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Oxidized Leukotrienes: Synthesis of 20-OH and 20-COOH LTD_4. Possible Metabolites in the Lipoxygenase Pathway

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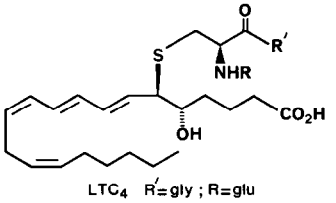
Summary: Leukotriene LTD_4 analogs 20-OH LTD_4 16 and 20-COOH LTD_4 17 were synthesized. These ω-oxidative derivatives are possible metabolites of the natural product.

The leukotrienes LTC_4, LTD_4 and LTE_4, also known as SRS-A (slow reacting substance of anaphalaxis) are all potent spasmogens, producing smooth muscle contractions in mammalian organisms. Typically, doses as low as 0.3 ng/mL produce observable contractions of the guinea pig ileum.[1] These compounds are produced from arachidonic acid via the 5-lipoxygenase cascade. Arachidonic acid is oxidized to give 5-hydroperoxy eicosatetraenoic acid (HPETE) and this compound is dehydrated stereospecifically producing the 5,6 expoxide, LTA_4, which upon nucleophilic opening with the thiopeptide glutathione gives rise to LTC_4. Specific enzymatic degradation of the peptide affords LTD_4 and LTE_4. The leukotrienes are believed to be important mediators in allergic reactions and are known to be formed during asthmatic states, inducing broncho-constriction. Our laboratories are primarily concerned with the biochemical and physiological roles of the leukotrienes and their metabolites.



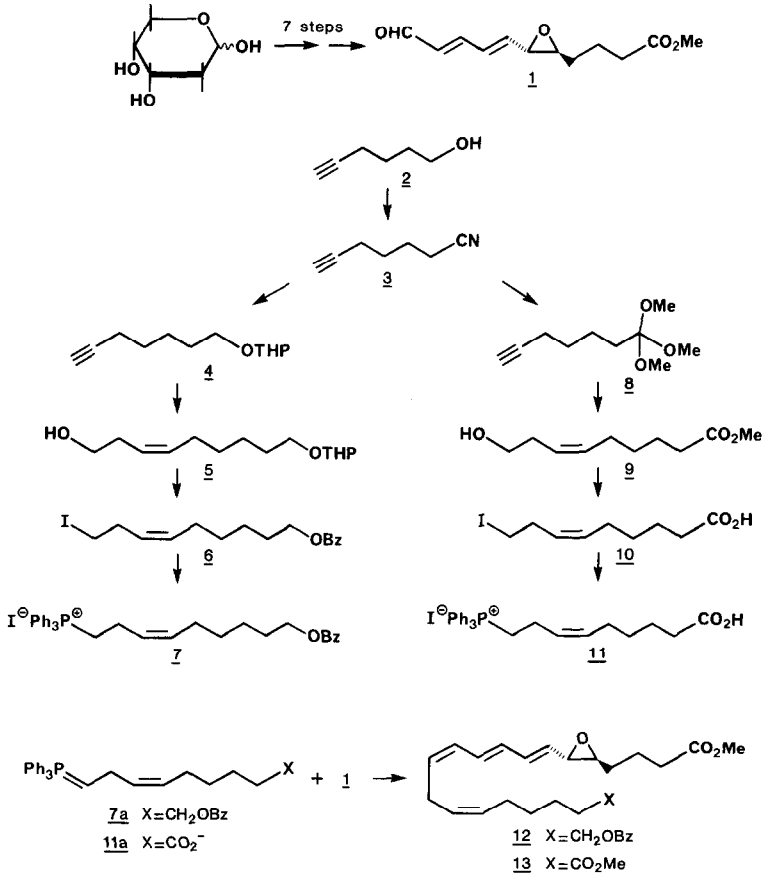
LTC_4 R'=gly ; R=glu
LTD_4 R'=gly ; R=H
LTE_4 R'=OH ; R=H

