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# Lipoxins A4 and B4
# Total Synthesis Including Deprotection Studies

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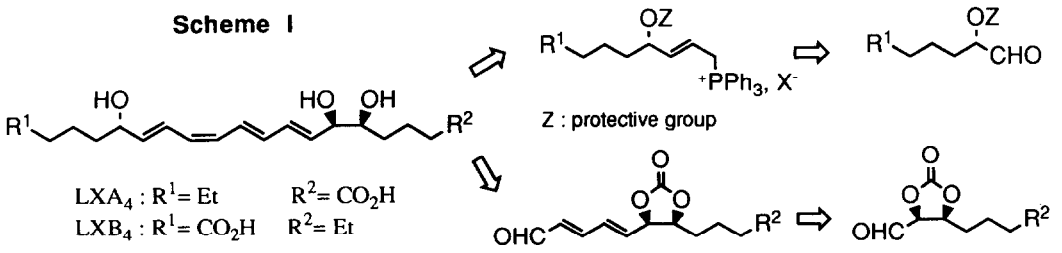
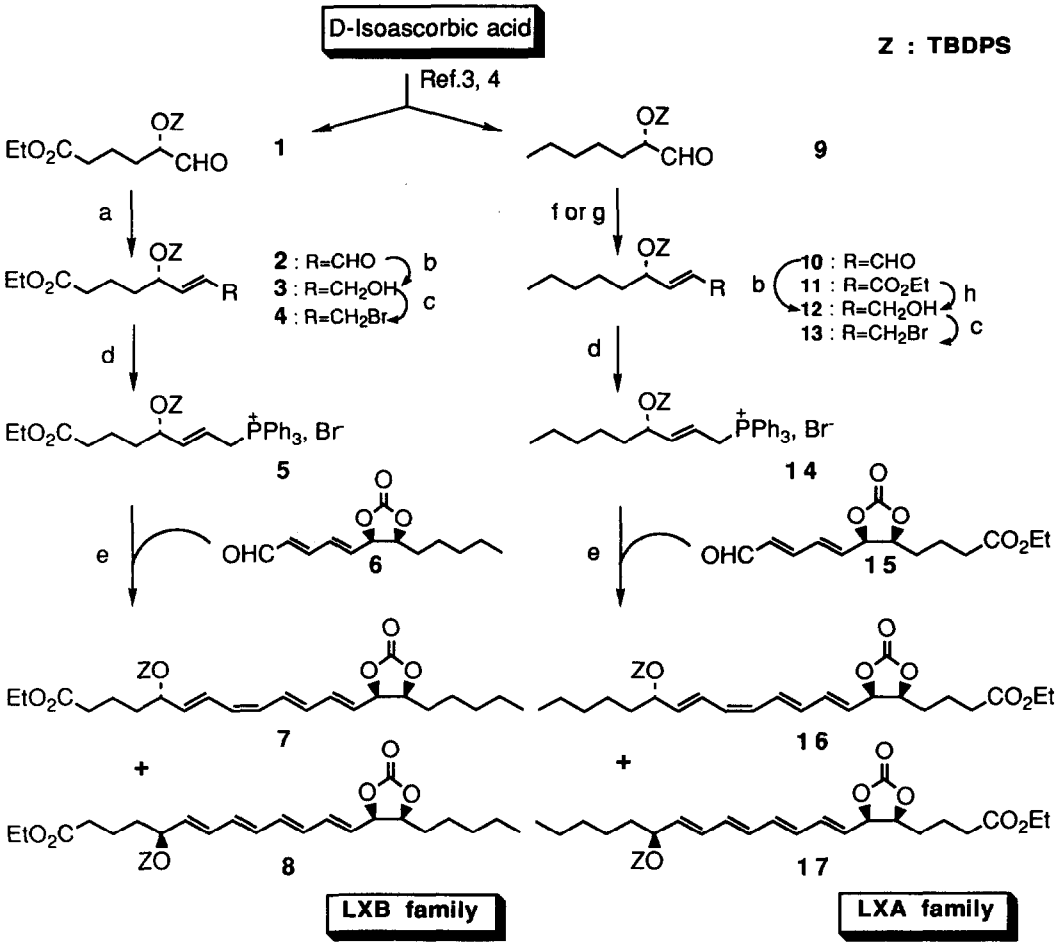
**Abstract** : The total synthesis of lipoxins $A_4$ and $B_4$ ($LXA_4$ and $LXB_4$) as well as of their all-*trans* isomers is reported. A study concerning the final steps of deprotection of silyl ether, cyclic carbonate and ethyl ester assisted by a high-speed scanning UV spectrophotometer has been carried out.

Lipoxins are a series of naturally occuring eicosanoids discovered by B. Samuelsson group[1]. These new metabolites of arachidonic acid isolated in human polymorphonuclear leukocytes are formed either through the interaction of 5- and 15-lipoxygenases or by cell-cell interactions. Compounds of this family contain a conjugated tetraenic structure and three asymmetric carbons. $LXA_4$ and $LXB_4$ (scheme I) are the major lipoxins formed *in vivo*, however their all-*trans* isomers have also been detected. These biologically active molecules[2] are implicated in the stimulation of human neutrophils, the modulation of immunological activities of lymphocytes and in processes involving inter-and intra-cellular exchanges. Due to their role in the regulation of various physiological responses and to their minute isolable quantities from natural sources, the availability of synthetic lipoxins is a prime target not only to fully evaluate their properties but also to study their metabolism. Thus, we developed a general strategy to reach these molecules starting from inexpensive, commercially available *D*-isoascorbic acid (IAA). The retrosynthetic analysis of $LXA_4$ and $LXB_4$ is outlined in scheme I.

**Scheme I**



**Scheme II**




(a) $Ph_3P$=CH-CHO, toluene, 80°C, 5h. (b) $NaBH_4$, $CeCl_3$, iPrOH, r.t., 1.5 h. (c) $CBr_4$, $(Ph_2PCH_2)_2$, $CH_2Cl_2$, -35°C, 2 h. (d) $PPh_3$, $CH_3CN$, r.t., 18 h. (e) KHMDS, -105°C, 5 min then **6** or **15**, -105°C, 15 min then HMPA and -105°C→-40°C, 1 h at -40°C. (f) **9** → **10** : $Ph_3P$=CH-CHO, toluene, 85°C, 6h. (g) **9**→**11** : $(EtO)_2P(O)CH_2CO_2Et$, LiCl, DBU, r.t., 2.5 h. (h) DIBAL, $CH_2Cl_2$, -78°C, 1.5 h.

