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A SYNTHON FOR C-20 TRIDEUTERATED EICOSANOIDS: PREPARATION OF [$^2H_3$]-ARACHIDONIC ACID

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SUMMARY

Starting from hex-5-yn-1-ol an efficient seven step synthesis of (Z)9-[$^2H_3$](non-3-en-1-yl) triphenylphosphonium bromide is described. This Wittig reagent is a key intermediate for the synthesis of C-20 trideuterated eicosanoids. Introduction of deuterium at C-20 provides standards that are stable to a wide range of reagents and reaction conditions. The utility of the Wittig synthon was demonstrated by the preparation of C-20 trideuterated arachidonic acid.

Key Words: Wittig reagent, deuterated arachidonic acid, deuterated eicosanoids, leukotrienes, mass spectrometry, stable isotope dilution.

INTRODUCTION

Arachidonic acid (AA) is metabolized by cyclooxygenase and lipoxygenase enzymes to a wide variety of oxygenated metabolites including prostaglandins, thromboxanes, prostacyclin, leukotrienes (LTs), hydroxyeicosatetraenoic acids (HETEs) and lipoxins (1-3). In addition, AA is metabolized by cytochrome P-450 to a series of epoxyeicosatrienoic acids (EETs) (4-6). The metabolic products of these various pathways (known as eicosanoids) possess potent and diverse biological activities (7).

Quantification of eicosanoids that are present in biological samples using highly sensitive and specific methods based on GC/MS requires appropriate heavy isotope labelled standards. These can be prepared either by total synthesis or by biosynthesis from deuterated AA (8). $^2H_8$-AA has been synthesized previously for this purpose by the reduction with deuterium of 5,8,11,14-eicosatetraynoic acid (ETYA) with P-2 nickel (9) or Lindlar catalyst (10). Deuterium scrambling that occurs during reduction

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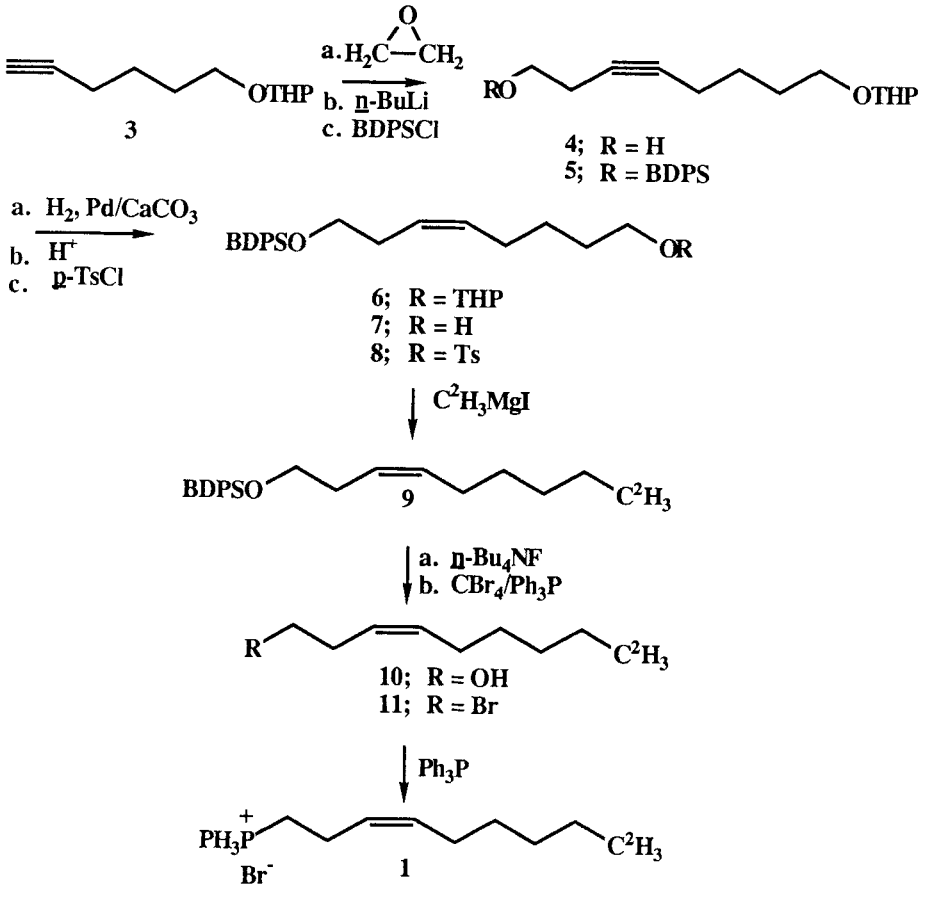
complicates the use of deuterated internal standards prepared by this methodology. In addition, deuteriums are introduced at sites that are susceptible to exchange in biochemical processes. The aim of the current study was to introduce deuterium at C-20 so that it would be remote from the double bonds and thus be stable to both chemical and biosynthetic manipulations. We have developed a convergent route that allows the ready preparation of these standards either by total synthesis or by biosynthesis from deuterated AA. The synthetic strategy involved the preparation of a key deuterated phosphonium salt **1** that can serve as a synthon for the preparation of various eicosanoids (11-16) by Wittig condensation with an appropriate aldehyde. This report details the synthesis of (Z)9-[$^2H_3$](non-3-en-1-yl)triphenylphosphonium bromide **1** and its use in the preparation of trideuterated AA **2.**

