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An efficient approach to the synthesis of LTB_4 and ω-substituted LTB_4 metabolites

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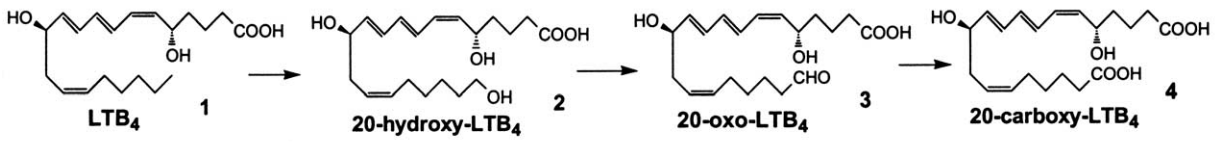
Abstract—The first total synthesis of the methyl ester of 20-oxo-LTB_4 **26** is described. The key synthon **6** is an advanced new intermediate which has been used in the synthesis of LTB_4 **1**, 20-oxo-LTB_4 methyl ester **26**, and 20-hydroxy-LTB_4 **2**. The synthetic **26** has been used to study the cytosolic aldehyde dehydrogenase-catalyzed oxidation of LTB_4 to its ω-carboxy metabolite.

Leukotriene B_4 (LTB_4) is a product of the lipoxygenase pathway of arachidonic acid (AA) metabolism, and is derived from leukotriene A_4 (LTA_4) via the enzymatic addition of water at C-12 of LTA_4. LTB_4 is a mediator of inflammation and one of the most potent chemotactic agents produced by human polymorphonuclear leukocytes.[1] It has been implicated in a variety of human inflammatory diseases and allergic reactions. The proinflammatory activity of LTB_4 is mediated by the BLT_1 receptor.[2] It induces chemotaxis,[3] aggregation[1] and the adhesion[4] of inflammatory cells, especially neutrophils, to endothelial cells. Furthermore, LTB_4 synergizes with other chemotactic factors, to amplify the inflammatory response. Recently, a second LTB_4 receptor (BLT_2 receptor) has been identified and cloned, its expression is broad and highest in liver, intestine, spleen and kidney.[5] Also it has broader ligand specificity for various eicosanoids such as 12(*S*)-hydroxyeicosatetraenoic acid (12-HETE) and 15(*S*)-hydroxyeicosatetraenoic acid (15-HETE). Thus, LTB_4-R2 receptor provides a novel target for anti-inflammatory therapy and should help us expand our knowledge of LTB_4 function.

A substantial amount of synthetic work on the metabolites of LTB_4 has been accomplished.[6–8] Earlier on we reported on the synthesis of two ω-metabolites of LTB_4 **2** and **4** made by neutrophils,[9] and confirmed[10] that LTB_4-20-hydroxylase ($P\text{-}450_{LTB}$) is the cytochrome P-450 in the microsomes of human polymorphonuclear leukocytes that catalyze the ω-oxidation of LTB_4 to 20-oxo-LTB_4. ω-Oxidation of LTB_4 (Scheme 1) in the neutrophil is critical because it results in the biological inactivation of this extremely potent chemoattrac-



Scheme 1. Metabolism of C20-LTB4.

