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Synthesis of (9Z,12E)-, (9E,12Z)-[1-^{14}C]-linoleic acid, (9Z,12Z,15E)-, (9E,12Z,15Z)-[1-^{14}C]-linolenic acid and (5Z,8Z,11Z,14E)-[1-^{14}C]-arachidonic acid

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SUMMARY

Synthesis of (9Z,12E)-, (9E,12Z)-[1-^{14}C]-linoleic acid, (9Z,12Z,15E)-, (9E,12Z,15Z)-[1-^{14}C]-linolenic acid and (5Z,8Z,11Z,14E)-[1-^{14}C]-arachidonic acid

Trans polyunsaturated fatty acids are produced in vegetable oils during heat treatment (240-250 °C).In order to study the metabolic pathway of 9c,12t and 9t,12c linoleic acid and 9c,12c,15t and 9t,12c,15c linolenic acid, these products were prepared labelled with carbon 14 in the carboxylic position. 5c,8c,11c,14t-Arachidonic acid was also labelled on the carboxylic position with carbon 14 in order to study its physiological effects. To introduce the labelling (E)-bromo precursors with a 17 carbons chain or a 19 carbon chain were needed. The different syntheses were done by elongation steps and creation of *cis* double bonds via highly stereospecific Wittig reactions. The radioactive carbon atom was introduced from [^{14}C]-potassium cyanide. The final radioactive fatty acids had a specific activity greater than 50 mCi/mmol and a radioactive purity better than 99 % for linoleic and linolenic and better than 98.6 % for arachidonic acid

*KEY-WORDS: [^{14}C]-fatty acids – **trans**-arachidonic acid – **trans**-linoleic acid – **trans**-linolenic acid – Wittig reaction*

The general routes to introduce a carbon-14 into the carboxylic position of a fatty acid from a bromo compound is, to make the Grignard reagent then to carbonate it with [^{14}C]-carbon dioxide «Liu (1988)» or, from the same bromo derivative, to introduce a [^{14}C]-cyano group then to hydrolyse it to a carboxylic compound «Stoffel (1964); Rakoff (1982)».Due to the small quantity of unlabelled bromo precursor available we chose the second route according to the scheme 1.

$$R\text{-}CH_2\text{-}Br + K^{14}CN \longrightarrow R\text{-}CH_2\text{-}^{14}CN$$

$$R\text{-}CH_2\text{-}^{14}CN \longrightarrow R\text{-}CH_2\text{-}^{14}COOH$$

Scheme 1

The main difficulty concerning the E isomers was the synthesis of the unlabelled starting products which were not commercially available. Each precursor was obtained in multi-step syntheses.

A carbon-carbon double bond of (Z)-configuration can be created through the reduction of an acetylenic bond «Osbond (1961)» or via a Wittig reaction which usually is not stereoselective «Rakoff (1982)».We chose the Wittig route under highly stereospecific conditions for (Z) bond formation. The Wittig reactions were done between a phosphonium salt and an aldehyde using n-butyl lithium as a base, tetrahydrofuran-hexamethylphosphoramide as solvent and a temperature between -40°C and -78°C. The purity of each compound was controlled by ^{1}H and ^{13}C NMR and gas chromatography.

The labelling was introduced as described in scheme 1: [^{14}C]-potassium cyanide reacted with the corresponding (E)-bromo precursor in dimethylsulfoxide; the [^{14}C]-cyano compound was hydrolysed, in a water/ethanol solution containing potassium hydroxide, into the corresponding *trans* fatty acid., which was purified by preparative liquid chromatography or preparative HPLC.

The radiochemical purity was checked by TLC and HPLC. The specific activity was measured by mass spectrometry and the structural analyses were done by mass spectrometry and proton NMR. We previously described these syntheses «Eynard (1994); Berdeaux (1995)».

