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Large-scale preparation of (9Z,12E)-[1-^{13}C]-octadeca-9,12-dienoic acid, (9Z,12Z,15E)-[1-^{13}C]-octadeca-9,12,15-trienoic acid and their [1-^{13}C] all-*cis* isomers

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Abstract

Several grams of labelled *trans* linoleic and linolenic acids with high chemical and isomeric purities (> 97%) have been prepared for human metabolism studies. A total of 12.5 g of (9Z,12E)-[1-^{13}C]-octadeca-9,12-dienoic acid and 6.3 g of (9Z,12Z,15E)-[1-^{13}C]-octadeca-9,12,15-trienoic acid were obtained in, respectively, seven steps (7.8% overall yield) and 11 steps (7% overall yield) from 7-bromo-heptan-1-ol. The *trans* bromo precursors used for the labelling were synthesised by using copper-catalysed couplings. The *trans* fatty acids were then obtained via the nitrile derivatives. A total of 23.5 g of (9Z,12Z)-[1-^{13}C]-octadeca-9,12-dienoic acid and 10.4 g of (9Z,12Z,15Z)-[1-^{13}C]-octadeca-9,12,15-trienoic acid were prepared in five steps in, respectively, 32 and 18% overall yield. Large quantities of bromo and chloro precursors were synthesised from the commercially available acid according to Barton's procedure. In all cases, the main impurities (> 0.5%) of each labelled fatty acid have been characterised.

Keywords: Carbon 13; *cis*; Labelling; Linoleic; Linolenic; *trans*

1. Introduction

Geometrical polyunsaturated fatty acid isomers are formed during oil processing such as deodorization (Grandgirard et al., 1984; Sébédio et al., 1988; Ackman and Mag, 1998). Consequently, *trans* isomers of linoleic and linolenic acids are found in human foods such as refined oils, margarines, human milk and infant formulas (Wolff and Sébédio, 1991; Wolff, 1993; Chardigny et al., 1996; Ratnayake et al., 1997).

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Previous studies (Brétillon et al., 1998a,b; Sébédio and Chardigny, 1998) reported the use of *trans* isomers of linoleic acid labelled with carbon 14 for metabolic studies in animals. In order to investigate further the metabolism of dietary *trans* linoleic acid for comparison with that of the all-*cis* compound in healthy volunteers, we synthesised (9Z,12E)-, (9Z,12Z)-[1-^{13}C]-linoleic acid and (9Z,12Z,15E)-, (9Z,12Z,15Z)-[1-^{13}C]-linolenic acid on a large scale (several grams) with high chemical and isomeric purities (> 97%). Furthermore, as these labelled fatty acids were used for human metabolism studies after agreement of an ethics committee, the main impurities (> 0.5%) of each acid had to be identified.

Fatty acids labelled at the carboxylic position can be obtained from a halide by well-established methods (Tulloch, 1979) as shown in Fig. 1.

Several reports have described the syntheses of all-*cis* bromo intermediates using a fatty acid as starting material. The bromo precursors of [1-^{13}C]- and [1-^{14}C]-oleic acid and (9Z,12Z)-[1-^{14}C]-linoleic acid could be prepared via a silver salt degradation (Bergstrom et al., 1952; Howton et al., 1954). (9Z,12Z)-[1-^{13}C]-Linoleic acid was obtained from an α-bromolinoleate intermediate (Campbell and Clapp, 1989). Some all-*cis* fatty acids labelled with carbon 11 have been synthesised by using Barton's bromo-decarboxylation (Barton et al., 1983; Channing and Simpson, 1993). The all-*cis* bromo precursor can also be prepared by total organic synthesis. Stoffel synthesised (9Z,12Z)-[1-^{14}C]-linoleic acid by reaction of an alkali acetylide with a propargylic halide followed by partial hydrogenation (Stoffel, 1964). More recently, a few papers have described the preparation of *trans* fatty acids labelled with carbon 14. The *trans* bromo precursors were synthesised in small quantities via stereoselective Wittig reactions (Eynard et al., 1994, 1998; Berdeaux et al., 1995). Unfortunately, the experimental procedures of these stereoselective Wittig reactions were not practical in the case of a large-scale preparation.

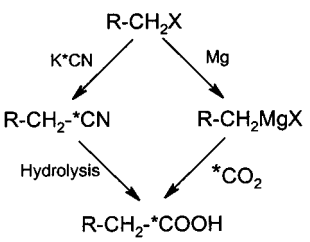

Fig. 1. Labelling of organic acids from halides. * Denotes labelling. X = Br, Cl, I.

Here, we now report convenient large-scale syntheses of (9Z,12E)-[1-^{13}C]-octadeca-9,12-dienoic acid and (9Z,12Z,15E)-[1-^{13}C]-octadeca-9,12,15-trienoic acid (respectively **7** and **19**). These syntheses are suitable for the introduction of a labelled carbon atom. The *trans* bromo intermediates **5** and **18** were prepared by synthetic schemes involving copper-catalysed couplings of terminal acetylenes with allylic bromides and selective partial reductions with Lindlar catalyst. As the all-*cis* isomers were needed for the human metabolism studies, we also describe the large-scale preparations of (9Z,12Z)-[1-^{13}C]-octadeca-9,12-dienoic acid and (9Z,12Z,15Z)-[1-^{13}C]-octadeca-9,12,15-trienoic acid (respectively **22** and **23**) by using Barton's bromo-decarboxylation.

2. Experimental

Both 7-bromo-1-heptanol and (E)-oct-2-en-1-ol were from Sigma. [1-^{13}C] potassium cyanide (isotopic enrichment > 99%) was from Isotec. Other starting materials and chemical reagents were from Aldrich. 4-(Dimethylamino) pyridine, 2-mercaptopyridine *N*-oxide, sodium salt and K^{13}CN were stored in a desiccator with phosphorus pentoxide prior to use. Unless otherwise stated, flash chromatography was performed using silica gel 60 (0.040–0.063 mm) from Merck. Potassium hydroxide solution, hydrochloric acid solution and sodium chloride were from Labosi. Magnesium sulphate, ethanol and dimethylsulphoxide were from Prolabo. Ammonium chloride and other solvents were from SDS. Acetone and methanol used for preparative reversed-phase chromatography were from Merck. Solvents were purified before use: dimethylsulphoxide, bromotrichloromethane and toluene were distilled; diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. All purified

solvents were stored over molecular sieves. Unless otherwise stated, reactions were carried out under argon.

Analytical TLC was performed with silica gel 60F254 (Merck) and visualisation was carried out with an anisaldehyde solution (anisaldehyde 12.5 ml; acetic acid 5 ml; sulphuric acid 17 ml; 95% ethanol 450 ml). NMR spectra were recorded on a Bruker AC 300 spectrometer (7.05 T; 300.13 MHz (^{1}H); 75.47 MHz (^{13}C)) and the chemical shifts were reported in ppm. Isotopic enrichment was determined by mass spectrometry (DCI/NH_3) on a Finnigan instrument (Model 4600). GC/MS analyses were effected on an HP 6890 Series gas chromatograph system coupled to an HP 5973 mass selective detector. GC analyses were carried out on a Varian 3300 gas chromatograph (N_2, 10 ml/min) fitted with a flame ionisation detector. Analytical HPLC analyses were obtained with a Shimadzu LC-9A pump and a Sedex 65 evaporative light scattering detector. Preparative reversed-phase chromatography was conducted with a NovaPrep 5000 apparatus from Merck fitted with a YRD-883 RI monitor from Uniflows.

2.1. (E)-1-Bromo-oct-2-ene **1**

(E)-Oct-2-en-1-ol (95% pure; 105.2 g; 779 mmol) in 1200 ml of anhydrous diethyl ether and 15 ml of anhydrous pyridine were placed in an oven-dried three-necked flask equipped with a magnetic stirrer and a thermometer. The solution was cooled to −10°C and phosphorus tribromide (185.9 g; 686 mmol; 0.9 eq.) was added dropwise. The reaction mixture was stirred at −10°C for 4 h after which it was poured into cold water. The aqueous layer was extracted four times with diethyl ether. The combined organic layers were washed twice with brine then with water until the washes were of neutral pH, dried over $MgSO_4$ and filtered. Partial distillation of diethyl ether gave a yellow solution (178.9 g) containing compound **1** (124.7 g; 653 mmol; 83% yield). This ethereal solution of **1** was used without any further purification in the next step.

