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CARBON-11 LABELLED ANALOGS OF ALANINE BY THE STRECKER SYNTHESIS.

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SUMMARY

Derivatives of alanine, α-[2-^{11}C]aminoisobutyric acid **1a** and α-(N-methyl)-[2-^{11}C]aminoisobutyric acid **1b** were prepared for the *in-vivo* study of amino acid transport phenomena by positron-emission-tomography (PET). Compounds **1a** and **1b** were obtained by a Zelinski-Stadnikoff variant of the Strecker α-amino acid synthesis from *in-situ* formed [^{11}C]acetone in presence of sodium cyanide and either ammonium sulfate (for **1a**) or methylamine hydrochloride (for **1b**). The complete preparation required 50 min from the end of [^{11}C]CO_2 production, and delivered 1.2 - 2 GBq of labelled product for application (2.4 - 4%; not corrected for decay; related to trapped [^{11}C]CO_2). The specific activity of the labelled products was 16 to 20 GBq·μmol^{-1}. The radiochemical and chemical purity of the preparations was greater than 98%.

Key Words: Amino acid synthesis, [^{11}C]acetone, α-[2-^{11}C]aminoisobutyric acid, α-(N-methyl)-[2-^{11}C]aminoisobutyric acid, amino acid transport tracer, PET.

INTRODUCTION

The amino acid transport system A was postulated to be a functionally relevant target of oncogene encoded protein kinases and regulator of cellular growth. The system A activity is responsive to conditions of growth and cellular energy levels, as well as to hormonal stimulation and exogenous amino acid availability [1 - 4]. Thus it is expected that changes in system A activity will reflect the biochemical environment of malignant transformation [1, 2]. Due to the lack of high

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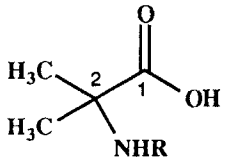
affinity substrates for specifically labelling the transport components, the system can only be evaluated and identified by kinetic means [3]. Key indicators for the system A carrier are non-racemic synthetic aminoisobutyric acids **1**. These compounds are recognized predominantly or exclusively by the sodium dependent system A [4]. Therefore, labelling of **1** with the positron emitting carbon isotope ^{11}C would allow the *in-vivo* examination of this important amino acid transport protein in tumors by positron emission tomography. We suggest that the Strecker synthesis is a practical route to the target compounds, with [^{11}C]acetone as the labelled precursor. It allows the no carrier added incorporation of the radioactive carbon into the 2-position for both compounds.



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1a: R = —H

1b: R = —CH_3

Figure 1: Two indicators for a functional study of the sodium dependent amino acid transport system A in neoplastic desease, labelled in the 2-position. α-[2-^{11}C]Aminoisobutyric acid **1a**, α-(N-methyl)-[2-^{11}C]aminoisobutyric acid **1b**.

DISCUSSION

The Bucherer-Strecker method using ammonium carbonate, acetone and cyanide in presence of labelled [^{11}C]CN^- anions was first used for producing the α-[1-^{11}C]aminoisobutyric acid [5]. The procedure essentially required the addition of cyanide carrier, and delivered primarily a labelled heterocyclic dimethyl hydantoin, from which the amino acid could be obtained by hydrolysis. Formation of the hydantoin resulted from the reaction of the corresponding α-aminoisobutyronitrile with CO_2, which was also the original procedure for obtaining dimethyl hydantoin [6]. Generally α-aminonitriles could not be isolated in the presence of CO_2. Furthermore, their synthesis required the cyanide carrier, specifically equimolar amounts of ammonia and cyanide [7, 8], but not of the carbonyl component. This was confirmed when [^{11}C]acetone was used as the labelled precursor in a Zelinski-Stadnikoff variation of the Strecker synthesis. The procedure allowed the preparation of α-[2-^{11}C]aminoisobutyric acid **1a** and α-(N-methyl)-[2-^{11}C]aminoisobutyric acid **1b**, and the isolation of the corresponding α-[2-^{11}C]aminoisobutyronitriles as well, with maintaining the required and appropriate equimolar proportion of ammonia and cyanide. A similar variant, the

Knoevenagel-Bucherer modification, was reported recently by reacting the bisulfite adduct of acetaldehyde with [^{11}C]CN$^-$ and ammonia. It produced α-[1-^{11}C]aminopropionitrile in the first step. Addition of cyanide carrier here apparently improved the yield [9].

