup> The structure of LTB<sub>4</sub> (**1**) was reported by Borgeat and Samuelsson in 1979.<sup>4</sup> Shortly thereafter the first total synthesis by Corey and co-workers established the stereochemistry of the conjugated triene to be 6Z,8E,10E.<sup>5</sup> The name leukotriene was coined to reflect two attributes of this class of lipid mediators. The first relates to those white blood cells, like the polymorphonuclear leukocytes, that synthesize this class of eicosanoids, while the second part of the name reflects the conjugated triene present in the leukotrienes.<sup>6</sup> LTB<sub>4</sub> (**1**) is produced by enzymatic conversion of arachidonic acid (**2**) as outlined in Figure 1. First, insertion of molecular oxygen at the 5-position of **2** by 5-lipoxygenase (5-LO) produces 5-(S)-hydroperoxyeicosatetraenoic acid (5-HPETE, **3**), which is converted to leukotriene A<sub>4</sub> (LTA<sub>4</sub>, **4**) by the second catalytic activity of 5-LO, see Figure 1. LTA<sub>4</sub> (**4**) is the precursor of LTB<sub>4</sub>, as well as leukotriene C<sub>4</sub>, D<sub>4</sub> and E<sub>4</sub>. The latter three belongs to the cysteinyl leukotriene class of natural products. These are formed by addition of glutathione on LTA<sub>4</sub> (**4**) to form first LTC<sub>4</sub>, which is further converted successively by peptidases to LTD<sub>4</sub> and then LTE<sub>4</sub>. Enzymatic hydrolysis of LTA<sub>4</sub> (**4**) by LTA<sub>4</sub> hydrolase produces LTB<sub>4</sub> (**1**). On the other hand, nonenzymatic hydrolysis of LTA<sub>4</sub> (**4**) produces two all *trans* isomers of LTB<sub>4</sub> (**1**) and two 4,5-dihydroxy eicosatetraenoic acids,<sup>7</sup> all of which are lacking significant biological activity.<sup>8</sup> Further metabolism of LTB<sub>4</sub> (**1**) produce 20-OH and 20-COOH leukotriene B<sub>4</sub>,<sup>9</sup> and these metabolites have significantly lower biological activity than LTB<sub>4</sub> (**1**).<sup>10</sup>



**Figure 1.** Outline of the biosynthesis of leukotriene B<sub>4</sub> (**1**).

Although several syntheses of LTB<sub>4</sub> (**1**) have been reported in the literature,<sup>11</sup> this highly potent lipid mediator is still of interest to the scientific community. The stability of the conjugated *Z,E,E*-triene found in **1** is a challenge for all synthesis of **1**. This moiety is prone to undergo isomerization in the presence of light, oxygen and acidic conditions. The instability of the *Z,E,E*-triene is further intensified by the presence of the two chiral allylic alcohols, rendering this moiety prone to water elimination. Based on our success with the acetate-aldol reaction in recent synthesis of some lipid mediators,<sup>12</sup> we were motivated to demonstrate this strategy in an efficient and convergent synthesis of LTB<sub>4</sub> (**1**). The retrosynthetic analysis, shown in Figure 2, allows for the installment of the sensitive *Z,E,E*-triene at a late stage of the synthesis.

