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Synthesis of (6*Z*,9*Z*,11*E*)-octadecatrienoic and (8*Z*,11*Z*,13*E*)-eicosatrienoic acids and their [1-^{14}C]-radiolabeled analogs

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Abstract

In order to study the metabolic pathway and the physiological effects of 9*c*,11*t*-18:2 (major isomer of conjugated linoleic acid) and its $C_{18:3}$ and $C_{20:3}$ metabolites, 6*c*,9*c*,11*t*-18:3 and 8*c*,11*c*,13*t*-20:3 and their [1-^{14}C]-radiolabeled analogs were prepared stereoselectively by total synthesis. The 8*c*,11*c*,13*t*-20:3 was obtained in 11 steps. The synthesis involves a highly stereoselective Wittig reaction between 3-(*t*-butyldiphenylsilyloxy)propanal and the ylide of 7-(2-tetrahydropyranyloxy)heptanylphosphonium salt which gave (3*Z*)-1-(*t*-butyldiphenylsilyloxy)-10-(2-tetrahydropyranyloxy)dec-3-ene in a first step. Then the *t*-butyldiphenylsilyl derivative was deprotected selectively and the resulting alcohol function was converted via a bromide into a phosphonium salt. The second stereoselective Wittig condensation between the phosphonium salt and commercial (2*E*)-non-2-enal under *cis*-olefinic conditions using Lithium hexamethyldisilazide as base afforded the (7*Z*,10*Z*,12*E*)-1-(2-tetrahydropyranyloxy)nonadeca-7,10,12-triene in a very good isomeric purity. The intermediate product was brominated and transformed by reaction with magnesium into Grignard reagent, which was one-carbon elongated by unlabeled or labeled carbon dioxide to obtain the 8*c*,11*c*,13*t*-20:3 in good isomeric purity (95%) and high radiochemical purity for its [1-^{14}C]-radiolabeled analog (99%). 6*c*,9*c*,11*t*-18:3 was synthesized in a similar way by using 5-(2-tetrahydropyranyloxy)pentanylphosphonium salt in place of 7-(2-tetrahydropyranyloxy)heptanylphosphonium salt in a first step. Other reactions were unchanged and products were obtained in similar yields. Similar to 8*c*,11*c*,13*t*-20:3, the 6*c*,9*c*,11*t*-18:3 was obtained in a very good isomeric purity (95%) and its [1-^{14}C]-radiolabeled analog in a high radiochemical purity (95%).

Keywords: Conjugated linoleic acid; Fatty acid; Wittig reaction; [1-^{14}C]-labeled fatty acids; Stereoselective syntheses

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1. Introduction

Conjugated linoleic acid (CLA) is a collective term that refers to a mixture of positional and geometrical isomers of linoleic acid (9*c*,12*c*-18:2), which have two conjugated double bonds in *cis* or *trans* configuration. The most important isomer of CLA is the 9*c*,11*t*-18:2 as it is found naturally in ruminant animal tissues, ruminant animal fat and milk fat, as major sources of CLA in human nutrition (Chin et al., 1992). It is considered that CLA is formed as a consequence of microbial hydrogenation in the rumen and by Δ9 desaturation of *trans* vaccenic acid in the mammary gland (Kepler et al., 1966; Griinari et al., 2000). For example, about 85–90% of total CLA found in milk fat is 9*c*,11*t*-18:2 (Jiang et al., 1996; Lin et al., 1995).

CLA has reached scientific interest because of possible beneficial effects for human health. It has been reported to have anticarcinogenic and antiatherogenic effects in various animal experiments (Banni and Martin, 1998; Sébédio et al., 1999a,b; Pariza et al., 2000). Furthermore, it has been shown in several feeding studies that CLA influenced body composition in different animals, as it reduced body fat and increases lean body mass (Park et al., 1999; West et al., 1998).

Until now, the way of action of CLA is still unclear. To ensure the effects of CLA, including possible interactions of one CLA-isomer to another and to clarify their reaction pathways, the syntheses of pure CLA-isomers were necessary. Recently, methods to synthesize large quantities of purified isomers, in particular, 9*c*,11*t*-18:2 and its stable isotopes were developed in order to carry out animal and human experiments on isolated isomers (Berdeaux et al., 1997; Lie Ken Jie et al., 1997; Adlof, 1997).

It was recently shown by the working groups of Sébédio and Banni that CLA could be metabolized in vivo into conjugated C18:3 and C20:3 fatty acids by desaturation and followed elongation (Banni et al., 1999; Sébédio et al., 1997, 1999a,b). Also a diminution of the PGE_2 level after CLA-treatment was found by several working groups (Liu and Belury, 1998). These results hypothesized an interaction of CLA with the arachidonic acid pathway and prostaglandin metabolism as one possible way of action.

In order to clarify the metabolic pathway and to study the physiological effects of 9*c*,11*t*-18:2 and its desaturation and elongation metabolites, synthesis of 9*c*,11*t*-18:2; 6*c*,9*c*,11*t*-18:3 and 8*c*,11*c*,19*t*-20:3 and their [1-^{14}C]-radiolabeled analogs is necessary. Synthesis of also small quantities of pure metabolites allow the use in biological in vitro experiments. Moreover, the utilization of radiolabeled analogs permits the detection of small changes in metabolism products.

The aim of this work was to develop two efficient stereoselective syntheses for 8*c*,11*c*,13*t*-20:3 and 6*c*,9*c*,11*t*-18:3 which allowed their preparation up to a gram scale, in high chemical and isomeric purities.

Moreover, these syntheses should give the possibility to prepare the corresponding [1-^{14}C] analogs.

2. Experimental procedures

2.1. Laboratory materials

Starting materials and chemical reagents were purchased from Acros Organic (Noisy-Le-Grand, France) or Sigma–Aldrich (Saint Quentin Fallavier, France) and [^{14}C]-barium carbonate (55 mCi mmol^{-1}) from Mayak Production Association. Silica gel (35–70 mesh) was purchased from SDS (Peypin, France).

All solvents were purified before use: dichloromethane, hexamethylphosporamide (HMPA) and acetonitrile were distilled from calcium hydride; tetrahydrofuran (THF) and diethyl ether from sodium benzophenone ketyl.

2.2. Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker FT-NMR spectrometer operating at 500 MHz for ^{1}H and 125.8 MHz for ^{13}C. Unless otherwise stated spectra were recorded in $CDCl_3$, and chemical shifts were reported (in ppm) downfield from tetramethylsilane (δ).

2.3. Gas chromatography

2.3.1. Analytical gas chromatography analysis

Two gas chromatographs were used for gas chromatography (GC) analyses. The first one was a Carlo Erba HRGC 5300 Mega Series equipped with a 'on column' injector and a flame ionization detector. Helium was the carrier gas (1 ml min^{-1}). The column used was a DB5 fused silica capillary column (J & W Scientific, Folsom, CA; 0.25 μm of film thickness, 8-m length, 0.25-mm i.d.). It was programmed from 60 to 250 °C at 20 °C min^{-1} and held at this temperature until completion of the analysis. The second one was a Packard model 438A equipped with a 'Ross' injector and a flame ionization detector. Helium was the carrier gas (1 ml min^{-1}). The column used was a HP1 fused silica capillary column (Hewlett-Packard, Les Ulis, France; 0.25-μm film thickness, 30-m length, 0.25-mm i.d.). The oven temperature was programmed from 60 to 200 °C at 10 °C min^{-1}.

2.4. Gas chromatography–mass spectrometry

A Hewlett-Packard 5890 gas chromatograph coupled to an Hewlett-Packard model 5970 mass spectrometer instrument was used for the gas chromatography–mass spectrometry (GC–MS) analyses. The latter was operated in the electron impact mode at 70 eV with a source temperature of 250 °C. The GC separation was performed on a DB5 capillary column as described above, and helium was used as carrier gas. The oven temperature was programmed from 60 to 190 °C at 20 °C min^{-1}. Splitless injection was used with the injection port maintained at 250 °C. GC–MS analysis of 4,4-dimethyloxazoline (DMOX) were carried out using a BPX column (Melbourne, Australia; 30-m length, 0.25-mm i.d. and 0.25-μm film thickness), which was programmed from 60 to 170 °C at 20 °C min^{-1}.

2.5. Gas–liquid chromatography–Fourier-transform infrared spectroscopy

Samples were analyzed by GC (HP 5890 gas chromatograph; column BPX 70, 30 m × 0.25 mm i.d., film thickness 0.25 μm, SGE, Melbourne, Australia). The instrument was fitted with a splitless injector maintained at 250 °C and coupled with a Fourier-transform infrared spectrometer (FTS 60A, Bio-Rad, Cambridge, MA). The two instruments were connected by a Digibal tracer® direct-deposition interface. The oven temperature was programmed from 60 to 200 °C (20 °C min^{-1}).