RESULTS AND DISCUSSION

The synthesis of **1** was accomplished by starting from the readily available 5-hexyn-1-ol (Scheme 1). Reaction of this alcohol with dihydropyran (2 equiv) and a catalytic amount of p-toluenesulfonic acid gave the corresponding tetrahydropyranyl ether **3** (17). Condensation of its lithio derivative (prepared by reaction with n-BuLi) with ethylene oxide gave the acetylenic alcohol **4** in 75% yield. The primary alcoholic group of **4** was protected as the t-butyldiphenylsilyl ether by reaction with t-butylchlorodiphenylsilane and 4-dimethylaminopyridine and the resultant silyl ether **5** was reduced using Lindlar catalyst in hexane containing 3% pyridine to give the cis-olefin **6** in excellent yield. Under these conditions no over reduced product or trans-isomer could be detected.

Removal of the THP group by treatment with acetic acid in THF/water gave the alcohol **7**. Introduction of the $^2H_3$ methyl group was carried out by converting the alcohol **7** to its tosyl derivative **8** followed by displacement with ($^2H_3$) methyl-magnesium iodide in the presence of a catalytic amount of dilithium tetrachlorocuprate to yield the deuterated compound **9.** It was observed that displacement of the tosyl group of **8** with [$^2H_3$]-methylmagnesium iodide using standard procedures resulted in the formation of the corresponding iodide as a major product. However, when the reaction was carried out with a larger excess of the Grignard reagent (2.5 equiv) the required product was obtained. The silyl group was removed by tetrabutylammonium

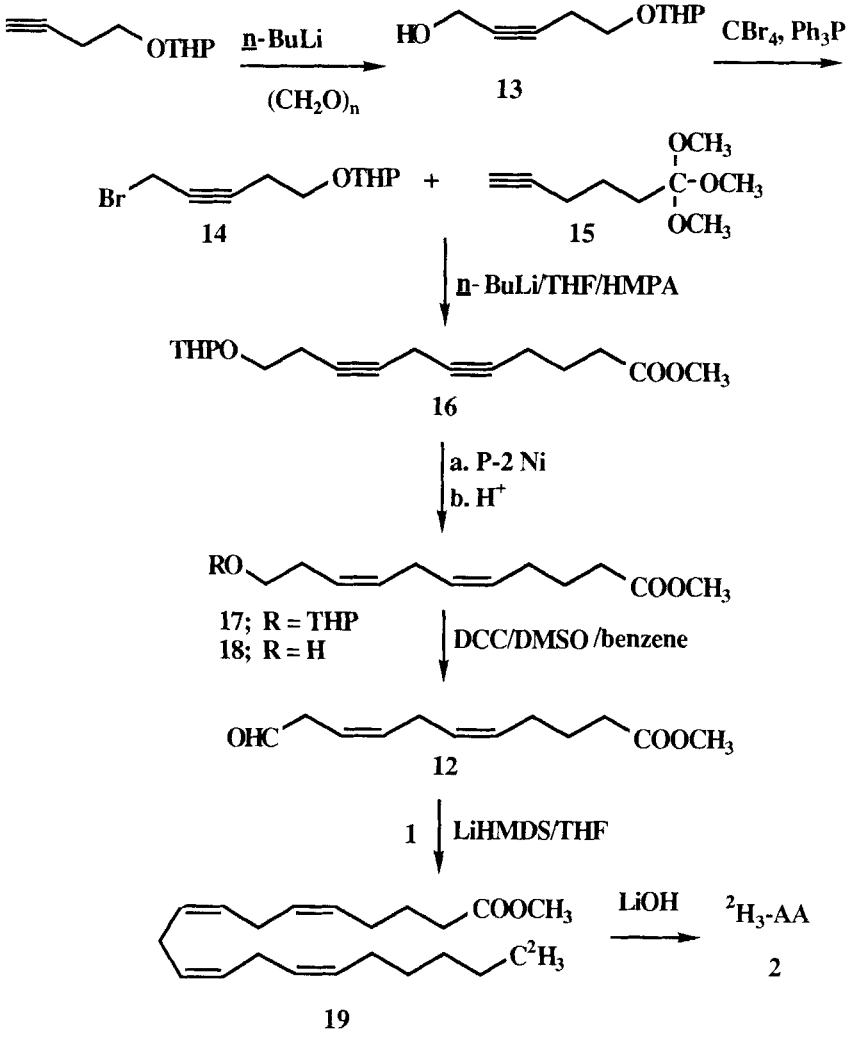
fluoride to afford the alcohol **10** which was then converted to bromide **11**. Reaction of bromide **11** with triphenylphosphine in toluene yielded the required phosphonium salt **1**.



