SYNTHESIS OF [19-^{11}C]ARACHIDONIC ACID

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SUMMARY

The preparation of (all Z)-1,17-dichloro-4,7,10,13-heptadecatetraene is reported. The synthesis was performed in five steps with a total yield of 22 %, starting from 5-chloro-1-pentyne. The corresponding bisGrignard reagent was used in a copper-mediated coupling reaction with [1-^{11}C]ethyl iodide followed by a carbonation with CO_2 to afford [19-^{11}C]arachidonic acid in 23 % decay corrected radiochemical yield within 52 min. The radiochemical purity of the final product was 98 %. In a typical run starting with 20 GBq [^{11}C]O_2, 760 MBq of [19-^{11}C]arachidonic acid was obtained, with a specific activity of 1.6 GBq / μmol.

Keywords: (all Z)-1,17-dichloro-4,7,10,13-heptadecatetraene, [19-^{11}C]-arachidonic acid.

INTRODUCTION

During the last years we have been engaged in a project concerning the development of PET-tracers by ^{11}C-labelling of fatty acids in selected positions.[1] The ^{11}C-labelling has also been combined with deuterium substitution in order to investigate if kinetic isotope effects related to fatty acid metabolism can be observed *in vivo* by PET.[1] Both the approach of specific labelling in various positions and multiple isotopically labelling, has previously been used in PET experiments with other types of ^{11}C-labelled substances.[2,3] By development of these principles we are investigating the possibility of tuning the PET-tracer for a certain biological address.[1] This approach is based on the postulate, that isotopic substitution and change of the position of the label can alter the measured kinetics but not the actual biochemical pathway. We have previously shown that this methodology has the potential to highlight different aspects of a complex biochemical system by PET.[1,2,4] In line

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with this, we have developed a synthesis of [19-^{11}C]arachidonic acid in order to complement the synthesis of [1-^{11}C]arachidonic acid previously reported by Channing and Simpson.[5] We thus report the synthesis of (all Z)-1,17-dichloro-4,7,10,13-heptadecatetraene (**5**) and its use in the preparation of [19-^{11}C]arachidonic acid.

Arachidonic acid labelled with ^{11}C is of interest not only as a tracer as such, but also as a potential precursor for ^{11}C-labelled eicosanoids. It may be possible to use freely prepared enzymes of the arachidonic acid cascade[6] in the synthesis of ^{11}C-labelled eicosanoids. These enzymes has previously been used in synthesis of ^{14}C-labelled prostaglandins from ^{14}C-labelled arachidonic acid.[7] Moreover, the approach of multi-enzymatic catalysis is known to be useful for synthesis of ^{11}C-labelled tracers.[8]

EXPERIMENTAL

General. The [^{11}C]carbon dioxide was produced at the Uppsala University PET Centre from the ^{14}N(p,α)^{11}C reaction using a nitrogen gas (AGA, 6.0) target containing 0.1 % oxygen (AGA, 5.0) and 17 MeV protons produced from the Scanditronix MC-17 Cyclotron.

Analytical LC was performed on a Hewlett-Packard 1090 liquid chromatograph equipped with a u.v. diode-array detector in series with a β$^+$-particle flow detector and the following column: (A) Beckman Ultrasphere ODS C$_{18}$, 5 μm, 250 × 4.6 mm ID. The following mobile phases were used: (B) 0.05 M ammonium formate pH 3.5 and (C) acetonitrile/water (50/7: v/v). Semi-preparative HPLC was performed using a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV-detector in series with a β$^+$-particle flow detector, and the following column: (D) Beckman Ultrasphere ODS C$_{18}$ (250 × 10 mm, 5 μm). The mobile phases used, were (C), and pre made mixtures of (E) methanol, (F) THF and (G) 0.01 M KH_2PO_4. Data collection was performed using the Beckman System Gold Chromatography Software Package. In the LC-analysis of the ^{11}C-labelled compounds, authentic reference substances were co-injected in all runs. Preparative HPLC was performed using a Waters Prep LC/ System 500 Liquid Chromatograph with a Prep PAK-500 C$_{18}$ Cartridge. Acetonitrile/water (70/30: v/v), flow 0.25 L / min, was used as mobile phase. NMR spectra were in most cases recorded on a Varian XL 300 spectrometer, ^{1}H at 300 MHz and ^{13}C at 75.4 MHz, with chloroform-d_1 as internal standard. For the ^{13}C NMR analysis of (19-^{13}C)arachidonic acid and arachidonic acid a Varian Unit 400 spectrometer was used; ^{13}C at 100.5 MHz, with chloroform-d_1 as internal standard. GC-MS was performed by a Finnigan MAT INCOS

50 instrument in the electron impact mode using a potential of 70 eV. The LC-MS analyses were performed by a VG Platform, Fisons instrument with negative electo spray ionisation using an applied spray voltage of 3.2 kV.