It reveals the key role of enantiomerically pure α-hydroxy and α,β-dihydroxyaldehydes which are readily available from IAA as previously described[3]. Starting from these chiral synthons, the total convergent syntheses of both $LXA_4$ and $LXB_4$ has been carried out notably involving Wittig type reactions. Our approach has already been disclosed in a preliminary form[4] and we wish to detail herein our results including a study concerning the deprotection, assisted by a high-speed scanning UV spectrophotometer, of silyl ether, cyclic carbonate and ethyl ester which led to LX's potassium salts. This method allowed the desired tetraenic molecules to be discriminated from non-tetraenic by-products.

## RESULTS AND DISCUSSION

The synthesis of fully protected $LXA_4$ and $LXB_4$ as well as of their all-*trans* isomers is depicted in scheme II.

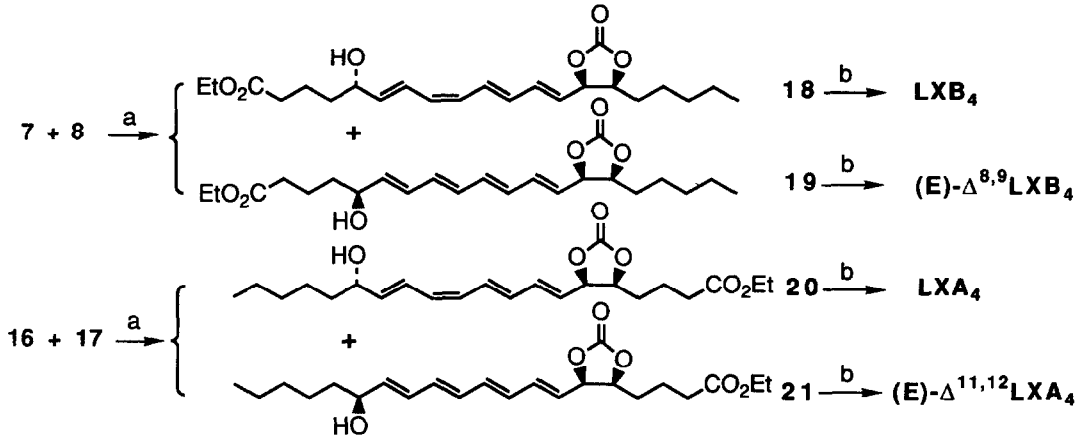
The silicon-protected enantiomerically pure α-hydroxyaldehyde **1**[3,4] was homologated into α,β-ethylenic aldehyde **2** in 48% yield by a Wittig reaction with (formylmethylene) triphenylphosphorane ($Ph_3P$=CH-CHO 1.2 eq, toluene, 80°C, 5h). Then, selective reduction of the aldehydic carbonyl function smoothly occured under Luche conditions[5] ($NaBH_4$, $CeCl_3$, iPrOH, r.t.) and afforded the primary allylic alcohol **3** in 92% yield. Its transformation into the phosphonium salt **5** involved a two-step sequence : first bromination at low temperature ($CBr_4$, $(Ph_2PCH_2)_2$, -35°C) which led to the unstable allylic bromide **4** in quantitative yield and then nucleophilic substitution by triphenyl phosphine ($PPh_3$, $CH_3CN$, 97% yield).

The ylide of **5**, generated at -100°C by additon of potassium bis(trimethylsilyl) amide, was reacted with the dienic aldehyde **6**[3,4] and afforded after HMPA addition and increase of the temperature to -78°C a 1:1 mixture of the fully protected $LXB_4$ **7** and of its all-*trans* [(*E*)-$\Delta^{8,9}$] isomer **8** in 55% yield. Both isomers can be separated by HPLC (hexane : ethylacetate 80:20).

In a similar way, the protected $LXA_4$ **16** and its (*E*)-$\Delta^{11,12}$ isomer **17** were prepared starting from the aldehydes **9** and **15**. The silicon-protected enantiomerically pure α-hydroxyaldehyde **9**[3,4] could be converted into the allylic alcohol **12** according to two different routes. On one hand, the condensation of (formylmethylene) triphenylphosphorane on the aldehyde **9** afforded the α,β-ethylenic aldehyde **10** in 50% yield[6], then its reduction in Luche conditions led to the allylic alcohol in 98% yield. On the other hand, the aldehyde **9** was converted in the α,β-ethylenic ester **11** in 83% yield by a Wittig-Horner reaction with the anion of triethylphosphonoacetate smoothly generated by DBU in presence of LiCl[7]. The unsaturated ester **11** was then reduced into the alcohol **12** in presence of DIBAL at low temperature. The overall yield under the latter conditions was 69% (**9**→**11**→**12**) compared to 49% (**9**→**10**→**12**) under the previous ones. It reveals the poor control of the alkylidenation[6] of the sterically hindered α-silyloxyaldehyde **9** by the phosphorane and demonstrates that the use of a more reactive phosphonate under less drastic conditions is more appropriate.

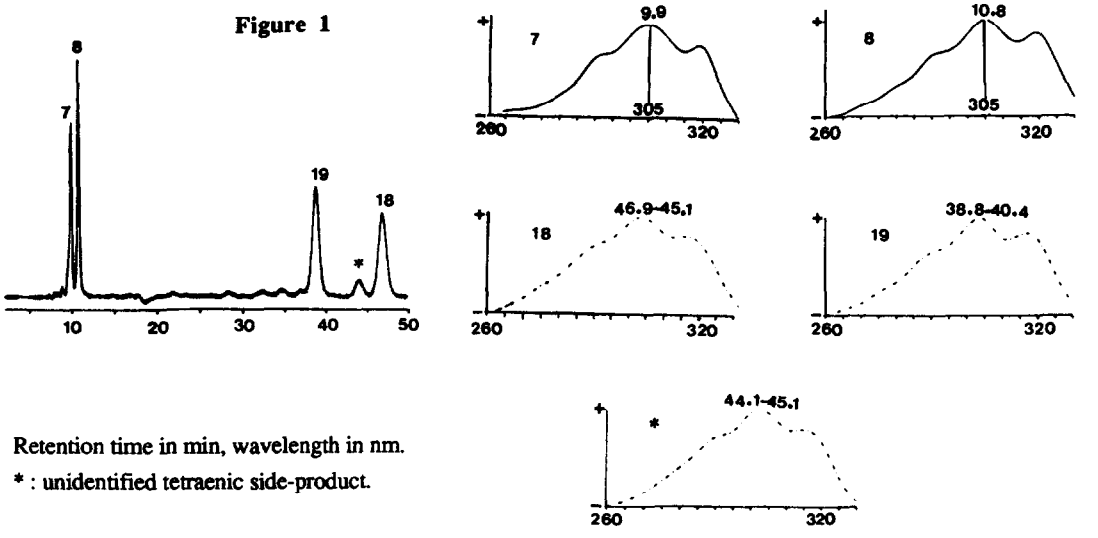
The phosphonium salt **14** was then obtained from the allylic alcohol **12** under the same conditions as **5** and its ylide, generated at -100°C, was reacted with the dienic aldehyde **15**[3,4] as above to give a 1:1 mixture of the fully protected $LXA_4$ **16** and of its all-*trans* [(*E*)-$\Delta^{11,12}$] isomer **17**. The separation of both isomers by HPLC (hexane : ethylacetate 80:20) can easily be carried out (purity ≥ 98 %). Attempts to increase the *Z*:*E* ratio of the last Wittig reaction by maintaining the temperature at -100°C, even after HMPA addition, failed and only resulted in a poorer yield (25%).

