PREPARATION OF α–[3-^{11}C]AMINOISOBUTYRIC ACID FROM AN AZADISILOLIDINE DERIVATIVE OF ALANINE

Franz Oberdorfer, Axel Zobeley, Klaus Weber, Christian Prenant, Uwe Haberkorn and Wolfgang Maier-Borst.

Deutsches Krebsforschungszentrum, Radiologie, Abteilung Radiochemie und Radiopharmazeutische Chemie, im Neuenheimer Feld 280, D-6900 Heidelberg, FRG.

SUMMARY

Alkylation of ethyl 2-(2,2,5,5-tetramethyl-[1,2,5,]azadisilolidine-1-yl)propionate with [^{11}C]CH_3I afforded, following acid hydrolysis and chromatographic purification, the non-physiological α-[3-^{11}C]aminoisobutyric acid within 40 min in yields of 5 - 15% (not corrected for decay) related to initially produced [^{11}C]CH_3I. Usually $1.2 \cdot 10^9$ Bq of the tracer molecule were prepared for use in amino acid transport studies. Dilution with a known amount of unlabelled α-aminoisobutyric acid (10 μmol) provided a constant specific radioactivity of $1.2 \cdot 10^8$ Bq·μmol^{-1} for each application. The radiochemical purity of that product was better than 98%, whereas the final carrier-added solution typically contained 10 - 45 μmol of alanine.

Key words: ^{11}C-Labelled amino acids, α-[3-^{11}C]aminoisobutyric acid, [^{11}C]CH_3I, amino acid transport tracer, PET.

INTRODUCTION

Due to a generally limited blood supply, tumour cells compensate nutritional deficiency by development of a rapid and parasitic access to carbohydrates and amino acids. The required nutrients usually enter the tumour cell through the operation of a specialized transport system whose expression is distinctly increased in agressive proliferating neoplastic tissue. A most active and specific transport system is that involved in the transport and intracellular accumulation of amino acids. Hence it has been suggested that a significant alteration of amino acid transport phenomena would reflect the early response of a tumour to chemotherapy or radiation therapy.

The non-physiological branched chain amino acids **1** and **2**, derived from alanine, and the straight chain 2,4-diaminobutyric acid **3** were reported as unique tools for a selective study of the

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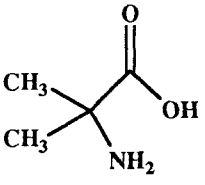
sodium dependent amino acid transport system A (alanine type) which is assumed to regulate the intracellular amino-acid uptake [1-4].



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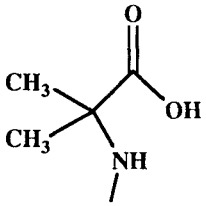
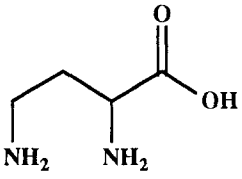


2 a: R = -CH_3 b: R = -$C(O)CH_3$



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Moreover, the system A transporter appeares to be a key for the examination of actions by which protooncogenes and oncogenes control biochemical pathways which regulate growth [1,2]. We decided to first evaluate the title compound **1** for several reasons. α-Aminoisobutyric acid is the most intensively studied substrate sharing the same transport system as alanine, it is clearly not be incorporated into proteins and its transport is very high in fast proliferating tissue where it accumulates rapidly [5]. Furthermore it has proven its utility in various oncological PET studies [6,11] and it may provide a stronger correlation to tumour grade than natural amino acids [6,7] or than substrates **2** and **3**. In addition, **1** was chosen for our studies because it advantageously could be prepared from the most frequently used precursor [^{11}C]CH_3I applying an extrapolation of the glycine Schiff base methodology of α-amino acid synthesis of Stork [8]. This alternate route to a labelled α-[^{11}C]methyl amino acid involved the reaction of an azadisylolidinyl enolate of the parent amino acid which exhibited an increased reactivity towards the electrophilic [^{11}C]CH_3I, as compared to the respective conjugated Schiff base enolates. Although the procedure delivered the α-[3-^{11}C]-**1** as a racemic mixture, with different isotopes of carbon as substituents at C-2, we assume that this fact will not alter its efficency as an *in vivo* amino acid transport marker.

