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Cuprate-Mediated ^{11}C–C Coupling Reactions Using Grignard Reagents and ^{11}C-Alkyl Iodides

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Various ^{11}C-labelled alkyl iodides were reacted with Grignard reagents in syntheses of labelled fatty acids, alkenes and aromatic compounds. Fatty acids were labelled in a number of positions by the combination of specifically labelled alkyl iodides and Grignard reagents of different chain lengths. The positions of the label were confirmed by ^{13}C-labelling and NMR spectroscopy. Dilithium tetrachlorocuprate was used, except in the coupling reaction with short-chain bis-Grignard reagents, where 2-thienylcuprate was employed. In the cases when [^{11}C]methyl iodide and dilithium tetrachlorocuprate were used, the coupling reaction proceeded in nearly quantitative yield within a minute, while the reaction rate and yield using the other labelled alkyl iodides varied considerably. The decay-corrected radiochemical yields of the ^{11}C-labelled alkenes, fatty acids and aromatic compounds were 70–90, 15–55 and 98%, respectively. The radiochemical purities were 94–99% and the specific activity, as determined for [α-^{11}C]toluene, was 880 MBq μmol^{-1}.

Biomolecules and pharmaceuticals labelled with short-lived positron-emitting radionuclides in combination with the positron emission tomography (PET) technique has become a powerful tool for studies of regional distribution and pharmaco-kinetics *in vivo*.[1] Further development of the PET method is, however, much dependent on the advent of new synthetic methods and techniques, which can be used for the production of specifically labelled radiotracers of high specific radioactivity. Owing to the radioactivity and the short half-life of the radionuclides used (e.g. ^{11}C: $t_{1/2} = 20.3$ min), extraordinary synthetic methods are required.[2] The production of the labelled tracer has to be fast (total synthesis time preferably < 1 h in the case of ^{11}C) and preferably be carried out by remote control or be automated. One-pot procedures and on-line systems are useful approaches for solving some of these problems.

Another feature of interest using these radiotracers is the application of position specific labelling.[3] The option of labelling substances of interest in selected positions, offers possibilities to extract additional information from the PET data with a potential to distinguish normal from diseased function. For example, labelling a fatty acid in various selected positions, gives the possibility of following both the β-oxidation pathway and the TCA pathway. Depending on whether an even-chain fatty acid is labelled in an even or an odd position, acetate labelled in the carboxy or methyl position will be formed as a result of the β-oxidation. This variance will, in turn, result in differences in the radioactivity measurements due to differences in the elimination rate of radioactivity by labelled carbon dioxide.[4a,b]

Methods used in ordinary synthetic chemistry are not usually directly applicable to synthesis of ^{11}C compounds. The reason is partly due to the time constraint mentioned and partly to the fact that the amount of the ^{11}C-labelled substance is small (usually $< 10^{-6}$ mol). Using such small amounts of substance sometimes leads to problems with side reactions or lack of reactivity. However, it can also be beneficial, for example when an extraordinary stoichiometric relationship is utilized, to shorten the synthesis time or change the proportions of the product composition. These aspects are important parts of the investigation presented here.

It is of particular interest to find new reaction pathways for the use of ^{11}C-labelled alkyl iodides in labelling synthesis, since they are conveniently prepared in medium to high yields and with short synthesis times.[5] [^{11}C]Methyl iodide, for example, is, at present, the most frequently used ^{11}C-labelled precursor. The coupling reaction between alkyl halides and organocopper intermediates has been known for several decades and has been well investigated.[6a,b] Its use in ^{11}C-labelling, however, has

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been limited to the coupling reactions of [^{11}C]methyl iodide with dialkyl lithium cuprates and diaryl lithium cuprates.[7] An objective of this investigation was the development of methods for ^{11}C-labelling of saturated and unsaturated fatty acids (e.g., palmitic and linoic acid) in the methyl and methylene positions. The present work shows the use of organocopper intermediates, obtained *in situ* from Grignard reagents, in the synthesis of some ^{11}C-labelled 1-alkenes, saturated fatty acids and aromatic hydrocarbons.

Experimental

General. [^{11}C]Carbon dioxide was prepared by the ^{14}N(p,α)^{11}C reaction using a nitrogen gas target and 10 or 17 MeV protons. The production was achieved either by use of the Scanditronix MC-17 Cyclotron at the Uppsala University PET Centre or by the tandem Van de Graaff accelerator at The Svedberg Laboratory, Uppsala University. In the latter case the [^{11}C]carbon dioxide obtained was trapped in a lead-shielded oven containing 4 Å molecular sieves and transported to the chemistry laboratory.

