SYNTHESIS OF RACEMIC [2-^{11}C]PHENYLGLYCINE

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SUMMARY

The synthesis of racemic [2-^{11}C]phenylglycine, starting with $^{11}CO_2$, is reported. The [^{11}C]benzaldehyde, prepared as previously described in a two-step reaction from the corresponding [^{11}C]benzoic acid salt, was used in a modified Bücherer-Strecker reaction. The radiochemical yield of [2-^{11}C]phenylglycine in benzaldehyde-added experiments was 20 %, and in non-added experiments 6 % (corrected for decay). The overall synthesis time, starting with $^{11}CO_2$, was 50 min (including LC separation). The radiochemical purity was higher than 99 %.

Key Words: [2-^{11}C]phenylglycine, [^{11}C]phenylglycine, ^{11}C-amino acid, [2-^{11}C]amino acid, aromatic ^{11}C-amino acid

INTRODUCTION

Interest in the in vivo study of amino acid metabolism in general, and in brain in particular, has been one incentive for us to label amino acids in various positions with ^{11}C for use in positron emission tomography. Studies with ^{11}C-labelled amino acids[1] suggest that these tracers are promising agents for investigation of pancreatic function. Amino acids labelled with ^{11}C, ^{13}N and ^{18}F have been prepared and used in studies of human protein synthesis[2] or as precursors of neurotransmitters.[3]

We have been particularly interested in the possibility of synthesizing amino acids labelled with ^{11}C in various

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positions, in enantiomeric pure or enriched form. The only ^{11}C-amino acid so far obtained by organic synthetic methods in pure enantiomeric form is D or L-[methyl-^{11}C]methionine.[4] L-[3-^{11}C]Alanine was prepared in 48 % e.e. (enantiomeric excess = L/D 74/26 %).[5]

The asymmetric synthesis of L-[3-^{11}C]phenylalanine using chiral hydrogenation catalysts was recently reported;[6] the synthetic route was similar to the one used in the synthesis of racemic [3-^{11}C]phenylalanine and [3-^{11}C]DOPA.[7]

However, employing enzymatic reactions, L-[4-^{11}C]aspartic acid was prepared from $^{11}CO_2$ by the use of phosphoenolpyruvate carboxylase and glutamic/oxaloacetic acid transaminase immobilized on Sepharose supports, as reported by Barrio et al.[8]

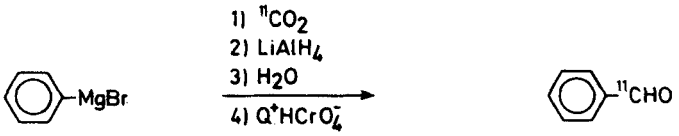
The development of a high-pressure, high-temperature modification of the Bücherer-Strecker synthesis for production of [1-^{11}C]amino acids by Hayes et al.[9] has opened interesting ways of preparing ^{11}C-amino acids, labelled in different positions.

In this work, the Bücherer-Strecker approach was used to label the amino acid with ^{11}C in a position other than the carboxylic. This was attained by the use of ^{11}C-aldehyde instead of ^{11}C-cyanide as the labelled precursor.

In the Bücherer-Strecker reaction, a racemic mixture of the amino acid is obtained, but the ^{11}C-labelled racemic amino acid mixture can be resolved by oxidative deamination,[10] LC separation employing a chiral mobile phase[11] or by using human serum albumin coupled to a Sepharose resin.[12] DL-[1-^{11}C]DOPA was recently resolved using a chiral stationary phase consisting of a polymer-bound (L-proline)$_2$-Cu complex.[13]

SYNTHETIC PATHWAYS

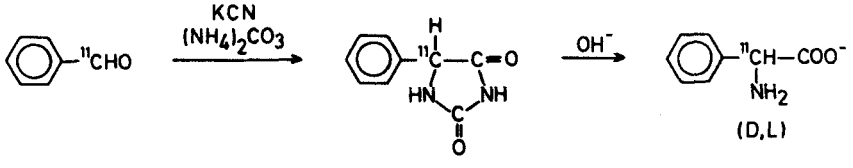
[^{11}C]Benzaldehyde was prepared from the corresponding [^{11}C]benzoic acid salt via the [^{11}C]benzyl alcohol, starting with $^{11}CO_2$, according to Scheme 1.