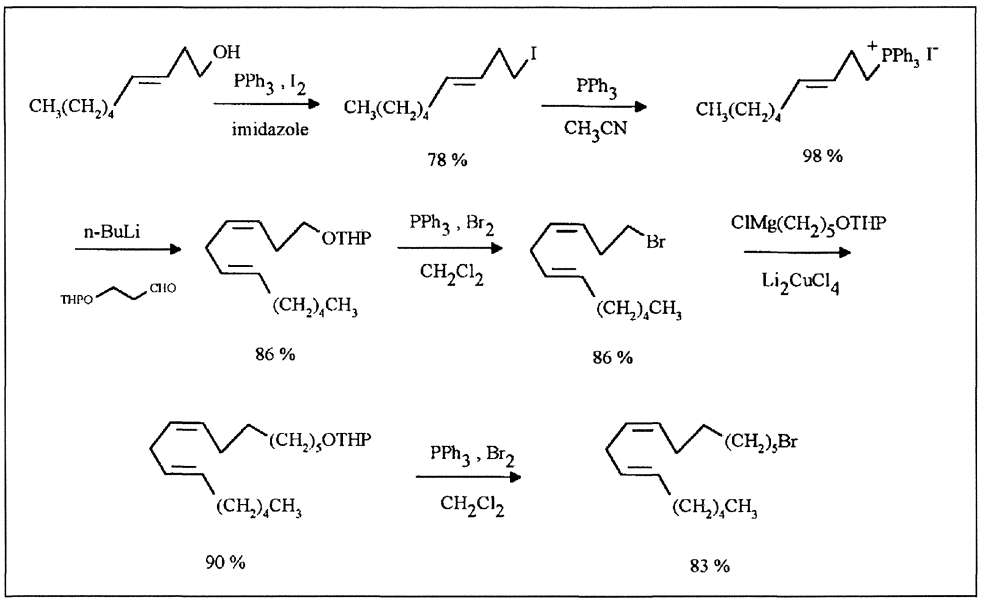
The main results are summarized in the Figure 1.

Synthesis of (9Z,12E)-[1-^{14}C]-linoleic acid

(9Z,12E)-[1-^{14}C]-Linoleic acid was synthesised according to the Scheme 2, via two elongation steps. The first one involved a Wittig reaction between the ylide of ((E)-non-3-enyl)) triphenylphosphonium iodide and 3-(2-tetrahydropyranyloxy)propanal to afford (3Z,6E)-1-(2-tetrahydropyranyloxy)dodeca-3,6-diene in 86 % yield. The second one was effected by a cross-coupling reaction between (3Z,6E)-1-bromododeca-3,6-diene and 5-(2-tetrahydropyranyloxy)-pentyl magnesium chloride with dilithiumchlorocuprate in 90 % yield. The radioactive labelling was effected from the bromo derivative with [^{14}C]-potassium cyanide according to the general method.

product	number of steps	bromo derivative yield (%)	specific activity (mCi/mmol)	radioactive yield (%)
(9Z,12E)-[1-^{14}C] linoleic acid	8	42.2	54.1	55
(9E,12Z)-[1-^{14}C] linoleic acid	6	38.3	54.1	64
(9Z,12Z,15E)-[1-^{14}C] linolenic acid	7	54.8	52.5	64
(9E,12Z,15Z)-[1-^{14}C] linolenic acid	9	36.2	50.8	40
(5Z,8Z,11Z,14E)-[1-^{14}C] arachidonic acid	11	27.5	52.5	55

