Synthesis of L- and D-[Methyl-^{11}C]Methionine

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This report describes the synthesis of L- and D-[methyl-^{11}C]methionine in pure enantiomeric forms. The compounds were prepared routinely ~1,000 times with less than 20 failures. Starting with carbon-11 (^{11}C) methyl iodide, a simple one-carbon precursor produced from a one-pot or a two-pot apparatus, L- and D-[methyl-^{11}C]methionine were prepared, respectively, with an optical purity higher than 99% in 40%–90% radiochemical yields. The total time for synthesis, starting from [^{11}C]carbon dioxide, was 12–15 min. The crude product usually had a radiochemical purity >95%. The total time for synthesis, including LC purification, was 20–30 min. The radiochemical purity of the product in each case was >98%.

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Both the D and the L forms of [methyl-^{11}C]methionine have been used in nutritional studies of experimental animals such as pigs (*1*) and pregnant Rhesus monkeys (*2*). Carbon-11–labeled L-methionine also has been applied in studies of tumors of the type of supratentorial gliomas in the human brain (*3*) using positron emission tomography (PET), and in studies of hyperammonemia in children using simple external detectors in combination with blood analysis (*4*). In another study of tumors in the human brain, L-[methyl-^{11}C] methionine was used in combination with [^{11}C]glucose (*5*). Investigations are now in progress using D-[methyl-^{11}C]methionine (*6*) in PET studies of brain and pituitary tumors in humans (*7*).

In this synthesis, previously reported for L-[methyl-^{11}C]methionine (*10*), the amino acid precursors L- or D-S-benzyl homocysteine (*11*) were converted to the corresponding sodium salts of the sulfide anions by sodium in liquid ammonia (Fig. 1). The enantiomeric purity was >99%, as determined by a method utilizing aminoacylation of tRNA (*10*) or by liquid chromatography enantiomeric analysis (*11*).

Other methods of generating the homocysteine sulfide anion have been reported (*12,13*). In our laboratory, however, liquid ammonia/sodium was the method of choice, because ammonia is an excellent solvent in other synthetic applications (i.e., enkephalins and other peptides have been labeled with ^{11}C by a similar reaction route) (*14,15*). The same solvent has been applied in the synthesis of [methyl-^{11}C]selenomethionine (*16*). The generation of the sulfide anion and the removal of the protective groups of the appropriate amino acid or peptide precursor were carried out prior to the synthesis of [^{11}C]methyl iodide.

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MATERIALS AND METHODS

General

Nitrogen gas was irradiated with 10 MeV protons at the tandem Van de Graaff accelerator at the University of Uppsala. By slow flushing of the target, the [^{11}C]carbon dioxide produced was trapped in 4 Å molecular sieves kept in a lead shield. The trap was then transported to the chemistry laboratory. When the trap had been connected to the four-way valve in the synthesis system (Fig. 2), the [^{11}C]carbon dioxide was released from the molecular sieves by heating to 250°C.

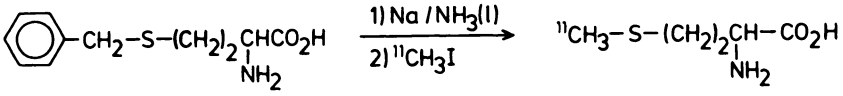
Carbon-11 Methyl Iodide (One-Pot Apparatus)

The [^{11}C]carbon dioxide was carried in a stream of nitrogen and trapped at room temperature in the glass reaction vessel (vessel I) containing 0.2–0.4 ml of 1.0*M* lithium aluminium hydride (LAH) in tetrahydrofuran (THF). After trapping, the THF was removed by increasing nitrogen gas flow and the temperature to 80°C. The LAH complex was hydrolyzed by addition of 1.5 ml of 54% hydriodic acid. The [^{11}C]methyl iodide produced was transferred by the carrier gas through drying towers (sodium hydroxide and phosphorous pentoxide) to vessel II (Fig. 2).

