

Chemistry and Physics of Lipids 78 (1995) 71-80 Chemistry and Physics of LIPIDS

Synthesis of (9Z,12E)- and (9E,12Z)- $[1-^{14}C]$ linoleic acid and (5Z,8Z,11Z,14E)- $[1-^{14}C]$ arachidonic acid

Olivier Berdeaux^a, Jean- Michel Vatèle^{*b}, Thierry Eynard^b, Mohammed Nour^a, Didier Poullain^c, Jean- Pierre Noël^c, Jean- Louis Sébédio^a

"INRA, Unité de Nutrition Lipidique, 17 rue Sully, 21034 Dijon Cédex, France ^bLaboratoire de Chimie Organique I, Associé au CNRS, Université Claude Bernard, CPE-Lyon, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cédex, France °CEA Saclay, Service des Molécules Marquées, 91191 Gif-sur-Yvette, France

Received 10 April 1995; revision received 21 July 1995; accepted 26 July 1995

Abstract

(3Z,6E)-1-Bromododeca-3,6-diene, a common intermediate in the synthesis of labelled 12t linoleic acid and 14t arachidonic acid, was obtained from (E)-3-nonyl-1-ol, in four steps, via conventional functional manipulations. Cross-coupling of this bromododecadiene with the Grignard reagent of 1-chloro-5-(tetrahydropyranyloxy)pentane gave a C17 dienic ether which was further transformed in three steps to 12t-[1-¹⁴C]linoleic acid in 22% overall yield from 3-nonyl-1-ol (eight steps). The synthesis of 14t arachidonic acid involves a Wittig reaction between (Z)-7-(t-butyldiphenylsilyloxy)hept-3-enal and the yilde of (3Z,6E)-dodeca-3.6-dienyl-triphenylphosphonium bromide. The resulting C19 tetraenic ether was transformed in three steps to 14t [1-¹⁴C]arachidonic acid (isomeric and radiochemical purities > 99%). 9t Linoleic acid was obtained by a stepwise six-carbon elongation chain of both ends of (E)-6-(2-tetrahydropyranyloxy)-hex-3-enyltriphenylphosphonium salt in 20% overall yield.

Keywords: n-6 Trans polyunsaturated fatty acid synthesis; [1-14C]-Labelled fatty acids; Wittig reaction

Abbreviations: DMSO, dimethylsulfoxide; GC, gas chromatography; HMPA, hexamethylphosphoramide; HPLC, high-performance liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; TFA, trifluoroacetic acid; THF, tetrahydrofuran; THP, tetrahydropyranyl; TLC, thin-layer chromatography; TMS, tetramethylsilane.

1. Introduction

Edible oil processing results in the conversion of variable amounts of the naturally occuring polyunsaturated C18 fatty acids into a variety of isomers, including large quantities of *trans* fatty acids. Hydrogenation of oils is a widely used process to increase the melting range of oils so as to obtain solid fats and to improve the stability of oils towards oxidation. In the course of the hy-

^{*} Corresponding author, Phone: (+33) 7243 1151; Fax: (+33) 7243 1214.

drogenation many geometrical and positional isomers of linoleic are formed [1]. Geometrical isomers of linoleic acid are subsequently found in hydrogenated vegetable oil-containing foods like margarines, shortenings cooking oils, parfried French fries, cookies [2] and infant formulas [3,5]. *Trans* isomers of linoleic acid are also found in deodorized or fried vegetable oils [6,8].

It has been shown in rats and mice fed a diet supplemented with *trans* linoleic acids that these *trans* fatty acids could be incorporated into most tissue lipids like liver, heart and brain [9,10]. The presence of 9c,12t and 9t,12c 18:2 was also detected in human tissue lipids [11]. The metabolism of *trans* isomers of linoleic acid and their effects on the metabolism of linoleic acid have been studied in both the intact animal [9,12–15] and at enzyme level [16]. These studies have shown that *trans* isomers of linoleic acid did not affect the level of arachidonate [14] and that only the 9c,12tisomer could be desaturated and elongated to 5c,8c,11c,14t 20:4 by the same pathway as *all-cis* linoleic acid [9,13–14].

In order to study further the metabolic pathways of 9c,12t and 9t,12c isomers of linoleic acid and the physiological effects of 14t 20:4, we needed these compounds and their $[1^{-14}C]$ -labelled analogs in pure form and sizable amount.

In the literature, only a few total syntheses of geometrical isomers of arachidonic or linoleic acids are reported. H. Rakoff et al. have described a practical and stereoselective synthesis of 9c, 12t18:2 in two steps from 9,10-dihydroxyoctadecanoate isolated from Vernonia anthelmintica seed oil [17]. They also reported a non-stereoselective preparation of deuterated 9c, 12t and 9t, 12c 18:2 using the Wittig reaction as a key step [18]. Lastly, G.J.N. Egmond et al. used the 'acetylenic approach' developed by Osbond [19] to synthesize 5c, 8c, 11c, 14t 20:4 in low overall yield (< 1%) [20]. None of these synthesis of mono trans fatty acids of the n-6 series are suitable for the introduction of the label carbon atom at the last stage of the synthesis.

Here, we report a divergent synthesis of 9c,12t18:2 and 5c,8c,11c,14t 20:4 and of their [1-¹⁴C]-labelled analogs and a highly stereoselective synthesis of the 9t,12c isomer of linoleic acid.

2. Experimental procedures

2.1. Laboratory materials

Starting materials and chemical reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis. [1-¹⁴C]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: alcohols were distilled from magnesium metal. NMR spectra were measured on a Brucker AC 200 Fourier transform spectrophotometer with proton observation at 200 MHz and carbon observation at 50 MHz. Unless otherwise stated spectra were recorded in CDCl₃, and chemical shifts are reported (in ppm) downfield form TMS (δ). Gas chromatography analyses were effected on a OV 1701 capillary column (25 m \times 0.32 mm i.d.) using a Varian 3300 gas chromatograph (N_2 , 2 ml/min) fitted with a flame detector. Analytical thin-layer chromatography (TLC) was performed on 0.25-mm pre-coated silica gel containing a fluorescent indicator. Unless otherwise stated, all reactions were carried out under an inert atmosphere.

