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Synthesis of (9Z,12Z,15E)- and (9E,12Z,15Z)-octadecatrienoic acids and their [1-^{14}C]-radiolabelled analogs

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Abstract

In order to study the effect of the double bonds geometry of linolenic acid (18:3 *n*-3) on its biological activities, (9*Z*,12*Z*,15*E*)- and (9*E*,12*Z*,15*Z*)-octadecatrienoic acids, found in many refined vegetable oils, were made by total synthesis. Synthesis of 18:3 Δ9*c*, 12*c*, 15*t* involves a Wittig reaction between 3-(2-tetrahydropyranyloxy)-propylphosphonium salt and (*E*)-3-hexenal which gave (3*Z*,6*E*)-1-(2-tetrahydropyranyloxy)-nonadiene in 66% yield. The transformation of the ether function to a phosphonium salt, followed by a Wittig reaction with 8-(*t*-butyldimethylsilyloxy)-octanal afforded a C17 trienic ether. A one-carbon homologation of its corresponding bromide with potassium cyanide followed by hydrolysis in basic medium furnished 18:3 Δ9*c*, 12*c*, 15*t* in high isomeric purity and high radiochemical purity for its [1-^{14}C]-labelled analog. In the synthesis of 18:3 Δ9*t*, 12*c*, 15*c*, a Wittig reaction between (*E*)-6-(2-tetrahydropyranyloxy)-hex-3-enylphosphonium salt, obtained in three steps from 6-(2-tetrahydropyranyloxy)-hex-3-yn-1-ol and (*Z*)-3-hexenal afforded a C12 (*E*,*Z*,*Z*)-trienic ether. After a six-carbon homologation of this ether, in three steps, the resulting nitrile was hydrolyzed to (9*E*,12*Z*,15*Z*)-octadecatrienoic acid (99% purity) (99% radiochemical purity of its [1-^{14}C]-labelled analog).

Keywords: *Trans* linolenic acids synthesis; [1-^{14}C]-labelled fatty acids; Wittig reaction

Abbreviations: DHA, docosahexaenoic acid; DMSO, dimethylsulfoxide; EPA, eicosapentaenoic acid; GC, gas chromatography; HMPA, hexamethylphosphoramide; HPLC, high-performance liquid chromatography; IR, infrared; MS, mass spectrometry; NMR, nuclear magnetic resonance; PDC, pyridinium dichromate; TBDMS, *t*-butyldimethylsilyl; THP, tetrahydropyranyl; TLC, thin-layer chromatography; TMS, tetramethylsilane.

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1. Introduction

Linolenic acid, an essential fatty acid in the human diet [1,2], is a precursor of higher polyunsaturated fatty acids, EPA and DHA, which have important biological activities [3–6]. Plant oils are the major dietary source of linolenic acid, but it is also found in most foods in small quantities [7,8].

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Vegetable oil refining at the deodorization step, realized at high temperature (245–250°C) [9], induces geometrical isomerization of unsaturated fatty acids, particularly of linolenic acid [10]. In deodorized oils, two main geometrical isomers of linolenic acid are found, Δ9*t*, 12*c*, 15*c* 18:3 and Δ9*c*, 12*c*, 15*t* 18:3, along with variable amounts of Δ9*t*, 12*c*, 15*t* 18:3. These *trans* isomers are also present in partially hydrogenated soybean oil [11], fried oils [12], low-calorie spreads [13] and margarines [14]. Recent in vivo studies have shown that one isomer of linolenic acid, Δ9*c*, 12*c*, 15*t* 18:3, could be converted after desaturation and elongation to mono-*trans* isomer of DHA and EPA [15] which are then incorporated in most rat tissue lipids [16,17]. Preliminary studies on the effect of dietary *trans* isomers of linolenic acid on the biosynthesis of arachidonic in rat have indicated an increase in the rate of Δ6 desaturation [18]. In order to study further the effect of mono-*trans* isomers of linolenic acid on the metabolism of essential fatty acids and to gain some insight into their own metabolism pathways, pure synthetic *trans* linolenic acids and their [1-^{14}C]-labelled analogs were needed in sizable amount. Very few reports have appeared in the literature concerning the preparation of geometrical isomers of linolenic acid. In these papers [19,20] were described the synthesis of eight geometrical isomers of linolenic acid using a non-stereoselective Wittig reaction as a key step.

Herein, we described a practical and highly stereoselective synthesis of two geometrical isomers of linolenic acid, (*E,Z,Z*)- and (*Z,Z,E*)-9,12,15-octadecatrienoic acids and of their [1-^{14}C]-labelled analogs where the label carbon was introduced by well-established methods [21,22].

2. Experimental procedures

Starting materials and chemical reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis. [1-^{14}C] Potassium cyanide (spec. act. between 50 and 55 mCi/mmol) was obtained from Slava (Russia). Silica gel (35–70 mesh) was purchased from Amicon (Lausanne, Switzerland). All solvents were purified before use: dichloromethane, dimethylsulfoxide, hexamethylphosphoramide and acetonitrile were distilled from calcium hydride; tetrahydrofuran was distilled from sodium benzophenone ketyl; methyl alcohol was distilled from magnesium metal. 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 AC 200 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. 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 spectrophotometer coupled to a Delsi-DI700 gas chromatograph fitted with a OV1 capillary column (25 m × 0.32 mn i.d.).

Gas chromatography analyses were effected on a 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. The evaporative bulb-to-bulb distillations were done with a Büchi-Kügelrohr oven at the pressure and oven temperature indicated. Analytical thin-layer chromatography (TLC) was performed on 0.25 mm pre-coated silica gel containing a fluorescent indicator. Spots were realized using one or more of the following techniques: (a) UV illumination; (b) spraying with Kägi-Miescher reagent [23]; (c) iodine vapor. Unless otherwise stated, all reactions were carried out under an inert atmosphere.