2.6. Thin layer chromatography

Thin layer chromatography (TLC) was performed on a 0.25 mm pre-coated silica gel plate containing a fluorescent indicator. Spots were visualized using one or more of the following techniques: (a) UV illumination; (b) spraying with a solution containing anisaldehyde (Kägi-Miescher reagent: anisaldehyde 12.5 ml; acetic acid 5 ml; sulfuric acid 17 ml; 95% ethanol 450 ml); and (c) iodine vapor.

2.7. Reversed phase-high performance liquid chromatography (Banni et al., 1994)

Separation of the 6*c*,9*c*,11*t*-18:3 and 6*c*,9*t*,11*t*-18:3 were performed with a Varian 9010 liquid chromatograph, equipped with a JASCO MD-1510 photodiode array detector. A C-18 Nucleosil (SFCC, Eragny, France; 250-mm length, 10-mm i.d. and 5-μm particle size) was used for separation. The mobile phase was $CH_3CN/H_2O/CH_3CO_2H$ (70:30:0.12, v/v/v), flowing at a rate of 4 ml min^{-1}. The photodiode array detector was set at a primary monitoring wavelength of 234 nm to detect fatty acids containing conjugated diene and a secondary monitoring wavelength of 200 nm (to detect non-conjugated dienic fatty acids).

2.8. Reversed phase-high performance liquid chromatography for labeled compounds

Analytical reversed phase-high performance liquid chromatography (RP-HPLC) analyses were obtained with a Shimadzu LC-10AS pump fitted with a Shimadzu SCL-10A system controller. The

radioactive detector was a Berthold LB 503 monitor (liquid scintillation cocktail, MS2 from SDS). A Zorbax SB C18 column (250×4.6 mm^2) was used and the mobile phase was ethanol/water/trifluoroacetic acid (70:30:0.1, v/v/v) flowing at a rate of 1.5 ml min^{-1}).

Preparative RP-HPLC was conducted with a Gilson pump (model 303) fitted with a Gilson manometric module (model 802C) and a Berthold LB 2040 nuclear spectrometer. A Zorbax SB C18 column (250×21.2 mm^2) was used for all labeled separations. The mobile phase was acetonitrile/water/acetic acid (70:30:0.1, v/v/v); flowing at rate of 10 ml min^{-1}.

3. Synthetic procedure

Unless otherwise stated, all reactions were carried out under nitrogen as inert atmosphere.

3.1. Synthesis of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid

3.1.1. 3-(t-Butyldiphenylsilyloxy)propanol

To a mixture of propane-1,3-diol (22.13 g, 0.29 mol), dimethylaminopyridine (1.28 g, 0.01 mmol) and triethylamine (9.6 g, 0.095 mol) in 250 ml of CH_2Cl_2 was added dropwise at room temperature *t*-butyldiphenylchlorosilane (20 g, 0.072 mol) in 60 ml of CH_2Cl_2. After stirring at room temperature for 18 h, the mixture was diluted with diethyl ether, washed with brine, several times with water and then dried over Na_2SO_4. After concentration in vacuo, flash chromatography of the residue on silica gel (petroleum ether/diethyl ether, 60:40, v/v) gave the monoprotected diol (19.02 g, 60.5 mmol, 84% yield) as a colorless oil. TLC: Rf = 0.68 (petroleum ether/diethyl ether, 50:50, v/v). GC/MS: $m/z = 257$ [M-57]$^+$.

3.1.2. 3-(t-Butyldiphenylsilyloxy)propanal

To a mixture of pyridinium dichromate (PDC; 26.95 g, 70 mmol) and powdered molecular sieves 4 Å (90 g) in 190 ml of CH_2Cl_2, was added dropwise 3-(*t*-butyldiphenylsilyloxy)propan-1-ol (15 g, 47 mmol) in 130 ml of CH_2Cl_2 at 0 °C. The mixture was stirred at room temperature for 45 min, diluted with a mixture of petroleum ether/diethyl ether (3:1, v/v) and filtered on a pad of silica gel. After evaporation of solvents, chromatography of the residue on silica gel using petroleum ether/diethyl ether (90:10, v/v) gave the aldehyde **4** as a colorless oil (11.28 g, 36 mmol, 75.5% yield). TLC: Rf = 0.84 (petroleum ether/diethyl ether, 60:40, v/v). GC/MS: $m/z = 255$ [M-57]$^+$.

3.1.3. 1-Iodo-7-(2-tetrahydropyranyloxy)heptane

To a solution containing triphenylphosphine (20.1 g, 76.3 mmol), imidazole (10.34 g, 152 mmol) and 7-(2-tetrahydropyranyloxy)heptan-1-ol **1** (10.67, 50.8 mmol) in 140 ml of THF, iodine (19.36 g, 76.3 mmol) was added at −10 °C. The solution was allowed to warm up to room temperature and then stirred for 30 min. The reaction mixture was diluted with diethyl ether, washed successively with a saturated solution of sodium thiosulfate and a saturated solution of sodium hydrogen carbonate. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was stirred with petroleum ether to precipitate triphenyl phosphine oxide. After filtration and concentration, the residue was chromatographed on silica gel (petroleum ether/diethyl ether, 97:3, v/v) to give **2** (13.1 g, 40.2 mmol, 79.1% of yield) as a clear oil. TLC: Rf = 0.43 (petroleum ether/diethyl ether, 95:5, v/v). GC/MS: $m/z = 101$ [OTHP]$^+$, 325 [M–H]$^+$, 326 [M]$^+$. GC/FTIR: 2932.7, 2855.5, 1136.1, 1078.3, 1026.6 cm^{-1}, ^{1}H NMR ($CDCl_3$): 1.25–1.35 (m, 6H, 3,4,5-H_2), 1.4–1.9 (m, 10H, 2, 6-H_2, 3′,4′,5′-H_2 (THP)), 3.11 (t, 2H, $J = 7$ Hz, 1-H_2) 3.25–3.85 (m, 4H, 7-H_2, 6′-H_2 (THP)), 4.5 (t, 1H, $J = 3.4$ Hz, 2′H (THP)). ^{13}C NMR ($CDCl_3$): 98.6 (THP C2′), 67.3 (C7), 62.1 (THP C6′), 33.3 (C2), 30.6 (THP C3′), 30.3 (C6), 29.5 (C3), 28.2 (C4), 25.9 (THP C5′), 25.3 (C5), 19.5 (THP C4′), 7.0 (C1).

3.1.4. [7-(2-Tetrahydropyranyloxy)heptyl]triphenylphosphonium iodide

A solution containing triphenylphosphine (19.3 g, 73.6 mmol), 1-iodo-7-(2-tetrahydropyranyloxy)heptane **2** (12 g, 36.7 mmol) and calcium carbonate (2 g) in 100 ml of acetonitrile was heated at 80–85 °C for 22 h. After filtration of

calcium carbonate and concentration, the oily residue was dissolved in a minimum of CH_2Cl_2 and precipitated by addition of diethyl ether. The phosphonium salt **3**, obtained as a gum (19.9 g, 34 mmol, 92.6% yield), was dried under vacuum (0.05 mmHg) at room temperature. TLC: Rf = 0.69 (CH_2Cl_2/MeOH, 95:5, v/v).

3.1.5. (3Z)-1-(t-butyldiphenylsilyloxy)-10-(2-tetrahydropyranyloxy)dec-3-ene

To a solution of phosphonium salt **3** (15.48, 26.3 mmol) in a mixture of THF/HMPA (150 ml, 5:1, v/v), *n*-BuLi (2.5 M in hexane, 10.52 ml, 26.3 mmol) was added dropwise at −78 °C. After stirring for 1 h at −78 °C, 3-(*t*-butyldiphenylsilyloxy)propanal **4** (8.61 g, 27.5 mmol) in 25 ml of THF was added slowly, and the temperature was allowed to warm up to 0 °C within 90 min. After stirring at room temperature for 1 h, the reaction mixture was diluted with diethyl ether/petroleum ether (1:3, v/v) and filtered on a pad of silica gel. After evaporation of the solvents, the residue was chromatographed on silica gel (petroleum ether/diethyl ether, 95:5, v/v) and afforded 8.31 g of **5** as an oil (16.8 mmol, 63% of yield). TLC: Rf = 0.42 (petroleum ether/diethyl ether, 95:5, v/v). GC/MS: $m/z = 101$ $[OTHP]^+$, 437 $[M\text{-}57]^+$.