Analytical LC was performed on a Waters system (pump 501, data and control system 840, UV absorbance detector 440) in series with a β^+-flow detector and one of the following columns: (A) Spherisorb C_6, 5 μm, 250 × 4.6 mm ID, or (B) Beckman Ultrasphere ODS C_{18}, 5 μm, 250 × 4.6 mm ID. The following mobile phases were used: (C) 0.05 M ammonium formate pH 3.5 and (D) methanol, at a flow of 2 ml min^{-1}. Semipreparative HPLC was performed at room temperature using a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV-detector in series with a β^+-flow detector, and one of the following columns: (E) Beckman Ultrasphere ODS C_{18} (250 × 10 mm, 5 μm) or (F) Spherisorb C_1 (250 × 10 mm, 5 μm); (D), (G) ethanol–H_2O (80:20, v/v), (H) acetonitrile–water (50:7, v/v), (I) 0.01 M KH_2PO_4, (J) THF and (K) hexane were used as mobile phases,[4b] at a flow of 5 ml min^{-1}. Data collection was performed using the Beckman System Gold Chromatography Software Package. Analytical GC was performed on a Shimadzu GC-14A in combination with a Raytest, Raga 93, radiodetector using one of the following columns: (L) a 2 m × 3 mm column packed with 10% FFAP on Chromosorb W AW 100/120 DMCS with 50 ml min^{-1} flow of hydrogen gas or (M) a 50 m × 0.548 mm GS-Alumina, J & W Scientific with a 12 ml min^{-1} flow of hydrogen gas. In the analysis of the ^{11}C-labelled compounds, authentic, unlabelled reference substances were used for comparison in all GC runs and in the HPLC runs when compounds suitable for UV detection were analysed. NMR spectra were recorded on a Varian XL 300 spectrometer, ^{1}H at 300 MHz and ^{13}C at 75.4 MHz, with tetramethylsilane in chloroform-d_1 as the internal standard. Mass spectra were recorded on a Finnigan MAT INCOS 50 instrument in the electron impact mode using a potential of 70 eV.

18-Crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane) was obtained from Aldrich. Tetrahydrofuran (THF) was dried by distillation over sodium–benzophenone under N_2, prior to use. CuI was prepared according to Ref. 8. In the syntheses of the ^{13}C-substituted compounds, ^{11}C was used concomitantly in order to monitor the products during production and analysis.

Preparation of the Grignard reagents. The Grignard solutions (0.3–0.5 M) were prepared by reacting the corresponding bromide with an excess of magnesium turnings (*purum* quality) in THF or diethyl ether. When the reaction was complete, the solutions were transferred to 1.5 ml membrane-equipped vials kept under argon and stored at −18 °C. In the case of 1,3-bis(bromomagnesio)propane, the THF contained 1% pyridine in order to increase the solubility.

Preparation of the cuprate reagents. 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 stored in the same way as the Grignard reagents. 2-Thienylcuprate[9] (0.5 M) was prepared in the following way. To a stirred solution of thiophene (1.2 ml, 15 mmol) in THF (3 ml) at 0 °C, was added slowly *n*-butyllithium (14 mmol in 9 ml hexane). After 30 min the mixture was transferred to a stirred slurry of CuI (2.66 g, 14 mmol) in THF (5 ml) at 0 °C. After another 30 min of vigorous stirring the mixture was allowed to settle and the supernatant was removed. THF (23 ml) was added to the residue, and the solution was transferred to 250 μl membrane-equipped vials and stored in the same way as the Grignard reagents.

1,14-Dibromotetradecane. A solution of 1,6-bis(bromomagnesio)hexane in THF (150 ml) was prepared from 1,6-dibromohexane (4.9 g, 20 mmol) and magnesium (0.95 g, 40 mmol). The solution was cooled to −10 °C and Li_2CuCl_4 (2 ml, 1 M in THF), followed by 1,4-dibromobutane (17.3 g, 80 mmol), was added. The mixture, kept in the cooling bath, was allowed to reach room temperature overnight. Sulfuric acid (100 ml, 2 M) was added and the resulting solution was extracted with diethyl ether (2 × 100 ml). The combined ethereal fractions were dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was recrystallized from diethyl ether–ethanol and dried at 0.1 torr to yield 1,14-dibromotetradecane (4.5 g, 13 mmol, 63%). MS, *m/z* (rel. int.): 277 (11), 275 (11), 137 (16), 135 (17), 69 (60), 55 (100).

Short chain [^{11}C]alkyl iodides. [^{11}C]Methyl iodide was prepared according to Ref. 5(a). [1-^{11}C]Ethyl iodide and [1-^{11}C]propyl iodide were prepared according to Ref. 5(c), with the exception that diethyl ether was used instead of THF.

[1-^{11}C]Nonyl iodide. Octyl magnesium bromide (1 ml, 0.3 M in diethyl ether) was placed in a reaction vessel of

the same type as used in the synthesis of [^{11}C]methyl iodide. [^{11}C]Carbon dioxide was transferred to the Grignard solution in a stream of nitrogen gas (flow 40–60 ml min^{-1}) via a perforated Teflon tube devised to increase the trapping of [^{11}C]carbon dioxide. The reaction mixture was briefly heated to reflux and lithium aluminium hydride (LAH) (0.5 ml, saturated diethyl ether solution) was added. The solvent was evaporated off, the reaction mixture was cooled to −72°C, and hydriodic acid (3.5 ml, 57%) was added. The cooling bath was removed, and when the mixture had become homogenous, it was heated to reflux for 4 min while kept at 220°C. After being cooled to room temperature, the solution was extracted with pentane, (3 × 2 ml) and the organic phase was passed through a drying column (5 × 0.5 cm column filled with sodium bisulfite–magnesium sulfate–calcium sulfate, approximately 1:2:2 by volume). The pentane was removed by heating at 80°C and flushing with nitrogen gas. The residue was dissolved in 0.3 ml THF and transferred to the vial used for the subsequent reactions. The procedure is a modification of the synthesis used in the preparation of substituted [α-^{11}C]benzyl iodides.[10] Analytical LC: column A, mobile phase C–D 20:80 at 52°C, wavelength 254 nm, t_R = 2.9 min.