^{1}H NMR ($CDCl_3$): 0.87 (t; $J = 6.9$ Hz; 8-H_3), 1.15–1.4 (m; 5,6,7-H_2), 2.03 (m; 4-H_2), 3.92 (d; $J = 7.3$ Hz; 1-H_2), 5.55–5.8 (m; 2,3-H_1).

2.2. Non-8-yn-1-ol **2**

Lithium acetylide, ethylenediamine complex (90% pure; 61.4 g; 600 mmol; 1.15 eq.) and 350 ml of dry DMSO were placed in an oven-dried three-necked flask equipped with a magnetic stirrer and a thermometer. The mixture was cooled to 10°C and 7-bromo-heptan-1-ol (101.4 g; 520 mmol) was added dropwise. After the addition, the dark reaction mixture was stirred vigorously at room temperature for 4 h after which it was slowly added to a mixture of water and ice. The aqueous solution was then extracted four times with diethyl ether. The combined ethereal layers were washed with saturated sodium chloride solution and water, dried over $MgSO_4$ and filtered. After concentration, the residue was chromatographed on silica gel with pentane/diethyl ether (75/25) giving 35.4 g of a mixture of compound **2** (31.9 g; 228 mmol; 44% yield) and 7-bromo-heptan-1-ol. **2** was obtained in 48% yield for the synthesis of compound **19** by using the same protocol.

^{1}H NMR ($CDCl_3$): 1.25–1.65 (m; 2,3,4,5,6-H_2), 1.95 (t; $J = 2.6$ Hz; 9-H_1), 2.2 (dt; $J = 6.9$, 2.6 Hz, 7-H_2), 3.65 (t; $J = 6.9$ Hz; 1-H_2).

2.3. (E)-Heptadeca-11-en-8-yn-1-ol **3**

Anhydrous THF (46 ml) and **2** (31.9 g; 228 mmol) were placed in an oven-dried 2-l three-necked flask equipped with a magnetic stirrer, a thermometer and a reflux condenser. The mixture was cooled to 5°C and treated for 1.75 h with 167 ml of 2.94 M CH_3MgCl in THF (491 mmol; 2.15 eq.). After the addition, the reaction mixture was stirred vigorously at 65°C for 1 h and then cooled to room temperature. CuCl (1.7 g; 17.1 mmol; 0.075 eq.) was added all at once and then 159 g of the ethereal solution of **1** (110.8 g; 580 mmol; 2.5 eq.) were added over 1 h. The mixture was heated at 65°C for 2 h giving a homogeneous solution, cooled to 25°C and quenched with 360 ml of 20% NH_4Cl solution. Sodium cyanide (5.22 g) was added and the solution became slightly yellow. The aqueous layer was extracted three times with diethyl ether and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified on

silica gel with pentane/diethyl ether (80/20) giving compound **3** (45.8 g; 183 mmol; 80% yield) as a pale yellow oil.

^{1}H NMR ($CDCl_3$): 0.87 (t; $J = 6.9$ Hz; 17-H_3), 1.2–1.65 (m; 2,3,4,5,6,14,15,16-H_2), 2 (m; 13-H_2), 2.16 (tt; $J =$ 6.9, 2.3 Hz; 7-H_2), 2.86 (m; 10-H_2), 3.63 (t; $J = 6.8$ Hz; 1-H_2), 5.4 (m; 11-H_1), 5.65 (m; 12-H_1).

Several attempts were made to optimise the formation of **3**. The different crude materials were first purified on silica gel with pentane/diethyl ether (80/20) and then subjected to reversed-phase chromatography using Partisil ODS 3 with methyl alcohol/water (85/15) providing pure **3** (8.8 g; 35 mmol)).

The different purified materials were combined to give 32.3 g of **3** (129 mmol) which were used for the next step.

2.4. (8Z,11E)-Heptadeca-8,11-dien-1-ol ***4***

Compound **3** (32.3 g, 129 mmol), Lindlar catalyst (5% wt. Pd; 1.3 g), quinoline (1.3 ml) and 150 ml of ethyl acetate were shaken in hydrogen. After the absorption of hydrogen had ceased, the catalyst was removed and the mixture was concentrated. Crude material (33.7 g) was combined with residues obtained from previous partial hydrogenation of **3** to give 54.4 g of dienol **4**. Chromatography on silica gel with pentane/diethyl ether (80/20) yielded **4** (51.2 g; 203 mmol; 97% yield).

^{1}H NMR ($CDCl_3$): 0.87 (t; $J = 6.9$ Hz; 17-H_3), 1.2–1.4 (m; 3,4,5,6,14,15,16-H_2), 1.5–1.65 (m; 2-H_2 and OH), 1.9–2.1 (m; 7,13-H_2), 2.71 (bt; $J =$ 5.3 Hz; 10-H_2), 3.63 (m; 1-H_2), 5.25–5.5 (m; 8,9,11,12-H_1).

2.5. (6E,9Z)-17-Bromo-heptadeca-6,9-diene ***5***

The bromide **5** was prepared from compound **4** (50 g; 198 mmol) using phosphorus tribromide (43 g; 159 mmol; 0.8 eq.), anhydrous pyridine (5 ml) and 300 ml of anhydrous diethyl ether as described previously for bromide **1**. Purification by chromatography on silica gel with pentane gave **5** (19.3 g; 61 mmol; 31% yield) as a colourless oil.

^{1}H NMR ($CDCl_3$): 0.87 (t; $J = 6.9$ Hz; 1-H_3), 1.2–1.5 (m; 2,3,4,12,13,14,15-H_2), 1.85 (quint.; $J = 6.9$ Hz; 16-H_2), 1.9–2.1 (m; 5,11-H_2), 2.71 (bt; $J = 5.4$ Hz; 8-H_2), 3.4 (t; $J = 6.9$ Hz; 17-H_2), 5.25–5.5 (m; 6,7,9,10-H_1).

2.6. (9Z,12E)-[1-^{13}C]-Octadeca-9,12-dienenitrile ***6***

A mixture of compound **5** (19.3 g; 61 mmol) dissolved in 190 ml of dry DMSO and $K^{13}CN$ (5.1 g; 77 mmol; 1.2 eq.) was stirred overnight at 80°C, and then cooled to room temperature. The reaction mixture was diluted with 300 ml of diethyl ether and 350 ml of saturated sodium chloride solution. The aqueous layer was extracted twice with diethyl ether. The combined organic fractions were washed with water, dried over $MgSO_4$, filtered and concentrated. The residue was eluted through silica gel with pentane/diethyl ether (95/5) to give the nitrile **6** (14 g, 53 mmol; 87% yield).

^{1}H NMR ($CDCl_3$): 0.87 (t; $J = 6.9$ Hz; 18-H_3), 1.2–1.5 (m; 4,5,6,7,15,16,17-H_2), 1.63 (m; 3-H_2), 1.9–2.1 (m; 8,14-H_2), 2.32 (dt; $J = 9.5$, 7 Hz; 2-H_2), 2.71 (bt; $J = 5.5$ Hz; 11-H_2), 5.25–5.5 (m; 9,10,12,13-H_1).

2.7. (9Z,12E)-[1-^{13}C]-Octadeca-9,12-dienoic acid ***7***

Compound **6** (14 g; 53 mmol) was placed in 225 ml of ethyl alcohol and 225 ml of 40% KOH. The mixture was heated at 80°C for 5 h and then cooled to room temperature. Ground ice was placed in the flask and the reaction mixture was acidified to pH 1 with 6 N HCl. The aqueous layer was extracted three times with 500 ml of diethyl ether. The combined organic layers were washed with water until washes were of neutral pH, dried over $MgSO_4$, filtered and concentrated. The crude material was chromatographed on silica gel with pentane/diethyl ether/acetic acid (90/10/1). Compound **7** (12.5 g; 44 mmol; 83% yield) was obtained as a yellow oil.

