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Synthesis of methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*E*)-eicosapentaenoate and methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*E*)-docosahexaenoate

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Abstract

(3*Z*,6*Z*,9*Z*,12*E*)-Pentadecatetraenal, a common intermediate in the synthesis of methyl Δ17*t* EPA and methyl Δ19*t* DHA, was obtained by copper(I)-catalyzed coupling between the Grignard reagent of 1,1-diethoxy-3-butyne and (*Z*,*E*)-1-bromo-5,8-undecadien-2-yne followed by semi-hydrogenation of the resulting diendyne. The tetraenic aldehyde was subjected to a Wittig reaction with the ylide of (3-carboxybutyl)triphenylphosphonium bromide or of [6-(2,6,7-trioxabicyclo [2.2.2] octyl)-hex-3-*Z*-enyl] triphenylphosphonium iodide to give, respectively Δ17*t* EPA and the protected Δ19*t* DHA in high isomeric purity.

Keywords: Wittig reaction; Catalyzed acetylenic coupling; β,γ-Unsaturated aldehydes; *n*-3 *trans* Polyunsaturated fatty acids

1. Introduction

Geometrical isomers of *n*-3 or *n*-6 polyunsaturated fatty acids are formed during heat treatment of fish oils [1] or vegetable oils, particularly during deodorization [2,3] and deep frying processes [4]. These isomers are subsequently found in frying oils [4], refined vegetable oils [2], some low-calorie spreads [5] and in encaspulated fish oils (Sébédio, J.L., unpublished data). On the other hand, animal experiments have shown that geometrical isomers of C—18 PUFA, when present in the diet, can be metabolized in isomers of arachidonic acid [6] and of EPA and DHA [7]. *n*-3 *trans* PUFA have been found in the liver [7] but also in other tissues like heart, kidney and neural structures [8,9]. Studies on the biological properties of dietary *trans* fatty acids have shown that they can effect serum lipids and lipoproteins levels

Abbreviations: DHA, docosahexaenoic acid; DME, 1,2-dimethoxyethane; EPA, eicosapentaenoic acid; GC, gas chromatography; HMPA, hexamethylphosphoramide; IR, infrared; MS, mass spectrometry; NMR, nuclear magnetic resonance; OBO, 2,6,7-trioxabicyclo[2.2.2] octyl; PUFA, polyunsaturated fatty acids; TFA, trifluoroacetic acid; THF, tetrahydrofuran; THP, tetrahydropyranyl; TLC, thin-layer chromatography; TMS, tetramethylsilane.

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[10,11]. Moreover, mono-*trans* isomers of EPA and DHA (Δ17*t* EPA and Δ19*t* DHA) have been shown to alter platelet aggregation [12]. In order to fully evaluate the physiological properties of Δ17*t* EPA and Δ19*t* DHA and to have a better understanding of their anti-aggregating properties, significant quantities of these fatty acids were needed. Given the scarcity of (**12**) and (**19**) in their biological source (liver lipids of rats) [17], total synthetic materials were clearly required.

Natural all-*cis* EPA and DHA have already been synthesized [13–15]. In most of these syntheses the 'acetylenic approach', developed by Osbond et al. [16], was used, which involved the stereoselective partial hydrogenation of the corresponding polyacetylenic ester of the desired polyenic acid. Only a few reports have appeared in the literature concerning the synthesis of *trans* PUFA. Rakoff described the synthesis of *trans* isomers of α- and γ-linolenate using as a key step a non-stereoselective Wittig reaction [17,18]. *Trans* analogs of arachidonic acid have also been recently synthesized [19,20].

In this paper, we described the convergent synthesis of Δ17*t* EPA and Δ19*t* DHA, from a common intermediate the tetraenic aldehyde (**10**), using a combination of successive highly stereoselective Wittig reactions on β,γ-unsaturated aldehydes and copper-catalyzed acetylenic coupling.

2. Experimental procedures

2.1. Reagents

If not otherwise specified, all chemicals and reagents were obtained from Aldrich Chemical Company (France). All solvents were purified before use: dichloromethane, acetonitrile, hexamethylphosphoramide were distilled from calcium hydride; tetrahydrofuran and dimethoxyethane were distilled from sodium benzophenone ketyl; ethyl alcohol was distilled from magnesium metal. Silica gel (35–70 mesh) was purchased from Amicon (Lausanne, Switzerland).

2.2. Methods

IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer and were calibrated with the 1601 cm^{-1} absorption of polystyrene. NMR spectra were measured on either a Brucker AC200 Fourier transform spectrophotometer with proton observation at 200 MHz and carbon observation at 50 MHz, or a Brucker AM 300 instrument with proton observation at 300 MHz and carbon observation at 75 MHz or a Varian EM 360 with proton observation at 60 MHz.

Unless otherwise stated, spectra were recorded in $CDCl_3$ and chemical shifts are reported (in ppm) downfield from TMS (δ). GC/MS analyses were carried out on a Nermag R10–10S quadrupole mass spectrometer coupled to a Delsi-DI 700 gas chromatograph fitted with a OV1 capillary column (25 m × 0.32 mm i.d.).

Gas chromatographic analyses were effected on an OV 1701 capillary column (25 m × 0.32 mm i.d.) using a Varian 3300 gas chromatograph (N_2, 2 ml/min) fitted with a flame detector. Analytical thin-layer chromagraphy (TLC) was performed on 0.25 mm pre-coated silica gel containing a fluorescent indicator. Spots were visualized using one or more of the following techniques: (a) UV illumination; (b) spraying with Kägi-Miescher reagent [35]; (c) iodine vapor.

