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Synthesis of 19,19,20,20,20-Pentadeuteriolipoxin A₄ 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 A₄ 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 A₄ 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 A₄, 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 A₄ methyl ester (**25**). The present findings will permit the detection of LXA₄, which may be present in various biological sources (i.e., including patient-derived samples), by selected ion monitoring on gas chromatography-mass spectroscopy.

(1) *CRC Handbook of Eicosanoids and Related Lipids*; Willis, A. L., Ed.; CRC Press, Inc.: Boca Raton, FL, 1987; Vol. 1.

(2) Samuelsson, B.; Dahlen, S. E.; Lindgren, J. A.; Bouzer, L. A.; Serhan, C. N. *Science* 1987, 237, 1171.

(3) Serhan, C. N.; Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* 1984, 81, 5335.

(4) Wong, P. Y.-K.; Serhan, C. N. In *Advances in Experimental Medicine and Biology*; Plenum Press: New York, 1988; Vol. 229.

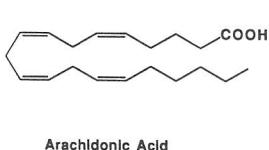
(5) Barrow, S. E.; Taylor, G. W. In *Prostaglandins and Related Substances*; Benedetto, C., McDonald-Gibson, R. G., Nigam, S., Slater, T. F., Eds.; IRL Press: Oxford, 1987; pp 99-141.

(6) Haskins, N. J. *Biomed. Mass. Spectrum* 1982, 9, 269.

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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

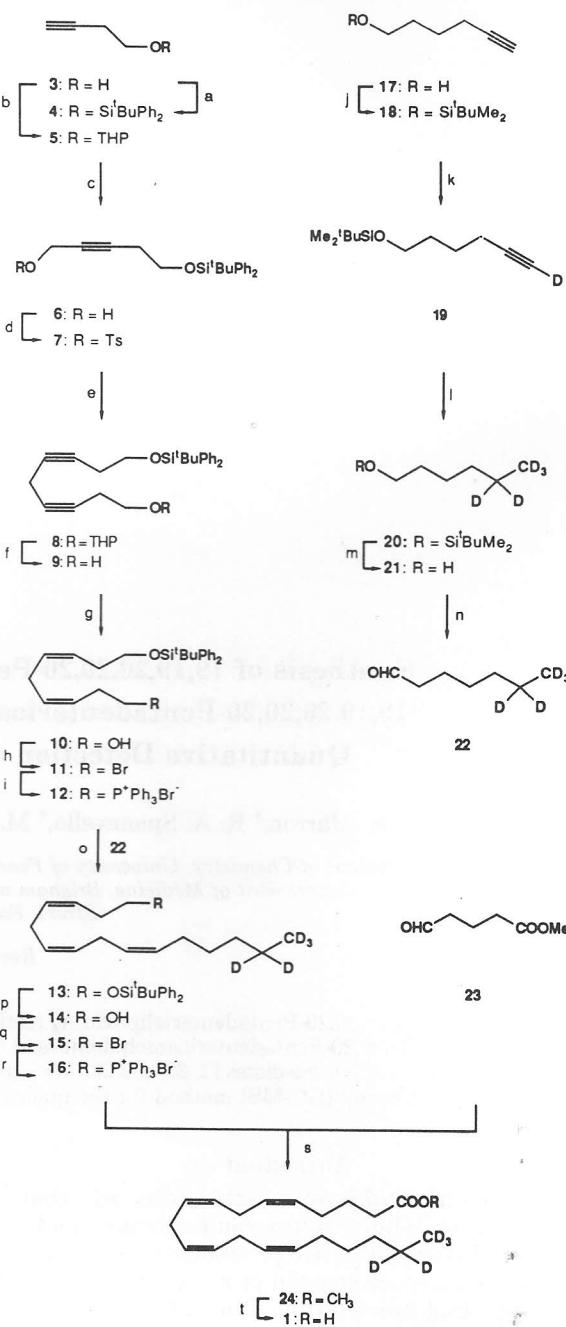
The synthesis of 19,19,20,20,20-pentadeuterioarachidonic acid (1) and its methyl ester (24) is detailed in Scheme I. Thus, 3-butyn-1-ol (3) was converted to both its silyl ether (4, 98%) and its tetrahydropyranyl ether (5, 96%) by standard conditions. The acetylenic silyl derivative 4 was then hydroxymethylated using nBuLi-(CH₂O)_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 CBr₄-PPh₃ (85%) followed by heating with PPh₃ 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 (D₂O, 99% yield, >99% deuterated by 500-MHz ¹H NMR spectroscopy). Exhaustive catalytic deuteration of 19 (D₂, Rh/alumina)⁹ gave 20 (100% yield, >87% pentadeuterated by mass spectroscopy). Finally desilylation of 20 (95%) using fluoride ion followed by CrO₃-pyridine oxidation led to the requisite aldehyde 22 (85%).