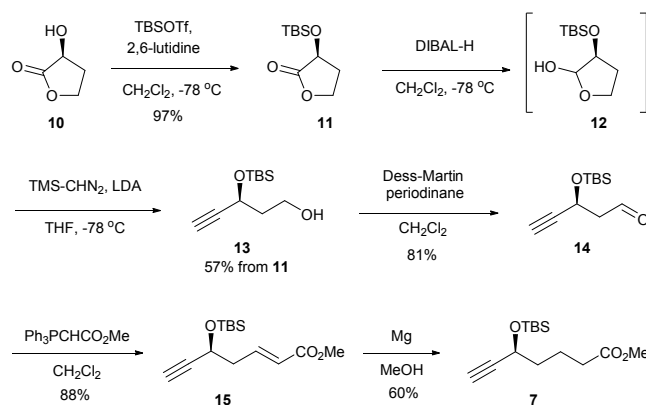


**Figure 2.** Retrosynthetic analysis of leukotriene B<sub>4</sub> (**1**).

## Results and discussion

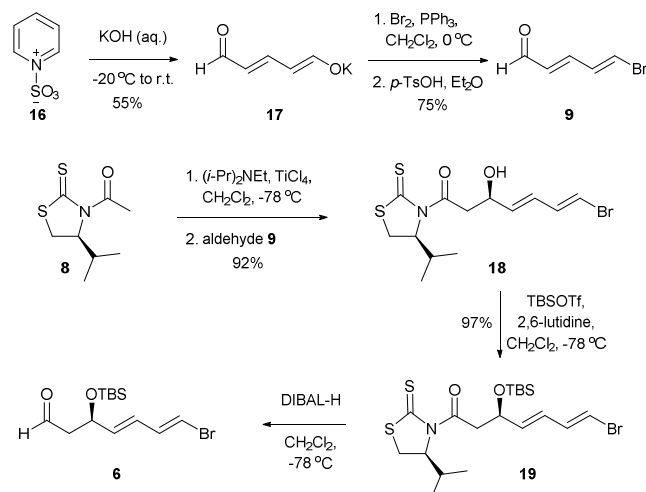
Our synthesis of LTB<sub>4</sub> (**1**) commenced with the preparation of terminal alkyne **7** in six steps and 24% overall yield from commercially available (*S*)-(-)- $\alpha$ -hydroxy- $\gamma$ -butyrolactone (**10**), as outlined in Scheme 1. Protection of **10** using TBS-triflate<sup>13</sup> in the presence of 2,6-lutidine in dichloromethane at -78 °C afforded the protected alcohol **11**. Reduction of the lactone in **11** with DIBAL-H produced the corresponding lactol **12** which was directly converted in a Colvin rearrangement to give the terminal alkyne **13** in 57% yield over the two steps.<sup>12d</sup> Oxidation with Dess-Martin periodinane afforded the aldehyde **14**, which was treated with methyl (triphenylphosphoranylidene)acetate to afford the  $\alpha,\beta$ -unsaturated ester **15** in 88% yield. Various procedures for the 1,4-reduction of the  $\alpha,\beta$ -unsaturated ester **15** were attempted. Tsuda and co-workers have reported that selective 1,4-reductions of  $\alpha,\beta$ -unsaturated esters may be achieved using DIBAL-H in the presence of MeCu in a mixture of HMPA and THF as solvent.<sup>14</sup> In our hands, this procedure gave low conversion of the starting material **15**. We then tried this reduction using the Stryker reagent.<sup>15</sup> This gave the ester **7** in 41% yield.

Reduction using magnesium in methanol<sup>16</sup> proved to be the best method of those attempted, affording ester **7** in 60% yield from **15**.



**Scheme 1.** Synthesis of the terminal alkyne **7**.

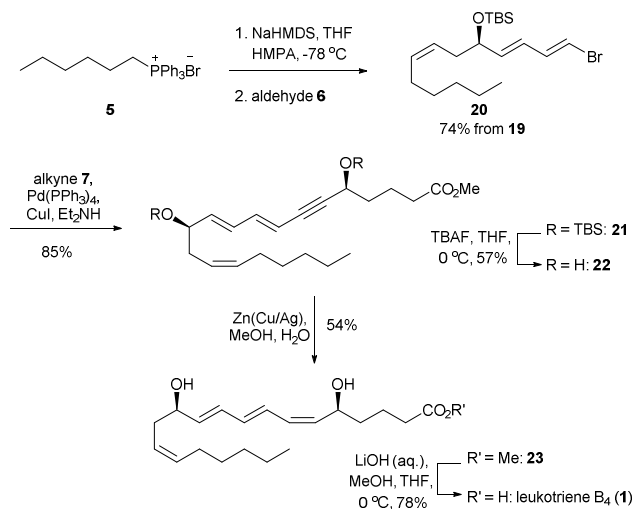
Aldehyde **6** was prepared in six steps from salt **16** essentially as previously reported (Scheme 2).<sup>12a, b</sup> Commercially available pyridinium-1-sulfonate (**16**) was treated with aqueous potassium hydroxide to yield potassium salt **17**,<sup>17</sup> which was first treated with  $\text{PPh}_3/\text{Br}_2$  in dichloromethane, and then *p*-TsOH in diethyl ether, to form (*2E,4E*)-5-bromopenta-2,4-dienal **9** in 75% yield over the two steps.<sup>18</sup> The aldehyde **9** was reacted with thiazolidinone **8**<sup>19</sup> in an acetate-aldol reaction to produce intermediate **18**<sup>20</sup> in a 15.3:1 diastereomeric ratio. Purification by chromatography yielded diastereomeric pure **18** in 92% yield. Next, protection of the secondary alcohol to give **19**, followed by removal of the chiral auxiliary with DIBAL-H afforded aldehyde **6** which was used immediately in the next step.



