diene in 18% yield each by GC analysis, starting with diene 7.

Addition of Chlorofluorocarbene to Diene 7. A 4-mL heavy-walled freshly silylated Pyrex ampule was filled with 0.5 g (1.3 mmol) of phenyl(dichlorofluoromethyl)mercury[26] and 0.64 g (2.5 mmol) of diene 7. The tube was frozen at 77 K, evacuated, and sealed with a flame. The white slurry was heated to 130–135 °C for 1 h in a rocker bomb. After cooling, the bomb was cracked open and the volatiles were dynamically transferred to a U-bulb to yield 0.7 g of colorless oil. GC analysis (25-m methylsilicone capillary column, 15 psi, 40 °C for 15 min and then 10°/min to 100 °C) revealed starting material (74%) and products 31–34 (26%, 50% yield based on mercurial). Also found was a significant amount of benzene, apparently present in the starting mercurial. The mixture was purified by preparative GC (10 ft × $^1/_4$ in. 10% SF-96 on Chromasorb W HP, 100 °C, 160 °C injection and detection temperature, 160 mL/min). The four desired products were collected together and had retention times of 5 to 6 min. Boiling point of mixture: 158_{755} °C (inverted capillary). IR (vapor phase): 1750, 1370, 1325, 1200 cm^{-1}. ^{19}F NMR (CD_3CN): **31** (60% of mixture) 119.7, 134.6, 135.3, 144.6, 151.8, 180.0, 206.5 ppm; **32** (30% of mixture) 119.2, 113.2, 137.0, 141.1, 148.9, 195.7, 208.2 ppm. MS: **31** *m/e* 252 ($-CF_2Cl$), 237, 221, 202, 186, 85 (CF_2Cl^+, 100); **32** *m/e* 221 ($-CFCl_2$), 202, 186, 101 ($CFCl_2^+$, 100). Anal. of mixture. Calcd: C, 26.00; F, 41.10; Cl, 32.90. Found: C, 26.18; F, 41.10; Cl, 32.49.

***cis*-6,7-Dichlorohexafluoro-2-oxabicyclo[4.1.0]hept-4-ene (36).** To an ice-cooled mixture of 1.0 mL of 80% H_2O_2 (23 mmol) in 10 mL of methylene chloride was added dropwise with good stirring 5.7 mL (39 mmol) of trifluoroacetic anhydride over the course of 10 min. After it had warmed to room temperature, 10 mL of this approximately 2.0 M solution of peroxytrifluoroacetic acid solution was vigorously stirred with 1.5 g of 85% diene 7 (5 mmol) for 24 h. The light yellow solution was diluted with Freon 11 and washed with saturated sodium bisulfite solution followed by saturated sodium bicarbonate solution. After drying, the solvent was removed and the product was purified by preparative GC (10 ft × $^1/_4$ in. 10% SF-96 on Chromasorb W HP 80/100, 50 °C, 160 mL/min) to give 0.30 g (22%) of colorless liquid. IR (gas phase): 1745, 1385, 1225, 995, 885 cm^{-1}. ^{19}F NMR: 109.6, 139.0, 142.6, 145.5, 161.8, 170.1 ppm. MS: *m/e* 272 (M^+), 237, 225, 209, 171 (100). Anal. Calcd: C, 26.38; F, 41.77. Found: C, 26.50; F, 42.01.

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Synthesis of 19,19,20,20,20-Pentadeuteriolipoxin A_4 Methyl Ester and 19,19,20,20,20-Pentadeuterioarachidonic Acid. Agents for Use in the Quantitative Detection of Naturally Occurring Eicosanoids

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19,19,20,20,20-Pentadeuteriolipoxin A_4 methyl ester (**2**) was synthesized from key intermediates **31** and **32**. 19,19,20,20,20-Pentadeuterioarachidonic acid (**1**) and its methyl ester (**24**) were also synthesized by sequential coupling of intermediates **12**, **22**, and **23**. With use of the pentadeuterated derivative **2**, a gas-chromatography–mass spectroscopy (GC-MS) method for the quantitative detection of lipoxin A_4 was developed.

Introduction

Arachidonic acid is an essential fatty acid that, when released from cellular stores, can be transformed to a variety of biologically active products.[1,2] The lipoxins are a novel series of oxygenated products of arachidonic acid that were first isolated from human leukocytes.[2,3] These compounds display a unique profile of biological activities that are different from those of either prostaglandins or leukotrienes (for reviews, see ref 4). Therefore, the detection and quantitation of the lipoxins, which, like other products of arachidonic acid metabolism, may be present in low levels in human fluids, is of considerable interest.

Since it is now clear that radioimmunologic detection of eicosanoids may, with complex biological fluids, give misleading results, the use of stable isotopes has become extremely helpful in the quantitative analysis of eicosanoids from biological samples.[5] In particular, deuterium-containing stable isotopes were used to quantitate both prostaglandins and thromboxanes in biological samples by methods utilizing gas chromatography–mass spectrometry detection.[5]

In order to assist in studies of the arachidonic acid cascade, and to develop analytical techniques (as in ref 5 and 6) that utilize mass spectroscopy for detection of products of arachidonic acid metabolism, in particular lipoxin A_4, we undertook the syntheses of 19,19,20,20,20-pentadeuterioarachidonic acid (**1**), its methyl ester (**24**), as well as 19,19,20,20,20-pentadeuteriolipoxin A_4 methyl ester (**25**). The present findings will permit the detection of LXA_4, which may be present in various biological sources (i.e., including patient-derived samples), by selected ion monitoring on gas chromatography–mass spectroscopy.

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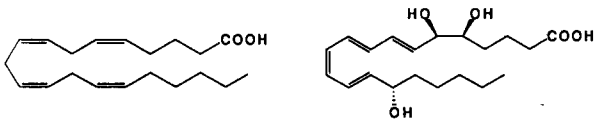


Arachidonic Acid Lipoxin A_4

Moreover, the availability of 19,19,20,20,20-pentadeuterioarachidonic acid will enable the biosynthetic generation of other deuterium-containing eicosanoids as well as facilitate stereochemical analysis of the enzymatic events associated with ω-oxidation of eicosanoids.