2.3. 1-(2-Tetrahydropyranyloxy)-5-iodo-2-pentyne (4)

To a solution of the acetylenic alcohol (**3**) [23] (4g; 21.7 mmol) in 50 ml of THF, triphenylphosphine (8.54 g; 32 mmol) and imidazole (4.43 g; 65 mmol) were successively added under nitrogen at room temperature. The solution was cooled at −20°C and 8.24 g (32 mmol) of iodine were added in one portion. The brownish solution was then stirred for 2 h at room temperature. The solution was diluted with ether and washed successively with water, a saturated solution of sodium thiosulfate, water again and dried (Na_2SO_4). After removal of volatiles on the rotatory evaporator, the residue was triturated with hexane (2 × 25 ml). After concentration in vacuo, the residue was flash chromatographed on silica gel (80 g) (petroleum ether/ether, 6:1 v/v) to furnish the iodo compound (**4**) (5.7 g; 89% yield) as an oil. ^{1}H-NMR (60 MHz):1.26–1.9 (m, 6H, $3CH_2$), 2.83 (m, 2H, $C\underline{H}_2$-CH_2I), 3.2 (t, 2H, J = 7 Hz, $C\underline{H}_2$-I), 3.43–4

(m, 2H, $C\underline{H}_2OCHOR$), 4.2 (t, 2H, J = 2Hz, $C\underline{H}_2OTHP$), 4.8 (m, 1H, $OC\underline{H}O$).

2.4. [5-(2-Tetrahydropyranyloxy)-pent-3-ynyl]triphenylphosphoniun iodide (**2**)

The iodide (**4**) (4.49 g, 15 mmol) dissolved in 30 ml of acetonitrile containing triphenylphosphine (7.9 g, 30 mmol) was stirred for 2 days at 50–55°C in the presence of 0.75 g of calcium carbonate. After filtration of calcium carbonate and evaporation of acetonitrile in vacuo, the residue was dissolved in a minimum of CH_2Cl_2 and dilution with ether caused the precipitation of the phosphonium salt (**2**) (8.0 g; 94% yield). ^{1}H NMR (300 MHz): 1.4–1.9 (m, 6H, $3CH_2$), 2.85 (td, 2H, J = 6 and J_{PH} = 21 Hz, $C\underline{H}_2$-CH_2P), 3.5 (m, 1H, $C\underline{H}_2OCHOR$), 3.73 (m, 1H, $C\underline{H}_2OCHOR$), 3.78 (d, 1H, J = 15 Hz, $C\underline{H}_2OTHP$), 3.88 (d, 1H, $C\underline{H}_2OTHP$), 3.92 (dt, 2H, J_{PH} = 12.5 Hz, $C\underline{H}_2P$), 4.55 (s, 1H, $OC\underline{H}O$), 7.6–7.9 (m, 15 H, aromatic). ^{13}C-NMR (75.47 MHz): C1, 22.62 (J_{CP} = 52.6 Hz); C2, 13.30 (J_{CP} = 4.75 Hz); C3, 80.73; C4, 82.11 (J_{CP} = 7.3 Hz); C5, 53.93. THP group: C1, 96.8; C2, 30.17; C3, 19.02; C4, 25.2; C5, 62.01. Phenyl groups: C *quaternary* 117.5 (J_{CP} = 86.08 Hz); C *ortho*, 130.05; C *meta*, 133.83; C *para*, 135.31.

2.5. (Z,E)-1-(2-Tetrahydropyranyloxy)-5,8-undecadien-2-yne (**5**)

To the stirred slurry of the phosphonium salt (**2**) (1.61 g; 2.9 mmol) in 10 ml of THF, under nitrogen at −78°C, was added dropwise 1.2 ml of *n*-BuLi (2.35 M in hexanes) (2.8 mmol). The suspension was allowed to warm up to −30°C over 1 h. The red solution was cooled at −78°C and 2 ml of HMPA followed by 0.23 g (2.3 mmol) of (E)-3-hexenal (**1**) in 3 ml of THF was added. The mixture was allowed to warm to 0°C (1 h) and diluted with a mixture of ether-petroleum ether (20 ml; 1:3 v/v). The suspension was filtered on a bed of silica gel and washed twice (2 × 10 ml) with the same mixture of petroleum ether-ether. After evaporation of the solvents, the residue was flash chromatographed on silica gel (petroleum ether/ether, 95:5 v/v) to afford compound (**5**) (0.428 g, 73% yield) as a yellow oil. IR (film) 3020 (m, CH=CH), 2280 (w, C≡C), 2230 (w, C≡C), 1650 (w, C=C), 970 (*trans* double bond). ^{1}H-NMR (300 MHz): 0.97 (t, 3H, J = 7 Hz, CH_3), 1.45–1.9 (m, 6H, $3CH_2$), 2.0 (qd, 2H, J = 1 Hz, $C\underline{H}_2$—CH_3), 2.7 (t, 2H, J = 6 Hz, CH=CH—$C\underline{H}_2$—CH=CH), 2.95 (m, 2H, CH=CH—$C\underline{H}_2$—C≡C), 3.47 (m, 1H, $C\underline{H}_2OCHOR$), 3.8 (m, 1H, $C\underline{H}_2OCHOR$), 4.02 (dt, 1H, J = 15 and 2 Hz, $C\underline{H}_2OTHP$): 4.27 (dt, 1H, $C\underline{H}_2OTHP$), 4.77 (t, 1H, J = 3 Hz, $OC\underline{H}O$), 5.27–5.55 (m, 4H, olefinic H). ^{13}C-NMR (75.47 MHz): C1, 54.64; C2, 75.69; C3, 77.57; C4, 17.26; C7, 30.30; C10, 25.57; C11, 13.80; olefinic C: 124.50, 126.31, 129.76, 132.94. THP group: C1, 96.69; C2, 30.30; C3, 19.11; C4, 25.42; C5, 61.92. GC/MS = *m/e* 163 $(M-85)^+$.

