

A NEW PRACTICAL METHOD FOR THE PREPARATION OF [³H₆]-LEUKOTRIENES C₄ AND D₄

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Summary

A new method for the synthesis of highly deuterated or tritiated leukotrienes was developed. The higher reactivity of a terminal alkene compared to a 1,2-disubstituted one permitted the selective deuteration or tritiation of the diyne 14,15,19,19,20,20-hexadehydro LTC₄ triester **1**. After hydrolysis, LTC₄ was obtained in 36% overall yield. An average of seven deuterium or tritium atoms was incorporated and the specific activity of the tritiated LTC₄ was greater than 180 Ci/mmol. **1** was obtained from the addition of glutathione to 14,15,19,19,20,20-hexadehydro LTA₄ ethyl ester which was the product of a Wittig reaction between (3,8-nonanediyn-1-yl)triphenylphosphonium iodide and 5(S), 6(S), 7(E), 9(E) ethyl 5,6-epoxy-11-oxo-7,9-undecadienoate.

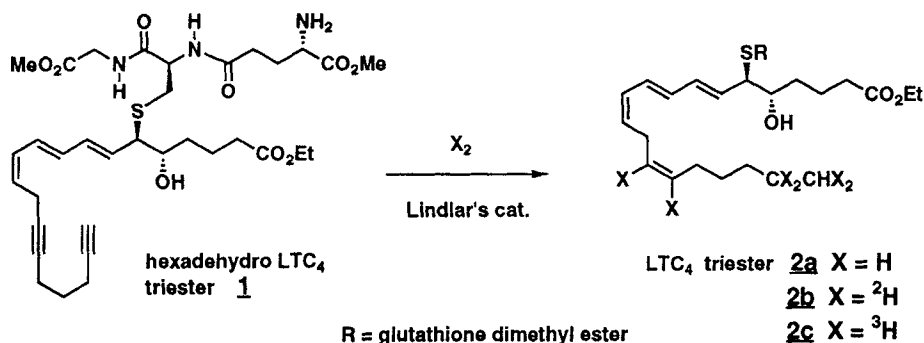
Key Words: Leukotriene C₄ (LTC₄), 14,15,19,19,20,20-Hexadehydro LTC₄ triester, 14,15,19,19,20,20-Hexadehydro LTA₄ ethyl ester, [³H₆]-LTC₄, [³H₆]-LTD₄, glutathione dimethyl ester

Introduction

In our current research program aimed at finding new leukotriene D₄ (LTD₄) antagonists for the treatment of diseases such as asthma and allergies¹, the new drug candidates are evaluated in a LTD₄ receptor binding assay. In order to diminish the effect of non-specific binding and hence, increase the reliability and sensitivity of the assay, radioactive LTD₄ having a specific activity greater than 150 Ci/mmol was required.

Radioactive leukotrienes are usually obtained by the catalytic hydrogenation of 14,15-didehydro LTA₄ to give [14,15-³H₂]-LTA₄, which can subsequently be converted to LTC₄, LTD₄ and LTE₄ by known methods.¹ LTE₄ having a specific activity of 40 Ci/mmol was reported using this route.² This method was also applied to the synthesis of [³H₆]-hepoxilin B₃,³ which belongs to a new class of acyclic arachidonic acid metabolites, to [²H₄]-LTA₄,⁴ [²H₂]-LTB₄,⁵ and the tritiated metabolites of LTE₄.⁶

Some methods for the synthesis of highly deuterated leukotrienes have been published⁷, but they all incorporate the deuterium atoms early in the sequence. In order to avoid the manipulation of tritiated intermediates and introduce more than six tritium atoms into LTC₄ and D₄, the selective reduction of a diyne such as **1** to the triester of LTC₄ **2** was envisaged (Scheme 1).