3.1.6. (3Z)-10-(2-tetrahydropyranyloxy)dec-3-en-1-ol (Freeman and Kim, 1992)

To a solution of **5** (8.10 g, 16.4 mmol) in 60 ml of THF, was added at room temperature to a 1 M solution of tetra-*n*-butylammonium fluoride in THF (9.75 and 32.8 ml). The resulting solution was stirred for 1 h, diluted with diethyl ether and washed with water. The aqueous layer was extracted with diethyl ether. The organic layers were combined and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was chromatographed on silica gel (petroleum ether/diethyl ether, 60:40, v/v) to give **6** (4.18 g, 16 mmol, 99% of yield) as a clear oil. TLC: Rf = 0.65 (petroleum ether/diethyl ether, 50:50, v/v). GC/MS: $m/z = 101$ $[OTHP]^+$, 255 $[M\text{-}1]^+$, 256 $[M]^+$. GC/FTIR: 3386.0, 3005.3, 2934.9, 2856.4, 1138.9, 1025.4 cm^{-1}, ^1H NMR ($CDCl_3$): 1.2–1.85 (m, 14H, 6,7,8,9-H_2, 3′,4′,5′-H_2 (THP)), 1.85–2.40 (m, 5H, O*H*, 2,5-H_2), 3.25–3.9 (m, 6H, 1,10-H_2, 6′-H_2 (THP)) 4.5 (t, 1H, $J = 3.5$ Hz, 2′-H (THP)), 5.3–5.55 (m, 2H, *H*C=C*H*). ^{13}C NMR ($CDCl_3$): 133.1 (C4), 125.1 (C3), 98.8 (THP C2′), 67.5 (C10), 62.3 (THP C6′), 62.2 (C1), 30.7 (THP C3′, C9), 29.6 (C9), 29.5 (C6), 29.0 (C2), 27.2 (THP C5′), 26.0 (C5), 25.4 (C8), 19.6 (THP C4′).

3.1.7. (3Z)-1-iodo-10-(2-tetrahydropyranyloxy)dec-3-ene

To a solution containing triphenylphosphine (6.14 g, 23.4 mmol), imidazole (3.18 g, 46.8 mmol) and (3*Z*)-10-(2-tetrahydropyranyloxy)dec-3-en-1-ol **6** (5.94 g, 23.4 mmol) in 45 ml of THF, cooled to −30 °C, iodine (5.94 g, 23.4 mmol) was added. The solution was allowed to warm up to room temperature and stirred at this temperature for 30 min. The reaction mixture was diluted with diethyl ether (250 ml), washed successively with a saturated solution of sodium thiosulfate (250 ml) and water (2 × 250 ml). The organic layer was dried (Na_2SO_4) and evaporated. The residue was stirred with petroleum ether to precipitate triphenylphosphine oxide and filtered. After concentration in vacuum, flash chromatography of the residue on silica gel (petroleum ether/diethyl ether, 95:5, v/v) gave the iodide **7** (4.83 g, 13.1 mmol, 84.2% of yield) as an oil. TLC: Rf = 0.55 (petroleum ether/diethyl ether, 95:5, v/v). GC/MS: $m/z = 101$ $[OTHP]^+$, 239 $[M\text{-}I]^+$, 366 $[M]^+$. GC/FTIR: 3007.1, 2931.9, 2854.1, 1137.1, 1032.2 cm^{-1}, ^1H NMR ($CDCl_3$): 1.2–1.9 (m, 14H, 6,7,8,9-H_2, 3′,4′,5′-H_2 (THP)), 2.0 (m, 2H, 5-H_2), 2.59 (q, 2H, $J = 7.3$ Hz, 2-H_2) 3.1 (t, 2H, $J = 7.3$ Hz, 1-H_2), 3.3–3.9 (m, 4H, 10-H_2, 6′-H_2 (THP), 4.55 (t, 1H, $J = 2.5$ Hz, 2′-H (THP)), 5.2–5.6 (m, 2H, *H*C=C*H*).

3.1.8. (3Z)-[10-(2-tetrahydropyranyloxy)dec-3-enyl]triphenylphosphonium iodide

A solution containing triphenylphosphine (6.87 g, 26.2 mmol) (3*Z*)-1-iodo-10-(2-tetrahydropyranyloxy)dec-3-ene **7** (4.82 g, 13.1 mmol) and calcium carbonate (0.7 g) in 36 ml of acetonitrile was heated at 75 °C for 20 h. After filtration of calcium carbonate and evaporation of acetoni-

trile, the oily residue was dissolved in a minimum of CH_2Cl_2 and precipitated by addition of diethyl ether. The phosphonium salt **8** obtained as a gum (7.47 g, 11.9 mmol, 91% yield) was dried under vacuum (0.05 mmHg) for 12 h at room temperature. TLC: Rf = 0.80 (CH_2Cl_2/MeOH, v/v).

3.1.9. (7Z,10Z,12E)-1-(2-tetrahydropyranyloxy)nonadeca-7,10,12-triene

At −78 °C, 3.66 ml (9.15 mmol) of a 2.5 M solution of *n*-BuLi in hexane was added to a solution of 1.67 g (10.3 mmol) of hexamethyldisilazane (HMDS) in 32 ml of THF and 8 ml of HMPA. The reaction temperature was raised to 0 °C for 3 min, and cooled back to −78 °C, then a solution of 6.4 g (10.2 mmol) of the phosphonium salt **8** in 42 ml of THF was added. The reaction mixture turned to orange. After stirring at −78 °C for 30 min, (2*E*)-non-2-enal (1.86 g, 1.33 mmol) in 5 ml of THF was added (the orange reaction mixture became yellow). After 45 min at −78 °C, the cooling bath was removed and the reaction was stirred at room temperature for 90 min. The mixture was diluted with diethyl ether/petroleum ether (1:3, v/v) and filtered on a pad of silica gel. After evaporation of the solvents, the residue was chromatographed on silica gel (petroleum ether/diethyl ether, 98:2, v/v) and afforded 1.9 g of **9** as an oil (5.25 mmol, 51% of yield). TLC: Rf = 0.60 (petroleum ether/diethyl ether, 95:5, v/v). GC/MS: $m/z = 101$ $[OTHP]^+$, 278 $[M]^+$. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, $J = 7$ Hz, 19-H_3), 1.18–1.90 (m, 22H, 2,3,4,5,15,16,17, 18-H_2, 3′,4′,5′-H_2 (THP)), 2.0–2.15 (m, 4H, 6,14-H_2), 2.90 (t, 2H, $J = 7$, 9-H_2), 3.33–3.91 (m, 4H, 1-H_2, 6′-H_2 (THP)), 4.57 (t, 1H, $J = 2.8$ Hz, 2′-H (THP)), 5.25 (dt, 1H, $J_{7-6} = 7.6$ Hz, $J_{7-8} = 10.6$ Hz, 7-H), 5.37 (m, 2H, 8,10-H), 5.68 (dt, 1H, $J_{13-12} = 15$ Hz, $J_{13-14} = 7$ Hz, 13-H), 5.95 (dd, 1H, $J_{11-10} = 10.9$ Hz, $J_{11-12} = 10.9$ Hz, 11-H), 6.32 (dd, 1H, $J_{12-11} = 10.9$ Hz, $J_{12-13} = 15.0$ Hz, 12-H). ^{13}C NMR ($CDCl_3$): 135.3 (C13), 130.4 (C7), 128.8 (C11), 127.7 (C12), 127.6 (C10), 125.3 (C8), 98.8 (THP C2′), 67.6 (C1), 62.3 (THP C6′), 32.9 (C14), 31.7 (THP C3′), 30.8 (C2), 29.7 (C17), 29.6 (C15), 29.36 (C4), 19.2 (C5), 28.9 (C16), 27.2 (THP C5′), 26.2 (C6), 26.0 (C3), 25.5 (C9), 22.6 (C18), 19.7 (THP C4′), 14.1 (C19).