DISCUSSION

The most widely used syntheses of ^{11}C-labelled amino acids have briefly been reviewed recently [9]. Among these, the modified Strecker synthesis appeared as a method of choice for [^{11}C]carboxyl labelled non-racemic, branched chain synthetic amino acids [10]. Compound **1** was previously labelled by that route [11]. It required strong reaction conditions, temperatures above

200 ^{0}C, and the addition of cyanide carrier. The yield of this reaction was inconsistent in our experience and the complete procedure was outward to handle in a remotely operated apparatus. Therefore we examined a new procedure for the preparation of **1** which allowed the reaction of [^{11}C]CH_3I with a suited glycine anion equivalent. The general efficacy of this method for the synthesis of ^{11}C-labelled amino acids has yet been demonstrated [12].

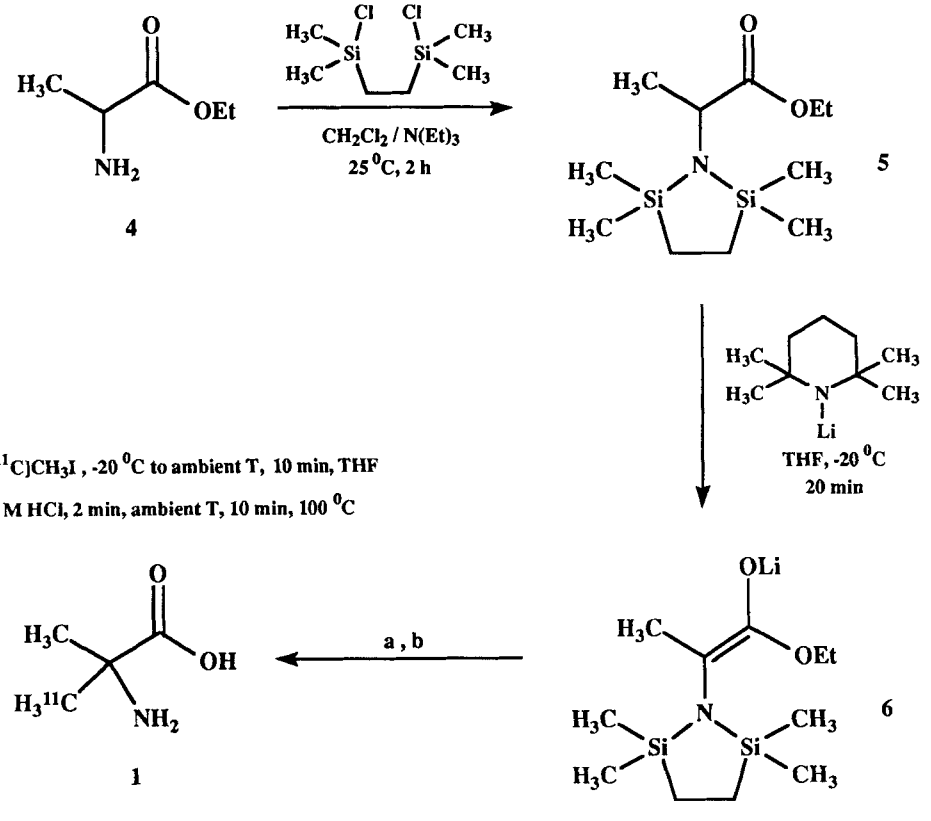

Figure 1: Novel route to α-[3-^{11}C]aminoisobutyric acid through the "stabase" adduct **6** of the parent amino acid alanine.

We chose a silicon-based group for protection of the primary amine function to prepare the precursor to the enolate **6** (Fig.1). Ethyl alanate **4** was reacted with 1,1,4,4-tetramethyl-1,4-dichlorodisilethylene which left 90% of the [1,2,5]azadisilolidinyl derivative **5**, the "stabase" adduct [13] of ethyl alanate. This had several advantages over the corresponding Schiff base derivative of **4**. It was much more readily hydrolyzed under mild acidic conditions. It also was very stable against bases which were applied for deprotonation of the α-carbon, thus allowing an increased

temperature for the reaction to the enolate **6**. Although **6** seemed to be a quite stable α-carbanion analog, it retained a stronger nucleophilic potency for the quick reaction with [^{11}C]CH_3I when compared to the more delocalized conjugated Schiff base species. This was also examined for our previously reported synthesis of racemic α-[^{11}C]methylornithine [14]. The lithiation and methylation procedure through the [1,2,5]azadisilolidinyl intermediate evidently was the more reliable labelling process. It clearly exceeded the Strecker synthesis from [^{11}C]CN- and acetone too.