**Scheme III**




(a) TBAF, THF, r.t. then HPLC separation. (b) $K_2CO_3$, MeOH:$H_2O$ 3:1, r.t., 15 h.

**Figure 1**




Retention time in min, wavelength in nm.

* : unidentified tetraenic side-product.

Desilylation of the mixture of protected $LXB_4$ **7** and of its all-*trans* isomer **8** was monitored at 305 nm by normal phase HPLC using a Spectra-Focus (Spectra Physics) high-speed scanning UV spectrophotometer.

HPLC conditions : μ-Porasil column (length 30 cm, i.d. 7.8 mm), Eluent : hexane : AcOEt : $NEt_3$ 60:40:0.1

The deprotection of the molecules and the obtention of their potassium salts is outlined in scheme III. The desilylation of the protected $LXA_4$ **16** or $LXB_4$ **7** (TBAF, THF, r.t.) resulted in a mixture of (*E,E,Z,E*) : (*E,E,E,E*) desilylated compounds, exhibiting the instability of the tetraenic (*E,E,Z,E*) system. The mixture of the desilylated products could be separated by HPLC (hexane:ethylacetate:triethylamine 60:40:0.1), so that it was more efficient to carry out only one HPLC separation after the desilylation step, due to the observed isomerisation.

The desilylation of the mixture of protected LX's (**7** and **8** or **16** and **17**) was carefully monitored by normal phase HPLC using high-speed scanning photometric detection. Particularly, the typical UV absorption of tetraenes (λmax 290, 302, 320 nm) allowed the differentiation from eventual non-tetraenic impurities. The figure 1 shows a typical HPLC profile obtained during the desilylation (5eq TBAF, THF, r.t.) of the mixture of fully protected $LXB_4$ **7** and of its all-*trans* isomer **8** and indicates the nature of the various observed peaks. It is noteworthy that the best efficiency of this reaction required to stop the desilylation after 70% of conversion of the starting material to avoid substantial degradation of the products. HPLC separation of the desilylated compounds was done under those conditions and led to the pure products **18** and **19**. The δ-lactonisation observed in some cases by Nicolaou *et al.*[8] during this reaction arose neither during the desilylation nor during the HPLC separation. Using the same protocol, the desilylated compounds **20** and **21** were respectively obtained from the mixture of fully protected $LXA_4$ **16** and of its all-*trans*- isomer **17**.

The deprotection of both carbonate and ester groups of **18**, **19**, **20** or **21** was carried out immediately after their isolation in a single step (excess $K_2CO_3$, MeOH:$H_2O$ 3:1, r.t.) and respectively gave the potassium salts of lipoxin $B_4$ and of its (*E*)-$\Delta^{8,9}$ isomer as well as of lipoxin $A_4$ and of its (*E*)-$\Delta^{11,12}$ isomer. Again, the progress of the reaction was monitored by RP-HPLC using high-speed scanning photometric detection analysis. The deprotection of **18** (figure 2) revealed the formation of two intermediates (**X** and **Y**) prior to the obtention of fully deprotected $LXB_4$. Each intermediate **X** and **Y** exhibited the typical UV pattern of a conjugated tetraene. The structure of the compound **X** has been attributed according to HPLC comparison with the methyl ester of $LXB_4$ which has been prepared from $LXB_4$ potassium salt ($CH_2N_2$, $Et_2O$, r.t.). The monitoring of the saponification of **18** at different reaction times shows that the deprotection of the cyclic carbonate is longer than those of the ester moiety, as observed after 4h of reaction. The HPLC profile obtained after 24 h suggests a very good conversion.

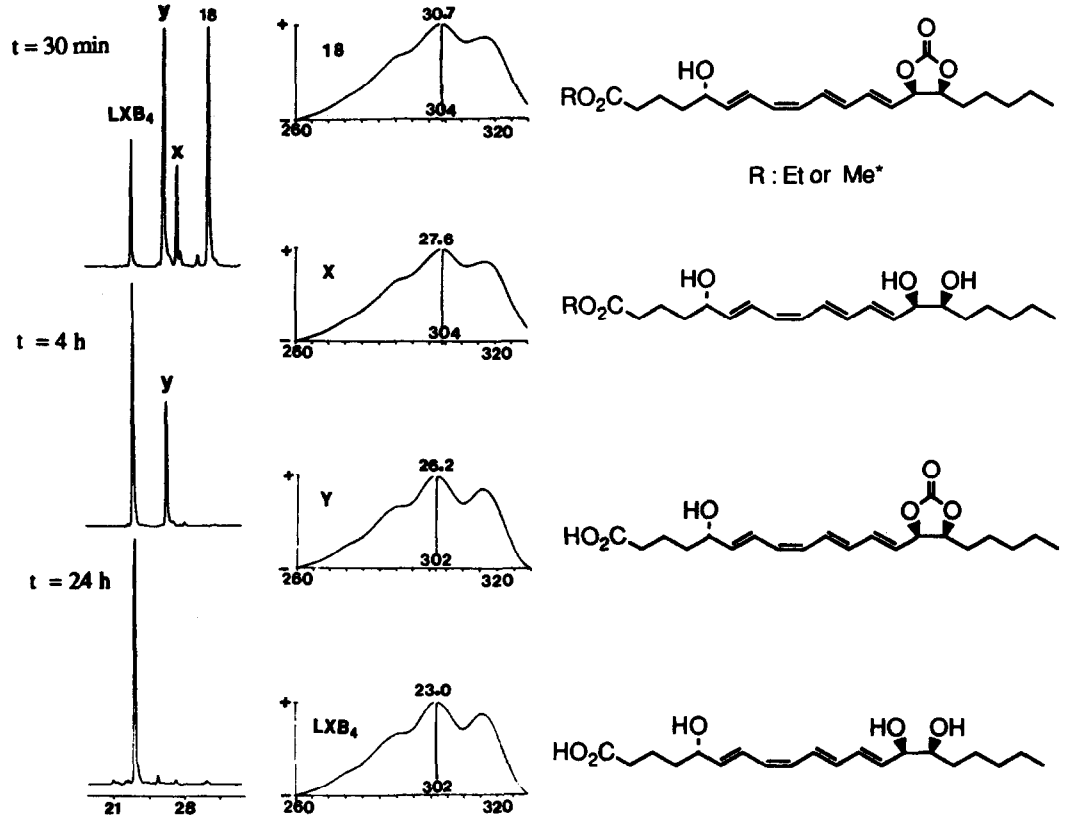
LX's potassium salts ($LXA_4$, $LXB_4$ and their all-*trans*-isomers) were obtained with over than 95% purity (RP-HPLC analysis). Our synthetic $LXA_4$ coelutes with a sample supplied by Euromedex ; an identical result was also obtained for their methyl esters (see experimental section).

