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Synthesis of Methyl (5Z,8Z,11Z,14Z,17Z)-and (5Z,8Z,11Z,14Z,17E)-[18-^{14}C] Eicosapentaenoate

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Summary

In order to have a better insight of the effect of the double bond geometry of EPA on its anti-aggregating properties, methyl ester of [18-^{14}C] EPA and of its Δ17*t* isomer were synthesized from a common intermediate [(3Z,6Z,9Z)-12,12-diethoxydodeca-3,6,9-trienyl]triphenylphosphonium iodide **11** via three sequential Wittig olefination. Labelled methyl ester of EPA **16b** and EPAΔ17*t* **17b** were obtained in near 100% isomeric and radiochemical purities.

Keywords : n-3 Labelled fatty acids, Wittig reaction.

Introduction

Geometrical isomers of polyunsaturated fatty acids are formed during heat treatment of edible oils, particularly during deodorization[1,2] and deep frying processes[3]. Thus, α-linolenic acid, found in many vegetable oils, [4] is partially transformed during heat treatment to 18:3 Δ9*c*,12*c*,15*t* and Δ9*t*,12*c*,15*c* accompanied by variable amounts of 18:3 Δ9*t*,12*c*,15*t*.[1,2,5] Geometrical isomers of linolenic acid are subsequently present in human foods including low-calorie spreads,[6] margarines[7] and infant formulas.[8,9] Consequently, *trans* isomers of linolenic acid are found in human tissues like serum[10] and milk.[8,9] Animal experiments have shown that, when present in the diet, geometrical isomers of linolenic acid could be desaturated and elongated to isomers of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[11,12] These latter were detected in human platelets[13] suggesting that humans were able to desaturate and elongate *trans* isomers of linolenic acid. Studies

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on human platelets have highlighted that EPA Δ17*t* and DHA Δ19*t* exhibit different physiological effects than their respective *cis* analogs on platelet function, altering platelet aggregation.[14,15].

With the aim of having a better insight on the mechanism of action of EPA **16a** and EPA Δ17*t* **17a** on platelet function particularly by identifying their oxygenated metabolites, we synthesized their [^{14}C]-radiolabelled analogs. In order to avoid the loss of radioactivity during the catabolism of these labelled fatty acids through β-oxidation which involves an enzymatic reaction sequence by which fatty acids are oxidized in such way that acetates are split off successively from the carboxyl end of the molecule, [16] the labelling was effected on the C-18 position far away from the carboxylic function.

Consequently, the strategy of synthesis of fatty acids **16b,17b** was dictated by the position of the labelling and by the fact that the incorporation of the isotope must be effected near the end of the synthetic sequence.

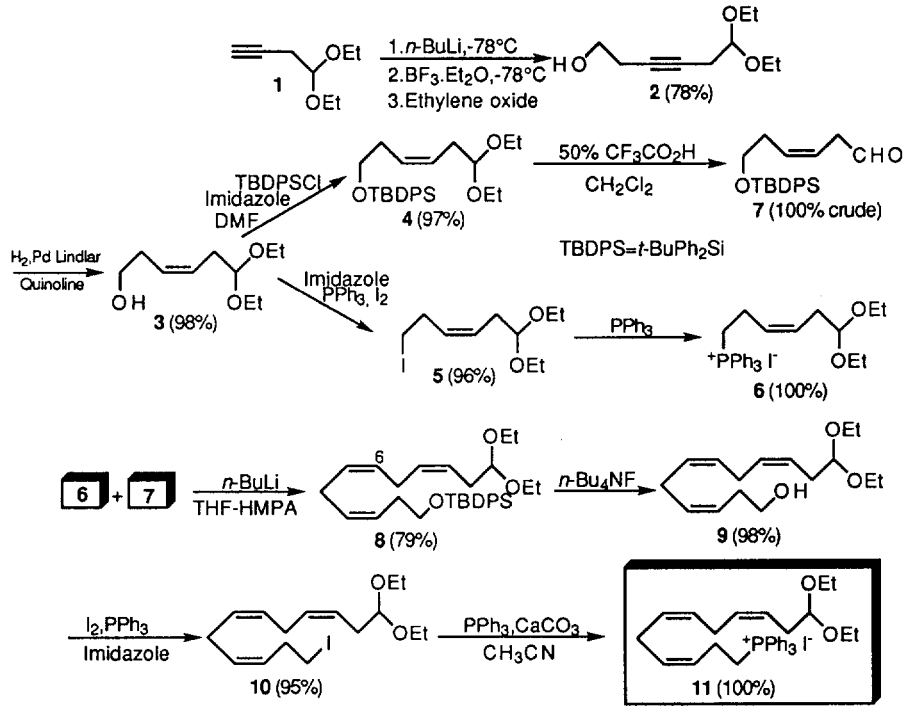
The main features of the preparation of labelled polyunsaturated fatty acids **16b,17b** are : (1) elaboration of (1,4)-diene units by highly stereoselective Wittig reactions ; (2) use of a common C-3 labelled precursor **15b** ; (3) preparation of the advanced intermediate **11** from only one precursor, compound **3** ; (4) efficient synthesis of C_3 [1-^{14}C] labelled phosphonium salt **15b**.