2.2. (E)-1-Iodonon-3-ene (2)

To a solution containing triphenylphosphine (10.62 g, 40.5 mmol), imidazole (5.51 g, 81 mmol) and (E)-3-nonyl-1-ol (3.8 g 27.5 mmol) in 60 ml of THF, cooled to -10° C, iodine (10.28 g, 40.5 mmol) was added. The solution was allowed to warm up to room temperature and stirred at this temperature for 45 min. The reaction mixture was diluted with ether (200 ml), washed successively with water, a saturated solution of sodium thiosulfate and water. The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was stirred with petroleum ether and triphenylphosphine oxide filtered. After evaporation of the solvent, the residue was distilled in a Büchi-Kugelrohr apparatus (10⁻² mmHg, 70°C) to give the iodo compound 2 as an oil (5.33 g, 78%) yield). ¹H-NMR: 0.88 (t, 3H, J = 7 Hz, CH₃), 1.17–1.45 (m, 6H, 3CH₂), 1.97 (quint., 2H, J = 6Hz, (CH₂)₃–CH₂CH=CH) 2.45 (q, 2H, J = 7 Hz CH₂–CH₂I), 3.14 (t, 2H, J = 7 Hz, CH₂I), 5.34 (qt, 1H, J = 14, 6, 1 Hz, CH=CH), 5.52 (qt, 1H, CH=CH). ¹³C-NMR: 133.6 (C4), 128.2 (C3), 36.8 (C2), 32.5 (C5), 31.4 (C7), 28.9 (C6), 22.5 (C8), 14.1 (C9), 6.1 (C1). GC/MS: m/e = 252 M⁺.

2.3. ((E)-Non-3-enyl))triphenylphosphonium iodide (3)

A suspension containing triphenylphosphine (9.84 g, 37.5 mmol) the iodo compound **2** (4.73 g, 18.7 mmol) and calcium carbonate (0.3 g) in 40 ml of acetonitrile was heated at 60°C for 48 h. After filtration of calcium carbonate and evaporation of acetonitrile, the phosphonium salt **3**, obtained as a gum (9.35 g, 98% yield), was dried under vacuum (0.05 mmHg) for 24 h at room temperature.

2.4. (3Z,6E)-1-(2-Tetrahydropyranyloxy)dodeca-3,6-diene (5)

To a solution of the phosphonium salt, 3(7.7 g)15.2 mmol) in a mixture of THF-HMPA (100 ml, 9:1 v/v), cooled to -78° C, *n*-BuLi (2.6 M in hexanes, 5.8 ml) was added dropwise. After stirring for 1 h at -78° C, 3-(2-tetrahydropyranyloxy)propanal (4) [21] (2.36 g, 15.1 mmol) in 10 ml of THF was added and the temperature was allowed to warm up to 0°C (90 min). After stirring the mixture at room temperature for 30 min, a mixture of petroleum ether/ether (100 ml, 3:1 v/v) was added and the suspension was filtered on a bed of silica gel. After evaporation of the solvents, the residue was chromatographed on silica gel (petroleum ether/ether, 95:5 v/v) to afford the (E,Z)-dienic ether, 5, as an oil (3.48 g, 86% yield). ¹³C-NMR: 131.0 (C7), 129.9, 128.0, 126.1 (olefinic C), 98.7 (THP C2), 67.0 (C1), 62.2 (THP C6), 32.6 (C8), 31.4 (C10), 30.7 (C5), 30.6 (THP C3), 29.2 (C9), 27.9 (C2), 25.5 (THP C5), 22.6 (C11), 19.6 (THP C4), 14.1 (C12).

2.5. (3Z,6E)-1-Bromododeca-3,6-diene (6)

The diene 5 (1.5 g, 5.6 mmol), in 12 ml of CH_2Cl_2 , was added to a slurry of triphenylphosphine dibromide (5 g, 11.3 mmol) in 12 ml of

CH₂Cl₂ cooled to 0°C. The mixture was stirred at room temperature for 15 min, diluted with ether, washed with a saturated solution of sodium bicarbonate, water and dried (Na₂SO₄). After concentration in vacuo, flash chromatography of the residue on silica gel (petroleum ether) gave the bromide 6 (1.18 g, 86% yield) as an oil. ¹H-NMR: 0.9 (t, 3H, J = 7 Hz, CH₃), 1.15–1.43 (m, 6H, $3CH_2$), 1.94 (quint., 2H, J = 6 Hz, $(CH_3)_3 - CH_2 - CH_3$ CH=CH), 2.61 (q, 2H, J = 7 Hz, $CH_2 - CH_2Br$), 2.72 (t, 2H, J = 6.3 Hz, $CH = CH - CH_{2}$ CH=CH), 3.35 (t, 2H J = 7 Hz, CH₂Br), 5.23– 5.62 (m, 2H, CH=CH). ¹³C-NMR: 131.4 (C4) 131.0 (C7), 127.5 (C6), 126.4 (C3), 32.5 (C8), 32.3 (C1), 31.4 (C10), 30.8 (C5), 30.6 (C2), 29.2 (C9), 22.6 (C11), 14.1 (C12). GC/MS: m/e = 244 M⁺, 246 M⁺.

