SYNTHESIS OF D/L- AND L-SE-[METHYL-^{11}C]SELENOMETHIONINE

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SUMMARY

The syntheses of D/L- and L-Se-[methyl-^{11}C]selenomethionine are reported. The Se-benzyl selenohomocysteines were deprotected in sodium/liquid ammonia and the selenide anions generated *in situ* were alkylated with [^{11}C]methyl iodide to give, after purification by LC, the products in 80 to 85 % radiochemical yield, with a radiochemical purity higher than 99 % within 30 min and with a specific radioactivity of 20 to 200 mCi/μmol. The enantiomeric purity of the L-compound, determined by LC, was higher than 99 %.

Key words: D/L- and L-Se-[methyl-^{11}C]selenomethionine, ^{11}C-labelled D/L- and L-selenomethionine.

INTRODUCTION

Trends in positron emission tomography in recent years have indicated that this technique is becoming a very useful tool for *in vivo* biochemistry.[1-3] Progress in the synthesis of compounds labelled with the short-lived radionuclides has played an important role in this development. Labelled amino acids, peptides, carbohydrates, nucleosides, fatty acids, neurotransmitters, ketone bodies and pharmaceuticals labelled with a positron emitting nuclide are thus available.[4]

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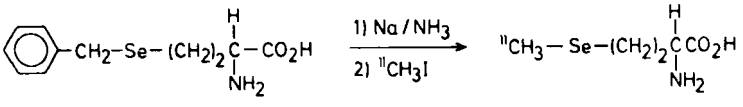
^{11}C-Labelled alkyl halides have frequently been used in various N-, O-, C- and S-alkylation reactions.[5] Using S-alkylation on sulphide anions, prepared in situ, D- or L-Methionine[6] and methionine-containing peptides[7-9] have been prepared in high yields.

Interest in selenium as a tracer element is well documented[10] and the positron-emitting ^{73}Se is a radionuclide of some potential. In this report the synthesis of L-Se-[methyl-^{11}C]selenomethionine by a reaction similar to the one used in the preparation of L- or D-S-[methyl-^{11}C]methionine[6] is described.

RESULTS AND DISCUSSION

The strategy, originally developed for the synthesis of ^{11}C-labelled methionine,[6] is equally suitable for the alkylation of selenide anions for preparing D/L- or L-Se-[methyl-^{11}C]selenomethionine as shown in Scheme 1. The proper selenohomocysteine anion (L- or D/L-) was generated from the corresponding Se-benzyl compound by deprotection in sodium/liquid ammonia shortly before the start of the [^{11}C]methyl iodide synthesis. When transfer of this reagent to the reaction vessel was completed, ammonia was removed by flushing with nitrogen gas and gentle heating.



Scheme 1

After semipreparative LC purification, the product was obtained in 80 to 85 % radiochemical yield, decay corrected, within 30 min from start of the [^{11}C]methyl iodide synthesis, in a sterile, pyrogen-free solution ready for administration in a PET investigation.

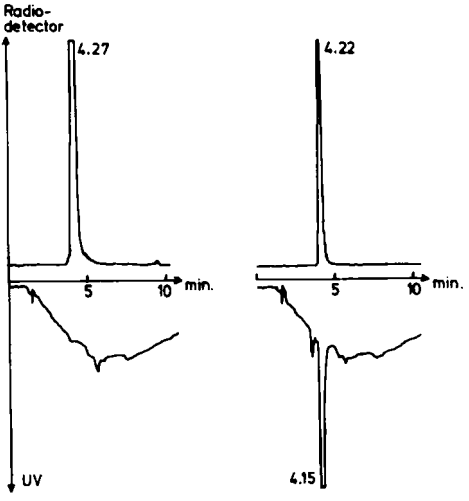


Figure 1. LC-analysis of L-Se[methyl-^{11}C]selenomethionine before and after addition of a reference.

The specific radioactivity of the product, which is correlated to the specific radioactivity of the [^{11}C]methyl iodide produced, has been in the order of 20 to 200 mCi/µmol. The chemical identity was confirmed by LC and addition of a reference which co-chromatographed with the radioactive peak as shown in Figure 1. For determination of the enantiomeric purity of L-Se[methyl-^{11}C]selenomethionine, the product was derivatized according to the procedure by Marfey[11] and analysed on an LC system which separated the two diastereomers formed. The identity of the derivatized ^{11}C-products was confirmed by addition of cold references as shown in Figure 2.