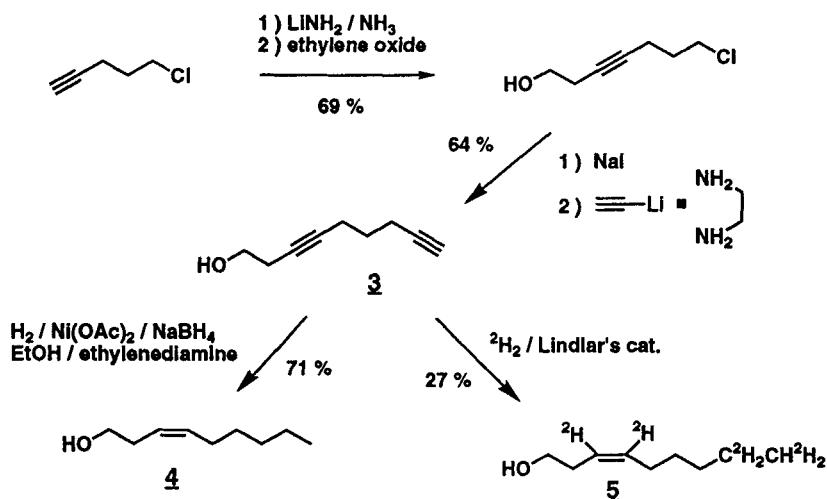


Scheme 1

Results and Discussion

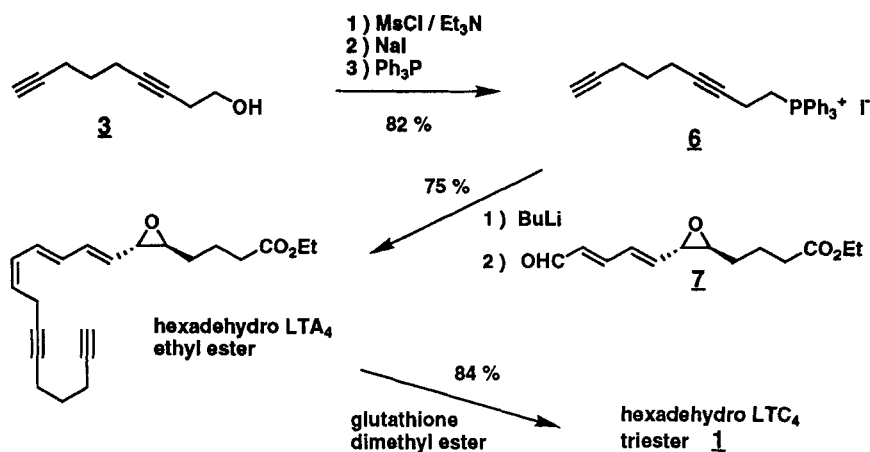
The reduction of a terminal alkyne is known to be difficult to stop at the alkene stage. We believed that we might be able to reduce it completely and selectively in the presence of other internal double bonds to give **2** in a reasonable yield. The feasibility of this approach was tested on compound **3**. This diyne was prepared by the addition of the lithium anion of 5-chloro-1-pentyne on ethylene oxide and then substitution via the iodide by lithium acetylide as outlined in Scheme 2. A very good selectivity and a high yield of **4** were obtained with the nickel boride reduction⁸, but this method requires too many labelled reagents to be practical for deuteration or tritiation. Deuteration

of **3** was achieved with Lindlar's catalyst to give the hexadeuterated alkene **5** in 27% unoptimized yield. These results prompted us to test the method on 14,15,19,19,20,20-hexadehydro LTC $_4$ **1**.