Scheme 1

The [^{11}C]benzoic acid salt was obtained by trapping [^{11}C]carbon dioxide in the phenylmagnesium bromide solution. The labelled acid salt was reduced directly with lithium aluminium hydride (LAH) to give the corresponding alcohol. The [^{11}C]benzaldehyde was then produced by using tetrabutylammonium hydrogen chromate with 3 M sulfuric acid/ethyl acetate as solvent in an ion-pair oxidation.

The solution containing the labelled aldehyde was washed with diluted base and was then concentrated. It was used as such, with ammonium carbonate and potassium cyanide, in the hydantoin formation step, according to Scheme 2. The labelled hydantoin was hydrolysed with strong base to give the amino acid.



Scheme 2

RESULTS

[^{11}C]Benzaldehyde was prepared routinely in a radiochemical yield > 95 % within 5 min, starting from the corresponding [^{11}C]acid salt, as reported elsewhere.[14] The trapping efficiency of [^{11}C]carbon dioxide in the Grignard solution depends on the concentration of the latter, the choice of solvent, temperature and gas flow. The trapping efficiency varied between 83 % and 87 % of total radioactivity when carried out with 0.20 M phenylmagnesium bromide in ether at 0 °C.[14]

From the end of trapping the [^{11}C]carbon dioxide in the Grignard solution, the total time was 50 min (including 10-12 min for the transportation of the Grignard reaction vessel to the chemistry laboratory, and approximately 15-20 min for LC separation, evaporation, pH adjustment and sterile filtration).

As reported by other workers,[15] radiochemical yields of the hydantoin formation were found to be dependent on the reaction temperature. Reaction temperatures above 240 °C resulted in decomposition of the hydantoin, while lowering the temperature below 200 °C decreased the yield. No significant increase in yield was obtained with reaction times greater than 5 min in either the hydantoin formation step or in the hydrolysis.

Increasing the amount of added benzaldehyde from 0.1 mmol to 0.3 mmol did not significantly increase the yield of hydantoin. However, addition of 0.05 mmol benzaldehyde lowered the radiochemical yield to the level of the non-carrier added experiments. The decay corrected radiochemical yield was 20 % for [2-^{11}C]phenylglycine in carrier-added experiments, and 6 % in non-carrier-added experiments. In a typical production run, about 4-7 mCi [2-^{11}C]phenyl-

glycine was isolated, starting with 100-200 mCi [^{11}C]carbon dioxide. The radiochemical purity was higher than 99 %.

This method may be applied for other 2-labelled amino acids if the appropriate ^{11}C-labelled aldehyde can be prepared.

EXPERIMENTAL

General. The ^{11}C was produced at the Tandem Van de Graaff accelerator at the University of Uppsala by means of the ^{14}N(p,α)^{11}C reaction on a nitrogen gas target. The [^{11}C]-carbon dioxide was trapped in a reaction vessel containing the phenylmagnesium bromide solution. When trapping was completed, the reaction vessel was transported to the chemistry laboratory.

The Bücherer-Strecker reaction was performed in a stain-less-steel reaction vessel (Figure 1).

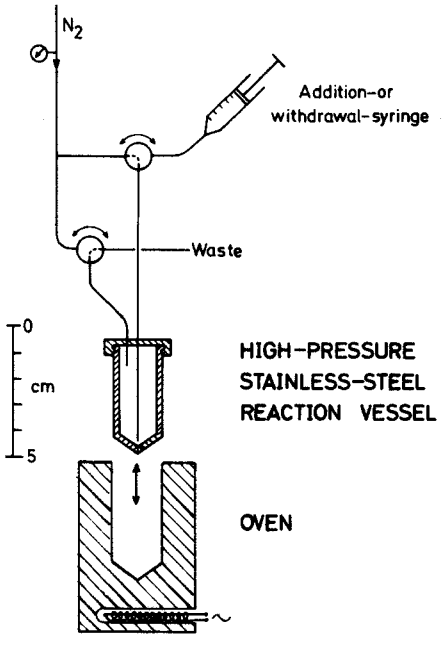


Figure 1. High-pressure, stain-less-steel reaction vessel used in the Bücherer-Strecker synthesis.

Analytical LC was performed on a Hewlett-Packard 1084B equipped with a 250 x 4.6 mm Spherisorb C-18 μm column or a 150 x 3.9 mm Spherisorb C-18 μm column (for OPA derivatization), a variable wavelength detector, a Waters fluorescence detector (model 420, e.g., 440 nm) in series with a β-flow detector.[16] Preparative LC was carried out on a Waters system equipped with a 250 x 10 mm Spherisorb C-18 30 μm column, UV detector M-441 and GM detector. The following solvents were used in the LC applications: (A) aqueous 0.1 M ammonium formate, pH 3.5, (B) methanol, (C) methanol/THF/0.05 M sodium acetate, 0.05 M disodium hydrogen phosphate, pH 7.5, 2/2/96, (D) methanol/water, 65/35 and (E) 0.01 M ammonium formate, pH 3.5.