The ylide derived from 12 and NaN(SiMe₃)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 \rightarrow 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 B₄, human neutrophils (*in vitro*) do not ω -oxidize either lipoxin A₄ or lipoxin B₄.¹⁰ The carbon-20 position of lipoxin A₄ was selected as the site for incorporation of deuterium label

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



(7) Strife, R. J.; Murphy, R. C. *Prostaglandins, Leukotrienes Med.* 1984, 13, 1.

(8) For a review on deuterated eicosanoids, see: Meese L. O. J. *Labeled Compd. Radiopharm.* 1986, 23, 295.

(9) Burnell, R. L., Jr. *Acc. Chem. Res.* 1969, 2, 289.

(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.

^a Reagents and conditions: (a) 1.2 equiv of ^tBuPh₂SiCl, 1.5 equiv of imidazole, 25 °C, 12 h, 98%; (b) 1.3 equiv of dihydropyran, pyridinium *p*-toluenesulfonate (PPTS) cat., CH₂Cl₂, 25 °C, 3 h, 96%; (c) 1.25 equiv of n-BuLi, THF, 1.3 equiv of (CH₂O)_n, -78 → 25 °C, 12 h, 85%; (d) 5 equiv of KOH, 1.25 equiv of TsCl, Et₂O, -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 7, THF, 0 → 25 °C, 1 h, 94%; (f) 0.1 equiv of PPTS, MeOH, 25 °C, 6 h, 73%; (g) H₂, Lindlar cat., CH₂Cl₂-hexane 1:1, 25 °C, 10 h, 99%; (h) 1.2 equiv of CBr₄, 1.5 equiv of PPh₃, CH₂Cl₂, -40 → -30 °C, 45 min, 85%; (i) 24 equiv of PPh₃, CH₃CN, reflux, 12 h, 97%; (j) 1.2 equiv of ^tBuMe₂SiCl, 1.5 equiv of imidazole, 25 °C, 12 h, 98%; (k) 1.5 equiv of n-BuLi, THF, -78 °C, 2 equiv of D₂O, 99%; (l) D₂, 5% Rh/alumina (20% w/w), THF, 25 °C, 12 h, 100%; (m) 1.1 equiv of n-Bu₄NF, THF, 0 → 25 °C, 4 h, 95%; (n) 6 equiv of CrO₃, 12 equiv of pyridine, CH₂Cl₂, 25 °C, 0.5 h, 85%; (o) 1.1 equiv of NaN(Si(C₂H₅)₃)₂, DME, 1.1 equiv of 22, -35 °C, 1.5 h, 89%; (p) 1.3 equiv of n-Bu₄NF, THF, 0 → 25 °C, 0.5 h, 95%; (q) 1.2 equiv of CBr₄, 1.5 equiv of PPh₃, CH₂Cl₂, -40 → -30 °C, 45 min, 86%; (r) 2.4 equiv of PPh₃, CH₃CN, reflux, 12 h, 95%; (s) 1.1 equiv of NaN(Si(C₂H₅)₃)₂, DME, 1.3 equiv of 23, -35 °C, 1.5 h, 89%; (t) 3 equiv of LiOH, THF/H₂O 1:1, 0 → 25 °C, 8 h, 98%.

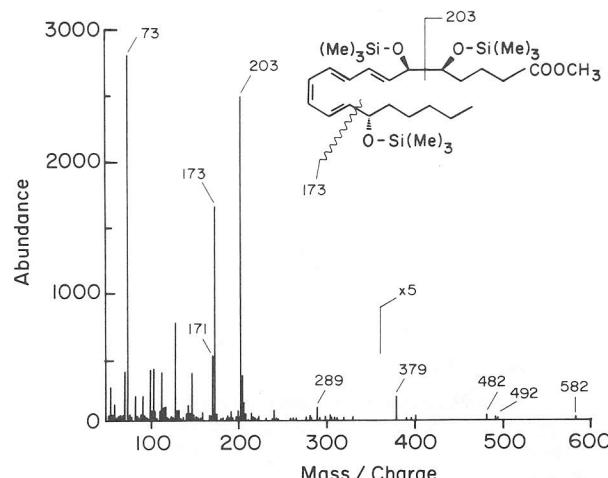


Figure 1. Mass spectra of Me_3Si derivative of the methyl ester of synthetic LXA_4 .