2.1. (3Z,6E)-1-(2-Tetrahydropyranyloxy)-nona-3-6-diene (3)

To a suspension of 3-(2-tetrahydropyranyloxy)-propylphosphonium salt (**1**) (7.8 g, 14.7 mmol) in a mixture of THF-HMPA (66 ml; 4:1 v/v), cooled at −78°C, was added dropwise *n*-butyllithium (2.2 M in hexane; 6.7 ml, 1 eq.). The solution was allowed to warm up slowly to −40°C, cooled down at −78°C and (3*E*)-hexenal (**2**) (1.2 g, 14.7 mmol) was added. The mixture was allowed to warm up to 0°C (1 h), diluted with a mixture of ether-petroleum ether (1:3 v/v). After evaporation of the solvents, the residue was chromatographed on

silica gel (petroleum ether-ether, 95:5 v/v) and afforded the (*Z,E*)-diene (**3**) (1.82 g, 66% yield) as an oil. IR (film): 3020, 1650, 970. ^{1}H-NMR (200 MHz): 0.95 (t, 3H, J = 7.4 Hz, CH_3), 1.4–1.9 (m, 6H, $3CH_2$), 2.0 (quintuplet, 2H, J = 7 Hz, $C\underline{H}_2$—CH_3), 2.35 (q, 2H, J = 7 Hz, CH=CH—$C\underline{H}_2$—CH_2), 2.75 (t, 2H, J = 5.6 Hz, CH=CH—$C\underline{H}_2$—CH=CH), 3.4 (dt, 1H, J = 9.5 and 7 Hz, CH_2OTHP), 3.5 (m, 1H, $C\underline{H}_2$O—CH—OR), 3.75 (dt, 1H, J = 7.1 and 9.5 Hz, CH_2OTHP), 3.9 (m, 1H, $C\underline{H}_2$O—CH—OR), 4.6 (t, 1H, J = 3.5 Hz, OCHO), 5.3–5.6 (m, 4H, olefinic H). ^{13}C-NMR (50 MHz): 132.5, 129.9, 127.2, 126.2 (olefinic C), 98.7 (THP C2), 67.0 (C1), 62.2 (THP C6), 30.8 (THP C3), 30.5 (C5), 27.9 (C2), 25.6 (C8 and THP C5), 19.6 (THP C4), 13.8 (C9). GC/MS: m/e = 245 M^+. Elemental analysis for $C_{14}H_{24}O_2$; found: C, 74.88; H, 10.71; O, 14.41; calculated: C, 74.95; H, 10.78; O, 14.27.

2.2. *(3Z,6E)-1-Bromonona-3,6-diene* (4)

To a slurry of triphenylphosphine dibromide (13.7 g, 32.5 mmol) in 80 ml of CH_2Cl_2, cooled at 0°C, was added (3*Z*,6*E*)-1-(2-tetrahydropyranyloxyl-nona-3,6-diene (**3**) (3.49 g, 15.6 mmol) in 10 ml of CH_2Cl_2. The mixture was stirred for 1 h at room temperature, diluted with water, washed with a saturated solution of sodium bicarbonate, with water and dried (Na_2SO_4). After concentration in vacuo, a flash chromatography on silica gel (petroleum ether) gave the bromide (**4**) as an oil (3.02 g, 95% yield). IR (film): 3020, 1650, 965. ^{1}H-NMR (200 MHz): 0.9 (t, 3H, J = 7.5 Hz, CH_3), 2.0 (quint., 2H, J = 7 Hz, $C\underline{H}_2$—CH_3), 2.65 (q, 2H, J = 7 Hz, $C\underline{H}_2$—CH_2Br), 2.75 (t, 2H, J = 6 Hz, CH=CH—$C\underline{H}_2$—CH=CH), 3.35 (t, 2H, J = 7 Hz, CH_2Br), 5.3–5.6 (m, 4H, olefinic H). ^{13}C-NMR (50 MHz):132.9, 131.0, 126.6, 126.4 (olefinic C), 32.3 (C1), 30.8 and 30.6 (C2 and C5), 25.6 (C8), 13.8 (C9). GC/MS: m/e = 204 M^+, 202 M^+.

2.3. *(3Z,6E)-Nona-3,6-dienylphosphonium bromide* (5)

A solution containing triphenylphosphine (3.85 g, 14.7 mmol), (3*Z*,6*E*)-1-bromonona-3,6-diene (**4**) (1.49 g, 7.35 mmol) and calcium carbonate (0.38 g) in 16 ml of CH_3CN was heated at 90°C for 2 days. After evaporation of the solvents, the oily residue was chromatographed on silica gel (CH_2Cl_2: MeOH, 95:5 v/v) to furnish the phosphonium salt (**5**) as a solid (3.36 g, 98% yield). IR (KBr) 3060–3000, 1650–1600, 1590. ^{1}H-NMR (200 MHz): 0.9 (t, 3H, J = 7.5 Hz, CH_3), 1.95 (quint., 2H, J = 7.5 Hz, $C\underline{H}_2$—CH_3), 2.4–2.6 (m, 4H) 3.7–3.9 (m, 2H, CH_2P), 5.2–5.7 (m, 4H, olefinic, H), 7.6–7.9 (m, 15H, aromatic). ^{13}C-NMR (50 MHz): 135.2, 133.9 (phenyl C), 132.7 (olefinic C), 130.7 (phenyl C), 130.2, 127.1, 126.4 (olefinic C), 118.3 (phenyl C), 30.4 (C5), 25.6 (C8), 23.1 (C1), 20.5 (C2), 13.8 (C9).