Tetrahydrofuran (THF) was dried by distillation over sodium/benzophenone under N_2, prior to use. 5-Chloro-1-pentyne was obtained from Aldrich. Arachidonic acid (chemical purity grade > 90 %) and sodium hydrogen (^{13}C)carbonate were obtained from Sigma. Palladium on barium sulphate was prepared according to ref. 7. Tween 80 was obtained from Apoteksbolaget, Sweden.

The Li_2CuCl_4-solution (0.2 M) was prepared by drying equimolar quantities of LiCl and $CuCl_2$ at reduced pressure (1 torr, 200 °C) and then dissolving the salts in THF. The solution was transferred to 250 µL membrane-equipped vials kept under argon atmosphere and stored at -18 °C.

During the synthesis of the alkenyl halides, all solutions were purged with argon. All reaction- and purification steps were carried out under argon atmosphere.

Synthesis of alkenyl halides

1-Chloro-4,7-oktadiyn (1). The synthesis was performed according to ref. 10 in 77 % yield

9-Chloro-2,5-nonadiyn-1-ol (2). 1-Chloro-4,7-oktadiyn (**1**) (36 g 91 %, 230 mmol) was converted to its Grignard derivative with ethyl magnesium bromide prepared from magnesium (5.9 g, 0.25 g atom) and ethyl bromide (28.2 g, 257 mmol) in THF (400 mL). The solution was cooled to 0 °C and paraformaldehyde (13.1 g, 440 mmol) was sublimated into the reaction mixture in a stream of argon gas. The solution was warmed to 35 °C for 30 min and was then cooled to 0 °C again. The reaction mixture was worked up by pouring it into 400 mL of a saturated NH_4Cl- ice water solution and extracting the aqueous layer with diethyl ether. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was applied to a column consisting of approximately 200 g silica, and conditioned with pentane. The column was first eluted with 500 mL pentane/diethyl ether 80/20 and then 800 mL of diethyl ether/methanol 50/50. The latter fraction was concentrated under reduced pressure to leave a residue which on destillation under reduced pressure (0.1 torr bp. 124 °C) afforded **2** (32.9 g, 193 mmol, 83 %). ^{1}H NMR δ 4.26 (t, J = 2.2 Hz, 2 H), 3.65 (t, J = 6.4 Hz, 2 H), 3.20 (p, J = 2.3 Hz, 2 H), 2.37 (tt, J = 2.3, 6.7 Hz, 2 H), 2.27 (s, 1 H), 1.95 (p, J = 6.5 Hz, 2 H); ^{13}C NMR δ 80.4, 79.1, 78.6, 74.6, 51.1, 43.7, 31.3, 16.1, 9.8; MS: m/z 170 (M^+, 1.2 %), 142 (11), 115 (16), 107 (28), 91 (32), 77 (99), 39 (85), 27 (100).

1-Bromo-9-chloro-2,5-nonadiyne (3). To an ice-cooled solution of **2** (32.5 g, 190 mmol) and pyridine (0.8 g, 10 mmol) in dry diethyl ether (250 mL) was added PBr_3 (20 g, 71 mmol) during 1 h. The mixture was refluxed for 3 h and was again cooled on ice. The reaction mixture was worked up by pouring it into ice water and extracting the aqueous layer with additional ether. The combined organic layers were washed with H_2O saturated with $NaHCO_3$, and dried ($MgSO_4$). Concentration under reduced pressure afforded **3** (41.4 g, GC 90 %, 160 mmol, 84 %). ^1H NMR δ 3.92 (t, J = 2.4 Hz, 2 H), 3.65 (t, J = 6.3 Hz, 2 H), 3.22 (p, J = 2.4 Hz, 2 H), 2.37 (tt, J = 2.4, 6.9 Hz, 2 H), 1.95 (p, J = 6.6 Hz, 2 H); ^{13}C NMR δ 81.7, 79.3, 75.4, 74.0, 43.7, 31.3, 16.1, 14.8, 10.1; MS m/z 236 (M^+, 0.8 %), 234 (M^+, 2.5), 232 (M^+, 1.7), 206 (18), 125 (88), 115 (68), 103 (22), 91 (100).

