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Synthesis of [18-^{11}C/(^{13}C)]linoleic acid

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Summary

A method for the preparation of [18-^{11}C]linoleic acid is described. A highly reactive zerovalent copper complex was prepared from lithium (2-thienyl)iodocuprate reduced by lithium naphtalenide. This copper complex was used in a coupling reaction between 17-iodo-*cis*,*cis*-9,12-heptadecadienoic acid *tert*-butyl ester and [^{11}C]methyl iodide to form [18-^{11}C]linoleic acid *tert*-butyl ester as intermediate. The *tert*-butyl ester protecting group was rapidly removed with trifluoroacetic acid, affording [18-^{11}C]linoleic acid in 48% radiochemical yield. In a typical run starting with 27 GBq of [^{11}C]methyl iodide, 2.9 GBq [18-^{11}C]linoleic acid was obtained within 44 min from the end of radionuclide production.

Key words: [18-^{11}C]linoleic acid, ^{11}C, ^{11}C-labelled, fatty acids, lithium (2-thienyl)iodocuprate

Introduction

The essential fatty acids linoleic- and linolenic acid can not be synthesized by mammals due to the lack of enzymes to introduce double bonds beyond the C-9 carbon in the fatty acid carbon chain (1). In humans, these fatty acids are incorporated

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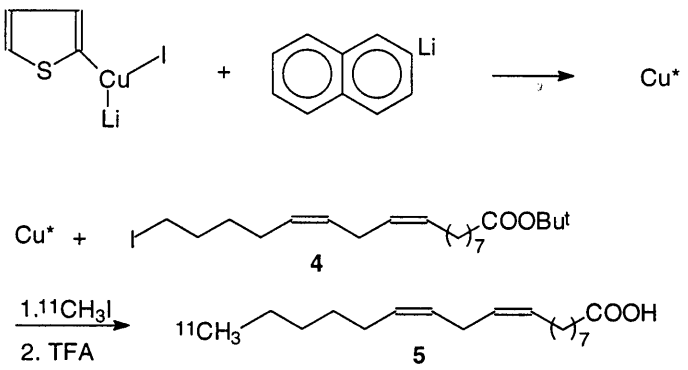
into phospholipids in the cell membranes or act as precursors for other unsaturated fatty acids such as arachidonic acid and prostaglandins (2).

Fat emulsions, containing the essential fatty acids, are given intravenously to prematurely born children for total parenteral nutrition. One such emulsion, Intralipid®, has linoleic acid (53%) and linolenic acid (16%) as its main constituents. Although the use of Intralipid® is well established, the biological mechanisms involved in treatment with Intralipid® are not clearly understood. To provide a possibility to study Intralipid® in a positron emission tomography (PET) (3) investigation, a synthetic procedure for [18-^{11}C]linoleic acid was developed.

The synthesis of saturated fatty acids ^{11}C-labelled in selected positions, and their use in PET studies of myocardial metabolism, has been described previously (4-7). The unsaturated fatty acid arachidonic acid has also been labelled with ^{11}C, both in the carboxyl (8) and the 19-position (9). In order to synthesise ^{11}C-labelled linoleic acid, previously described methods(6,7) for labelling of fatty acids were investigated. Since the carboxyl labelling of linoleic acid was not successful, the possibility to introduce the label in the methyl position was investigated. In the methyl ^{11}C-labelling of saturated fatty acids (6) and [19-^{11}C]arachidonic acid (9), an α,ω-(bis)Grignard reagent was used together with dilithium tetrachlorocuprate and [^{11}C]methyl iodide, followed by addition of carbon dioxide. [18-^{11}C]Linoleic acid could not be labelled by this method since the double bonds are located unsymmetrically (*i.e.* in the 9 and 12 position). Likewise in another method (7), an ω-Grignard reagent with a furane protected carboxyl group was coupled with [^{11}C]methyl iodide, followed by oxidative cleaved of the furane ring with ruthenium tetraoxide and sodium periodate, affording the methyl labelled fatty acid. Since the oxidative conditions used would cleave the double bonds of linoleic acid, this method was also ruled out (10).