TLC: pentane/diethyl ether/acetic acid (90/10/1). Rf = 0.15 (visualisation with an anisaldehyde solution).

^{1}H NMR ($CDCl_3$): 0.87 (t; $J = 6.9$ Hz; 18-H_3), 1.2–1.4 (m; 4,5,6,7,15,16,17-H_2), 1.62 (m; 3-H_2),

1.9–2.1 (m; 8,14-H_2), 2.34 (dt; $J = 7.3$, 7.3 Hz, 2-H_2), 2.71 (bt; $J = 5.4$ Hz; 11-H_2), 5.25–5.5 (m; 9,10,12,13-H_2).

^{13}C NMR ($CDCl_3$): 13.82 (C18), 22.31 (C17), 24.42 (C3), 26.83 (C8), 28.81 and 28.88 (C4, C5 and C6), 28.99 (C15), 29.34 (C7), 30.21 (C11), 31.19 (C16), 32.29 (C14), 33.48 and 34.2 ($J = 54.3$ Hz, C2), 127.62 (C10), 128.01 (C12), 130.05 (C9), 130.61 (C13), 180.13 (C1).

HPLC: column: Hypersil HS C18 (250×4.6 mm). Solvent system: acetone/methanol/water/acetic acid (50/25/25/0.5). Flow rate: 1.5 ml/min. Chemical purity: unknown impurity (r.t. = 24.5 min; 0.4%), (9Z,12Z)-[1-^{13}C]-linoleic acid (r.t. = 26 min; 0.9%), (9Z,12E)-[1-^{13}C]-linoleic acid **7** (r.t. = 27.7 min; 97.7%), (9E,12E)-[1-^{13}C]-linoleic acid (r.t. = 30.6 min; 0.9%), (9Z)-[1-^{13}C]-oleic acid (r.t. = 42 min; 0.1%).

GC/MS: column: HP-23 *cis*/*trans* FAME (60 m × 0.25 mm × 0.25 μm). Column temperature: 200°C. Carrier gas: He (1 ml/min). Injector temperature: 250°C. Identification (methyl ester derivative): unknown impurity (r.t. = 7.1 min; $m/z = 295$ M^+), (9Z)-[1-^{13}C]-oleic acid, methyl ester (r.t. = 8.2 min; $m/z = 297$ M^+), (9E,12E)-[1-^{13}C]-linoleic acid, methyl ester (r.t. = 8.5 min; $m/z = 295$ M^+), (9Z,12E)-[1-^{13}C]-linoleic acid, methyl ester (r.t. = 8.8 min; $m/z = 295$ M^+), (9Z,12Z)-[1-^{13}C]-linoleic acid, methyl ester (r.t. = 9 min; $m/z = 295$ M^+).

MS (DCI/NH_3): m/z (%): 298 (2.7), 299 (100), 300 (16.7), 301 (3.7). Isotopic enrichment: 97.4%.

2.8. Discoloration of **7**

Two typical procedures which can be used for the discoloration are described:

1. Compound **7** (10.4 g; 37 mmol) was dissolved in 70 ml of freshly distilled diethyl ether and a small amount of activated charcoal was added to the solution. The mixture was stirred at room temperature for 1.5 h, filtered and concentrated to give acid **7** (10.3 g; 99% yield) as a pale yellow oil.
2. Compound **7** (1 g) was chromatographed on Partisil ODS 3 with acetone/methyl alcohol/water/acetic acid (50/25/25/0.5) giving in a quantitative yield the acid **7** as a colourless oil.

2.9. (E)-1-Bromo-pent-2-ene **8**

A total of 155 g of a mixture of bromide **8** (84.9 g; 570 mmol; 84% yield) and diethyl ether were prepared from (E)-pent-2-en-1-ol (95% pure; 58.5 g; 679 mmol) using phosphorus tribromide (142.5 g; 526 mmol; 0.8 eq.), 2.3 ml of dry pyridine and 900 ml of anhydrous diethyl ether as described previously for bromide **1**. This solution of **8** was used without any further purification in the next step.

^1H NMR ($CDCl_3$): 0.94 (t; $J = 7.4$ Hz; 5-H_3), 2 (m; 4-H_2); 3.87 (d; $J = 7.4$ Hz; 1-H_2), 5.6 (m; 2-H_1), 5.75 (m; 3-H_1).

2.10. (E)-Oct-5-en-2-yn-1-ol **9**

A total of 47 g of a mixture of alcohol **9** (44.6 g; 360 mmol; 94% yield) and diethyl ether were prepared from prop-2-yn-1-ol (21.3 g; 380 mmol), 260 ml of 3 M methylmagnesium chloride in THF (780 mmol; 2.05 eq.), Cu(I)Cl (2.7 g; 27.3 mmol; 0.07 eq.) and compound **5** in diethyl ether (570 mmol; 1.5 eq.) as described previously for compound **3**.

^1H NMR ($CDCl_3$): 0.95 (t, $J = 7.4$ Hz, 8-H_3), 2 (m, 7-H_2), 2.9 (m, 4-H_2), 4.25 (m, 1-H_2), 5.35 (m, 5-H_1), 5.7 (m, 6-H_1).

2.11. (E)-1-Bromo-oct-5-en-2-yne **10**

Compound **10** was obtained from 2.71 g of a mixture of compound **9** (2.57 g; 20.7 mmol) and diethyl ether, anhydrous pyridine (0.2 ml), phosphorus tribromide (4.3 g; 15.8 mmol; 0.8 eq.) and anhydrous diethyl ether (20 ml) as described for bromide **1**. After concentration of the ethereal fractions, the residue was eluted through silica gel with pentane giving bromide **10** (2.38 g; 12.7 mmol; 61% yield).

^1H NMR ($CDCl_3$): 0.95 (t, $J = 7.4$ Hz, 8-H_3), 2 (m, 7-H_2), 2.9 (m, 4-H_2), 3.95 (t, $J = 2.4$ Hz, 1-H_2), 5.35 (m, 5-H_1), 5.7 (m, 6-H_1).

2.12. (E)-Heptadec-14-ene-8,11-diyn-1-ol **11**

Bromide **10** (1.68 g; 9 mmol) and terminal acetylene **2** (1.26 g; 9 mmol) were placed in 20 ml

of dry DMF. K_2CO_3 (2.88 g; 20 mmol), NaI (1.52 g; 10 mmol) and CuI (1.93 g; 10 mmol), each finely ground and anhydrous, were added. The suspension was stirred overnight at 40°C, then quenched with 100 ml of a saturated solution of NH_4Cl. The mixture was extracted three times with diethyl ether, dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography on silica gel with pentane/diethyl ether (75/25) giving **11** (1.52 g; 61 mmol; 67%). Then 1 g of purified **11** was eluted through 200 g of C18 Partisil Prep 20 ODS 3 with methanol/water (85/15) and only 0.53 g of **11** were recovered.

^{1}H NMR ($CDCl_3$): 0.95 (t; $J = 7.4$ Hz, 17-H_3), 1.2–1.7 (m; 2,3,4,5,6-H_2), 2 (m; 16-H_2), 2.1 (m; 7-H_2), 2.85 (m; 13-H_2), 3.1 (quint.; $J = 2.4$ Hz; 10-H_2), 3.6 (t; $J = 6.7$ Hz; 1-H_2), 5.35 (m; 14-H_1), 5.65 (m; 15-H_1).

2.13. (8Z,11Z,14E)-Heptadeca-8,11,14-trien-1-ol ***12***

The triene **12** was prepared from compound **11** (520 mg; 2.1 mmol), purified on silica gel, Lindlar catalyst (5% wt. Pd; 48 mg), quinoline (40 μl) and ethyl acetate (15 ml) as described for diene **4**. Chromatography on silica gel with pentane/diethyl ether (75/25) gave **12** (170 mg, 0.68 mmol; 32% yield).