Results and discussion

As depicted in Scheme 1, synthesis of the methyl ester of labelled EPA and its Δ17*t* isomer **16b,17b** commenced by hydroxyethylation of 1,1-diethoxyprop-3-yne **1**[17] with ethylene oxide in the presence of boron trifluoride etherate[18] to give **2** in 78% yield. (Z)-Stereoselective semihydro genation of the triple bond of compound **2** in the presence of Lindlar catalyst and quinoline, followed by protection of the alcohol function of the resulting **3** as its *t*-butyldiphenylsilyl ether[19] yielded **4** in 95% overall yield. Alcohol **3** was also transformed in two steps to phosphonium salt **6** through the iodide[20] **5** in 96% yield.

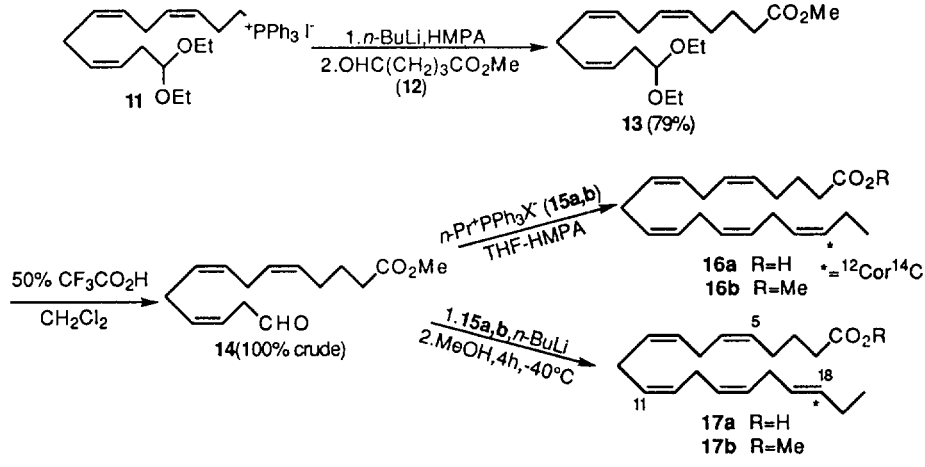
The stage was now set up for the installation of Δ^{11} unsaturation of **16b** and **17b**. To this goal, treatment of acetal **4** by aqueous trifluoroacetic acid afforded quantitatively the crude homoconjugated aldehyde **7**, which was condensed with the corresponding ylide of **6**, under *cis*-olefination conditions[21], to give as a sole product the triene **8** in 79% yield. The chemical shifts for C5 and C8 of compound **8** which appeared at 25.85 and 25.71 ppm in the ^{13}C NMR spectrum supported the configuration assigned for the newly formed double bond (Δ^6 unsaturation).[22] Transformation of the silylated ether **8** to phosphonium salt **11** was effected in three steps by standard functional group manipulations in 90% overall yield.

According to Scheme 2, introduction of the C1-C5 fragment bearing the acid function and of the (Z)-Δ^5 unsaturation was done by stereoselective Wittig reaction between the ylide of the trienic phosphonium salt **11** and methyl 5-oxopentanoate **12**, prepared from commercially available methyl-4-(chloroformyl)butyrate by Rosenmund reduction procedure,[23] provided the C_{17} tetraenic ester **13** in 79% yield. Acidic hydrolysis of the acetal **13** to homoconjugated aldehyde **14** set the stage for the introduction of a ^{14}C atom via a Wittig reaction. This reaction, which can give rise either to *Z* or *E* double bonds, depending on the experimental conditions, was first realized with the unlabelled phosphonium salt **15a**.



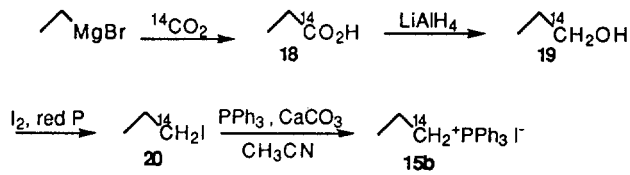
Scheme 1. Preparation of the common precursor (**11**) of Labelled EPA and EPA Δ17*t* synthesis.

Uneventfully, *cis*-olefination reaction between aldehyde **14** and ylide **15a**, in the presence of HMPA,[21] furnished natural EPA as its methyl ester in 43% yield and 94% isomeric purity. By contrast the (*E*)-stereoselective Wittig reaction was troublesome and needed extensive experiments before finding that the Henrick procedure,[24] which involves controlled partial equilibration of the intermediate adducts, obtained by addition of methanol at -40°C, gave the best *E*/*Z* ratio (85/15).



Scheme 2. Synthesis of methylester of [18-^{14}C]EPA and [18-^{14}C]EPA Δ17*t* from **11**.

Having devised satisfactory experimental conditions to introduce (*Z*) or (*E*)-Δ^{18} unsaturation by the Wittig reaction, we turned our attention to the synthesis of the [1-^{14}C] labelled phosphonium salt **15b** (Scheme 3).To accomplish this objective, ethylmagnesium bromide was carbonated by $^{14}CO_2$ in ether and the resulting acid **18** was reduced with $LiAlH_4$ to give labelled propanol **19** contaminated by 1-octanol used as a proton source. Partial conversion of the alcohol **19** to iodide was effected at 70°C in the presence of iodine and red phosphorus. Heating a 2:1 mixture of **20** and **19** in the presence of triphenylphosphine gave pure phosphonium salt **15b** in 37% yield from $Ba^{14}CO_3$, after purification by chromatography on silica gel.