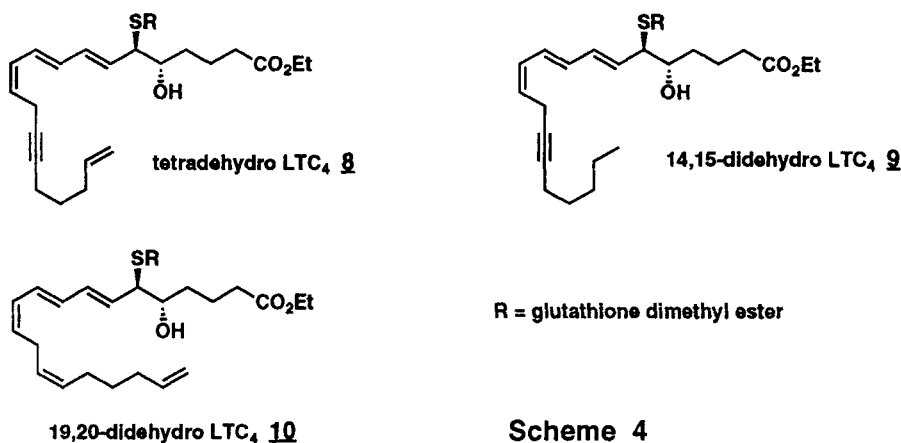
Scheme 2

The synthesis of **1** is described in Scheme 3. A Wittig reaction between the known epoxyaldehyde **7** and the phosphonium salt **6** (obtained from the diynol **3**) afforded the ethyl ester of hexadehydro LTA $_4$. Addition of glutathione dimethyl ester yielded the triester of hexadehydro LTC $_4$ **1**.



Scheme 3

Hydrogenation of **1** over the Lindlar's catalyst was monitored by HPLC. Using EtOAc as the reaction solvent, the product obtained was found to be almost exclusively the tetrahydro LTC₄ **8** (Scheme 4). In a more polar solvent such as methanol, **1** was reduced to the desired LTC₄ **2a** and two dihydro LTC₄ intermediates **9** and **10** were identified in the process.



Using this procedure, the deuteration of **1** afforded the triester of [²H₆]-LTC₄ **2b** in 17% isolated yield. A better yield of [²H₆]-LTC₄ was obtained by hydrolysis of the crude reaction mixture followed by HPLC purification. [²H₆]-LTC₄ was shown to co-elute with LTC₄ in two different HPLC systems. Mass spectra showed an average of seven deuterium atoms incorporated with some allylic exchange occurring during the hydrogenation¹⁰.

Tritiation of **1** and hydrolysis of the triester afforded [³H₆]-LTC₄, with specific activity greater than 180 Ci/mmol. [³H₆]-LTC₄ was then converted to [³H₆]-LTD₄ by known enzymatic methods.^{1,2}

This methodology should be useful for the preparation of other multiply labelled cis alkenes such as fatty acids and their metabolites and derivatives.

Experimental

7-CHLORO-3-HEPTYN-1-OL. At -78°C , a solution of *n*-butyllithium 2.4 M in hexanes (88 mL) was added to ~500 mL of ammonia and the mixture was stirred at this temperature for 20 min. To the suspension of LiNH_2 so obtained, a solution of 5-chloro-1-pentyne (20.10 g, 19.5 mmol) in 200 mL of THF was added dropwise from a -78°C cooled addition funnel and the suspension was stirred at -78°C for 30 min. Then, ethylene oxide (100 mL) was added and the reaction mixture was allowed to warm to room temperature and stirred for 16 hours. 25% aq. NH_4OAc was then added and the product was extracted in EtOAc, dried over Na_2SO_4 and distilled ($124\text{--}7^\circ\text{C}$ / 12 mmHg). Yield: 17.5 g, 69%. ^1H NMR (CDCl_3) δ 1.80 (1H, t, OH), 1.96 (2H, m), 2.40 (4H, m), 3.77 (4H, m).

7-IODO-3-HEPTYN-1-OL. A mixture of 7-chloro-3-heptyn-1-ol (2.375 g, 16.2 mmol) and NaI (14.54 g, 6 equiv.) in 30 mL of acetone was heated to reflux for 12 hours. The mixture was then cooled to room temperature and 25% aq. NH_4OAc was added. The product was extracted in EtOAc, dried over Na_2SO_4 and filtered through silica to give the pure iodide. Yield: 3.56 g, 92%. ^1H NMR (CDCl_3) δ 1.90–2.06 (3H, m), 2.32 (2H, m), 2.43 (2H, m), 3.29 (2H, t), 3.69 (2H, t).