^{1}H NMR ($CDCl_3$): 0.95 (t; $J = 7.4$ Hz; 17-H_3), 1.2–1.45 (m; 3,4,5,6-H_2), 1.55 (m, 2-H_2), 1.9–2.1 (m; 7,16-H_2), 2.7–2.85 (m; 10,13-H_2), 3.6 (t; $J =$ 6.7 Hz; 1-H_2), 5.25–5.55 (m; 8,9,11,12,14,15-H_1).

2.14. (2Z,5E)-Octa-2,5-dien-1-ol ***13***

Compound **13** was obtained from 33.2 g of a mixture of **9** (31.5 g, 254 mmol) and diethyl ether, Lindlar catalyst (5% wt. Pd; 5.5 g), quinoline (3.4 ml) and ethanol (410 ml) as described for diene **4**. Chromatography on silica gel with pentane/diethyl ether (85/15) gave 32 g of a mixture of the alcohol **13** (28.2 g; 224 mmol; 88% yield) and residual quinoline.

^{1}H NMR ($CDCl_3$): 0.95 (t; $J = 7.4$ Hz; 8-H_3), 1.6 (bt; $J = 5.1$ Hz; OH), 2 (m; 7-H_2), 2.75 (bt; $J = 6.5$ Hz; 4-H_2), 4.2 (m; 1-H_2), 5.25–5.7 (m; 2,3,5,6-H_1).

2.15. (2Z,5E)-1-Bromo-octa-2,5-diene ***14***

A mixture of bromide **14** (32 g; 170 mmol; 94% yield) and diethyl ether was prepared from 25.8 g of a mixture of dienol **13** (22.7 g; 180 mmol) and quinoline, dry pyridine (1.9 ml), anhydrous diethyl ether (230 ml) and phosphorus tribromide (39.3 g; 145 mmol; 0.8 eq.) as described for bromide **1**. This solution was used for the next step without any further purification.

^{1}H NMR ($CDCl_3$): 0.9 (t; $J = 7.4$ Hz; 8-H_3), 1.9 (m; 7-H_2), 2.75 (bt; $J = 6.7$ Hz; 4-H_2), 3.9 (d; $J = 8.2$ Hz; 1-H_2), 5.2–5.75 (m; 2,3,5,6-H_1).

2.16. 2-Non-8-ynyloxy-tetrahydro-pyran ***15***

A total of 28.5 g of a mixture of **2** (25 g, 178 mmol) and 7-bromo-heptan-1-ol, and 3.8 g of *p*-toluene sulphonic acid were placed in 600 ml of dry dichloromethane. Dihydropyran (27.8 g; 331 mmol) was added dropwise at room temperature. The reaction mixture was stirred for 5.5 h and then 500 ml of a saturated $NaHCO_3$ solution were added. The aqueous layer was extracted with dichloromethane. The combined organic fractions were washed with water, dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel with pentane/diethyl ether (95/5) giving 2-non-8-ynyloxy-tetrahydropyran **15** (34.8 g; 155 mmol; 87% yield).

^{1}H NMR ($CDCl_3$): 1.25–1.9 (m; 2,3,4,5,6-H_2; 3,4,5-H_2 (THP)), 1.95 (t; $J = 2.6$ Hz; 9-H_1), 2.15 (dt; $J = 6.9$, 2.6 Hz, 7-H_2), 3.36 (dt, $J = 9.5$, 6.6 Hz; 1-H_a), 3.5 (m; 6-H_a (THP)), 3.71 (dt; $J = 9.5$, 6.9 Hz; 1-H_b), 3.85 (m; 6-H_b (THP)), 4.56 (m; 2-H_1 (THP)).

2.17. 2-((11Z,14E)-Heptadeca-11,14-dien-8-ynyloxy)-tetrahydro-pyran ***16a*** *and 2-((E)-10-vinyl-pentadec-12-en-8-ynyloxy)-tetrahydro-pyran* ***16b***

A mixture of **16a** and **16b** was obtained from compound **15** (27.2 g; 121 mmol), 42 ml of 3 M CH_3MgCl in THF (126 mmol; 1.04 eq.), CuCl (1 g; 10.1 mmol; 0.08 eq.) and the ethereal solution of **14** (74.6 g; 170 mmol; 1.4 eq.) as described for

compound **3**. The residue was purified by chromatography on silica gel with pentane/diethyl ether (95/5) giving 31.9 g of a 79:21 mixture of **16a** and **16b** (96 mmol; 79% yield).

^{1}H NMR ($CDCl_3$): 0.95 (t, $J = 7.4$ Hz, 17-H_3 (**16a**)), 0.96 (t, $J = 7.4$ Hz, 17-H_3 (**16b**)), 1.25–1.9 (m; 2,3,4,5,6-H_2 (**16a** and **16b**); 3,4,5-H_2 (THP)), 1.9–2.05 (m; 16-H_2 (**16a**); 14-H_2 (**16b**)), 2.1–2.25 (m, 7-H_2 (**16a**); 7,11-H_2 (**16b**)), 2.7 (m; 13-H_2 (**16a**)), 2.9 (m; 10-H_2 (**16a**)), 3.05 (m; 10-H_1 (**16b**)), 3.36 (dt, $J = 9.5$,6.6 Hz; 1-H_a (**16a** and **16b**)), 3.5 (m; 6-H_a (THP)), 3.71 (dt; $J = 9.5$, 6.9 Hz; 1-H_b (**16a** and **16b**)), 3.85 (m; 6-H_b (THP)), 4.56 (m; 2-H_1 (THP)), 5.04 (d; $J = 10$ Hz; 2-H_{cis} (**16b**: vinyl)), 5.25 (d, $J = 17.5$ Hz, 2-H_{trans} (**16b**: vinyl)), 5.3–5.55 (m; 11,12,14,15-H_1 (**16a**); 12,13-H_1 (**16b**)), 5.75 (ddd, $J = 17.5$, 10, 6 Hz; 1-H_1 (**16b**: vinyl)).

GC/MS: column: HP-5MS (30 m × 0.25 mm × 0.25 μm). Column temperature: 42°C (2 min) to 300°C (3 min) at 18°C/min. Carrier gas: He (0.9 ml/min). Injector temperature: 250°C. Compound **16a** (r.t. = 15.3 min; $m/z = 332$ M^+), compound **16b** (r.t. = 14.7 min; $m/z =$ 332 M^+).

Several purified materials were combined giving a mixture of **16a** and **16b** (39.7 g; 119 mmol) which was used for the next step.

*2.18. 2-((8Z,11Z,14E)–Heptadeca-8,11,14-trienyloxy)-tetrahydro-pyran **17a***

A mixture of **16a** and **16b** (35.7 g; 107 mmol), Lindlar catalyst (5% wt. Pd; 2.94 g), quinoline (2.95 ml) and 225 ml of ethyl acetate were shaken in hydrogen. After the absorption of hydrogen had ceased, the catalyst was removed and the mixture was concentrated. Purification on silica gel with pentane/diethyl ether (95/5) gave a mixture of **17a** and **17b** and an analytical sample of pure **16b**. Pure compound **17a** was obtained by successive silver ion flash chromatography runs (silica gel: 300 g; $AgNO_3$: 60 g) with a pentane/diethyl ether gradient ((95/5): 2 l; (85/15): 1 l; diethyl ether: 1 l). Traces of silver nitrate were removed by washes with saturated sodium chloride solution and elution through silica gel with pentane/diethyl ether (95/5). Compound **17a** (21.8 g; 65.2 mmol; 61% yield) was obtained as a colourless oil.

^{1}H NMR ($CDCl_3$): 0.95 (t; $J = 7.4$ Hz; 17-H_3), 1.2–1.45 (m; 3,4,5,6-H_2), 1.95–2.1 (m; 7,16-H_2), 2.65–2.85 (m; 10,13-H_2), 3.36 (dt, $J = 9.5$, 6.6 Hz; 1-H_a), 3.5 (m; 6-H_a (THP)), 3.71 (dt; $J = 9.5$, 6.9 Hz; 1-H_b), 3.85 (m; 6-H_b (THP)), 4.56 (m; 2-H_1 (THP)), 5.25–5.55 (m; 8,9,11,12,14,15-H_1).