Scheme 1: Preparation of (Z)9-[$^2H_3$](non-3-en-1-yl) triphenylphosphonium bromide **1**.

The use of $^2H_3$-labelled phosphonium salt **1** in the total synthesis of $^2H_3$- AA **2** is outlined in Scheme 2. The aldehyde **12** required for the synthesis was obtained from 3-butyn-1-OTHP by a modification of the method of Bhanu and Scheinmann (18). Condensation of the anion, prepared from but-3-yn-1-OTHP by reaction with n-BuLi, with paraformaldehyde gave alcohol **13** in 75% yield. It was then converted to bromide **14** with carbon tetrabromide and triphenylphosphine. Alkylation of the ortho ester **15** (19) in THF with bromide **14** in the presence of n-BuLi in hexane gave poor yields. It was found that by carrying out the reaction in a mixture of HMPA and THF (1:5, v/v), the required product **16** was formed in excellent yield (20). Semi-hydrogenation of **16**

with P-2 nickel (21) followed by removal of THP ether from 17 and then oxidation of resulting alcohol 18 afforded the required aldehyde 12.



Scheme 2: Preparation of C-20 $^2H_3$-AA.

Condensation of the ylide, prepared from 1 by the reaction of lithium bis(trimethylsilyl) amide in THF: HMPA (4:1), with the aldehyde 12 under cis-olefination conditions (22) yielded the $^2H_3$-AA methyl ester 19 as the major product. Purification using silica gel chromatography afforded the deuterated methyl arachidonate as a single homogenous compound. Saponification of the methyl ester with 2 N LiOH gave the required C-20 $^2H_3$-AA 2. The structure of this compound was confirmed by a

combination of NMR, HPLC and GC/MS. The ^{1}H-NMR spectrum was identical to an authentic sample of AA except that the signal for the C-20 methyl group was absent from the spectrum. The compound eluted as a single peak on HPLC with the same retention volume as AA. The methyl ester of C-20-$^2H_3$-AA gave a single peak on capillary GC that eluted 0.2 sec ahead of the non-deuterated compound. The EI mass spectrum of $^2H_3$-AA methyl ester showed a molecular ion ($M^{+\cdot}$) at m/z 321 together with the fragmentation pattern similar to that observed for non-deterated AA. The negative ion chemical ionization (NICI) mass spectrum of the pentafluorobenzyl (PFB) derivative of **2** showed an intense ion at m/z 306 corresponding to the expected loss of $C_7F_5H_2$ (PFB) from the molecular ion.

In summary, an efficient method for the preparation of the deuterated phosphonium salt **1** has been developed. Its use in the synthesis of labelled AA has been described. The content of unlabelled AA was below the detection limit of NICI/MS methodology. The C-20 $^2H_3$-AA is now available for the biosynthesis of eicosanoids for use as internal standards in stable isotope dilution assays. The Wittig synthon **1** has proved to be particularly useful for the preparation of stable isotope analogs of the sulfidopeptide LTs. This work will be reported separately.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker AM 400 or an IBM NR 300. Chemical shifts (δ ppm) are reported relative to Me_4Si as an internal standard. Fast atom bombardment (FAB) mass spectra were obtained on a VG 70/250 double focusing magnetic sector instrument at a resolving power of 2,000. Accurate mass measurements were obtained in the EI mode at a resolving power of 10,000. GC/MS was carried out on a Nermag R-1010C quadrupole instrument interfaced to a Varian Vista gas chromatograph. Injections were made in the splitless mode on a SPB 5 fused silica capillary column (0.32 mm int. diam., 0.25 μm coating thickness, Supelco, Bellefonte, PA). Under standard GC conditions, the column was temperature programmed from 100 °C to 320 °C at 15 °C/min with a helium as carrier gas at a flow rate of 1 mL/min. Methane was used as the reagent gas for negative ion chemical ionization at an analyzer pressure of 6.4 x 10^{-6} Torr.