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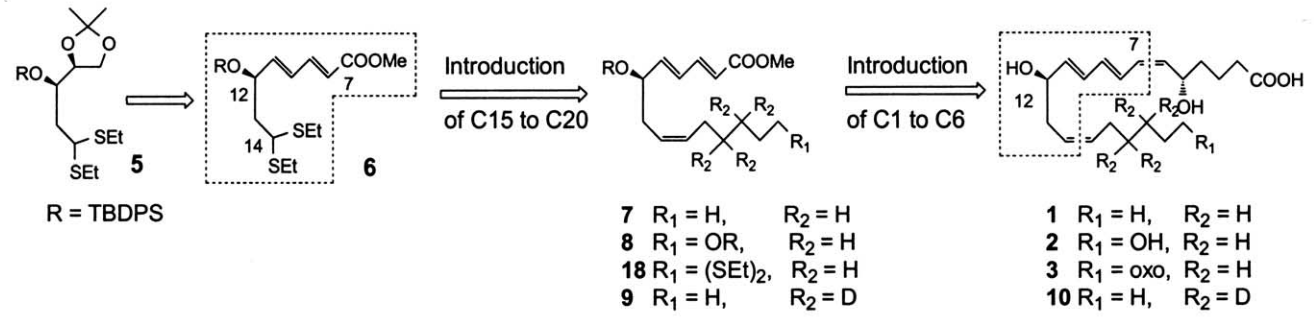
tant.[11–19] Investigation of the steps in the ω-oxidation of LTB_4 in the neutrophil will provide clarification on the balance between the synthesis of LTB_4 and its catabolism. To study the cytosolic aldehyde dehydrogenase, which catalyzes the final step in the oxidation of LTB_4 to ω-carboxy-LTB_4 **4** we needed synthetic 20-oxo-LTB_4 **3** as well as 20-OH-LTB_4 **2**. Since **3** is extremely unstable, we decided to use its stable methyl ester derivative 20-oxo-LTB_4 methyl ester **26**.

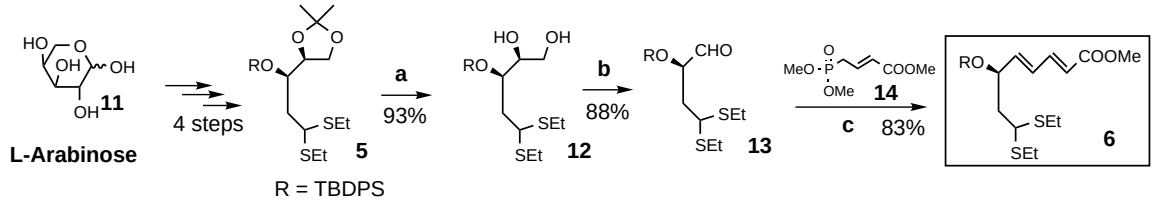
The object of this communication is a new strategy we developed for a more efficient synthesis of LTB_4 derivatives, exemplified by the synthesis of LTB_4 **1**, 20-oxo-LTB_4 methyl ester **3**, 20-hydroxy-LTB_4 **2** (Scheme 2). The synthesis of 17,17,18,18-tetradeutero-LTB_4 **10** has also been approached by the same method.[20] Our earlier syntheses of LTB_4 were linear, starting from the ω-end of the molecule.[21] We were looking for a synthetic strategy which will allow maximal flexibility for the synthesis of many of the LTB_4 analogs planned and provide a more effective and shortened approach to LTB_4 structural modification (Scheme 2). The advanced intermediate **6**, which will provide the C_7–C_{14} part of the backbone of the LTB_4 was our target and was prepared first. This new synthetic approach will also help for the synthesis of LTB_4 derivatives for the chemical characterization of the new LTB_4 receptor.

The derivatives shown in Scheme 2 were prepared using the improved synthesis. The synthesis of pivotal synthon **6** was performed as shown in Scheme 3. Compound **5**, which contains the would-be 12*R*-hydroxy of the target LTB_4 derivatives, was prepared as described previously.[21,22] The selective deprotection of acetonide in **5** was carried out by treatment with trifluoroacetic acid in tetrahydrofuran at room temperature and gave the diol **12** in 93% yield. The lead tetraacetate oxidation of **12** gave aldehyde **13** in 88% yield. The four carbon diene units were introduced by performing a Wittig–Horner reaction using phosphonate **14**[23] to afford **6** in 83% yield. Synthon **6**, which is now the starting point of many of our LTB_4 syntheses, is prepared in multigram scale and is stable for prolonged periods of time at −20°C.