Scheme 3. Synthesis of [1-^{14}C] propyltriphenylphosphonium iodide (**15b**).

Synthesis of labelled EPA **16b** and EPA Δ17*t* **17b** methyl ester from aldehyde **14** was realized according to the protocol described for the cold synthesis except that the ratio aldehyde/phosphonium salt was different in order to improve the radioactive yield. The methyl esters of [18-^{14}C] EPA and [18-^{14}C] EPA Δ17*t* were thus obtained in low chemical overall yield but in stereoisomerically pure form and 99% radiochemical purity. Labelled EPA Δ17*t* **17b** was purified by reversed-phase HPLC chromatography using as a mobile phase water-acetonitrile (23/77) (Figure 1). In this case, the use of silver resin chromatography was unnecessary.

In summary, we have developed an efficient and concise synthetic sequence for the preparation of isomerically pure [18-^{14}C] EPA and its EPA Δ17*t* isomer from a common advanced precursor **14**, obtained in 13 steps from the readily available 1,1-diethoxyprop-3-yne **1**. Other applications of the interesting building block **11** (10 steps from **1**, 52% yield) in the field of labelled or not polyunsaturated fatty acids synthesis are currently underway in our laboratory.

Experimental

General. Starting materials and chemical reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis. Barium [^{14}C] carbonate was purchased from Slavia (Russia). Silica gel (35-70 mesh) was purchased from Amicon (Lausanne, Switzerland). All solvents were purified before use : acetonitrile, dichloromethane, dimethylformamide, hexamethylphosphoramide and hexane were distilled from calcium hydride ; diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl ; methanol was distilled from magnesium metal. Boron trifluoride etherate was distilled just before use from calcium hydride. NMR spectra were measured on a Bruker AC 200 or AM 400 Fourier spectrometers or Varian EM 360 spectrometer with proton observation at 60, 200 or

300 MHz and carbon observation at 50 or 75 MHz. Unless otherwise stated spectra were recorded in $CDCl_3$ and chemical shifts were reported (in ppm) downfield from TMS (δ). IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer. Gas chromatography analyses were effected on a OV 1701 capillary column (25m x 0.32 mm i.d.) using a Varian 3300 gas chromatograph (N_2, 2 ml/min) fitted with a flame detector. Radiochemical purity was determined on thin layer chromatography with a Berthold Model LB 2821 and by HPLC (Merck L 6200 system) on a Zorbax ODS semi-preparative reversed phase column using a Merck Model L 4250 UV-detector. Specific activities were measured with a Finnigan Model 4600 mass spectrometer. Combustion analyses were performed by the Service Central de Microanalyse, CNRS (Solaize, France). Unless otherwise stated, all experiments were conducted under anhydrous conditions in an atmosphere of nitrogen.

1,1-Diethoxyhex-3-yn-6-ol (2). To a solution of 1,1-diethoxyprop-3-yne **1** (0.25 g, 1.8 mmol) in THF (3 ml), cooled to -78°C, was added *n*-BuLi (2.4 M in hexanes, 0.75 ml, 1.8 mmol). After stirring the solution for 10 min at -78°C, $BF_3.Et_2O$ (0.25 ml, 1.95 mmol) was added. The solution was stirred for 10 min at -78°C and ethylene oxide (1 ml, 20 mmol) was added. After stirring the solution for 1 h at -78°C, Et_2O was added then saturated NH_4Cl solution. The organic layer was washed once with saturated $NaHCO_3$ solution then water. The ethereal layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash chromatography (Et_2O-petroleum ether, 1:4 then 1:1) to give the homopropargylic alcohol **2** as an oil (0.26 g, 78% yield). IR (neat) 3560-3200, 1120, 1060 ; ^{1}H NMR (60 MHz) 1.2 (t, 6H, J=7Hz, $2CH_3$), 2.3-2.9 (m, 5H, OH, $C\underline{H}_2$-C≡C-$C\underline{H}_2$), 3.4-3.9 (m, 6H, $2C\underline{H}_2CH_3$, $C\underline{H}_2OH$), 4.7 (t, 1H, J=6Hz, $C\underline{H}(OEt)_2$).

(*Z*)-1,1-Diethoxyhex-3-en-6-ol (3). To a solution of compound **2** (0.465 g, 2.5 mmol) in hexane (14 ml) were added quinoline (0.1 ml) and Lindlar catalyst (0.1 g) and the suspension was vigorously stirred under H_2 at room temperature and atmospheric pressure. After an uptake of 56 ml of hydrogen (1 h), the reaction was stopped, the mixture was filtered on a pad of celite and the filtrate concentrated. The residue was chromatographed on silica gel (Et_2O-petroleum ether, 1:4) to give **3** as an oil (0.46g, 98% yield). IR (neat) 3560-3200, 3020, 1660, 1120, 1060 ; ^{1}H NMR (60MHz) 1.1 (t, 6H, J=7Hz, $2CH_3$), 2.1-2.5 (m, 5H, OH, $C\underline{H}_2$-CH=CH-$C\underline{H}_2$), 3.3-3.8 (m, 6H, $2C\underline{H}_2CH_3$,$C\underline{H}_2OH$), 4.5 (t, 1H, J=5Hz, $C\underline{H}(OEt)_2$), 5.4-5.7 (m, 2H, $C\underline{H}$=$C\underline{H}$) ; ^{13}C NMR (50MHz) 15.3, 31.0, 32.3, 61.7, 61.9, 102.4, 126.9, 128.4. Anal. Calcd for $C_{10}H_{20}O_3$: C, 63.79 ; H, 10.71. Found : C, 63.47 ; H, 10.71.