Compounds **1a** and **1b** were obtained without addition of carrier. Accordingly, [^{11}C]acetone was reacted *in-situ* with an excess of the respective amine giving the corresponding [^{11}C]azomethine. This was immediately trapped by the addition of cyanide yielding α-[2-^{11}C]aminoisobutyronitriles which readily liberated the target compounds following hydrolysis. In particular, ammonium sulfate was added for **1a**, or methylamine hydrochloride for **1b**, and sodium cyanide, dissolved in dilute alcaline solution, to the primarily formed dry dilithium [^{11}C]ketal (Fig.2).

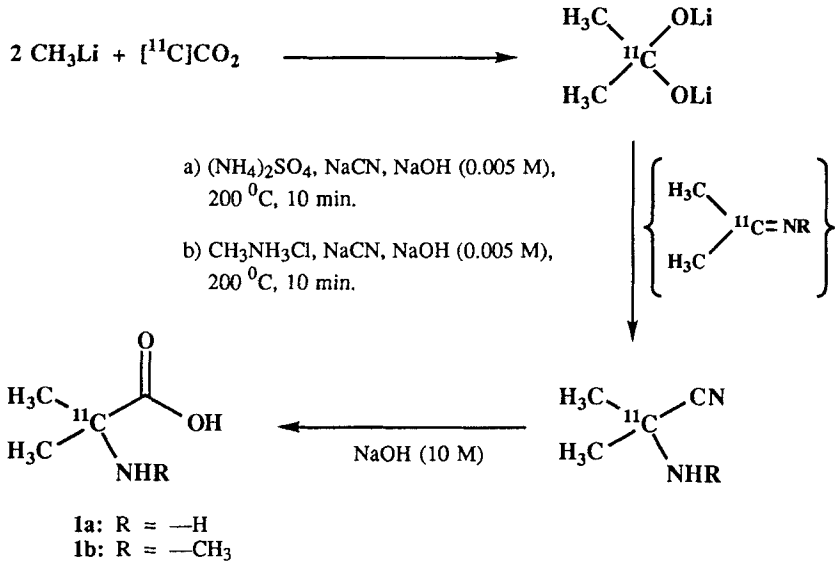

Figure 2: Complete reaction sequence for the Zelinski-Stadnikoff synthesis of α-[2-^{11}C]aminoisobutyric acid **1a** and α-(N-methyl)-[2-^{11}C]aminoisobutyric acid **1b**.

Our novel synthetic route to the [2-^{11}C]-labelled aminoisobutyric acid species captivates by simplicity and reliability. It was shown to be superior to the hydantoin reaction pathway - hydrolysis of aminonitriles usually is rapid and quantitative as compared to a hydantoin [6] - and even superior to our formerly suggested method through the lithiated azadisilolidine derivative of ethyl alanate [10]. Compared to the latter this new procedure passed off completely in aqueous solution, practically in a one-step and one-pot reaction. Therefore the synthesis of labelled aminoisobutyric acids can be handled with ease using remote control devices. The reaction also allowed

the preparation of a variety of N-mono- and N-di-alkyl substituted derivatives using a suitable amine component in the reaction mixture [11]. Within this report we demonstrated this effectively by the synthesis of compound **1b**, under conditions totally identical to the synthesis of **1a**. Compound **1b** is a most important and highly selective substrate for the *in-vivo* determination of the system A carrier. It was not available before as an ^{11}C-labelled tracer.

A preponderantly observed side product of the procedure was [1-^{11}C]acetic acid. It was the result of an incomplete conversion of [^{11}C]CO_2 to dilithium [^{11}C]ketal, resulting when only one equivalent of CH_3Li reacted with [^{11}C]CO_2. Formation of [1-^{11}C]acetic acid was favoured by a weak CH_3Li solution. An increase of [1-^{11}C]acetic acid in the crude product usually indicated that the concentration of the CH_3Li solution in use had diminished. Conversely, an excessive concentration of the CH_3Li solution produced ^{11}C-labelled *tert*-butanol along with the elimination of Li_2O at an elevated temperature. This happened in the very first step, when ether was not removed instantaneously from the solute, or by excessively long heating. The optimal balance between these extremes was found to be when more than 20 µmol and less than 25 µmol of CH_3Li were used in a total volume of 150 µL to 160 µL of ether, and with a temperature of 200 ^{0}C for a period of 1 sec inside of the heating bath.