L- or D-[Methyl-^{11}C]Methionine

Approximately 10 min before initiation of the synthesis of [^{11}C]methyl iodide, 1.5 ml of ammonia was condensed in the reaction flask (vessel II) containing 3 mg of L- or D-S-benzyl homocysteine (*9*) and 2 mg of sodium. Ammonia (g) was

FIGURE 1
Synthesis of L- and D-[methyl-^{11}C] methionine.



dried by passing through a tower containing sodium hydroxide. The blue color characteristic of the sodium/liquid ammonia solution was observed at once, and if it persisted for more than 10–30 sec, ammonium chloride was added or nitrogen gas passed through until the solution became colorless. The [^{11}C]methyl iodide produced was trapped in the ammonia solution. After trapping, the ammonia was removed by increasing the nitrogen flow and heating the flask to room temperature. The solid residue that remained was dissolved in a buffer.

For the semipreparative liquid chromatography (LC) purification, 1.5 ml of phosphate buffer (0.1*M*, pH 7.40) was added and the solution transferred to a LC loop for injection. Preparative LC was carried out on a Waters system with a constant wavelength detector in series with a GM tube. A 250 × 10 mm, 30 microns Spherisorb, C-18 column was used, flow 4.0 ml/min, ultraviolet 254 nm, column temperature 25°C, solvent 90/10 0.1*M* ammonium formate pH 3.50/ethanol. The retention times for homocysteine and methionine were 4.5 min and 6.0 min, respectively.

After evaporation of the appropriate radioactive LC fractions, 10–15 ml of volume, addition of sterile phosphate buffer, pH adjustment and sterile filtration, the ^{11}C-amino acid was ready for administration. LC analysis of the final product was carried out by any of the following three systems using the conditions specified:

1. 250 × 4.6 mm C-18 column, 10 microns Nucleosil, flow 1.0 ml/min, ultraviolet 240 or 230 nm, column temperature 40°C, solvent 87/13 (v/v) isocratic 0.1*M* ammonium formate pH 3.50/methanol.

2. 200 × 4.6 mm LiChrosorb-NH_2 column, 5 microns, flow 2.0 ml/min, ultraviolet 220 nm, column temperature 35°C, solvent A = 0.01*M* potassium dihydrogen phosphate pH 4.30 B = acetonitrile/water 500/70 (v/v), linear gradient 0–10 min B/A 95/5-50/50. A typical example of a [^{11}C] methionine LC analysis is shown in Figure 3.

3. 150 × 4.6 mm C-18 column, 10 microns, flow 1.5 ml/min, fluoroscence 440 nm OPA-derivate (*17*), column temperature 35°C, solvent A = methanol/tetrahydrofuran/0.05*M* sodium acetate, 0.05*M* disodium hydrogen phosphate pH 7.5 (20/20/960) (v/v/v) B = methanol/water 65/35 (v/v), gradient 0–14 min A/B 90/10-50/50, 14–23 min A/B 50/50-0/100, 23–28 min A/B 0/100-90/10.

Determination of Optical Purity (tRNA Method and LC-Enantiomer Column)

tRNA method. The tRNA method for determination of enantiomeric purity of ^{11}C-amino acids, described in detail elsewhere (*8*), was used for [methyl-^{11}C]methionine. The following mixture was used in the amino-acylation: 70 or 80 μl H_2O; 35 μl buffer a or b (see *8*); 10 μl tRNA; 10 μl L-[methyl-^{14}C]methionine and 10 μl [methyl-^{11}C]methionine; 5 μl aminoacyl-tRNA ligase.

The final volume was incubated at 37°C for 10 min. The solution was immediately placed on a PD-10 column of Sephadex G-25 for gel filtration and eluted with 0.05*M* cacodylate buffer, pH 6.9. In order to optimize the separation between the high- and the low molecular weight substances, fractions of 0.5 or 1 ml were taken. The fractions were counted with respect to ^{11}C in a gamma counter as soon as possible and the resultant data was corrected for background and decay. The ^{14}C radioactivity was measured on the following day. The percentage of the total radioactivity of ^{11}C and ^{14}C in the tRNA fraction was then calculated. The amount of ^{11}C-labeled amino acid was chosen to give the same range of percentage incorporation obtained with ^{14}C-labeled amino acid.