(*Z*)-6-(t-Butyldiphenylsilyloxy)-1,1-diethoxyhex-3-ene (4). To a solution of the alcohol **3** (2.05 g, 10.9 mmol) in DMF (50 ml) was added imidazole (1.5 g, 22 mmol) then *t*-butyldiphenyl chlorosilane (3.1 ml, 11.9 mmol). After stirring for 30 min at room temperature, the reaction mixture was diluted with Et_2O and washed with water. After concentration *in vacuo*, the residue was purified by chromatography on silica gel (Et_2O-petroleum ether, 5:95) to give **4** as an oil (4.51 g, 97% yield). IR (neat) 3060, 3040, 3020, 1660, 1585, 1110, 1085, 1060 ; ^{1}H NMR (200MHz) 1.0 (s, 9H, *t*-BuSi), 1.2 (t, 6H, J=7Hz, $2CH_3$), 2.3-2.4 (m, 4H, $C\underline{H}_2$-CH=CH-$C\underline{H}_2$), 3.4-3.7 (m, 6H, $C\underline{H}_2CH_3$,$C\underline{H}_2OSi$), 4.45 (t, 1H, J=5.8Hz, $C\underline{H}(OEt)_2$), 5.4-5.56 (m, 2H, $C\underline{H}$=$C\underline{H}$) ; 7.3-7.5 (m, 6H, Ph), 7.6-7.7 (m, 4H, Ph). ^{13}C NMR (50MHz) 15.3, 19.2, 26.8, 31.1, 32.1, 61.1, 63.5, 102.5, 125.7, 127.6, 128.2, 129.5, 133.9, 135.6. Anal. Calcd for $C_{26}H_{38}O_3Si$: C, 73.19 ; H, 8.98. Found : C, 73.16 ; H, 9.03.

***(Z)*-1,1-Diethoxy-6-iodohex-3-ene (5).** To a stirred solution of compound **3** (0.435 g, 2.3 mmol) in THF (30 ml) was successively added triphenylphosphine (0.9 g, 3.4 mmol), and imidazole (0.47 g, 6.9 mmol). The solution was cooled to -30°C and iodine (0.88 g, 3.4 mmol) was added. The solution was allowed to warm up to room temperature, diluted with ether, washed with saturated bicarbonate solution, then water. The ethereal layer was dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (Et_2O-petroleum ether, 2:98) to afford the iodide **5** as an oil (0.66 g, 96% yield). IR (neat) 3010, 1650, 1120, 1060 ; ^{1}H NMR (200MHz) 1.1 (t, 6H, J=7Hz, $2CH_3$), 2.4 (t, 2H, J=6Hz, $C\underline{H}_2CH(OEt)_2$), 2.6 (q, 2H, J=7Hz, $C\underline{H}_2CH_2I$), 3.15 (t, 2H, J=7Hz, CH_2I), 3.4-3.75 (m, 4H, $C\underline{H}_2CH_3$), 4.5 (t, 1H, J=6Hz, $C\underline{H}(OEt)_2$), 5.4-5.65 (m, 2H, $C\underline{H}$=$C\underline{H}$) ; ^{13}C NMR (50MHz) 5.0, 15.3, 31.7, 32.4, 61.4, 102.3, 126.6, 130.3.

[*(Z)*-6,6-Diethoxyhex-3-en-1-yl]triphenylphosphonium iodide (6). A mixture of **5** (4.14 g, 13.9 mmol), triphenylphosphine (7.3 g, 27.8 mmol) and calcium carbonate (0.75 g) in acetonitrile (30 ml) was heated at 40°C for 3 days. After filtration of calcium carbonate, the filtrate was concentrated. The residue was diluted in a minimum of dichloromethane and ether was added to precipitate the phosphonium salt **6**. The precipitate was filtered, washed twice with ether and dried (7.78 g, 100% yield). IR (KBr) 3040, 3020, 1585, 1110, 1055 ; ^{1}H NMR (200MHz) 1.1 (t, 6H, J=7Hz, $2CH_3$), 2.2 (t, 2H, J=6Hz, $C\underline{H}_2CH(OEt)_2$), 2.35-2.55 (m, 2H, $C\underline{H}_2CH_2P$), 3.5-3.7 (m, 4H, $2C\underline{H}_2CH_3$) ; 3.7-3.85 (m, 2H, $C\underline{H}_2P$), 4.4 (t, 1H, J=5.6Hz, $C\underline{H}(OEt)_2$), 5.4-5.5 (m, 1H, $C\underline{H}$=CH), 5.7-5.85 (m, 1H, $C\underline{H}$=CH), 7.6-7.9 (15H, Ph) ; ^{13}C NMR (50MHz) 15.4, 20.5 (d, J=3.4Hz), 23.2 (d, J=48.5Hz), 32.7, 62.1, 102.3, 117.9 (d, J=85.8Hz), 126.8, 128.6 (d, J=15.3Hz), 130.7 (d, J=12.5Hz), 133.7 (d, J=10Hz), 135.3 (d, J=2.9Hz). Anal. Calcd for $C_{28}H_{34}O_2PI$: C, 60.0 ; H, 6.12. Found : C, 60.08 ; H, 6.15.