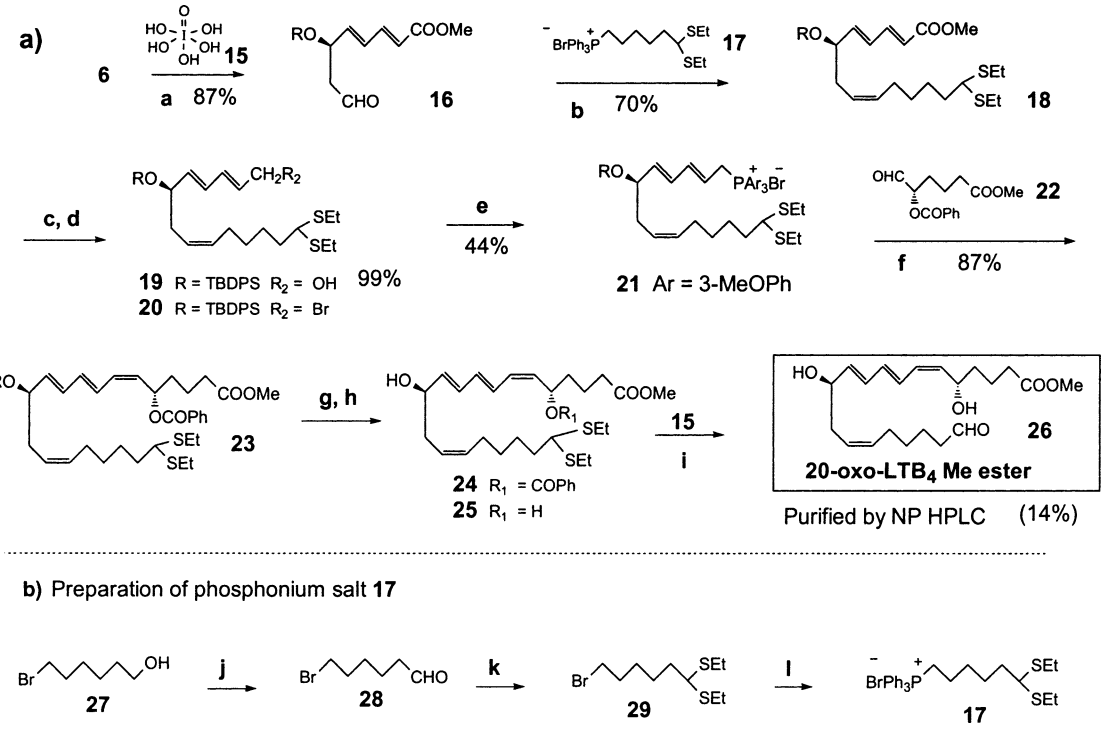
We are showing in Scheme 4 the detailed synthesis of **26** which has not been reported before. Intermediates **7**, **8**, and the tetradeutero derivative **9** were prepared similarly by modifying the Wittig reagent in step **b** (Scheme 4). The dithioacetal group in **6** was cleaved using our recently developed periodic acid method under anhydrous conditions to yield aldehyde **16** in 87% yield after purification by column chromatography over silica gel. Aldehyde **16** was reacted with the ylide generated from phosphonium salt **17** and sodium bis(trimethylsilyl)amide at −78°C in THF. Flash column chromatography afforded **18** in 70% yield. The reduction of ester **18** with DIBAL-H in methylene chloride at 0°C, followed by aqueous acidic work-up, gave alcohol **19** in quantitative yield, which was converted to bromide **20** and used in the preparation of phosphonium salt **21**. The Wittig reaction to introduce the C_6–C_7 *cis* double bond was performed using aldehyde **22**[23,24] and sodium hexamethyldisilazide at −98°C to room temperature to give **23**. The minor 6 *trans*-isomer of **23** (10%, not shown) was easily separated by flash column chromatography. Note that the high yield of the *cis* isomer contrasts with our earlier syntheses of LTB_4 in which the *cis*:*trans* ratio is 2:1. The use of the particular methoxy phosphonium **21** shown here is responsible for the increase of the *cis* to *trans* ratio from 2:1 to 9:1. This reaction is currently under further



Scheme 2. Synthetic approach.



Scheme 3. *Reaction conditions*: (a) TFAA, THF, water 50°C 4 days; (b) $Pb(OAc)_4$, Na_2CO_3, 3 equiv., CH_2Cl_2 −40 to −30°C 10 min then at −30°C 10 min; (c) phosphonate **14** in THF, cooled to −78°C, $LiN(SiMe_3)_2$ added at −78°C, 30 min at −78°C, allowed to warm to −20°C during 1.5 h then added aldehyde **13** at −20°C, 30 min at −20°C then room temperature overnight.