3,8-NONANEDIYN-1-OL 3. To an ice cooled solution of lithium acetylide, ethylenediamine complex, (96 mg, 1.04 mmol) in 2 mL of anhydrous DMSO was added a solution of 7-iodo-3-heptyn-1-ol (89 mg, 373 μmol) in 0.5 mL of DMSO and the mixture was stirred at room temperature for an hour. 25% aq. NH_4OAc was then added and the product was extracted with ether, washed with brine, dried over Na_2SO_4 and purified by flash chromatography on silica with EtOAc:hexane 20:80. Yield: 35 mg, 69%. ^1H NMR (CD_3COCD_3) δ 1.65 (2H, m), 2.18–2.35 (7H, m), 3.58 (2H, td), 3.76 (1H, t, OH).

3-NONEN-1-OL 4. Nitrogen was bubbled into a suspension of $\text{Ni}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ (58 mg) in 1 mL 95% EtOH. A 0.5 M suspension of NaBH_4 in 95% EtOH (465 μL) was then added, followed by 23 μL of ethylenediamine and the mixture was stirred for ~5 min. The bubbling of N_2 was

stopped and a solution of 3,8-nonanediyn-1-ol **3** (30.2 mg, 222 μmol) in 1 mL of 95% EtOH was added. The mixture was stirred vigorously under an atmosphere of hydrogen for 2.5 h. CH_2Cl_2 was added and the mixture was filtered through silica with EtOAc:hexane 40:60. Purification of the product by HPLC (μ Porasil column, 12 mm diameter, flow rate 8.9 mL/min) with EtOAc:hexane 15:85 yielded 23 mg (71%) of 3-nonen-1-ol. ^1H NMR (CDCl_3) δ 0.89 (3H, t), 1.23-1.43 (6H, m), 1.53 (1H, s, OH), 2.07 (2H, td), 2.34 (2H, td), 3.64 (2H, t), 5.38 (1H, td), 5.57 (1H, td).

[3,4,8,8,9,9- $^2\text{H}_6$]-3-NONEN-1-OL **5**. A mixture of 3,8-nonanediyn-1-ol **3** (17.1 mg), 50 μL of pyridine and 8.8 mg of Lindlar's catalyst in 10 mL of hexane was stirred under an atmosphere of deuterium for 40 min. CH_2Cl_2 was added and the mixture was filtered through silica with EtOAc:hexane 40:60. The product was purified by HPLC (12 mm diameter μ Porasil column, flow rate 8.9 mL/min) with EtOAc:hexane 15:85. Yield 5 mg, 27%. ^1H NMR (CDCl_3) δ 0.86 (1H, m), 1.23-1.46 (4H, m), 2.07 (1H, s, OH), 2.07 (2H, t), 2.34 (2H, t), 3.66 (2H, t).

9-iodo-1,6-NONANEDIYNE. At -78°C , methanesulfonyl chloride (310 μL , 1.2 equiv.) and triethylamine (740 μL , 1.6 equiv.) were added successively to a solution of 3,8-nonanediyn-1-ol **3** (452 mg, 3.32 mmol) in 16 mL of CH_2Cl_2 and the reaction mixture was allowed to stir at room temperature for an hour. 25% aq. NH_4OAc was then added and the mesylate was extracted in CH_2Cl_2 , dried over Na_2SO_4 and purified by filtration through silica with EtOAc:hexane 15:85 and 30:70. ^1H NMR (CDCl_3) δ 1.70 (2H, tt), 1.96 (1H, t), 2.29 (4H, m), 2.62 (2H, tt), 3.04 (3H, s), 4.27 (2H, t). A mixture of this mesylate and sodium iodide (2.55 g, 5 equiv.) in 11 mL of acetone was heated to reflux for 2 hours. At room temperature, CH_2Cl_2 was added and the product was filtered through silica with EtOAc. The organic solution was washed with 25% aq. NH_4OAc and sodium bisulfite and was dried over Na_2SO_4 . The iodide was purified by flash chromatography on silica with hexane and EtOAc:hexane 2.5:97.5. Yield: 750 mg, 91%. ^1H NMR (CDCl_3) δ 1.74 (2H, tt), 1.97 (1H, t), 2.24-2.40 (4H, m), 2.75 (2H, tt), 3.23 (2H, t).