***(3Z,6Z,9Z)*-12-(t-Butyldiphenylsilyloxy)-1,1-diethoxydodeca-3,6,9-triene (8).** To a solution of the phosphonium salt **6** (2.65 g, 4.8 mmol) in THF (23 ml) cooled to -78°C, was added *n*-BuLi (2.3M in hexanes, 2.1 ml, 4.8 mmol). The reaction mixture was stirred for 30 min at -45°C. The solution was cooled down to -78°C and HMPA (3 ml) was added. After stirring the solution for 30 min at -78°C, (Z)-6-(*t*-butyldiphenylsilyloxy)hex-3-enal **7** (1.73 g, 4.9 mmol), obtained by hydrolysis of the acetal **4**,[26] in THF (7 ml) was added. The reaction mixture was allowed to warm up to room temperature and a mixture of petroleum ether/Et_2O (30 ml, 3:1, v/v) was added. After filtration of triphenylphosphine oxide, the filtrate was concentrated. The residue was purified by chromatography on silica gel (Et_2O-petroleum ether, 5:95) to yield the triene **8** as an oil (1.91 g, 79% yield). IR (film) 3060, 3040, 3020, 1585, 1110, 1085, 1060 ; ^{1}H NMR (200MHz) 1.05 (s, 9H, *t*-BuSi), 1.2 (t, 6H, J=7Hz, $2CH_3$), 2.3-2.45 (m, 4H, $C\underline{H}_2$-$CH(OEt)_2$, $C\underline{H}_2CH_2OSi$), 2.75-2.85 (m, 4H, 2CH=CH-$C\underline{H}_2$-CH=CH), 3.4-3.7 (m, 6H, $C\underline{H}_2CH_3$,CH_2OSi), 4.5 (t, 1H, J=5.8Hz, $C\underline{H}(OEt)_2$), 5.3-5.5 (m, 6H, $3C\underline{H}$=$C\underline{H}$), 7.3-7.4 (m, 6H, Ph), 7.6-7.7 (m, 4H, Ph). ^{13}C NMR (50MHz) 15.3, 19.2, 25.7, 25.8, 26.8, 30.8, 32.1, 61.2, 63.5, 102.4, 124.3, 126.3, 127.6, 127.9, 128.3, 129.5, 130.1, 133.9, 135.6. Anal. Calcd for $C_{32}H_{46}O_3Si$: C, 75.84 ; H, 9.15. Found : C, 75.73 ; H, 9.22.

***(3Z,6Z,9Z)*-1,1-Diethoxydodeca-3,6,9-trien-12-ol (9).** To a solution of compound **8** (1.91 g, 3.8 mmol) in THF (20 ml) was added at room temperature tetra-*n*-butylammonium fluoride (1M in THF, 7.6 ml, 7.6 mmol). After stirring the solution for 30 min at room temperature, ether was added and the organic layer was washed with water. The ethereal layer was dried (Na_2SO_4) and

concentrated *in vacuo*. The residue was chromatographed on silica gel (Et_2O-petroleum ether, 1:1) to give **9** as an oil (1.0 g, 98% yield). IR (film) 3500-3200, 3010, 1650, 1120, 1060 ; ^{1}H NMR (200MHz) 1.2 (t, 6H, J=7.1Hz, $2CH_3$), 1.7 (s, 1H, OH), 2.3-2.5 (m, 4H, $C\underline{H}_2CH(OEt)_2$), 2.8-2.9 (m, 4H, $2CH{=}CH\text{-}C\underline{H}_2\text{-}CH{=}CH$), 3.4-3.7 (m, 6H, $2C\underline{H}_2CH_3$, $2C\underline{H}_2OH$), 4.5 (t, 1H, J=5.8Hz, $CH(OEt)_2$), 5.4-5.6 (m, 6H, $3C\underline{H}{=}C\underline{H}$) ; ^{13}C NMR (50MHz) 15.3, 25.8, 25.9, 30.9, 32.1, 61.3, 62.1, 102.5, 124.3, 125.8, 128.10, 128.15, 130.1, 130.7.

***(3Z,6Z,9Z)*-1,1-Diethoxy-12-iodododeca-3,6,9-triene (10).** A mixture of **9** (0.79 g, 2.94 mmol), triphenylphosphine (0.93 g, 3.5 mmol), imidazole (0.48 g, 7.1 mmol) and iodine (1.09 g, 4.3 mmol) in THF (30 ml) was stirred for 1 h at room temperature. After an usual work-up, chromatography on silica gel of the residue (Et_2O-petroleum ether, 2:98) gave **10** as an oil (1.06 g, 95% yield). IR (film) 3010, 1650, 1120, 1060 ; ^{1}H NMR (200MHz) 1.2 (t, 6H, J=7.1Hz, $2CH_3$), 2.4 (t, 2H, J=5.7Hz, $C\underline{H}_2CH(OEt)_2$), 2.65 (q, 2H, J=7Hz, $C\underline{H}_2CH_2I$), 2.75-2.9 (m, 4H, $2CH{=}CH\text{-}C\underline{H}_2\text{-}CH{=}CH$), 3.15 (t, 2H, J=7Hz, $C\underline{H}_2I$), 3.45-3.75 (m, 4H, $2C\underline{H}_2CH_3$), 4.5 (t, 1H, J=5.8Hz, $C\underline{H}(OEt)_2$), 5.3-5.6 (m, 6H, $3CH{=}CH\text{-}C\underline{H}_2\text{-}CH{=}CH$) ; ^{13}C NMR (50MHz) 5.2, 15.4, 25.87, 25.93, 32.2, 33.5, 61.3, 102.5, 124.5, 127.7, 128.4, 130.0, 130.4. Anal. Calcd for $C_{16}H_{27}O_2I$: C, 50.80 ; H, 7.19. Found : C, 50.73 ; H, 7.17.