1,17-Dichloro-4,7,10,13-heptadecatetrayne (4). 1-Chloro-4,7-oktadiyn (**1**) (18 g 91 %, 116 mmol) was converted to its Grignard derivative with ethyl magnesium bromide prepared from 2.78 g (0.116 g atom) of magnesium and 12.53 g (114 mmol) of ethyl bromide in 170 mL of THF. A suspension of CuBr (0.7 g, 5 mmol) in THF (10 mL) was added, and the mixture was stirred at room temperature for 15 min before being cooled to 0 °C. A solution of **3** (40 g, 90 %, 154 mmol) in 40 mL THF was rapidly added and the mixture was stirred for 1 h. The ice bath was removed and the mixture was stirred for an additional 3.5 h at room temperature. The reaction mixture was then poured out in 500 mL NH_4Cl saturated ice water. The organic phase was separated and the aqueous layer was extracted with diethyl ether. The combined organic layers were washed with NaCN (2 g) in H_2O (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was heated to 150 °C at reduced pressure (0.001 torr) for 20 min. The residue was applied to a silica-column (ca. 200 g) conditioned with pentane. The column was first eluted with 500 mL pentane/diethyl ether 85/15 and then 500 mL of pentane/diethyl ether 70/30. The latter fraction was concentrated under reduced pressure and again purified as above. The combined fractions from the first elution (85/15) were concentrated under reduced pressure to afford **4** (25.9 g, 88 mmol, 76 % with respect to **1**). ^1H NMR δ 3.64 (t, J = 6.4 Hz, 4 H), 3.17-3.12 (m, J = 1.2, 2.1 Hz, 6 H), 2.36 (tt, J = 2.2, 6.7 Hz, 4 H), 1.94 (p, J = 6.6 Hz, 4 H); ^{13}C NMR δ 78.8, 74.91, 74.87, 74.3, 43.7, 31.4, 16.1, 9.7; MS m/z 292 (M^+, 1 %), 264 (20), 229 (21), 193 (16), 179 (38), 165 (75), 152 (26), 115 (18), 65 (23), 27 (47), 14 (100).

(all Z)-1,17-Dichloro-4,7,10,13-heptadecatetraene (5). In a 500 mL flask, a solution of **4** (7.7 g, 26 mmol), quinoline (6 g) and Pd on $BaSO_4$ (6 g, 0.3 g Pd) in 100 mL pyridine was stirred vigorously under H_2 at room temperature and atmospheric pressure. The reaction was followed by GC-analysis, and the reaction was stopped when all **4** had been consumed. The mixture was filtered

on Celite, concentrated under reduced pressure and dissolved in 50 mL diethyl ether/ pentane (50/50: v/v). The solution was washed with 4 × 30 mL of 0.5 M HCl, 30 mL H_2O saturated with $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by two successive runs on the preparative LC using 3 g in each injection. The appropriate fractions were combined and concentrated under reduced pressure by evaporating the acetonitrile. The aqueous residue was saturated with NaCl and extracted with diethyl ether (3 × 100 mL). The combined ether fractions were dried ($MgSO_4$) and concentrated under reduced pressure to afford **5** (4.7 g, GC 91 %, 14.2 mmol, 55 %). ^{1}H NMR δ 5.49-5.28 (m, 8 H), 3.54 (t, J = 6.5 Hz, 4 H), 2.84 (t, J = 5.5 Hz, 6 H), 2.22 (q, J = 6.4 Hz, 4 H), 1.84 (p, J = 6.6 Hz, 4 H); ^{13}C NMR δ 129.4, 128.2, 44.5, 32.3, 25.7, 24.4; MS m/z 300 (M^+, 0.7 %), 246 (1), 223 (4), 209 (4), 196 (10), 156 (25), 119 (21), 105 (30), 91 (66), 79 (100).