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-pentadeuteriolipoxin A₄ 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 A₄ methyl ester.¹¹

Next, aliquots of synthetic LXA_4 and pentadeuterio- LXA_4 methyl esters ($\text{LXA}_4\text{-Me}$) 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 A₄ methyl ester (C value = 24.1). Ions of high intensity were at m/e 203 ($\text{Me}_3\text{SiO}^+ = \text{CH}(\text{CH}_2)_3\text{COOCH}_3$), 173 ($\text{Me}_3\text{SiO}^+ = \text{CH}(\text{CH}_2)_4\text{CH}_3$), 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-pentadeuterated counterpart is given in Figure 2 (C value = 24.0). Prominent ions in its mass spectrum were at m/e 203, 178 ($\text{Me}_3\text{SiO}^+ = \text{CH}(\text{CH}_2)_3\text{CD}_2\text{CD}_3$), 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).

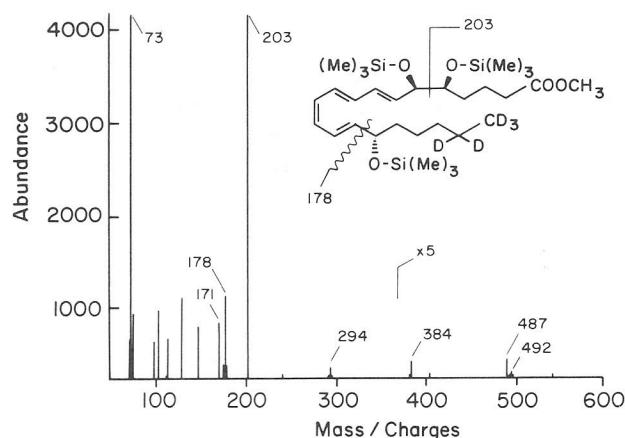


Figure 2. Mass spectra of Me_3Si derivative of the methyl ester of 19,19,20,20-pentadeuterio- LXA_4 .