[*(3Z,6Z,9Z)*-12,12-Diethoxydodeca-3,6,9-trienyl]triphenylphosphonium iodide (11) A solution of the iodide **10** (0.915 g, 2.4 mmol) in acetonitrile (5.5 ml) containing triphenylphosphine (1.27 g, 4.8 mmol) was heated for 3 days at 50°C in the presence of calcium carbonate (0.13 g). After filtration, the filtrate was concentrated and the residue was chromatographed on silica gel (CH_2Cl_2-MeOH, 95:5) to give the phosphonium salt **11** as a foam (1.54 g, 100% yield). IR (film) 3050, 3010, 1585, 1110, 1060 ; ^{1}H NMR (200MHz) 1.2 (t, 6H, J=7.1Hz, $2CH_3$), 2.3-2.7 (m, 8H, $2CH{=}CH\text{-}C\underline{H}_2\text{-}C{=}CH$, $C\underline{H}_2$, $CH(OEt)_2$,$C\underline{H}_2CH_2P$),3.4-3.8 (m, 6H, $2C\underline{H}_2CH_3$, CH_2P), 4.45 (t, 1H, J=5.7Hz, $C\underline{H}(OEt)_2$), 5.1-5.5 (m, 5H,$C\underline{H}{=}C\underline{H}$), 5.55-5.7 (m, 1H, $C\underline{H}{=}C\underline{H}$), 7.6-8.0 (m, 15H, Ph) ; ^{13}C NMR (75MHz) 15.4, 20.4 (d, J=3.4Hz), 23.4 (d, J=48.9Hz), 25.7, 25.9, 32.3, 61.6, 102.6, 117.9, 124.6, 126.5 (d, J=14.6Hz), 127.3, 128.6, 129.7, 130.7, (d, J=12.6Hz), 133.8, (d, J=10Hz), 135.3 (d, J=2.7Hz).

Methyl (*5Z,8Z,11Z,14Z*)-17,17-diethoxyheptadeca-5,8,11,14-tetraenoate (13). To a solution of the phosphonium salt **11** (0.61 g, 0.95 mmol) in THF (5 ml), cooled to -78°C, was added *n*-BuLi (2.4M in hexanes, 0.4 ml, 0.96 mmol). The temperature of the reaction mixture was allowed to warm up to -40°C (45 min). The solution was cooled to -78°C and HMPA (0.6 ml) was added.

After stirring for 30 min at -78°C, methyl-5-oxopentanoate **12** (0.25 g, 1.9 mmol) in THF (2 ml) was added and the mixture was allowed to warm up to room temperature (90 min). A mixture of petroleum ether/ether (10 ml, 3:1) was added and the precipitate (Ph_3PO) filtered. After evaporation of the solvents, the residue was purified by chromatography on silica gel (petroleum ether-Et_2O, 95:5) to give the tetraenic ester **13** as an oil (0.275 g, 79% yield). IR (film) 3010, 2970, 2920, 2880, 1740, 1650, 1110, 1060 ; ^{1}H NMR (200 MHz) 1.1 (t, 6H, J=7Hz, $2CH_3$), 1.7 (quint, 2H, J=7.3Hz, $C\underline{H}_2CH_2CO_2Me$), 2.1 (q, 2H, J=6.7Hz, $C\underline{H}_2(CH_2)_2(CO_2Me)$, 2.3 (t, 2H, J=7.5Hz, $C\underline{H}_2CO_2Me$), 2.4 (t, 2H, J=5.8Hz, $C\underline{H}_2CH(OEt)_2$), 2.75-2.85 (m, 6H, $3CH{=}CH\text{-}C\underline{H}_2\text{-}CH{=}CH$), 3.6-3.7 (m, 4H, 2 $C\underline{H}_2CH_3$), 3.65 (s, 3H, OMe), 4.5 (t, 1H, J=5.8Hz, $C\underline{H}$ $(OEt)_2$), 5.3-5.5 (m, 8H, $4C\underline{H}{=}C\underline{H}$) ; ^{13}C NMR (50MHz) 15.2, 24.7, 25.5, 25.8, 26.5, 32.0, 33.3, 51.3, 61.1, 102.4, 124.3, 128.0,

128.08, 128.1, 128.7, 128.8, 130.0, 173.9. Anal. Calcd for $C_{22}H_{36}O_4$: C, 72.49 ; H, 9.95. Found : C, 72.49 ; H, 10.03.