2.4. *8-(t-Butyldimethylsilyloxy)-octanal* (6)

To a mixture of 1,8-octanediol (2 g, 13.7 mmol), dimethylaminopyridine (0.054 g, 0.5 mmol) and triethylamine (0.45 g, 4.4 mmol) was added dropwise at room temperature *t*-butyldimethylchlorosilane (0.51 g, 3.4 mmol) in 2 ml of CH_2Cl_2. After stirring the mixture at room temperature overnight, ether was added, washed several times with water and dried (Na_2SO_4). After evaporation of solvents, the residue was stirred with pentane and the unreacted crystalline diol filtered. The residue was purified by chromatography on silica gel (ether:petroleum ether, 1:1 v/v) and gave the mono-protected diol (0.64 g, 72% yield). ^{1}H-NMR (200 MHz): 0.04 (s, 6H, $Si(CH_3)_2$), 0.9 (s, 9H, SitBu), 1.3 (m, 8H, $4CH_2$), 1.4–1.6 (m, 5H, $2CH_2$ and OH), 3.6 (m, 4H). To a mixture of PDC (9.2 g, 24.5 mmol) and powdered molecular sieves 4 Å (12.6 g) in 90 ml of CH_2Cl_2, was added dropwise at room temperature 8-(*t*-butyldimethylsilyloxy)-octan-1-ol (3.98 g, 15.3 mmol) in 5 ml of CH_2Cl_2. The mixture was stirred at room temperature for 15 min, diluted with a mixture of petroleum ether/ether (3:1 v/v) and filtered on a pad of silica gel. After evaporation of solvents, chromatography of the residue on silica gel (petroleum ether:ether, 90:10, then 50:50 v/v) gave the aldehyde (**6**) as an oil (2.7 g, 68% yield). IR (film): 2710, 1725. ^{1}H-NMR (200 MHz): 0.4 (s, 6H, $SiMe_2$), 0.9 (s, 9H, SitBu), 1.3 (s, 6H, $3CH_2$), 1.4–1.7 (m, 4H, $2CH_2$), 2.4 (dt, 2H, J = 3 and 7 Hz, $C\underline{H}_2$—CHO), 3.6 (t, 2H, J = 7 Hz, CH_2OR), 9.75 (t, 1H, J = 3 Hz, CHO). ^{13}C-NMR (50 MHz): 202.5 (C1), 63.0 (C8), 43.8 (C2), 32.7 (C7),

29.1 ($2CH_2$), 25.9 (SitBu), 25.6 and 22.0 ($2CH_2$), 18.3 (SitBu), −5.3 ($SiMe_2$).

2.5. (8Z,11Z,14E)-1-(t-Butyldimethylsilyloxy)-heptadeca-8,11,14-triene (**7**)

To a solution of (3*Z*,6*E*)-nona-3,6-dienylphosphonium bromide (**5**) (4.37 g, 9.4 mmol) in a mixture of THF-HMPA (9:1 v/v) (50 ml), was added dropwise at −78°C *n*-BuLi (2.5 M in hexanes, 3.8 ml). After stirring for 1 h at −78°C, 8-(*t*-butyldimethylsilyloxy)-octanal (**6**) (2.68 g, 10.4 mmol) in 10 ml of THF was added and the temperature was allowed to warm up to −10°C and stirred at this temperature for 30 min. After precipitation of triphenylphosphine oxide with a mixture of petroleum ether/ether (3:1 v/v) and filtration on a bed of silica gel, the residue was purified by chromatography on silica gel (petroleum ether:ether, 98:2 v/v) to give the (*Z,Z,E*)-triene (**7**) (3.2 g, 93% yield) obtained as an oil. IR (film): 3020, 1660–1640, 965. ^{1}H-NMR (200 MHz): 0.05 (s, 6H, $SiMe_2$), 0.9 (s, 9H, SitBu), 0.95 (t 3H, J = 7.5 Hz, CH_3), 1.3–1.4 (s, 8H, $4CH_2$), 1.45–1.55 (m, 2H, $C\underline{H}_2$—CH_2OR), 1.95–2.1 (m, 4H, $2CH_2$), 2.75 (t, 2H, J = 5.3 Hz, CH=CH—$C\underline{H}_2$—CH=CH), 2.8 (t, 2H, J = 5.3 Hz, CH=CH—$C\underline{H}_2$—CH=CH), 3.6 (t, 2H, J = 6.4 Hz, CH_2OR), 5.3–5.5 (m, 6H, olefinic H). ^{13}C-NMR (50 MHz): 132.5, 130.3, 128.6, 128.0, 127.8, 127.1 (olefinic C), 63.3 (C1), 32.9 (C2), 30.5 (C13), 29.7, 29.4, 29.35 ($3CH_2$), 27.3 (C7), 26.0 (SitBu), 25.85 (C10), 25.6 (C16), 18.4 (SitBu), 13.85 (C17), −5.3 ($SiMe_2$). GC/MS: *m/e* = 364 M^+. Elemental analysis for $C_{23}H_{44}$-OSi; found: C, 75.75; H, 12.16; calculated: C, 75.40; H, 12.20.

2.6. (8Z,11Z,14E)-1-Bromoheptadeca-8,11,14-triene (**8**)

The triene ether (**7**) was converted into the bromide (**8**) in 96% yield by the procedure described for the preparation of (**4**). ^{1}H-NMR (200 MHz): 0.95 (t, 3H, J = 7.4 Hz, CH_3), 1.2–1.5 (m, 8H), 1.85 (quint, 2H, J = 6.9 Hz, $C\underline{H}_2$—CH_2Br), 2.0–2.1 (m, 4H), 2.75 (t, 2H, J = 5.4 Hz), 2.8 (t, 2H, J = 5.4 Hz), 3.4 (t, 2H, J = 6.9 Hz, CH_2Br), 5.3–5.6 (m, 6H, olefinic H). ^{13}C-NMR (50 MHz): 132.5, 130.1, 128.5, 128.0, 127.9, 127.1 (olefinic C), 33.8 (C1), 32.8 (C2), 30.5 (C13), 29.5, 29.1, 28.7, 28.2 ($4CH_2$), 27.2 (C7), 25.6 (C10 and C16), 13.8 (C17). Elemental analysis; found: C, 65.26, H, 9.38; calculated for $C_{17}H_{29}Br$: C, 65.17, H, 9.37.