2.6. (8Z,11E)-1-(2-Tetrahydropyranyloxy)heptadeca-8,11-diene (8)

A solution of 1-chloro-5-(2-tetrahydropyranyloxy)pentane (1 g, 4.8 mmol) in 6 ml of THF was added carefully to a suspension of magnesium turnings (0.24 g, 9.9 mmol) in 2 ml of THF. The reaction mixture was then refluxed for 30 min and cooled to room temperature. The Grignard reagent (7) was canulated to a solution of (3Z,6E)-1-bromododeca-3,6-diene (6) (0.4 g, 1.63 mmol) in 6 ml of THF, cooled to 0°C, and Li₂CuCl₄ (0.2 M in THF, 0.1 ml) was added. The solution was stirred at 0°C for 45 min and overnight at room temperature. The reaction mixture was diluted with ether (50 ml), washed successively with a saturated solution of NH₄Cl (10 ml) water (2 \times 10 ml) and dried (Na₂SO₄). After evaporation of volatiles the residue was heated in a Kugelrohr apparatus at 90-100°C (0.05 mm/ Hg) to remove the hydrolysis product of the Grignard reagent in excess ((2-tetrahydropyranyloxy)pentane). Chromatography of the residue on silica gel (petroleum ether/ether, 98:2 v/v) gave the C17 dienic ether 8 as an oil (0.49 g, 90% yield). ¹H-NMR: 0.91 (t, 3H, J = 6.4 Hz, CH₃), 1.05–1.4 (m, 12H), 1.4-1.6 (m, 8H), 1.8-2.04 (m, 4H, $2CH_2-CH=CH$), 2.65 (t, 2H, J = 6.5 Hz, CH=CH-CH₂-CH=CH), 3.21-3.91 (m, 4H, CH₂OTHP, THPH6), 4.6 (t, 1H, J = 3 Hz, THPH2), 5.18-5.45 (m, 4H, 2CH=CH). ¹³C-

NMR: 130.8 (C12), 130.3 (C8), 128.2 (C11), 127.7 (C9), 98.8 (THPC2), 67.6 (C1), 62.2 (THP C6), 32.5 (C13), 31.4 (C15), 30.7 (THP C3), 30.4 (C10), 29.7, 29.5, 29.3, 29.2, 27.0 (C7), 26.2 (C2), 25.5 (THP C5), 22.5 (C16), 19.6 (THPC4), 14.0 (C17). GC/MS: m/e = 336 M⁺. Elemental analysis: Found: C, 78.16; H, 12.02. Calculated for C₂₂H₂₄O₂: C, 78.51; H, 11.98.

2.7. (8Z,11E)-1-Bromoheptadeca-8,11-diene (9)

The bromide **9** was obtained from the tetrahydropyranylether **8** in 83% yield using the procedure previously described for the preparation of **6**. ¹H-NMR: 0.88 (t, 3H, J = 7 Hz, CH₃), 1.1–1.6 (m, 7CH₂), 1.86 (quint. 2H, J = 6.9 Hz, CH₂CH₂Br) 1.96–2.17 (m, 4H, 2CH₂–CH=CH), 2.72 (t J = 6.5 Hz, CH=CH–CH₂–CH=CH), 3.41 (t 2H, J = 7 Hz, CH₂Br), 5.2–5.6 (m, 4H 2CH=CH). ¹³C-NMR: 130.8 (C12), 130.2 (C8), 128.2 (C11), 127.8 (C9), 33.9 (C1), 32.8 (C2), 32.5 (C13), 31.4 (C15), 30.4 (C10), 29.5, 29.2, 29.0 28.6, 28.1, 27.0 (C7), 22.5 (C16), 14.05 (C17). GC/MS: m/e = 314 M⁺, 316 M⁺.

2.8. (8Z,11E)-1-Cyanoheptadeca-8,11-diene (10)

A solution containing potassium cyanide (0.065 g, 1 mmol) and (8Z,11E)-1-bromoheptadeca-8,11diene (9) (0.21 g, 0.64 mmol) in 5 ml of DMSO was heated at 60°C for 20 min. Water was added (10 ml) and the nitrile was extracted three times with ether. The combined etheral extracts were dried (Na₂SO₄) and evaporated. Chromatography on silica gel of the residue (petroleum ether/ether, 90:10 v/v) afforded compound 10 as an oil (0.14 g 84% yield). GC/MS: m/e = 261 M⁺.

2.9. Me (9Z,12E)-octadeca-9,12-dienoate (11)

46% Anhydrous methanolic HCl (3 ml) was added dropwise to a solution of (8Z,11E)-1cyanoheptadeca-8,11-diene (10) (0.14 g, 0.53 mmol) in 3 ml of dry methanol, cooled to 0°C. The solution was stirred at 0°C for 2 h and at room temperature for 7 h. The solution was then poured into water (6 ml) and stirred at 0°C for 30 min. The ester 11 was extracted three times (3 × 15 ml) with ether and the combined organic extracts were dried (Na₂SO₄) and evaporated. The residue was chromatographed on silica gel (petroleum ether/ether, 95:5 v/v) to give the mono *trans* isomer of linoleic acid, compound 11 (0.14 g, 89% yield, 95% purity). GC/MS: m/e = 294 M⁺. Spectral data of 11 are in perfect agreement with those reported in the literature [17,22].

2.10. (9Z,12E)-[1-¹⁴C]Octadeca-9,12-dienoic acid (12)

2.10.1. Synthesis of the $[1^{-14}C]$ nitrile 10, from the bromide 9. The reaction was effected on 0.5 mmol scale with K¹⁴CN in the same conditions as the 'cold' synthesis. After a usual work-up, the residue was purified by column chromatography on silica gel 40–63 μ m (hexane/ether 9:1 v/v) to give $[1^{-14}C]$ -labelled 10 in 85% yield.

2.10.2. Hydrolysis of the $[1^{-14}C]$ nitrile (10). A solution of the nitrile (0.066 g, 0.25 mmol) in a mixture of ethanol and 40% aqueous solution of KOH (32 ml, 1:1) was heated at 80°C for 4 h. After cooling the mixture to room temperature, a 10% HCl solution was added and the acid was extracted with ether, dried (MgSO₄) and the solvents evaporated. The residue was purified by column chromatography on silica gel Merck lichroprep (40–63 μ m) (ether/petroleumether/ acetic acid, 85:15:1) to give the acid 12 in 65% yield (specific activity: 54.1 mCi/mmol). The radioactive purity determined by radiochromatography and HPLC was found to be > 99%.