3,8-NONANEDIYN-1-YL TRIPHENYLPHOSPHONIUM IODIDE **6**. A mixture of 9-iodo-1,6-nonanediyn-1-ol (750 mg, 3.05 mmol) and triphenylphosphine (1.26 g, 1.5 equiv.) was heated

to reflux in 6 mL of CH_3CN for 3 hours. Ether was added and an oil separated. The oil was washed twice with ether and dried. Yield: 1.43 g, 90%. ^1H NMR (CDCl_3) δ 1.42 (2H, tt), 1.91 (2H, br t), 1.97 (1H, t), 2.13 (2H, td), 2.83 (2H, br td), 3.92 (2H, td), 7.65-7.92 (15H, m).

GLUTATHIONE, REDUCED, DIMETHYL ESTER. A mixture of 4.51 g of glutathione, reduced, and 23 mL of 2N HCl in MeOH was stirred at room temperature overnight. The reaction mixture was then added slowly to cold aq. 25% NH_4OAc and the pH was adjusted to 9 with NaOH. The product was extracted with EtOAc:THF 1:1 five times, dried over Na_2SO_4 and purified by flash chromatography on silica with a gradient of MeOH: CH_2Cl_2 : NH_4OH 2.5:97.5:1 to 10:90:1. Yield: 1.39 g, 28%. ^1H NMR (CDCl_3) δ 1.93-2.20 (2H, m), 2.29-2.43 (1H, m), 2.47-2.59 (1H, m), 2.69 (1H, dd), 3.28 (1H, dd), 3.48 (1H, dd), 3.76 (6H, s), 3.92 (1H, dd), 4.15 (1H, dd), 4.79 (1H, td), 6.94 (1H, d, NH), 7.58 (1H, br t, NH).

(5S,6S,7E,9E,11Z) ETHYL 5,6-EPOXY-7,9,11-EICOSATRIENE-14,19-DIYNOATE (or 14,15,19,19,20,20-hexadehydro LTA_4 ethyl ester). At -78°C , 1.6 M n-BuLi in hexane (100 μL) was added dropwise to 1.26 mL of a 0.14 M solution of (3,8-nonanedien-1-yl)triphenylphosphonium iodide **6** in THF:HMPA 5:1 and the mixture was stirred at this temperature for 1.3 hours. A solution of 5(S), 6(S), 7(E), 9(E) ethyl 5,6-epoxy-11-oxo-7,9-undecadienoate **7^{9b}** (34.5 mg, 145 μmol) in 1.5 mL of THF was slowly added and the stirring continued for 45 min. at -78°C and 2 hours at 0°C . Then, 25% aq. NH_4OAc and triethylamine (~100 μL) were added and the product was extracted in EtOAc, dried over Na_2SO_4 and purified by flash chromatography on silica (deactivated with hexane: Et_3N 80:20) with EtOAc:hexane: Et_3N 5:95:2. Yield: 35 mg, 71%. ^1H NMR (CD_3COCD_3) δ 1.20 (3H, t), 1.48-1.80 (6H, m), 2.20-2.39 (7H, m), 2.84 (1H, td, $J = 2.3, 5.3$ Hz), 3.10 (2H, br d), 3.16 (1H, dd, $J = 2.3, 7$ Hz), 4.08 (2H, q), 5.47 (2H, td, $J = 7, 10$ Hz and dd, $J = 7, 15$ Hz), 6.08 (1H, dd, $J = 10, 10$ Hz), 6.30 (1H, dd, $J = 10, 15$ Hz), 6.57 (1H, dd, $J = 15, 18$ Hz), 6.62 (1H, dd, $J = 15, 18$ Hz).