[methyl-^{11}C]-1-Alkenes **1a**, **2a** *and* **3a**. The [^{11}C]methyl iodide was transferred in a stream of nitrogen gas to a specially designed one-pot reaction vessel[5c] containing THF (200 µl) cooled to −72°C. The corresponding ω-(bromomagnesio)-1-alkenyl compound in diethyl ether (50 µl, 0.5 M) and Li_2CuCl_4 (50 µl, 0.1 M in THF) was added. The solution was mixed by means of a stream of nitrogen gas and allowed to react for 1 min at 0°C. After addition of benzaldehyde (20 µl), the reaction mixture was heated to 50–60°C and the product distilled off by a stream of nitrogen gas into cooled THF (−72°C). The labelled butene and pentene were allowed to pass through an alumina SPE column before being trapped. Analytical LC: column B, mobile phase C–D 50:50 at 50°C, t_R: 4.7, 7.2, and 7.8 for **1a**, **2a** and **3a** respectively.

(methyl-^{13}C)-1-Alkenes **1b**, **2b** *and* **3b**. The syntheses and analyses were performed as above with the following exceptions. [^{11}C]Methyl iodide was trapped in dibutyl ether and the appropriate Grignard reagent (150 µl, 0.2 M) in dibutyl ether was used together with Li_2CuCl_4 (10 µl, 0.5 M) in THF and $^{13}CH_3I$ (20 µl 20% in heptane). The labelled alkene was trapped in dibutyl ether (200 µl) via an alumina SPE column. MS: **1b**: *m/z* 57 (M^+, 64%), 56 (36), 42 (100), 41 (76), 40 (31), 39 (47), 29 (27), 28 (59), 27 (27). **2b**: *m/z* 71 (M^+, 23%), 56 (27), 55 (32), 43 (51), 42 (50), 39 (53), 28 (100). **3b**: *m/z* 85 (M^+, 24%), 70 (15), 57 (64), 55 (38), 43 (51), 42 (100).

*General procedures for synthesis of methyl and methylene-^{11}C-labelled fatty acids (***4–9***) (Scheme 2). Method A.* [^{11}C]Methyl iodide was trapped in a 3 ml reaction vessel containing THF (400 µl) and cooled to −72°C. A THF-solution of thienyl cuprate (20 µl, 0.5 M) followed by the corresponding α,ω-bis(bromomagnesio)alkane (200 µl, 0.5 M) were added, producing a thick precipitate. The cooling bath was removed, and when the precipitate had disappeared and the solution had become dark (ca. 4 min), the vial was put back into the cooling bath and a stream of CO_2 was introduced. The cooling bath was removed, and after 2 min, the vial was put in a 60°C heating block for 1 min. HCl (0.5 ml, 0.5 M) was added and the mixture was allowed to become homogeneous (1 min), and LC-mobile phase (1 ml) was added. The solution was injected into the semipreparative LC-system together with LC-mobile phase (1 ml) that was used to rinse the reaction vial.

Method B. The corresponding [^{11}C]alkyl iodide was trapped in a 3 ml, specially designed reaction vessel containing THF (200 µl) and Li_2CuCl_4 (20 µl, 0.2 M) cooled to −72°C. The corresponding α,ω-bis(bromomagnesio) alkane (200 µl, 0.5 M) was added and the vial was placed in an ice bath. After 1 min (or a few minutes in the case of labelled alkyl iodides other than [^{11}C]methyl iodide) a stream of CO_2 was introduced into the solution and THF (0.5 ml) was added. The vial was heated at 70°C for 2 min and HCl (0.5 ml, 0.5 M) was added. When the mixture was homogeneous (1 min), LC-mobile phase (1 ml) was added and the solution, combined with LC-mobile phase (1 ml) that was used to rinse the reaction vial, was injected into the semipreparative LC-system.

*[5-^{11}C]Pentanoic acid (***4a***).* Method A was used for 1,3-bis(bromomagnesio)propane. Semipreparative LC: column (E), mobile phase H–I 30:70. Analytical LC: column B, mobile phase C–D 50:50, at room temperature, t_R = 3.2 min. Analytical GC: column L at 170°C, t_R = 4.4 min.