**Scheme 2.** Synthesis of the aldehyde **6**.

As for the assembly of the fragments, aldehyde **6** was reacted in a *Z*-selective Wittig reaction with the ylide of commercially available hexyltriphenylphosphonium bromide (**5**), the latter

obtained by reaction with NaHMDS, to afford vinyl bromide **20** in 74% yield from **19** (Scheme 3). Using low temperature and HMPA as a co-solvent in the Wittig-reaction, only the *Z*-isomer could be detected by  $^1\text{H}$  or  $^{13}\text{C}$  NMR analyses after purification.<sup>12a</sup> The vinyl bromide **20** was reacted in a Sonogashira cross-coupling reaction<sup>21</sup> with terminal alkyne **7** at room temperature in the presence of catalytic  $\text{Pd}(\text{PPh}_3)_4$  and  $\text{CuI}$  using diethyl amine as solvent. This afforded the conjugated dienyne **21** in 85% yield. Removal of the two TBS-groups was achieved with five equivalents of TBAF in THF at 0 °C to give the corresponding diol **22** in 57% yield. The conjugated alkyne **22** was then stereoselectively reduced. We first attempted a modified Lindlar hydrogenation reaction.<sup>22</sup> This gave a low yield of the desired *Z,E,E*-triene **23** that was contaminated with by-products which we were unable to remove by chromatography. The Boland reduction<sup>23</sup> was then attempted. Gratifyingly, this produced chemically pure LTB<sub>4</sub> methyl ester (**23**) in an acceptable 53% yield after chromatography. Finally, saponification of the methyl ester **23** with dilute aqueous  $\text{LiOH}$  in a mixture of methanol and THF at 0 °C followed by work up with aqueous  $\text{NaH}_2\text{PO}_4$  afforded LTB<sub>4</sub> (**1**) in 78% yield (Scheme 3). All spectroscopic data of **1** were in agreement with those previously reported.<sup>11a, c, d, 24</sup>



**Scheme 3.** Synthesis of leukotriene B<sub>4</sub> (**1**).

## Conclusions

In summary, a short and efficient total synthesis of the potent inflammatory lipid mediator leukotriene B<sub>4</sub> (**1**) has been achieved over 10 steps (longest linear sequence) using 19 synthetic operations in a non-optimized 5% overall yield. Our synthesis of leukotriene B<sub>4</sub> (**1**) compares well with those previously reported.<sup>11</sup> In particular, the acetate-aldol reaction was central for this synthesis.

## Experimental

### (S)-3-((*tert*-Butyldimethylsilyloxy)pent-4-ynal (**14**)

A solution of the alcohol **13** (0.52 g, 2.4 mmol, 1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (8.0 mL) was added to a stirring solution of Dess-Martin periodinane (1.3 g, 3.0 mmol, 1.3 eq.) in dry CH<sub>2</sub>Cl<sub>2</sub> (15 mL). The mixture was stirred at room temperature for four hours before it was quenched with a solution of sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (16 mL) and sat. aq. NaHCO<sub>3</sub> (16 mL). The layers were separated and the aq. layer was extracted with Et<sub>2</sub>O (3×16 mL). The combined organic layers were washed with brine (9.0 mL), dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The crude product was passed through a silica plug using hexane/EtOAc 8:2 as eluent to afford the aldehyde **14** as a colorless oil. Yield: 0.41 g (81%). TLC (hexane/EtOAc 8:2, KMnO<sub>4</sub> stain): R<sub>f</sub> = 0.40. Spectroscopic and physical data were agreement with those reported in the literature.<sup>25</sup> <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 9.82 (t, *J* = 2.1 Hz, 1H), 4.86 (ddd, *J* = 6.9, 5.0, 2.2 Hz, 1H), 2.75 (m, 2H), 2.49 (d, *J* = 2.2 Hz, 1H), 0.88 (s, 9H), 0.16 (s, 3H), 0.13 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 200.18, 83.85, 73.85, 58.19, 51.45, 25.75 (3C), 18.19, -4.48, -5.09.