In a previous paper we described the synthesis of ω-oxidative metabolites of leukotriene LTB_4 20-OH LTB_4 and 20-COOH LTB_4.[2] While the role of these natural products is not fully understood, it is likely that the normal course of fatty acid oxidation, also present in prostaglandins, serves to de-toxify the organism of these biologically potent mediators. These more polar metabolites have decreased lipophilicity and may be "flushed" out of tissues due to their enhanced aqueous solubility. Recently, it has been reported that 20-OH LTB_4 is present in the urine of monkeys.[3]

By analogy to the LTB_4 series, we anticipated that ω-oxidation is a likely possibility for metabolism of leukotrienes C,D and E. We undertook unambiguous syntheses

of 20-OH LTD_4 and 20-COOH LTD_4, potential new natural products for two reasons. Firstly, with the belief that these compounds may be discovered to occur naturally, we are very interested in having authentic samples for comparison. This is particularly important since biological sources produce very small amounts of leukotrienes. Secondly, we are interested in the biological properties of these metabolites - particularly agonist or antagonist activity to the parent leukotrienes.

The synthetic strategy is derived from our efficient stereospecific chiral synthesis of LTA_4.[5] Epoxy-aldehyde 1 is prepared from 2-deoxy-D-ribose in 7 steps. This common intermediate is suitable for coupling with an appropriate phosphorane in a Wittig reaction to form the desired triene system with the correct double bond geometry. By varying the phosphonium salt having either ω-OH or ω-COOH functions we can obtain directly the LTA_4 analogue which can then be converted to LTD_4 using the protected cys-gly dipeptide.



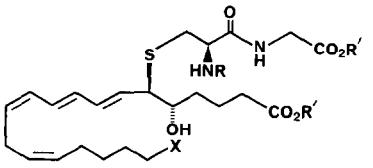
The synthesis of the two phosphonium salts begins from a common bi-functional acetylene, 1-hexyn-6-ol, which is homologated to 6-cyano-1-hexyne 3 (1. MsCl, Et_3N/ CH_2Cl_2; 2. NaI/ acetone; 3. NaCN/acetone-H_2O) in 64% overall yield. In the 20-OH series the nitrile 3 is

hydrolyzed to the acid, reduced to the corresponding alcohol and protected as its THP ether (1. 10N KOH/EtOH, Δ; 2. EtOCOCl, Et_3N/CH_2Cl_2; 3. $NaBH_4$/EtOH; 4. dihydropyran, $TsOH/CH_2Cl_2$) giving acetylene 4 in 59% yield. Condensation of the acetylide anion with ethylene oxide to give the homopropargyl alcohol followed by semi-hydrogenation produced the cis olefin 5 in good yield, 67% (1. 4, BuLi/NH_3; 2. ethylene oxide/THF; 3. $Ni(OAc)_2$, $NaBH_4$,$NH_2CH_2CH_2NH_2$,H_2/EtOH[6]). The alcohol 5 was converted to the iodide and the THP-group was replaced by a benzoate, deemed more compatible for the later stages in the synthesis, giving 6. (1. MsCl,Et_3N/CH_2Cl_2; 2. NaI/acetone; 3. PPTS/EtOH; 4. BzBr,Et_3N,DMAP/CH_2Cl_2). Finally the phosphonium salt 7 was formed by heating the iodide 6 in the presence of triphenylphosphine (ϕ_3P 1.6 aq, 6/ϕCH_3, 60-70°C) providing a 48% yield from 5.

In the 20-CO_2H series 6-cyano-1-hexyne was converted to orthoester 8 (MeOH,HCl/Et_2O)[7] in 36% yield. Condensation with ethylene oxide, hydrolysis and semi-hydrogenation afforded the homo-allylic alcohol 9 in 35% yield (1. 8, $LiNH_2/NH_3$; 2. ethylene oxide; 3. 1N HCl, 2 min; 4. $Ni(OAc)_2$, $NaBH_4$,$NH_2CH_2CH_2NH_2$,H_2/EtOH). The alcohol was transformed to the corresponding iodide and the ester was hydrolyzed to the free acid 10. Finally the phosphonium salt 11 was generated, (1. MsCl,Et_3N/CH_2Cl_2; 2. NaI/acetone; 3. LiOH/DME,H_2O; 4. ϕ_3P 1.6 eq, 10/ϕCH_3, 60-70°) in 58% overall yield from 9.