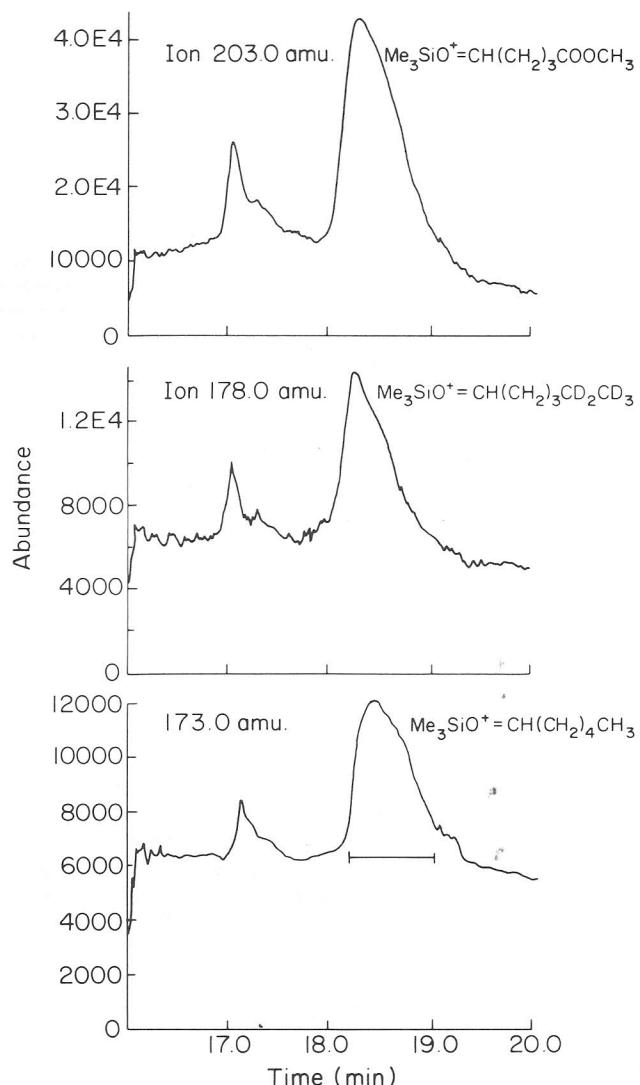
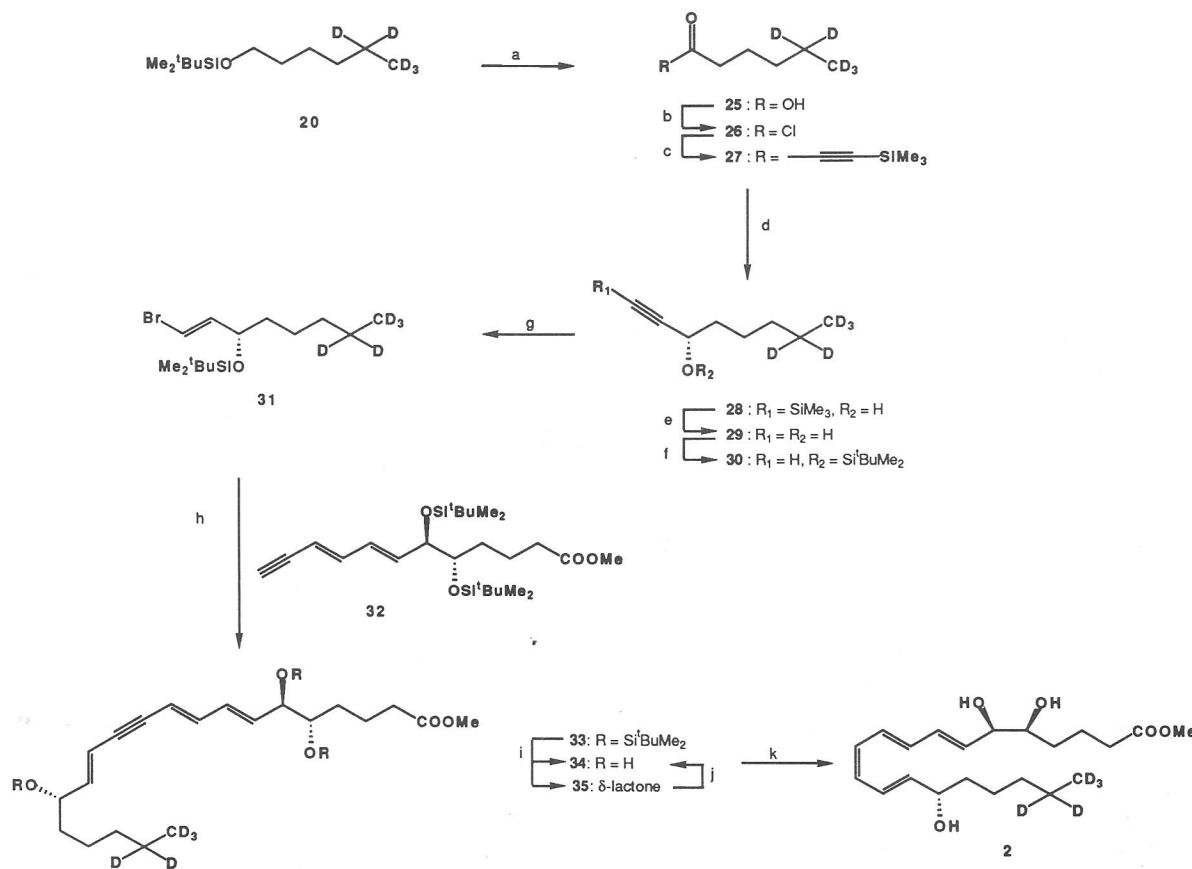


Figure 3. Representative selected ion monitoring chromatograms of the Me_3Si derivatives of $\text{LXA}_4\text{-Me}$ and pentadeuterio- $\text{LXA}_4\text{-Me}$. Following injection on GC-MS, selected ion monitoring was carried out at m/z 203.0, 173.0, and 178.0, respectively. The deuterium-containing ion at m/z 178 represents addition of 7.5 ng of 19,19,20,20-pentadeuterio- $\text{LXA}_4\text{-Me}$ as an internal standard before workup. The ion at m/z 173.0 from synthetic $\text{LXA}_4\text{-Me}$ 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

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(12) Serhan, C. N.; Nicolaou, K. C.; Webber, S. E.; Veale, C. A.; Dahlén, S.-E.; Puustinen, T. J.; Samuelsson, B. *J. Biol. Chem.* 1986, 261, 16340.