Results and Discussion

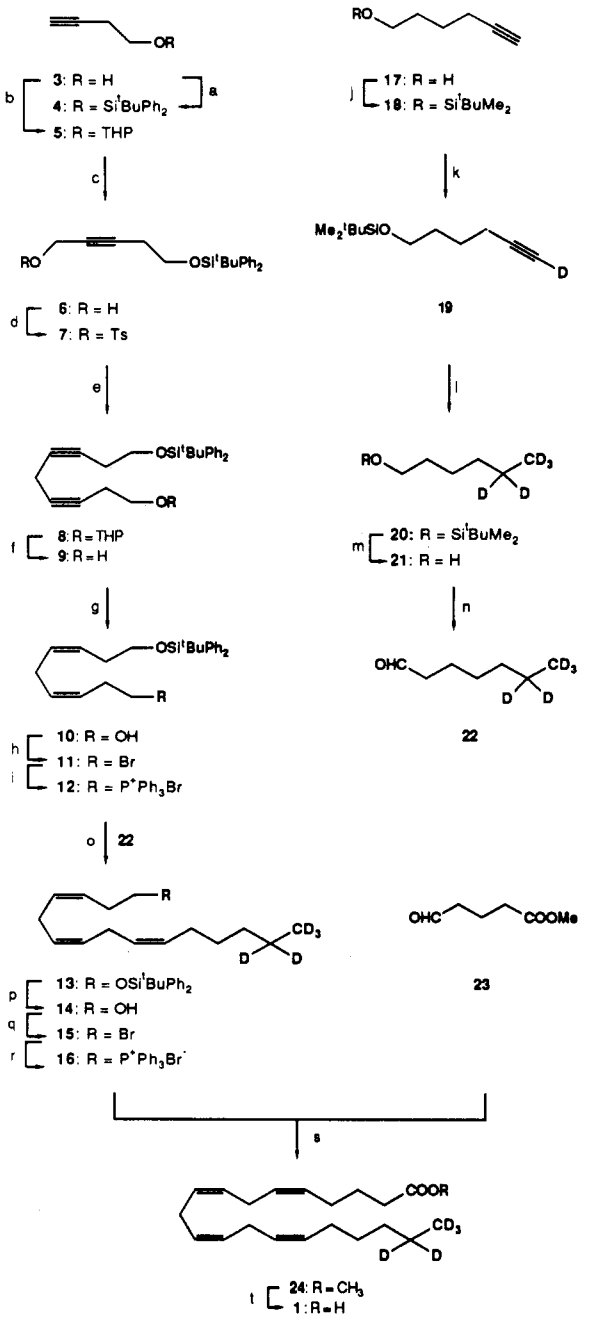
The synthesis of 19,19,20,20,20-pentadeuterioarachidonic acid (1) and its methyl ester (**24**) is detailed in Scheme I. Thus, 3-butyn-1-ol (**3**) was converted to both its silyl ether (**4**, 98%) and its tetrahydropyranyl ether (**5**, 96%) by standard conditions. The acetylenic silyl derivative **4** was then hydroxymethylated using nBuLi–$(CH_2O)_n$ (85%) and tosylated to give compound **7** (96%) via alcohol **6**. Reaction of **7** with the magnesium reagent derived from **5** and EtMgBr in the presence of CuI as a catalyst resulted in the formation of the diacetylene **8** in 94% yield. Selective removal of the tetrahydropyranyl group from **8** was achieved by treatment with pyridinium *p*-toluenesulfonate (PPTS) in methanol (73%). Controlled hydrogenation of **9** using Lindlar's catalyst resulted in the formation of the *cis,cis*-diene **10** in 99% yield. Bromination of **10** by CBr_4–PPh_3 (85%) followed by heating with PPh_3 gave the desired phosphonium salt **12** (97%) via bromide **11**.

The deuterated fragment **22** required for the synthesis of **1** and **24** was obtained from 5-hexyn-1-ol (**17**) by the sequence shown in Scheme I. Thus, silylation of **17** under standard conditions led to **18** (98%), which was deprotonated with nBuLi and deuterated (D_2O, 99% yield, >99% deuterated by 500-MHz ^1H NMR spectroscopy). Exchaustive catalytic deuteration of **19** (D_2, Rh/alumina)[9] gave **20** (100% yield, >87% pentadeuterated by mass spectroscopy). Finally desilylation of **20** (95%) using fluoride ion followed by CrO_3–pyridine oxidation led to the requisite aldehyde **22** (85%).

The ylide derived from **12** and $NaN(SiMe_3)_2$ in dimethoxyethane (DME) were then reacted with aldehyde **22** to afford triene **13** (89% yield, only cis isomer detected). Standard functional group manipulation as summarized in Scheme I then gave, sequentially, compounds **14** (95%), **15** (86%), and **16** (95%). The final coupling of the ylide derived from **16** and aldehyde **23** under the conditions described above for **12** + **22** → **13** gave 19,19,20,20,20-pentadeuterioarachidonic acid methyl ester (**24**) (89% yield, >87% pentadeuterated by mass spectroscopy). Alkaline hydrolysis of **24** (LiOH) finally led to 19,19,20,20,20-pentadeuterioarachidonic acid (**1**).