*(5-^{13}C)Pentanoic acid (***4b***).* Method A was used for 1,3-bis(bromomagnesio)propane, with the exceptions that fivefold amounts of Grignard reagent and cuprate were used together with $^{13}CH_3I$ (20 µl, 20%) in heptane. The analysis was performed as described for **4a**. After purification by semipreparative LC, the collected fraction was rendered alkaline with KOH (5 M) and evaporated. Conc. HCl was added, and the residue was extracted with 0.7 ml $CDCl_3$. The ^{13}C NMR spectrum showed a single peak at δ 13.7 ($CDCl_3$ used as reference, δ 77.0) which is consistent with the methyl carbon of pentanoic acid.[11]

*[6-^{11}C]Hexanoic acid (***5***).* Method A was used for 1,4-bis(bromomagnesio)butane. Semipreparative LC: column (E), mobile phase H–I 40:60. The analysis was performed as described for **4a**. Analytical LC: t_R = 5.0 min. GC: t_R = 7.5 min.

*[8-^{11}C]Octanoic acid (***6***).* Method B was used for 1,6-bis-(bromomagnesio)hexane and [^{11}C]methyl iodide. Semi-

preparative LC: column F, mobile phase, premixed (44% I, 36% J and 20% D). Analytical LC: column A, mobile phase C–D 30:70, at room temperature, $t_R = 2.4$ min. Analytical GC: column L, temperature gradient 150–200°C, 10°C min^{-1}, $t_R = 12.2$ min.

[7-^{11}C]Octanoic acid (**7**). Method B was used for 1,5-bis-(bromomagnesio)pentane and [1-^{11}C]ethyl iodide. The analysis was performed as described for **6**.

[16-^{11}C]Palmitic acid (**8a**). Method B was used for 1,14-bis(bromomagnesio)tetradecane and [^{11}C]methyl iodide. Semipreparative LC: column F, mobile phase 100% of premixed (36% I, 44% J, 19% D, 1% K) for 3 min and then a gradient over 7 min to 70% C. Analytical LC: column A, mobile phase C–D 20:80, at room temperature, $t_R = 3.4$ min. Analytical GC: column H, temperature gradient 200–270°C, 10°C min^{-1}, $t_R = 13.2$ min.

(16-^{13}C)Palmitic acid (**8b**). Method B was used for 1,14-bis(bromomagnesio)tetradecane (400 µl, 0.5 M) and [^{11}C]methyl iodide, with the following exceptions. After the reaction with [^{11}C]methyl iodide. $^{13}CH_3I$ (20 µl, 20% in heptane) was added, and the mixture was allowed to react for 5 min before addition of carbon dioxide. After semipreparative LC the collected fraction was analysed, as described for **8a**. The fraction was evaporated to dryness and redissolved in 0.7 ml $CDCl_3$. The ^{13}C NMR spectrum showed a single peak at δ 14.1 ($CDCl_3$ used as reference, δ 77.0) which is consistent with the methyl carbon of palmitic acid.[11]

[14-^{11}C]Palmitic acid (**9a**). Method B was used for 1,12-bis(bromomagnesio)dodecane and [1-^{11}C]propyl iodide. The analysis was performed as described for **8a**.

(14-^{13}C)Palmitic acid (**9b**). Method B was used for 1,12-bis(bromomagnesio)dodecane and [1-^{11}C]propyl iodide together with (1-^{13}C)propyl iodide produced in the following way. In the synthesis of [1-^{11}C]propyl iodide, $^{13}CO_2$, produced by acidification of $NaH^{13}CO_3$ (10 mg) was transferred in a stream of nitrogen gas and trapped together with the [^{11}C]carbon dioxide in ethylmagnesium bromide (2 ml, 0.5 M) in diethyl ether. The synthesis was then performed as described, with the exception that an increased amount of 1,12-bis(bromomagnesio)dodecane (1 ml, 0.25 M) was used and allowed to react for 10 min before addition of CO_2. The analysis was performed as described for **8a**. The collected fraction was evaporated to dryness and redissolved in $CDCl_3$ (0.7 ml). The ^{13}C NMR spectrum showed a single peak at δ 32.0 ($CDCl_3$ used as reference, δ 77.0) which is consistent with position C14 of palmitic acid.[11]

[8-^{11}C]Palmitic acid (**10a**). Method B was used for 1,6-bis(bromomagnesio)hexane and [1-^{11}C]nonyl iodide. Semipreparative LC: column E, mobile phase 100% of premixed (36% I, 44% J, 19% D, 1% K) for 2 min and then a gradient over 5 min to 90% C. The analysis was performed as described for **8a**.

(8-^{13}C)Palmitic acid (**10b**). Method B was used for 1,6-bis(bromomagnesio)hexane and [1-^{11}C]nonyl iodide together with (1-^{13}C)nonyl iodide produced in the following way. In the synthesis of [1-^{11}C]nonyl iodide, $^{13}CO_2$, produced by acidification of $NaH^{13}CO_3$ (10 mg), was transferred in a stream of nitrogen gas and trapped together with the [^{11}C]carbon dioxide in octylmagnesium bromide (2 ml, 0.3 M) in diethyl ether. The synthesis was then performed as described for **10a**, with the exception that an increased amount of 1,6-bis(bromomagnesio)hexane (1.5 ml, 0.5 M) was used and allowed to react for 15 min before addition of CO_2. The analysis was performed as described for **8a**. The collected fraction was evaporated to dryness and redissolved in $CDCl_3$ (0.7 ml). The ^{13}C NMR spectrum showed a single peak at δ 29.7 ($CDCl_3$ used as reference, δ 77.0) which is consistent with position C8 of palmitic acid.[11]