Semi-preparative ion-pair chromatography of the complete reaction mixture, using a polymer based reversed phase column with 0.002 M H_3PO_4 as eluant in presence of heptane sulfonate, showed only one major radioactive fraction for products **1a** or **1b** (Fig. 3). The radioactivity appearing with the solvent front was [1-^{11}C]acetic acid (component 2 in Fig.3). It was followed from small contaminations which were occasionally detected at retention volumes of 30 mL to 40 mL (components 3 and 4 in Fig.3). These appeared to be ^{11}C-labelled *tert*-butanol and [^{11}C]acetone which had the same V_R in a corresponding analytical system. Finally a residue of incompletely hydrolyzed α-amino nitrile may be present in the crude product which eluted at 110 to 120 mL (not shown in Fig.3). The analytical identification of **1a** and **1b** was possible by comparing it with commercial ^{14}C-labelled reference material. Each [^{11}C]-labelled product was collected and placed into two separate vials. The contents of one vial were mixed with the corresponding [^{14}C]-labelled reference compound for a chromatographic analysis, after decay of the ^{11}C-radioactivity. The other one was analyzed immediately. No difference was observed between the retention volumes of the [^{11}C]-labelled species and their coordinated [^{14}C]-labelled reference compounds. The detection limit for unlabelled α-aminoisobutyric acid and α-(N-methyl)aminoisobutyric acid was 0.07 µmol

at 214 nm with the system used. No solute was detected below this amount. The specific activity of most of the preparations was in this range, giving values of around 0.05 μmol/GBq.

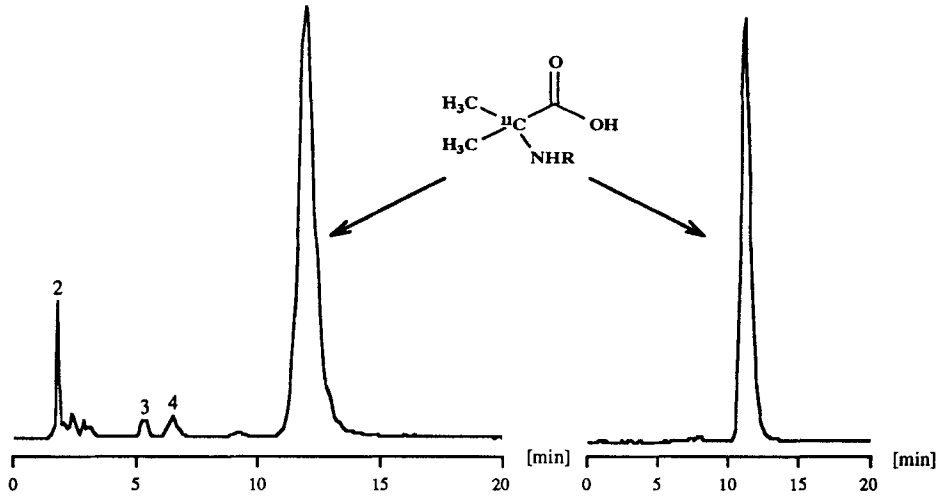

Figure 3: Ion-Pair chromatography of α-[2-^{11}C]aminoisobutyric acids **1a** and **1b**. The crude reaction product is on the left. Analytical chromatography on the right confirmed the purity of the final product after separation. Compound **1a** eluted close to **1b** (at 60 mL and 75 mL, respectively). Solute 2 was [1-^{11}C]acetic acid, 3 appeared as ^{11}C-labelled *tert* -butanol, and 4 was [^{11}C]acetone.

Some of the syntheses of **1a** were carried out - for analytical purposes - by adding 100 MBq of Na[^{14}C]CN (5 GBq/mmol; 66 μmol) and $(NH_4)_2SO_4$ to dilithium [^{11}C]ketal. This resulted in the double labelled α-[1-^{14}C, 2-^{11}C]aminoisobutyric acid, following hydrolysis and semi-preparative fractionated separation. Autoradiographic analysis of this sample revealed one radioactive peak for the α-[1-^{14}C, 2-^{11}C]aminoisobutyric acid. After decay of ^{11}C, only the crude product contained a significant amount of radioactivity which exclusively appeared to be [^{14}C]CN^-. From this experiments we concluded, that CN^- is not a contamination in the product fraction and finally in the radiopharmaceutical preparation.

EXPERIMENTAL

Reagents. Chemicals and solvents were of analytical grade and used as delivered, except ether which was dried over sodium and distilled. CH_3Li (1.6 M in ether) was partitioned into 5 mL vials

and separated from inorganic precipitates by centrifugation just before use. The [^{14}C]-labelled reference compounds, α-[1-^{14}C]aminoisobutyric acid, α-(N-methyl)-[1-^{14}C]aminoisobutyric acid and Na[^{14}C]CN, were obtained from NEN DuPont, Dreieich, Germany.

Apparatus. All radioactive procedures were carried out in an automated apparatus using a Simatic S5-95U programmable controller which was operated through the WinLab Simatic driver S5DRV in connection with the WinLab PRO software (Graf Electronic Systems; Kempten, Germany) using a standard personal computer. The complete installation allowed controlled program cycles or a step by step manual operation on a user defined Windows surface. Input/output of analog data and the final print-out of a complete synthesis protocol was possible.