3.1.10. (7Z,10Z,12E)-1-bromononadeca-7,10,12-triene

9 (2.33 g, 6.43 mmol) diluted in 20 ml of CH_2Cl_2, was added at 0 °C to a slurry of triphenylphosphine dibromide (5.41 g, 12.82 mmol) in 20 ml of CH_2Cl_2. The mixture was stirred at room temperature for 15 min, diluted with diethyl ether, washed with a saturated solution of sodium bicarbonate, then with water. The organic layer was dried over Na_2SO_4 and concentrated. Flash chromatography of the residue on silica gel (hexane) gave the bromide **10** (1.7 g, 4.98 mmol, 77.4% yield) as a colorless oil. TLC: Rf = 0.94 (petroleum ether/diethyl ether, 95:5, v/v). GC/MS: $m/z = 262/264$ $[M–Br]^+$, 340/342 $[M]^+$. ^{1}H NMR ($CDCl_3$): 0.85 (t, 3H, $J = 6.9$ Hz, 19-H_3), 1.1–1.5 (m, 14H, 3,4,5,15,16,17,18-H_2), 1.8–1.9 (m, 2H, 2-H_2), 2.0–2.15 (m, 4H, 6,14-H_2), 2.90 (t, 2H, $J = 6.5$, 9-H_2), 3.38 (t, 2H, $J = 6.5$, 1-H_2), 5.23 (dt, 1H, $J_{7-6} = 7.6$ Hz, $J_{7-8} = 10.6$ Hz, 7-H), 5.36 (m, 2H, 8,10-H), 5.67 (dt, 1H, $J_{13-12} = 15$ Hz, $J_{13-14} = 7$ Hz, 13-H), 5.94 (dd, 1H, $J_{11-10} = 10.9$ Hz, $J_{11-12} = 10.9$ Hz, 11-H), 6.30 (dd, 1H, $J_{12-11} = 10.9$ Hz, $J_{12-13} = 15.0$ Hz, 12-H). ^{13}C NMR ($CDCl_3$): 135.3 (C13), 130.1 (C7), 128.8 (C11), 127.8 (C12), 127.6 (C10), 125.3 (C8), 33.9 (C2), 32.9 (C14), 32.8 (C17), 31.7 (C1), 29.4 (C15), 29.33 (C5), 28.9 (C16), 28.4 (C4), 28.0 (C3), 27.1 (C6), 26.0 (C9), 22.6 (C18), 14.1 (C19).

3.1.11. (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid

Bromide **10** (670 mg, 1.96 mmol) dissolved in 1.5 ml of anhydrous diethyl ether was added to magnesium turnings (120 mg, 4.93 mmol) and a crystal of iodine in 3.5 ml of anhydrous diethyl ether. The reaction mixture was refluxed for 90 min at 35 °C. The resulting Grignard compound was carbonated at −20 °C with CO_2 (liberated from 500 mg (2.5 mmol) of barium carbonate by addition of concentrated sulfuric acid). After stirring at −20 °C for 2.5 h, 10 ml of 5% NH_4Cl were added. The mixture was diluted with 50 ml of saturated NH_4Cl solution and 50 ml of ether. A 1 N H_2SO_4 solution was added dropwise until the aqueous layer was acidified to pH 3. After decantation, the etheral layer was washed with water, dried (Na_2SO_4), filtered and concentrated. Flash chromatography of the residue on silica gel

(hexane/diethyl ether/acetic acid, 90:10:0.1, v/v/v) gave the free fatty acid **11** (328.5 mg, 1.07 mmol, 55% yield) as an oil. TLC: Rf = 0.25 (hexane/diethyl ether/acetic acid, 90:10:0.1, v/v/v). ^{1}H NMR ($CDCl_3$): 0.85 (t, 3H, $J = 7$ Hz, 20-H_3), 1.2–1.45 (m, 14H, 4,5,6,16,17,18,19-H_2), 1.62 (quint, 2H, $J = 7.5$ Hz, 3H_2), 2.0–2.15 (m, 4H, 7,15-H_2), 2.34 (t, 2H, $J = 7.5$, 2-H_2), 2.88 (t, 2H, $J = 6.8$, 10-H_2), 5.23 (dt, 1H, $J_{8-7} = 7.6$ Hz, $J_{8-9} = 10.6$ Hz, 8-H), 5.28 (s, 1H, COO*H*), 5.36 (m, 2H, 9,11-H), 5.66 (dt, 1H, $J_{15-14} = 15$ Hz, $J_{15-16} = 7$ Hz, 15-H), 5.94 (dd, 1H, $J_{12-11} = 10.9$ Hz, $J_{12-13} = 10.9$ Hz, 12-H), 6.30 (dd, 1H, $J_{13-12} = 10.9$ Hz, $J_{13-14} = 15.0$ Hz, 13-H). ^{13}C NMR ($CDCl_3$): 180.3 (C1), 135.3 (C14), 130.2 (C8), 128.7 (C12), 127.7 (C13), 127.6 (C11), 125.3 (C9), 34.0 (C2), 32.9 (C15), 31.7 (C18), 29.4 (C16), 29.3 (C6), 28.9 (C5), 28.9 (C17), 28.8 (C4), 27.1 (C7), 26.0 (C3), 24.6 (C10), 22.6 (C19).

3.1.12. (8Z,11Z,13E)-[1-^{14}C]-eicosa-8,11,13-trienoic acid

(8*Z*,11*Z*,13*E*)-[1-^{14}C]-eicosa-8,11,13-trienoic acid **11′** (30 mCi; specific activity: 50 mCi mmol^{-1}, 0.6 mmol, 60% yield from 10) was obtained from bromide 10 (340 mg; 1 mmol), magnesium turnings (65 mg) and $Ba^{14}CO_3$ (216 mg, 1.1 mmol, 60.5 mCi) according to the procedure described for the preparation of the unlabeled acid **11**. The radiochemical purity of the [1-^{14}C] acid was found to be 93.9% by analytical RP-HPLC.

3.2. Synthesis of (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid

3.2.1. 1-Iodo-5-(2-tetrahydropyranyloxy)pentane

7.85 g (26.34 mmol) of 1-iodo-5-(2-tetrahydropyranyloxy)pentane **13** was obtained from 7-(2-tetrahydropyranyloxy) pentan-1-ol in 72.6% yield using the procedure previously described for the preparation of **2**. TLC: Rf = 0.43 (petroleum ether/diethyl ether, 95:5, v/v). GC/MS: $m/z = 101$ [OTHP]$^+$, 298 [M]$^+$. GC/FTIR: 2938, 2863.9, 1135.6, 1030.1 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.45–2.0 (m, 10H, 3,4-H_2, 3′,4′,5′-H_2 (THP)), 2.4 (m, 2H, 2-H_2), 3.20 (t, 2H, $J = 7$, 1-H_2), 3.70–3.90 (m, 4H, 5-H_2, 6′-H_2 (THP)), 4.57 (t, 1H, $J = 3.3$ Hz, 2′-H (THP)). ^{13}C NMR ($CDCl_3$): 98.8 (THP C2′), 67.6 (C5), 62.3 (THP C6′), 33.1 (C2), 31.0 (THP C3′), 28.5 (C4), 27.2 (THP C5′), 25.5 (C3), 19.7 (THP C4′), 7.1 (C1).

3.2.2. [5-(2-Tetrahydropyranyloxy)pentyl]triphenylphosphonium iodide

14.03 g (25.03 mmol) [5-(2-tetrahydropyranyloxy)pentyl]triphenylphosphonium iodide **14** was obtained in 95% yield using the procedure previously described for the preparation of the phophonium salt **3**. The reaction time was 24 h and temperature was 80–85 °C. TLC: Rf = 0.69 (CH_2Cl_2/MeOH, v/v). ^{1}H NMR ($CDCl_3$): 1.4–1.85 (m, 12H, 2,3,4-H_2, 3′,4′,5′-H_2 (THP)), 3.30–3.85 (m, 4H, 5-H_2, 6′-H_2 (THP)), 4.5 (m, 1H, 2′-H (THP)), 5.3 (m, 2H, 1-H), 5.37 (m, 2H, 8,10-H), 7.68–7.88 (m, 15H, –C_6H_5).

3.3. (3Z)-1-(t-butyldiphenylsilyloxy)-8-(2-tetrahydropyranyloxy)oct-3-ene

A Wittig reaction between the ylide of **14** and the aldehyde **4** was effected as described for the preparation of compound **5**. The (3*Z*)-1-(*t*-butyldiphenylsilyloxy)-8-(2-tetrahydropyranyloxy) oct-3-ene **15** was obtained in 54.7% yield (4.35 g, 9.33 mmol). TLC: Rf = 0.44 (petroleum ether/diethyl ether, 90:10, v/v). ^{1}H NMR ($CDCl_3$): 1.05 (s, 9H, –CH_3), 1.15–1.9 (m, 10H, 3,4-H_2, 3′,4′,5′-H_2 (THP)), 2.0 (q, 2H, $J = 7$ Hz, 5-H_2), 2.3 (q, 2H, $J = 6.8$, 2-H_2), 3.65 (t, 2H, $J = 7$, 1-H_2), 3.32–3.90 (m, 4H, 8-H_2, 6′-H_2 (THP)), 4.57 (t, 1H, $J = 3.3$ Hz, 2′-H (THP)), 5.34–5.50 (m, 2H, –*H*C=C*H*–), 7.3–7.75 (m, 10H, –C_6H_5).