### Methyl (*S,E*)-5-((*tert*-butyldimethylsilyloxy)hept-2-en-6-ynoate (**15**)

To a solution of aldehyde **14** (0.27 g, 1.3 mmol, 1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (8.0 mL) was added methyl (triphenylphosphoranylidene) acetate (0.56 g, 1.7 mmol, 1.3 eq.). After stirring for three hours the solvent was removed *in vacuo*. The residue was passed through a silica plug using hexane/EtOAc 8:2 as eluent to give the α,β-unsaturated methyl ester **15** as a colorless oil. Yield: 0.30 g (88%); TLC (hexane/EtOAc 9:1, KMnO<sub>4</sub> stain): R<sub>f</sub> = 0.48; [α]<sub>D</sub><sup>20</sup> = -42 (c = 0.20, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.97 (dt, *J* = 15.6, 7.3 Hz, 1H), 5.91 (dt, *J* = 15.8, 1.5 Hz, 1H), 4.45 (td, *J* = 6.2, 2.1 Hz, 1H), 3.73 (s, 3H), 2.57 (ddd, *J* = 7.5, 6.2, 1.4 Hz, 2H), 2.43 (d, *J* = 2.1 Hz, 1H), 0.89 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 166.78, 144.08, 123.95, 84.35, 73.28, 61.73, 51.62, 41.45, 25.83 (3C), 18.31, -4.47, -4.98. HRMS (TOF ES<sup>+</sup>): Exact mass calculated for C<sub>14</sub>H<sub>24</sub>O<sub>3</sub>Si<sub>2</sub>Na [M+Na]<sup>+</sup>: 291.1392, found 291.1388.

### Methyl (*S*)-((*tert*-butyldimethylsilyloxy)hept-6-ynoate (**7**)

Magnesium turnings (0.27 g, 11 mmol, 10 eq.) (pre dried in an oven at 120 °C) was added to a solution of the α,β-unsaturated methyl ester **15** (0.33 g, 1.2 mmol, 1.0 eq.) in MeOH (9.0 mL). The mixture was stirred for two hours. The crude product was passed through a short

silica plug using hexane/EtOAc 8:2 as eluent before the solvent was removed *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc 98:2) to afford the title compound **7** as a clear oil. Yield: 0.20 g (60%). Spectroscopic and physical data were in agreement with those reported in the literature.<sup>26</sup> TLC (hexane/EtOAc 9:1, KMnO<sub>4</sub> stain):  $R_f = 0.48$ ;  $[\alpha]_D^{20} = -36$  ( $c = 0.20$ , MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  4.37 (td,  $J = 6.0, 2.1$  Hz, 1H), 3.67 (s, 3H), 2.35 (m, 3H), 1.83-1.66 (m, 4H), 0.90 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  173.97, 85.31, 72.46, 62.51, 51.63, 37.90, 33.81, 25.90 (3C), 20.76, 18.33, -4.44, -4.96. HRMS (TOF ES<sup>+</sup>): Exact mass calculated for C<sub>14</sub>H<sub>26</sub>O<sub>3</sub>SiNa [M+Na]<sup>+</sup>: 293.1548, found 293.1552.