Labelled precursors. Radioactive [^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction in a stainless steel target chamber. It contained N_2 at 30 kg·cm^{-2} and was irradiated for about 30 min with a 20 μA proton beam of 20 MeV. The radioactive target gas was expanded into a stainless steel capillary, immersed in liquid argon. Typically 50 GBq of radioactivity were obtained. Yield numbers were related to this radioactivity and were not corrected for decay.

[^{11}C]Acetone was prepared through the reaction of [^{11}C]CO_2 with excess CH_3Li (24 μmol, 15 μL of 1.6 M solution) in ether (150 μL) at -10 °C [12]. Subsequent strong heating for 1 sec in a bath of 200 °C, under a stream of He, left the dry dilithium salt of the expected [^{11}C]ketal for the preparation of compounds **1a** or **1b**.

*α-[2-^{11}C]aminoisobutyric acid (**1a**) and α-(N-methyl)-[2-^{11}C]aminoisobutyric acid (**1b**)*. A solution of 3 mg (61 μmol) sodium cyanide and 3 mg (23 μmol) ammonium sulfate, for the synthesis of **1a**, or 4 mg (77 μmol) of methylamine hydrochloride, for synthesis of **1b**, in 250 μL of 0.005 M NaOH was added to the dry dilithium salt of the [^{11}C]ketal. The mixture was allowed to react for 10 min at 200 °C in a closed vial (total volume 2 mL) which was mounted, instead of a sample loop, on a remotely actuated HPLC-injection valve (Rheodyne 7010), using Peek capillaries with an inner diameter of 0.2 mm. After cooling, an amount of 300 μL of 10 M NaOH was added, and the mixture was again heated for 10 min (200 °C) with the vial closed, effecting hydrolysis of the primarily formed α-[2-^{11}C]aminonitrile. The resulting solution of the product was cooled and made acidic, by the addition of 400 μL of 2 M H_3PO_4 and 400 μL of 5 M HCl prior to chromatographic purification. An average yield of 1.5 to 2 GBq of labelled **1a** was finally isolated (3 - 4%). Labelled compound **1b** was obtained with a yield of about 1.2 - 1.5 GBq (2.4 - 3%). The specific activity for

both products ranged from 16 to 20 GBq·μmol^{-1}. The total preparation time, including chromatography and pharmaceutical dressing, was 50 min after [^{11}C]CO_2 trapping.

Chromatography and analytical procedures. Pure products of **1a** and **1b** were separated from the crude reaction mixture by ion-pair HPLC (Hamilton PRP-1 column, 300 x 7 mm, 10 μ). A solution of 0.002 M H_3PO_4 containing a heptyl sulfonate counter anion (0.005 M, PIC B7, Waters) was used as eluant at a flow rate of 5 mL·min^{-1}. Compound **1a** eluted at V_R = 60 mL (k' = 4.2), compound **1b** eluted at V_R = 75 mL (k' = 5.5), both showed sharp and symmetrical peaks. The volume collected with the products was between 12 and 15 mL. It was neutralized with sterile $NaHCO_3$ solution and prepared for injection by sterile filtration. Radioactivity was detected qualitatively together with optical density at 214 nm having a Waters M481 UV-detector in line with a shielded Geiger-Müller tube. Analytical HPLC using the same type of column, with quantitation of the radioactivity on a Canberra FLOW-ONE A200 detection system (cell size 500 μL), confirmed the identity of **1a** and **1b** by comparing the respective retention volumes with those of the unlabelled and ^{14}C-labelled reference samples. Chromatography without addition of the ion-pairing reagent delivered the radioactivity totally within 0 < k' < 1.8, except the α-aminonitrile with a k' of 7.5 (V_R = 98 mL). Addition of the ion-pairing reagent moved only the labelled amino acids to significantly higher retention volumes. This behaviour also may be used to identify the amino acid components of the solution.

A radio-TLC method for the recognition of labelled amino acid products was also possible. Labelled material and reference samples (2 μL of 0.001M solutions) were spotted alternatively on a cellulose HPTLC plate (Merck). The plate was first developed with 1-butanol/acetone/water/acetic acid (5/5/3.3/1; v/v), then with acetone/1-butanol (1/1; v/v) containing ninhydrin (75 mg/mL). It was analyzed by digital autoradiography using a multiwire proportional chamber (20 x 20 cm, LB 287 DAR, EG & G Berthold) and then heated to 80 °C. The amino acid refernces components exactly matched the 1:1 copy of the autoradiographic image.

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