3.3.1. (3Z)-8-(2-tetrahydropyranyloxy)oct-3-en-1-ol

To a solution of the THP ether **15** (4.32 g, 9.27 mmol) in 60 ml of THF, was added at room temperature to a 1 M solution of tetra-*n*-butylammonium fluoride in THF (9.75 ml, 32.8 mmol). The solution was stirred for 90 min, diluted with 500 ml of diethyl ether and washed with H_2O (3×500 ml). The water layer was extracted with diethyl ether, and the organic layers were com-

bined and dried (Na_2SO_4). After concentration, the residue was chromatographed over silica gel (petroleum ether/diethyl ether, 60:40, v/v) to give **16** (2.07 g, 9.1 mmol, 98.1% yield) as a clear oil. TLC: Rf = 0.16 (petroleum ether/diethyl ether, 90:10, v/v). GC/MS: $m/z = 101$ $[OTHP]^+$, 228 $[M]^+$. GC/FTIR: 3366.5, 3006.1, 1657.27, 1139.4, 1032.9 cm^{-1}. ^1H NMR ($CDCl_3$): 1.35–1.9 (m, 11H, 6,7-H_2, 3′,4′,5′-H_2 (THP), –O*H*), 2.1 (q, 2H, $J = 7.3$ Hz, 5-H_2), 2.32 (q, 2H, $J = 6.7$, 2-H_2), 3.3–3.95 (m, 6H, 1,8-H_2, 6′-H_2 (THP)), 4.55 (t, 1H, $J = 3.6$ Hz, 2′-H (THP)), 5.3–5.6 (m, 2H, –*H*C=C*H*–).

3.3.2. (3Z)-1-iodo-8-(2-tetrahydropyranyloxy)oct-3-ene

2.58 g (7.64 mmol) of (3*Z*)-1-iodo-8-(2-tetrahydropyranyloxy)oct-3-ene **17** was obtained from alcohol **16** in 84% yield using the procedure previously described for the preparation of **7**. TLC: Rf = 0.58 (petroleum ether/diethyl ether, 50:50, v/v). GC/MS: $m/z = 101$ $[OTHP]^+$, 211 $[M–I]^+$, 338 $[M]^+$. GC/FTIR: 2938.7, 2862.9, 1719.6, 1652.1, 1137.1, 1032.5 cm^{-1}. ^1H NMR ($CDCl_3$): 1.17–1.90 (m, 10H, 3,4-H_2, 3′,4′,5′-H_2 (THP)), 2.06 (q, 2H, $J = 7.2$ Hz, 5-H_2), 2.63 (q, 2H, $J = 7.3$ Hz, 2-H_2), 3.13 (t, 2H, $J = 7.3$, 1-H_2), 3.35–3.92 (m, 4H, 8-H_2, 6′-H_2 (THP)), 4.57 (t, 1H, $J = 3.5$ Hz, 2′-H (THP)), 5.1–5.6 (m, 2H). ^{13}C NMR ($CDCl_3$): 131.9 (C4), 127.7 (C3), 98.5 (THP C2′), 67.0 (C8), 62.0 (THP C6′), 31.1 (THP C3′), 30.4 (C7), 29.0 (C2), 26.9 (THP C5′), 25.8 (C5), 25.1 (C6), 19.3 (THP C4′), 5.1 (C1).

3.3.3. (3Z)-[8-(2-tetrahydropyranyloxy)oct-3-enyl] triphenylphosphonium iodide

5.61 g (9.35 mmol) (3*Z*)-[8-(2-tetrahydropyranyloxy)oct-3-enyl]triphenylphosphonium iodide **18** was obtained in 90% yield using the procedure previously described for the preparation of the phophonium salt **8**. Reaction time was 20 h. TLC: Rf = 0.53 (CH_2Cl_2/MeOH, 95:5).

3.3.4. (5Z,8Z,120E)-1-(2-tetrahydropyranyloxy) heptadeca-5,8,10-triene

A Wittig reaction between the ylide of **18** and (2*E*)-non-2-enal was effected as described for the preparation of compound **9**. The (5*Z*,8*Z*,12*E*)-1-(2-tetrahydropyranyloxy)heptadeca-5,8,10-triene **19** was obtained in 67.8% (1.74 g, 5.21 mmol). TLC: Rf = 0.49 (petroleum ether/diethyl ether, 98:2, v/v). GC/MS: $m/z = 101$ $[OTHP]^+$, 334 $[M]^+$. ^1H NMR ($CDCl_3$): 0.89 (t, 3H, $J = 6.7$ Hz, 17-H_3), 1.20–1.90 (m, 18H, 2,3,13,14,15,16-H_2, 3′,4′,5′-H_2 (THP)), 2.07–2.18 (m, 4H, 4,12-H_2), 2.91 (t, 2H, $J = 7$, 7-H_2), 3.35–3.90 (m, 4H, 1-H_2, 6′-H_2 (THP)), 4.6 (m, 1H, 2′-H (THP)), 5.25 (dt, 1H, $J_{5-4} = 7.6$ Hz, $J_{5-6} = 10.6$ Hz, 5-H), 5.37 (m, 2H, 6,8-H), 5.68 (dt, 1H, $J_{11-10} = 15$ Hz, $J_{11-12} = 7$ Hz, 11-H), 5.95 (dd, 1H, $J_{9-8} = 10.9$ Hz, $J_{9-10} = 10.9$ Hz, 9-H), 6.35 (dd, 1H, $J_{10-9} = 10.9$ Hz, $J_{10-11} = 15.0$ Hz, 10-H). ^{13}C NMR ($CDCl_3$): 135.3 (C11), 130.4 (C5), 128.8 (C9), 127.7 (C10), 127.6 (C8), 125.3 (C6), 98.8 (THP C2′), 67.6 (C1), 62.3 (THP C6′), 32.9 (C12), 31.7 (THP C3′), 30.8 (C2), 29.6 (C13), 29.36 (C14), (THP C5′), 26.2 (C4), 26.0 (C3), 22.6 (C16), 19.7 (THP C4′), 14.1 (C17).

3.3.5. (5Z,8Z,10E)-1-bromoheptadeca-5,8,10-triene

The tetrahydropyranyl ether **19** was converted to the bromide **20** in 53.3% yield (> 97% purity by GC) by the procedure described for the preparation of **10**. TLC: Rf = 0.84 (petroleum ether/diethyl ether, 98:8, v/v). ^1H NMR ($CDCl_3$): (t, 3H, $J = 6.7$ Hz, 17-H_3), 1.2–1.60 (m, 10H, 3,13,14,15,16-H_2), 1.8–2.2 (m, 6H, 2,4,12-H_2), 2.90 (t, 2H, $J = 7$, 7-H_2), 3.4 (t, 2H, 1-H_2), 5.25 (dt, 1H, $J_{5-4} = 7.6$ Hz, $J_{5-6} = 10.6$ Hz, 5-H), 5.37 (m, 2H, 6,8-H), 5.68 (dt, 1H, $J_{11-10} = 15$ Hz, $J_{11-12} = 7$ Hz, 11-H), 5.95 (dd, 1H, $J_{9-8} = 10.9$ Hz, $J_{9-10} = 10.9$ Hz, 9-H), 6.35 (dd, 1H, $J_{10-9} = 10.9$ Hz, $J_{10-11} = 15.0$ Hz, 10-H). ^{13}C NMR ($CDCl_3$): 135.2 (C11), 129.0 (C5), 128.8 (C9), 128.4 (C10), 127.3 (C8), 125.0 (C6), 33.8 (C2), 33.0 (C12), 32.0 (C1), 31.6 (C15), 29.0 (C13), 28.9 (C14), 28.0 (C3), 26.1 (C4), 26.0 (C7), 22.5 (C16), 14.0 (C17).

3.3.6. (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid

Bromide **20** (420 mg, 1.3 mmol) dissolved in 2 ml of anhydrous diethyl ether was added to magnesium turning (86 mg, 3.5 mmol) and a crystal of

iodine in 1 ml of anhydrous diethyl ether. The reaction was refluxed for 90 min at 35 °C. The resulting Grignard compounds was carbonated at −20 °C with CO_2 (liberated from 2.4 g (12.1 mmol) of barium carbonate by addition of concentrated sulfuric acid). After stirring at −20 °C for 2 h, 7 ml of 5% NH_4Cl were added. The mixture was diluted with saturated NH_4Cl solution. A 1 N H_2SO_4 solution was added dropwise until the aqueous layer was acidified to pH 3. After decantation, the ethereal layer was washed with water, dried over Na_2SO_4, filtered and concentrated. Flash chromatography of the residue on silica gel (hexane/diethyl ether/acetic acid, 80:20:0.1, v/v/v) gave the acid **21** (106 mg, 0.38 mmol, 30% yield) as an oil.