In this study, the synthesis of [18-^{11}C]linoleic acid is described. It was performed *via* a coupling reaction between 17-iodo-*cis*-*cis*-9,12-heptadecadienoic acid

tert-butyl ester **4** and [^{11}C]methyl iodide, using a highly reactive zerovalent copper complex (Cu*) (11,12,13). This procedure was originally developed for saturated fatty acids (14), but we now report the first ^{11}C-labelling of unsaturated fatty acids (Scheme 1).



Scheme 1.

Results and Discussion

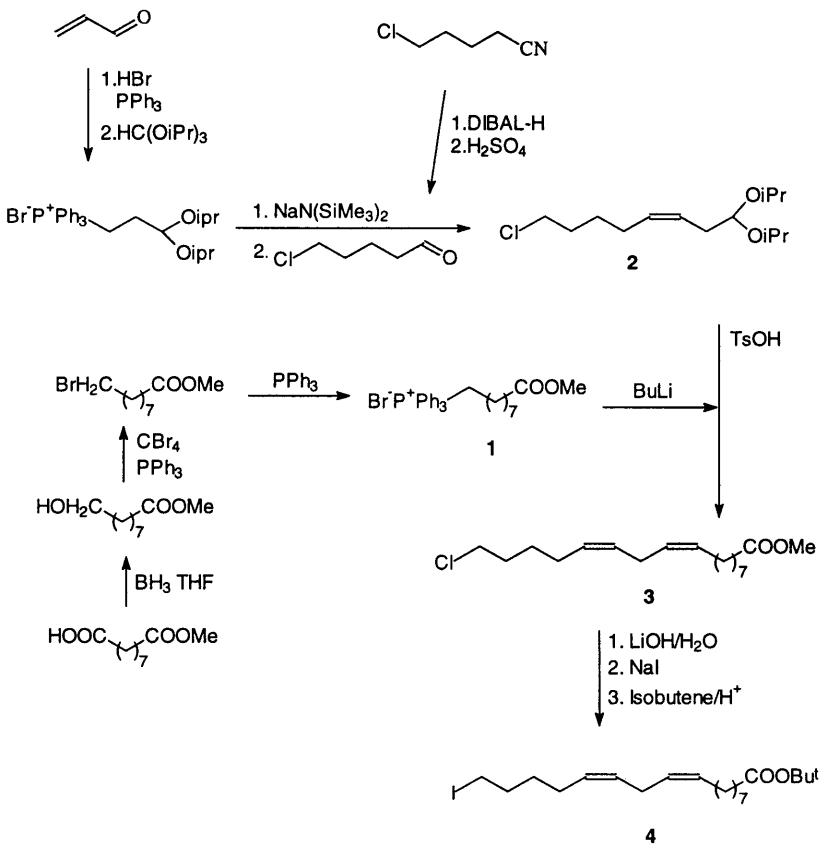
Initial attempts to synthesize [18-^{11}C]linoleic acid involved an ω-Grignard reagent in a cuprate mediated reaction with [^{11}C]methyl iodide, using a 1,3-oxazine for the protection of the carboxyl function (15). Synthesis of the unsaturated carbon skeleton was performed using well established alkyne coupling chemistry (16), where the triple bonds were reduced to *cis* double bonds using a modified Lindlar procedure (17). In an attempt to react the ω-Grignard reagent in a copper mediated reaction with [^{11}C]methyl iodide no coupling product was observed. Thus a different labelling method was therefore employed (14) using ω-iodo carboxylic acid *tert*-butyl ester **4** as starting material. The starting material was obtained by cleavage of the 1,3-oxazine precursor, with the observation that the cleavage was too time consuming, to be useful

in ^{11}C-labelling chemistry. The obtained carboxylic acid was esterified to a *tert*-butyl ester and the chloride was substituted with an iodide. The precursor **4** obtained was suitable for ^{11}C-labelling of linoleic acid, but the total chemical yield was less than 1%.