**(R,1E,3E,7Z)-1-Bromo-5-((tert-butyldimethylsilyl)oxy)trideca-1,3,7-trien-5-yl (20)**

Commercially available hexyltriphenylphosphonium bromide (**5**) (1.8 g, 4.2 mmol, 2.0 eq.) was added THF (40 mL) and HMPA (5.0 mL), and the mixture was cooled to -78 °C before NaHMDS (7.0 mL, 0.6 M in toluene, 2.0 eq.) was added dropwise. The resulting mixture was stirred for one hour. Then a solution of freshly prepared aldehyde **6** in THF (5.0 mL) was added dropwise at -78 °C. The solution was allowed to slowly warm up to room temperature in the dry ice/acetone bath over 24 hours before it was quenched with aq. phosphate buffer (30 mL, pH = 7.2). Et<sub>2</sub>O (50 mL) was added and the layers were separated. The aq. layer was extracted with Et<sub>2</sub>O (2×50 mL) and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc 97:3) to afford the title compound **20** as a yellow oil. Yield: 0.60 g (74% yield over two steps from **19**). TLC (hexane/EtOAc, KMnO<sub>4</sub> stain):  $R_f = 0.66$ ;  $[\alpha]_D^{20} = -17$  ( $c = 0.10$ , MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.68 (dd,  $J = 13.5, 10.9$  Hz, 1H), 6.27 (d,  $J = 13.5$  Hz, 1H), 6.14 – 6.03 (m, 1H), 5.72 (dd,  $J = 15.2, 5.8$  Hz, 1H), 5.50 – 5.41 (m, 1H), 5.38 – 5.29 (m, 1H), 4.14 (q,  $J = 5.8$  Hz, 1H), 2.31 – 2.19 (m, 1H), 2.00 (q,  $J = 6.9$  Hz, 2H), 1.38 – 1.23 (m, 6H), 0.93 – 0.86 (m, 12H), 0.05 (s, 3H), 0.03 (s, 3H). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  138.15, 137.20, 132.40, 126.54, 124.86, 108.17, 72.76, 36.30, 31.71, 29.44, 27.60, 26.02 (3C), 22.74, 18.42, 14.23, -4.37, -4.60. HRMS (CI<sup>+</sup>): Exact mass calculated for C<sub>19</sub>H<sub>35</sub>BrOSi [M+1]<sup>+</sup>: 387.1719, found 387.1708.

**Methyl (5S,8E,10E,12R,14Z)-5,12-bis((tert-butyldimethylsilyl)oxy)icosa-8, 10, 14-trien-6-ynoate (21)**



To a solution of vinyl bromide **20** (0.29 g, 0.74 mmol, 1.0 eq.) in Et<sub>2</sub>NH (3.3 mL) and benzene (0.6 mL), Pd(PPh<sub>3</sub>)<sub>4</sub> (26 mg, 0.02 mmol, 3.0 mol%) was added and the reaction was stirred for 45 min in the dark. CuI (7.0 mg, 0.04 mmol, 5.0 mol%) in a minimum amount of Et<sub>2</sub>NH was added followed by dropwise addition of alkyne **7** (0.20 mg, 0.74 mmol, 1.0 eq.) in Et<sub>2</sub>NH (1.5 mL). After stirring for 20 hours at room temperature, the reaction was quenched by addition of saturated aq. NH<sub>4</sub>Cl (20 mL). Et<sub>2</sub>O (15 mL) was added and the layers were separated. The aq. layer was extracted with Et<sub>2</sub>O (2×20 mL) and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc 98:2) to afford the title compound **21** as a yellow oil in 85% yield (0.36 g). Spectroscopic and physical data were in agreement with those reported in the literature.<sup>26</sup> TLC (hexane/EtOAc 95:5, KMnO<sub>4</sub> stain): R<sub>f</sub> = 0.28; [α]<sub>D</sub><sup>20</sup> = -33 (c = 0.14, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.51 (dd, *J* = 15.5, 10.8 Hz, 1H), 6.18 (dd, *J* = 15.2, 10.9 Hz, 1H), 5.77 (dd, *J* = 15.2, 5.9 Hz, 1H), 5.57 (d, *J* = 15.6 Hz, 1H), 5.45 (m, 1H), 5.34 (m, 1H), 4.50 (t, *J* = 5.2 Hz, 1H), 4.17 (q, *J* = 6.0 Hz, 1H), 3.67 (s, 3H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.25 (m, 2H), 2.00 (q, *J* = 7.2 Hz, 2H), 1.75 (m, 4H), 1.27-1.10 (m, 6H), 0.82-0.74 (m, 21H), 0.13 (s, 3H), 0.11 (s, 3H), 0.05 (s, 3H), 0.03 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 174.05, 141.30, 139.48, 132.33, 128.51, 124.97, 110.48, 93.07, 83.77, 72.94, 63.27, 51.63, 38.09, 36.42, 33.88, 31.70, 29.44, 27.60, 26.03 (3C), 25.97 (3C), 22.73, 20.95, 18.43, 18.39, 14.23, -4.27, -4.34, -4.60, -4.85. HRMS (TOF ES<sup>+</sup>): Exact mass calculated for C<sub>33</sub>H<sub>60</sub>O<sub>4</sub>Si<sub>2</sub>Na [M+Na]<sup>+</sup>: 599.3930, found 599.3963.