While 19,19,20,20,20-pentadeuterioarachidonic acid and products derived from this compound may be useful in ω-oxidation studies of various eicosanoids, results of recent studies indicate that, unlike leukotriene B_4, human neutrophils (in vitro) do not ω-oxidize either lipoxin A_4 or lipoxin B_4.[10] The carbon-20 position of lipoxin A_4 was selected as the site for incorporation of deuterium label

Scheme I. Synthesis of 19,19,20,20,20-Pentadeuterioarachidonic Acid (1)



[a] Reagents and conditions: (a) 1.2 equiv of ^{t}BuPh$_2$SiCl, 1.5 equiv of imidazole, 25 °C, 12 h, 98%; (b) 1.3 equiv of dihydropyran, pyridinium *p*-toluenesulfonate (PPTS) cat., CH_2Cl_2, 25 °C, 3 h, 96%; (c) 1.25 equiv of n-BuLi, THF, 1.3 equiv of $(CH_2O)_n$, −78 → 25 °C, 12 h, 85%; (d) 5 equiv of KOH, 1.25 equiv of TsCl, Et_2O, −20 → 0 °C, 1 h, 96%; (e) 1.3 equiv of **5**, 1.25 equiv of EtMgBr, reflux, 1 h, then 0.2 equiv of CuI, 1.0 equiv of CuI, 1.0 equiv of **7**, THF, 0 → 25 °C, 1 h, 94%; (f) 0.1 equiv of PPTS, MeOH, 25 °C, 6 h, 73%; (g) H_2, Lindlar cat., CH_2Cl_2–hexane 1:1, 25 °C, 10 h, 99%; (h) 1.2 equiv of CBr_4, 1.5 equiv of PPh_3, CH_2Cl_2, −40 → −30 °C, 45 min; 85%; (i) 24 equiv of PPh_3, CH_3CN, reflux, 12 h, 97%; (j) 1.2 equiv of ^{t}BuMe$_2$SiCl, 1.5 equiv of imidazole, 25 °C, 12 h, 98%; (k) 1.5 equiv of n-BuLi, THF, −78 °C, 2 equiv of D_2O, 99%; (l) D_2, 5% Rh/alumina (20% w/w), THF, 25 °C, 12 h, 100%; (m) 1.1 equiv of n-Bu_4NF, THF, 0 → 25 °C, 4 h, 95%; (n) 6 equiv of CrO_3, 12 equiv of pyridine, CH_2Cl_2, 25 °C, 0.5 h, 85%; (o) 1.1 equiv of $NaN(Si(CH_3)_3)_2$, DME, 1.1 equiv of **22**, −35 °C, 1.5 h, 89%; (p) 1.3 equiv of n-Bu_4NF, THF, 0 → 25 °C, 0.5 h, 95%; (q) 1.2 equiv of CBr_4, 1.5 equiv of PPh_3, CH_2Cl_2, −40 → −30 °C, 45 min, 86%; (r) 2.4 equiv of PPh_3, CH_3CN, reflux, 12 h, 95%; (s) 1.1 equiv of $NaN(Si(CH_3)_3)_2$, DME, 1.3 equiv of **23**, −35 °C, 1.5 h, 89%; (t) 3 equiv of LiOH, THF/H_2O 1:1, 0 → 25 °C, 8 h, 98%.

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(10) See: Serhan, C. N. "On the relationship between leukotriene and lipoxin production by human neutrophils: Evidence for differential metabolism of 15-HETE and 5-HETE", paper submitted for publication.

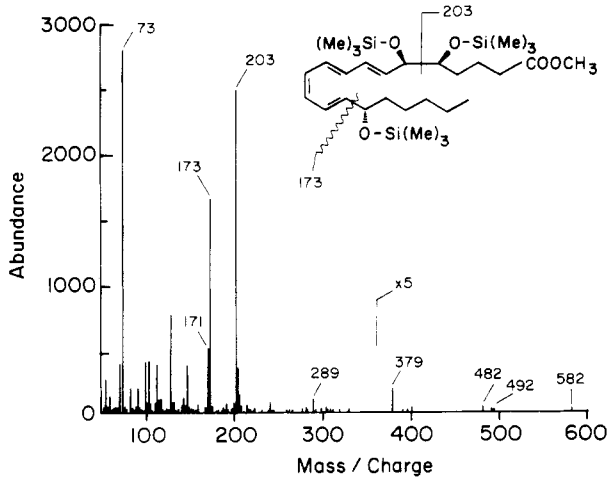

Figure 1. Mass spectra of Me_3Si derivative of the methyl ester of synthetic LXA_4.

because the C-15 through C-20 fragment gives rise to a prominent ion at m/e 173 (Figure 1). Addition of five atoms of deuterium at this site would be expected to shift the ion to m/e 178. This ion is highly suitable for selected ion monitoring because fragmentation of the native compound would not lead to potential interference with ions at m/e 178.

The 19,19,20,20,20-pentadeuteriolipoxin A_4 methyl ester (**2**) was synthesized as summarized in Scheme II. Thus, the pentadeuterated compound **20** was oxidized directly with Jones reagent to afford the carboxylic acid **25** (83%), which was then converted to its acid chloride **26** with oxalyl chloride. Condensation of **26** with bis(trimethylsilyl)-acetylene in the presence of $AlCl_3$ then led to the acetylenic ketone **28** (52% overall yield from **25**). The conversion of compound **28** to intermediates **29–31**, the coupling of **31** to the known acetylene **32** to afford **33**, and the elaboration of **33** to the target molecule **2** via intermediates **34** and **35** proceeded along the same lines as previously described for lipoxin A_4 methyl ester.[11]

Next, aliquots of synthetic LXA_4 and pentadeuterio-LXA_4 methyl esters (LXA_4-Me) were converted to Me_3Si derivatives and subjected to analysis by GC-MS. As we have previously reported,[12] synthetic LXA_4 and biosynthetically derived LXA_4 give identical prominent ions in their mass spectra. Figure 1 shows the mass spectrum of the Me_3Si derivative of synthetic lipoxin A_4 methyl ester (C value = 24.1). Ions of high intensity were at m/e 203 ($Me_3SiO^+{=}CH(CH_2)_3COOCH_3$), 173 ($Me_3SiO^+CH(CH_2)_4CH_3$, 171 (203 - 32), 289 (379 - 90), and 379 (M - 203). Ions of lower intensity were at m/e 482 (M - 100), 492 (M - 90), and 582 (M). The mass spectrum of its 19,19,20,20,20-pentadeuterated counterpart is given in Figure 2 (C value = 24.0). Prominent ions in its mass spectrum were at m/e 203, 178 ($Me_3SiO^+{=}CH(CH_2)_3CD_2CD_3$), 171, and 294. Ions of lower intensity were at 384, 487, 492, and 487 (M). Since the compound carried five atoms of deuterium in the C-15 through C-20 fragments, ions originating from these fragments were shifted plus 5 amu. Mass spectral analysis of the synthesized **2** revealed that it carried >87% pentadeuterated material in its C-15 through C-20 fragment (i.e., m/e 178 versus 173).