2.7. (8Z,11Z,14E)-1-Cyanoheptadeca-8,11,14-triene (**9**)

A solution containing potassium cyanide (0.34 g, 3.6 mmol) and (8*Z*,11*Z*,14*E*)-1-bromoheptadecatriene (**8**) (0.805 g, 2.6 mmol) in 17 ml of DMSO was heated at 80°C for 20 min. Water was added (20 ml) and extracted three times with ether (40 ml). The combined ethereal extracts were dried (Na_2SO_4) and evaporated. Chromatography of the residue on silica gel (petroleum ether:ether, 95:5 v/v) gave the nitrile (**9**) as an oil (0.64 g, 96% yield). IR (film): 3010, 2240, 1660–1640, 965. ^{1}H-NMR (200 MHz): 0.95 (t, 3H, J = 7.4 Hz, CH_3), 1.2–1.5 (m, 8H, $4CH_2$), 1.6–1.7 (m, 2H, $C\underline{H}_2$—CH_2CN), 1.9–2.1 (m, 4H, $2CH_2$), 2.3 (t, 2H, J = 7 Hz, CH_2CN), 2.75 (t, 2H, J = 5.4 Hz), 2.8 (t, 2H, J = 5.4 Hz), 5.3–5.6 (m, 6H, olefinic H). ^{13}C-NMR (50 MHz): 132.5, 130.0, 128.4, 128.0, 127.9, 127.0 (olefinic C), 119.7 (CN), 30.4 (C13), 29.4, 28.9, 28.6, ($3CH_2$), 27.1 (C7), 25.5 (C10), 25.3 (C16), 17.1(C1),13.8(C7). GC/MS: *m/e* = 259M^+. Elemental analysis; found: C, 83.47, H, 11.31; calculated for $C_{18}H_{29}N$: C, 83.33, H, 11.27.

2.8. Methyl (9Z,12Z,15E)-octadeca-9,12,15-trienoate (**10**)

To a solution of (8*Z*,11*Z*,14*E*)-1-cyanoheptadeca-8,11,14-triene (**9**) (0.075 g, 0.39 mmol) in 1.2 ml of methanol was added 46% anhydrous methanolic HCl (12 ml) at 0°C. The solution was stirred at 0°C for 4 h and overnight at room temperature. The solution was then poured into water and stirred at 0°C for 25 min. The product was extracted three times with ether (20 ml). The combined etheral extracts were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (petroleum ether:ether, 98:2 v/v) to give the *trans* isomer of linolenic (**10**) (0.15 g, 89% yield and 98.7% purity determined by GC. IR (film): 3010, 1735, 1660–1640, 965. ^{1}H-NMR (200 MHz): 0.95 (t, 3H, J = 7.4 Hz, CH_3), 1.2–1.4 (m, 8H), 1.6–1.7 (m, 2H, $C\underline{H}_2$—CH_2—CO_2Me), 1.9–2.1 (m, 4H), 2.3 (t, 2H, J = 7.4 Hz, CH_2—CO_2Me), 2.75 (t, 2H; J = 5.3 Hz), 2.8 (t, 2H,

J = 5.3 Hz), 3.7 (s, 3H, CO_2Me), 5.3–5.5 (m, 6H, olefinic H). ^{13}C-NMR (50MHz): 174:2 (CO), 132.5, 130.2, 128.6, 128.0, 127.9, 127.2 (olefinic C), 51.4 (CO_2CH_3), 34.1 (C2), 30.5 (C14), 29.6 (C7), 29.23, 29.2, 29.16 ($3CH_2$), 27.25 (C8), 25.6 (C11), 25.0 (C17), 13.9 (C18). GC/MS: m/e = 292M$^+$.

2.9. (9Z,12Z,15E)-Octadecatrienoic acid (**11**)

To a solution of the nitrile (**9**) (0.041 g, 0.16 mmol) in ethanol (10 ml) was added a 40% aqueous solution of KOH. The mixture was heated at 80–85°C for 7 h, diluted with cold water, acidified with a 10% HCl solution. The product was extracted with ether, dried (Na_2SO_4) and the solvents evaporated. Purity of the acid (**11**) was determined by GC, as its methyl ester, as being 97.5%.

2.10. [1-^{14}C]-(9Z,12Z,15E)-Octadecatrienoic acid (**11**)

2.10.1. Synthesis of the [1-^{14}C] nitrile (9) from the bromide (8). The reaction was realized on 0.5 mmol scale with K^{14}CN in the same reaction conditions as the 'cold' synthesis. After a usual work-up, the residue was purified by column chromatography on silica gel (40–63 mesh) (hexane:ether, 9:1 v/v) to give [1-^{14}C] (**9**) in 80% yield.

2.10.2. Hydrolysis of the [1-^{14}C] nitrile (9). The same procedure was used as for its non-radioactive analog (0.25 mmol of [1-^{14}C] (**9**) 80°C for 3 h). The acid was purified by reversed phase HPLC (column: Zorbax ODS 7 mesh, 250 mm × 21.2 mm i.d.) (acetone:water:methanol:acetic acid, 60:20:20:0.05 v/v) and obtained in 80% yield (specific activity: 52.5 mCi/mmol). The radioactive purity was determined by radiochromatography and HPLC and found >99%.