2.11. (Z)-1,1-Diethoxy-7-(t-butyldiphenylsilyloxy)hept-3-ene (15)

n-BuLi (2.4 in hexanes, 6.6 ml) was added dropwise solution of (3,3-dito а ethoxypropyl)triphenylphosphonium bromide (13) [23,24] (7.48 g, 15.8 mmol) in THF-HMPA (100 ml 9:1 (v/v)), cooled to -78° C. After stirring for 1 h at -78° C, *t*-butyldiphenylsilyloxybutanal (14) (5.2 g, 15.8 mmol) in 15 ml of THF was added slowly. The temperature of the reaction mixture was allowed to warm up to 0°C (60 min) and stirred for 10 min at room temperature. A mixture of petroleum ether/ether (100 ml, 3:1 v/v) was added and the mixture was filtered on a bed of silica gel. After concentration in vacuo, flash chromatography of the residue on silica gel (petroleum ether/ether, 85:15 v/v) afforded compound 15 (4.8 g, 65% yield) obtained as an oil. ¹H-NMR: 1.05 (s, 9H, C(CH₃)₃), 1.22 (t, 6H, J = 7 Hz, 2CH₃), 1.65 (quint., 2H, J = 6.8 Hz CH₂-CH₂OSi), 2.15 (q, 2H, J = 6.6 Hz, CH₂-(CH₂)₂OSi), 2.38 (t, 2H, J = 5.7 Hz, CH₂-CH(OR)₂), 3.38-3.75 (m, 6H, 2CH₂CH₃, CH₂OSi), 4.46 (t, 1H, J = 5.7 Hz, CH(OR)₂), 5.31-5.57 (m, 2H, CH=CH), 7.28-7.42 (m, 6H, phenyl H), 7.6-7.71 (m 4H, phenyl H). ¹³C-NMR: 135.6, 134.0 (aromatic C), 131.7 (C4), 129.5, 127.6 (aromatic C), 124.3 (C3), 102.6 (C1), 63.4 (C7), 61.4 (2OCH₂CH₃), 32.5, 32.0 (C2 and C5), 26.9 (SiC(CH₃)₃), 23.8 (C6), 19.2 (SiC(CH₃)₃), 15.3 (CH₃).

2.12. (3Z,6E)-Dodeca-3,6-dienyltriphenylphosphonium bromide (16)

A mixture containing (3Z, 6E)-1-bromododeca-3,6-diene (6) (1.62 g 6.7 mmol), triphenylphosphine (3.4 g, 13.3 mmol) and calcium carbonate (0.3 g) in 18 ml of acetonitrile was heated at 85°C for 3 days. After filtration of calcium carbonate and evaporation of acetonitrile in vacuo, the oily residue was triturated with petroleum ether in order to remove triphenylphosphine in excess. The resulting gummy phosphonium salt (16) (3.21 g, 95% yield) was dried in vacuo (0.05 mmHg). ¹H-NMR: 0.86 (t, 3H, J = 6.4 Hz, CH₃), 1.2–1.4 (m, 6H, J = 7 Hz, 3CH₂), 1.9 (m, 2H (CH₂)₃-CH₂-CH=CH), 2.3-2.6 (m, 4H, CH=CH- CH_2 -CH=CH, CH₂-CH₂P), 3.7-3.9 (m, 2H $CH_{2}P$), 5.1–5.7 (m, 4H, 2CH=CH), 7.6–7.9 (m, ¹³C-NMR: 135.3, 133.6 15H, aromatic H). (phenyl C), 131.7 (C7), 130.6 (phenyl C), 130.3 (C4), 127.1 (C6), 126.7 (C3 $J_{cp} = 15$ Hz), 118.0 (phenyl C), 32.4 (C8), 31.3 (C10), 30.3 (C5), 29.1 (C9), 22.9 (C2 $J_{cp} = 51$ Hz), 22.4 (C11), 20.3 (C1 $J_{\rm cp} = 3.4$ Hz), 14.0 (C12).

2.13. (4Z,7Z,10Z,13E)-1-(t-Butyldiphenylsilyloxy)nonadeca-4,7,10,13- tetraene (18)

To a solution of the phosphonium salt 16 (1.9 g, 5 mmol) in a mixture of THF-HMPA (25 ml, 9:1 v/v), cooled to -78°C, was added n-BuLi (2.4 M in hexane, 2.1 ml). The reaction mixture was stirred at -78°C for 90 min while the acetal 15 (2.2 g 5 mmol) dissolved in 6 ml of CH₂Cl₂ was hydrolyzed, at 0°C, by a 50% aqueous trifluor-acetic acid solution (3 ml). After stirring for 1 h,

the mixture was diluted with pentane and washed successively with water, a saturated solution of sodium bicarbonate and water. The organic layer was dried (Na₂SO₄), the solvents evaporated and the residue throughly dried twice by azeotropic distillation (cyclohexane) on a rotatory evaporator. The crude aldehyde 17 (1.8 g, 5 mmol) was used in the next step without further purification. ¹H-NMR (200 MHz): 1.05 (s, 9H C(CH₃)₃), 1.63 (quint., 2H, J = 6.6 Hz, CH₂-CH₂OSi) 2.13 (q, 2H, J = 6.6 Hz, CH₂-(CH₂)₂OSi) 3.18 (dd, 2H, J = 5.5 and 1.5 Hz, CH₂-CHO), 3.65 (t, 2H, J = 6.5 Hz, CH₂OSi), 5.3-5.6 (m, 2H, CH=CH) 7.3-7.45 (m, 6H, phenyl H), 7.6-7.75 (m, 4H phenyl H), 9.62 (t, 1H, J = 1.5 Hz, CHO).