The Wittig reaction to form 20-OBz LTA_4 12 produced an unexpected 3:1 cis-trans mixture in 40% yield. (1. 7, $[(CH_3)_3Si]_2NLi$, 1.2 eq/THF-HMPA @ 0°C 1 h cool -78°C; 2. aldehyde 1 THF-HMPA, 20 min, warm to 0°C 30 min, quench NH_4OAc; chromatography on silica gel eluting with 2:1 hexanes/EtOAc/3% Et_3N).

The Wittig reaction to produce 20-CO_2Me LTA_4 13, proceeded much better to give a 10:1 cis-trans mixture in 60% yield (1. 11, $[(CH_3)_3Si]_2NLi$, 2.2 eq/THF-HMPA, 0°C, 1 h, cool -78°C; 2. aldehyde 1/THF-HMPA 20 min, warm 0°C, 30 min, quench $NaHCO_3/(CH_3O)_2SO_2$; chromatography on silica gel eluting 4:1 hexanes/EtOAc/3% Et_3N). Ester 13 was also purified by HPLC to remove the 11-trans contaminant. However benzoate 12 (cis-trans mixture 3:1) could not be separated at this stage and was carried through until the last step in the synthesis.



14 X=CH_2OBz; R=$COCF_3$; R'=Me
15 X=CO_2Me ; R=$COCF_3$; R'=Me
16 X=CH_2OH ; R=R'=H
17 X= COOH ; R=R'=H

Opening of the epoxides 12 and 13 with the thiopeptide N-trifluoroacetyl-L-cysteinyl-glycine methyl ester proceeded quantitatively and hydrolysis of the protecting groups (1M

K_2CO_3/MeOH) was achieved to produce the final products 20-OH LTD_4 16 and 20-COOH LTD_4 17 respectively. Final purification of these compounds was achieved using reverse phase HPLC[8] to give the desired isomerically correct analogs of LTD_4.[9] Both 20-OH LTD_4 and 20-COOH LTD_4 demonstrated agonist activity in the guinea pig ileum assay although both were 50 and 1,000 times respectively less potent than the parent LTD_4. This suggests a fair degree of specificity with regards to the lipophilic chain in LTD_4 eliciting the muscular contractions.[10]

References

1. Guinea pig ileum assay: G. Holme, G. Brunet, H. Piechuta, P. Masson, Y. Girard and J. Rokach, Prostaglandins 20, 717 (1980).

2. R. Zamboni and J. Rokach, Tet. Lett., 23, 4751 (1982).

3. J.A. Oates, Vanderbilt University, Tenn., U.S., Private communication.

4. 20-OH and 20-COOH LTC_4 and LTE_4 analogs are made in a similar fashion.

5. J. Rokach, R. Zamboni, C.K. Lau and Y. Guindon, Tet. Lett., 22, 2759 (1981).

6. C.A. Brown and V.K. Ahuja. J. Chem. Soc. Chem. Comm., 553 (1973).

7. G. Just and C. Luthe, Tet. Lett., 23, 1331 (1982) ref. 9.

8. HPLC Conditions: Waters reverse-phase C_{18} semi-prep column. Elute with 50% MeOH/H_2O contained 0.1% HOAc, buffered to pH 5.6.

9. Satisfactory spectral data was obtained for all purified intemediates: ^1H NMR 250 MHz and 400 MHz, UV, HPLC and IR.

10. We are currently studying the oxidative metabolism of LTD_4 using radio-labelled 14,15 $^3H_2$-LTD_4, obtained from New England Nuclear. The full pharmacological profile of the metabolites is being pursued and will be disclosed at a later date.

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