The metabolism of $LXA_4$, $LXB_4$ and of their all-*trans* isomers by human leukocytes and rat liver microsomes has been studied in our laboratory[9]. Similarities between the metabolism of LX's and that of leukotriene $B_4$ ($LTB_4$) suggest that $LXA_4$ and $LXB_4$ are mainly hydroxylated on ω or ω-1 position, by human leukocytes and rat or human liver microsomes, whereas their all-*trans* isomers are mainly reduced into conjugated trienic compounds (λmax 270 nm).

In summary, $LXA_4$, $LXB_4$ and their all-*trans* isomers were obtained in a straightforward manner from *D*-isoascorbic acid *via* enantiomerically pure hydroxyaldehydes, which allows a strict control of asymmetric carbon atoms configuration. The careful HPLC monitoring assisted by high-speed scanning UV-spectrophotometer ensures optimal conditions for the ultimate two steps of deprotection resulting in a purity over than 95%. According to the flexible strategy[4] we describe, other isomers or analogs of LX's useful for biological screening could easily be produced.

**Figure 2**

Deprotection of both carbonate and ester groups of **18** was monitored at 305 nm by reverse phase HPLC using a Focus (Spectra Physics) high-speed scanning UV spectrophotometer.
HPLC conditions : O.D.S.2 silica (5 µm, Nucleosil, length 25cm, i.d. 4.6 mm); eluent : A/0.05M aq. $H_3PO_4$, B/$CH_3CN$ ; gradient : from A:B 85:15 to 0:100 in 35 min.




Retention time in min, wavelength in nm.

* : a transesterification leading to the methyl ester can occur in the conditions of the reaction ($K_2CO_3$, MeOH : $H_2O$ 3 :1).

Our synthetic LX's have been tested in various biological systems[9], and further biological investigations are currently in progress.

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**Experimental Section**

Prior to use, tetrahydrofuran (THF) and diethylether ($Et_2O$) were distilled from sodium-benzophenone and dichloromethane ($CH_2Cl_2$) from $P_2O_5$. $CH_2Cl_2$ and ethyl acetate (AcOEt) were filtered on $K_2CO_3$ prior to use. $^1H$ NMR (250MHz) and $^{13}C$ NMR spectra were recorded in $CDCl_3$ (unless indicated). Chemical shifts are reported in δ (ppm) and coupling constants are given in Hertz. High Resolution Mass Spectra were recorded in Service de Spectrométrie de Masse, Université Pierre et Marie Curie. Specific rotations were measured on a Perkin Elmer 241C polarimeter with sodium (589 nm) or mercury (365 nm) lamps. All reactions were recorded under argon atmosphere, and were monitored by thin-layer chromatography with Merck 60F-254 precoated silica (0.2 mm) on glass. Chromatography was performed with Merck Kieselgel 60 (200-500 μm) or 60H (5-40 μm) or with labochrom HP-FPGC prepacked column (Labomatic, 20-45 μm, 37x338 mm, 5 bars). Spectroscopic ($^1H$ and $^{13}C$ NMR, MS) and/or analytical data were obtained using chromatographically homogeneous samples.

*Ethyl (5S)-(6E)-5-tert-butyldiphenylsilyloxy-7-formyl-6-heptenoate* **2.**

A mixture of aldehyde **1** (610 mg, 1.48 mmol) and formylmethylene triphenylphosphorane (562 mg, 1.78 mmol) in toluene (4.5 mL was heated at 80°C for 5h. It was then cooled to 20°C and filtered through a celite pad (cyclohexane:AcOEt 1:1). The fractions containing **2** were purified by chromatography (Labochrom, $CH_2Cl_2$:$Et_2O$ 97:3) and lead to 310 mg (48% *Rf* 0.38) of α,β-ethylenic aldehyde **2** together with 90 mg (15%, *Rf* 0.45) of starting material **1** and 35 mg (5% *Rf* 0.26) of dienic aldehyde. **2** : Mp 80°C; $[\alpha]_D$-13° (c1.135, $CH_2Cl_2$); $^1H$ NMR δ 1.08 (s, 9H, tBu), 1.21 (t, 3H, *J*=7, OEt), 1.42-1.72 (m, 4H, H-3,4), 2.16 (m, 2H, H-2), 4.08 (q, 2H, *J*=7, OEt), 4.48 (m, 1H, H-5), 6.12 (ddd, 1H, $J_{7,6}$=16, $J_{7,8}$=8, $J_{7,5}$=1.5, H-7), 6.65 (dd, 1H, $J_{6,7}$=16, $J_{6,5}$=5, H-6), 7.30-7.70 (2m, 10H, Ph), 9.43 (d, 1H, $J_{8,7}$=8, H-8); $^{13}C$ NMR δ 14.9 (OEt), 19.3, 27.0 (tBu), 19.5, 33.9, 35.9 (C-2-4), 60.2 (OEt), 72.0 (C-5), 127.6, 129.9, 133.1, 133.4, 135.7 (Ph), 131.2, 158.4 (C-6,7), 173.0 (C-1), 193.3 (C-8).

*Ethyl (5S)-(6E)-5-tert-Butyldiphenylsilyloxy-8 hydroxy-6-octenoate* **3.**

To a solution of the α,β-ethylenic aldehyde **2**[4] (370 mg, 0.84 mmol) at 0°C were successively added a solution of cerous chloride in isopropanol ($CeCl_3$ 0.1M, 17 mL) and sodium borohydride (112 mg, 2.94 mmol). After a 1.5 h stirring at 20°C, the mixture was poured into a suspension of $CH_2Cl_2$ (50 mL) and water (10 mL). The pH was then adjusted to neutrality by the addition of aqueous $NH_4Cl$. Usual work-up followed by flash chromatography of the oily residue (cyclohexane:AcOEt 2:1) afforded 342 mg (92%, *Rf* 0.26) of the oily colorless allylic alcohol **3** : $[\alpha]_D$ -3.6° (c 1.07, $CH_3Cl_2$), $[\alpha]_{365}$ -16.5° (c 1.07, $CH_2Cl_2$); $^1H$ NMR δ 1.03 (s, 9H, tBu), 1.21 (t, 3H, *J*=7, OEt), 1.30-1.55 (m, 4H, H-3,4), 2.8 (t, 2H, *J*=7, H-2), 3.87 (d, 2H, $J_{8,7}$=5, H-8), 4.08 (q, 2H, *J*=7, OEt), 4.19 (m, 1H, H-5), 5.37 (dt, 1H, $J_{7,6}$=15.5, $J_{7,8}$=5, H-7), 5.52 (ddt, 1H, $J_{6,7}$=15.5,

$J_{6,5}$=6.5, $J_{6,8}$=1, H-6), 7.30-7.70 (m, 10H, Ph); MS *m/e* (relative intensity) 425 ($M^+$-$CH_3$, 20), 409 ($M^+$-$CH_2OH$, 20), 395 ($M^+$-OEt, 30), 383 (80), 199 ($Ph_2SiOH^+$, 100); HRMS calcd. for $C_{24}H_{31}O_3Si$ ($M^+$-OEt) 395.2043, found 395.2042.