The aldehyde 17 dissolved in 5 ml of THF was added to the solution of the ylide of 16, cooled to -78° C, and the temperature of the reaction mixture was allowed to warm up to 0°C (1 h) and then stirred at room temperature for 10 min. A mixture of petroleum ether/ether (20 ml, 3:1 v/v) was added and the suspension was filtered on a bed of silica gel. After concentration in vacuo, the residue was purified by chromatography on silica gel (petroleum ether/ether, 80:20 v/v) to furnish the tetraenic ether 18 as an oil (1.37 g 54% yield).

2.14. (4Z,7Z,10Z,13E)-1-Bromononadeca-4,7,10,13-tetraene (19)

The bromide **19** was obtained from the silylether **18** in 95% yield using the procedure previously described for the preparation of **6** except that the the reaction time was 7 h. ¹H-NMR: 0.88 (t, 3H, J = 6.5 Hz, CH₃), 1.16–1.41 (m, 6H, 3CH₂), 1.84–2.03 (m, 4H, CH₂–CH=CH CH₂–CH₂Br), 2.24 (q, 2H, J = 7 Hz, CH₂–(CH₂)₃Br) 2.7–2.9 (m, 6H, 3CH=CH-CH₂–CH=CH), 3.4 (t, 2H, J = 7 Hz, CH₂Br), 5.26–5.5 (m, 8H 4CH=CH). ¹³C-NMR: 131.1, 129.5, 128.3, 128.1, 128.0, 127.9, 127.8 (olefinic C), 33.3 (C1), 32.5 (C2 and C15), 31.4 (C17), 30.4 (C12), 29.2 (C16), 25.7, 25.6, 25.5 (C3, C6, C9), 22.5 (C18), 14.0 (C19). GC/MS: m/e = 338 M⁺, 340 M⁺.

2.15. (4Z,7Z,10Z,13E)-1-Cyanononadeca-

4,7,10,13-tetraene (20)

The bromide 19 was converted to the nitrile 20 in 85% yield by the procedure described for the

preparation of **10.** ¹H-NMR: 0.88 (t, 3H, J = 6.8Hz, CH₃) 1.12–1.45 (m, 6H, 3CH₂), 1.72 (quint., 2H, J = 7.1 Hz, CH₂–CH₂CN), 1.97 (q, 2H J = 6 Hz, CH₂–CH=CH), 2.24 (q, 2H, J = 7Hz, CH₂–(CH₂)₂CN), 2.34 (t, 2H, J = 7.1 Hz, CH₂CN), 2.65–2.92 (m, 6H, 3CH=CH–CH₂– CH=CH), 5.2–5.56 (m, 8H, 4CH=CH). ¹³C-NMR: 131.1, 130.3, 128.5, 128.3, 128.0, 127.8, 127.6, 127.2 (olefinic C), 119.5 (CN), 32.7 (C15), 31.4 (C17), 30.4 (C12), 29.2 (C16), 26.0, 25.6, 25.5 25.2 (4CH₂), 22.5 (C18), 16.4 (C1), 14.0 (C19). GC/MS: m/e = 285 M⁺. Elemental analysis: Found: C, 84.36; H, 10.80; N, 4.71. Calculated for C₂₀H₃₁N: C, 84.15; H, 10.94; N, 4.94.

2.16. Methyl (5Z,8Z,11Z,14E)-eicosa-5,8,11,14tetraenoate (21)

The isomer of methyl arachidonate 21 was obtained from the nitrile 20 in 85% yield and 98% purity (GC) by the procedure previously described for the preparation of 11. ¹H-NMR: 0.88 (t, 3H, J = 7 Hz, CH₃), 1.2–1.4 (m, 6H), 1.7 (quint., 2H, J = 7.2 Hz, CH₂-(CH₂)₂CO₂Me) 1.97 (q, 2H, J = 6 Hz, CH₂-CH=CH), 2.10 (q, 2H, J = 7.2Hz CH₂-(CH₂)₂CO₂Me), 2.31 (t, 2H, J = 7.2 Hz CH_2-CO_2Me , 2.68–2.86 (m, 6H, 3CH=CH– $CH_2-CH=CH$), 3.65 (s, 3H, OMe), 5.25-5.50 (m, 8H, 4CH=CH). ¹³C-NMR: 174.1 (CO), 131.2 (C15), 128.9 (3CH), 128.2, 128.1 (2CH), 127.8 (olefinic), 51.2 (CO₂Me), 33.5 (C2), 32.1 (C16), 31.0 (C18), 30.2 (C13), 29.0 (C17), 26.5 (C4), 25.5 (C7, C10), 24.5 (C3), 22.3 (C19), 14.0 (C20). GC/MS: m/e = 318 M⁺.

2.17. (5Z,8Z,11Z,14E)-[1-¹⁴C]Eicosa-5,8,11,14tetraenoic acid (**22**)

The radiolabelled 14t arachidonic acid 22 was obtained from the bromide 19 using the same protocol as for 12. It has a radiochemical purity of 99% (specific activity 52.5 mCi/mmol).

2.18. (3E,6Z)-1-(2-Tetrahydropyranyloxy)dodeca-3,6-diene (25)

Wittig reaction between the ylide of 23 [25] and hexanal 24 was effected exactly as described for the preparation of compound 5. The (E,Z)-diene 25 was obtained as an oil in 63% yield. GC/MS: m/e = 266 M⁺.