Flash chromatography was carried out on S/P silica gel 60 A°. Thin layer chromatography (TLC) was performed on Analtech silica gel GF uniplates. THF was distilled from sodium benzophenone ketyl immediately prior to use. HMPA and DMSO were vacuum distilled from CaH_2 and stored over 3 A^0 molecular sieves. Dry CH_2Cl_2 was obtained by distillation from P_2O_5. All other solvents were reagent grade and were used directly. Reactions were carried out under a dry N_2 atmosphere. The PFB derivative of AA was prepared using a published procedure (23). Dilithium tetrachlorocuprate solution was prepared by the method of Tamura et al. (24) by reaction of LiCl (0.2 mol) with $CuCl_2$ (0.1 mol) in THF (100 mL).

8-(Tetrahydropyran-2'-yloxy)oct-3-yn-1-ol **4.** To the magnetically stirred solution of hex-5-yn-1-THP ether **3** (3.64 g, 20 mmol) in THF (6 mL) was added a solution of n-BuLi (12.5 mL, 1.6 M, 20 mmol) at -78 °C. The reaction mixture was warmed to -20 °C and stirred at that temperature for 15 min. It was then re-cooled to -78 °C and treated dropwise with a solution of ethylene oxide (1.5 g, 35 mmol) in ether. The reaction mixture was slowly warmed to 25 °C, stirred at that temperature for 16 h. It was then quenched with a mixture of a saturated aqueous NaCl and ether (100 mL). The organic layer was separated and washed successively with 1M $CuSO_4$ solution (2x50 mL), water (25 mL), brine and dried ($MgSO_4$). Concentration of the solvents followed by chromatographic purification on a silica gel column using 20% ether in petroleum ether afforded **4** as an oil (3.7 g, 82%). NMR 4.54 (m, 1H, H-2'), 3.82 (m, 2H, H-1), 3.62 (m, 2H, H-8), 3.40 (m, 2H, H-6'), 2.32 (m, 2H, H-2), 2.04 (m, 2H, H-5), and 1.70 (m, 10H, H-6 , H-7, H-3' to H-5'). Mass spectrum (m/z) 226 ($M^{+\cdot}$), 209 (M-OH), 181 (M-CH_2CH_2OH), 125 (M-OTHP); high-resolution mass spectrum calculated for $C_{13}H_{22}O_3$ ($M^{+\cdot}$) 226.1562, found 226.1549.

1-O-t-Butyldiphenylsilyl-8-(tetrahydropyran-2'-yloxy)oct-3-yne **5.** Alcohol **4** (2.26 g, 10 mmol) in CH_2Cl_2 (30 mL) was stirred with 4-dimethylaminopyridine (2.44 g, 20 mmol) and t-butylchlorodiphenylsilane (3.6 g, 13 mmol) at 0 °C for 1 h. The reaction mixture was diluted with water (10 mL). The organic layer was separated, washed with brine and dried. Removal of the solvents and purification on a silica gel column using 5% ether in petroleum ether provided the pure compound **5** as a colorless oil (3.95 g, 84.7%). NMR 7.67 (d, 4H, J=7.6 Hz, aromatic), 7.39 (m, 6H, aromatic), 4.55 (brs, 1H, H-2'), 3.74 (m, 2H, H-1), 3.62 (m, 2H, H-8), 3.42 (m, 2H, H-6'), 2.32 (m, 2H, H-2), 2.12

(m, 2H, H-5), 1.48 to 1.72 (m, 10H, H-6, H-7, H-3' to H-5') and 1.02 (s, 9H, t-butyl); high resolution mass spectrum calculated for $C_{20}H_{23}O_2Si$ (MH-THP-C_4H_9) 323.1440, found 323.1470.

1-O-t-Butyldiphenylsilyl-8-(tetrahydropyran-2'-yloxy)oct-3Z-ene **6**. Lindlar catalyst (400 mg, $Pd/CaCO_3$, 5%) in hexane (15 mL) containing 3% pyridine was charged with H_2 gas for 15 min. The acetylene **5** (3.95 g, 8.5 mmol) in hexane (10 mL) was added and the hydrogenation was continued until the uptake of H_2 ceased (30 min, 190 mL). The catalyst was removed by filtration and washed with more hexane. The combined solution was washed with cold dilute HCl, aqueous sodium bicarbonate (5%), water and dried ($MgSO_4$). Evaporation of the hexane gave the required olefin **6** as an oil (3.9 g, 98%). NMR 7.66 (d, 4H, J=7.6 Hz, aromatic), 7.38 (m, 6H, aromatic), 5.39 (m, 2H, H-3 and H-4), 4.55 (brs, 1H, H-2'), 3.72 (m, 2H, H-1), 3.61 (m, 2H, H-8), 3.42 (m, 2H, H-6'), 2.32 (m, 2H, H-2), 2.12 (m, 2H, H-5), 1.48 to 1.70 (m, 10H, H-6, H-7, H-3' to H-5') and 1.02 (s, 9H, t-butyl). Mass spectrum (m/z) 409 (M^+-Bu), 325 (MH-THP-C_4H_9), 199 (Ph_2SiOH); high resolution mass spectrum calculated for $C_{25}H_{33}O_3Si$ (M^+-Bu) 409.2248, found 409.2224.