LC-Enantiomeric analysis

[Methyl-^{11}C]methionine was analyzed with respect to optical purity by use of a 250 × 4.6 mm CHIRALPAK WH column* 5 microns, flow 1.5 ml/min, ultraviolet 254 nm, column temperature 50°C, solvent 0.25 m*M* copper sulphate (Fig. 3).

RESULTS AND DISCUSSION

Carbon-11 methyl iodide has been prepared routinely in our laboratories by utilizing a simple remotely con-

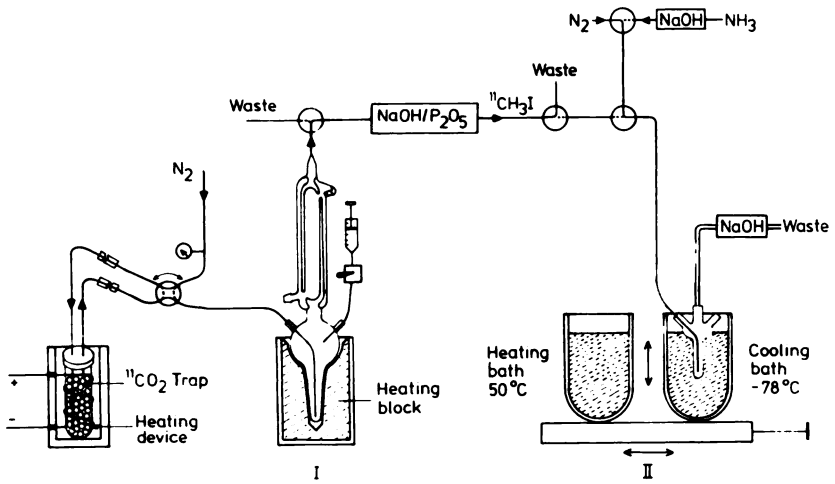


FIGURE 2
One-pot apparatus for synthesis of [^{11}C]methyl iodide.

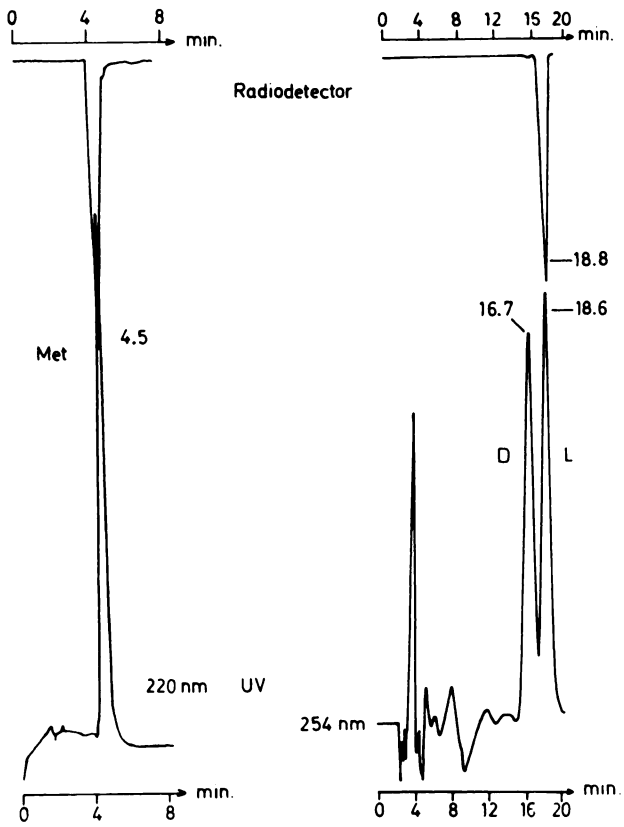


FIGURE 3
LC-chromatograms of L-[methyl-^{11}C]methionine showing radiochemical and chemical purity (left) and enantiomeric purity with added D/L methionine (right).

trolled chemical chart containing a one- or two-pot system. In most of the preparations the labeled methyl iodide was prepared by using the two-pot system previously described (*18*). A simple one-pot apparatus, based on the same chemical chart system, was then developed (*19*). With access to a tandem accelerator using a lead-shielded hood, we have been able to carry out one synthesis per hour.