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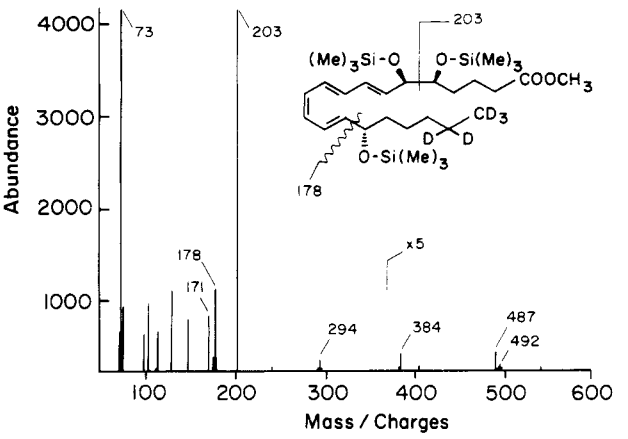


Figure 2. Mass spectra of Me_3Si derivative of the methyl ester of 19,19,20,20,20-pentadeuterio-LXA_4.

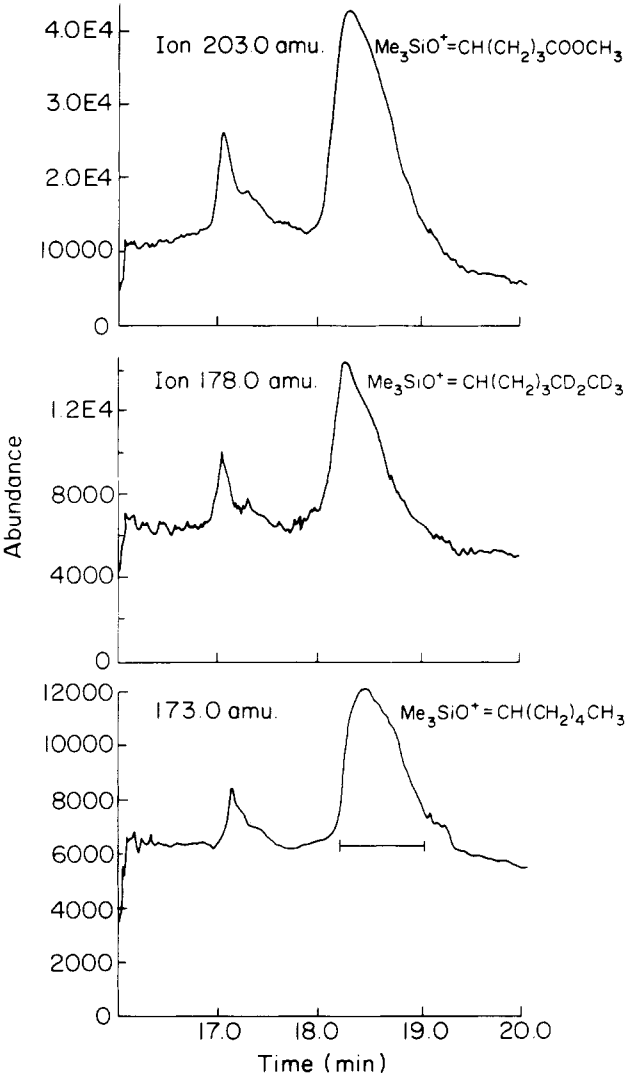
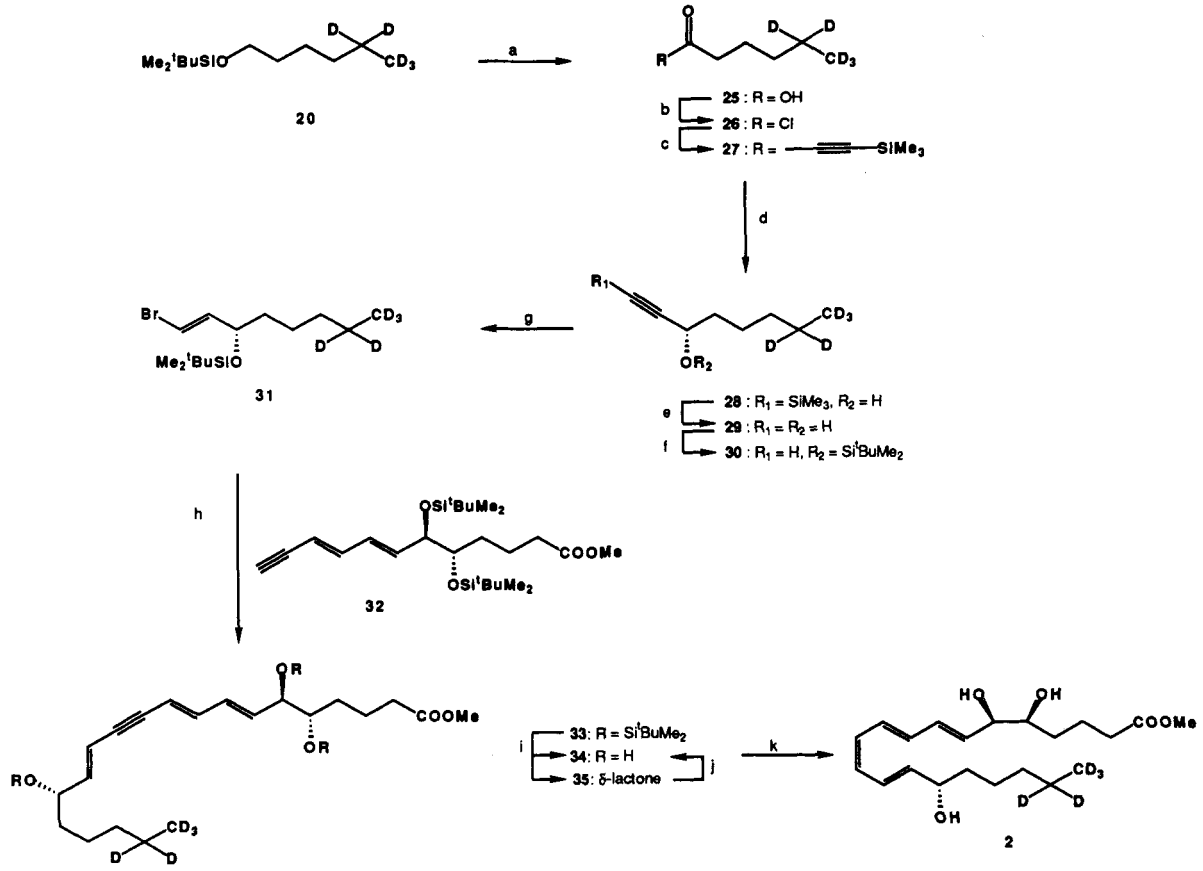