1-O-t-Butyldiphenylsilyloct-3Z-en-8-ol **7**. A mixture of THP derivative **6** (3.9 g, 8.37 mmol) in acetic acid: THF: water (35 mL, 3:2:2) was magnetically stirred for 12 h at 45 °C. The mixture was cooled, diluted with water (10 mL) and extracted with CH_2Cl_2 (3x50 mL). The combined extracts were washed sequentially with H_2O (3x10 mL), saturated aqueous $NaHCO_3$ (3x10 mL), brine and dried ($MgSO_4$). Concentration of the solvent followed by purification on a silica gel column yielded pure alcohol **7** as an oil (2.9 g, 90%). NMR 7.67 (d, 4H, J=7.6 Hz, aromatic), 7.38 (m, 6H, aromatic), 5.40 (m, 2H, H-3 and H-4), 3.65 (t, 2H, J=6.9 Hz, H-1), 3.61 (t, 2H, J=6.7 Hz, H-8), 2.31 (q, 2H, J=6.6 Hz, H-2), 2.01 (q, 2H, J=6.7 Hz, H-5), 1.38 to 1.52 (m, 4H, H-6 and H-7) and 1.02 (s, 9H, t-butyl). Mass spectrum (m/z) 325 (M^+-Bu), 307 (M-Bu-H_2O), 199 (Ph_2SiOH); high resolution mass spectrum calculated for $C_{20}H_{25}O_2Si$ (M^+-Bu) 325.1623, found 325.1605.

1-O-t-Butyldiphenylsilyl-8-O-p-toluenesulfonyloct-3Z-ene **8**. To a stirred solution of alcohol **7** (2.80 g, 7.3 mmol) in CH_2Cl_2 (20 mL) was added pyridine (1.52 mL, 18.6 mmol) and p-toluenesulfonyl chloride (2.7 g, 14 mmol) at 0 °C. The reaction mixture was stirred for 2 h and then quenched with H_2O (10 mL). It was extracted with ether

(3x50 mL). The combined ether extracts were washed with HCl (20 mL, 1N), 5% $NaHCO_3$ (20 mL), saturated NaCl (20 mL) and dried ($MgSO_4$). Evaporation of the solvent followed by purification on a silica gel column gave pure tosyl derivative **8** as an oil (3.19 g, 81%). NMR 7.78 (d, 2H, J=9.5 Hz, aromatic), 7.66 (d, 4H, aromatic), 7.34 (m, 8H, aromatic), 5.36 (m, 2H, H-3 and H-4), 3.98 (t, 2H, J=6.7 Hz, H-8), 3.64 (t, 2H, J=6.9 Hz, H-1), 2.41 (s, 3H, CH_3), 2.22 (q, 2H, J=6.6 Hz, H-2), 1.90 (q, 2H, J=6.7 Hz, H-5), 1.36 to 1.54 (m, 4H, H-6 and H-7) and 1.02 (s, 9H, t-butyl); high resolution mass spectrum calculated for $C_{27}H_{31}O_4SiS$ (M^+-Bu) 479.1711, found 479.1687.

1-O-t-Butyldiphenylsilyl-9-[$^2H_3$]non-3Z-ene **9**. To a solution of tosyl derivative **8** (3.19 g, 5.9 mmol.) in THF (20 mL) was added dropwise, with stirring, a solution of [$^2H_3$] methylmagnesium iodide (14.75 mL, 1 M, 14.75 mmol) and a catalytic amount of dilithium tetrachlorocuprate (59 μL,0.059 mmol) at 0^0C. After stirring for 4 h at room temperature the reaction mixture was diluted with brine and extracted with ethyl acetate. The organic extracts were washed with water, dried ($MgSO_4$) and concentrated. Purification on a silica gel column using hexane provided pure [$^2H_3$] compound **9** as an oil (1.7 g, 75.2%). NMR 7.70 (d, 4H, aromatic), 7.38 (m, 6H, aromatic), 5.38 (m, 2H, H-3 and H-4), 3.62 (t, 2H, J=6.9 Hz, H-1), 2.31 (q, 2H, J=6.6 Hz, H-2), 2.05 (q, 2H, J=6.6 Hz, H-5), 1.28 (m, 6H, H-6 to H-8) and 1.02 (s, 9H, t-butyl); high resolution mass spectrum calculated for $C_{21}H_{24}O^2H_3Si$ (M^+-Bu) 326.2019, found 326.2025.