14,15,19,19,20,20-HEXADEHYDRO LTC_4 DIMETHYL ETHYL ESTER **1**. To a solution of 14,15,19,19,20,20-hexadehydro LTA_4 ethyl ester (221 mg, 0.65 mmol) in 2 mL of MeOH and 0.5

ml of Et₃N was added 1.2 mg of 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy, free radical, followed by 305 mg (1.4 equiv.) of glutathione, reduced, dimethyl ester and the mixture was stirred at room temperature for 5 hours. The solvents were evaporated and the product was purified by flash chromatography on silica with Et₃N:MeOH:Hexane:EtOAc 5:7:20:80 (revelation of TLC with KMnO₄) to give 368 mg (84%) of pure hexadehydro LTC₄ triester. This compound was kept in solution in EtOAc or MeOH containing 0.1% Et₃N at -70°C. ¹H NMR (CD₃OD) δ 1.23 (3H, t, J = 7 Hz), 1.40-1.83 (5H, m), 1.90 (1H, m), 2.07 (1H, m), 2.20-2.44 (9H, m), 2.65 (1H, dd, J = 10, 14 Hz), 2.91 (1H, dd, J = 4, 14 Hz), 3.07 (2H, br d), 3.37 (1H, dd, J = 4, 10 Hz), 3.52 (1H, dd, J = 6, 7 Hz), 3.67 (1H, m), 3.72 (6H, 2s), 3.95 (2H, AB system, J = 17 Hz), 4.10 (2H, q, J = 7 Hz), 4.55 (1H, dd, J = 4, 9 Hz), 5.43 (1H, m), 5.68 (1H, m), 6.05 (1H, t, J = 11 Hz), 6.27 (2H, m), 6.58 (1H, m). ¹H NMR (CDCl₃) δ 1.35 (3H, t), 1.50 (2H, m), 1.59-2.07 (5H, m), 1.97 (1H, t), 2.15 (1H, m), 2.23-2.40 (7H, m), 2.47 (1H, m), 2.79 (1H, dd, J = 6, 14 Hz), 3.50 (2H, m), 3.75 (6H, 2s), 3.77 (1H, m), 3.95 (1H, dd, J = 5, 22 Hz), 4.12 (3H, q), 4.12 (1H, dd, J = 22 Hz), 4.71 (1H, br q), 5.48 (1H, td, J = 7, 10 Hz), 5.67 (1H, dd, J = 10, 14.0 Hz), 6.06 (1H, dd, J = 11, 11 Hz), 6.22 (1H, dd, J = 10, 14 Hz), 6.31 (1H, dd, J = 10, 14 Hz), 6.49 (1H, dd, J = 12, 14 Hz), 7.13 (1H, d, NH, J = 10 Hz), 7.42 (1H, br t, NH, J = 5 Hz). MS, m/e 676 (M+1), 446, 336, 286.

[14,15,19,19,20,20-²H₆]-LTC₄ DIMETHYL ETHYL ESTER 2b. To a solution of 10 mg of 1 and triethylamine (1 µL) in 3 mL of methanol was added the Lindlar's catalyst (99 mg) and the mixture was stirred under an atmosphere of deuterium for 2.5 hours. Another portion of the Lindlar's catalyst (41 mg) was then added and the mixture was stirred for 2 hours. The deuteration was followed by HPLC (column µBondapak C18, solvent MeOH:0.2% aq. NH₄OAc, pH 7.0 75:25, flow rate 1.2 mL/min, λ 280 nm). The first product formed, 14,15,19,20-tetradehydro LTC₄ 8 appeared at 9.8 min. Then 14,15 and 19,20-didehydro LTC₄ 9 and 10 appeared at 12.5 and 13.7 min respectively. Finally, 2b, eluted at 19.2 min. When the reaction was completed, 2 mL of CH₂Cl₂ were added and the mixture was filtered through celite with methanol and concentrated. The product was purified by HPLC (small cartridge NovaPak C18, solvent MeOH:0.2% aq. NH₄OAc pH

