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# TOTAL SYNTHESIS OF LIPOXIN B: ASSIGNMENT OF STEREOCHEMISTRY

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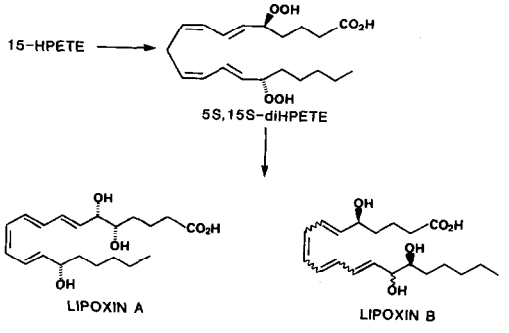
SUMMARY: Four isomers of lipoxin B were synthesized using carbohydrate precursors. An authentic sample of lipoxin B (from human leukocytes) was found to be a mixture of all-trans isomers 1a and 1c.

The lipoxins are a new class of oxidative metabolites derived from arachidonic acid. The first report on lipoxins by Samuelsson and co-workers,[1,2] described the incubation of 15-hydroperoxyeicosatetraenoic acid (15-HPETE) with purified human leukocytes to produce lipoxins A and B, two isomeric conjugated trihydroxy tetraenes. Preliminary studies revealed that the lipoxins have interesting biological profiles, including the stimulation of superoxide release from cells and immuno-regulant activities of natural killer (NK) lymphocytic cells. At the time of the initial communication on the structure of lipoxins, the skeletal structure, but not the relative stereochemistry of the trihydroxy tetraenes was known.

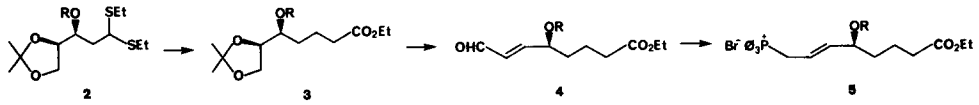
We have recently completed the synthesis of 6 isomeric 5, 6, 15 trihydroxy tetraenes and subsequently shown that lipoxin A has the structure 5S, 6S, 15S, 7E, 9E, 11Z, 13E trihydroxy eicosatetraenoic acid by comparison with an authentic natural sample of lipoxin A.[3] Our recent efforts have been devoted to the synthesis of the second member of this class of arachidonic acid metabolites, lipoxin B.

As shown in Scheme I, it is postulated that 15S-HPETE undergoes a second lipoxygenation reaction giving rise to 5S, 15S-diHPETE and this is the common precursor for lipoxins A and B. The second oxidation, thus establishes the stereochemistry of the hydroxyl groups at $C_5$ and $C_{15}$ as S. The synthetic lipoxin B targets consisted of preparing both 14S and 14R hydroxyl isomers having the 8-cis, 6, 10, 12-trans olefin geometry as well as the all-trans isomers for comparison to the naturally produced lipoxin B. It is important to prepare isomers not only for the purposes of characterization but also to study structure-activity relationships when evaluating the biological effects of lipoxin B.