Figure 3. Representative selected ion monitoring chromatograms of the Me_3Si derivatives of LXA_4-Me and pentadeuterio-LXA_4-Me. Following injection on GC-MS, selected ion monitoring was carried out at m/z 203.0, 173.0, and 178.0, respectively. The deuterium-containing ion at m/z 173 represents addition of 7.5 ng of 19,19,20,20,20-pentadeuterio-LXA_4-Me as an internal standard before workup. The ion at μ/z 173.0 from synthetic LXA_4-Me was also 7.5 ng.

Since the pentadeuterated compound contained a specific ion at m/e 178 that was not present in high intensity in the native molecule, the pentadeuterio-LXA_4 serves as

Scheme II. Synthesis of 19,19,20,20,20-Pentadeuteriolipoxin A_4 Methyl Ester



[a] Reagents and conditions: (a) Jones oxidation, acetone, 0 °C, 30 min, 83%; (b) 1.1 equiv of $(COCl)_2$, DMF, CH_2Cl_2, 25 °C, 15 min; (c) 1.2 equiv of bis(trimethylsilyl)acetylene, 1.2 equiv of $AlCl_3$, 0 °C, 1.5 h, 52%; (d) 1.5 equiv of (*X*)-Alpine Borane, 25 °C, 2 h, 98%, 97% ee; (e) 1.3 equiv of n-Bu_4NF, THF, 25 °C, 20 min, 97% (f), 1.2 equiv of $^tBuMe_2SiOTf$, 1.5 equiv of 2,6-lutidine, CH_2Cl_2, 0 °C, 98%; (g) 1.3 equiv of n-Bu_3SnH, 130 °C, 2 h, then 1.3 equiv of Br_2, CH_2Cl_2, −40 °C, 86%; (h) 1.2 equiv of **32**, 0.05 equiv of $Pd(PPh_3)_4$, 0.16 equiv of CuI, n-$PrNH_2$, benzene, 25 °C, 2 h, 78%; (i) 10 equiv of HF·pyr, THF, 0 → 25 °C, 4 h, **34** 47%, **35** 11%; (j) 3.0 equiv of Et_3N, MeOH, 25 °C, 15 min, 100%; (k) H_2, 10% by weight Lindlar cat., 30 μL of quinoline, CH_2Cl_2, 25 °C, 2 h, 54%.

an appropriate internal standard for the quantitation of LXA_4 from a variety of biological sources. Representative selected ion chromatograms of a mixture of Me_3Si derivatives of LXA_4-Me and pentadeuterio-LXA_4-Me are given in Figure 3. Selected ion monitoring was carried out at *m/e* 203.0, 173.0, and 178.0. Figure 4 gives the calibration curve obtained when pentadeuterio-LXA_4 was used as internal standard and varying amounts of lipoxin A_4 were added to the mixtures prior to derivatization and subsequent analysis by GC-MS. The areas beneath the ions at *m/e* 173 and 178 were obtained following selected ion monitoring (Figure 3) and integration. Here, the isotope ratio (*m/e* 173/178) is expressed as a function of the native LXA_4 present in each mixture. Thus, the levels of native LXA_4 were obtained with pentadeuterio-LXA_4 as internal standard to monitor the presence and magnitude of the ion at *m/e* 173. The limit of detection of LXA_4 was approximately 75 pg with bronchoalveolar lavage fluids obtained from patients.[13]

In summary, the present syntheses of deuterated eicosanoids enable the detection and quantitation of lipoxin A_4 by selected ion monitoring on GC-MS. Utilizing these methods and deuterated lipoxin A_4 as internal standard, we have recently obtained evidence for the levels of LXA_4 present in bronchial lavage fluids from patients with selected preliminary diseases including sarcoidosis.[13] The results of these studies will be presented elsewhere.[13]

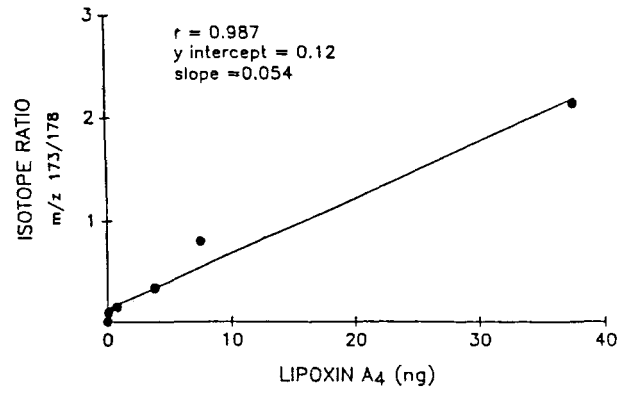


Figure 4. Typical calibration curve for the quantitation of LXA_4 utilizing pentadeuterio-LXA_4-Me as an internal standard. LXA_4-D_5 (7 ng) was mixed with varying concentrations of native LXA_4. EI-MS of the Me_3Si derivatives was employed and ion currents at *m/z* 173 and 178 were measured for the native and labeled compounds. The area beneath the peaks (Figure 3) was integrated and is represented as the peak ratio *m/z* 173/178.