2.11. (3E)-6-(2-Tetrahydropyranyloxy)-hex-3-en-1-ol (**13**)

To a suspension of lithium aluminium hydride (0.91 g, 24 mmol) in 40 ml of THF, cooled at 0°C, was added 6-(2-tetrahydropyranyloxy)-hex-3-yn-1-ol (**12**) (1.19 g, 6 mmol) in 10 ml of THF. The mixture was refluxed for 39 h. The excess of hydride was hydrolyzed carefully with cold water, the precipitate was filtered and washed with ether. The etheral phase was dried (Na_2SO_4) and evaporated. Column chromatography on silica gel (petroleum ether:ether, 40:60 v/v) afforded the (*E*)-homoallylic alcohol (**13**) as an oil (1.15 g, 95% yield) which displayed identical spectral properties to those described in ref. [33].

2.12. (3E)-1-Iodo-6-(2-tetrahydropyranyloxy)-hex-3-ene (**15**)

To a solution containing triphenylphosphine (12.2 g, 47 mmol), imidazole (6.35 g, 93 mmol) and (3*E*)-6-(2-tetrahydropyranyloxy)-hex-3-en-1-ol (**13**) (6.2 g, 31 mmol) in 85 ml of THF, cooled at −30°C, was added iodine (11.8 g, 47 mmol). The solution was allowed to warm up to room temperature and stirred at this temperature for 1 h. The reaction mixture was diluted with ether (100 ml), washed successively with water, a saturated solution of sodium bicarbonate and water. The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed on silica gel (petroleum ether:ether, 95:5 v/v) to give the oily iodide (**15**) (8.6 g, 89% yield) which has identical spectral data to those described in the literature [33].

2.13. (3E)-6-(2-Tetrahydropyranyloxy)-hex-3-enylphosphonium iodide (**16**)

A mixture of (3*E*)-1-iodo-(2-tetrahydropyranyloxy)-hex-3-ene (**15**) (4 g, 12.9 mmol), triphenylphosphine (6.8 g, 26 mmol) and calcium carbonate (1.1 g) in 30 ml of acetonitrile was heated at 65°C for 41h. After filtration on a sintered funnel, the reaction mixture was concentrated and the residue purified by column chromatography on silica gel (CH_2Cl_2-MeOH, 98:2 then 95:5). The phosphonium salt (**16**) was obtained as a solid (7.1 g, 96% yield) which has identical spectra data to those described in the literature [33].

2.14. (3E,6Z,9Z)-1-(2-Tetrahydropyranyloxy)-dodeca-3,6,9-triene (**18**)

To a solution of (3*E*)-6-(2-tetrahydropyranyloxy-hex-3-enylphosphonium iodide (**16**) (0.63 g, 1.1 mmol) in 6 ml of THF, cooled at −78°C, was added *n*-butyllithium (2.4 M in hexanes; 0.5 ml, 1.2 mmol) then HMPA (0.6 ml). The solution was stirred for 1 h at −78°C and then (3*Z*)-hexenal (0.22 g, 2.2 mmol) in 2 ml of THF was added. The reac-

tion mixture was allowed to warm up to room temperature and diluted with a mixture of petroleum ether:ether (3:1 v/v). Triphenylphosphine oxide was filtered on a bed of silica gel and the filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:ether, 98:2 v/v) to afford the trienic ether (**18**) as an oil (0.15 g, 52% yield). ^{1}H-NMR (200 MHz): 0.95 (t, 3H, J = 7.5 Hz, CH_3), 1.5–1.8 (m, 6H, THP H3, H4, H5), 2.0–2.15 (m, 2H, $C\underline{H}_2$—CH_3), 2.25–2.35 (m, 2H, CH_2—CH_2-OTHP), 2.75–2.80 (m, 4H, 2CH=CH—$C\underline{H}_2$—CH=CH), 3.4 (dt, 1H, J = 7 and 9.6 Hz, $C\underline{H}_2$—OTHP), 3.45–3.60 (m, 1H, THP C6), 3.75 (dt, 1H, J = 7 and 9.6 Hz, CH_2OTHP), 3.8–3.9 (m, 1H, THP H6), 4.6 (t, 1H, J = 3 Hz, THP H2), 5.3–5.5 (m, 6H, olefinic H). ^{13}C-NMR (50MHz): 131.9, 130.3, 128.8, 127.7, 127.2, 127.0 (olefinic C), 98.7 (THP C2), 67.3 (C1), 62.1 (THP C6), 33.15 (C2), 30.8 (THP C3), 30.6 (C5), 25.7 (THP C5), 25.5 (C8), 20.6 (C11), 19.6 (THP C4), 14.3 (C12).

2.15. (3E,6Z,9Z)-1-Bromododeca-3,6,9-triene (**19**)

The bromide (**19**) was obtained from the tetrahydropyranyl ether (**18**) in 95% yield using the procedure previously described for the preparation of (**4**). ^{1}H-NMR (200 MHz): 0.95(t, 3H, J = 7.5 Hz, CH_3), 2.05 (quint., 2H, J = 7.2 Hz, $C\underline{H}_2$—CH_3), 2.55 (q, 2H, J = 7 Hz, $C\underline{H}_2$—CH_2Br), 2.75–2.8 (m, 4H, 2CH=CH—$C\underline{H}_2$—CH=CH), 3.35 (t, 2H, J = 7.1 Hz, CH_2Br), 5.2–5.6 (6H, m, olefinic H). ^{13}C-NMR (50MHz): 132.0, 131.7, 129.2, 127.2, 127.1, 127.0 (olefinic C), 36.1 (C1), 35.2 (C2), 30.8 (C5), 25.5 (C8), 20.6 (C11), 14.3 (C12).