*2.18.1. 2-((E)-10-Vinyl-pentadec-12-en-8-ynyloxy)-tetrahydro-pyran **16b**:*

^{1}H NMR ($CDCl_3$): 0.96 (t; $J = 7.4$ Hz; 15-H_3), 1.25–1.9 (m; 2,3,4,5,6-H_2; 3,4,5-H_2 (THP)), 1.95–2.1 (m; 14-H_2), 2.1–2.25 (m; 7,11-H_2), 3 (m; 10-H_1), 3.36 (dt; $J = 9.5$, 6.6 Hz; 1-H_a), 3.5 (m; 6-H_a (THP)), 3.71 (dt; $J = 9.5$, 6.9 Hz; 1-H_b), 3.85 (m; 6-H_b (THP)), 4.56 (m; 2-H_1 (THP)), 5.04 (d; $J = 10$ Hz; 2-H_{cis} (vinyl)), 5.25 (d; $J = 17.5$ Hz; 2-H_{trans} (vinyl)), 5.35–5.55 (m; 12,13-H_1), 5.75 (ddd; $J = 17.5$, 10, 6 Hz; 1-H_1 (vinyl)).

GC/MS: column: HP-5MS (30 m × 0.25 mm × 0.25 μm). Column temperature: 42°C (2 min) to 300°C (3 min) at 18°C/min. Carrier gas: He (0.9 ml/min). Injector temperature: 250°C. Compound **16b** (r.t. = 14.7 min; $m/z =$ 332 M^+), compound **17a** (r.t. = 15 min; $m/z = 334$ M^+), compound **17b** (r.t. = 14.5 min; $m/z =$ 334 M^+).

*2.19. (3E,6Z,9Z)-17-Bromo-heptadeca-3,6,9-triene **18***

Compound **17a** (21.3 g; 63.7 mmol) in 65 ml of CH_2Cl_2 was added at 0°C to a slurry of dibromotriphenylphosphorane (57.3 g; 136 mmol) in 325 ml of CH_2Cl_2. After the addition, the mixture was stirred at room temperature for 1 h, washed with a saturated solution of sodium bicarbonate, then with water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on silica gel with pentane. Bromide **18** (18.3 g, 58.5 mmol, 91% yield) was obtained as a colourless oil.

^{1}H NMR ($CDCl_3$): 0.96 (t; $J = 7.4$ Hz; 1-H_3), 1.2–1.5 (m; 12,13,14,15-H_2), 1.84 (quint; $J = 6.9$ Hz; 16-H_2), 1.95–2.15 (m; 2,11-H_2), 2.65–2.85 (m; 5,8-H_2), 3.4 (t, $J = 6.9$ Hz, 17-H_2), 5.25–5.55 (m; 3,4,6,7,9,10-H_1).

2.20. (9Z,12Z,15E)-[1-^{13}C]-Octadeca-9,12,15-trienoic acid **19**

A total of 12.5 g of compound **19** contaminated with 9% (RP-HPLC, see below for experimental conditions) of a geometrical isomer with two *trans* double bonds were obtained as a pale yellow oil from the bromide **18** (18.3 g; 58.5 mmol) as described for acid **7**. Successive preparative reversed-phase chromatography runs using 1-g samples with 200 g of Hyperprep 100A HS C18 10μ using acetonitrile/water/acetic acid (75/25/0.5) as mobile phase gave pure acid **19** (6.3 g; 22.6 mmol, 38% yield from compound **18**).

TLC: pentane/diethyl ether/acetic acid (90/10/1). Rf = 0.3 (visualisation with an anisaldehyde solution).

^{1}H NMR ($CDCl_3$): 0.95 (t; $J = 7.4$ Hz; 18-H_3), 1.2–1.4 (m; 4,5,6,7-H_2), 1.6 (m; 3-H_2), 1.9–2.1 (m; 8,17-H_2), 2.34 (dt; $J = 7.3$, 7.3 Hz, 2-H_2), 2.65–2.85 (m; 14,11-H_2), 5.25–5.55 (m; 9,10,12,13,15,16-H_2).

^{13}C NMR ($CDCl_3$): 13.57 (C18), 24.42 (C3), 25.32 (C11 and C17), 26.94 (C8), 28.82 and 28.88 (C4, C5 and C6), 29.32 (C7), 30.17 (C14), 33.49 and 34.22 ($J = 55.7$ Hz, C2), 126.82 (C15), 127.58 (C10), 127.75 (C13), 128.25 (C12), 129.93 (C9), 132.25 (C16), 180.13 (C1).

HPLC: column: Zorbax C18 SB (250 × 4.6 mm). Solvent system: acetone/methanol/water/acetic acid (45/27.5/27.5/0.055). Flow rate: 1.5 ml/min. Chemical purity: (9Z,12Z,15Z)-[1-^{13}C]-linolenic acid (r.t. = 30.2 min; 0.7%), (9Z,12Z,15E)-[1-^{13}C]-linolenic acid **19** (r.t. = 31.4 min; 97.6%), [1-^{13}C]-18:3 fatty acid with two *trans* double bonds (r.t. = 34.2 min; 1.7%).

GC/MS: column: HP-23 *cis*/*trans* FAME (60 m × 0.25 mm × 0.25 μm). Column temperature: 200°C. Carrier gas: He (1 ml/min). Injector temperature: 250°C. Identification (methyl ester derivative): [1-^{13}C]-18:3 fatty acid with two *trans* double bonds, methyl ester (r.t. = 10.5 min; $m/z = 293$ M^+), (9Z,12Z,15E)-[1-^{13}C]-linolenic acid, methyl ester (r.t. = 10.8 min; $m/z = 293$ M^+), (9Z,12Z,15Z)-[1-^{13}C]-linolenic acid, methyl ester (r.t. = 11.2 min; $m/z = 293$ M^+).

MS (DCI/NH_3): m/z (%): 296 (3.4), 297 (100), 298 (26.9), 299 (3.8). Isotopic enrichment: 97.4%.

2.21. (9Z,12Z)-Octadeca-9,12-dienoyl chloride **20**

(9Z,12Z)-Octadeca-9,12-dienoic acid (50 g; 178 mmol) and 400 ml of anhydrous toluene were placed in an oven-dried 2-l three-necked flask equipped with a magnetic stirrer, a thermometer and a gas absorption trap containing 2 N NaOH. Freshly distilled oxalyl chloride (86 ml; 980 mmol; 5.5 eq.) was added dropwise at 2°C and the yellow solution was stirred overnight at room temperature. The solution was then concentrated. Then 3 × 100 ml of anhydrous toluene were added to the crude acyl chloride **20** and evaporated under vacuum to remove traces of oxalyl chloride. The residue (53 g of **20**; 177 mmol; 99% yield) was used without further purification.

2.22. (6Z,9Z)-17-Bromo-heptadeca-6,9-diene **21a** and (6Z,9Z)-17-chloro-heptadeca-6,9-diene **21b**

4-(Dimethylamino)pyridine (1.74 g; 14.2 mmol; 0.1 eq.), 2-mercaptopyridine *N*-oxide, sodium salt (26.5 g; 178 mmol; 1.2 eq.) and 610 ml of bromotrichloromethane were introduced into an oven-dried 2-l three-necked flask equipped with a magnetic stirrer, a reflux condenser and an oil bubbler. The mixture was heated to reflux and **20** (43.8 g; 146 mmol) in 220 ml of bromotrichloromethane was added dropwise. After the addition, the mixture was heated to reflux for 1 h and then cooled to room temperature. The reaction mixture was diluted with 500 ml of diethyl ether and 500 ml of saturated sodium chloride solution. The aqueous layer was extracted twice with diethyl ether. The combined ethereal fractions were washed with water, dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel with pentane giving 22.8 g of a mixture of **21a** and **21b** in 50% yield ((**21a**: 20.8 g, 66 mmol); (**21b**: 2 g, 7 mmol)).