TLC: Rf = 0.26 (hexane/diethyl ether/acetic acid, 90:10:0.1, v/v/v). ^{1}H NMR ($CDCl_3$): 0.9 (t, 3H, $J = 7$ Hz, 18-H_3), 1.2–1.5 (m, 10H, 4,14,15,16,17-H_2), 1.68 (m, 2H, 3H_2), 2.05–2.15 (m, 4H, 7,13-H_2), 2.37 (t, 2H, $J = 7.5$, 2-H_2), 2.9 (t, 2H, $J = 6.8$, 8-H_2), 5.25 (dt, 1H, $J_{6-5} = 7.6$ Hz, $J_{6-7} = 10.6$ Hz, 6-H), 5.4 (m, 2H, 7,9-H), 5.69 (dt, 1H, $J_{13-12} = 15$ Hz, $J_{13-14} = 7$ Hz, 13-H), 5.94 (dd, 1H, $J_{12-11} = 10.9$ Hz, $J_{12-13} = 10.9$ Hz, 12-H), 6.30 (dd, 1H, $J_{11-10} = 10.9$ Hz, $J_{11-12} = 15.0$ Hz, 11-H), 7.3 (s, 1H, COOH).

3.3.7. (6Z,9Z,11E)-[1-^{14}C]-octadeca-6,9,11-trienoic acid

A mixture of (6*Z*,9*Z*,11*E*)-[1-^{14}C]-octadeca-6,9,11-trienoic acid and its geometrical isomers (8.5 mCi, 53 mCi mmol^{-1}, 0.16 mmol, 19.3% yield from **20**) was obtained from bromide 20 (260 mg; 0.83 mmol), magnesium turnings (58 mg) and $Ba^{14}CO_3$ (370 mg, 1.88 mmol, 103.4 mCi) according to the procedure described for the preparation of the unlabeled acid **21**. The radiochemical purity of (6*Z*,9*Z*,11*E*)-[1-^{14}C]-octadeca-6,9,11-trienoic acid was found to be 46% by RP-HPLC. 0.2 mCi of (8*Z*,11*Z*,13*E*)-[1-^{14}C]-eicosa-8,11,13-trienoic acid **21′** (radiochemical purity: 95%) were obtained by preparative RP-HPLC.

3.3.8. Preparation of fatty acid methyl esters

Free fatty acids were converted to fatty acid methyl esters using boron trifluoride in methanol (14%). To free fatty acids in pentane 500 μl BF_3/MeOH were added. The solution was stirred for 10 min at room temperature. The solution was diluted with 2 ml of pentane and washed with a saturated solution of sodium bicarbonate. The organic layer was removed and was used for GC analysis.

3.3.9. Preparation of 4,4-dimethyloxazoline derivatives

FAME (500 μg) were converted to their DMOX derivatives by treatment with 2-amino-2-methylpropanol (0.25 ml) in a sealed ampoule under N_2 at 170 °C for 8 h (Fay and Richli, 1991; Luthria and Sprecher, 1993; Zhang et al., 1988). The reaction mixture was cooled, dissolved in 3 ml of dichloromethane and washed twice with 1 ml of water. After drying the organic phase, the solvent was removed under a stream of nitrogen and the sample was dissolved in hexane. The sample was applied to a short column of Florisil which was subsequently washed with hexane prior to elute the DMOX derivatives with a mixture of hexane/acetone (96:4, v/v; Dobson and Christie, 1996).

4. Results and discussion

Our synthetic strategy was to form the conjugated double bond system at the end of the synthesis and also just before labeling in order to avoid degradation and isomerization.

4.1. Synthesis of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid

As shown in Fig. 1, the synthesis of the eicosatrienoic acid **11** used heptane-1,7-diol as a starting material, which is readily available in large quantities. Heptane-1,7-diol was tetrahydropyranylated in the presence of pyridinium *p*-toluenesulfonate to give the monoprotected heptane-1,7-diol **1** according to Danieli et al. (1984). The phosphonium salt **3** was prepared from the monoprotected heptanediol **1** by reaction with iodine in modified Garegg–Samuelsson conditions (Garegg and Samuelsson, 1980; Berlage et al., 1987) to give **2** (79.1% yield) followed by reaction with

triphenylphosphine in the presence of calcium carbonate. 1,3-Propanediol was monoprotected with *tert*-butyldiphenylsilyl chloride to give 3-(*t*-butyldiphenylsilyloxy)propanol (84% yield) which was oxidized to 3-(*t*-butyldiphenylsilyloxy)propanal **4** with PDC in 79.1% yield (Abdel-Baky and Giese, 1986; Moody et al., 1992; Herscovici et al., 1982). Highly stereoselective Wittig condensation between the ylide of the phosphonium salt **3** and aldehyde **4** in presence of HMPA (Maryanoff and Reitz, 1989) furnished the (3*Z*)-1-(*t*-butyldiphenylsilyloxy)-10-(2-tetrahydropyranyloxy)dec-3-ene **5** in 63% yield. Selective deprotection (desilylation) of compound **5** afforded alcohol **6** (Freeman and Kim, 1992) which was transformed to iodide **7** using modified Garegg–Samuelsson conditions (Garegg and Samuelsson, 1980; Berlage et al., 1987). Displacement of the iodine atom of **7** by triphenylphosphine in refluxing acetonitrile gave the ten-carbon chain unsaturated phosphonium salt **8**. The ylide from **8** was reacted with commercially available (2*E*)-non-2-enal to give (5*Z*,8*Z*,10*E*)-1-(2-tetrahydropyranyloxy)heptadeca-5,8,10-triene **9** in 51% yield. Therefore, the synthesis of the conjugated double bond system with specific *cis* stereochemistry at the newly formed double bond was carried out using a Wittig reaction. Lithium hexamethyldisilazide was chosen as a weak base in the presence of HMPA in THF at −78 °C according to Labelle et al. (1990), to avoid isomerization of the existing *trans*-double bond of the (2*E*)-non-2-enal. Replacement of the tetrahydropyranyloxy function of **9** by a bromine atom was accomplished by triphenylphosphine dibromide (Sonnet, 1976) in 77.4% yield. At this stage, no geometrical

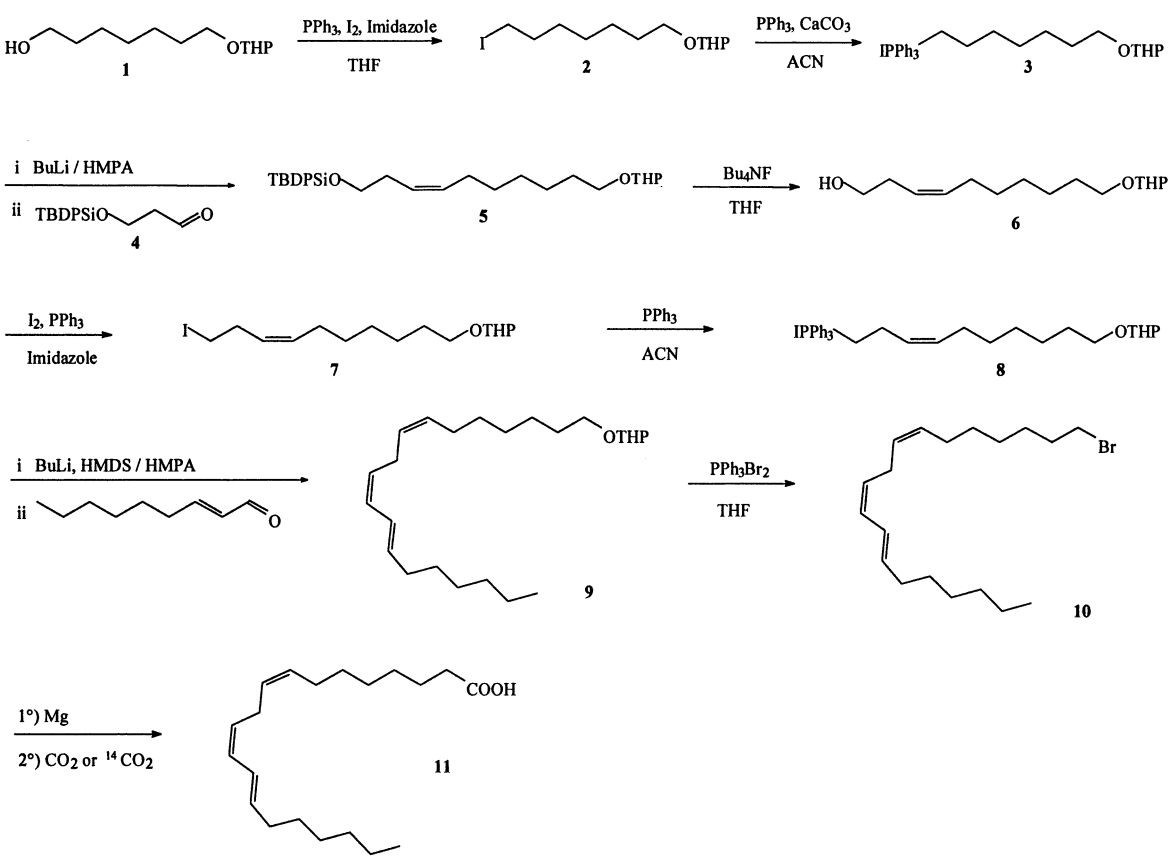

Fig. 1. Synthesis of (8*Z*,11*Z*,13*E*)-eicosa-8,11,13-trienoic acid and of its corresponding [1-^{14}C]-labeled acid.

isomers of compounds **9** and **10** were detected by GC, ^{1}H and ^{13}C NMR.