A more attractive synthetic route using successive Wittig reactions was investigated (18-21). The method for preparation of *cis* double bonds involves the use of a phosphonium ylide, obtained from a phosphonium bromide and a sodium containing base, condensed with an aldehyde or ketone in a dilute solution (22). By using (3,3-diisopropoxypropyl)triphenylphosphonium bromide in the Wittig reaction, 1,3-double bonds may be introduced into the target molecule. The isopropyl acetal tolerated the Wittig conditions and was easily cleaved using *para*-toluensulfonic acid. Incorporation of a ω-iodo group and protection of the carboxyl function as a *tert*-butyl ester would give a suitable starting material.

The method described in this paper followed the previously described methods (18,20,21,23) for the synthesis of unsaturated compounds, including fatty acids, with a few exceptions as shown in Scheme 2.

The first double bond was introduced *via* a condensation between the ylide from (3,3-diisopropoxypropyl)-triphenylphosphonium bromide and sodium hexamethyl silylamide with 5-chlorovaleroaldehyde. The chloro valeraldehyde was obtained from 5-chlorovaleronitrile and used without purification. The second double bond was introduced using the ylide from nonanedioic acid monomethyl ester phosphonium bromide **1** (23), using butyl lithium as base and the aldehyde from the cleaved isopropyl acetal **2** obtained in the first reaction sequence. The 17-chloro-*cis,cis*-9,12-heptadecadienoic acid methyl ester **3**, was hydrolyzed to the acid and converted to the *tert*-butyl ester (24). A Finkelstein reaction afforded the iodide **4** (25), yielding the starting material used in the ^{11}C-labelling synthesis. Surprisingly, when sodium hexamethylsilylamide was used as base in the second condensation the *trans* isomer was formed.



Scheme 2.

The ^{11}C-labelling of linoleic acid was performed *via* a coupling reaction between 17-iodo-*cis,cis*-9,12-heptadecanoic acid *tert*-butyl ester **4** and [^{11}C]methyl iodide, using a highly reactive zerovalent copper complex (Cu*) as coupling agent (Scheme 1) (14). The copper complex was obtained from the reduction of lithium(2-thienyl)iodocuprate (26) with lithium naphtalenide. After cleavage of the *tert*-butyl ester with trifluoroacetic acid (TFA), the crude product was purified by solid phase extraction and semi-preparative HPLC, formulated and sterile filtered. The decay-corrected radiochemical yield was 36-48% based on trapped [^{11}C]methyl iodide, with high chemical purity and a radiochemical purity higher than 98%. The specific activity

was estimated to about 20 GBq/μmol. The synthesis time was 45 min counted from end of radionuclide production.

The position of the label was confirmed by analysis of the ^{13}C-NMR spectrum of (^{13}C-methyl)linoleic acid. The ^{13}C-labelled fatty acid was synthesized using the same method as for the ^{11}C-labelled compound, by a simultaneous addition of (^{13}C)methyl iodide with the [^{11}C]methyl iodide. The ^{13}C signal at δ 14.1 ppm corresponded to the methyl group of authentic linoleic acid.

In conclusion, the ^{11}C-labelling method and the synthesis of the starting material reported herein are general and should be valuable for the synthesis of various unsaturated fatty acids as linolenic- and arachidonic acid as well as for other unsaturated fatty acids labelled in the methyl position.

Experimental

General

Radionuclide production was performed on a Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. [^{11}C]Carbon dioxide was prepared by the ^{14}N(p,α)^{11}C reaction using a gas target containing nitrogen and oxygen (AGA, 0.05% oxygen in nitrogen 6.0) bombarded with 17 MeV protons. Synthia, an automated synthesis system, was used for [^{11}C]methyl iodide production, SPE handling, HPLC injection, fraction collection, and formulation (27).