GC: column: Megabore DB5 (30 m × 0.53 mm × 1.5 μm). Column temperature: 150–250°C (8°C/min). Injector and detector temperature: 250°C. r.t.(**21a**) = 10.1 min, r.t.(**21b**) = 8.9 min.

^{1}H NMR ($CDCl_3$): 0.9 (t; $J = 6.9$ Hz; 1-H_3, (**21a** and **21b**)), 1.2–1.5 (m; 2,3,4,12,13,14,15-H_2, (**21a** and **21b**)), 1.75 (quint.; $J = 6.9$ Hz; 16-H_2, (**21b**)), 1.85 (quint.; $J = 6.9$ Hz; 16-H_2, (**21a**)), 1.9–2.1 (m; 5,11-H_2, (**21a** and **21b**)), 2.77 (bt; $J = 5.9$ Hz; 8-H_2, (**21a** and **21b**)), 3.4 (t; $J = 6.8$ Hz; 17-H_2 (**21a**)), 3.52 (t; $J = 6.8$ Hz; 17-H_2 (**21b**)), 5.25–5.45 (m; 6,7,9,10-H_1,(**21a** and **21b**)).

Previous attempts were made to optimise the formation of **21a** and the different purified mixtures of **21a** and **21b** were combined to give 39.4 g of halides ((**21a**: 29.4 g, 93.2 mmol); (**21b**: 10 g, 37.1 mmol)) which were used for the preparation of the [1-^{13}C] labelled acid.

*2.23. (9Z,12Z)-[1-^{13}C]-Octadeca-9,12-dienoic acid **22***

Compound **22** (31.5 g; 112 mmol; 86% yield from (**21a** and **21b**)) was obtained as a yellow oil containing conjugated acids from the mixture of **21a** and **21b** as described for the acid **7**.

TLC: pentane/diethyl ether/acetic acid (85/15/1). Rf = 0.33 (UV-active spot (254 nm)).

GC: column: Stalbiwax-DA (30 m × 0.53 mm × 0.25 μm). Column temperature: 150–250°C (8°C/min). Injector and detector temperature: 250°C. Chemical purity: **22** (r.t. = 12 min; 96.6%), conjugated acids (r.t. = 12.7 min; 3.4%)

*2.24. Chemical purification of **22***

Compound **22** (31.5 g; 112 mmol) containing conjugated acids, maleic anhydride (43.9 g; 448 mmol; 4 eq.) and 240 ml of anhydrous *o*-xylene were placed in an oven-dried 1-l three-necked flask equipped with a magnetic stirrer and a reflux condenser. The mixture was heated to reflux for 4.5 h, then cooled to room temperature and diluted with 330 ml of diethyl ether. Hydrolysis with 230 ml of water, addition of LiOH until the pH was basic and acidification to pH 3 with 4 N HCl gave a mixture which was extracted three times with diethyl ether. The combined ethereal fractions were washed with water, dried over $MgSO_4$, filtered and concentrated. The crude pale yellow oil was chromatographed on silica gel with pentane/diethyl ether/acetic acid (85/15/1) giving pure acid **22** (23.5 g; 84 mmol; 75% yield).

TLC: pentane/diethyl ether/acetic acid (85/15/1). Rf = 0.33 (visualisation with an anisaldehyde solution).

^{1}H NMR ($CDCl_3$): 0.9 (t; $J = 6.9$ Hz; 18-H_3), 1.2–1.4 (m; 4,5,6,7,15,16,17-H_2), 1.62 (m; 3-H_2), 1.95–2.1 (m; 8,14-H_2), 2.34 (dt; $J = 7.3$, 7.3 Hz, 2-H_2), 2.77 (bt; $J = 5.9$ Hz; 11-H_2), 5.25–5.45 (m; 9,10,12,13-H_2).

^{13}C NMR ($CDCl_3$): 13.82 (C18), 22.33 (C17), 24.41 (C3), 25.39 (C11), 26.95 (C8 and C14), 28.83 and 28.89 (C4, C5 and C6), 29.12 (C15), 29.34 (C7), 31.29 (C16), 33.49 and 34.22 ($J = 55.7$ Hz, C2), 127.66 (C12), 127.83 (C10), 129.76 (C9), 129.95 (C13), 180.26 (C1).

HPLC: column: Zorbax SB C18 (250 × 4.6 mm). Solvent system: acetone/methanol/water/acetic acid (60/20/20/0.4). Flow rate: 1.5 ml/min. Chemical purity: (9Z,12Z)-[1-^{13}C]-linoleic acid **22** (r.t. = 14 min; > 99%).

MS (DCI/NH_3): *m*/*z* (%): 299 (100), 300 (18.1), 301 (10.4). Isotopic enrichment: 99%.

*2.25. (9Z,12Z,15Z)-[1-^{13}C]-Octadeca-9,12,15-trienoic acid **23***

Pure acid **23** (10.4 g; 37 mmol) free of conjugated acids was prepared in 18% overall yield from (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (57.5 g; 206 mmol) by using the procedure described for compound **22**.

TLC: pentane/diethyl ether/acetic acid (90/10/1). Rf = 0.3 (visualisation with an anisaldehyde solution).

^{1}H NMR ($CDCl_3$): 0.96 (t; $J = 7.4$ Hz; 18-H_3), 1.2–1.4 (m; 4,5,6,7-H_2), 1.6 (m; 3-H_2), 2–2.15 (m; 8,17-H_2), 2.34 (dt; $J = 7.3$, 7.3 Hz, 2-H_2), 2.75–2.85 (m; 14,11-H_2), 5.2–5.45 (m; 9,10,12,13,15,16-H_2).

^{13}C NMR ($CDCl_3$): 14.04 (C18), 20.33 (C17), 24.42 (C3), 25.30 (C14), 25.39 (C11), 26.98 (C8), 28.85 and 28.92 (C4, C5 and C6), 29.35 (C7), 33.52 and 34.25 ($J = 55.3$ Hz, C2), 126.89 (C15), 127.53 (C10), 128 (C12 and C13), 129.95 (C9), 131.66 (C16), 180.39 (C1).

HPLC: column: Zorbax SB C18 (250 × 4.6 mm). Solvent system: acetone/methanol/water/acetic acid (50/25/25/0.125). Flow rate: 1.5 ml/min. Chemical purity: > 99%.

MS (DCI/NH_3): *m*/*z* (%): 296 (0.2), 297 (100), 298 (17.5), 299 (4.4). Isotopic enrichment: 99%.

3. Results and discussion

3.1. Synthesis of [1-^{13}C]-(9Z,12E)-octadeca-9,12-dienoic acid 7 (Fig. 2)

The synthesis of the mono *trans* dienoic acid **7** used (E)-oct-2-en-1-ol and 7-bromoheptan-1-ol as starting materials.

The *trans* octenol was converted to its corresponding allylic bromide **1** with phosphorus tribromide in 83% yield (van Liemt et al., 1994) and the 7-bromoheptan-1-ol was transformed in 45% yield to non-8-yn-1-ol **2** by reaction with lithium acetylide, ethylenediamine complex (Porwoll and Leete, 1985). Compound **2** was treated with methylmagnesium chloride and coupled with a molar excess of **1** in the presence of a catalytic amount of Cu(I)Cl giving the enyne **3** in 80% yield (Snider and Burbaum, 1983). Partial hydrogenation of **3** over Lindlar's catalyst poisoned with quinoline in ethyl acetate (Rakoff, 1990) afforded the dienol **4** (97% yield) which was converted into the bromide **5** with PBr_3 (31% yield). As we obtained an unsatisfying yield with phosphorus tribromide, dibromotriphenyl phosphorane was used with good yield as bromination reagent at the similar step during the synthesis of (9Z,12Z,15E)-[1-^{13}C]-octadeca-9,12,15-trienoic acid. Reaction of **5** with K^{13}CN in DMSO at 80°C provided the nitrile **6** in 87% yield. (9Z,12E)-[1-^{13}C]-Octadeca-9,12-dienoic acid **7** was obtained by alkaline hydrolysis of **6** with 40% KOH in ethanol at 80°C (83% yield). After discoloration of the acid **7** with activated charcoal, its chemical purity was determined by RP-HPLC as 97.7% (isotopic enrichment: 97.4%). NMR data were in agreement with those reported in the literature for the methyl ester derivative (Bus et al., 1976; Berdeaux et al., 1995). The main impurities were identified by RP-HPLC on an (9Z,12E)-[1-^{13}C]-linoleic acid sample and GC/MS on its methyl ester derivative by comparison with authentic samples. (9Z,12Z)-[1-^{13}C]-Linoleic acid (0.9%), (9E,12E)-[1-^{13}C]-linoleic acid (0.9%), (9Z)-[1-^{13}C]-oleic acid (0.1%) were found to be present. One impurity (0.4%) was identified as a 18:2 fatty acid but its complete structure remains unknown.