Methyl *(5Z,8Z,11Z,14Z)*-17-oxoheptadeca-5,8,11,14-tetraenoate (14). To a solution of the acetal **13** (0.08 g, 0.22 mmol) in dichloromethane (2 ml) was added at 0°C a 50% aqueous trifluoroacetic acid solution (0.8 ml). After stirring for 30 min, the mixture was diluted with pentane and washed successively with water, saturated sodium bicarbonate solution and water. The organic layer was dried (Na_2SO_4), the solvents evaporated and the residue thoroughly dried twice by azeotropic distillation (cyclohexane) on a rotary evaporator. The crude aldehyde **14** (0.064 g, 100% yield) was used in the next step without further purification. IR (film) 3010, 1750-1720 ; ^{1}H NMR (200Mhz) 1.7 (quint, 2H, J=7.2Hz, C$\underline{H}_2$CH$_2$CO$_2$Me), 2.05-2.15 (m, 2H, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$Me), 2.33 (t, 2H, J=7.5Hz, C$\underline{H}_2$CO$_2$Me), 2.75-2.85 (m, 6H, 3CH=CH-C$\underline{H}_2$-CH=CH), 3.23 (dd, 2H, J=6.5Hz, C$\underline{H}_2$CHO), 3.65 (s, 3H, OMe), 5.25-5.5 (m, 6H, 2C$\underline{H}$=C$\underline{H}$), 5.5-5.8 (m, 2H, 2C$\underline{H}$=C$\underline{H}$), 9.7 (t, 1H, J=1.8Hz, CHO) ; ^{13}C NMR (50MHz) 24.5, 25.35, 25.4, 25.7, 26.3, 33.8, 42.2, 51.2, 126.9, 127.6, 128.1, 128.2, 128.5, 128.6, 128.8, 133.2, 173.8, 199.

Methyl *(5Z,8Z,11Z,14Z,17Z)*-eicosa-5,8,11,14,17-pentaenoate (EPA) (16b) and its [18-^{14}C] analog. To a suspension of propyltriphenylphosphonium bromide **15a** (0.4 g, 0.92 mmol) in THF (5 ml), cooled to -78°C, was added *n*-BuLi (2.3M in hexanes, 0.4 ml, 0.92 mmol) and the orange solution was allowed to warm up to room temperature. After cooling down the solution to -78°C, HMPA (0.5 ml) and the aldehyde **14** (0.064 g, 0.22 mmol) in THF (2 ml) were successively added. The mixture was allowed to warm up to room temperature (60 min), diluted with a mixture of petroleum ether-Et$_2$O (7 ml, 3:1) and the precipitate (PPh_3O) filtered. The residue was purified by chromatography on silica gel (petroleum ether- Et$_2$O, 95:5) to give EPA ester **16b** (0.03 g, 43% yield, 94% purity). ^{1}H NMR (200 Mhz) 1.0 (t, 3H, J=7.5Hz, CH$_3$), 1.7 (quint, 1H, J=7.3Hz, C$\underline{H}_2$CH$_2$CO$_2$Me), 2.0-2.15 (m, 4H, C$\underline{H}_2$CH$_3$, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$Me), 2.32 (t, 2H, J=7.4Hz, C$\underline{H}_2$CO$_2$Me), 2.7-2.9 (m, 8H, 4CH=CH-C$\underline{H}_2$-CH=CH), 3.66 (s, 3H, OMe), 5.3-5.5 (m, 10H, 5C$\underline{H}$=C$\underline{H}$) ; ^{13}C NMR (75MHz) 14.3, 20.6, 24.8, 25.56, 25.64, 25.65, 25.656, 26.6, 33.5, 51.5, 127.0, 127.9, 128.12, 128.15, 128.17, 128.25, 128.3, 128.6, 128.9, 129.0, 174.0.

The preparation of [18-^{14}C] EPA was effected on 0.57 mmol scale (aldehyde) using essentially the same procedure as for the unlabelled EPA except that the ratio phosphonium salt **15b**/aldehyde **14** was 1/2.3. [18-^{14}C] EPA **16b** was obtained in 20% yield from **15b** and has a radiochemical purity > 99% (specific activity 53 mCi mmol). MS (DCI/NH$_3$) *m/z* 336.

Methyl *(5Z,8Z,11Z,14Z,17E)*-eicosa-5,8,11,14,17-pentaenoate 17b and its [18-^{14}C] analog. To a suspension of the phosphonium salt **15a** (0.05 g, 0.13 mmol) in ether (1 ml), cooled to -50°C, was added *n*-BuLi (2.2M in hexanes, 0.06 ml, 0.13 mmol) and the mixture was allowed to warm up to room temperature. After cooling the orange suspension to -40°C, the aldehyde **14** (0.014 g, 0.05 mmol) in ether (0.5 ml) was added and the mixture was stirred for 90 min at -40°C. Dry methanol (0.7 ml) was added and the mixture was stirred at -40°C for 4 h. The mixture was diluted with ether and washed with brine. The ethereal extracts were dried (Na_2SO_4) and evaporated to dryness. Purification of the residue by chromatography on silica gel (petroleum ether-ether, 98:2) gave EPA Δ17*t* methyl ester **17b** as an oil (6.5 mg, 41% yield, 85% purity). IR (film) 3010, 1740, 1650, 965 ; ^{1}H NMR (200MHz) 0.96 (t, 3H, J=7.4Hz, CH$_3$), 1.7 (quint, 2H, J=7.2Hz,

$C\underline{H}_2CH_2CO_2Me$), 2.12 (quint, 2H, J=6.6Hz, $C\underline{H}_2CH_3$), 2.32 (t, 2H, J=7.5Hz, $C\underline{H}_2$ CO_2Me), 2.7-2.9 (m, 8H, 4CH=CH-$C\underline{H}_2$-CH=CH), 3.65 (s, 3H, OMe), 5.3-5.5 (m, 10H, 5CH=CH ; ^{13}C NMR (50MHz) 13.7, 24.7, 25.5, 25.6, 26.3, 30.3, 33.4, 51.4, 126.9, 128.0, 128.1, 128.2, 128.24, 128.8, 128.9, 132.5, 173.9.