In previous syntheses of labeled fatty acids (Vatèle et al., 1994; Eynard et al., 1994; Berdeaux et al., 1995), [1-^{14}C] fatty acids were prepared by reaction at 80 °C of bromo precursors with $K^{14}CN$ and alkaline hydrolysis at 80 °C of the resulting nitriles. Unfortunately, in the case of conjugated fatty acids, isomerization of the conjugated system occurred with the heat treatment. Therefore, to avoid degradation and isomerization of the *cis–trans* conjugated double bond system, the carbonation of the corresponding Grignard reagent with ^{14}C-carbon dioxide was chosen for the labeling (Channing and Simpson, 1993).

Firstly, the unlabeled fatty acid was prepared. The bromide-compound **10** was transformed into its corresponding nonadecatrienylmagnesium bromide by reaction with magnesium turnings in refluxing anhydrous diethyl ether (90 min). The Grignard reagent was carbonated with CO_2 liberated from barium carbonate (Howton et al., 1954). (5*Z*,8*Z*,10*E*)-eicosa-8,11,13-trienoic acid **11** was obtained in 51% yield after purification by flash chromatography. GC analysis showed a 95% purity and small quantities of an impurity. The structure of (8*Z*,11*Z*,13*E*)-eicosa-8,11,13-trienoic acid was confirmed by GC–MS and NMR. An aliquot of the product was converted into its DMOX derivative for GC–MS analysis which gave a spectrum with a molecular ion of $m/z =$ 359. A mass interval of 12 units (instead of 14) occurred between m/z 182 (C_7) and 194 (C_8), between m/z 222 (C_{10}) and 234 (C_{11}) and between 248 (C_{12}) and 260 (C_{13}), indicating the presence of a double bond in the position 8 and two conjugated double bonds in positions 11 and 13. The ^{1}H NMR spectrum of free **11** (Fig. 2) showed four distinct signals for olefinic protons, one multiplet and one double-triplet ($\delta_H = 5.36$ and 5.66 ppm), which correspond to the shifts of the protons of the outer *trans–cis* conjugated diene system (14-H and 11-H), and two doublet–doublet ($\delta_H = 5.94$ and 6.30 ppm), which correspond to the shifts of the protons of the inner *trans–cis* diene system (13-H and 12-H). The 9*c*,11*t*-18:2 showed a double-triplet for the proton 11-H at $\delta_H = 5.36$ (Lie Ken Jie et al., 1997; Berdeaux et al., 1998). For the 8*c*,11*c*,13*t*-20:3 the same shift was found for the 11-H, but it was overlaid by the shift of the 10-H.

Analysis of the impurity showed that it was a geometrical isomer of (8*Z*,11*Z*,13*E*)-eicosa-8,11,13-trienoic acid. In fact, the DMOX derivative of the impurity and of (8*Z*,11*Z*,13*E*)-eicosa-8,11,13-trienoic acid gave the same spectrum. Moreover, traces of (8*Z*,11*E*,13*E*)-eicosa-8,11,13-trienoic acid were detected by ^{1}H NMR (two minor multiplets at $\delta_H = 5.55$ for the outer positioned protons (11-H, 14-H) and $\delta_H = 6$ ppm for the inner positioned protons (12-H, 13-H). ^{1}H NMR showed a minor triplet at $\delta_H = 2.77$ for the protons 10-H of the methylene carbon which is between the *E*,*E*-diene system and the *Z*-double bond of (8*Z*,11*E*,13*E*)-eicosa-8,11,13-trienoic acid, while the triplet at $\delta_H = 2.88$ corresponded at the protons 10-H of the methylene carbon which is between the *E*,*Z*-diene system and the *Z*-double bond of (8*Z*,11*Z*,13*E*)-octadeca-8,11,13-trienoic acid (Fig. 2). These two triplets allowed a quantitation of the (8*Z*,11*E*,13*E*)-eicosa-8,11,13-trienoic acid which represented 5% of the mixture, according to the GC and HPLC results.

The synthesis of the [1-^{14}C]-radiolabeled analog **11′** from the bromo precursor **10** followed the same procedure of that described for the fatty acid, using $^{14}CO_2$ liberated from ^{14}C barium carbonate. Its radiochemical purity was determined by TLC and RP-HPLC as being 94% (specific activity: 31.3 mCi mmol^{-1}). However, 0.4 mCi of (8*Z*,11*Z*,13*E*)-[1-^{14}C]-eicosa-8,11,13-trienoic acid with a radiochemical purity greater than 99% were obtained by preparative RP-HPLC.

4.2. Synthesis of (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid

The octadecatrienoic acid was synthesized in a similar way by using pentane-1,5-diol instead of heptane-1,7-diol as starting material (Fig. 3). A highly stereoselective Wittig condensation between the 5-(2-tetrahydropyranyloxy) pentylphosphonium salt **14** and the aldehyde **4** furnished the