HPLC was performed using a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV absorbance detector at 222 nm in series with a β$^+$-flow detector (28). Data collection and HPLC control were performed with Beckman System Gold. Authentic reference substances were used for comparison in the HPLC analyses using UV absorbance detection at 222 nm.

Flash chromatography was performed using silica gel 60, 230-400 mesh. TLC was performed on silica 60 plates and developed with 10% phosphomolybdic acid hydrate in ethanol. NMR spectra were recorded on a XL 300 spectrometer at 300 MHz for ^{1}H and 75.4 MHz for ^{13}C with chloroform-d_1 as internal standard. All shifts are reported in ppm. THF was distilled under nitrogen from sodium/benzophenone. 5-Chlorovalero nitrile, sodium hexamethyl silylamide, thiophene and copper(I) iodide, lithium were purchased from Sigma-Aldrich, Sweden. Sodium iodide, acetone and silica gel were purchased from Merck, Germany. Butyllithium and naphtalene were purchased from Lancaster, Great Britain. Isobutene was purchased from AGA, Sweden. Polysorbatum 80 was purchased from Apoteksbolaget, Sweden.

Lithium naphtalenide was prepared by reacting lithium and naphthalene in THF under an atmosphere of argon. (3,3-diisopropoxypropyl)triphenylphosphonium bromide (20) was prepared as previously described and obtained as white crystals in 90 % yield. 5-Chlorovaleraldehyde was prepared as described from 5-chlorvaleronitrile (29). Methyl 8-(triphenylphosphonium) nonanoate bromide **1** was prepared as described starting from acelaicacid monomethylester in 44 % yield (23). [^{11}C]Methyl iodide was prepared by reacting [^{11}C]carbon dioxide with lithium aluminum hydride. After evaporation of THF, hydroiodic acid was added and [^{11}C]methyl iodide was transferred in a stream of nitrogen to the reaction vessel (30).

Lithium(2-thienyl)iodocuprate (31)

To a stirred solution of thiophene (0.82 ml, 10.2 mmol) in THF (10 ml) at -72°C, butyllithium (10.0 mmol, 1.6 M, 6.4 ml) was added and the mixture slowly warmed to room temperature under an atmosphere of argon. After 30 min, the mixture was slowly transferred by cannula to a stirred slurry of copper(I) iodide (1.90 g, 10.0 mmol) in THF (10 ml) at -72°C. The mixture was stirred vigorously at -72 °C and was allowed to warm to room temperature overnight. THF (13 ml) was added, and the resulting solution was stored in the reaction flask and used as such.

8-Chloro-1,1-diisopropoxy-octane-2-ene **2**

To a suspension of (3,3-diisopropoxypropyl)triphenylphosphonium bromide (6.5 g, 12.7 mmol) in THF (100 ml), a solution of sodium hexamethylsilylamide (13 ml, 13 mmol, 1 M in THF) was added at -40°C. The resulting orange ylide was stirred at -40 for 1 hour and then at room temperature for 1 hour. Meanwhile, 5-chorovaleraldehyde was prepared and dissolved in THF (1 ml) and slowly added to the ylide at -72°C. After 5 hours the mixture was neutralised with saturated NH_4Cl (50 ml) and dilution with water (50 ml), the solution was extracted with ether (3 x 75 ml). The organic extracts were washed with brine (50 ml), dried and concentrated under reduced pressure. The resulting dark-yellow oil was purified by flash chromatography on silica gel (ca. 50 g) (ether/pentane 1:30) giving a colourless oil of **2** (2.9 g, 11.1 mmol) in 87 % yield. TLC ether/pentane 5:95, $R_f = 0.45$.