6.2 84:16, flow rate 12 mL/min, λ 280 nm), the solvents were partially evaporated and the product was extracted in EtOAc, dried over Na_2SO_4 and concentrated to afford 4.6 mg of **2b** containing some aliphatic impurities from the column. Pure **2b** was obtained by flash chromatography on silica with $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$ 1:10:330 and 1:10:160. Yield 1.7 mg, 17%. ^1H NMR (CDCl_3) characteristic peaks δ 0.84 (1H, m, CHD_2), 3.75 (6H, s), 4.13 (2H, q), 4.72 (1H, br q), 5.45 (1H, br q), 5.66 (1H, dd), 6.03 (1H, t), 6.27 (2H, m), 6.56 (1H, dd), 7.08 (1H, NH, br d), 7.38 (1H, NH, br t). MS, m/e (relative intensity) 694.5 (0.58), 693.5 (1.25), 692.5 (1.28), 691.5 (1.47), 690.5 (2.67), 689.4 (4.14), 688.4 ($M+1$, 4.42), 687.4 (2.36), 686.4 (1.03), 690.5 (2.67), 689.4 (4.14), 688.4 ($M+1$, 4.42), 687.4 (2.36), 686.4 (1.03).

[14,15,19,19,20,20- $^2\text{H}_6$]-LTC $_4$. The deuteration of **1** (10 mg), was performed as previously described. The crude product was dissolved in 1 mL of MeOH and 500 μL of freshly prepared 1.0 M K_2CO_3 and 2-3 small crystals of 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy, free radical, were added. The mixture was stirred under N_2 in the dark overnight. Water (~ 500 μL) was then added to give a clear solution and the pH was neutralized with AcOH. The product was purified by HPLC on a small cartridge Nova Pak C18 with MeOH:0.2% aq. NH_4OAc pH 6.75 60:40 (flow rate 10 mL/min, λ 280-310 nm). The methanol was evaporated and water was removed by freeze-drying to yield a white solid. Dissolution in MeOH and filtration gave 3.6 mg of [$^2\text{H}_6$]-LTC $_4$. ^1H NMR (CD_3OD) δ 0.87 (1H, m, CHD_2), 1.10-1.53 (m), 1.60 (2H, m), 1.76 (1H, m), 1.92 (3H, s), 1.97-2.28 (m), 2.56 (2H, t), 2.70 (1H, dd), 2.95 (2H, m), 3.35 (1H, m), 3.67 (2H, m), 3.73 (2H, AB system), 4.55 (1H, dd), 5.37 (1H, br q), 5.67 (1H, dd), 6.02 (1H, t), 6.24 (2H, m), 6.58 (1H, dd). UV, λ_{max} 280.2 nm, MS, m/e (relative intensity) 660.4 (2.31), 659.4 (2.52), 658.5 (2.62), 657.4 (4.28), 656.5 (6.05), 655.5 ($M+\text{Na}$, 10.41), 654.5 (9.89), 653.4 (6.40), 635.5 (4.04), 634.6 (4.70), 633.5 (7.67), 632.5 ($M+1$, 7.61), 631.5 (3.90), 630.4 (2.19).