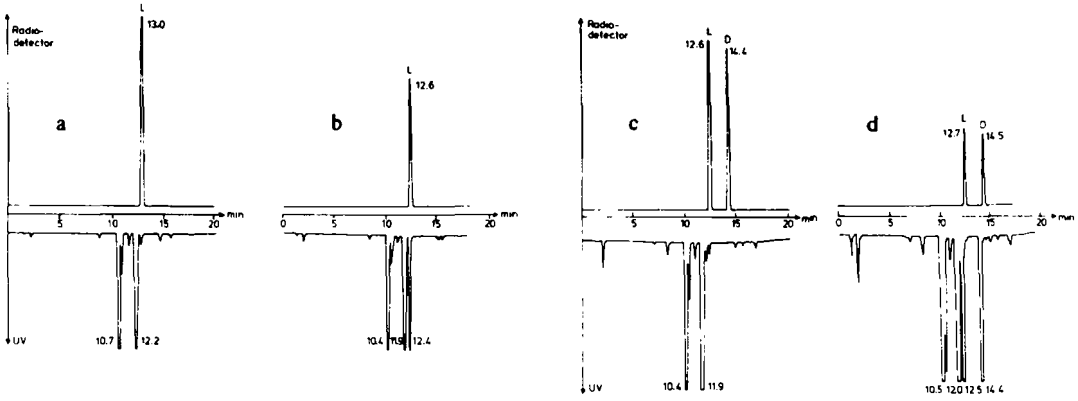


Figure 2. LC analysis of Marfey's derivative of L- and D/L-Se[methyl-^{11}C]selenomethionine before and after addition of a reference.
a: L, b: L + reference, c: D/L, d: D/L + reference.

EXPERIMENTAL

General: The ^{11}C was produced at the tandem Van de Graaff accelerator at the University of Uppsala by the $^{14}N(p,\alpha)^{11}C$ reaction using a nitrogen gas target. The [^{11}C]carbon dioxide was trapped in 4 Å molecular sieves and transported to the radiochemical laboratory in a lead shield. [^{11}C]Methyl iodide was prepared according to the routine laboratory procedure.[6] The derivatization according to Marfey's procedure[11] was performed, with minor modifications, as follows: A sample of the final product (100 µL) was added to a solution of Marfey's reagent, purchased from Pierce Chemical Company, (400 µL, 1 % in EtOH, w/v) and sodium hydrogen carbonate (80 µL, 1.0 M) and the reaction solution was heated at 75 ^{o}C for 10 min. The reaction was stopped by addition of hydrochloric acid (40 µL, 2M).

LC analysis of chemical purity was carried out on a Hewlett-Packard 1090 system equipped with a diode-array detector in series with a β-flow detector[12] and a Supelco 250 x 3.9 mm LC-NH_2 5 µm column using

a gradient of potassium dihydrogen phosphate (0.01 M, pH 4.6) (A) and acetonitrile/water (500/70, v/v) (B). The analysis of the enantiomeric purity was carried out on a Waters system with an M-680 automated gradient controller, two M-501 pumps and an M-441 absorbance detector using a 250 x 4.6 mm Alltech Lichroma C-18 10 µm column and a gradient of triethylammonium dihydrogen phosphate (0.025 M) (C) and acetonitrile (D).

Preparative LC was performed on a Waters system equipped with a M 441 UV detector in series with a GM tube and a Nucleosil 250 x 10 mm C-18, 30 µm column using a mixture of aqueous ammonium formate (0.05 M, pH 3.5) (E) and ethanol (F). Sterile septum-equipped flasks and sterile solutions of aqueous phosphate buffer (pH 7.4, physiological buffer), sodium hydroxide (0.1 M) and hydrochloric acid (0.1 M) were obtained from the Hospital Pharmacy, University Hospital of Uppsala.

D/L- or L-Se[methyl-^{11}C]Selenomethionine (Scheme 1): Prior to the arrival of the trap containing the [^{11}C]carbon dioxide at the radiochemistry laboratory, dry ammonia was condensed at -78 ^{o}C in a reaction vessel[6] containing about 1 mg of D/L- or L-Se-benzyl selenohomocysteine[13] and 1 mg of sodium (dispersed and stored under pentane). After condensation of 2-3 mL of ammonia, the persistent blue colour was removed by addition of a small amount of ammonium chloride. [^{11}C]-Methyl iodide was prepared by our routine procedure[6] and transferred to the reaction vessel in a stream of dry nitrogen gas. After 1-2 min, a steady state of radioactivity was reached and the ammonia was removed by increasing the nitrogen gas flow from 7 mL/s to 20 mL/s and by gentle heating of the reaction vessel at 45 ^{o}C. The solid residue obtained was dissolved in 2 mL of the physiological buffer solution and purified on the preparative LC system using a flow of 4 mL/min of a mixture of solvents E and F (90/10, v/v). After evaporation of the collected LC-fractions, the residue was dissolved in physiological buffer, adjusted to pH 7.0 and sterile filtered.

Analysis of radiochemical purity was performed using the following linear gradients of A/B (v/v): time 0-8, 95-60; time 8-12, 60-45; time 12-14, 45; time 14-14.9, 45-95 with a flow of 1.5 mL/min and an oven temperature of 40 ^{o}C. The eluate was monitored with the UV response at 254 nm and the product eluted at 4.0 min (Figure 1). The optical purity was determined (for the diastereomers formed on reaction with Marfey's reagent) using the following linear gradient of C/D (v/v): time 0, 80/20; time 15, 50/50; time 16, 50/50 with a flow of 2.0 mL/min. and an oven temperature of 25 ^{o}C. The eluate was monitored with the UV response at 313 nm and the diastereomers eluted at 12.4 min (L,L) and 14.2 min (D,L) (Figure 2).

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