Experimental Section

General. NMR spectra were recorded on an IBM AF-250 or a Bruker AM-500 instrument. IR spectra were recorded on a Perkin-Elmer Model 781 infrared spectrophotomer.

High resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under chemical ionization (Cl) conditions or on a VG ZAB E instrument under FAB conditions.

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Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN, or Robertson Laboratories, Inc., Madison, NJ.

All reactions were monitored by thin layer chromatography carried out on 0.25-mm E. Merck silica gel plates (60F-254), using UV light and 7% ethanolic phosphomolybdic acid–heat as developing agent. Preparative layer chromatography was performed on 0.5 or 0.25 mm × 20 cm × 20 cm E. Merck silica gel plates (60F-254). E. Merck silica gel (60, particle size 0.040–0.063) was used for flash column chromatography.

Al reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise noted. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated.

1-(*tert*-Butyldiphenylsiloxy)-9-[(tetrahydropyran-2′-yl)-oxy]nona-3,6-diyne-1,9-diol (8). To a magnetically stirred solution of compound **5** (2.40 g, 15.6 mmol) in THF (50 mL) was added EtMgBr (2 M solution in THF, 7.5 mL, 15 mmol) at ambient temperature under argon. The mixture was stirred at 65 °C for 1 h and then cooled to 0 °C. Cuprous iodide (457 mg, 2.4 mmol) was added and stirring was continued for 30 min, followed by addition of tosylate **7** (5.90 g, 12.0 mmol) in THF (20 mL) and further stirring for 30 min at 0 °C. Saturated aqueous NH_4Cl (20 mL) was added and the reaction mixture was extracted with ether (3 × 50 mL). The combined organic phase was washed with brine (30 mL) and dried ($MgSO_4$). Concentration and purification by flash chromatography (silica, 5–20% ether in petroleum ether) gave pure **8** (5.33 g, 94%). **8**: colorless oil; R_f 0.38 (silica, 20% ether in petroleum ether); IR (neat) ν_{max} 3070, 2940, 2860, 1590, 1430 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 4 H, Ar), 7.42 (m, 6 H, Ar), 4.62 (t, J = 8.5 Hz, 1 H, OC*H*O), 3.76 (m, 4 H, CH_2O), 3.50 (m, 2 H, CH_2O), 3.08 (m, 2 H, bis-propargylic), 2.44 (m, 4 H, CH_2-allylic) 1.54 (m, 6 H, CH_2), 1.04 (s, 9 H, Si^t*Bu*); HRMS (CI) calcd for $C_{30}H_{38}O_3Si$ (M + NH_4^+) 492.293, found 492.295.

(3*E*,5*E*)-1-(*tert*-Butyldiphenylsiloxy)nona-3,5-diene-1,9-diol (10). Diacetylene **9** (2.19 g, 5.6 mmol) was dissolved in hexane/CH_2Cl_2 (1:1, 150 mL) and stirred with Lindlar catalyst (Fluka, 440 mg) under a hydrogen atmosphere at 25 °C for 10 h. The reaction progress was followed by HPLC (Altex reverse phase-ODS C-18 column, MeOH/H_2O 70:30, flow rate 3 mL/min, t_R(product) = 10 min, t_R(starting material) = 5.7 min). The catalyst was removed by filtration through a pad of $MgSO_4$ and washed with CH_2Cl_2 (100 mL). Solvent evaporation afforded essentially pure diene **10** (2.18 g, 99%). **10**: colorless oil; R_F 0.21 (silica, 20% ether petroleum ether, three developments); IR (neat) ν_{max} 3350, 3070, 3010, 2930, 2860, 1710, 1430, 1115 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.68 (m, 4 H, Ar), 7.42 (m, 6 H, Ar), 5.41 (m, 4 H, olefinic), 3.65 (m, 4 H, CH_2O), 2.77 (t, J = 6.1 Hz, 2 H, bis-allylic), 2.33 (m, 4 H, allylic), 1.05 (s, 9 H, Si^t*Bu*); HRMS (CI) calcd for $C_{25}H_{34}O_2Si$ (M$^+$ + H) 395.241, found 395.244.

(3*E*,5*E*,7*E*)-19,19,20,20,20-Pentadeuterio-1-(*tert*-butyldiphenylsiloxy)pentadeca-3,5,7-trien-1-ol (13). To a stirred suspension of phosphonium salt **12** (719 mg, 1.0 mmol) in DME (10 mL) at −35 °C was added $NaN[Si(CH_3)_3]_2$ (1 M solution in THF, 1.1 mL, 1.1 mmol) under argon. The mixture was stirred at that temperature for 45 min and then aldehyde **22** (118 mg, 1.1 mmol) in DME (10 mL) was added. Stirring was continued at −35 °C for 45 min and then the reaction was quenched with saturated NH_4Cl solution (10 mL). Extraction with ether (3 × 50 mL) followed by washing with brine (25 mL), drying ($MgSO_4$), and concentration gave the crude product, which was flash chromatographed (silica, 5% ether in petroleum ether) to give pure **13** (414 mg, 89%). **13**: colorless oil; R_f 0.61 (silica, 5% ether in petroleum ether); IR (neat) ν_{max} 3080, 3020, 2940, 2860, 1435, 1115 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 4 H, Ar), 7.42 (m, 6 H, Ar), 5.37 (m, 6 H, olefinic), 3.67 (t, J = 13.8 Hz, 2 H, CH_2O), 2.78 (m, 4 H, bis-allylic), 2.35 (m, 2 H, allylic), 2.06 (m, 2 H, allylic), 1.29 (m, 4 H, CH_2), 1.04 (s, 9 H, Si^t*Bu*).