Lithiation of **5** was complete within 20 min at -20 ^{0}C, methylation of **6** occured within 10 min from -20 ^{0}C to room temperature. Hydrolysis of the heterocycle took 2 min at room temperature and cleavage of the ester function needed 100 ^{0}C for 10 min. The radiochemical yield for the methylation reaction reached its maximum at 10 ± 1 min (58%). Hydrolysis, purification, and preparation of the final solution left at best a 15% yield (not corrected for decay) for **1**, related to [^{11}C]CH_3I. The total preparation time took 40 min.

The identification and characterization of α-[3-^{11}C]aminoisobutyric **1** was possible by TLC and HPLC procedures [15] by comparision with the ^{14}C-labelled analogs obtained commercially ([1-^{14}C]-**1**) or as synthesized from [^{14}C]CH_3I and **6** ([3-^{14}C]-**1**). Randomly selected preparations were hydrolyzed for 2 min at room temperature to cleave selectively the azadisilolidine ring which yielded ethyl α-[3-^{11}C]aminoisobutyrate. This was analyzed by column chromatography with the same eluent strength as applied for the final semi-preparative separation of the product (LiChrosorb RP-18, 250 x 4 mm, 7 μ, 2 mM NaH_2PO_4 buffer in 0.9% sodium chloride solution 0.5 mL·min^{-1}). The ethyl ester of **1** showed only one major peak at a retention time of 12 min under these conditions. This could be reproduced in preparations where [^{14}C]CH_3I was added to the reaction before the ^{11}C-methylation was effected. Additionally the resulting ^{11}C/^{14}C-labelled solute was collected and hydrolyzed completely (100 ^{0}C, 10 min) after one day, when the ^{11}C radioactivity had decayed. The radioactivity was then observed at the same retention time (9 min) as it was for the [1-^{14}C]-labelled NEN-standard. The solutes of the crude reaction mixture of a complete ^{11}C-labelling procedure were detected in accord with these chromatographic results. The method was then extended to a semi-preparative scale allowing the purification and separation of compound **1**. Analysis of the final parenteral solution was made by ion-pair chromatography which had a better selectivity for α-[3-^{11}C]aminoisobutyric acid (see experimental part and inset **B** in Fig. 2). Ion-pair chromatography also is suggested as the method of choice for the examination of the drug product.