[14,15,19,19,20,20- $^3\text{H}_6$]-LTC $_4$. To a solution of 4 mg (5.9 μmol s) of **1** and 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy, free radical, (0.3 mg) in 3 ml of methanol was added the Lindlar's catalyst (75 mg) and the mixture was stirred under 1.1 atmospheres of tritium gas for 2 hours. After

removal of labile tritium the reaction mixture was filtered and the crude 2c was thus obtained, 900 mCi, 85% yield based on a S.A. of 180 Ci/mmol. The crude 2c was dissolved in 10 ml methanol and 10 ml of 1M LiOH were added. The mixture was stirred under N₂ for 30 minutes at room temperature and was neutralized with acetic acid. The product was purified by HPLC on a μ Bondapak C18 column, 9.4 x 250 mm, with MeOH:H₂O:AcOH 65:35:0.1 (pH 5.8 adjusted with NH₄OH; flow rate 3 min/ml, λ 280 nm). The fractions containing [³H₆]-LTC₄ were combined to afford 150 mCi of product, 14% yield.

References

1. For a review on the chemistry and biology of leukotrienes, see: Rokach, J., *Bioactive molecules, Vol. 11, Leukotrienes and Lipoxygenases*, Elsevier, (1989).
2. Cohen, N., Banner, B.L., Lopresti, R.J., Wong, F., Rosenberger, M., Liu, Y.-Y., Thom, E. and Liebman, A.A. - J. Am. Chem. Soc. 105: 3661 (1983) and references cited.
3. Shevchenko, V.P., Demin, P.M., Nagaev, I.Yu., Pivnitsky, K.K., Myagkova, G.I. and Myasoedov, N.F. - *Full Chemical Synthesis of [5,6,8,9,14,15-³H₆]-Hepoxylin B₃*, 4th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, September 3-7, 1991, Toronto, Canada.
4. Lellouche, J.P., Aubert, F. and Beaucourt, J.P. - Tetrahedron Lett., 29: 3069 and 3073 (1988).
5. Leblanc, Y., Fitzsimmons, B.J., Zamboni, R. and Rokach, J. - J. Org. Chem. 53: 265 (1988).
6. Delorme, D., Girard, Y. and Rokach, J.- J. Org. Chem. 54: 3635 (1989).
7. Meese, C.O. - J. Lab. Comp. Radiopharm. 23: 295 (1985). Prakash, C. and Blair, I.A. - Prostaglandins, 37: 251 (1989).
8. Brunet, J.J., Gallois, P. and Caubère, P. - Tetrahedron Lett. 45: 3955 (1977).
9. a) Corey, E.J., Clark, D.A., Goto, G., Marfat, A., Mioskoski, C., Samuelsson, B. and Hammarstrom, S. - J. Am. Chem. Soc. 102: 1436 and 3663 (1980).

- b) Rokach, J. Zamboni, R., Lau, C.K. and Guindon, Y. - *Tetrahedron Lett.* **22**: 2759 (1981).
 - c) Rokach, J., Young, R.N., Kakushima, M., Lau, C.K., Séguin, R., Frenette, R. and Guindon, Y. - *Tetrahedron Lett.* **22**:, 979 (1981).
 - d) Ernest, I., Main, A.J. and Menassé, R. - *Tetrahedron Lett.* **23**: 167 (1982).
 - e) Corey, E.J. and Albright, J.O. - *J. Org. Chem.* **48**: 2114 (1983).
10. a) Evans, E.A. - *Tritium and Its Compounds*, 2nd ed., Butterworths, London (1974). Evans, E.A., Warrell, D.C., Elvidge, J.P. and Jones, J.R. - *Handbook of Tritium NMR Spectroscopy and Applications*, Wiley (1985).
- b) Vader, J., Sperling, E., Kaspersen, F., Wagenaars, G. and Funke, C. - 4th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, September 3-7, 1991, Toronto, Canada.
- c) Shevchenko, V.P., Nagaev, I.Yu and Myasoedov, N.F. - The Producing of Triple and Quadruple-Labelled Biologically Active Compounds by the Hydrogenation of Terminal Double Bonds, 4th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, September 3-7, 1991, Toronto, Canada.