2.16. (8E,11Z,14Z)-1-(2-Tetrahydropyranyloxy)-heptadeca-8,11,14-triene (**21**)

To a suspension of magnesium turnings (0.24 g, 10 mmol) in 1 ml of THF containing a crystal of iodine, was added a solution of 1-chloro-5-(2-tetrahydropyranyloxy)-pentane (1 g, 4.8 mmol) in 5 ml of THF. After the addition, the reaction mixture was refluxed for 30 min and cooled at room temperature. The Grignard solution was cannulated, to a solution of (3*E*,6*Z*,9*Z*)-1-bromododeca-3,6,9-triene (**19**) (0.4 g, 1.65 mmol) in 6 ml of THF. The reaction mixture was cooled at 0°C, and Li_2CuCl_4 (0.2 M in THF; 0.1 ml) [39] was added. The solution was stirred for 1 h at 0°C and overnight at room temperature. The reaction mixture was diluted with ether (50 ml), and washed with water (2 × 10 ml), dried. After evaporation of volatiles, the residue was heated at 90–100°C (0.05 mmHg) to remove the hydrolysis product of the Grignard reagent in excess [(2-tetrahydropyranyloxy)-pentane]. Chromatography on silica gel of the residue (petroleum ether:ether, 98:2 v/v) gave the C17 trienic ether (**21**) (0.52 g, 94% yield). IR (film): 3010, 1660–1640, 965. ^{1}H-NMR (200 MHz): 1.0 (t, 3H, J = 7.5 Hz, CH_3), 1.3–1.4 (m, 8H, $4CH_2$), 1.5–1.8 (m, 8H, $4CH_2$), 1.9–2.1 (m, 4H), 2.7–2.85 (m, 4H, 2CH=CH—$C\underline{H}_2$—CH=CH), 3.35 (dt, 1H, J = 6.6 and 9.6 Hz, CH_2—OTHP), 3.4–3.55 (m, 1H, THP H6), 3.75 (dt, 1H, J = 6.8 and 9.6 Hz, CH_2OTHP), 3.8–3.95 (m, 1H, THP H6), 4.55 (t, 1H, J = 3 Hz, THP H2), 5.2–5.5 (m, 6H, olefinic H). ^{13}C-NMR (50MHz): 131.9, 130.95, 128.6, 128.1, 128.0, 127.2 (olefinic C), 98.8 (THP C2), 67.7 (C1), 62.3 (THP C6), 32.6 (C7), 30.8 (THP C3), 30.5 (C10), 29.8, 29.5, 29.4, 29.2, 26.3 ($5CH_2$), 25.6 (THP C5), 25.5 (C13), 20.6 (C16), 19.7 (THP C4), 14.3 (C17). Elemental analysis; found: C, 79.07, H, 11.54; calculated for $C_{17}H_{38}O_2$: C, 78.98, H, 11.45.

2.17. (8E,11Z,14Z)-1-Bromoheptadeca-8,11,14-triene (**22**)

The trienic ether (**21**) was converted into the bromide (**22**) in 96% yield by the procedure described for the preparation of (**4**). ^{1}H-NMR (200 MHz): 0.95 (t, 3H, J = 7.5 Hz, CH_3), 1.2–1.5 (m, 8H, $4CH_2$), 1.85 (quint., 2H, J = 6.9 Hz, $C\underline{H}_2$—CH_2Br), 1.95–2.15 (m, 4H), 2.7–2.85 (m, 4H, 2CH=CH—$C\underline{H}_2$—CH=CH), 3.4 (t, 2H, J = 6.9 Hz, CH_2Br), 5.3–5.45 (m, 6H, olefinic H). ^{13}C-NMR (50MHz): 131.9, 130.8, 128.6, 128.2, 127.9, 127.2 (olefinic C), 33.9 (C1), 32.8 (C2), 32.5 (C7), 30.5 (C10), 29.4, 28.9, 28.6, 28.15 (CH_2), 25.5 (C13), 20.5 (C16), 14.3 (C17). Elemental analysis; found: C, 64.69, 9.37; calculated for $C_{17}H_{29}Br$: C, 65.17, H, 9.33.

2.18. (8E,11Z,14Z)-1-Cyanoheptadeca-8,11,14-triene (**23**)

The nitrile (**23**) was obtained from the bromide (**22**) in 99% yield using the procedure previously

described for the preparation of (**9**). ^{1}H-NMR (200 MHz): 1.0 (t, 3H, J = 7.5 Hz, CH_3), 1.25–1.5 (m, 8H), 1.65 (m, 2H, CH_2—CH_2CN), 1.95–2.15 (m, 4H), 2.35 (t, 2H, J = 7 Hz, CH_2CN), 2.7–2.85 (m, 4H, 2CH=CH—CH_2—CH=CH), 5.3–5.5 (m, 6H, olefinic H). ^{13}C-NMR (50MHz): 131.9, 130.7, 128.6, 128.3, 127.9, 127.2 (olefinic C), 119.7 (CN), 32.5 (C7), 30.4 (C10), 29.3, 28.8, 28.6 ($3CH_2$), 25.5 and 25.4 (C13 and CH_2), 20.6 (C16), 17.1 (C1), 14.3 (C17). GC/MS: m/e = 259 M^+. Elemental analysis; found: C, 83.07; H, 11.25; calculated for $C_{18}H_{29}N$: C, 83.33; H, 11.27.