(all Z)-1,17 Bis(chloro magnesium) 4,7,10,13-heptadecatetraene (6). The Grignard solution was prepared by reacting **5** (0.4 mL, 1.5 mmol) with an excess of magnesium turnings (0.5 g 22 mmol) in THF (3 mL). The reaction was started by an addition of 1,2-dibromo ethane (0.1 mL), and was then stirred vigorously for 1.5 h. The solution was transferred to 250 µL membrane equipped vials kept under argon atmosphere and stored at -18 °C.

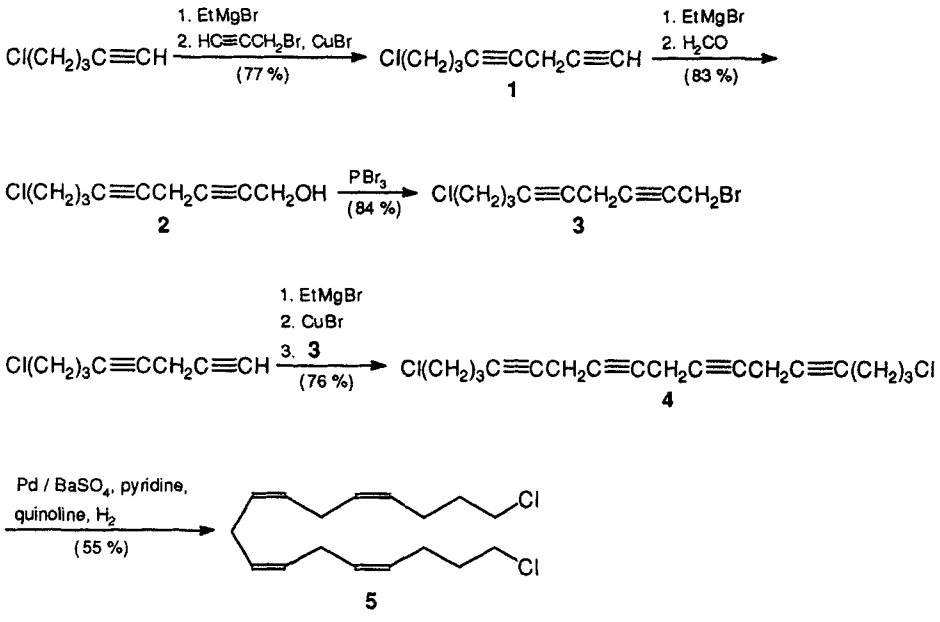
[19-^{11}C]Arachidonic acid (7). [1-^{11}C]Ethyl iodide[11] was trapped in a 3 mL reaction vessel containing THF (200 µL) and Li_2CuCl_4 (4 µmol) cooled to -72 °C. **6** (200 µL 0.5 M) was added and the vial was put in an ice bath. After 1 min a stream of CO_2 was introduced to the solution and 0.5 mL THF was added. The vial was heated at 70 °C for 2 min and then HCl (0.5 mL 0.5 M) was added. When the mixture was homogeneous, LC-mobile phase (1 mL) was added and the solution was injected on the semi-preparative LC-system together with LC-mobile phase (1 mL) that was used to rinse the reaction vial. Semi-preparative LC: column D, mobile phase 100 % of the premixed (36 % G, 44 % F, 19 % E and 1 % H) for 2 min and then a gradient during 5 min to 80 % C, finally at 12 min a gradient to 100 % C during 0.5 min, R_t = 13.9 min. The fraction containing **7** was collected and evaporated under reduced pressure. The residue was dissolved in 1 mL ethanol/Tween 80 (9/1: v/v) followed by a mixture of 6 mL saline and 1 mL phosphate buffer, pH 7.4. The pH of the solution was adjusted to ca. 7 and then sterilised by passage through a sterile 0.22 µm filter into a sterile vial. Analytical LC: column A, mobile phase B/C 15/85, at room temperature, wavelength 210 nm, R_t = 5.7 min. LC-MS m/z 302,9 (M^-, deprotonated).

(19-^{13}C)Arachidonic acid (8). The synthesis was performed in analogy with (**7**) with the following modifications. In the synthesis of [1-^{11}C]ethyl iodide, $^{13}CO_2$, produced by acidification of sodium

hydrogen (^{13}C)carbonate (15 mg, 176 μmol), was transferred in a stream of nitrogen gas and trapped together with the [^{11}C]carbon dioxide in methyl magnesium bromide (1 mL, 0.5 M) in THF. The synthesis was then performed as described, with the exception that an increased amount of **6** (800 μL, 0.5 M) was used and allowed to react for 10 min before addition of CO_2. The collected fraction was evaporated to ca. 1 mL and then again injected on the semi-preparative LC. The collected fraction from the second run was analysed as described for (**7**) and then evaporated to dryness and redissolved in $CDCl_3$ (0.7 mL). The yield of (**8**) was determined using data from the LC-analysis; 11 % (6 mg). The product was analysed by ^{13}C and ^{1}H NMR and LC-MS. LC-MS m/z 303,9 (M^-, deprotonated).