2.6. (Z,E)-1-Bromo-5,8-undecadien-2-yne (**6**)

To a solution of triphenylphosphine (0.55 g; 2.1 mmol) in 4 ml of CH_2Cl_2 was added, at −20°C under nitrogen, 0.1 ml of a solution of bromine (19M in CH_2Cl_2). The solution was allowed to warm up to −5°C (formation of a precipitate) and 0.4 g (1.6 mmol) of the compound (**5**) in 4 ml of CH_2Cl_2 was added dropwise at this temperature. The mixture was then allowed to warm to room temperature and stirred for 2 h. The solution was diluted with ether, washed with an aqueous solution of sodium bicarbonate, with water and dried (Na_2SO_4). After concentration in vacuo, flash chromatography of the residue on silica gel (petroleum ether/ether, 98:2 v/v) afforded the bromide (**6**) as a yellow oil (0.356 g; 97% yield), 99.5% purity as determined by GC analysis. IR (film): 3020 (m), 2300 (w), 2230 (m), 1645 (w). ^{1}H-NMR (300 MHz): 0.96 (t, 3H, J = 7 Hz, CH_3), 2.0 (qd, 2H, J = 1 Hz, $C\underline{H}_2$—CH_3), 2.75 (t, 2H, J = 6 Hz, CH=CH—$C\underline{H}_2$-CH=CH), 3.0 (m, 2H, CH=CH—$C\underline{H}_2$—C≡C), 3.90 (t, 1H, 2H, CH_2Br), 5.27–5.55 (m, 4H, olefinic H). ^{13}C-NMR (75.47 MHz): C1, 15.5; C2, 75.2; C3, 86.1; C4, 17.33; C7, 30.28; C10, 25.55; C11, 13.79; olefinic C: 123.71, 126.12, 130.26, 133.0 GC/MS: *m/e* = 198 $(M-29)^+$.

2.7. (Z,E)-1,1-Diethoxy-9,12-pentadecadien-3,6-diyne (**8**)

To the solution of 1,1-diethoxy-3-butyne (**7**) [27]

(0.57 g: 4 mmol) in 4 ml of anhydrous THF, was added 2.7 ml (3.8 mmol) of ethylmagnesium bromide (1.4 M in THF) at room temperature under nitrogen. The solution was then refluxed 30 min and allowed to cool to room temperature. After the addition of CuBr (0.02 g; 0,14 mmol), the bromide (*6*) (0.365 g; 1.6 mmol) in 4 ml of THF was added and the stirring was continued for 2 h at room temperature. The mixture was diluted with ether, washed with a saturated solution of NH_4Cl, then by water and the organic layer was dried on Na_2SO_4. After evaporation of the solvents in vacuo, the residue was flash chromatographed on silica gel (petroleum ether/ether/Et_3N, 95:5:1 v/v) to furnish 0.415 g (88% yield) of (**8**) as a yellow oil purity > 99% (GC).

Because of its instability, the diyne has to be used for the next step as quickly as possible. ^{1}H-NMR (300 MHz): 0.96 (t, 3H, J = 7 Hz, CH_3), 1.22 (t, 6H, J = 7 Hz, $OCH_2—C\underline{H}_3$), 2.0 (qd, 2H, J = 1 Hz, $C\underline{H}_2—CH_3$), 2.5 (dt, 2H, J = 6 and 2 Hz, $C\underline{H}_2—CH(OEt)_2$), 2.73 (t, 2H, J = 6 Hz, $CH=CH—C\underline{H}_2—CH=CH$), 5.92 (m, 2H, $CH=CH—C\underline{H}_2—C\equiv C—$), 3.12 (q, 2H, $C\equiv C—C\underline{H}_2—C\equiv C$), 3.48–3.75 (m, 4H, $2CH_2$), 4.60 (t, 1H, $C\underline{H}(OEt)_2$), 5.3–5.55 (m, 4H, olefinic protons). ^{13}C-NMR (75.47 MHz): C1, 100.98; C2, 25.08; C5, 9.87; C8, 17.08; C11, 30.24; C14, 25.52; C15, 13.76; acetylenic C: 74.06, 75.68, 76.02, 78.63; olefinic C: 124.77, 126.34, 129.54, 132.82. Diethoxy group: CH_2, 61.87; CH_3, 15.22.