2.19. Methyl (9E,12Z,15Z)-octadeca-9,12,15-trienoate (**24**)

The nitrile (**23**) was converted into the ester (**24**) in 92% yield by the procedure described for the preparation of (**10**). IR (film): 3010, 1740, 1660–1640, 965. ^{1}H-NMR (300 MHz): 0.95 (t, 3H, J = 7.5 Hz, CH_2—CH_3), 1.3 (s, 8H, $4CH_2$), 1.55–1.7 (2H, m, CH_2—CH_2CO_2Me), 1.95 (q, 2H, J = 6.3 Hz), 2.1 (quint, 2H, J = 7.4 Hz, CH_2CH_3), 2.3 (t, 2H, J = 7.4 Hz, CH_2CO_2Me), 2.7–2.8 (m, 4H, CH=CH—CH_2—CH=CH), 3.65 (s, 3H, OCH_3), 5.25–5.5 (m, 6H, olefinic H). ^{13}C-NMR (50MHz): 174.2 (C1), 131.9, 130.9, 128.6, 128.16, 128.0, 127.2 (olefinic C), 51.4 (CO_2CH_3), 34.1 (C2), 32.6 (C8), 30.5 (C11), 29.5 (C7), 29.2 (C4 and C5), 29.0 (C6), 25.5 (C14), 25.0 (C3), 20.6 (C17), 14.3 (C18). GC/MS: m/e = 292 M^+.

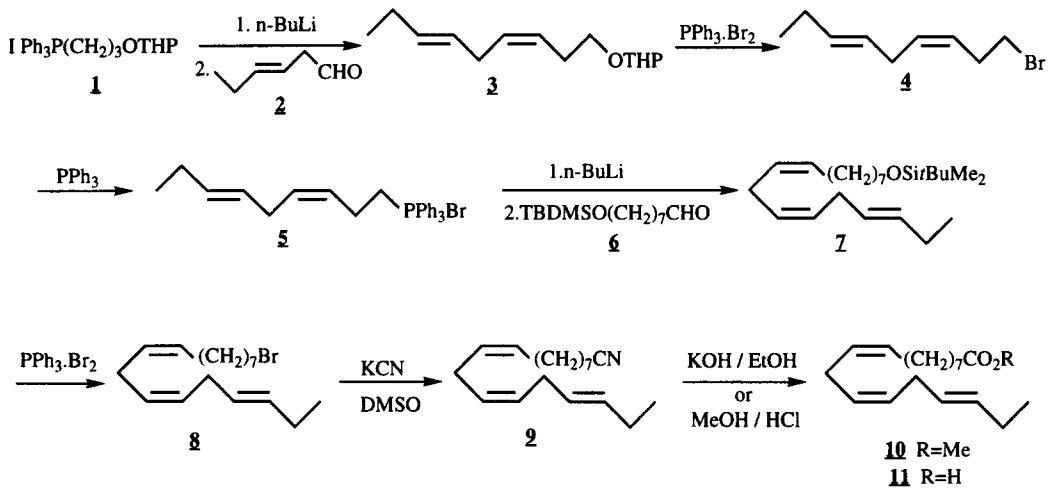
2.20. [1-^{14}C]-(9E,12Z,15Z)-Octadeca-9,12,15-trienoic acid (**25**)

The radiolabelled (*E,Z,Z*)-isomer of linolenic acid (**25**) was obtained from the bromide (**22**) using the same protocol as for its (**Z,Z,E**)-isomer (**11**). It has a radiochemical purity >99% (specific activity: 50.8 mCi/mmol).

3. Results and discussion

3.1. Synthesis of (9Z,12Z,15E)-octadecatrienoic acid (11) and its [1-^{14}C]-labelled analog

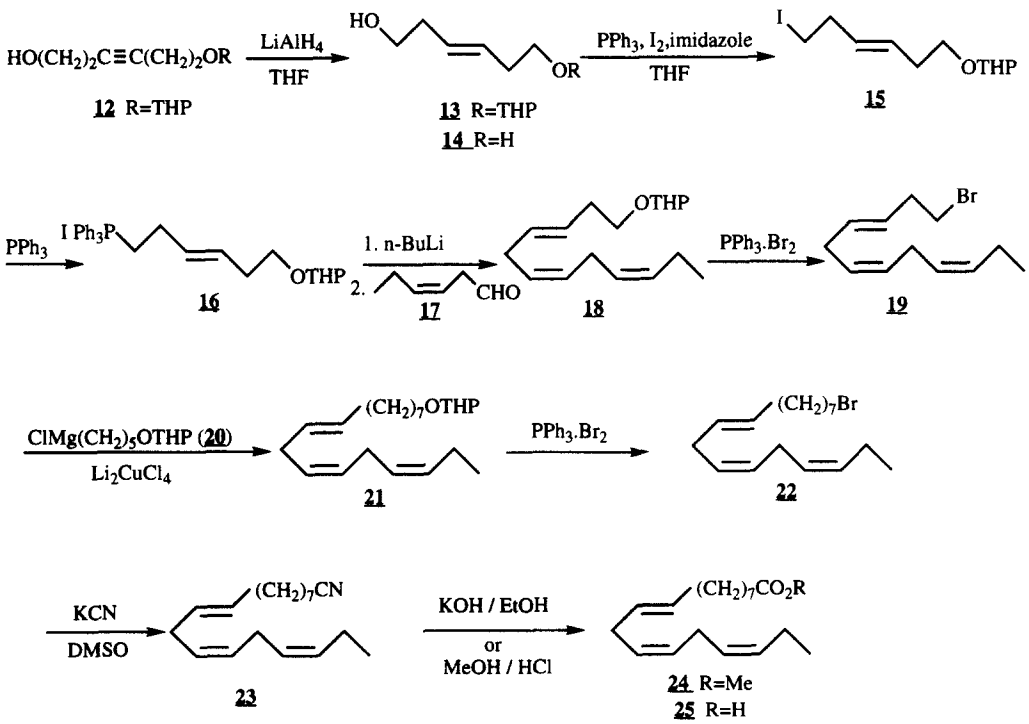
Synthesis of the trienoic fatty acid (**11**), outlined in Scheme 1, starts by a Wittig reaction under *cis*-olefination conditions [24] between (*E*)-3-hexenal (**2**) [25] and the ylide of the three-carbon phosphonium salt (**1**) [26] to afford the (3*Z*,6*E*)-diene (**3**) in 66% yield (>98% purity by GC). The structure and the isomeric purity of compound (**3**) were confirmed by ^{13}C-NMR spectroscopy. The chemical shifts for C5 and C8 which appeared, respectively at 30.5 and 25.6 ppm supported the configuration assigned for the 3,4 and 6,7 double bonds [19,20]. Compound **3** was transformed to the bromide (**4**) by reaction with triphenylphosphine dibromide



Scheme 1. Synthesis of methyl (9*E*,12*Z*,15*Z*)-octadecatrienoate acid (**24**).