^{1}H NMR: δ 1.11 (d, J = 6.0 Hz, 6H), 1.19 (d, J = 6.0 Hz, 6H), 1.52 (m, J = 7.3 Hz 2H), 2.08 (m, J = 7.3 Hz, 2H), 2.36 (m, J = 5.3 Hz, 2H), 2.49 (t, J = 5.3 Hz, 2H), 3.5 (t, J = 6.6 Hz, 2H), 3.85 (septet, J = 6.3 Hz, 2H), 4.52 (t, J = 5.8 Hz, 1H), 5.45 (m, J = 5.5 Hz, 2H). ^{13}C NMR: δ 22.2, 23.0, 26.3, 31.8, 33.4, 44.6, 67.5, 99.5, 124.7, 130.7

17-Chloro-cis,cis-9,12-heptadecadienoic acid methyl ester **3**

The phosphonium salt **1** (610 mg, 1.2 mmol) was dried by azeotropic distillation by dissolving the salt in CH_2Cl_2 (10 ml) before addition of dry benzene (15 ml) and thereafter heated at reduced pressure at 120-130°C for several hours. To a suspension of **1** in THF (25 ml) n-BuLi (1.6 ml, 1.5 mmol, 1.6 M in hexane) was added at -40°C. The resulting orange ylide was stirred at -40°C for 0.5 h and then at room temperature for 0.5 hours before cooling to -72°C. Simultaneously, the isopropylacetal **2** (378 mg, 1.8 mmol) was cleaved with p-toluensulfonic acid (0.1 M, 25 µl) in refluxing THF (15 ml) to the aldehyde (TLC ether/pentane 5:95, $R_f = 0.3$). The crude aldehyde was dried 3 times by azeotropic distillation with dry benzene, and

diluted in THF (1 ml) before slow addition to the ylide of **1**. After reaction for 5 hours the reaction was quenched with saturated NH_4Cl (25 ml), diluted with water (25 ml) and extracted with ether (3 x 50 ml). The organic extracts were washed with brine (25 ml), dried and concentrated under reduced pressure. The resulting dark oil was purified by flash chromatography (ether/pentane 1:30) to give **3** as a colourless oil (140 mg, 0.44 mmol) in 37% yield. TLC ether/pentane 5:95, $R_f = 0.42$.

^{1}H NMR: δ 1.30 (bs, 8H), 1.47 (m, J = 7.4 Hz, 2H), 1.63 (t, J = 7.3 Hz 2H), 1.84 (m, J = 7.4 Hz, 2H), 2.07 (m, J = 7.4 Hz, 4H), 2.30 (t, J = 6.9 Hz, 2H), 2.77 (t, J = 5.1 Hz, 2H), 3.52 (t, J = 6.6 Hz, 2H), 3.66 (s, 3H), 5.36 (m, J = 5.5 Hz, 4H), ^{13}C NMR: δ 24.9, 25.6, 26.4, 26.8, 27.2, 29.1, 29.1, 29.5, 32.1, 34.1, 45.0, 51.4, 127.7, 128.7, 129.1, 130.2, 174.0.

17-Iodo-(cis, cis)-9,12-heptadecadienoic acid tert-butyl ester **4**

To the methyl ester **3** (330 mg, 1.1 mmol) in THF (12 ml) LiOH (5 ml, 0.5 M) was added at 0°C. The mixture was stirred overnight, acidified with 2M HCl to pH 1, saturated with NaCl and extracted with ether/pentane 1:1 (3 x 25 ml). The organic layer was washed with brine (25 ml), dried and concentrated under reduced pressured to yield the crude carboxylic acid as a pale oil (320 mg, 1.1 mmol). The crude carboxylic acid (320 mg, 1.1 mmol), NaI (500 mg, 3.3 mmol) in aceton (25 ml) were refluxed overnight. The solvent was removed and the resulting solution dissolved in water (25 ml) and extracted with ether (3 x 25 ml). The combined organic layers was washed with brine (25 ml) and sodium thiosulfate (10%, 25 ml), dried with sodium sulphate and concentrated under reduced pressure.