Figure 1



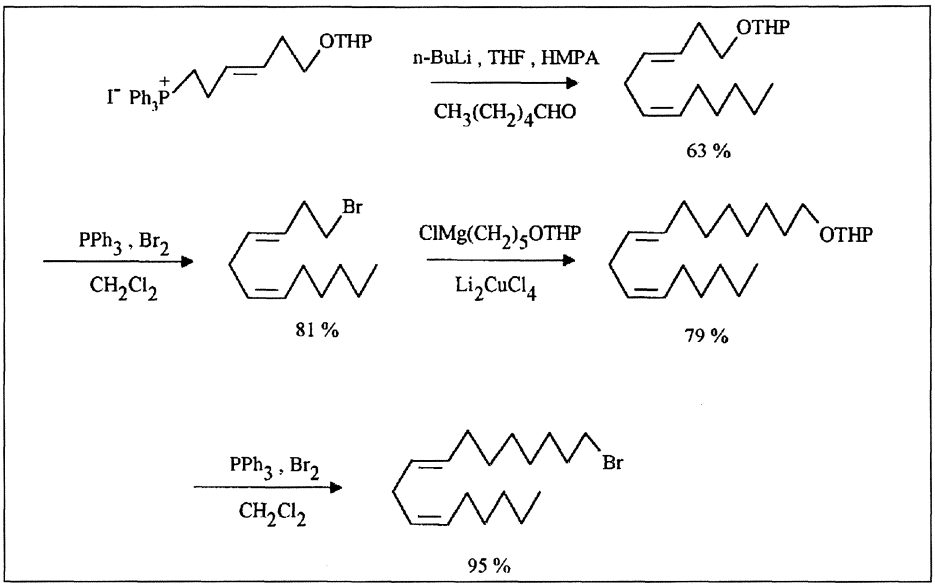
Scheme 2

Synthesis of (9E,12Z)-[1-^{14}C]-linoleic acid

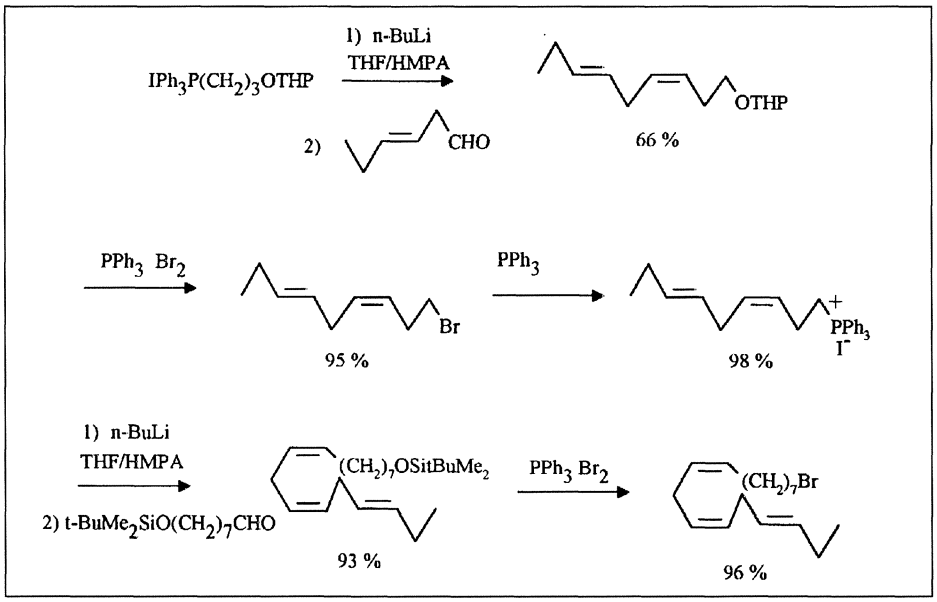
The synthesis of (9E,12Z)-[1-^{14}C]-linoleic acid is outline in the Scheme 3. As described for the synthesis of (9Z,12E)-[1-^{14}C]-linoleic acid the key-steps are two chain elongations. The first one was a cis-Wittig condensation of (3E)-6(2-tetrahydropyranyloxy)-hex-3-enyl-phosphonium iodide and hexanal, in 63 % yield; the second one was the same cross-coupling between (3E,6Z)-1-bromododeca-3,6-diene and the Grignard reagent of 1-chloro-5-(2-tetrahydropyranyloxy)pentane with dilithiumchlorocuprate as described in Scheme 2; the cross-coupling yield was 79 %.The labelling was done by the classical route.

Synthesis of (9Z,12Z,15E)-[1-^{14}C]-linolenic acid

The synthesis of (9Z,12Z,15E)-[1-^{14}C]-linolenic acid was accomplished as in the Scheme 4. Two Wittig reactions under cis-olefination conditions afforded the final product. The first one between 3-(2-tetrahydropyranyloxy)-propylphosphonium iodide and (E)-3-hexenal gave the (3Z,6E)-diene in 66 % yield; the second one, between (3Z,6E)-nona-3,6-dienylphosphonium bromide and 8-(t-butyldimethylsilyloxy)-octanal, afforded the (8Z,11Z,14E)-trienic derivative having a 17-carbon chain in 93 % yield . The labelling was obtained from the bromo compound by the general method previously described.