RESULTS AND DISCUSSION

The individual steps used in the synthesis of compound **5** (Scheme 1), are all based on established alkyne chemistry procedures.[12] Compound **1**, synthesised according to ref. 10, was transformed to the corresponding Grignard reagent by an exchange reaction and allowed to react with formaldehyde. The alcohol **2** obtained was brominated with phosphorus tribromide to yield the chloro, bromo diyne **3**. The Grignard reagent of **1** was then coupled with **3** using Cu^I-catalysis to form the dichloro tetrayne **4**. This compound was finally transformed into the corresponding all-cis tetraene by the use of a Lindlar type reduction. All steps except the last proceeded in good to high yield. In the last step, considerable amounts of the corresponding triene and diene were formed as side products. This was surprising, since the Lindlar reduction is an established method for the synthesis of similar compounds in high yields.[12,13] The standard procedure according to Lindlar gave a very slow reaction and low yield, why a number of modifications were investigated. Using palladium on barium sulphate (without lead) and pyridine together with quinoline as solvent[14] gave less overreduction and a faster reaction rate resulting in less polymerisation. However, the ratio between the different alkenes was largely constant during the whole reaction and was not much affected by the modifications. The different alkenes could not be separated by ordinary flash chromatography on silica gel. However, using reverse phase chromatography with a preparative C_{18} column and acetonitrile/water (70/30) as eluent, compound **5** was obtained in a chemical purity higher than 91 %. The main impurity (ca. 6 %) was identified by MS as the corresponding triene compound. The total yield of **5**, starting from the commercially available 5-chloro-1-pentyne, was 22 %.

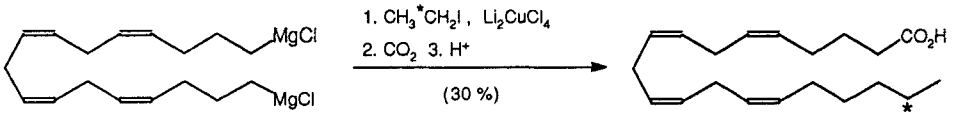


Scheme 1. Synthesis of the precursor for [19-^{11}C]arachidonic acid.

A number of different approaches preceded the development of the present synthesis. For example, attempts to build the carbon chain from the middle outwards, starting from 1,4 dibromo-(or dichloro) 2-butyne, resulted in severe polymerisation. We thus switched over to the approach of building the carbon chain from the ends, and then fusing the two halves. However, when 5-pentyn-1-ol, protected with a methylthiomethyl or a tetrahydropyrane group, was employed in this strategy, the synthesis failed since the protective groups impaired the halogenation of the diyne propargylic alcohol. Finally, the realisation that alkyl chlorides, in contrast to propargylic halides, do not couple with alkynyl cuprates[10], made it possible to develop the present synthesis.

In some modern syntheses of unsaturated fatty acids, the Wittig synthesis has been utilised with good selectivity in the double bond formation.[15] With regard to the poor selectivity of the palladium reduction, the question can be raised if that would be a useful method also for the synthesis of compound **5**. However, an important aspect of the presented synthesis, that would be less easily achieved by the Wittig method, is the possibility of substituting the hydrogen atoms connected to the double bonds with deuterium. This should be easily done by changing the hydrogen gas used, with deuterium gas in the reduction of the triple bonds. The option of using deuterium substitution in combination with ^{11}C-labelling has a potential value in revealing the biochemical fate of a substance.[1,3,4]

The general labelling synthesis using bisGrignard reagents is described and discussed elsewhere.[16] The synthesis was achieved by reacting the corresponding bisGrignard reagent of **5** by a copper mediated reaction with [1-^{11}C]ethyl iodide followed by carbonation with carbon dioxide (Scheme 2). After purification by semi-preparative LC, the product was dissolved in a physiological solution by the aid of the emulsion agent Tween 80. [19-^{11}C]arachidonic acid (**6**) was obtained in 23 % decay corrected radiochemical yield within 52 min and with a radiochemical purity of 98 %. In a typical run starting with 20 GBq [^{11}C]O_2, 760 MBq of **6** was obtained, with a specific activity of 1.6 GBq / μmol. The relative low value of the specific activity, was related to the quality of the methyl Grignard reagent used in the synthesis of [1-^{11}C]ethyl iodide.