**Methyl (5S,6Z,8E,10E,12R,14Z)-5,12-dihydroxyicosan-8,10,14-triene-6-ynoate (22)**  
TBAF (0.67 mL, 1.0 M in THF, 0.66 mmol, 5.0 eq.) was added to a solution of **21** (76 mg, 0.13 mmol, 1.0 eq.) in THF (3.5 mL) at 0 °C. The reaction was stirred for five hours at 0 °C, before it was quenched with phosphate buffer (pH = 7.2, 2.0 mL). Brine (3.5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (7.0 mL) were added and the layers were separated. The aq. layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2×7.0 mL) and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc 6:4) to afford the diol **22** as a pale yellow oil. Yield: 26 mg (57%). Spectroscopic and physical data of **22** were in agreement with those reported in the literature.<sup>26-27</sup> TLC (hexane/EtOAc 4:6, KMnO<sub>4</sub> stain): R<sub>f</sub> = 0.39; [α]<sub>D</sub><sup>20</sup> = -10 (c = 0.05, MeOH); <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 6.55 (dd, *J* = 15.5, 10.8 Hz, 1H), 6.27 (dd, *J* = 15.3, 10.9 Hz, 1H), 5.81 (dd, *J* = 15.2, 6.3 Hz, 1H), 5.66 (d, *J* = 15.5, 1H), 5.43 (m, 2H), 4.45 (dd, *J* = 6.5, 1.7 Hz, 1H), 4.12 (q, *J* = 6.2 Hz, 1H)

3.66 (s, 3H), 2.38 (t,  $J = 7.0$  Hz, 2H), 2.29 (m, 2H), 2.04 (q,  $J = 6.8$  Hz, 2H), 1.84-1.62 (m, 4H) 1.40-1.25 (m, 6H), 0.90 (t,  $J = 6.9$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  175.62, 142.53, 139.92, 133.19, 130.24, 125.85, 111.64, 93.66, 84.37, 72.80, 62.88, 52.01, 38.23, 36.25, 34.38, 32.67, 30.40, 28.38, 23.64, 21.93, 14.45; HRMS (TOF  $\text{ES}^+$ ): Exact mass calculated for  $\text{C}_{21}\text{H}_{32}\text{O}_4\text{Na}$   $[\text{M}+\text{Na}]^+$ : 371.2198, found 371.2205.