2.19. (3E,6Z)-1-Bromododeca-3,6-diene(26)

The tetrahydropyranyl ether **25** was converted to the bromide **26** in 81% yield (>97% purity GC) by the procedure described for the preparation of **6**. ¹H-NMR: 0.88 (t, 3H, J = 6.8 Hz, CH₃), 1.2–1.45 (m, 6H, 3CH₂), 2.02 (q, 2H, J = 6Hz, CH₂–CH=CH), 2.55 (q, 2H, J = 6.2 Hz, CH₂–CH₂Br), 2.76 (t, 2H, J = 6 Hz, CH=CH– CH₂–CH=CH), 3.37 (t, 2H, J = 7 Hz, CH₂Br), 5.27–5.6 (m, 4H, 2CH=CH). ¹³C-NMR: 131.8 (C3), 131.0 (C7), 126.5 (C4,C6), 36.0 (C2), 32.8 (C1), 31.4 (C10), 30.2 (C5), 29.3 (C9), 27.0 (C8), 22.5 (C 11), 14.0 (C12). GC/MS: m/e = 244 M⁺, 246 M⁺.

2.20. (8E,11Z)-1-(2-Tetrahydropyranyloxy)heptadeca-8,11-diene (27)

Cross-coupling between the bromide **26** and Grignard reagent **7** of 1-chloro-5-(2-tetrahydropy-ranyloxy)pentane was effected by the same protocol of that described for the preparation of **8**. The C17 dienic ether **27** was obtained as an oil in 79% yield. ¹H-NMR: 0.88 (t, 3H, J = 7 Hz, CH₃) 1.21–1.47 (m, 12H), 1.47–1.66 (m, 8H), 1.66–1.92 (m, 2H), 1.92–2.13 (m, 4H, 2CH₂–CH=CH), 2.72 (t 2H, J = 6 Hz, CH=CH–CH₂–CH=CH), 3.37 (dt, 1H, J = 6.6, 10 Hz, CH₂OTHP), 3.43–3.55 (m, 1H, THP H6), 3.62 (dt, 1H CH₂OTHP), 3.8–3.93 (m, 1H, THP H6'), 4.58 (t, 1H, J = 3 Hz, THP H2), 5.29–5.55 (m, 4H, 2CH=CH). GC/MS: m/e = 336 M⁺.

2.21. (8E,11Z)-1-Bromoheptadeca-8,11-diene (28)

The bromide **28** was obtained from the tetrahydropyranyl ether **27** in 95% yield using the procedure described for the preparation of compound **6.** ¹H-NMR: 0.87 (t, 3H, J = 7 Hz, CH₃), 1.18–1.5 (m, 14H, 7CH₂), 1.83 (quint., 2H, J = 7Hz, CH₂–CH₂Br), 1.92–2.1 (m 4H,2CH₂Br), 2.71 (t, 2H, J = 6 Hz, CH=CH–CH₂–CH=CH), 3.41 (t, 2H, J = 7 Hz, CH₂Br), 5.26–5.48 (m 4H=CH). GC/MS: m/e = 314 M⁺, 316 M⁺. Elemental analysis: Found: C, 65.08; H, 9.81. Calculated for C₁₇H₃₁Br: C, 64.70; H 9.78.

2.22. (8E,11Z)-1-Cyanoheptadeca-8,11-diene (29)

The nitrile **29** was obtained from the bromide **28** in 83% yield (>97\% purity (GC)) by the

procedure described for the synthesis of **10.** ¹H-NMR: 0.88 (t, 3H, J = 7 Hz, CH₃), 1.18–1.55 (m, 14H, 7CH₂), 1.62 (quint., 2H, J = 7 Hz, CH₂– CH₂CN), 1.86–2.15 (m, 4H, 2CH₂–CH=CH), 2.33 (t, 2H, J = 7 Hz, CH₂CN), 2.62 (t, 2H, J = 6 Hz, CH=CH–CH₂–CH=CH), 5.26–5.55 (m 4H,2CH=CH). ¹³C-NMR: 130.5 (C12, C8), 128.6 (C9), 127.6 (C11), 119.7 (CN), 32.4 (C7), 31.5 (C15), 30.4 (C10), 29.3 (2CH₂), 28.7 (C14), 28.6 (2CH₂), 27.0 (C13), 25.3 (C3), 22.5 (C16), 17.1 (C1), 14.0 (C17). GC/MS: m/e = 261 M⁺. Elemental analysis: Found: C, 82.81; H, 11.82; N, 5.23. Calculated for C₁₈H₃₁N: C, 82.68; H, 11.95; N, 5.35.

2.23. Methyl (9E,12Z)-octadeca-9,12-dienoate (30)

The nitrile **29** was converted to 9*t* linoleic acid (**30**) in 98% yield by the procedure described for the preparation of compound **11**. ¹H-NMR: 0.87 (t, 3H, J = 7 Hz, CH₃), 1.2–1.4 (m, 14H, 7CH₂), 1.61 (quint., 2H, CH₂–CH₂CO₂Me), 1.9–2.07 (m, 4H, 2CH₂–CH=CH), 2.27 (t, 2H, J = 7 Hz, CH₂–CO₂Me), 2.69 (t, 2H J = 6 Hz, CH=CH– CH₂–CH=CH), 3.62 (s, 3H, OMe), 5.27–5.47 (m, 4H, 2CH=CH). ¹³C-NMR: 130.7 (C9), 130.5 (C13), 128.1 (C10), 127.5 (C12), 51.3 (OMe), 34.0 (C2), 32.5 (C8), 31.5 (C16), 30.3 (C11), 29.5 (C7), 28.5 (4CH₂), 27.0 (C14), 24.9 (C3), 22.5 (C17), 14.0 (C18). GC/MS: m/e = 294 M⁺.

2.24 (9E,12Z)-[1-¹⁴C]Octadeca-9,12-dienoic acid (**31**)

The radiolabelled (E,Z)-isomer of linoleic acid was obtained from the bromide **28** in 64% overall yield using the same protocol than its (Z,E)-isomer **12.** It has a radiochemical purity of >99% (spec. act., 54.1 mCi/mmol).