p-*Bromophenacyl derivatization of ^{11}C-labelled fatty acids.* The derivatization procedure is based on Ref. 12. After semipreparative LC, a sample (ca. 1 ml) was taken from the fraction containing the radioactive product. The pH of the solution was adjusted to ca. 12 (KOH, 5 M) and evaporated by heating at 130°C and flushing with nitrogen gas. Acetonitrile (1 ml), α,*p*-dibromoacetophenone (51 mg, 200 µmol) and 18-crown-6 (2.6 mg, 10 µmol) was added and the mixture was heated and shaken at 80°C for 4 min. The resulting solution was analysed by LC: column A, mobile phase C–D, gradient 0–8 min 60:40–100:0, at 50°C, λ = 254 nm. $t_R = 5.5$, and 7.6 min for the *p*-bromophenacyl derivative of ^{11}C-octanoate and ^{11}C-palmitate, respectively and the corresponding unlabelled substances, produced according to Ref. 12.

[α-^{11}C]Toluene (**11**). [^{11}C]Methyl iodide was trapped in a 1.5 ml vial containing THF (200 µl) and Li_2CuCl_4 (50 µl, 0.2 M) kept at −72°C. Phenylmagnesium bromide (50 µl, 2 M in diethyl ether) was added and the vial was transferred to an ice bath. After 1 min, the vial was put back into the −72°C bath and a stream of CO_2 was introduced. Ethanol–2 M HCl (50:50, 0.5 ml) was added and the mixture was injected into the semipreparative LC. Semipreparative LC: column E, 100% of premixed (36% I, 44% J, 19% D, 1% K) for 1 min and then a gradient over 4 min to 80% C. Analytical LC: column A, mobile phase C–D 30:70, at room temperature, $t_R = 3.6$ min.

[β-^{11}C]Ethylbenzene (**12**). The synthesis and analysis were performed in analogy with that of [α-^{11}C]toluene, except that benzylmagnesium bromide was used. $t_R = 3.8$ min.

Identification of ^{11}C-labelled alkanes and alkenes, formed in reactions of ^{11}C-labelled alkyl iodides with Grignard reagents. The ^{11}C-labelled alkyl iodide was trapped at −72°C in a 2 ml reaction vial containing THF (400 µl).

The cooling bath was removed and 1,6-bis(bromomagnesio)hexane (200 µl, 0.5 M) was added. After 10 min at room temperature, 100 µl ethanol were added and the vial was shaken. The gas phase in the vial was then analysed by radio GC: column M, at 50°C, t_R = 1.0; 1.2; 1.6; 2.2 and 5.7 for methane, ethane, ethene, propane and propene, respectively.

Results and discussion

The cuprate-mediated coupling reactions of ^{11}C-labelled alkyl iodides and Grignard reagents were generally run in THF at 0°C. Other solvents can be used, as exemplified by the synthesis of ^{13}C-labelled alkenes, where dibutyl ether was used. The choice of reaction temperature was based on the facts that at this temperature, most organocuprates are stable for at least 10 min and most Grignard reagents are soluble up to concentrations of ca. 0.1 M. The reaction with [^{11}C]methyl iodide was very fast, and thus it should be possible to omit the use of an ice-bath in order to simplify automatization.

In most reactions, the cuprate reagent of choice was Li_2CuCl_4.[6c] It is stable, convenient to handle, and easy to separate from the final product. The cuprate reagent should be added to the solution of the labelled alkyl iodide before or at the same time as the Grignard reagent. The reason is the occurrence of a metal–halogen exchange reaction that transforms the labelled alkyl iodide into the corresponding labelled Grignard reagent. This reaction is, however, considerably slower than the coupling reaction with organocuprate intermediates, and it is therefore of little importance when Cu^I is present. The occurrence of the exchange reaction was confirmed by reacting ^{11}C-labelled alkyl iodides with 1,6-bis(bromomagnesio)hexane and phenylmagnesium bromide, respectively. After quenching with ethanol the samples were analysed by GC. Labelled alkanes were formed in 70–80% yield in the case of 1,6-bis(bromomagnesio)hexane, but not in the case of phenylmagnesium bromide. This is consistent with the fact that the equilibrium constant for the metal–halogen exchange reactions between aryllithium and alkyl halides is small.[13]

The [*methyl*-^{11}C]-1-alkenes (**1a**, **2a** and **3a**) were synthesized by a Li_2CuCl_4-facilitated reaction using [^{11}C]-methyl iodide and alkenyl Grignard reagents. At the end of the reaction, benzaldehyde was added in order to quench the excess of Grignard reagent before the product was distilled off. (Scheme 1, Table 1.) In the case of

$$CH_2=CH(CH_2)_nMgBr \xrightarrow[2.\ *CH_3I]{1.\ Li_2CuCl_4} CH_2=CH(CH_2)_n{*}CH_3$$

a; * = 11
b; * = 13

1a, **1b**; n = 1
2a, **2b**; n = 2
3a, **3b**; n = 3

Scheme 1.