### **Methyl (5S,6Z,8E,10E,12R,14Z)-5,12-dihydroxy-6,8,10,14-icosatetraenoate (23)**

The Zn(Cu/Ag) mixture was prepared as described by Boland et al.<sup>23</sup> Zinc dust (1.8 g) was stirred under argon for 15 min.  $\text{Cu}(\text{OAc})_2$  (0.18 g) was then added and the reaction mixture was stirred for 15 min, before  $\text{AgNO}_3$  (0.18 g) was added and the solution was stirred for an additional 30 minutes. The mixture was filtered and washed successively with  $\text{H}_2\text{O}$ , MeOH, acetone and  $\text{Et}_2\text{O}$  before it was transferred to a flask containing the reaction solvents (MeOH/ $\text{H}_2\text{O}$ , 1:1, 7.4 mL). A solution of alkyne **22** (26 mg, 0.080 mmol) in MeOH/ $\text{H}_2\text{O}$  (1:1, 1.0 mL) was added and the mixture was stirred at room temperature and in the dark for five hours. The reaction mixture was filtered through a pad of Celite and washed with  $\text{Et}_2\text{O}$ . Water was added to the filtrate, and the organic layers were separated and the aq. layer was extracted with  $\text{Et}_2\text{O}$  (2 $\times$ 5.0 mL). The combined organic layers were washed with brine and dried ( $\text{Na}_2\text{SO}_4$ ). The solvent was removed *in vacuo* and the crude product was purified by column chromatography on silica gel (hexane/ $\text{EtOAc}$  1:1) to afford the methyl ester **23** in 54% yield (14 mg). Spectroscopic and physical data for **23** were in agreement with those reported in the literature.<sup>11c, 26-27</sup> TLC (heptane/ $\text{EtOAc}$  2:8,  $\text{KMnO}_4$  stain):  $R_f = 0.60$ ;  $[\alpha]_D^{20} = 5.6$  ( $c = 0.050$ ,  $\text{CCl}_4$ ); UV (MeOH)  $\lambda_{\text{max}}$  261, 270, 281 nm;  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.54 (m, 1H), 6.27 (m, 2H), 6.08 (t,  $J = 11.1$  Hz, 1H), 5.75 (dd,  $J = 14.5, 6.6$  Hz, 1H), 5.53-5.32 (m, 3H), 4.56 (q,  $J = 8.0$  Hz, 1H), 4.12 (q,  $J = 6.6$  Hz, 1H), 3.65 (s, 3H), 2.39-2.21 (m, 4H), 2.04 (q,  $J = 6.9$  Hz, 2H), 1.70-1.58 (m, 2H), 1.50-1.28 (m, 8H), 0.90 (t,  $J = 6.9$  Hz, 3H);  $^{13}\text{C}$  NMR (151 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  175.75, 138.11, 135.10, 135.08, 133.08, 131.33, 130.56, 128.65, 126.00, 73.14, 68.12, 51.99, 37.91, 36.36, 34.61, 32.68, 30.41, 28.39, 23.65, 22.01, 14.43; HRMS (TOF  $\text{ES}^+$ ): Exact mass calculated for  $\text{C}_{21}\text{H}_{34}\text{O}_4\text{Na}$   $[\text{M}+\text{Na}]^+$ : 373.2354, found 373.2362.

### **(5S,6Z,8E,10E,12R,14Z)-5,12-Dihydroxy-6,8,10,15-icosatetraenoic acid (LTB<sub>4</sub>, 1)**

To a solution of methyl ester **23** (5.1 mg, 0.014 mmol, 1 eq.) in THF/MeOH/ $\text{H}_2\text{O}$  (2:2:1, 1.7 mL), solid LiOH (10 mg, 0.43 mmol, 31 eq.) was added at 0 °C. The reaction mixture was stirred at 0 °C for three hours and then allowed to warm up to room temperature. The solution

was acidified with sat. aq. NaH<sub>2</sub>PO<sub>4</sub> (1.9 mL). EtOAc (2.0 mL) was added and the layers were separated. The aq. layer was extracted with EtOAc (2 × 2.0 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) to afford LTB<sub>4</sub> (**1**) in 78% yield (3.8 mg). Spectroscopic and physical data were in agreement with those reported in the literature.<sup>11a, c, d, 24</sup>; TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5, KMnO<sub>4</sub> stain): *R*<sub>f</sub> = 0.10; [α]<sub>D</sub><sup>20</sup> = 12 (c = 0.050, CHCl<sub>3</sub>); UV (MeOH) λ<sub>max</sub> 261, 270, 281 nm; <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 6.54 (dd, *J* = 14.1, 11.7 Hz, 1H), 6.33 – 6.19 (m, 2H), 6.08 (t, *J* = 11.2 Hz, 1H), 5.74 (dd, *J* = 14.7, 6.6 Hz, 1H), 5.51 – 5.31 (m, 3H), 4.60 – 4.53 (m, 1H), 4.11 (q, *J* = 6.5 Hz, 1H), 2.36–2.21 (m, 4H), 2.04 (q, *J* = 7.2 Hz, 2H), 1.70 – 1.59 (m, 2H), 1.51 – 1.42 (m, 1H), 1.38 – 1.25 (m, 7H), 0.90 (t, *J* = 6.9 Hz, 3H); <sup>13</sup>C NMR (151 MHz, CD<sub>3</sub>OD) δ 180.19, 138.00, 135.25, 135.00, 133.07, 131.41, 130.49, 128.78, 126.01, 73.15, 68.26, 38.28, 36.46, 36.35, 32.68, 30.42, 28.39, 23.65, 22.63, 14.43; HRMS (TOF ES<sup>+</sup>): Exact mass calculated for C<sub>20</sub>H<sub>32</sub>O<sub>4</sub>Na [M+Na]<sup>+</sup>: 359.2198, found 359.2203.

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