2.8. (Z,Z,Z,E)-1,1-Diethoxy-3,6,9,12-pentadecatetraene (**9**)

2.8.1. Obtained from (8) by partial hydrogenation. To the well-stirred solution of $Ni(OAc)_2 \cdot 4H_2O$ (0.056 g; 0.22 mmol) dissolved in 3 ml of absolute ethanol, under nitrogen at room temperature, was added a solution of freshly prepared sodium borohydride (0.45 M in ethanol) (1 ml; 0.45 mmol). Then, ethylenediamine (60 μl; 0.9 mmol) was added and the system was evacuated and flushed several times with H_2. Then, the acetal (**8**) (0.65 g; 2.2 mmol) in 3 ml of absolute ethanol was added. When absorption of H_2 has ceased, the reaction mixture was evaporated to dryness. The residue was triturated with ether and filtered through a pad of celite and washed twice with ether. The combined extracts were concentrated and the residue was flash chromatographed on silica gel (CH_2Cl_2/ petroleum ether/Et_3N, 20:30:-0.5 v/v) to give 0.541 g of (**9**) as a colorless oil (83% yield), 92% purity (GC). ^{1}H-NMR (300 MHz): 0.96 (t, 3H, J = 7 Hz, CH_3), 1.18 (t, 6H, J = 7 Hz, $2OCH_2—C\underline{H}_3$), 2.0 (qd, 2H, J = 1 Hz, $CH_3—C\underline{H}_2—CH=CH$), 2.4 (t, 2H, J = 6 Hz, $C\underline{H}_2—CH(OEt)_2$), 2.65–2.85 (m, 6H, $3CH=CH—C\underline{H}_2—CH=CH$), 3.42–3.72 (m, 4H, $2OC\underline{H}_2—CH_3$), 4.47 (t, 1H, J = 6 Hz, $CH(OEt)_2$), 5.27–5.52 (m, 8H, olefinic H). ^{13}C-NMR (50.32 MHz): C1, 102.51; C2, 32.15; C5 and C8, 25.61 and 25.90; C11, 30.44; C14, 25.60; C15, 13.83; olefinic C: 124.34, 127.01, 127.97, 128.17, 128.28, 128:35, 130.16, 132.55. Diethoxy group: CH_2, 61.23; CH_3, 15.33.

2.8.2. Obtained from (8) by hydroboration. To the solution of cyclohexene (0.66 ml; 6.5 mmol) in 2 ml of distilled THF, was added under nitrogen at 0°C, a solution of $BH_3.Me_2S$ (2M in THF) (1.64 ml; 3.3 mmol). The mixture was stirred at 0°C for 90 min (apparition of a white precipitate), cooled at −30°C and 0.345 g (1.2 mmol) of the acetal (**8**) in 5 ml of THF was added. The suspension was allowed to warm to room temperature and stirred for 75 min and cooled at 0°C. Acetic acid 0.2 ml (3.5 mmol) was added at room temperature. Ether was added and the organic layer was washed with a solution of sodium bicarbonate, water and dried (Na_2SO_4). Purification of the residue by flash chromatography on silica gel (ether/petroleum ether/Et_3N, 4:96:1 v/v) afforded 0.13 g (38% yield) of the tetraene (**9**), 99.5% purity (GC). Its physical data (^{1}H- and ^{13}C-NMR, GC retention time) were found identical to those of the compound prepared by semi hydrogenation.

2.9 1-(6-Hydroxyhex-3-ynyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**14**)

To the acetylenic OBO orthoester (**13**) (1 g, 5.5 mmol) in 15 ml of anhydrous DME, cooled at −55°C under nitrogen, was added 2.5 ml (5.5 mmol) of *n*-BuLi (2.3 M in hexanes). The mixture was allowed to warm up to −30°C and stirred at this temperature for 1 h. Then, 2.7 ml (55 mmol)

of ethylene oxide was added and the mixture was stirred for 48 h at room temperature. The reaction was quenched with water and extracted twice with ether. The combined extracts were washed with brine, dried (Na_2SO_4) and evaporated to dryness. Chromatography of the residue on silica gel (ethyl acetate/hexane/Et_3N, 60:40:1 v/v) gave 0.788 g (64% yield) of the acetylenic alcohol (**14**). ^{1}H-NMR (200 MHz): 0.79 (s, 3H, CH_3), 1.92 (t, 2H, J = 7 Hz, $2OC\underline{H}_2—C(OR)_3$), 2.22–2.45 (m, 5H, $2CH_3—C\underline{H}_2—C\underline{H}=CH$ and OH), 3.65 (t, 2H, J = 6 Hz, $C\underline{H}_2OH$), 3.88 (s, 6H, $3CH_2$). ^{13}C-NMR (50.32 MHz): C1′, 36.08; C2′, 13.09; C3′, 82.26; C4′, 76.36; C5′, 23.15; C6′, 61.18. Orthoester group: C1, 108.25, C3, C5, C8, 72.51, C4, 30.22, CH_3, 14.44. Elemental analysis: found: C, 63.97; H, 8.17; O, 28.20; calculated for $C_{12}H_{18}O_4$: C, 63.76; H, 8.02; O, 28.31.