Scheme II. Synthesis of 19,19,20,20,20-Pentadeuteriolipoxin A₄ Methyl Ester

^a Reagents and conditions: (a) Jones oxidation, acetone, 0 °C, 30 min, 83%; (b) 1.1 equiv of (COCl)₂, DMF, CH₂Cl₂, 25 °C, 15 min; (c) 1.2 equiv of bis(trimethylsilyl)acetylene, 1.2 equiv of AlCl₃, 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-Bu₄NF, THF, 25 °C, 20 min, 97%; (f) 1.2 equiv of ^tBuMe₂SiOTf, 1.5 equiv of 2,6-lutidine, CH₂Cl₂, 0 °C, 98%; (g) 1.3 equiv of n-Bu₃SnH, 130 °C, 2 h, then 1.3 equiv of Br₂, CH₂Cl₂, -40 °C, 86%; (h) 1.2 equiv of 32, 0.05 equiv of Pd(PPh₃)₄, 0.16 equiv of CuI, n-PrNH₂, 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 Et₃N, MeOH, 25 °C, 15 min, 100%; (k) H₂, 10% by weight Lindlar cat., 30 μL of quinoline, CH₂Cl₂, 25 °C, 2 h, 54%.

an appropriate internal standard for the quantitation of LXA₄ from a variety of biological sources. Representative selected ion chromatograms of a mixture of Me₃Si derivatives of LXA₄-Me and pentadeuterio-LXA₄-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-LXA₄ was used as internal standard and varying amounts of lipoxin A₄ 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 LXA₄ present in each mixture. Thus, the levels of native LXA₄ were obtained with pentadeuterio-LXA₄ as internal standard to monitor the presence and magnitude of the ion at *m/e* 173. The limit of detection of LXA₄ 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 A₄ by selected ion monitoring on GC-MS. Utilizing these methods and deuterated lipoxin A₄ as internal standard, we have recently obtained evidence for the levels of LXA₄ present in bronchial lavage fluids from patients with selected preliminary diseases including sarcoidosis.¹³ The

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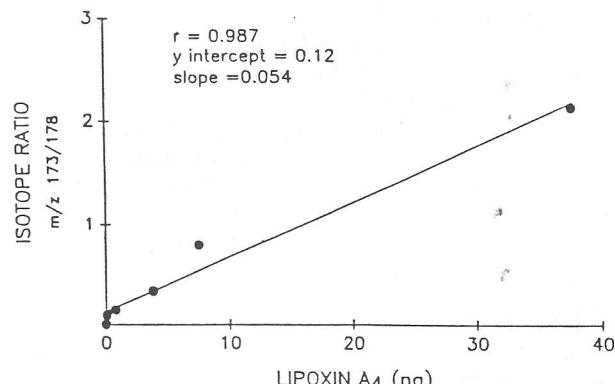


Figure 4. Typical calibration curve for the quantitation of LXA₄ utilizing pentadeuterio-LXA₄-Me as an internal standard. LXA₄-D₅ (7 ng) was mixed with varying concentrations of native LXA₄. EI-MS of the Me₃Si derivatives was employed and ion currents at *m/z* 173 and 178 were measured for the native and labeled compounds. The area beneath the peaks (Figure 3) was integrated and is represented as the peak ratio *m/z* 173/178.

results of these studies will be presented elsewhere.¹³

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 spectrophotometer.

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

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 (¹H 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 NH₄Cl (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 (MgSO₄). 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; ¹H NMR (500 MHz, CDCl₃) δ 7.68 (m, 4 H, Ar), 7.42 (m, 6 H, Ar), 4.62 (t, *J* = 8.5 Hz, 1 H, OCHO), 3.76 (m, 4 H, CH₂O), 3.50 (m, 2 H, CH₂O), 3.08 (m, 2 H, bis-propargylic), 2.44 (m, 4 H, CH₂-allylic) 1.54 (m, 6 H, CH₂), 1.04 (s, 9 H, Si^tBu); HRMS (CI) calcd for C₃₀H₃₈O₃Si (M + NH₄⁺) 492.293, found 492.295.