Table 1. Synthesis times, yields and purities for some ^{11}C-labelled alkenes and aromatic compounds.

Product	Synthesis time/min[a] I	Synthesis time/min[a] II	Radiochemical yield[b](%) I	Radiochemical yield[b](%) II	Radiochemical purity[c](%)
[4-^{11}C]-1-Butene	13	6	75	88	98
[5-^{11}C]-1-Pentene	16	9	73	86	98
[6-^{11}C]-1-Hexene	22	15	60	70	94
[α-^{11}C]Toluene	27	20	70	82	>98
[β-^{11}C]Ethylbenzene	27	20	70	82	>98

[a]From [^{11}C]O_2 (I) or [^{11}C]H_3I (II) to purified product. [b]Decay corrected. [c]Determined by LC.

labelled butene and pentene, the products were allowed to pass through a small alumina-column before trapping in order to remove impurities. All alkenyl Grignard reagents except vinylmagnesium bromide afforded high yields of labelled alkenes. The observation that vinyl magnesium bromide failed to react was surprising, since vinyl copper reagents are known to undergo alkylation reactions in the usual way.[14,6b]

The identity of the ^{11}C-labelled alkenes was assessed by running the corresponding ^{13}C-synthesis concomitantly with the ^{11}C-synthesis and then, after decay, analysing the product by GC–MS. Analysis of the radioactive product on LC, gave identical results as when the synthesis was run without the ^{13}C-substituted substance. The mass spectra were compared with spectra of authentic samples of unmodified alkenes. Thus it could be confirmed that the ^{11}C labels were present in the molecules of the modified alkenes. In the synthesis of the ^{13}C-labelled alkenes, dibutyl ether was used as solvent, since the occurrence of diethyl ether and THF in the product disturbed the GC–MS analysis. Although the cuprate reagent precipitated in this solvent, the reaction still proceeded in good yield.

The ^{11}C-labelled alkenes, and especially [4-^{11}C]-1-butene, have a potential value as precursors since they are conveniently obtained with short reaction times and high yields. The carbon–carbon double bond is a versatile functional group which can be converted into reagents such as 1,2-dihaloalkanes, organoboranes and epoxides. These reagents have the potential for further use in coupling reactions in the search for new routes to ^{11}C-labelled target molecules.

The preparation of ^{11}C-labelled fatty acids was achieved in two steps by a one-pot procedure. First, and [1-^{11}C]alkyl iodide was reacted with an α,ω-bis(bromomagnesio)alkane assisted by Li_2CuCl_4 or 2-thienyl cuprate. The labelled Grignard reagent formed was then converted into a fatty acid by a reaction with an excess of CO_2 and subsequent hydrolysis, as shown in Scheme 2. Synthesis time, yields and purities for the ^{11}C-labelled fatty acids are shown in Table 2.

Short-chain ^{11}C-labelled alkyl iodides were prepared from $^{11}CO_2$ as described previously[5] with the exception

$$R^*CH_2I + BrMg(CH_2)_nMgBr \xrightarrow{Cu^I} R^*CH_2(CH_2)_nMgBr \xrightarrow[2.\ H^+]{1.\ CO_2} R^*CH_2(CH_2)_nCO_2H$$

a; * = 11
b; * = 13

4a, **4b**; R = H, $n = 3$
5; R = H, $n = 4$
6; R = H, $n = 6$
7; R = CH_3, $n = 5$

8a, **8b**; R = H, $n = 14$
9a, **9b**; R = CH_3CH_2, $n = 12$
10a, **10b**; R = $CH_3(CH_2)_7$, $n = 6$

Scheme 2.

Table 2. Synthesis times, yields and purities for some ^{11}C-labelled fatty acids.

Product	Alkyl halide	Synthesis time/min[a] I	Synthesis time/min[a] II	Radiochemical yield[b] (%) I	Radiochemical yield[b] (%) II	Radiochemical purity[c](%)
[5-^{11}C]Pentanoic acid	[^{11}C]Methyl iodide	31	24	35	41	97
[6-^{11}C]Hexanoic acid	[^{11}C]Methyl iodide	31	24	47	55	98
[8-^{11}C]Octanoic acid	[^{11}C]Methyl iodide	32	25	45	53	>98
[7-^{11}C]Octanoic acid	[1-^{11}C]Ethyl iodide	37	25	33	44	94
[16-^{11}C]Palmitic acid	[^{11}C]Methyl iodide	34	27	37	43	96
[14-^{11}C]Palmitic acid	[1-^{11}C]Propyl iodide	42	29	20	36	>98
[8-^{11}C]Palmitic acid	[1-^{11}C]Nonyl iodide	61	31	15	25	98

[a]From [^{11}C]O_2 (I) or ^{11}C-labelled alkyl iodide (II) to purified product. [b]Decay corrected. [c]Determined by LC.