2.10.1-(6-Hydroxyhex-3-Z-enyl)4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**15**)

P-2 Nickel was prepared via borohydride reduction of $Ni(OAc)_2 \cdot 4\ H_2O$ (0.027 g, 0.11 mmol) in 1 ml of absolute ethanol as for the semi-hydrogenation of compound (**9**). The reactor was then purged with H_2 and ethylenediamine (0.03 ml, 0.45 mmol) was added followed by the acetylenic compound (**14**) (0.195 g, 0.86 mmol) in 2 ml of absolute ethanol. When absorption of H_2 had ceased, the reaction mixture was evaporated to dryness. The residue was triturated with ether and filtered through a pad of celite and washed twice with ether. The combined ether extracts were concentrated and the residue purified by chromatography on silica gel (ethyl acetate/hexane/Et_3N, 6:4:0.1 v/v) gave 0.18 g (92%) of the ethylenic compound (**15**) (97% purity by GC). IR (film): 3420 (s, OH), 3010 (m, CH=CH), 1650 (w, C=C), 1050 (s, C—0). ^{1}H-NMR (200 MHz): 0.8 (s, 3H, CH_3), 1.73 (m, 2H, $C\underline{H}_2C(OR)_3$), 2.0 (s, 1H, OH), 2.25 (q, 2H, J = 7 Hz, $C\underline{H}_2—CH_2OH$), 2.35 (q, 2H, J = 7 Hz, $C\underline{H}_2—CH_2C(OR)_3$), 3.62 (t, 2H, J = 7 Hz, $C\underline{H}_2OH$), 3.91 (s, 6H, $3CH_2$), 3.57 (dt, 1H, J = 7 and 10 Hz, $C\underline{H}=CH$), 5.58 (dt, 1H, $CH=C\underline{H}$). ^{13}C-NMR (50.32 MHz): C1′ 36.42; C2′, 30.73; C3′, 125.52; C4′, 132.40; C5′, 21.39; C6′, 62.16. Orthoester group: C1, 108.80; C3, C5, C8, 72.55; C4, 30.24; CH_3, 14.51. GC/MS: *m/e*(relative intensity) = 228 (M^+, 6). Elemental analysis: found: C, 63.3; H, 8.7; 0, 27.9; calculated for $C_{12}H_{20}O_4$: C, 63.2; H, 8.8; O, 28.07.

2.11. 1-(6-Iodohex-3-Z-enyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**16**)

To a THF solution (5 ml) of triphenylphosphine (0.39 g, 1.4 mmol), imidazole (0.188 g, 2.8 mmol) and the ethylenic alcohol (**15**) (0.21 g, 0.92 mmol) cooled at −10°C, was added under nitrogen, in one portion, 0.32 g (1.3 mmol) of iodine. The solution was allowed to warm to room temperature and stirred for 1 h. Then, ether was added, washed successively with water, a saturated solution of sodium thiosulfate and again with water. The organic was dried (Na_2SO_4) and concentrated in vacuo. The residue was flash chromatographed on silica gel (petroleum ether/ ether/ Et_3N, 3:2:0.1 v/v) to furnish 0.28 g of (**16**) as an oil [90% yield, 97% purity (GC)]. ^{1}H-NMR (200 MHz): 0.8 (s, 3H, CH_3), 1.76 (m, 2H, $C\underline{H}_2—C(OR)_3$), 2.17 (q, 2H, J = 7 Hz, $C\underline{H}_2—CH_2—C(OR)_3$), 2.65 (q, 2H, J = 7 Hz, $C\underline{H}_2—CH_2I$), 3.12 (t, 2H, J = 7 Hz, $C\underline{H}_2I$), 3.8 (s, 6H, $3CH_2$), 5.2–5.57 (m, 2H, $C\underline{H}=C\underline{H}$). ^{13}C-NMR (50.32 MHz): C1′, 36.24; C2′, 31.27; C3′, 128.07; C4′, 131.55; C5′, 21.41; C6′, 5.47. Orthoester group: C1, 108.53; C3, C5, C8, 72.46; C4, 30.15; CH_3, 14.51. GC/MS: *m/e* (relative intensity) = 338 (M^+, 12), 211 [$(M-127)^+$, 57].

2.12. 6-(2,6,7-Trioxabicyclo[2.2.2]octyl)-hex-3-Z-enyl)triphenylphosphonium iodide (**17**)

A mixture of triphenylphosphine (1.8 g, 6.9 mmol), the ethylenic iodide (**16**) (1.2 g, 3.5 mmol) and powdered calcium carbonate (0.5 g) in 15 ml of acetonitrile was stirred for 3 days at 50°C under nitrogen. After filtration of calcium carbonate, the solvent was evaporated. The oily residue was dissolved in a minimum of CH_2Cl_2, and the phosphonium salt precipitated by addition of ether (1.89 g, 89% yield). ^{1}H-NMR (200 MHz): 0.81 (s, 3H, CH_3), 1.59 (m, 2H, $C\underline{H}_2—C(OR)_3$), 1.98 (broad q, 2H, J = 7 Hz, $C\underline{H}_2—CH_2—C(OR)_3$), 2.47 (m, 2H, $C\underline{H}_2—CH_2P$), 3.62 (m, 2H, $C\underline{H}_2—P$), 3.87 (s, 6H, $3CH_2$), 5.34–5.66 (m, 2H,

$C\underline{H}_2{=}C\underline{H}_2$), 7.6–7.9 (m, 15H, aromatic). ^{13}C-NMR (50.32 MHz): C1, 23.38 (J_{CP} = 48 Hz); C2, 20.19 (J_{CP} = 3.5 Hz); C3, 126.09 (J_{CP} = 14.8 Hz); C4, 131.90; C5, 21.32; C6, 36.22. Orthoester group: C1′, 108.39; C3′, C5′, C8′, 72.53; C4′, 30.23; CH_3, 14.56. Phenyl group: C *quaternary*, 117.77, C *ortho*, 130.69, C *meta*, 133.62, C *para*, 135.32. Elemental analysis: found: C, 60.16; H, 5.58; O, 7.94; I, 21.07; P, 5.11; calculated for $C_{30}H_{34}O_3PI$: C, 60.05; H, 5.71; 0, 7.50; I, 21.58; P, 5.11.