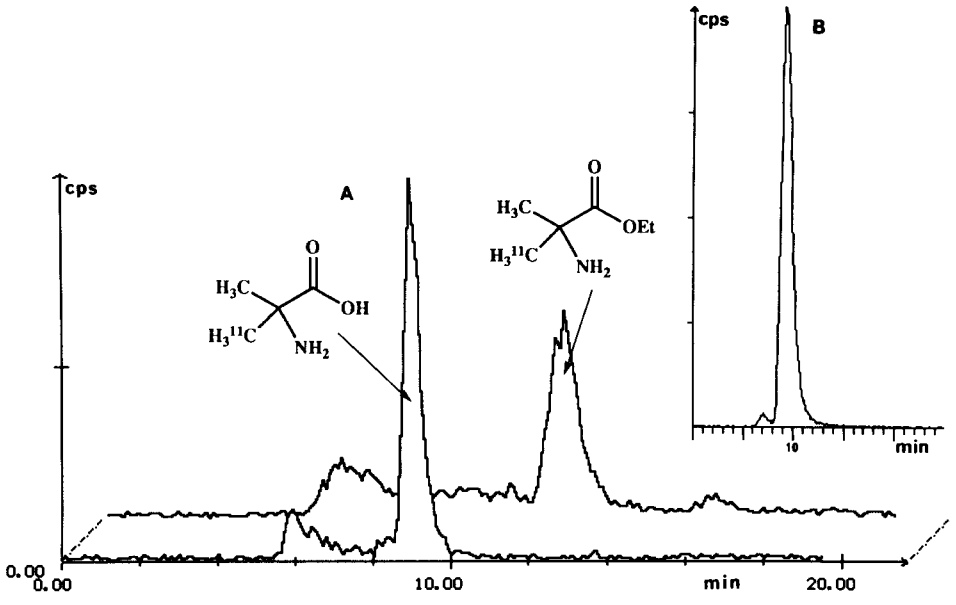

Figure 2: Radiochromatography of α-[3-^{11}C]aminoisobutyric acid and ethyl α-[3-^{11}C]aminoisobutyrate as obtained by selective hydrolysis of the protecting groups (**A**; the two traces are shifted by 1 min). Analytical ion-pair procedure of the separated and purified tracer (**B**) confirming the purity of the final product.

EXPERIMENTAL

Reagents. Chemicals, THF (dried over sodium), CH_2Cl_2, $N(Et)_3$, $LiAlH_4$, CS_2 (dried over P_4O_{10}), iodine (sublimed) and white phosphorus, were from Merck, Darmstadt, F.R.G. Ethyl alanate, 2,2,6,6tetramethyl piperidine and *n*-butyllithium (1.6 M in hexane; the molarity was examined by titration with 2-butanol against 2,2'-bipyridine as indicator [16]) were products from Fluka Chemie AG, Buchs, Switzerland. The 1,1,4,4-tetramethyl-1,4-dichlorodisilethylene was from Hüls America Inc., distributed by ABCR, Karlsruhe, F.R.G.. The ^{14}C reference materials α-[1-^{14}C]aminoisobutyric acid and [^{14}C]methyl iodide were obtained from NEN DuPont, Dreieich, F.R.G.. The corresponding α-[3-^{14}C]aminoisobutyric acid was prepared by the same method as the ^{11}C-labelled compound. Columns were from Merck, Darmstadt (LiChrosorb) or from Eurochrom Knauer, Berlin, F.R.G. (EUROCHROM SCEX).

Labelled precursor. The precursor [^{11}C]methyl iodide was produced as formerly described with a computer assisted remotely controlled apparatus (timed events, full scale 10 min) by methanolysis

of P_2I_4 [17]. The required P_2I_4 was prepared according to lit. [18]. The $[^{11}C]CO_2$ ($2.5 \cdot 10^{10}$ Bq) was reacted with $LiAlH_4$ (~5 μmol) in dry THF. Evaporation to dryness and hydrolysis using 100 μL of water left $[^{11}C]CH_3OH$ which was trapped on solid P_2I_4 (150 mg), affording $1.2 \cdot 10^{10}$ Bq of $[^{11}C]CH_3I$ when heated. It was subsequently distilled into dry THF (0.5 mL) for the following reaction with enolate **6**. Radioactive $[^{11}C]CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction from a routine stainless steel target chamber. It contained N_2 at 40 $kg \cdot cm^{-2}$ and was irradiated for 20 min with a 10-12 μA proton beam (incident energy 13 MeV). The radioactive target gas was expanded into a stainless steel capillary, immersed in liquid argon. Typically $2.5 \cdot 10^{10}$ Bq of radioactivity were obtained.

Preparation of the "stabase adduct" 2-methyl-2-(2,2,5,5-tetramethyl-[1,2,5]azadisilolidine-1-yl)-propionic acid ethyl ester **5**. 1,1,4,4-Tetramethyl-1,4-dichlorodisilethylene (10.75 g, 0.05 mol) dissolved in 20 mL CH_2Cl_2 was added to a cold (0 ^{0}C) mixture of **4**·HCl (7.7 g, 0.05 mol) and triethylamine (15.2 g, 0.15 mol) in 100 mL of CH_2Cl_2. The mixture was stirred for 2 h and allowed to reach room temperature, then filtered off from the ionic precipitate. Evaporation and vacuum distillation (80-82 ^{0}C, 0.05 torr) left 11.7 g (90%) of **5** as a colorless and stable liquid. High resolution MS (70 eV): 259.142022 (calc. 259.1423922) was in accord with $C_{11}H_{25}NO_2Si_2$.