Scheme I

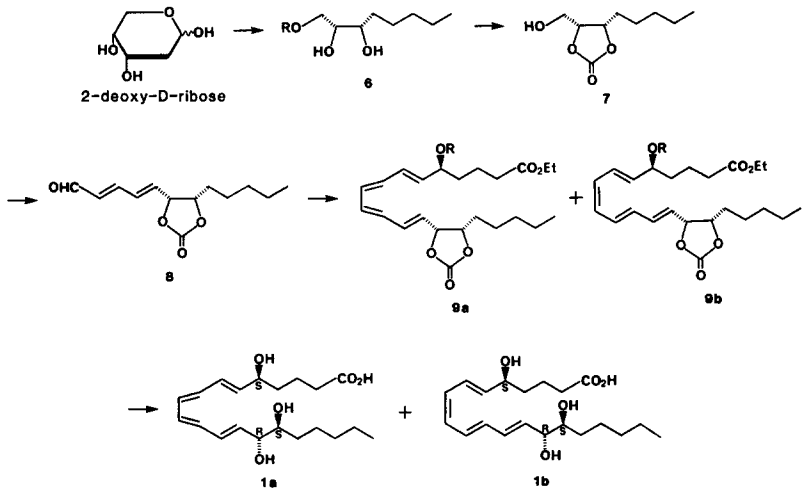


SCHEME II

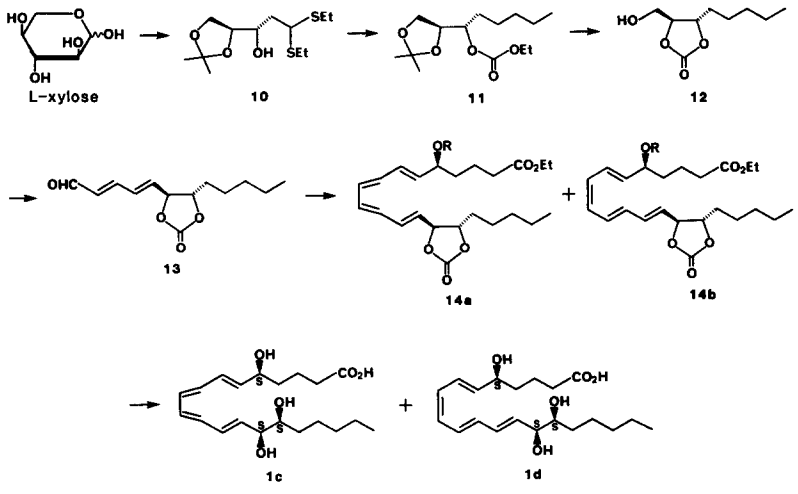


R = t-BuØ$_2$Si-

SCHEME III



SCHEME IV



To unambiguously predetermine the absolute stereochemistry of the hydroxyl groups of the lipoxin B isomers, carbohydrate precursors were utilized. The 5S stereocenter was obtained from D-arabinose for all of the lipoxin B isomers. The 14R,15S and 14S,15S vicinal diols were transposed from 2 deoxy-D-ribose and L-xylose respectively.

The C1-C8 fragment common to all of the lipoxin B isomers was prepared as shown in Scheme II. Treatment of the thioacetal 2,[3] prepared from D-arabinose , with N-chlorosuccinimide/silver nitrate in acetonitrile-water at -20°C gave the corresponding aldehyde which was condensed with 1 equivalent of (carboethoxylmethylene)triphenylphosphorane in dichloromethane to yield, after hydrogenation over 5% Pd-C, the ester 3 in a 71% yield. Hydrolysis of the acetonide of 3 with trifluoroacetic acid in THF-water, lead tetraacetate cleavage of the resultant diol in dichloromethane at -78°C, followed by condensation of the aldehyde with 1.2 equivalents of (formylmethylene)triphenylphosphorane in toluene at 80° for 5 h gave the $\alpha,\beta$ unsaturated aldehyde 4 in a 61% yield. Reduction of the aldehyde 4 with $NaBH_4$-$CeCl_3$ in isopropanol-water at 0°C,[4] treatment of the resulting alcohol with DIPHOS and carbon tetrabromide in dichloromethane at room temperature gave the corresponding bromide, which upon treatment with excess triphenylphosphine in acetonitrile yielded the phosphonium salt 5 in an 86% yield.

The 14R,15S isomers of lipoxin B were prepared as shown in Scheme III. Protection of 2-deoxy-D-ribose with t-butylchlorodiphenylsilane and triethylamine in dichloromethane gave the corresponding 5-O-silyl derivative in an 81% yield. Condensation of the lactol with excess (1-propylene)triphenylphosphorane in THF and hydrogenation of the resulting olefin over 5%-Pd-C gave the diol 6 in a 76% yield. Treatment of the diol 6 with excess 1,1'carbonyldiimidazole in methylethylketone at 100°C in a sealed tube and subsequent removal of the silyl group by treatment with tetra-n-butylammonium fluoride in THF gave the alcohol 7 in a 75% yield. Oxidation of the alcohol,[5] condensation of the resulting aldehyde with $Ph_3P{=}CH{-}CH{=}CHO$ in THF and photolysis of the resulting cis/trans mixture with a catalytic amount of iodine in dichloromethane[6] gave the trans,trans dienealdehyde 8 in a 47% yield. Condensation of the aldehyde 8 at -100°C with 1 equivalent of the ylide generated by treatment of the phosphonium salt 5 with 0.9 equivalents of LiHMDS in THF at -100°C, subsequent addition of HMPA and warming to -40°C for 2 h gave a 1:1 mixture of the two desired protected 14R,15S isomers of lipoxin B 9a and 9b in a 92% yield. The cis and trans isomers were separated easily by HPLC (12.5% EtOAc/hexane). Deprotection of the isolated isomers with tetra-n-butylammonium fluoride in THF and then potassium carbonate in aqueous methanol at 5°C gave the corresponding lipoxin B isomers 1a and 1b in an 83% yield.