*[(4S)-(2E)-4-tert-Butyldiphenylsilyloxy-7-carboxyethyl-2-hepten-1-yl] triphenyl phosphonium bromide* **5**.

To the allylic alcohol **3** (220 mg, 0.5 mmol) in $CH_2Cl_2$ (6 mL) at -35°C were successively added carbon tetrabromide (552 mg, 1.65 mmol) and bis-diphenylphosphino ethane (DIPHOS, 302 mg, 0.75 mmol) in $CH_2Cl_2$ (1 mL). After 2 h stirring at -35°C, the mixture was poured into hexane (100 mL). Removing of precipitated "DIPHOS monoxide" by filtration followed by concentration *in vacuo* afforded 250 mg (quantitative yield) of the unstable bromide **4** as a colorless oil which was used without purification in the next step.

To the bromide **4** (250 mg, 0.5 mmol) in $CH_3CN$ (10mL) was added triphenylphosphine (330 mg, 1.65 mmol). After 18 h stirring at 20°C, the mixture was concentrated *in vacuo* and the resulting sticky residue was washed with ether (3x5mL) to eliminate the excess of triphenylphosphine. The recovered amorphous phosphonium salt **5** (370 mg, 97%) was used without further purification : $^1H$ NMR δ 0.88 (s, 6H, tBu), 1.10-1.45 (m, 7H, H-3,4, OEt), 1.93 (m, 2H, H-2), 4.03 (q, 2H, *J*=7, OEt), 4.25 (m, 1H, H-5), 4.53 (dt, 1H, $J_{8,P}$=$J_{8,8'}$=15, $J_{8,7}$=7, H-8), 4.86 (dt, 1H, $J_{8',P}$=$J_{8',8}$=15, $J_{8',7}$=7, H-8'), 5.44 (dq, 1H, $J_{7,6}$=15.5, $J_{7,8}$=$J_{7,8'}$=$J_{7,P}$=7, H-7), 6.21 (dt, 1H, $J_{6,7}$=15.5, $J_{6,5}$=$J_{6,P}$=4, H-6), 7.20-8.00 (m, 25H, Ph).

*Ethyl (4S)-(2E)-4-tert-Butyldiphenylsilyloxy-2-nonenoate* **11**.

To a suspension of lithium chloride (64 mg, 1.5 mmol) in $CH_3CN$ (10mL), at 20°C were successively added ethyl (diethoxy-phosphinyl) acetate (0.297mL, 1.5 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (0.187mL, 1.25 mmol) and finally the α-silyloxy aldehyde **9**[4] (460 mg, 1.25 mmol). After 2h stirring, another addition of reagents allowed completion of the reaction (LiCl : 32 mg, 0.75 mmol; ethyl (diethoxy-phosphinyl) acetate : 0.149mL, 0.75 mmol; DBU : 94 µL, 0.63 mmol; 30 min more stirring). The mixture was then poured in a suspension of $Et_2O$ (50mL) and $H_2O$ (10mL). Usual work-up followed by flash chromatography (Kieselgel 60, cyclohexane:AcOEt 95:5) afforded 483 mg (83%, *Rf* 0.36) of the unsaturated ester **11** as a colorless oil : $[\alpha]_D$ -22° (c 1.17, $CH_2Cl_2$); $^1H$ NMR δ 0.84 (t, 3H, $J_{9,8}$=7, H-9), 1.06-1.55 (m, 20H, H-5-8,OEt,tBu), 4.20 (q, 2H, *J*=7, OEt), 4.41 (brq, 1H, $J_{4,3}$=$J_{4,5}$=5.5, $J_{4,2}$=1, H-4), 5.98 (dd, 1H, $J_{2,3}$=15.5, $J_{2,4}$=1, H-2), 6.95 (dd, 1H, $J_{3,2}$=15.5, $J_{3,4}$=5.5, H-3), 7.30-7.50 and 7.65-7.80 (2m, 10H, Ph); $^{13}C$ NMR δ 13.8, 14.1 (C-9, OEt), 19.2, 26.9 (tBu), 22.3, 23.6, 31.5, 36.7 (C-5-8), 60.0 (OEt), 72.4 (C-4), 127.4, 129.6, 133.3, 133.8, 135.7 (Ph), 120.1, 149.9 (C-2,3), 166.3 (C-1); MS *m/e* (relative intensity) 438 ($M^+$-tBu, 65), 227 (30), 199 ($Ph_2SiOH^+$, 100); HRMS calcd. for $C_{27}H_{38}O_3Si$ ($M^+$) 438.2590, found 438.2589.

*(4S), (2E)-4-O-tert-Butyldiphenylsilyloxy-2-nonenol* **12**.

The primary alcohol **12** could be obtained either from the α,β-ethylenic aldehyde **10**[4] by reduction under Luche conditions as described above for the preparation of **3** or from the unsaturated ester **11** as follows. To a solution of the ester **11** (420 mg, 0.96 mmol) in $CH_2Cl_2$ (32mL) at -78°C was slowly added diisobutyl aluminium hydride (1M in toluene, 2.8mL, 2.78 mmol). The mixture was stirred à -78°C for 1.5h. The reaction was then quenched by the addition of MeOH (1mL) and AcOEt (20mL). The organic layer was washed with a saturated aqueous solution of potassium and sodium tartrate, then with brine, dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (Kieselgel 60, $CH_2Cl_2$:$Et_2O$ 95:5) afforded 355 mg of pure alcohol **12** (93%, *Rf* 0.40) as a colorless oil : $[\alpha]_D$ -3° (c 1.08, $CHCl_3$), $[\alpha]_{365}$ -16° (c 1.08, $CHCl_3$); $^1H$ NMR δ 0.85 (m, 3H, H-9),