7; * : ^{11}C-labelled position **8**; * : ^{13}C-labelled position

Scheme 2. Synthesis of [19-^{11}C]arachidonic acid.

Identification of [19-^{11}C]arachidonic acid was achieved by radio-LC and LC-MS analysis using authentic arachidonic acid as reference. The product and the labelling position was further characterised by running the corresponding ^{13}C-synthesis concomitantly with the ^{11}C-synthesis and then, after decay, analysing the product by LC-MS and NMR. The carrier addition did not change the results obtained from LC-analysis of the radioactive product. The ^{1}H NMR spectra was consistent with the corresponding spectra of the reference. The ^{13}C NMR spectra (Fig. 1) differed with respect to the intensity of the peak at δ 22.0 and by the fact that two of the peaks were split into doublets. The intensive signal is consistent with position C_{19} of arachidonic acid, by comparison with assigned spectrums of similar compounds found in ref. 17. The two doublet peaks (Jcc = 30 Hz), related to position C_{18} and C_{20}, are split due to vicinity of the ^{13}C atom. The signals related to position C_1 and C_2 are broadened, and thus difficult to detect. The reason is probably ion-pairing resulting from impurities and the low concentration of the sample. The MS-analysis of (19-^{13}C)arachidonic acid showed a molecular ion peak with a m/z value of 303.9. The corresponding value for the reference was 302.9.

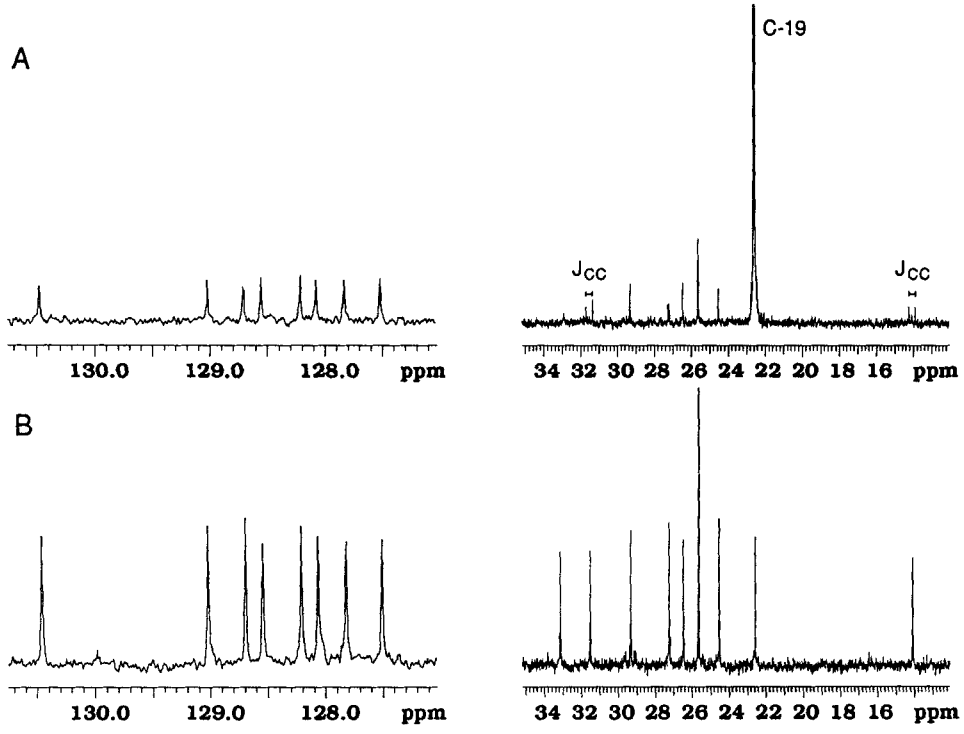


Figure 1. Selected parts of the ^{13}C NMR spectra of A) (19-^{13}C)arachidonic acid, and B) arachidonic acid.

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