[^{11}C]Benzaldehyde[14] (Scheme 1). [^{11}C]Carbon dioxide was trapped in a reaction vessel containing 2.5 ml of 0.20 M phenylmagnesium bromide in ether at 0 °C. The solution containing the magnesium salt of the labelled acid was reduced directly (1 min) by means of 0.4 ml of 1.0 M lithium aluminium hydride (LAH) in ether. The aqueous phase was removed after hydrolysis of the LAH complex with 2.0 ml water and addition of 1.0 ml ether. The ethereal phase containing the [^{11}C]benzyl alcohol was oxidized (1 min) with a two-phase mixture containing 2.0 ml ethyl acetate, 1.0 ml 3 M sulfuric acid, 0.25 g (0.74 mmol) tetrabutylammonium hydrogen sulfate and 0.50 g (1.7 mmol) sodium dichromate. The organic phase was washed once with 2.0 ml 0.25 M sodium hydroxide and twice with 2.0 ml 0.005 M sodium hydroxide.

LC analysis was carried out using the 250 x 4.6 mm C-18 column, employing A and B as solvents, and the following program: flow 3.0 ml/min, UV 257 nm, column temperature

60 °C, LC gradient time 0-4.5, A/B from 65/35-60/40; time 4.5-5.0, A/B from 60/40-20/80; time 5.0-9.0, A/B 20/80 isocratic. The retention times for benzoic acid, benzyl alcohol and benzaldehyde were about 2.4, 2.1 and 4.1 min, respectively.

[2-^{11}C]Phenylglycine (Scheme 2). The [^{11}C]benzaldehyde obtained as described above was then used as follows. To the solution containing the labelled aldehyde (and > 11 mg (0.10 mmol) benzaldehyde if carrier is added), concentrated by nitrogen flow for 2 min in the stainless-steel reaction vessel, a solution containing 2.0 ml 0.005 M sodium hydroxide, 320 mg (3.36 mmol) ammonium carbonate, 13.5 mg (0.25 mmol) ammonium chloride and 70 mg (1.07 mmol) potassium cyanide was added. The mixture was flushed with nitrogen for 1 min at approximately 70 °C. The reaction vessel was closed and heated at 220 °C for 5 min. The vessel was cooled and 1.0 ml of 6.25 M sodium hydroxide was added. The vessel was resealed and heated to 220 °C for another 5 min. After cooling, the mixture was neutralized with concentrated hydrochloric acid and filtered. The resulting homogeneous solution was chromatographed by preparative LC using an isocratic system and E as buffer to give [2-^{11}C]phenylglycine (UV 254 nm, flow 8 ml/min, column temperature 25 °C). After evaporation of the appropriate LC fractions, addition of sterile buffer, pH adjustment and sterile filtration, the [2-^{11}C]phenylglycine was ready for biomedical studies.

The radiochemical purity was determined by LC using the following programs: (1) (250 x 4.6 mm C-18 column) (A,B), flow 3.0 ml/min, UV 257 nm, column temperature 60 °C, LC gradient time 0-4.5, A/B 95/5 isocratic; time 4.5-5.0, A/B from 95/5-20/80; time 5.0-9.0, A/B 20/80 isocratic. (2) (150 x 3.9 mm C-18 column, OPA derivatization, fluorescence

detector) (C,D), flow 1.5 ml/min, column temperature 35 °C, LC gradient time 0-4.0, C/D from 70/30-60/40; time 4.0-15.0, C/D from 60/40-10/90; time 15.0-16.0, C/D from 10/90-70/30.

The OPA reagent[17] was made up of 1.07 g o-phthaldialdehyde and 0.85 ml 2-mercaptoethanol dissolved in 4.0 ml methanol. The volume was adjusted to 10 ml with 0.4 M sodium borate buffer, pH 10.0 (0.8 M). Samples of 250 µl were taken from the isolated [2-^{11}C]phenylglycine solution and derivatized by adding 250 µl of the OPA reagent (> 200-fold excess) and 500 µl 0.4 M sodium borate buffer. After a reaction time of 2 min at room temperature, 10 µl of the mixture was injected into the LC column.

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