1.00 -1.53 (m, 17H, H-5-8, tBu), 3.88 (d, 2H, $J_{1,2}$=5.5, H-1), 4.16 (q, 1H, $J_{4,5}$=$J_{4,5'}$=$J_{4,3}$=7, H-4), 5.40 (dt, 1H, $J_{2,3}$=15.5, $J_{2,1}$=$J_{2,1'}$=5.5, H-2), 5.54 (dd, 1H, $J_{3,2}$=15.5, $J_{3,4}$=7, H-3), 7.25-7.45 and 7.60-7.77 (m, 10H, Ph); $^{13}$C NMR δ 14.0 (C-9), 19.3, 27.0 (tBu), 22.5, 24.4, 31.7, 37.8 (C-5-8), 63.1 (C-1), 73.9 (C-4), 129.1, 134.6 (C-2,3), 127.3, 127.4, 129.4, 129.5, 134.4, 134.6, 135.9, 136.0 (Ph); **MS *m/e*** (relative intensity) 339 ($M^+$-tBu, 25), 239 (10), 199 ($Ph_2SiOH^+$, 100)

*1-Bromo-(4S)-(2E)-4-tert butyldiphenylsilyloxy-2-nonene* **13.**

The bromide **13** was prepared from the allylic alcohol **14** (198 mg, 0.5 mmol) in the same way as **4** in quantitative yield : $^1$H NMR (90 MHz) δ 0.87 (m, 3H, H-9), 1.00-1.65 (m, 17H, H-5-8,tBu), 3.83 (d, 1H, $J_{1,2}$=6.75, H-1), 4.17 (m, 1H, H-4), 5.30-5.90 (m, 2H, H-2,3), 7.18-7.45 and 7.55-7.80 (2m, 10H, Ph).

*[(4S)-(2E)-4-tert-Butyldiphenylsilyloxy-2-nonen-yl] triphenylphosphonium bromide* **14.**

The phosphonium salt **14** has been prepared in 95% yield from **13** (230 mg, 0.5 mmol) according to the already described protocol for the preparation of **5** : $^1$H NMR δ 0.75 (m, 3H, H-9), 0.80-1.30 (m, 17H, H-5-8, tBu), 4.20 (m, 1H, H-4), 4.48 (dt, 1H, $J_{1,P}$=$J_{1,1'}$=15, $J_{1,2}$=7, H-1), 4.82 (dt, 1H, $J_{1',P}$=$J_{1',1}$=15, $J_{1',2}$=7, H-1'), 5.43 (dq, 1H, $J_{2,3}$=15.5, $J_{2,P}$=$J_{2,1}$=$J_{2,1'}$=7, H-2), 6.15 (dt, 1H, $J_{3,2}$=15.5, $J_{3,P}$=$J_{3,4}$=4, H-3), 7.20-7.85 (m, 25H, Ph).

**Access to protected LX's** :

*(5S, 14R, 15S)-(6E, 8Z, 10E, 12E)-5-O-tert- Butyldiphenylsilyl-14,15-O-carbonyl lipoxin $B_4$ ethyl ester* **7** *and its 8E isomer* **8.**

To a suspension of phosphonium salt **5** (133 mg, 185 μmol) in THF (4mL) at -105°C was added potassium bis(trimethylsilyl) amide (0.5M in toluene, 336 μL, 168 μmol). After 5 min stirring, to the resulting homogeneous red solution was added at -105°C the dienic aldehyde **6**[3,4] (40 mg, 168 μmol) in THF (1.33mL). The temperature was maintained at -105°C for 15 min prior to the addition of HMPA (270 μL, 1.55 mmol) and increase of the temperature to -40°C. After 1h at -40°C, the mixture was hydrolysed by the addition at -40°C of a 25 % (w/v) aqueous solution of $NH_4OAc$ (3mL) and diluted with $Et_2O$ (20mL). After extraction with $Et_2O$ (2x5ml), the combined organic layers were successively washed with 25% aqueous $NH_4OAc$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography of the residue (Kieselgel 60H; cyclohexane:AcOEt : $Et_3N$ 65:35:0.1 afforded 55 mg (51%, *Rf* 0.38) of a 1:1 mixture of **7** and **8** which can be separated by HPLC (μ–porasil, 7.8 mm x 30 cm, hexane:AcOEt:$Et_3N$ 80:20:0.1, 1.8 mL/min, detection at 305 nm).

**7** : HPLC retention time : 21 min; UV (EtOH abs.) λmax 291, 304, 320 nm; $[\alpha]_D$ -9.8° (c 0.38, $CH_2Cl_2$); $^1$H NMR δ 0.87 (m, 3H, H-20), 1.10 (s, 9H, tBu), 1.18-1.90 (m, 15H, H-3,4,16-19,OEt), 2.18 (m, 2H, H-2), 4.10 (q, 2H, *J*=7, OEt), 4.25 (m, 1H, H-5), 4.69 (m, 1H, H-15), 5.15 (t, 1H, $J_{14,13}$=$J_{14,15}$=7.5, H-14), 5.62 (dd, 1H, $J_{13,12}$=15, $J_{13,14}$=7.5, H-13), 5.66 (dd, 1H, $J_{6,7}$=15, $J_{6,5}$=6, H-6), 5.83-6.02 (AB from ABMN system, 2H, $J_{A,B}$=10, H-8,9) 6.11-6.55 (m, 4H, H-7,10-12), 7.30-7.50 and 7.60-7.75 (2m, 10H, Ph); $^{13}$C NMR δ 13.9, 14.2 (C-20, OEt), 19.4, 27.1 (tBu), 20.1, 22.4, 25.1, 30.1, 31.3, 34.2, 37.1 (C-2-4,16-19), 60.2 (OEt), 73.6 (C-5), 80.3 (C-14,15), 122.8, 125.3, 128.2, 130.5, 131.3, 131.4, 136.7, 138.1 (C-6-13), 127.5, 129.6, 134.1 (Ph), 173.5 (C-1); HRMS calcd. for $C_{35}H_{43}O_6Si$ ($M^+$-*t*Bu) 587.2829, found : 587.2824.

**8** : HPLC retention time : 31 min; UV (EtOH abs.) λmax 292, 304, 320 nm; $[\alpha]_D$ -81° (c 0.43, $CH_2Cl_2$); $^1$H NMR δ 0.89 (m, 3H, H-20), 1.09 (s, 9H, tBu), 1.18-1.80 (m, 15H, H-3,4,16-19,OEt), 2.18 (m, 2H, H-2),

4.07 (q, 2H, *J*=7, OEt), 4.19 (m, 1H, H-5), 4.67 (m, 1H, H-15), 5.12 (t, 1H, $J_{14,15}$=$J_{14,13}$=7.5, H-14), 5.60 (dd, 1H, $J_{13,12}$=15, $J_{13,14}$=7.5, H-13), 5.66 (dd, 1H, $J_{6,7}$=15,$J_{6,5}$=7, H-6), 5.92 (dd, 1H, $J_{7,6}$=15, $J_{7,8}$=10, H-7), 6.05 (dd, 1H, $J_{9,8}$=15, $J_{9,10}$=10, H-9), 6.18 (m, 2H, H-11,18), 6.30 (dd, 1H, $J_{10,11}$=15, $J_{10,9}$=10, H-10), 6.38 (dd, 1H, $J_{12,13}$=15, $J_{12,11}$=10.5, H-12), 7.30-7.50 and 7.60-7.75 (2m, 10H, Ph); $^{13}$C NMR δ 13.9, 14.2, (C-20, OEt), 19.4, 27.1 (tBu), 20.1, 22.4, 25.1, 30.0, 31.3, 34.2, 37.2 (C-2-4,16-19), 60.2 (OEt), 73.6 (C-5), 80.3 (C-14,15), 127.4, 127.5, 134.0, 134.2, 135.9 (Ph), 122.3, 129.9, 130.1, 131.5, 134.7, 136.3, 136.8, 137.6, (C-6-13), 154.8 (C=0), 173.4 (C-1).