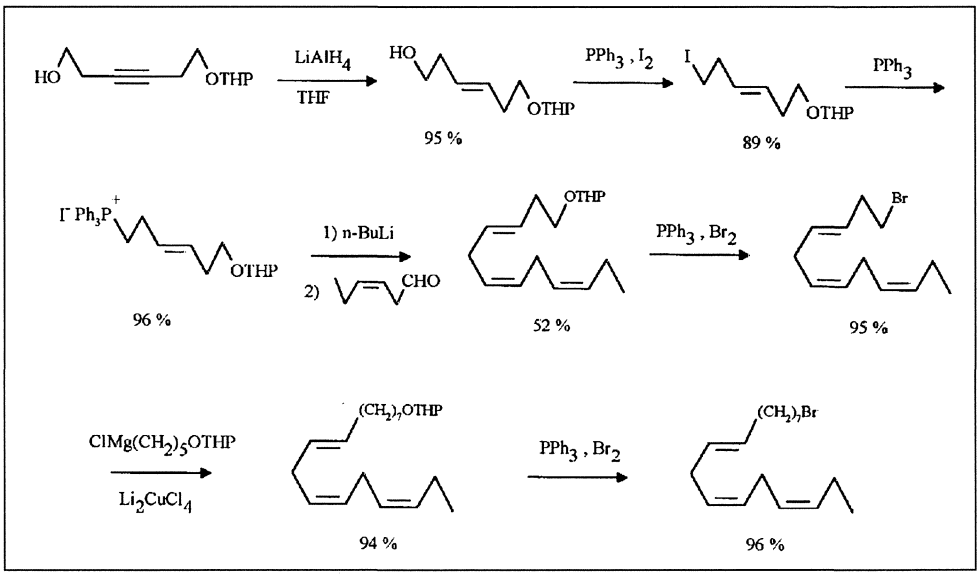
Scheme 3



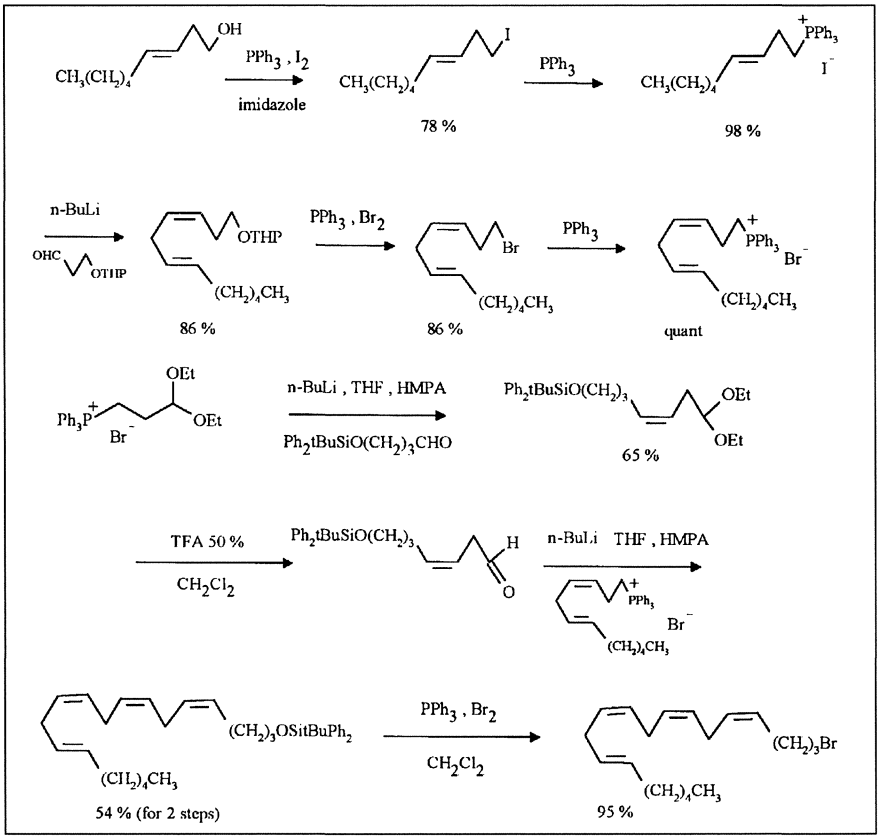
Scheme 4

Synthesis of (9E,12Z,15Z)-[1-^{14}C]-linolenic acid

(9E,12Z,15Z)-[1-^{14}C]-linolenic acid was synthesised as described in Scheme 5. The E double bond of the molecule was obtained by reduction of 6-(2-tetrahydropyranyloxy)-hex-3-yn-1-ol with lithiumaluminium hydride in 95 % yield. The two other cis double bonds were prepared by a highly stereoselective Wittig reaction between (3E)-6-(2-tetrahydropyranyloxy)-hex-3-enyl-phosphonium iodide and (3Z)-hexenal in 52 % yield. The final elongation from the trienic compound with 12 carbons to the 17 carbons chain was done by a coupling between the Grignard reagent of 1-chloro-5-(2-tetrahydropyranyloxy pentane and (3E,6Z,9Z)-1-bromododeca-3,6,9-triene in 94 % yield. The [^{14}C]-final product was obtained by the same route as previously described.



Scheme 5



Scheme 6

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

The synthesis of (5Z,8Z,11Z,14E)-[1-^{14}C]-arachidonic acid was achieved according to Scheme 6. The final product was obtained through three highly stereoselective Wittig reactions. The first one involved the condensation between ((E)-non-3-enyl)triphenylphosphonium iodide and 3-(2-tetrahydroxypyranyloxy) propanal in 86 % yield. The

corresponding phosphonium salt obtained via the bromo derivative was used later in the second part of the synthesis.

A second Wittig reaction between (3,3-diethoxy)propyltriphenylphosphonium bromide and 4-(t-butyldiphenylsilyloxy)butanal afforded (Z)-1,1-diethoxy-7-(t-butyldiphenylsilyloxy)hept-3-ene in 65 % yield. After deprotection of the acetal, the last Wittig reaction between the aldehyde and the phosphonium salt obtained in the first part of the synthesis gave the tetraenic compound with a 19 carbon chain in 54 % yield .The bromo compound obtained from the protected alcohol was used to introduce the radioactive labelling.

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