3. Results and discussion

3.1. Synthesis of methyl (9Z,12E)-octadecadienoate (11) and of its corresponding [1-¹⁴C]acid (12)

As shown in Scheme 1, the synthesis of the dienic fatty acid 12 used (E)-3-nonen-1-ol (1) as a starting material, readily available in large quantities by reduction of its corresponding acetylenic derivative with lithium aluminium hydride in refluxing THF [26]. Iodination of compound 1 in modified Garegg-Samuelsson conditions the [27,28] gave the iodo compound 2 which was transformed to the phosphonium salt 3 under standard conditions in 76% overall yield for the two steps. Wittig condensation between 3 and 3-(2-tetrahydropyranyloxy)propanal (4) [21] under cis-olefination conditions [29] furnished the C12 dienic ether 5 in 86% yield (99% purity determined by GC). The structure and the isomeric purity of 5 were confirmed by ¹³C-NMR spectroscopy. The chemical shifts for C5 and C8



Scheme 1. Synthesis of methyl (9Z, 12E)-octadecadienoate (11) and of its corresponding [1-¹⁴C]-labelled acid (12).



Scheme 2. Synthesis of methyl (5Z,8Z,11Z,14E)-eicosatetraenoate (21) and of its corresponding $[1-1^4C]$ -labelled acid (22).

which appeared, respectively at 30.7 and 32.6 ppm, supported the configuration assigned for the 3,4 and 6,7 double bonds of compound 5 [17,22]. Five-carbon homologation of 5 was effected by, its transformation to the bromide 6 (86% yield) [30], followed by a cross-coupling with the Grignard reagent 7 [31] in the presence of dilithium chlorocuprate [32]. Replacement of the tetrahydropyranyloxy function of 8 by a bromine atom was accomplished by triphenylphosphine dibromide in 83% yield. Treatment of the resulting bromide 9 with potassium cyanide in DMSO at 60°C for 20 min provided the nitrile 10 in 84% yield. Alcoholysis of 10 with 46% anhydrous methanolic HCl, at room temperature followed by hydrolysis of the resulting imino ether [33] afforded methyl (9Z,12E)-octadecadienoate (11) in 89% yield (99% purity determined by GC analysis). Spectral data of 11 are in perfect agreement with those reported for a synthetic sample [17,22]. The synthesis of the [1-¹⁴C]-labelled fatty acid 12 from the bromide 9 was accomplished by its transformation to a nitrile with K¹⁴CN followed by hydrolysis with potassium hydroxide in a mixture of water/ethanol at 80°C for 4 h [25]. After purification by HPLC, radiochemical purity of [1-¹⁴C]-labelled 12 was determined by HPLC and TLC as being > 99% (specific activity = 54.1 mCi/mmol).



Scheme 3. Synthesis of methyl (9*E*,12*Z*)-octadecadienoate (30) and its corresponding $[1-{}^{14}C]$ -labelled acid (31).

3.2. Synthesis of methyl (5Z,8Z,11Z,14E)eicosatetraenoate (21) and its corresponding [1-¹⁴C]acid (22)

The synthesis of the geometrical isomer of arachidonic acid 22 started by displacement of the bromine atom of 6, intermediate in the synthesis of 9c, 12t 18:2 (12) by triphenylphosphine in acetonitrile (85°C, 72h) to yield the phosphonium salt 16 in 95% yield (Scheme 2). Introduction of the double bonds in the $\Delta 5$ and $\Delta 8$ positions of 14t arachidonic acid (22) was effected by a highly stereoselective Wittig reaction between 16 and the C7 unsaturated aldehyde 17 in 54% yield. Preparation of the aldehyde 17 was accomplished by a Wittig reaction between the C3 acetal phosphonium salt (13) [23] and 4-(t-butyldiphenylsilyloxy)butanal (14) [34-36] in the presence of HMPA to give 15 (65% yield) followed by acidic hydrolysis of the acetal function [37]. The C17 tetraenic silvlether 18 was transformed to the bromide 19 in the presence of triphenylphosphine dibromide (95% yield) [38] and then homologated with potassium cyanide to furnish the C20 nitrile 20 in 81% overall yield.

Alcoholysis of the nitrile (20) afforded methyl (5Z,8Z,11Z,14E)-eicosatetraenoate (21) in 85% yield and 98% stereoisomeric purity. The analytical properties (¹³C-NMR, GC) of the fatty acid 21 were identical in all respects with those reported [39] or with a sample obtained from lipids of rats fed heated sunflower oil. From the bromide 19 was obtained in two steps 14t-[1-¹⁴C]arachidonic acid in 99% radiochemical purity (specific activity 52.5 mCi/mmol).

3.3. Synthesis of methyl (9E,12Z)-octadecadienoate (**30**) and its corresponding [1-¹⁴C]acid (**31**)

As depicted in Scheme 3, our synthetic strategy of the mono *trans* isomer of linoleic acid (31) involves a stepwise six-carbon elongation chain of both ends of symmetrically difunctionalized (*E*)-hexene 23 [25].

Introduction of the *cis* double bond at C12 of fatty acid **30** was effected by a Wittig condensation between **23** and hexanal **24** in the presence of HMPA to give the C12 dienic ether **25** in 63% yield and 95% isomeric purity. After conversion of the tetrahydropyranyl ether **25** to the bromide

26 with PPh₃-Br₂, the chain elaboration was achieved from the bromide 9 using a sequence of reactions described for the preparation of 9c,12t 18:2 (12) (Scheme 1). Thus, the (9*E*,12*Z*)-isomer of linoleic acid (30) was obtained from the bromide 26 in four steps and in 61% overall yield (97% purity). The synthesis of $[1-^{14}C]$ -radiolabelled fatty acid 31 from the bromide 28 followed the same protocol of that described for the synthesis of 12. Compound 31 has a radiochemical purity of >99% (determined by HPLC and radiochromatography) and a specific activity of 54 mCi/mmol.