*(5S, 6R, 15S)-(7E, 9E, 11Z, 13E)-15-O- tert-Butyldiphenylsilyl-5,6-O-carbonyl lipoxin $A_4$ ethyl ester* **16** *and its 11E isomer* **17**.

**16** and **17** were prepared from the phosphonium salt **14** and the dienic aldehyde **15**[4] as previously described for the preparation of **7** and **8** and they were obtained after flash chromatography in 55% yield as a 1:1 mixture which could be separated by HPLC (same conditions as above for the separation of **7** and **8**).

**16** : HPLC retention time : 40 min; UV (EtOHabs.) λmax 293, 305, 319 nm; $[\alpha]_D$ -4.7° (c 0.55, $CH_2Cl_2$); $^1$H NMR δ 0.83 (t, 3H, $J_{20,19}$=7, H-20), 1.08 (s, 9H, tBu), 1.10-1.90 (m, 15H, H-3,4,16-19,OEt), 2.38 (m, 2H, H-2), 4.12 (q, 2H, *J*=7, OEt), 4.21 (q, 1H, $J_{15,14}$=$J_{15,16}$=6.5, H-15), 4.70 (m, 1H, H-5), 5.15 (t, 1H, $J_{6,5}$=$J_{6,7}$=8, H-6), 5.61 (dd, 1H, $J_{7,8}$=15, $J_{7,6}$=8, H-7), 5.67 (dd, 1H, $J_{14,13}$=15, $J_{14,15}$=6.5, H-14), 5.90-5.93 (AB from ABMN system, 2H, $J_{A,B}$=10, H-11,12), 6.17 (dd, 1H, $J_{9,10}$=15, $J_{9,8}$=11, H-9), 6.25-6.42 (m, 2H, H-8,13), 6.51 (dd, 1H, $J_{10,9}$=15, $J_{10,11}$=11, H-10), 7.30-7.45 and 7.60-7.70 (2m, 10H, Ph); $^{13}$C NMR δ 14.0, 14.2 (C-20, OEt), 19.4, 27.1 (tBu), 21.0, 22.5, 24.3, 29.5, 31.8, 33.5, 37.8 (C-2-4,16-19), 60.5 (OEt), 74.1 (C-15), 79.9, 80.1 (C-5,6), 122.3, 124.9, 127.7, 130.2, 131.6, 137.0, 138.9 (C-7-14), 127.4, 129.5, 134.2 (Ph), 154.2 (C=O), 172.8 (C-1); MS *m/e* (relative intensity) 587 ($M^+$-tBu, 1), 353 (3), 311(8), 199 ($Ph_2SiOH^+$, 100); HRMS calcd. for $C_{35}H_{43}O_6Si$ ($M^+$-*t*Bu) 587.2829, found 587.2824.

**17** : HPLC retention time : 50 min; UV (EtOH abs.) λmax 292, 305, 320 nm; $[\alpha]_D$ -114° (c 0.51, $CH_2Cl_2$); $^1$H NMR δ 0.83 (m, 3H, H-20), 1.08 (m, 9H, tBu), 1.10-1.90 (m, 15H, H-3,4,16-19,OEt), 2.37 (m, 2H, H-2), 4.11 (q, 2H, *J*=7, OEt), 4.18 (m, 1H, H-15), 4.68 (m, 1H, H-5), 5.12 (t, 1H, $J_{6,7}$=$J_{6,5}$=8, H-6), 5.58 (dd, 1H, $J_{7,8}$=15, $J_{7,6}$=8, H-7), 5.67 (dd, 1H, $J_{14,13}$=15, $J_{14,15}$=6.5, H-14), 5.92 (dd, 1H, $J_{13,14}$=15, $J_{13,12}$=10.5, H-13), 6.05 (dd, 1H, $J_{11,12}$=15, $J_{10,11}$=10.5, H-11), 6.12-6.26 (m,2H, H-9,12) 6.31 (dd, 1H, $J_{10,9}$=15, $J_{10,11}$=10.5, H-10), 6.38 (dd, 1H, $J_{8,7}$=15, $J_{8,9}$=10.5, H-8), 7.30-7.45 and 7.60-7.70 (2m, 10H, Ph); $^{13}$C NMR δ 14.0, 14.2 (C-20,OEt), 19.3, 27.0 (tBu), 20.9, 22.5, 24.2, 29.5, 31.6, 33.4, 37.7 (C-2-4,16-19), 60.5 (OEt), 73.9 (C-15), 80.0, 80.4 (C-5,6), 135.9, 134.2, 134.0, 129.5, 127.3 (Ph), 121.8, 129.5, 131.1, 135.2, 136.5, 137.1, 138.5 (C-7-14), 154.3 (C=O), 178.9 (C-1).

**Desilylation protocol** :

To a mixture of **7** and **8** (resp. **16** and **17**) (1mg, 1.55 μmol) in THF (1mL) was added a solution of tetrabutylammonium fluoride in THF (1M in THF, 8μL, 7.75 μmol). The progress of the reaction was monitored by normal phase HPLC (μ–porasil, 7.8 mm x 30 cm, hexane:AcOEt:$Et_3N$ 60:40:0.1, 1.8 mL/min, detection at 305 nm) assisted by a high-speed scanning UV spectrophotometer. After 4h stirring at 20°C (about 70 % of conversion) the reaction was quenched by addition of water (500 μL) and the mixture was diluted with $Et_2O$ (2mL). The organic layer was then dried ($MgSO_4$), filtered through a millipore filter (pore size : 0.45 μm) and concentrated *in vacuo*. The residue was then purified by HPLC (same conditions as monitoring of desilylation).

Due to the instability of desilylated compounds, it was preferable to carry out the basic hydrolysis of both ester and carbonate groups immediately after the desilylation to avoid substantial degradation.

HPLC retention times (min) :

| $LXA_4$ | | $LXB_4$* | |
|---|---|---|---|
| silylated isomers | desilylated isomers | silylated isomers | desilylated isomers |
| **16** : 11 | **20** : 40 | **7** : 11.5 | **18** : 52 |
| **17** : 12 | **21** : 35 | **8** : 12.5 | **19** : 44 |

* 1.5 mL/min

*(5S, 6R, 15S)-(7E, 9E, 11Z, 13E)-5,6-O-carbonyl lipoxin $A_4$ ethyl ester* **20** *and (5S, 14R, 15S)-(6E, 8Z, 10E, 12E)-14,15-O-carbonyl lipoxin $B_4$ ethyl ester* **18**.