9-[$^2H_3$]Non-3Z-en-1-ol **10**. To a magnetically stirred solution of **9** (1.6 g, 4.2 mmol) in THF (20 mL) was added tetrabutylammonium fluoride (5.04 mL, 1 M, 5.04 mmol). The reaction mixture was stirred for 2 h (TLC monitoring) and diluted with ether and brine. The ether layer was separated and the aqueous solution was extracted with more ether (20 mL). The combined ether extracts were washed with water and dried ($MgSO_4$). Concentration and purification on a silica gel column using 10% ethyl acetate in hexane provided the alcohol **10** as a colorless oil (560 mg, 92%). The corresponding protium compound was isolated previously as an oil (13,20,25). NMR 5.54 (m, 1H, H-3), 5.35 (m, 1H, H-4), 3.62 (q, 2H, J=6.9 Hz, H-1), 2.31 (q, 2H, J=6.9 Hz, H-2), 2.04 (q, 2H, J=6.9 Hz, H-5), 1.32 (m, 6H, H-6 to H-8); high resolution mass spectrum calculated for $C_9H_{13}{}^2H_3$ (M^+-H_2O) 127.1440, found 127.1435. Relative intensities of [M^+-H_2O] cluster: m/z 124, < 0.05% ($^2H_0$); m/z 125, 2.05% ($^2H_1$); m/z 126, 7.9% ($^2H_2$); m/z 127, 90% ($^2H_3$).

1-Bromo-9-[$^2H_3$]non-3Z-ene **11.** A solution of alcohol **10** (2.90 g, 20 mmol) in CH_2Cl_2 (25 mL) was stirred magnetically and treated with CBr_4 (8.3 g, 25 mmol). The solution was then cooled (-40 °C) and treated with triphenylphosphine (6.25 g, 23 mmol) in four portions (15 min). The mixture was warmed slowly to 25 °C over 1.5 h. The solvent was then removed in vacuo and the purification of the residue on silica gel yielded the required bromide **11** as an oil (3.97 g, 95%). The corresponding protium compound was synthesized previously but no physical characteristics were reported (20,25). NMR 5.50 (m, 1H, H-3), 5.33 (m, 1H, H-4), 3.34 (t, 2H, J=7.2 Hz, H-1), 2.59 (q, 2H, J=7.1 Hz, H-2), 2.01 (q, 2H, J=7.1 Hz, H-5), 1.25 to 1.34 (m, 6H, H-6 to H-8); high resolution mass spectrum calculated for $C_9H_{14}{}^2H_3Br$ ($M^{+\cdot}$) 207.0681, found 207.0688. Relative intensities of $M^{+\cdot}$ cluster: m/z 204, < 0.05% ($^2H_0$); m/z 205, < 0.05% ($^2H_1$); m/z 206, 13.7% ($^2H_2$); m/z 207, 86.3% ($^2H_3$).

9-[$^2H_3$](non-3Z-en-1-yl)triphenylphosphonium bromide **1.** A solution of the bromide **11** (3.97 g, 19.0 mmol) and triphenylphosphine (5.47 gm, 20.3 mmol) in toluene (20 mL) was heated in an oil bath for 40 h at 80 °C. The reaction mixture was cooled to room temperature so that the phosphonium salt separated as an oil. The toluene was removed and discarded. The residue was stirred with dry ether overnight. Ether was then removed and the residue was washed three more times with ether to yield a thick oil, which was crystallized from CH_2Cl_2 and ether as white powder (5.36 g, 59%), m.p. 91-93 °C. The corresponding protium compound was synthesized previously but no physical characteristics were reported (20,25). NMR 7.75 (m, 15H, aromatic), 5.52 (m, 1H, H-3), 5.44 (m, 1H, H-4), 3.86 (m, 2H, H-1), 2.41 (m, 2H, H-2), 1.22 and 1.72 (m, 6H, H-6 to H-8). Positive FAB mass spectrum (m/z) 390 $[M-Br]^+$.