2.13. Methyl (Z,Z,Z,Z,E)-5,8,11,14,17-eicosapentaenoate (12)

To the suspension of (carboxybutyl)triphenylphosphonium bromide (**11**) (0.98 g, 2.2 mmol) in 10 ml of anhydrous THF, cooled at −78°C under nitrogen, was added 1.84 ml of *n*-BuLi (2.4 M in hexanes) (4.4 mmol) followed by 2 ml of HMPA. The temperature of the mixture was allowed to warm up to −30°C (90 min) while the tetraenic acetal (**9**) (0.50 g; 1.7 mmol) dissolved in 10 ml of CH_2Cl_2 was hydrolyzed, at 0°C under nitrogen, by a 50% aqueous trifluoroacetic acid solution (5 ml). After stirring for 1 h at 0°C, the mixture was diluted with pentane, and washed successively with water, a saturated solution of sodium bicarbonate, and water again. The organic layer was dried (Na_2SO_4), the solvents evaporated and the residue thoroughly dried twice by azeotopic distillation (cyclohexane) on a rotatory evaporator. The crude aldehyde (**10**) (0.35 g, 95% yield) was used in the next step without further purification: IR (film): 2720 (m, CHO), 1725 (s, CHO), 1650 (w, C=C) 970 (s, *trans* double bond). ^{1}H-NMR (200 MHz): 0.91 (t, 3H, J = 7 Hz, CH_3), 1.94 (q, 2H, $C\underline{H}_2{-}CH_3$), 2.7 (m, 6H, $3CH{=}CH{-}C\underline{H}_2{-}CH{=}CH$), 3.09 (broad d, 2H, $C\underline{H}_2{-}CHO$), 5.11–5.73 (m, 8H, $4C\underline{H}{=}C\underline{H}$), 9.52 (t, J = 2 Hz, $C\underline{H}O$).

To the red solution of the corresponding phosphorane of (**11**), cooled at −78°C, was added the aldehyde (**10**) (0.35 g) in 5 ml of THF. After 45 min of stirring at −78°C, the temperature was allowed to warm up to 0°C (1 h) and stirred at this temperature for 30 min.

The reaction mixture was quenched with an aqueous solution of HCl(1N) and extracted twice with ether. The combined ether extracts were washed once with brine, dried (Na_2SO_4) and concentrated. The residue, dissolved in ether, was filtered on a bed of silica and eluted with a mixture of petroleum ether/ethyl acetate (4:1 v/v) to give 0.26 g of a yellow oil (58% yield) which was esterified, at 0°C under nitrogen, with a freshly distilled solution of diazomethane in ether. A flash chromatography of the residue on silica gel (45 g) (petroleum ether/ether, 97:3 v/v) afforded 0.2 g of (**12**) as a colorless oil [75% yield, 95% purity (GC)]. IR (film): 3010 (m), 1740 (s), 1650 (w), 1050 (s), 970 (s). ^{1}H-NMR (200 MHz, C_6D_6): 0.93 (t, 3H, J = 7 Hz, CH_3), 1.60 (q, 2H, J = 7 Hz, $C\underline{H}_2{-}CH_2{-}CO_2Me$), 1.85–2.02 (m, 4H, $C\underline{H}_2{-}CH_3$ and $CH_2{-}C\underline{H}_2{-}CH{=}CH$), 2.1 (t, 2H, J = 7.3 Hz, $C\underline{H}_2{-}CO_2Me$), 2.67–2.94 (m, 8H, $4CH{=}CH{-}C\underline{H}_2{-}CH{=}CH$), 3.34 (s, 3H, OMe), 5.17–5.57 (m, 10H, $5C\underline{H}{=}C\underline{H}$). ^{13}C-NMR (50.32 MHz): C1, 173.92; C2, 33.41; C3, 24.81; C4, C7, C10, 25.65 and 26.58 ($3CH_2$); C16, 30.44; C19, 25.60; C20, 13.84; OCH_3, 51.42. Olefinic C: 127.04, 128.05, 128.16 (2), 128.20, 128.25, 128.32, 128.90, 128.94, 132.53.

2.14. 1-(4-Methyl-2,6,7-trioxabicyclo[2.2.2]octyl)-3,6,9,12,15,18-uncosahexaene (18)

To the slurry of the phosphonium salt (**17**) (0.9 g, 1.5 mmol) in a mixture of THF-HMPA (4:1 v/v, 6.25 ml), cooled at −78°C under nitrogen, was added 0.62 ml (1.5 mmol) of *n*-BuLi (2.4 M in hexanes). The red mixture was stirred for 1 h at −70°C and the aldehyde (**10**) added in 4 ml of THF prepared by hydrolysis of its diethylacetal (0.3 g, 1 mmol) as previously described.

The temperature was allowed to warm up to 0°C and the reaction mixture was quenched with water. The mixture was extracted twice with ether and the combined ether extracts were washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on silica gel, (petroleum ether/ether/Et_3N, 9:1:0.2 v/v) to give 0.29 g of (**18**) (69% yield) obtained as a yellow oil.