**20** : UV (HPLC solvent) λmax 291, 303, 318 nm; **18** : UV (HPLC solvent) λmax 291, 305, 320 nm; the $^1H$ NMR spectra of **20** and **18** were analogous. **20** : $^1H$ NMR δ 0.87 (m, 3H, H-20), 1.20-1.85 (m, 15H, H-3,4,16-19, OEt), 2.35 (m, 2H, H-2), 4.11 (q, 2H, *J*=7, OEt), 4.20 (m, 1H, H-15), 4.68 (m, 1H, H-5), 5.14 (t, 1H, $J_{6,5}=J_{6,7}$-8, H-6), 5.63 (dd, 1H, $J_{7,8}$=15, $J_{7,6}$=8, H-7), 5.79 (dd, 1H, $J_{14,13}$=15, $J_{14,15}$=6, H-14), 6.04 (AB, 2H, $J_{AB}$=11, $J_{A,13}$=11, $J_{B,10}$=10, H-11,12), 6.23 (dd, 1H, $J_{9,10}$=15, $J_{9,8}$=11, H-9), 6.44 (dd, 1H, $J_{8,7}$=15, $J_{8,9}$=11, H-8), 6.67 (dd, 1H, $J_{10,9}$=15, $J_{10,11}$=10, H-10), 6.77 (dd, 1H, $J_{13,14}$=15, $J_{13,12}$=11, H-13).

*(5S, 6R, 15S)-(7E, 9E, 11E, 13E)-5,6-O-carbonyl lipoxin $A_4$ ethyl ester* **21** and *(5S, 14R, 15S)-(6E, 8E, 10E, 12E)-14,15-O-carbonyl lipoxin $B_4$ ethyl ester* **19**.

**21** : UV (HPLC solvent) λmax 291, 305, 320 nm; **19** : UV (HPLC solvent) λmax 292, 304, 320 nm; the $^1H$ NMR spectra of **21** and **19** were analogous **21** : $^1H$ NMR δ 0.88 (m, 3H, H-20), 1.20-1.90 (m, 15H, H-3,4,16-19, OEt), 2.37 (m, 2H, H-2), 4.12 (q, 2H, *J*=7, OEt), 4.18 (m, 1H, H-15), 4.70 (m, 1H, H-5), 5.15 (t, 1H, $J_{6,5}=J_{6,7}$=8, H-6), 5.62 (dd, 1H, $J_{7,8}$=15, $J_{7,6}$=8, H-7), 5.79 (dd, 1H, $J_{14,13}$=15, $J_{14,15}$=6, H-14), 6.18-6.48 (m, 6H, H-8-13).

**Basic hydrolysis of carbonate and ester groups :**

To the desilylated compound **20** (resp. **21**, **18**, **19**) was added a solution of potassium carbonate (0.01M in MeOH : $H_2O$ 3:1, 1 mL). The mixture was kept without stirring for 15 h at 20°C. The solution of LX potassium salt was then concentrated *in vacuo* and filtered through a $C_{18}$ Sep-Pak cartridge (Waters). Elution with $H_2O$ (2 mL) removed the salts ($K_2CO_3$ and $KHCO_3$) and then elution with MeOH (2 mL) afforded $LXA_4$ potassium salt (resp. all-*trans* $LXA_4$, $LXB_4$ and all-*trans* $LXB_4$ potassium salts). The reaction has been monitored by RP-HPLC (ODS-2 silica, 5μm, Nucleosil, 4.6mm x 25cm, solvent : A-0.05M aqueous phosphoric acid, B-$CH_3CN$, gradient A/B=85/15 to A/B=0/100 in 35 min, 1mL/min, detection at 270 and 302 nm), see results and discussion. In spite of the minute quantities of products to be reacted (a few hundred(s) of μg) to give an accurate yield, the HPLC profile obtained suggests a very good conversion for this deprotection step. The purity of the products proved to be over than 95%. The UV spectra in MeOH of the four synthetized lipoxins showed maxima of absorption at 289, 302 and 317 nm ± 1nm according to the lipoxin. The RP-HPLC retention times (min) of the various LX's as well as those of the corresponding starting materials, in the same conditions as above for the monitoring of the reaction, were the followings :

| | Lipoxin $A_4$ | | | | Lipoxin $B_4$ | | | |
|---|---|---|---|---|---|---|---|---|
| | (7*E*, 9*E*, 11*Z*, 13*E*) | | (7*E*, 9*E*, 11*E*, 13*E*) | | (6*E*, 8*Z*, 10*E*, 12*E*) | | (6*E*, 8*E*, 10*E*, 12*E*) | |
| carbonate-ester | **20** | 32.0 | **21** | 31.5 | **18** | 30.2 | **19** | 30.0 |
| potassium salt | | 23.7* | | 23.4 | | 22.3 | | 22.1 |

Internal standard = $PGB_2$ 26.3 min.

*This sample coelutes with a $LXA_4$ sample provided by Euromedex. An identical result was also obtained for their methyl esters ($CH_2N_2$, $Et_2O$, 20°C, retention time : 25.9 min).

**$LXA_4$ and $LXB_4$ potassium salts** : $^1H$ NMR ($D_2O$) were analogous. $LXA_4$ potassium salt : $^1H$ NMR δ 0.88 (m, 3H, H-20), 1.00-1.60 (m, 12H, H-3,4,16-19), 2.15 (t, 1H, $J_{2,3}$=7, H-2), 3.57 (m, 1H, H-5), 3.97-4.20 (m, 2H, H-6,15), 6.00-6.11, 6.25-6.40, 6.65-6.85 (3m, 8H, H-7-14); MS of the $Me_3Si$ derivative of $LXB_4$ methyl ester (performed by Dr. Serhan, C.N.) *m/e* (relative intensity) 203 (C1-C5 portion of the molecule, 47), 173 (100), 159 (81), 133 (26), 81 (11).

**All-*trans*-$LXA_4$ and all-*trans*-$LXB_4$ potassium salts** : $^1H$ NMR ($D_2O$) were analogous. All-*trans*-$LXA_4$ $^1H$ NMR δ 0.85 (m, 3H, H-20), 1.06-1.78 (m, 12H, H-3,4,16-19), 2.18 (t, 3H, $J_{2,3}$=7, H-2), 3.62 (q, 1H, $J_{5,4}$=$J_{5,4'}$=$J_{5,6}$=5, H-5), 4.05-4.22 (m, 2H, H-6,15), 5.70-5.86 (m, 2H, H-7,14), 6.36-6.44 (m, 6H, H-8-13).

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