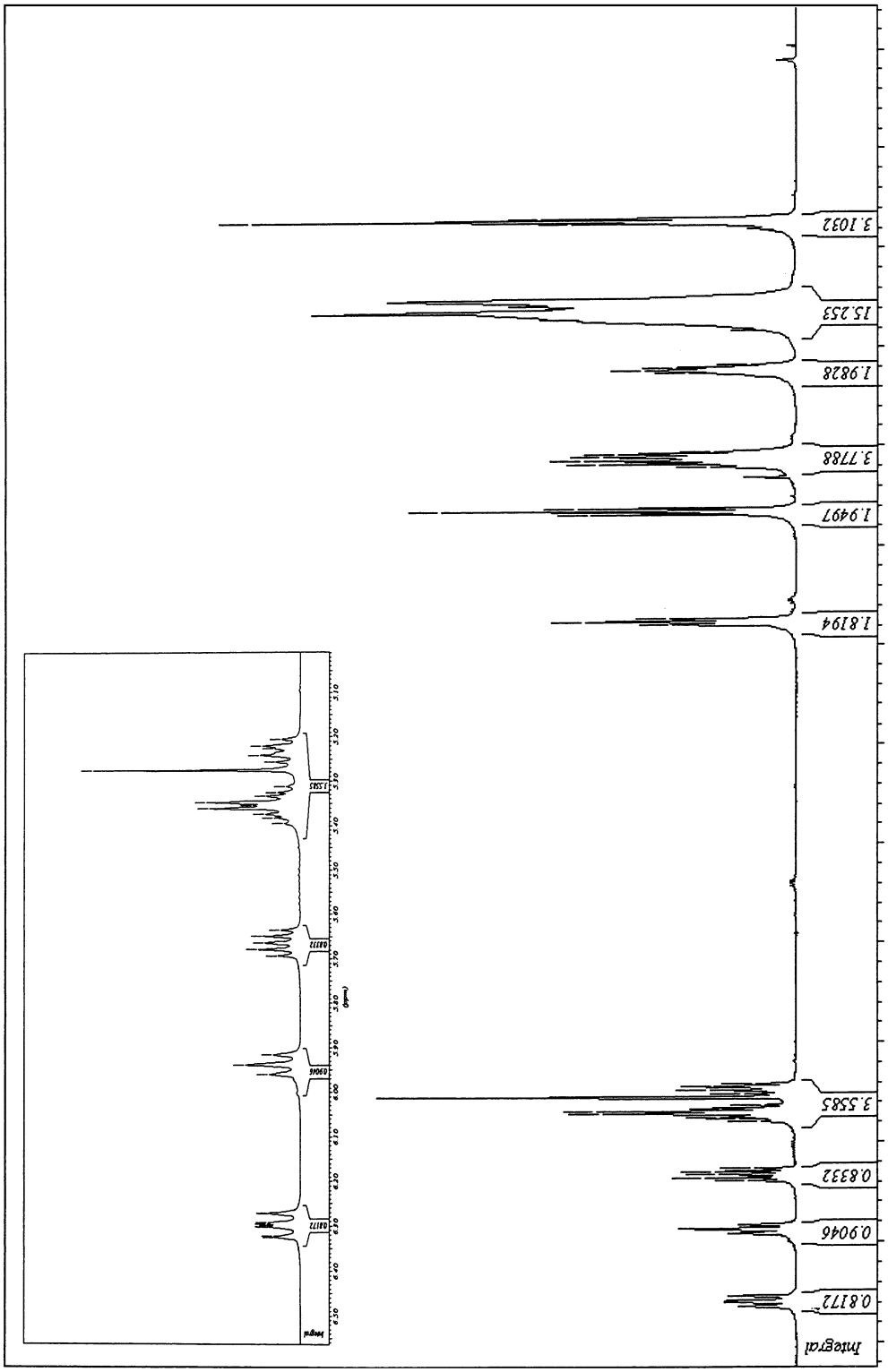

Fig. 2. ^{1}H NMR spectrum of (8*Z*,11*Z*,13*E*)-eicosa-8,11,13-trienoic acid.

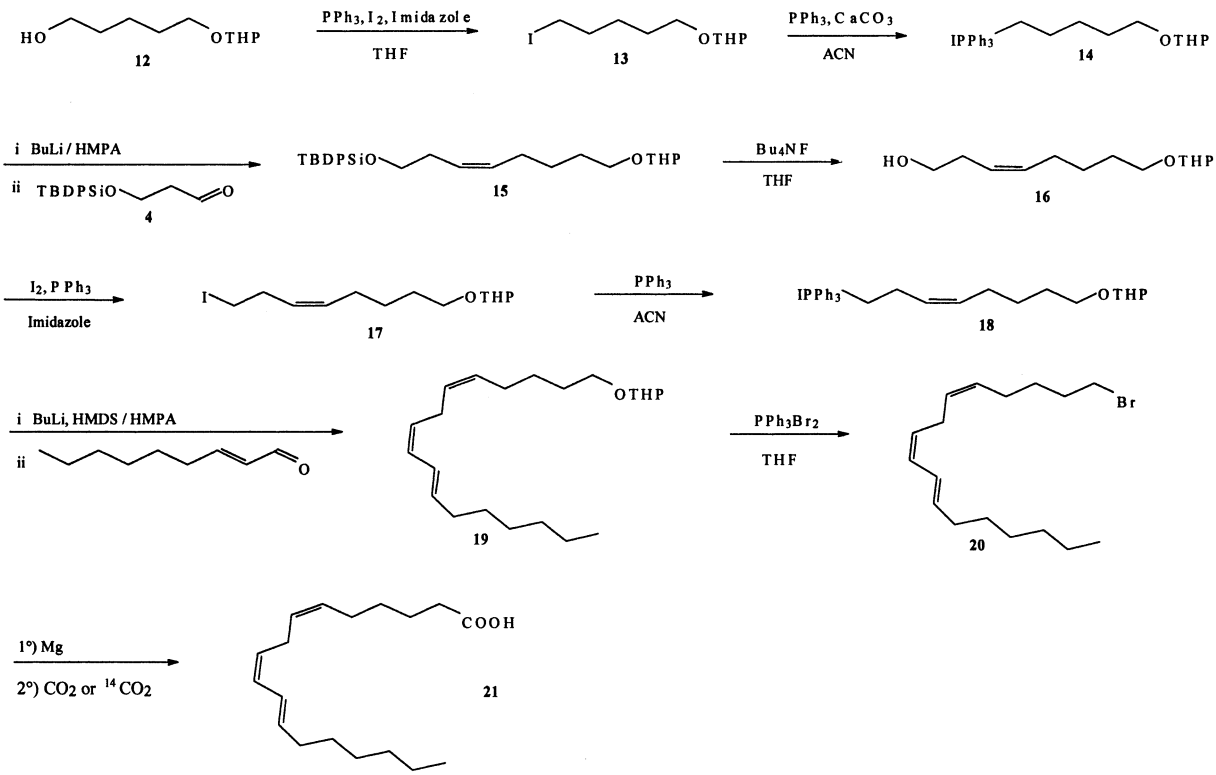

Fig. 3. Synthesis of (6*Z*,9*Z*,11*E*)-octadeca-6,9,11-trienoic acid and of its corresponding [1-^{14}C]-labeled acid.

(3*Z*)-1-(*t*-butyldiphenylsilyloxy)-8-(2-tetrahydropyranyloxy)oct-3-ene **15** in 55% yield. After preparation of the phosphonium salt **18** from **15** in 74% yield (three steps) as described for compound **8**, Wittig condensation of **18** with (2*E*)-non-2-enal in similar conditions as for the preparation of compounds **9** gave the (5*Z*,8*Z*,12*E*)-1-(2-tetrahydropyranyloxy)heptadeca-5,8,10-triene **19** in 68% yield (no geometric isomers was detected by GC, ^{1}H or ^{13}C NMR). The bromo precursor **20** was obtained in 53% yield by reaction with triphenylphosphine dibromide as described for the preparation of the bromide **10**. Formation of the corresponding heptadecatrienylmagnesium bromide (90 min in refluxing diethyl ether) followed by carbonation with CO_2 produced (6*Z*,9*Z*,11*E*)-octadeca-6,9,11-trienoic acid **21**. After purification by flash chromatography and derivatization to FAME, GC analysis showed that the 6*c*,9*c*,11*t*-18:3 had a purity of $>88\%$ and it was accompanied by the 6*c*,9*t*,11*t*-18:3 (11%) and small amounts of 6*c*,9*t*,11*c*-18:3 and 6*c*,9*c*,11*c*-18:3. Direct analysis on the free fatty acids by RP-HPLC confirmed this analysis. Structures of the 6*c*,9*c*,11*t*-18:3 and its isomers were determined by GC–MS. Therefore, an aliquot of the mixture was converted to its DMOX derivatives. GC–MS analysis of both isomers gave a spectrum with a molecular ion *m*/*z* 331, an intense ion at *m*/*z* 152 as well as ions of about equal intensity at *m*/*z* 166 and 167. A similar type of fragmentation has been reported for unsaturated fatty acids having the first double bond in Δ6 position (Dobson and Christie, 1996). The other two double bonds of the conjugated system in the positions 9 and 11 were located by fragments *m*/*z* 194 (C_8) and 206 (C_9), and fragments at *m*/*z* 220 (C_{10}) and 232 (C_{11}). The forma-

tion of the 6*c*,9*t*,11*t*-18:3 as an important by-product was surprising because the former synthesis of the 8*c*,11*c*,13*t*-20:3 only gave small amounts of the corresponding *trans–trans* isomer (isomerization rate in the last step was less than 5%). On the contrary, the isomerization rate for the 6*c*,9*c*,11*t*-18:3 was about 2.5-fold higher. The 6*c*,9*c*,11*t*-18:3 seems to be more sensitive to the heat treatment, which could be one cause of the high isomerization rate. To separate the 6*c*,9*c*,11*t*-18:3 from the 6*c*,9*t*,11*t*-18:3 isomer, HPLC purification was carried out. RP-HPLC allowed the separation of the different geometrical isomers. The purified product showed a 95% purity. The amounts of 6*c*,9*t*,11*c*-18:3 and of 6*c*,9*t*,11*t*-18:3 were 2.4 and 2.5%, respectively, and only traces of 6*c*,9*c*,11*c*-18:3 were detected. Structure of the purified 6*c*,9*c*,11*t*-18:3 was confirmed by NMR.

The synthesis of the [1-^{14}C]-radiolabeled analog **21** from the heptadecatrienylbromide **20** followed the same procedure of that described for the fatty acid synthesis. As for the unlabeled compound, an important formation of the '*trans–trans*' isomer was detected. RP-HPLC was carried out giving small amounts (0.2 mCi) of (6*Z*,9*Z*,11*E*)-[1-^{14}C]-octadeca-6,9,11-trienoic acid with a radiochemical purity of 95%.

In conclusion, we have developed two efficient and stereoselective syntheses of conjugated isomers of γ-linolenic and dihomo-γ-linolenic acids, in vivo metabolites of 9*c*,11*t*-18:2, in high isomeric purities. Conjugated 18:3 and 20:3 were prepared in nine steps and, respectively, in 8.1 and 3.3% overall yield from commercially available starting materials. Biological properties of compounds **11** and **21** are under investigation in our laboratory.

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