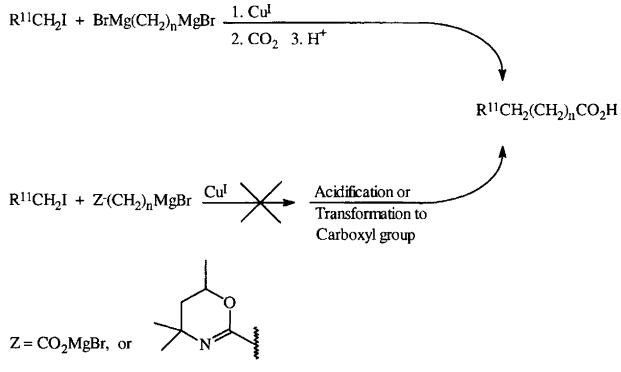
that THF was replaced by diethyl ether in the synthesis of [1-^{11}C]ethyl and propyl iodide. [1-^{11}C]Nonyl iodide was synthesized by a modification of the procedure described for the preparation of substituted [α-^{11}C]benzyl iodides.[10] The synthesis time from [^{11}C]carbon dioxide was 30 min, the decay corrected radiochemical yield was 60% and the radiochemical purity was higher than 98%.

When THF was used in the preparation of [1-^{11}C]propyl iodide and [1-^{11}C]nonyl iodide, only a small fraction of the [1-^{11}C]alkyl iodide reacted with the Grignard reagent. The reason was assumed to be the presence of 1,4-diiodobutane, which can be formed in the reaction of THF with hydriodic acid. In the preparation of the [1-^{11}C]alkyl iodides, THF is retained in the mixture of LAH and the Grignard reagent despite prolonged heating with flowing nitrogen gas. The problem was overcome by using diethyl ether instead of THF.

The properties of the long and the short α,ω-bis(bromomagnesio)alkanes differed markedly from each other with respect to the Li_2CuCl_4-mediated coupling reaction. Bis-Grignard reagents longer than six carbon atoms, gave similar radiochemical yields and analogous product composition. In contrast, 1,4-bis(bromomagnesio)butane yielded only a few percent of [6-^{11}C]hexane and 1,3-bis(bromomagnesio)propane did not yield any labelled coupling product. The reason for this is assumed to be related to the stability of the cuprate complex. In the search for a more stable cuprate, 2-thienyl cuprate was found useful. It is conveniently used in THF solution, it has increased thermal stability and it yields reactive mixed cuprates with alkyllithium or Grignard reagents.[9] This approach was successful, and [5-^{11}C]pentanoic acid and [6-^{11}C]hexanoic acid were obtained in moderate yields (Table 1).

A problem with 1,3-bis(bromomagnesio)propane is its low solubility in THF, which complicated the handling. However, the problem was overcome by the addition of a small amount of pyridine (1% of the THF volume), which markedly increased the solubility of the Grignard reagent, without affecting its reactivity. The reason for this effect may be that pyridine forms a complex with the Grignard reagent.

In the search for a route to methyl-^{11}C-labelled linoic acid, ω-Grignard reagents of undecanoic acid derivatives were used. The carboxy group was either masked as an oxazine[15] or used as a bromomagnesium salt[16] (Scheme 3). Attempts to perform a carbon–carbon coupling reaction with [^{11}C]methyl iodide were, however, unsuccessful in all these cases. The reason for the lack of reactivity can



Scheme 3.

be rationalized by assuming that a dimerisation takes place where the oxazine unit or the carboxy group forms a complex with the Grignard unit.[15]

Labelled propyl and nonyl iodide reacted more slowly than [^{11}C]methyl iodide, and also formed considerable amounts of by-products. Some of these products were identified as the corresponding alkenes. When [2-^{11}C]-isopropyl iodide[5b] was used in combination with Grignard and cuprate reagents, the expected fatty acid was not formed, but instead labelled propene was obtained in 80% yield.

To prevent oxygen from reacting with the Grignard reagent during the labelling synthesis, the Grignard reagents were added after the transfer of [^{11}C]methyl iodide. The reason for this is that the nitrogen gas used in the transfer of the [^{11}C]methyl iodide contains oxygen, due to diffusion through the Teflon tubing used in the production system.

The cuprate reagent is a potential source of by-product formation. (i) When Li_2CuCl_4 is used, one equivalent of the Grignard functionality is consumed in the reduction of Cu^{II} to Cu^{I}. (ii) Organocuprates are less reactive than Grignard reagents in reactions with carbonyl carbons. The amount of labelled alkane should thus increase at the expense of the labelled carboxylic acid, when the amount of cuprate reagent is increased. In order to investigate these relations, the synthesis of [8-^{11}C]octanoic acid was studied. Thienyl cuprate or Li_2CuCl_4 was used and the yield of labelled octanoic acid, as well as the labelled lipophilic by-product (alkane), was determined for different amounts of cuprate. In the case of Li_2CuCl_4 it was found that when the concentration ratio [Cu]:[Grignard] was less than 1:10, there was no obvious relationship between this ratio and the yield of octanoic acid or alkane. However, when the ratio was increased from 1:5 to 1:1 the yield of alkane was increased proportionally. Surprisingly, there was no relation between the ratio and the yield of octanoic acid or alkane, when thienyl cuprate was used. For example when the ratio varied from 1:5 to 5:9 the yield of octanoic acid was steady at about 65–70%.