(3E,5E)-1-(tert-Butyldiphenylsiloxy)nona-3,5-diene-1,9-diol (10). Diacetylene 9 (2.19 g, 5.6 mmol) was dissolved in hexane/CH₂Cl₂ (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/H₂O 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 MgSO₄ and washed with CH₂Cl₂ (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; ¹H NMR (250 MHz, CDCl₃) δ 7.68 (m, 4 H, Ar), 7.42 (m, 6 H, Ar), 5.41 (m, 4 H, olefinic), 3.65 (m, 4 H, CH₂O), 2.77 (t, *J* = 6.1 Hz, 2 H, bis-allylic), 2.33 (m, 4 H, allylic), 1.05 (s, 9 H, Si^tBu); HRMS (CI) calcd for C₂₅H₃₄O₂Si (M⁺ + H) 395.241, found 395.244.

(3E,5E,7E)-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(CH₃)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 NH₄Cl solution (10 mL). Extraction with ether (3 × 50 mL) followed by washing with brine (25 mL), drying (MgSO₄), 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; ¹H NMR (500 MHz, CDCl₃) 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, CH₂O), 2.78 (m, 4 H, bis-allylic), 2.35 (m, 2 H, allylic), 2.06 (m, 2 H, allylic), 1.29 (m, 4 H, CH₂), 1.04 (s, 9 H, Si^tBu).

1-(tert-Butyldimethylsiloxy)-6-deutero-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 D₂O (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 (MgSO₄), 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; ¹H NMR (250 MHz, CDCl₃) δ 3.64 (m, 2 H, CH₂OSi), 2.21 (m, 2 H, CH₂), 1.61 (m, 4 H, CH₂), 0.89 (s, 9 H, Si^tBu), 0.05 (s, 6 H, SiCH₃); MS *m/e* (rel intensity) 214 (M⁺ + H, 100), 156 (86); HRMS calcd for C₁₂H₂₄DOSi (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; ¹H NMR (250 MHz, CDCl₃) δ 3.60 (t, *J* = 3.6 Hz, 2 H, -CH₂OSi-), 1.51 (m, 2 H, -CH₂-), 1.31 (m, 4 H, CH₂), 0.90 (s, 9 H, Si^tBu), 0.05 (s, 6 H, SiCH₃); ¹³C NMR (125 MHz, CDCl₃) 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 C₁₂H₂₄D₅OSi (M⁺ + H) 222.2223, found 222.2314.

Methyl (3E,5E,7E,9E)-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(CH₃)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 NH₄Cl solution (10 mL) followed by extraction with ether (3 × 10 mL), washing the combined extract with brine (5 mL), drying (MgSO₄), 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 (CHCl₃) ν_{max} 3005, 2925, 2850, 1730, 1440, 1270 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.38 (m, 8 H, olefinic), 3.67 (s, 3 H, COOCH₃), 2.81 (m, 6 H, bis-allylic), 2.32 (t, *J* = 7.5 Hz, 2 H, CH₂COOCH₃), 2.11 (m, 2 H, allylic), 2.0 (m, 2 H, allylic), 1.71 (m, 2 H, CH₂), 1.35 (m, 2 H, CH₂), 1.27 (m, 2 H, CH₂); HRMS calcd for C₂₁H₂₉D₅O₂ (M⁺ + H) 323.2865, found 323.2872.

(3E,5E,7E,9E)-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/H₂O (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 H₂O (5 mL). Ether extraction (3 × 20 mL) followed by washing the combined ether extract with H₂O (5 mL) and brine (5 mL), drying (MgSO₄), 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 (CHCl₃) ν_{max} 3350–2500 (br, COOH), 3000, 2970, 2845, 1710, 1460, 1265 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.36 (m, 8 H, olefinic), 2.8 (m, 6 H, bis-allylic), 2.36 (t, *J* = 7.5 Hz, 2 H, CH₂COOH), 2.12 (m, 2 H, allylic), 2.02 (m, 2 H, allylic), 1.7 (m, 2 H, CH₂), 1.4–1.1 (m, 6 H, CH₂).

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 (MgSO₄), 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; ¹H NMR (250 MHz, CDCl₃) δ 9.39 (br s, 1 H, COOH), 2.35 (t, *J* = 7.4 Hz, 2 H, CH₂COOH), 1.64 (m, 2 H, CH₂), 1.41 (m, 2 H, CH₂); 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 A₄ 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 temperature 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); $[\alpha]^{24}_D +23.7^\circ$ (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, CHO), 4.15 (m, 1 H, CHO), 3.70 (m, 4 H, CHO, $COOCH_3$), 2.36 (t, $J = 6.6$ Hz, CH_2COOMe), 1.35–1.26 (multiplets 13 H, OH, 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 μ M 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.