2.15. Methyl (Z,Z,Z,Z,Z,E)-4,7,10,13,16,19-docosahexanoate(19)

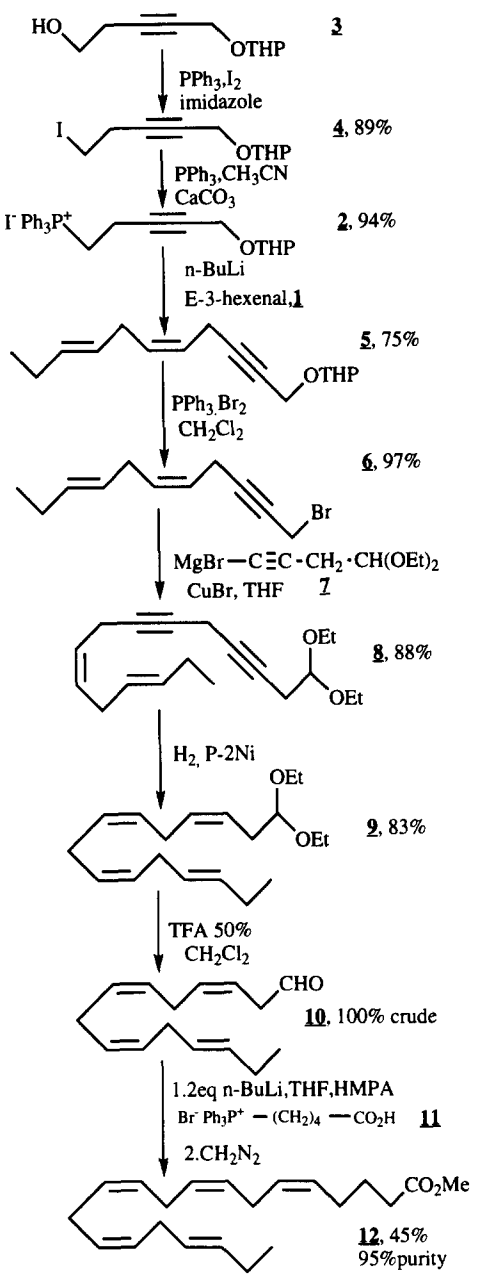
To a solution of the orthoester (**18**) (0.29 g, 0.7 mmol) in 5 ml of THF, was added 0.2 ml of 0.5 M aqueous oxalic acid under nitrogen. After stirring

for 1 h at room temperature, 0. 1 ml of aqueous oxalic acid was added and the stirring was continued for 30 min. The solution was diluted with ether, washed with a solution of sodium bicarbonate, and then brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was further dried by azeotropic distillation with cyclohexane (three times) and used in the next step without further purification.

To the solution of the crude ester (0.275 g) in 5 ml of MeOH was added 0.18 g of dried potassium carbonate. After 30 min of stirring at room temperature under nitrogen, the suspension was diluted with ether and washed successively with water, 0.1N HCl, and brine. After concentration in vacuo, the residue was purified by flash chromatography on silica gel (petroleum ether/ether, 97:3 v/v) to yield 0.162 g (67% yield for the two steps) of the hexanenic ester (**19**) (97% purity by GC). IR (film): 3010 (m, CH=CH), 1740 (s, C=O), 1650 (w, C=C), 970 (s,*trans* double bond). ^{1}H-NMR (200 MHz): 0.96 (t, 3H, J = 7.5 Hz, CH_3), 2.0 (q, 2H, J = 7 Hz, $C\underline{H}_2$—CH_3), 2.37 (broad s, 4H, $C\underline{H}_2$—$C\underline{H}_2CO_2Me$), 2.67–2.97 (m, 10H, 5CH=CH—$C\underline{H}_2$—CH=CH), 3.64 (s, 3H, OMe), 5.2–5.57 (m, 12H, olefinic H). ^{13}C-NMR (50.32 MHz): C1, 173.33; C2, 33.93; C3, 22.75; C6, C9, C12, C15, C18, C21, 25.52 and 25.59; C18, 33.93; C22, 13.84; OCH_3, 51.41. Ethylenic C: 126.93 (C19), 127.82, 127.96, 128.0, 128.02, 128.1, 128.2 (3), 128.25, 129.25, 132.45 (C20).