5-(Tetrahydropyran-2'-yloxy)pent-2-yn-1-ol **13.** To the magnetically stirred solution of but 3-yn-1-OTHP (5.10 g, 33.1 mmol) in THF (20 mL) was added dropwise a solution of n-butyllithium (21.2 mL, 1.52 M, 32.4 mmol) at -78°C over a period of 15 min. The reaction mixture was warmed to -20°C and stirred at that temperature for 15 min. The reaction mixture was re-cooled to -78°C and paraformaldehyde (1.0 g, 33.5 mmol) was added in one portion. The mixture was slowly warmed to 25 °C and stirred at that temperature for 1 h, then quenched with a mixture of ice (100 g) and ether (150 mL). The organic phase was washed sequentially with 1 M aqueous $CuSO_4$ (20 mL), water (20 mL), brine (50 mL) and dried. Removal of the solvent followed by column

chromatography on silica gel (20% ethyl acetate in hexane) afforded pure **13** (5.2 g, 85%) as an oil (26). NMR 4.58 (m, 1H, H-2'), 4.22 (m, 2H, H-1), 3.80 (m, 2H, H-5), 3.47 (m, 2H, H-6'), 2.50 (m, 2H, H-4) and 1.58 to 1.72 (m, 6H, H-3' to H-5').

1-Bromo-5-(tetrahydropyran-2'-yloxy)pent-2-yne **14.** A solution of the alcohol **13** (4.6 g, 25 mmol) in CH_2Cl_2 (25 mL) was stirred magnetically and treated with CBr_4 (10.37 g, 31.25 mmol). The solution was then cooled (-40°C) and Ph_3P (7.86 g, 30 mmol) was added in portions over 15 min. The mixture was warmed slowly to 25 °C over 2 h. The mixture was concentrated and chromatographed on a silica gel column (10% ethyl acetate in hexane) affording pure bromide **14** (4.2 g, 68.8%) as a colorless oil (26). NMR 4.59 (m, 1H, H-2'), 3.90 (m, 2H, H-1), 3.82 (m, 2H, H-5), 3.48 (m, 2H, H-6'), 2.51 (m, 2H, H-4) and 1.58 to 1.78 (m, 6H, H-3' to H-5').

Methyl 11-(tetrahydropyran-2'-yloxy)undeca-5,8-diynoate **16.** To the magnetically stirred solution of ortho ester **15** (1.72 g, 10 mmol) in THF:HMPA (9 mL, 5:1) was added n-BuLi (6.7 mL, 10 mmol) at -78 °C over a period of 20 min. The temperature was raised to -25 °C and the reaction mixture was stirred at that temperature for 15 min. Cuprous iodide (950 mg, 5 mmol) was then added. After stirring for 10 min a solution of bromide **14** (2.70 g, 11 mmol) in THF (2 mL) was added dropwise and the reaction was stirred at room temperature for 2 h. It was then quenched with ice and ether (100 mL). The ether solution was washed sequentially with aqueous $CuSO_4$ solution (2x15 mL), water (15 mL), brine (20 mL) and dried. Removal of the solvent and purification of the residue on silica gel column yielded pure **16** (1.81 g, 62%) as an oil. NMR 4.60 (m, 1H, H-2'), 3.82 (m, 2H, H-11), 3.67 (s, 3H, $COOCH_3$), 3.48 (m, 2H, H-6'), 3.08 (m, 2H, H-7), 2.48 (m, 4H, H-2 and H-10), 2.22 (m, 2H, H-4) and 1.52 to 1.78 (m, 8H, H-3, H-3'to H-5').

Methyl 11-(tetrahydropyran-2'-yloxy)undeca-5Z,8Z-dienoate **17.** $NiOAc.4H_2O$ (680 mg, 1.6 mmol) was added to a 100 mL flat-bottomed hydrogenation vessel equipped with a sidearm inlet and a magnetic stirring bar. Methanol (20 mL) was added and the flask purged three times with H_2 gas. $NaBH_4$ (75 mg, 1.76 mmol) in NaOH (8 mL, 0.2 M) was added in one portion, leading to instantaneous gas evolution and the formation of a thick black precipitate. After gas evolution had subsided, 0.8 mL of ethylenediamine was added and the mixture stirred for 5 min. Stirring was stopped, diyne **16** (1.60 g, 4 mmol) in methanol (2 mL) was added and hydrogenation was continued while gas uptake

was monitored. After consumption of 8 mmol, 180 mL of H_2, the reaction was stopped, the mixture was filtered through celite and the filtrate was washed with ethyl acetate (50 mL). The organic layer was then sequentially washed with HCl (2 N), water (20 mL), brine (20 mL) and dried. Concentration of organic solvent followed by purification on a silica gel column yielded the diene **17** (0.88 g, 75%) as an oil. NMR 5.36 (m, 4H, olefinic H), 4.58 (m, 1H, H-2'), 3.80 (m, 2H, H-11), 3.66 (s, 3H, $COOCH_3$), 3.47 (m, 2H, H-6'), 2.78 (m, 2H, H-7), 2.36 (m, 4H, H-2 and H-10), 2.10 (m, 2H, H-4) and 1.46 to 1.72 (m, 8H, H-3, H-3'to H-5').