In conclusion, we have developed concise sequences for preparing labelled mono *trans* isomers of fatty acids of the n-6 series in high isomeric purities. The $\Delta 12t$ and $\Delta 9t$ isomers of methyl linoleate were prepared respectively in 8 and 10 steps in 31% and 22% overall yield from commercially available starting materials. Starting from (*E*)-3-nonen-1-ol (1) 14t arachidonic acid has been efficiently synthesized in eight steps and 20% overall yield. Biological studies of compounds 12, 22 and 31 are in progress in our laboratories.

Acknowledgment

This work was supported in part by a grant (No. AIR1-CT92-0687) from the Commission of the European Communities.

References

- H.J. Dutton (1979) in: E.A. Emken and H.J. Dutton (Eds.) Geometrical and Positional Fatty Acid Isomers, AOCS Champaign, USA, pp. 17-52.
- [2] M.G. Enig, L.A. Pallansh, J. Sampugna and M. Keeney (1983) J. Am. Oil Chem. Soc. 60, 1788–1795.
- [3] R.M. Tomarelli (1988) in: J. Beare-Rogers (Ed.) Dietary Fat Requirement Health Development, AOCS, USA, Champaign, pp. 1–27.
- [4] B. Koletzko (1989) Acta Paediatr. Scand. 78, 513-521.
- [5] J. Boatella (1993) J. Pediat. Gastr. Nutr. 16, 432-434.
- [6] J.L. Sébédio, A. Grandgirard, C. Septier and J. Prevost (1987) Rev. Fr. Corps Gras 34, 15–18.
- [7] J.L. Sébédio, A. Grandgirard and J. Prévost (1988) J. Am. Oil Chem. Soc. 65, 362-366.
- [8] R.L. Wolff (1992) J. Am. Oil Chem. Soc. 69, 106-110.
- [9] E.C. Beyers and E.A. Emken (1991) Biochim. Biophys. Acta 1082, 275-284.

- [10] W.M.N. Ratnayake, Z.Y. Chen, G. Pelletier and D. Weber (1994) Lipids 29, 707-714.
- [11] R.O. Adlof and E.A. Emken (1986) Lipids 21, 543-546.
- [12] M.L. Blank and O. Privett (1963) J. Lipid Res. 4, 470-476.
- [13] O.S. Privett, E.M. Stearns and E.C. Nickell (1967) J. Nutr. 92, 303–310.
- [14] R.L. Anderson, C.S. Fullmer, Jr. and E.J. Hollenbach (1975) J. Nutr. 105, 393–400.
- [15] G. Bruckner, J. Shimp, S. Goswami, J. Mai and J.E. Kinsella (1982) J. Nutr. 112, 126-135.
- [16] R.R. Brenner and R.O. Peluffo (1969) Biochim. Biophys. Acta 176, 471-479.
- [17] H. Rakoff and E.A. Emken (1977) Lipids 12, 760-761.
- [18] H. Rakoff and E.A. Emken (1983) J. Am. Oil. Chem. Soc. 60, 546–552.
- [19] J.M. Osbond, P.G. Philpott and J.C. Wickens (1961) J. Chem. Soc. 2779–2787.
- [20] G.J.N. Egmond, H.J.J. Pabon and D.A. Van Dorp (1977) Recl. Trav. Chim. Pays-Bas 96, 172–175.
- [21] B. Danieli, G. Lesma, G. Palmiasano and S. Tollari (1984) J. Chem. Soc. Perkin Trans. I 1237-1240.
- [22] J. Bus, I. Sies and M.S.F. Lie Ken Jie (1976) Chem. Phys. Lipids 17, 501-518.
- [23] J. Viala and M. Santelli (1988) Synthesis 395-397.
- [24] A. Stoller, C. Mioskowski, J. Millet, C. Sepulchre and F. Bellamy (1990) Tetrahedron Lett. 31, 5035-5038.
- [25] T. Eynard, J.M. Vatèle, D. Poullain, J.P. Noël, J.M.

Chardigny and J.L. Sébédio (1994) Chem. Phys. Lipids 74 175-184.

- [26] R. Rossi, A. Carpita and G. Vita (1978) Gazz. Chim. Ital. 108, 709-712.
- [27] P.J. Garegg and B. Samuelsson (1980) J. Chem. Soc. Perkin Trans. I 2866–2869.
- [28] U. Berlage, J. Schmidt, U. Peters and P. Welzel (1987) Tetrahedron Lett. 27, 3091-3094.
- [29] B. Maryanoff and A. Reitz (1989) Chem. Rev. 89, 863– 927.
- [30] P.E. Sonnet (1976) Synth. Commun. 6, 21.
- [31] O.P. Vig, M.L. Sharma, A. Sabharwal and N. Vohra (1986) Indian J. Chem. 25B, 1042–1044.
- [32] M. Tamura and J. Kochi (1971) Synthesis 303-305.
- [33] J. March (1992) Advanced Organic Chemistry. Mc-Graw-Hill New York, p. 892.
- [34] S. Abdel-Baky and R.W. Giese (1986) J. Org. Chem. 51 3390–3391.
- [35] C.J. Moody, E.R. Sie and J.J. Kulagonski (1992) Tetrahedron 48, 3991–4004.
- [36] J. Herscovici, M.J. Egron and K. Antonakis (1982) J. Chem. Soc. Perkin Trans. I 1967-1973.
- [37] J.M. Vatèle, H.D. Doan, J.M. Chardigny, J.L. Sébédio and A. Grandgirard (1994) Chem. Phys. Lipids 74, 185 193.
- [38] J.M. Aizpurua, F.P. Cossio and C. Palomo (1986) J. Org. Chem. 51, 4941–4943.
- [39] J. Bus, I. Sies and M.S.F. Lie Ken Jie (1977) Chem. Phys. Lipids 18, 130–144.