Preparation of the lithium enolate **6**. To a cold (-20 ^{0}C) solution of 2,2,6,6-tetramethyl piperidine (0.38 ml, 0.32 g, 2.2 mmol) in 2 mL of dry THF was added 1.4 mL of *n*-butyllithium (1.6 M in hexane, equivalent to 2.2 mmol) during 10 min. It was allowed to warm up to room temperature for 10 min and cooled again to -20 ^{0}C. Compound **5** (0.57 g, 2.2 mmol) was added, through a syringe, to the solution which was then stirred for 20 min. An aliquot (1 mL) of this solution containing **6** was used for the reaction with $[^{11}C]CH_3I$.

Preparation of α-[3-^{11}C]aminoisobutyric acid **1**. Compound **6** was kept at -20 ^{0}C in a closed reaction vessel of 3 mL volume. Separately prepared $[^{11}C]CH_3I$ was trapped in 0.5 mL of dry THF and added in solution to the enolate **6** applying a smooth He pressure. The cooling was removed and the solution was stirred for 10 min. Then He was blown over the solution and heating to 100 ^{0}C was started 2 min after addition of 0.5 mL 5 M HCl to the reaction mixture. - At this point the heterocycle has been cleaved and an aliquot could be taken for examining the methylation yield by HPLC. It showed the labelled ethyl ester at t_R = 12 min (Fig. 2; **A**). - However, this was omitted for a routine preparation and instead, the methylated product was immediately taken to near dryness by heating in presence of a moderate He stream, before the hydrolysis with HCl was effected. After

neutralization of the cooled crude product with 0.5 mL of 5 M NaOH, the dark brown solution was loaded on a semi-preparative HPLC column (LiChrosorb RP-18, 250 x 10 mm, 7 μ) and eluted with 2 mM NaH_2PO_4 buffer in 0.9% sodium chloride solution at a flow rate of 3 mL·min^{-1}. The α-[3-^{11}C]aminoisobutyric acid **1** was collected at a V_R = 18 ± 2.6 mL. Radioactivity was continuously detected in the eluate by a lead shielded Geiger-Müller tube. A labelled product yield of 1.2·10^9 Bq was usually obtained in a volume of 5.2 mL at the end of synthesis. To this were added 100 μL of a 0.1 M solution of the unlabelled α-aminoisobutyric acid (equivalent to 10 μmol), in order to maintain a constant specific radioactivity for each application (2.4·10^7 Bq·μmol^{-1}·mL^{-1}). The final solution was prepared for injection by sterile filtration through a 0.25 μ filter.

Analytical procedures [15]. The ^{11}C-labelled compound **1** was analyzed, with the carrier added, by TLC: silica 60, $CH_3OH/CHCl_3/NH_3$(25%) 42/42/16 by volume, indicator ninhydrin, R_f **1**: 0.65, R_f alanine: 0.46. Radioactivity cochromatographed with the mass of α-aminoisobutyric acid. The quantitative interpretation using a Berthold TLC-Linear Analyzer showed a radiochemical purity of >98% for **1**. Analytical HPLC was run on a strong cation exchange column (EUROCHROM SCEX 125 x 4 mm, 300 Å, 7μ) with 0.01 M H_3PO_4 at 1 mL·min^{-1}. The α-[3-^{11}C]aminoisobutyric acid eluted at 3.1 min (k' = 3.4) as the first component. The system was suited for the selective determination of the final alanine contamination (α = 1.68; the alanine contamination was typically above 10 μmol and below 45 μmol). Analytical ion-pair chromatography (LiChrosorb RP-18, 250 x 4 mm, 7 μ) was also satisfactory with 1.5 mL·min^{-1} of 0.001 M H_3PO_4 (0.002 M PIC B, Waters) as eluant. It delivered the α-[3-^{11}C]aminoisobutyric acid at 10 min (k' = 5; Fig. 2, **B**). It was resolved from recovered alanine (k' = 3) and from traces of residual, unhydrolyzed α-[3-^{11}C]amino-isobutyric acid ethyl ester (k' = 7.2).

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