The crude 17-iodocarboxylic acid was dissolved in freshly distilled CH_2Cl_2 (20 ml), and isobutene added to double volume. Concentrated sulfuric acid (2 drops) was added. After approximately 48 hours the acid was neutralized with sodium carbonate (10%, 25 ml) and water (25 ml) was added. The CH_2Cl_2 layer was separated and

washed with water (25 ml), brine (25 ml), dried with sodium sulphate and concentrated under reduced pressure. The crude product was purified by flash chromatography ether/pentane 4:96 giving pure *tert*-butyl ester **4** (220 mg, 0.49 mmol) in 45% yield. TLC ether/pentane 5:95 R_f = 0.55. The dry **4** was dissolved in dry THF to a concentration of 0.5 M and stored in 250 µl vials.

^{1}H NMR: δ 1.30 (bs, 8H), 1.48 (s, 9H), 1.47 (m, J = 7.4 Hz, 2H), 1.63 (t, J = 7.3 Hz, 2H), 1.79 (m, J = 7.4 Hz, 2H), 2.07 (m, J = 7.4 Hz, 4H), 2.35 (t, J = 7.3 Hz, 2H), 2.77 (t, J = 5.1 Hz, 2H), 3.19 (t, J = 6.6 Hz, 2H), 5.36 (m, J = 5.5 Hz, 4H), ^{13}C NMR: δ 6.9, 25.1, 25.6, 26.1, 27.3, 28,2, 29.2, 29.3, 29.6, 29.6, 30.4, 33.1, 35.6, 80.3, 127.7, 128.8, 129.1, 130.3, 173.4

[18-^{11}C]Linoleic acid **5**

To a solution of lithium(2-thienyl)iodocuprate (100 µl, 0.25 M) in THF, lithium naphtalenide (100 µl, 0.25 M) was added at -72°C and the resulting mixture kept at -72°C for 10-20 min. Compound **4** (40 µl, 0.5 M in THF) was added to the solution shortly before (2-5 min) the transfer of [^{11}C]methyl iodide. After transfer of [^{11}C]methyl iodide, the mixture was heated to 70°C for 1 min. The vial was then rapidly cooled to 0°C and TFA (200 µl) added. The vial was heated for another 5 min at 70°C. The crude [18-^{11}C]linoleic acid was diluted to a final volume of 10 ml with acetonitrile/water 50:50. A SPE column (C_8, 500 mg, 3 ml, Applied Separations) was pre-conditioned with 5 ml ethanol and 5 ml acetonitrile/water 50:50, before the crude reaction mixture was applied. The SPE column was eluted with 3 ml acetonitrile. The eluate was diluted with 1.5 ml acetonitrile/water 50:50 solution and purified by HPLC (Beckman Ultrasphere Octyl C_8, 5 µm, 250 x 10 mm ID eluting with 0.05 M ammonium formate, pH 3.5/(acetonitrile/water (50:7 v:v)) 10:90 with a linear gradient to 100% acetonitrile/water (50:7 v:v) from 1-4 min, 5 ml/min). The collected fraction (t_R = 11-12 min) was concentrated at reduced pressure, and 1 ml

Polysorbatum 80 in ethanol (10%), was added followed by phosphate buffer (7 ml, 0.1 M, pH 7.4). The resulting solution was passed through a 0.22 μm sterile filter (Dynagard) into a sterile injection flask. [18-^{11}C]Linoleic acid was analysed by HPLC (Spherisorb C6, 5 μm, 250 x 4.6 mm ID eluting with 0.01 M KH_2PO_4/(acetonitrile/water (50:7, v:v) 30:70, 2 ml/min), t_R = 5.0 min.

(18-^{13}C)Linoleic acid

(18-^{13}C)Linoleic acid was prepared as described for [18-^{11}C]linoleic acid with the exception that (^{13}C)methyl iodide (62 μmol, 20 μl, 20% v:v in heptane) was added after trapping of [^{11}C]methyl iodide. The collected fraction was evaporated to dryness, dissolved in $CDCl_3$ (0.5 ml) and analyzed by ^{13}C-NMR.

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