[27] (95% yield). Displacement of the bromine atom of (**4**) by triphenylphosphine in refluxing acetonitrile gave the nine-carbon chain unsaturated phosphonium salt (**5**) in 98% yield. Introduction of the eight-carbon atoms C2 – C9 of the isomeric linolenic acid (**11**) to compound (**5**) was achieved by a highly stereoselective Wittig reaction with *t*-butyldimethylsilyloxyaldehyde (**6**), in the presence of HMPA [24], which led exclusively to the (8*Z*,11*Z*,14*E*)-trienic ether (**7**) in 93% yield. The geometry of the newly formed olefinic bond at C8 of compound (**7**) was supported by ^{13}C-NMR chemical shifts of C7 and C10 which were, respectively located at 27.3 and 25.85 ppm [19,20]. Aldehyde (**6**) was readily available in two steps from 1,8-octanediol in 49% overall yield [28–30]. Tranformation of the *t*-butyldimethylsilyl ether (**7**) to the bromide (**8**) was effected by triphenylphosphine dibromide in 96% yield [31]. The one-carbon homologation of compound (**8**) was accomplished by potassium cyanide in DMSO to give the unsaturated nitrile (**9**) in 96% yield. Alcoholysis of the nitrile (**9**) with 46% anhydrous methanolic HCl, at room temperature, gave intermediatly the hydrochloride salt of the corresponding imino ether of (**9**) [32] which was hydrolyzed with water to methyl (9*Z*,12*Z*,15*E*)-octadecatrienoate (**10**) in 89% yield (98.7% purity determined by GC analysis). The analytical properties (^{13}C-NMR, GC analysis) of the fatty acid (**10**) were identical in all respects to those of the synthetic (**10**) [19, 20] or to a sample isolated from heated linseed oil [12]. Methyl Δ15*t* linolenate could also be obtained in 68% yield (97.5% purity) by hydrolysis of the nitrile (**9**) at 80°C, in the presence of potassium hydroxide in a mixture of water/ethanol, followed by methylation with diazomethane.

The synthesis of [1-^{14}C]-radiolabelled fatty acid (**11**) from the trienic bromide (**8**) followed the same



Scheme 2. Synthesis of methyl (9*Z*,12*Z*,15*E*)-octadecatrienoate (**10**).

procedure of that described for the 'cold' synthesis. After purification by HPLC, radiochemical purity of [1-^{14}C]-(**11**) was determined by TLC and HPLC as being >99% (specific activity: 52.5 mCi/mmol).

3.2. Synthesis of (9E,12Z,15Z)-octadecatrienoic acid (25) and of its [1-^{14}C]-labelled analog

As shown in Scheme 2, our synthetic strategy of the fatty acid (**25**) involves a stepwise six-carbon elongation chain of both ends of the (*E*)-unsaturated symmetric diol (**14**). The synthesis of monoprotected (3*E*)-hexene-1,6-diol (**13**) has already been described from a commercially available (*E*)-β-hydromuconic acid [33]. In our work, the yield of this synthetic route to (**13**) did not exceed 28% and was difficult to scale up. Then, we developed a practical synthesis of compound (**13**) which used an highly stereoselective reduction of the acetylenic alcohol (**12**) with lithium aluminium hydride in refluxing THF (95% yield) [35]. The monoprotected hex-3-yne-1,6-diol (**12**) was readily prepared in two steps from the commercially available but-3-yne-1-ol in 78% overall yield [34]. The alcohol (**13**) was transformed to the phosphonium salt (**16**) by first iodination in the modified Garegg-Samuelsson conditions [36,37] followed by displacement of the iodine atom by triphenyl phosphine (85% overall yield). Introduction of the two bonds of Δ9*t* linolenic acid (**25**) in the 12 and 15 positions was achieved by an highly stereoselective Wittig reaction between the ylide of the phosphonium salt (**16**) and (*Z*)-3-hexenal (**17**) [25] to give the triene (**18**) in 52% yield. ^{13}C-NMR spectral analysis corroborated the structure assigned for compound (**18**). Direct transformation of the tetrahydropyranyl ether (**18**) to the bromide (**19**) was effected by triphenylphosphine dibromide and cross-coupling of (**19**) with the Grignard reagent **20**, prepared from 5-chloro-1-tetrahydropyranyloxypentane [38], in the presence of dilithium tetrachlorocuprate [39] yielded the C17 fatty ether (**21**) in 94% yield. Treatment of the trienic THP ether (**21**) with triphenylphosphine dibromide provided the bromide (**22**) which was transformed, via the nitrile (**23**), to methyl (9*E*,12*Z*,15*Z*)-octatrienoate (**24**) in 91% overall yield and 99% purity as determined by GC. The spectra data of (**24**) are in good agreement with those reported for a synthetic sample [19, 20].

The synthesis of the [1-^{14}C]-labelled fatty acid (**25**) from the bromide (**22**) used the same protocol as that described for the 'cold' synthesis of (**25**). It has a radiochemical purity of 99% (determined by HPLC and radiochromatography) and a specific activity of 50.8 mCi/mmol.

In conclusion, we have developed an efficient and highly stereoselective synthesis of two geometrical isomers of linolenic acid and of their [1-^{14}C]-labelled analogs, which allowed their preparation on a gram scale. Biological studies of compounds (**11**) and (**25**) are in progress.

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