1-(*tert*-Butyldimethylsiloxy)-6-deuterio-5-hexyn-1-ol (19). To a stirred solution of compound **18** (10.6 g, 50 mmol) in THF (167 mL) at −78 °C was slowly added a solution of nBuLi (1.6 M in hexanes, 46.9 mL, 75 mmol) under argon. The mixture was stirred at −78 °C for 45 min and then D_2O (2 mL, 99.96%, 100 mmol) was added. The reaction mixture was allowed to reach room temperature over a period of 1 h and then diluted with petroleum ether (300 mL). The solution was washed with brine (25 mL), dried ($MgSO_4$), and concentrated to give essentially pure **19** (10.5 g, 99%). **19**: colorless oil; R_f 0.28 (silica, 1% ether in petroleum ether); IR (neat) ν_{max} 2930, 2850, 2598, 1470, 1386, 1252, 1102 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.64 (m, 2 H, CH_2OSi), 2.21 (m, 2 H, CH_2), 1.61 (m, 4 H, CH_2), 0.89 (s, 9 H, Si^t*Bu*), 0.05 (s, 6 H, SiCH_3); MS m/e (rel intensity) 214 (M$^+$ + H, 100), 156 (86); HRMS calcd for $C_{12}H_{24}DOSi$ (M$^+$ + H) 214.1675, found 214.1686.

1-(*tert*-Butyldimethylsiloxy)-5,5,6,6,6-pentadeuteriohexan-1-ol (20). Acetylene **19** (2.13 g, 10 mmol) was dissolved in THF (100 mL) and stirred with 5% Rh on alumina (213 mg) under an atmosphere of deuterium (99.5%) at 25 °C for 12 h. Filtration through Celite followed by solvent removal and flash chromatography (silica, petroleum ether) gave pure compound **20** (2.21 g, 100%). **20**: colorless oil; R_f 0.26 (silica, petroleum ether); IR (neat) ν_{max} 2920, 2845, 2210, 1466, 1388, 1255, 1088 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.60 (t, J = 3.6 Hz, 2 H, -CH_2OSi-), 1.51 (m, 2 H, -CH_2-), 1.31 (m, 4 H, CH_2), 0.90 (s, 9 H, Si^t*Bu*), 0.05 (s, 6 H, SiCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 63.33, 32.92, 31.43, 25.98, 25.50, 22.2 (multiplet) 18.38, 12.8 (multiplet); MS m/e (rel intensity) 222 (M$^+$ + H, 42), 181 (70), 164 (100); HRMS calcd for $C_{12}H_{24}D_5OSi$ (M$^+$ + H) 222.2223, found 222.2314.

Methyl (3*E*,5*E*,7*E*,9*E*)-19,19,20,20,20-Pentadeuterio-3,5,7,9-eicosatetraenoate (24). To a stirred suspension of phosphonium salt **16** (22 mg, 0.04 mmol) in DME (0.5 mL) at −35 °C was added $NaN[Si(CH_3)_2]_2$ (1 M solution in THF, 44 μL, 0.044 mmol) under argon, and the mixture was stirred at that temperature for 45 min. Aldehyde **23** (8 mg, 0.052 mmol) in DME (0.5 mL) was then added at −35 °C and stirring was continued at that temperature for 45 min. Quenching with saturated NH_4Cl solution (10 mL) followed by extraction with ether (3 × 10 mL), washing the combined extract with brine (5 mL), drying ($MgSO_4$), and evaporation gave an oil, which was flash chromatographed (silica, 5% ether in petroleum ether) to give pure **24** (11.4 mg, 89%). **24**: colorless oil; R_f 0.37 (silica, 10% ether in petroleum ether); IR ($CHCl_3$) ν_{max} 3005, 2925, 2850, 1730, 1440, 1270 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (m, 8 H, olefinic), 3.67 (s, 3 H, COOCH_3), 2.81 (m, 6 H, bis-allylic), 2.32 (t, J = 7.5 Hz, 2 H, CH_2COOCH$_3$), 2.11 (m, 2 H, allylic), 2.0 (m, 2 H, allylic), 1.71 (m, 2 H, CH_2), 1.35 (m, 2 H, CH_2), 1.27 (m, 2 H, CH_2); HRMS calcd for $C_{21}H_{29}D_5O_2$ (M$^+$ + H) 323.2865, found 323.2872.

(3*E*,5*E*,7*E*,9*E*)-19,19,20,20,20-Pentadeuterio-3,5,7,9-eicosatetraenoic Acid (19,19,20,20,20-Pentadeuterioarachidonic Acid) (1). The methyl ester **24** (6.46 mg, 0.02 mmol in THF/H_2O (2:1, 0.5 mL) was cooled to 0 °C and LiOH (1 H, 60 μL, 0.06 mmol) was added with stirring. The mixture was stirred at ambient temperature for 8 h (TLC monitoring, silica, 20% EtOAc in petroleum ether). The reaction mixture was cooled to 0 °C and acidified to pH 5 with 1 N aqueous hydrochloric acid and diluted with H_2O (5 mL). Ether extraction (3 × 20 mL) followed by washing the combined ether extract with H_2O (5 mL) and brine (5 mL), drying ($MgSO_4$), and evaporation gave essentially pure 19,19,20,20,20-pentadeuterioarachidonic acid (**1**) (6.1 mg, 98%). **1**: colorless oil; R_f 0.22 (silica, 20% EtOAc in petroleum ether); IR ($CHCl_3$) ν_{max} 3350–2500 (br, COOH), 3000, 2970, 2845, 1710, 1460, 1265 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (m, 8 H, olefinic), 2.8 (m, 6 H, bis-allylic), 2.36 (t, J = 7.5 Hz, 2 H, CH_2COOH), 2.12 (m, 2 H, allylic), 2.02 (m, 2 H, allylic), 1.7 (m, 2 H, CH_2), 1.4–1.1 (m, 6 H, CH_2).