*3.2. Synthesis of (9Z,12Z,15E)-[1-^{13}C]-octadeca-9,12,15-trienoic acid **19** (Figs. 3 and 4)*

In a first synthetic scheme, we tried to prepare (E)-heptadeca-14-ene-8,11-diyn-1-ol **11** on a small scale by using a copper-catalysed coupling of the terminal acetylene **2** with the propargylic bromide

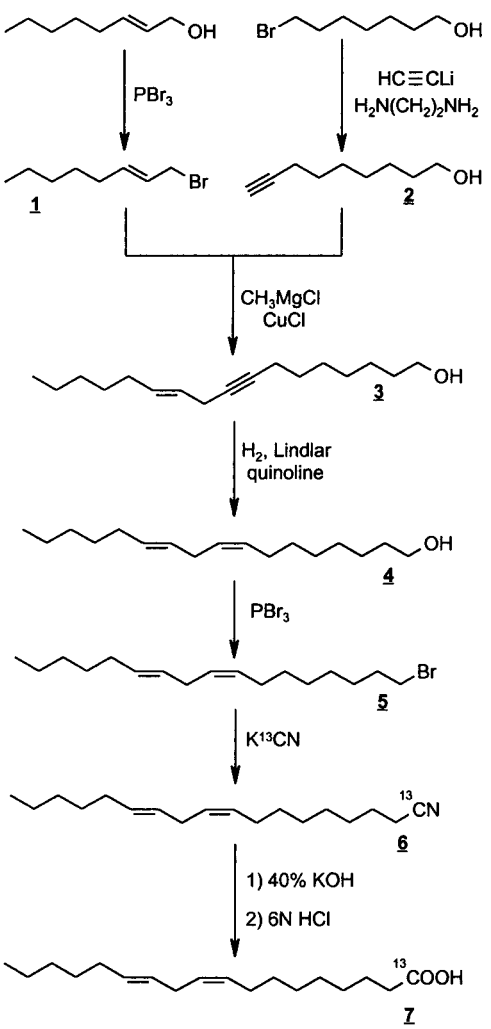

Fig. 2. Synthesis of (9Z,12E)-[1-^{13}C]-octadeca-9,12-dienoic acid.

10. Compound **11** could give the expected bromo intermediate **18** after partial hydrogenation and bromination (Fig. 3).

Prop-2-yn-1-ol was treated with methylmagnesium bromide in the presence of a catalytic amount of copper(I) chloride and coupled with 1.5 equivalents of (E)-1-bromo-pent-2-ene **8** (compound **8** was previously synthesised in 84% yield from (E)-pent-2-en-1-ol using phosphorus tribromide as bromination reagent). After purification, the alcohol **9** was obtained in 94% yield and then transformed into its bromo derivative **10** with PBr_3 (61% yield) by using the procedure described

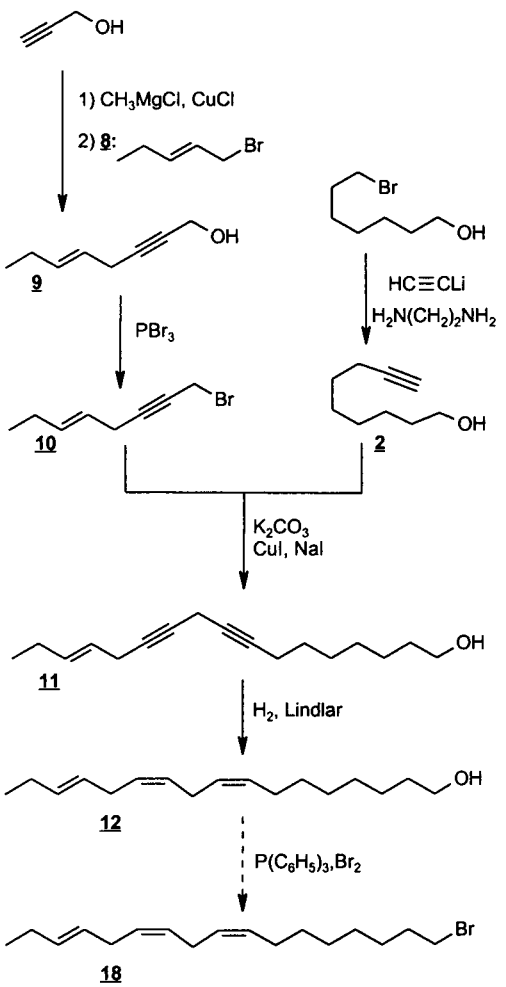

Fig. 3. Synthesis of (3E,6Z,9Z)-17-bromo-heptadeca-3,6,9-triene via (E)-heptadec-14-ene-8,11-diyn-1-ol.

for **8**. In Section 3.1, we described the synthesis of (9Z,12E)-[1-^{13}C]-linoleic acid via the coupling of **8** with 2.3 equivalents of (E)-1-bromo-oct-2-ene. Here, compound **2** was coupled with only one equivalent of propargylic bromide **10** under mild conditions in the presence of copper(I) iodide, sodium iodide and potassium carbonate (Lapitskaya et al., 1993; Durand et al., 1998). The alcohol **11** was obtained in 67% yield but was found to be unstable during purification by flash chromatography on silica gel or preparative RP-HPLC on Partisil ODS 3. Moreover, Lindlar reduction of purified compound **11** furnished the trienol **12** in only 32% yield.

So we decided to modify our synthetic pathway (Fig. 4).

Partial hydrogenation of (E)-oct-5-en-2-yn-1-ol **9** over Lindlar's catalyst poisoned with quinoline in ethanol (Dogra et al., 1989) afforded the dienol **13** (88% yield) which was converted into its bromo derivative **14** with PBr_3 (94% yield). Several attempts were made with terminal acetylenes **2** and **15** in order to reduce the molar excess of **14** used during the copper-catalysed coupling. The best results were obtained with the protected alcohol **15**. After coupling of compound **15** with 1.4 equivalents of the allylic bromide **14**, the crude residue was purified by flash chromatography on silica gel giving a 79:21 mixture of 1,1 and 1,3-coupling product **16a** and **16b** (79% yield). Lindlar reduction of this mixture furnished a residue containing the trienes **17a** and **17b** and a small amount of **16b**. This residue was first purified by flash chromatography on silica gel giving a mixture of trienes **17a** and **17b** and an analytical sample of pure **16b**. Compound **17a** was obtained after purification by successive silver-ion flash chromatography runs (61% yield) and transformed into its corresponding bromide **18** (91% yield) with dibromo triphenylphosphorane (Rakoff, 1982; Eynard et al., 1994). Then (9Z,12Z,15E)-octadeca-9,12,15-trienoic acid **19** (chemical purity: 90% (RP-HPLC); 76% yield from bromide **18**) was prepared by using the procedure described for the synthesis of the acid **7**. GC/MS was then performed on its methyl ester derivative. The main impurity (9% (RP-HPLC)) was identified as a geometrical isomer with two