3. Results and discussion

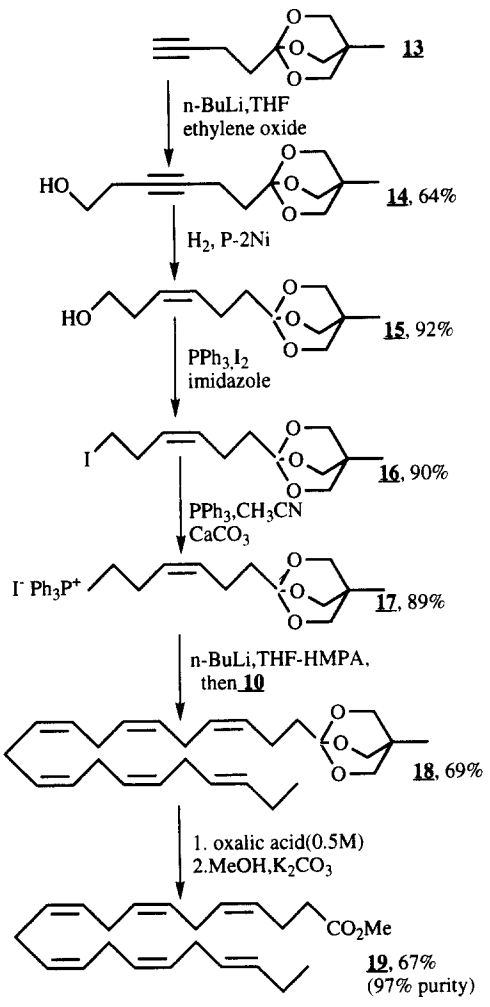
Synthesis of (*Z,Z,Z,E*)-3,6,9,12-pentadecatetraenal (**10**) started by a highly stereoselective Wittig reaction between (*E*)-3-hexenal (**1**) and the ylide of the acetylenic phosphonium salt (**2**), in the presence of HMPA [22], to afford exclusively the (*E,Z*) dienyne (**5**) in 75% yield (Scheme 1). The structure and the isomeric purity of compound (**5**) was confirmed by ^{13}C-NMR spectroscopy. Chemical shifts for C10 and C7 which appeared, respectively at 25.57 and 30.30 ppm supported the configuration assigned for the 5,6 and 8,9 double bonds [17]. The phosphonium salt (**2**), was prepared from the tetrahydropyranyl ether of the acetylenic alcohol (**3**) [23] by iodination in the modified Garegg-Samuelsson conditions [24,25] to give (**4**) (89% yield) followed by reaction with triphenylphosphine in the presence of calcium carbonate.



Scheme 1. Synthesis of methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*E*)-eicosapentaneoate (**12**).

The OTHP group of compound (**5**) was quantitatively transformed, with triphenylphosphine dibromide [26], to the bromide (**6**) which was then coupled with the Grignard reagent of the acetylenic acetal (**7**) [27] in the presence of cuprous bromide [16], to yield the diacetylenic diene (**8**) in 88% overall yield.

The next step, partial hydrogenation of the two triple bonds of (**8**) was quite troublesome. Hydrogenation, in the presence of Lindlar catalyst and quinoline, afforded the (*Z,Z,Z,E*)-tetraenic acetal (**9**) contaminated with 20–25% of an over-reduced product which is difficult to separate by flash chromatography. By using P-2Ni as catalyst [28] the formation of the by-product was reduced to the extent of 14% and it could be partially removed by flash chromatography on silica gel to afford (**9**) (83% yield, 92% purity) which was used for the next step without further purification.



Scheme 2. Synthesis of methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*E*)-docosahexaenoate (**19**).

Finally, the two triple bonds of compound (**8**) could be reduced chemioselectively, without any overreduced product formation, by reaction with 2.8 equiv. of dicyclohexylborane followed by protonolysis with acetic acid [29–31]. Unfortunately the isolated yield of the desired product was low (38%).

The final chain elaboration of Δ17*t* EPA was effected, by first deprotection of the unstable β,γ-olefinic aldehyde in a two phase aqueous trifluoroacetic acid-CH_2Cl_2 mixture [32], followed by Wittig reaction between the crude aldehyde (**10**) and the ylide of (4-carboxybutyl)triphenyl phosphonium bromide (**11**) (commercially available) to yield the isomer of EPA. Purified as its methyl ester (45% overall yield, 95% purity), compound (**12**) was found to be identical by GC analysis to an authentic sample obtained from liver lipids of rats fed heated linseed oil [7]. For bioassay, (**12**) could be obtained in 99.6% purity by preparative argentation TLC.

Next, we turned our attention to the seven-carbon phosphonium salt (**17**) (Scheme 2). Alkylation of the OBO orthoester of 4-pentynoic acid (**13**) [33] with ethylene oxide in dimethoxyethane, in the presence of *n*-BuLi, afforded the acetylenic alcohol (**14**) (64% yield). Partial hydrogenation of (**14**) over Lindlar catalyst and quinoline gave the *cis* isomer (**15**) contaminated by 4 to 10% of the *trans* isomer (determined by GC of the acetate derivative). Reduction of (**14**) with P-2 Ni catalyst gave more reproducible results and a better selectivity (ca. 98%) than with the Lindlar catalyst. The *Z*-geometry of the double bond of (**15**) was supported by ^{1}H-NMR spectroscopy ($J_{3,4}$ = 11 Hz). Then, transformation of the alcohol (**15**) to its corresponding phosphonium salt was achieved as described for compound (**3**), by iodination followed by reaction with triphenylphosphine in acetonitrile, in the presence of calcium carbonate, to give (**17**) in 77% overall yield. Ylide of the phosphoni-

um salt (**17**) underwent *cis*-Wittig coupling with the unsaturated aldehyde (**10**) to furnish the protected isomer of DHA (**18**) in 70% yield with a *Z*-selectivity > 95% at the newly formed olefin bond at C7.

The remaining step, deprotection of the acid function, was achieved by acidic hydrolysis of the orthoester [34] and, without purification of the resulting ester diol, transesterification with methanol in the presence of potassium carbonate gave after purification on silica gel methyl Δ19*t* DHA (**19**) in 69% overall yield and 97% purity. The synthetic Δ19*t* DHA was found to be identical by GC analysis to an authentic sample obtained from liver lipids of rats fed heated linseed oil [7].

In conclusion, we have developed an efficient and stereoselective synthesis of mono *trans* isomers of EPA and DHA which allowed their preparation on a scale ≥1 g. Some of the biochemical properties of (**12**) and (**19**) will be reported later.

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