5,5,6,6,6-Pentadeuteriohexanoic Acid (25). Jones reagent was added dropwise to a cooled (0 °C) solution of the silyl ether **20** (6.90 g, 36.6 mmol) in acetone (122 mL) until the reaction mixture turned light orange and TLC (silica, 1% ether in petroleum ether) indicated completion of the reaction. Isopropyl alcohol (3 mL) was added to reduce the excess Jones reagent and the reaction mixture was diluted with brine (150 mL) and extracted with ether (6 × 50 mL). The combined organic extract was dried ($MgSO_4$), filtered, and concentrated to give a pale yellow oil. Flash column chromatography (silica, 30% ether in petroleum ether) gave pure **25** (3.90 g, 88%). **25**: colorless oil; R_f 0.27 (silica, 30% ether in petroleum ether); IR (neat) ν_{max} 3352, 2922, 2850, 2203, 2113, 1710, 1410, 1250, 1053 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.39 (br s, 1 H, COO*H*), 2.35 (t, J = 7.4 Hz, 2 H, CH_2COOH), 1.64 (m, 2 H, CH_2), 1.41 (m, 2 H, CH_2); MS m/e (rel

intensity) 139 (M + NH_4^+, 78), 122 (M^+ + H, 50), 104 (100); HRMS calcd for $C_6H_8D_5O_2$ (M^+ + H) 122.1229, found 122.1223.

Methyl (7*E*,9*E*,11*Z*,13*E*,5*S*,6*R*,15*S*)-5,6,15-Trihydroxy-19,19,20,20,20-pentadeuterio-7,9,11,13-eicosatetraenoate (19,19,20,20,20-Pentadeuteriolipoxin A_4 Methyl Ester) (2). To a stirred solution of acetylene **34** (50.3 mg, 0.136 mmol) in CH_2Cl_2 (4.5 mL) was added Lindlar catalyst (Fluka, 15 mg, 30% w/w). The mixture was stirred under a hydrogen atmosphere at room temperture with monitoring by HPLC (Altex reverse phase-ODS C-18 column, MeOH/H_2O 70:30, flow rate 5 mL/min, λ_{max} 300 nm, UV detector) and allowed to proceed to ca. 85% completion (2.5 h). The catalyst was filtered off through Celite and the solvent was evaporated. The resulting oil was dissolved in MeOH (2 mL) and purified by reverse-phase HPLC (same conditions as above, t_R 11.6 min) to give, after removal of the solvents, compound **2** (27.3 mg, 54%). **2**: white waxy solid; R_f 0.31 (silica, 5% MeOH in CH_2Cl_2); $[\alpha]^{24}_D$ +23.7° (*c* 0.63, CH_2Cl_2); UV (MeOH) λ_{max} 288, 300, 315 nm; IR (CH_2Cl_2) ν_{max} 3558, 3027, 2960, 2867, 2210, 2115, 1740, 1613, 1460, 1440, 1240, 1005, 985 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.67 (m, 2 H, olefinic), 6.35 (m, 1 H, olefinic), 6.25 (m, 2 H, olefinic), 6.03 (m, 2 H, olefinic), 5.78 (m, 2 H, olefinic), 4.20 (m, 1 H, C*H*OH), 4.15 (m, 1 H, C*H*OH), 3.70 (m, 4 H, C*H*OH, COOCH_3), 2.36 (t, J = 6.6 Hz, CH_2COOMe), 1.35–1.26 (multiplets 13 H, O*H*, CH_2).

Gas Chromatography–Mass Spectroscopy Methods. Gas chromatography–mass spectrometry was performed with a Hewlett-Packard 5988A MS instrument equipped with a 59970A workstation and 5890 GC. A fused capillary column (SE-30 2-40004, 30 m, 0.25-mm i.d., 0.25 νM *df*; Supelco, Inc., Bellefonte, PA) was employed with a temperature program. The splitless on time was 0.9; initial temperature was 150 °C (1 min), followed by 230 °C (4 min), 250 °C (8 min), and 245 °C (12.0). The retention times for standard fatty acid methyl esters (carbons 20–26) in this system were 9.1 min (C20), 13.1 min (C22), 18.8 min C24), and 28.2 min (C26). Samples were treated with diazomethane, and trimethylsilyl (Me_3Si) derivatives were prepared (3) just prior to analysis on GC-MS.

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Supplementary Material Available: ^1H NMR and IR spectra of compounds **1**, **2**, **8**, **10**, **13**, **19**, **20**, **24**, **25**, **27–31**, **33**, and **33**; D and ^{13}C NMR spectra of compound **20** (17 pages). Ordering information is given on any current masthead page.

Total Synthesis of Novel Geometric Isomers of Lipoxin A_4 and Lipoxin B_4

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Methyl esters of the geometric isomers of lipoxin A_4 and lipoxin B_4 **1–4** were synthesized by stereoselective routes. Compound **1** was constructed from key intermediates **13** and **20** by phosphonate–aldehyde condensation followed by deprotection and Lindlar hydrogenation. Compound **2** was synthesized by Pd(0)–Cu(I)-catalyzed coupling of fragments **25** and **26** followed by deprotection and selective reduction. Compound **3** was prepared via phosphonate–aldehyde coupling of intermediates **34** and **35** followed by deprotection and selective reduction. Compound **4** was synthesized via Pd(0)–Cu(I) coupling of key segments **41** and **42** followed by deprotection and selective hydrogenation.

Introduction

In 1984, the isolation of a novel series of linear trihydroxy eicosanoids that contain a conjugated tetraene system in their structure was reported.[1] This series of oxygenated products of arachidonic acid was first isolated from human leukocytes incubated with 15(*S*)-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE).[1] Subsequent findings indicate that they can also be generated from 15(*S*)-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) by human neutrophils[2] and from endogenous arachidonic acid upon activation of human granulocytes from eosinophilic donors.[3]

Studies with isotopic oxygen ($^{18}O_2$) and intact human leukocytes revealed that lipoxin A_4 and lipoxin B_4 each carried an ^{18}O atom at carbon 5 and that oxygen substituents at carbon 6 of lipoxin A_4 and carbon 14 of lipoxin B_4 were not derived exclusively from molecular oxygen.[2,4] These results together with those obtained from alcohol trapping experiments indicated the involvement of a 5,6-epoxy tetraene intermediate (Scheme I) in the formation of lipoxin A_4 and lipoxin B_4 as well as their nonenzymatically derived isomers.[2,4,5] Total synthesis[6] of this epoxide followed by biogenetic studies with human leukocytes[5] as well as results obtained with 15-HETE and human neutrophils[7] revealed the generation of additional compounds

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