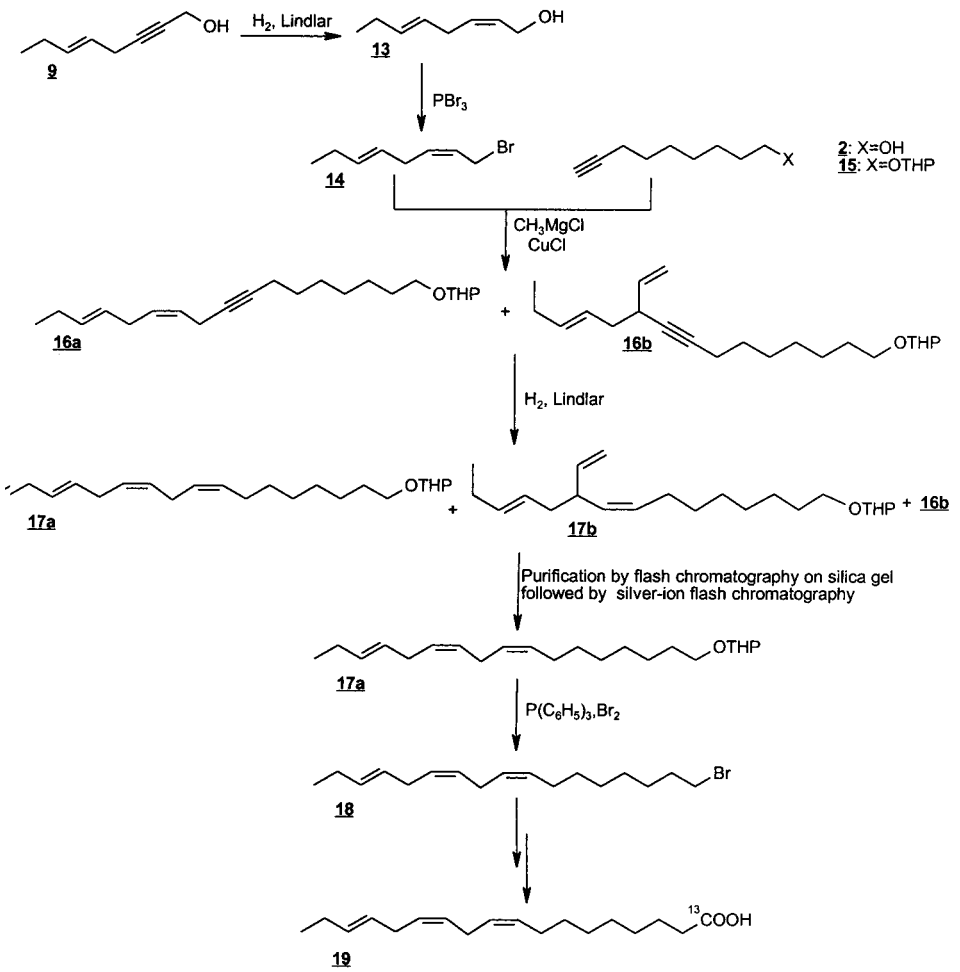

Fig. 4. Synthesis of (9Z,12Z,15E)-[1-^{13}C]-octadeca-9,12,15-trienoic acid.

trans double bonds by comparison with a commercial mixture of eight geometrical isomers of linolenic acid, methyl ester. Purification by successive preparative reversed-phase chromatography runs on a gram scale gave (9Z,12Z,15E)-[1-^{13}C]-linolenic acid **19** (chemical purity: 97.6%; isotopic enrichment: 97.4%) in 38% yield from bromide **18**. ^{1}H and ^{13}C NMR data were in agreement with those reported for methyl ester (Rakoff and Emken, 1982; Eynard et al., 1994). (9Z,12Z,15Z)-[1-^{13}C]-Linolenic acid (0.7%) and the 18:3 fatty acid with two *trans* double bonds previously identified (1.7%) were found to be present as impurities.

*3.3. Synthesis of (9Z,12Z)-[1-^{13}C]-octadeca-9,12-dienoic acid **22** and (9Z,12Z,15Z)-[1-^{13}C]-octadeca-9,12,15-trienoic acid **23** (Fig. 5)*

Commercially available (9Z,12Z)-octadeca-9,12-dienoic acid was transformed into its corresponding acyl chloride derivative **20** with freshly distilled oxalyl chloride in anhydrous toluene. According to the procedure of Barton et al. (1983), **20** was converted to a mixture of bromide **21a** and chloride **21b**.

Several attempts were made to optimise the formation of **21a** and we noted that we had a very low yield (7–13%) and a sharp increase in the

amount of **21b** (34–40% of **21a**; 60–66% of **21b** (wt.)) when we used concentrated reaction mixtures. Using more dilute conditions, we obtained a mixture of **21a** and **21b** (50% yield) containing 91.2% (wt.) of **21a**. Different purified mixtures of bromide and chloride compounds were combined giving a mixture of **21a** and **21b** (74.6% (wt.) of **21a**) which was transformed into [1-^{13}C]-linoleic acid **22** by using the procedure described for the synthesis of the mono *trans* isomers. Unfortunately, compound **22** was contaminated by a small amount of conjugated acids which were formed during the alkaline hydrolysis of the nitrile intermediate and detected by their UV-active spot on TLC. These conjugated acids were transformed into adducts by using a Diels Alder reaction with maleic anhydride in refluxing *o*-xylene (Viala and Labaudinière, 1993). These adducts were easily eliminated by flash chromatography on silica gel and pure acid **22** free of conjugated acids was obtained in 75% yield. The chemical purity of **22** was found to be $>99\%$ by RP-HPLC (isotopic enrichment: 99%). ^{13}C NMR data were in agreement with those described for the methyl ester derivative (Bus et al., 1976).

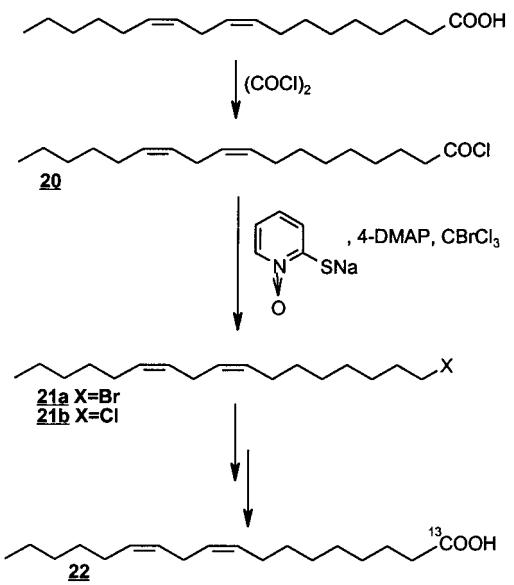

Fig. 5. Synthesis of (9Z,12Z)-[1-^{13}C]-octadeca-9,12-dienoic acid.

(9Z,12Z,15Z)-[1-^{13}C]-Linolenic acid **23** free of conjugated acids was prepared in 18% overall yield by using the synthetic pathway described for (9Z,12Z)-[1-^{13}C]-linoleic acid **22**. Chemical purity of **23** was found to be $>99\%$ by RP-HPLC (isotopic enrichment: 99%). ^{13}C NMR data were in agreement with those previously obtained for methyl ester (Rakoff, 1986).

In conclusion, we have developed practical and efficient large-scale syntheses giving [1-^{13}C] labelled (9Z,12E)-, (9Z,12Z)-linoleic acid and (9Z,12Z,15E)-, (9Z,12Z,15Z)-linolenic acid of high chemical and isomeric purities ($>97\%$). Our results show that both the 'acetylenic route' using copper-catalysed coupling and Barton's bromodecarboxylation are useful alternatives for the preparation of several grams of (9Z,12E)-, (9Z,12Z)-[1-^{13}C]-linoleic acid and (9Z,12Z,15E)-, (9Z,12Z,15Z)-[1-^{13}C]-linolenic acid intended for human studies. Indeed, by-products formed during these syntheses could be easily eliminated by either chromatography or chemical purification and all the main impurities ($>0.5\%$) present in the labelled fatty acids could be characterised.

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