Methyl 11-hydroxyundeca-5Z,8Z-dienoate **18.** THP ether **17** (735 mg, 25 mmol) in methanol (4 mL) was stirred with AG50-X8 (3.5 g) for 2 h. The resin was removed by filtration and washed with methanol (10 mL). Concentration of the solvent and purification by silica gel chromatography (30% ethyl acetate in hexane) gave pure alcohol **18** (420 mg, 80%) as an oil. NMR 5.50 (m, 1H, H-9), 5.38 (m, 3H, H-5, H-6 and H-8), 3.68 (m, 5H, H-11 and $COOCH_3$), 2.79 (m, 2H, H-7), 2.32 (m, 4H, H-2 and H-10), 2.10 (m, 2H, H-4) and 1.68 (m, 2H, H-3); high resolution mass spectrum calculated for $C_{12}H_{18}O_2$ ($M^{+\cdot}$-H_2O) 194.1306, found 194.1341.

Methyl 11-oxoundeca-5Z,8Z-dienoate **12.** To a stirred solution of alcohol **18** (420 mg, 2.0 mmol) in benzene:DMSO (13 mL, 10:1 v/v) was added DCC (1.2 g, 6.0 mmol) followed by dichloroacetic acid (0.5 mL). A thick white precipitate was obtained (10 min). Oxalic acid (560 mg) in methanol (1.5 mL) was then added dropwise and the reaction mixture was stirred for a further 10 min. It was then quenched with water, extracted with ethyl acetate and dried. Concentration of the solvent and then purification on a silica gel column using 10% ethyl acetate in hexane gave pure very unstable aldehyde **12** as an oil (320 mg, 75%). NMR 9.61 (s, 1H, CHO), 5.58 (m, 1H, H-9), 5.34 (m, 3H, H-5, H-6 and H-8), 3.70 (m, 2H, H-10), 3.67 (s, 3H, $COOCH_3$), 2.76 (m, 2H, H-7), 2.32 (m, 2H, H-2), 2.08 (m, 2H, H-4) and 1.68 (m, 2H, H-3).

Methyl 20-[$^2H_3$]5Z,8Z,11Z,14Z-eicosatetraenoate (arachidonic acid methyl ester) **19.** To a suspension of **1** (470 mg, 1 mmol) in THF: HMPA (2 mL, 4:1) was added lithium hexamethyldisilazide (1 mL, 1 M in THF) at 0 ^{0}C and the mixture was stirred for 10 minutes when a deep orange color was obtained. It was then cooled to -78 ^{0}C and aldehyde **12** (105 mg, 0.5 mmol) in THF (1 mL) was added. The solution was allowed to warm to 0 ^{o}C and was stirred slowly for 1 h. The reaction was diluted with 25%

ammonium acetate and extracted with 3x30 mL ethyl acetate. The combined ethyl acetate extracts were washed with water and dried ($MgSO_4$). Concentration of the solvent followed by purification on a silica gel column gave pure methyl ester as an oil (92 mg, 60%). NMR 5.34 (m, 8H, olefinic H), 3.68 (s, 3H, $COOCH_3$), 2.78 (m, 6H, H-7, H-10 and H-13), 2.31 (m, 2H, H-2), 2.08 (m, 4H, H-4 and H-16) and 1.28 to 1.72 (m, 8H, H-3, H-17 to H-19).

20-[$^2H_3$]5Z,8Z,11Z,14Z-eicosatetraenoic acid (arachidonic acid) **2**. To a solution of the deuterated methyl arachidonate **19** (70 mg, 0.29 mmol) in dimethoxyethane (4 mL) was added LiOH (3 N, 750 μL) and the mixture was stirred at 60 °C for 2 h. The reaction mixture was acidified with HCl (1 N) and extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by reversed-phase HPLC afforded deuterated AA **2** as a colorless oil. The corresponding protium compound was isolated previously as an oil (27). The methyl ester prepared from **2** was analyzed by GC/MS in the EI mode. Deuterium distribution was calculated from the relative intensities of the $M^{+\cdot}$ cluster as follows: m/z 318, < 0.05% ($^2H_0$); m/z 319, < 0.05% ($^2H_1$); m/z 320, 1.8% ($^2H_2$); m/z 321, 97.5% ($^2H_3$). Analysis of **2** as a PFB ester by GC/MS in the NICI mode showed a comparable isotope distribution for the corresponding $[M\text{-PFB}]^-$ ions at m/z 303-306.

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