Scheme 4. *Reaction conditions*: (a) ether:THF (4:1) 0°C to room temperature 3 min; (b) phosphonium salt **17** (6 mmol) in THF (80 mL) cooled to −78°C, *n*-BuLi (5 mmol), 30 min at −78°C then aldehyde **16**, −78°C to room temperature 2 h; (c) DIBAL-H, 2.4 equiv. in CH_2Cl_2, −60 to −20°C 1 h, then neutralized with cold 10% HCl; (d) DIPHOS, CBr_4, CH_2Cl_2, 0°C 20 min, reaction mixture filtered through a small silica gel pad and solution of bromide **20** in dry dichloromethane used as such in the next step; (e) **20** in dry dichloromethane $(3\text{-MeOPh})_3P$ room temperature overnight; (f) phosphonium salt **21** in THF cooled to −93°C, 0.95 equiv. $NaN(SiMe_3)_2$, 1 min, HMPA 10% added, then aldehyde **22**, −93 to −15°C 2 h; (g) TBAF, THF added at 0°C, room temperature overnight; (h) K_2CO_3, methanol, 0°C to room temperature 10 min; (i) periodic acid **15**, ether:THF (9:1) 0°C 20 min; (j) $PCC/Al_2O_3/CH_2Cl_2$ room temperature 30 min; (k) ethanethiol, $BF_3{\cdot}Et_2O$ room temperature overnight; (l) PPh_3 3 equiv., acetonitrile reflux 48 h.

investigation and will be described in detail in a future report. Finally, the pure *cis* isomer **23** was treated with tetrabutylammonium fluoride in THF to give the hydroxy compound **24**. The benzoate group was then removed by treatment with potassium carbonate in anhydrous methanol and the desired **26**[25] was prepared by deblocking of dithioacetal **25**[26] with periodic acid.[27] The analytical HPLC of the crude reaction product revealed that **26** was obtained in more than 50% yield from **25**. The pure product **26** was isolated in low yield (14%) after purification by normal phase HPLC.

The synthetic strategy described here for the synthesis of LTB_4 derivatives is a marked improvement over the one we have used previously. The synthetic material **26** was used to characterize the cytosolic aldehyde dehydrogenase that catalyzes the oxidation of 20-oxo-LTB_4 to ω-carboxy-LTB_4. This substance was found to be an excellent substrate for the enzymatic oxidation studies and remained stable during the 30 min incubation period with cell lysates. These results will be published separately.

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25. **20-Oxo-LTB_4-methyl ester**: ^{1}H NMR (d_6-acetone) δ 1.40 (m, 4H), 1.60 (m, 6H), 2.3 (m, 4H), 2.40–2.45 (m, 2H), 3.6 (s, 3H), 3.8 (d, $J=4.3$ Hz, 1H, OH), 3.9 (d, $J=4.5$ Hz, 1H, OH), 4.15 (m, 1H), 4.58 (m, 1H), 5.35–5.5 (m, 3H), 5.78 (dd, $J=14.7$ and 6.0 Hz, 1H), 6.0 (t, $J=11.0$ Hz, 1H), 6.20–6.35 (m, 2H), 6.57 (t, $J=12.0$ Hz, 1H), 9.7 (s, 1H).
26. **20-Dithioacetal derivative**: ^{1}H NMR (d_6-acetone) δ 1.20 (t, $J=7.0$ Hz, 6H), 1.4–1.8 (m, 12H), 2.3 (m, 4H), 2.5–2.7 (m, 4H), 3.6 (s, 3H), 3.8 (d, $J=4.3$ Hz, 1H, OH), 3.9 (m, 2H, C-20-H and OH), 4.17 (m, 1H), 4.57 (m, 1H), 5.35–5.5 (m, 3H), 5.8 (dd, $J=14.7$ and 6.0 Hz, 1H), 6.05 (t, $J=11.0$ Hz, 1H), 6.20–6.35 (m, 2H), 6.58 (t, $J=12.0$ Hz, 1H).
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