The 14S,15S isomers of lipoxin B were prepared as shown in Scheme IV. Treatment of the alcohol 10[3] with t-butylchlorodimethylsilane, DMAP (cat) and triethylamine in dichloromethane at room temperature gave the corresponding silylated alcohol in an 84% yield. Hydrolysis of the thioacetal with N-chlorosuccinimide and silver nitrate in acetonitrile-water at -20°C generated the free aldehyde which was condensed with (1-propylene)triphenylphosphorane in THF at 0°C to afford the corresponding olefin which was hydrogenated over 5% Pd-C to give the saturated compound. The silyl protecting group was then removed by treatment with excess tetra-n-butylammonium fluoride in THF at 0°C. Treatment of the resulting alcohol with

ethylchloroformate in pyridine gave the mixed carbonate 11 in a 67% yield from the thioacetal 10. Treatment of the mixed carbonate 11 with trifluoroacetic acid in THF-water caused hydrolysis of the acetonide with concurrent formation of the cyclic carbonate 12. The cyclic carbonate 12 was converted into the diene aldehyde 13 by the same sequence of reactions used to convert the alcohol 7 into the diene aldehyde 8 (Scheme III) in a similar yield. Condensation of the aldehyde 13 with the phosphorane generated from the salt 5 as described for the 14R,15S isomers gave the desired protected 14S,15S lipoxin B isomers 14a and 14b in an 87% yield. These isomers were separated and deprotected as described for the 14R,15S lipoxin B isomers to give the free 14S,15S lipoxin B isomers 1c and 1d in similar yield.

Using the procedure outlined by the Samuelsson group,[2] authentic samples of lipoxin A and B were prepared from human leukocytes. Reverse phase - HPLC comparison of authentic lipoxin B methyl ester and its free acid with our four synthetic lipoxin B methyl esters and acids were performed in two solvent systems. Using methanol-water (70:30 for the esters; 60:40, 0.05% HOAc for the acids) both the 14S and 14R all trans compounds 1a and 1c were not separable and both co-eluted with authentic lipoxin B as their Me-esters and free acids. However in $CH_3CN$-$H_2O$ (40:60 for the esters; 40:60, 0.05% HOAc for the acids) the 14S and 14R all trans compounds 1a and 1c were separable. Injection of authentic lipoxin B revealed that lipoxin B was a mixture of two isomers with peaks co-eluting with the synthetic standards 14S/14R-all trans isomers 1a and 1c.

Our previous work found lipoxin A isolated from human neutrophils to be the 5S, 6S, 15S 11-cis, 7, 9, 13-trans eicosatetraenoic acid.[3] The corresponding stereochemical pattern is clearly not observed in lipoxin B, suggesting that lipoxins A and B may be formed by differing mechanisms. We have recently proposed several possible routes to lipoxins[3] and are currently studying the biosynthesis of these compounds and will soon be reporting our findings in a separate communication.

References and Notes:

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8. Data on lipoxin B 1a
$^1$H-NMR (250 MHz, Acetone-$d_6$)$\delta$ 0.832 (t, 3H, $CH_2CH_3$); 1.36 - 1.69 (cm, 12H, $(CH_2)_4CH_3$ + $(CH_2)_2CH_2CO_2$); 2.3 (t, 2H J=8.0Hz, $CH_2CO_2$); 3.10 (m, 3H, 3-OH); 3.46 (m, 1H, =CHCH(OH)CH(OH)-); 4.00 (t(dd), 1H, =CHCH(OH)CH(OH), $J_{14,15}=J_{13,14}$=7.2 Hz); 4.14 (q(dt), 1H, =CHCH(OH)CH$_2$, $J_{56}=J_{4,5}$=8.0Hz); 5.79 (m, 2H, -CH=CHCH=CHCH=OHOH=CH-); 6.28 (bs, 6H, CH=CHCH=CHCH=CHCH=CH).
U.V. (MeOH) $\lambda$ max 287,301,316 nm
G.C.-M.S. lipoxin B 1a as its methyl ester tri-O-TMS derivative exhibited a G.C.-mass spectrum identical in all respects to the published spectrum.[2] ($M^+$ m/e=582; 100% peak m/e=173).
9. B.J. Fitzsimmons would like to thank NSERC (Canada) for an Industrial Fellowship.

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