The preparation of [18-^{14}C] EPA Δ17*t* was effected on a 0.44 mmol scale (aldehyde) using essentially the same protocol as for the unlabelled EPA Δ17*t* except that the ratio phosphonium salt **15b**/aldehyde **14** was 1/1.15. EPA Δ17*t* was separated from EPA by HPLC on Zorbax SB C_{18} column (acetonitrile-H_2O, 77:23). Labelled EPA Δ17*t* **17b** was obtained in 5% yield from **15b** and has a radiochemical purity of 99% specific activity 53 mCi/mmol). MS(DCI/NH_3) *m/z* 336.

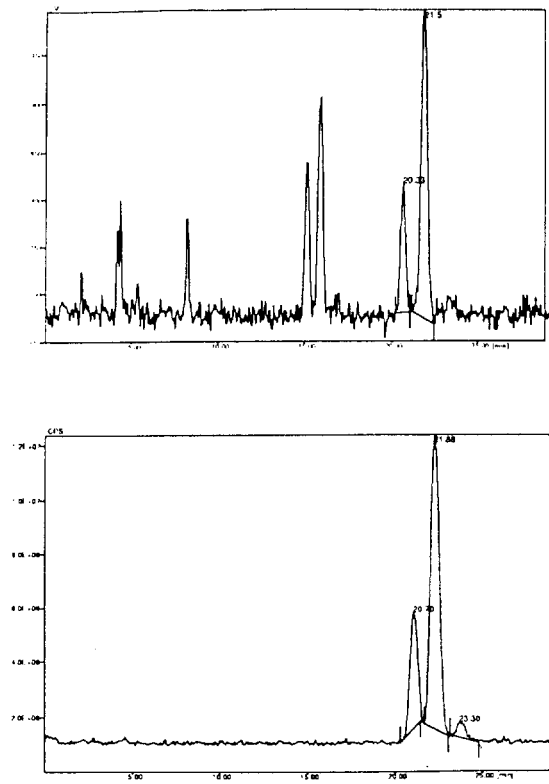


Figure 1. HPLC chromatograph of crude [18-^{14C}] EPA Δ17*t* after purification on silica gel ; column : SBC18, eluent : H_2O-acetonitrile (33/77), oven temperature : 40°C.

Synthesis of [1-^{14}C] propyltriphenylphosphonium iodide 15b.

[1-^{14}C] propionic acid (18). A carbonating tube containing barium [1-^{14}C] carbonate (1.83 g, 9.17 mmol) and a flask containing ethylmagnesium bromide (0.54 M in ether, 50 ml, 27 mmol) were frozen with liquid nitrogen under vacuum. To a cooled (-20°C) solution of degased Grignard reagent, was bubbled $^{14}CO_2$, generated by addition of H_2SO_4 to Ba[1-^{14}C] carbonate. The solution was stirred for 2 h at -20°C and quenched with 6N H_2SO_4 (15 ml). The ethereal layer was basified with 1N NaOH solution and the aqueous phase was washed with ether and acidified with 6N H_2SO_4. [^{14}C]-Propionic acid was extracted from the aqueous phase twice with ether and the organic layer was dried (Na_2SO_4). The solvent was carefully removed by distillation to leave **18** (0.558 g, 80% yield).

[1-^{14}C] Propan-1-ol (19). To a solution of [1-^{14}C] propionic acid (0.558 g, 7.3 mmol) in anhydrous ether (85 ml) was added by portions $LiAlH_4$ (0.56 g, 14.7 mmol). The reaction mixture was heated under reflux for 1 h, cooled down to room temperature and quenched with 1-octanol (bp 196°C) (10 ml). Ether was partially removed to a residual volume of ca. 30 ml and distillation of the residue gave [^{14}C] propanol (6.4 mmol, 70% yield) along with octanol (2 mmol).

[1-^{14}C]-1-Iodopropane (20). The above prepared impure [1-^{14}C] propanol (6.4 mmol) was transferred under vacuum to a flask containing iodine (1.14 g, 4.5 mmol) and red phosphorus (0.152 g, 4.9 mmol) (dessicated one night under vacuum). The reaction vessel was filled with nitrogen to atmospheric pressure and the mixture was heated for 16 h at 70°C. The reaction mixture was transferred under vacuum in a flask containing Cu turnings and dry K_2CO_3. GC analysis of the mixture showed that it was made up of 60% [1-^{14}C]-1-iodopropane and 30% of unreacted [1-^{14}C]-propanol. Impure **20** was used in the next step without purification.

[1-^{14}C]-Propyltriphenylphosphonium iodide (15b). To a solution of triphenylphosphine (2.77 g, 10 mmol) in acetonitrile (10 ml) containing dry K_2CO_3 (0.4 g) was transferred under vacuum the above prepared [1-^{14}C]-iodopropane **20**. The reaction vessel was filled with nitrogen to atmospheric pressure and the clear solution was refluxed for 40 h. After evaporation of the solvent and of residual propanol, the residue was purified by chromatography on silica gel (CH_2Cl_2-MeOH, 99:1) to give **15b** (1.47 g, 37% yield from $Ba^{14}CO_3$).

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