Carbon-11-methyl iodide was obtained in 70%–95% radiochemical yield from [^{11}C]carbon dioxide. Carbon-11-methyl iodide was routinely prepared within 3–5 min after release of [^{11}C]carbon dioxide. The ^{11}C-labeled methyl iodide was trapped in the freshly prepared ammonia solution containing the sodium salt of the sulfide anion of homocysteine. Because alkylation of the sulfide anion was very rapid, the ammonia was removed immediately after trapping of [^{11}C]methyl iodide. The crude product of [methyl-^{11}C]methionine, with a radiochemical purity usually >95%, was used in some animal experiments without further purification. The total synthesis time was ~12–16 min. In most of the experiments, however, including all human applications, purification by semipreparative LC was performed because small amounts of pyrogens were occasionally detected in the crude product by the limulus test. The synthesis system used is easily converted to a microprocessor-controlled one because all steps are performed in two reaction pots.

In the past 8 yr we have performed ~1,000 syntheses using these systems, with less than 20 failures. In these productions the starting radioactivity of [^{11}C]carbon dioxide was mostly in the range of 150–250 mCi. The [^{11}C]carbon dioxide was transferred by nitrogen gas and reduced by LAH in THF. THF was removed and 54% hydriodic acid added (in the two-pot system the [^{11}C] methanol was transferred to a flask containing boiling hydriodic acid). About 60%–90% of this radioactivity was obtained as [^{11}C]methyl iodide within 3–5 min of the release of [^{11}C]carbon dioxide.

A comparison between the one- and two-pot systems shows that the one-pot system is simpler to use and easier to service. In the synthesis of D- and L-[methyl-^{11}C]methionine, the classic approach by du Vigneaud (*20,21*), generating the sulfide anion of homocysteine with sodium in liquid ammonia, has been used. This approach has several advantages: The precursor is easy to generate, ammonia can easily be removed and the crude reaction product is very pure. The method has now also been extended to S-alkylation of homocysteine containing peptides giving labeled peptides, such as Met-enkephalin (*14,15*), and can be used successfully in the Se-alkylation to prepare [methyl-^{11}C]selenomethionine (*16*).

The enantiomeric purity of the final product was determined in some experiments by the use of tRNA method (*10*) and by LC-enantiomeric analysis. The aminoacyl coupling of L-amino acids to tRNA, followed by separation by means of gel filtration of the tRNA-amino acid complex and the free amino acid, is an adequate method for determining the enantiomeric purity of [methyl-^{11}C]methionine even in the pmol range. An enantiomeric purity >98% (subject to limitations of the method) was thus determined. Later, with the use of LC-enantiomeric analysis we showed that the enantiomeric purity was >99% (Fig. 3).

The question of specific radioactivity in relation to the preparation of ^{11}C-L- and D-methionine has not been considered in detail. However, we have occasionally shown by LC and GC analyses that the specific radioactivity in our hands of the ^{11}C-amino acids corresponds very well to the specific radioactivity of [^{11}C] methyl iodide. Our preparations of [methyl-^{11}C]methionine have been used in animal experiments; the radioactivity administered was between 0.5 and 80 mCi, depending on the type of experiment. In PET studies on humans the amount of radioactivity used was in the range of 2–10 mCi and the [methyl-^{11}C]methionine had a specific activity of 1–100 mCi/μmol at the time of administration. The value of the specific activity depended on the total amount of [^{11}C]carbon dioxide used in the synthesis and the elapsed time between synthesis

and administration. The radiochemical purity of the final product was generally >98%. The radiochemical purity in PET investigations, always confirmed by prior LC analysis, was never <92%.

NOTE

* Daicel Chemical Industries, LTD.

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