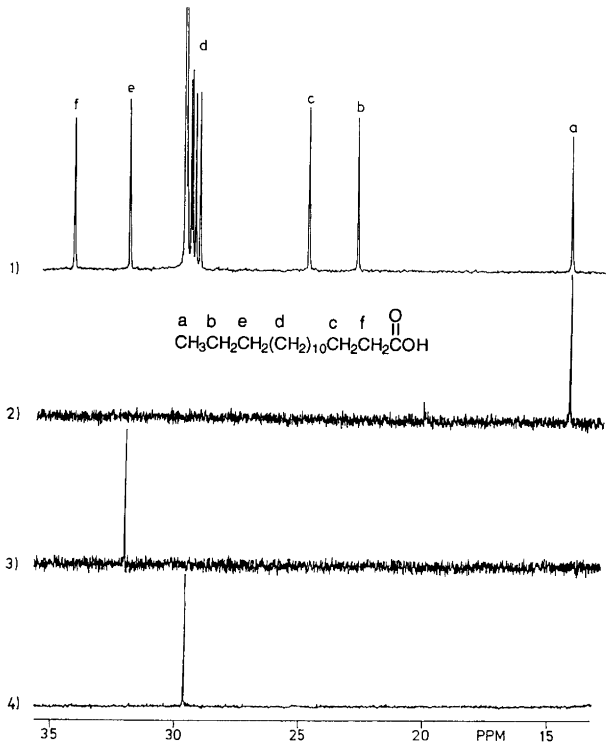


Fig. 1. Selected parts from ^{13}C NMR spectra of 1) palmitic acid, 2) (16-^{13}C)palmitic acid, 3) (14-^{13}C)palmitic acid and 4) (8-^{13}C)palmitic acid.

A valuable aspect of the fatty acid synthesis presented here is the availability of the unlabelled precursors. Most α,ω-dibromoalkanes shorter than 13 carbons are commercially available while the corresponding long chain compounds are easily synthesized. In this paper, a moderate-yielding synthesis of 1,14-dibromotetradecane is presented. However, the corresponding C_{16} and C_{18} compounds have also been produced by analogous procedures and with similar results. In this synthesis, an α,ω-bis(bromomagnesio)alkane was coupled with two equivalents of a α,ω-dibromoalkane with the aid of Li_2CuCl_4. The symmetrical properties also have been utilized in syntheses of other α,ω-dibromides. One example is the perdeuteriated α,ω-dibromoalkanes that have been used in the synthesis of ^{11}C-labelled deuterio fatty acids.[4b] Another example is the polyhomoallylic α,ω-dichloride used in the synthesis of [19-^{11}C]arachidonic acid.[17]

Identification of the labelled fatty acids was achieved in the following way.

- By LC-analysis of the *p*-bromophenacyl derivatives.
- By GC analysis, together with authentic reference samples.
- By ^{13}C NMR analysis of (16-^{13}C)-, (14-^{13}C)- and (8-^{13}C)-palmitic acid, synthesized by the same method as the corresponding ^{11}C-labelled palmitic acid.

The fatty acids were reacted with α,*p*-dibromo acetophenone[12] primarily in order to obtain a derivative that could be monitored by UV-detection. However, the derivatization also confirms that the product is a carboxylic acid. The ^{13}C-synthesis was performed concomitantly with the corresponding ^{11}C-synthesis for the reasons discussed in the case of the ^{13}C-substituted alkenes. Apart from being an important part of the identification, the ^{13}C syntheses together with the NMR analysis illustrates the possibilities of labelling alkyl chains in selected positions (Fig. 1).

The syntheses of labelled toluene and ethylbenzene are straightforward (Scheme 4). Carbon dioxide was added in order to quench the excess of phenylmagnesium bromide and thus simplify the purification of the product. The labelled products were obtained with a short synthesis times in high yields (Table 1). In a typical run start-

$$^{11}CH_3I + RMgBr \xrightarrow{Li_2CuCl_4} R^{11}CH_3$$

11; R = Ph
12; R = $PhCH_2$

Scheme 4.

ing with 6.3 GBq of [^{11}C]O_2, 2.0 GBq of [α-^{11}C]toluene was obtained with a specific activity of 880 MBq μmol^{-1}.

The synthesis of aliphatic and aromatic hydrocarbons by the use of conventional lithium organocuprates and $^{11}CH_3I$ has previously been described by Långström and Sjöberg.[7] This methodology, however, is less useful for the synthesis of methyl- and methylene-^{11}C-labelled fatty acids and cannot be applied in the coupling reactions involving alkyl iodides and benzylic carbons. The use of Li_2CuCl_4 and Grignard reagents is also advantageous in other cases owing to its convenience and reliability.

The synthetic approaches presented in this paper open up new possibilities for incorporation of ^{11}C into specific positions of a carbon chain. The option to select the position of the label is of importance for designing radiotracers, since it gives an additional dimension to PET as a tool for evaluating models describing degradation processes in biological systems.

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