SYNTHESIS OF RACEMIC [3-^{11}C]-LABELLED ALANINE, 2-AMINOBUTYRIC ACID, NORVALINE, NORLEUCINE, LEUCINE AND PHENYLALANINE AND PREPARATION OF L-[3-^{11}C]ALANINE AND L-[3-^{11}C]PHENYLALANINE

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SUMMARY

The syntheses of racemic [3-^{11}C]-labelled alanine, 2-aminobutyric acid, norvaline, norleucine, leucine and phenylalanine are reported. The reactions were performed by a phase-transfer alkylation reaction on *N*-(diphenylmethylene)-glycine *tert*-butyl ester with the appropriate ^{11}C-alkyl iodides followed by acidic hydrolysis and the labelled amino acids were obtained in 10-55 % radiochemical yield. L-[3-^{11}C]-Alanine and L-[3-^{11}C]phenylalanine were obtained in 99 % enantiomeric excess after treatment of the corresponding racemic mixture by D-amino acid oxidase immobilized on glutaraldehyde-activated glass beads.

In a typical run starting with 120 mCi [^{11}C]carbon dioxide, 25 mCi [1-^{11}C]benzyl iodide was prepared and used to give 6 mCi of DL-[3-^{11}C]phenylalanine within 50 minutes. Following treatment with D-amino acid oxidase, 0.3 mCi of L-[3-^{11}C]phenylalanine was obtained after a total synthesis time of about 100 minutes.

Key words: [3-^{11}C]-labelled amino acids, L-[3-^{11}C]-phenylalanine, L-[3-^{11}C]alanine.

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0362-4803/87/020125–19$09.50
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Received May 8, 1986
Revised June 12, 1986

INTRODUCTION

Synthetic work with short-lived positron-emitting nuclides, such as ^{11}C ($t_{1/2}$ = 20.4 min) and ^{18}F ($t_{1/2}$ = 110 min), is a great challenge to the organic chemist in view of the special conditions which have to be observed.[1]

Positron emission tomography (PET), together with the development of models for *in vivo* biochemical processes, particularly in the brain, has created a need for methods useful in the specific labelling of endogenic compounds and pharmaceuticals with these radionuclides.

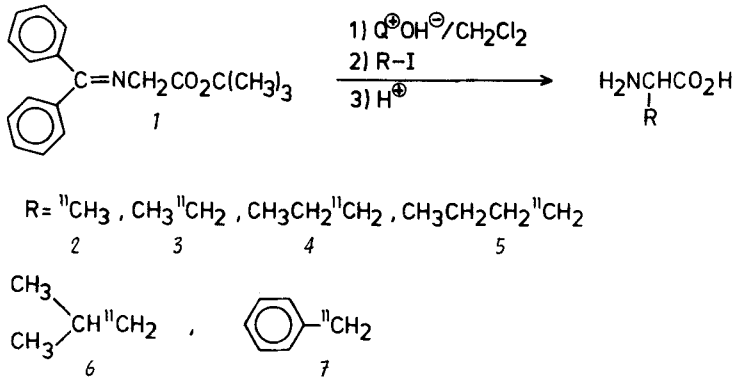
In our work, we are trying to develop general methods for the preparation of ^{11}C-amino acids labelled in various positions. The position of the label in the amino acids may be important in certain cases. We have mainly been focusing on labelling in the 3-position, using ^{11}C-alkyl iodides. Labelling in the carboxylic position can be obtained by, for example, a Bücherer-Strecker synthesis.[2]

The enantiomeric purity of the prepared ^{11}C-amino acids is of special interest. The synthesis of D and L-[methyl-^{11}C]-methionine illustrates the preparation of ^{11}C-labelled amino acids, where the stereochemistry is already known prior to the labelling synthesis.[3] Enantiomerically enriched ^{11}C-amino acids, such as L-[3-^{11}C]alanine[4a,b] and L-[3-^{11}C]phenylalanine,[5] have been prepared by different routes of asymmetric syntheses. Enzymatic methods have been utilized in the synthesis of L-[4-^{11}C]aspartic acid[6] by Barrio et al., and recently in the synthesis of L-[3-^{11}C]-serine by Svärd et al.[7] In these reactions the required stereochemical form was produced during the synthesis.

Racemic ^{11}C-amino acids labelled in various positions have also been prepared.[8a-k] By using fast resolving

methods, such as LC or enzymatic procedures, the enantiomeric pure ^{11}C-amino acids have been obtained.[8d,e,f,j]

This paper presents the syntheses of [3-^{11}C]-labelled amino acids by a phase-transfer alkylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester *1* with the corresponding ^{11}C-alkyl iodides, [^{11}C]methyl,[1,9] [1-^{11}C]ethyl, [1-^{11}C]-propyl, [1-^{11}C]butyl, [1-^{11}C]isobutyl, and [1-^{11}C]benzyl iodide (Scheme 1).[10] The synthesis of [1-^{11}C]benzyl iodide will be presented elsewhere. The racemic ^{11}C-amino acids: [3-^{11}C]alanine *2*, 2-amino[3-^{11}C]butyric acid *3*, [3-^{11}C]-norvaline *4*, [3-^{11}C]norleucine *5*, [3-^{11}C]leucine *6* and [3-^{11}C]phenylalanine *7*, were prepared in 10-55 % radiochemical yields with 93-99 % radiochemical purity.



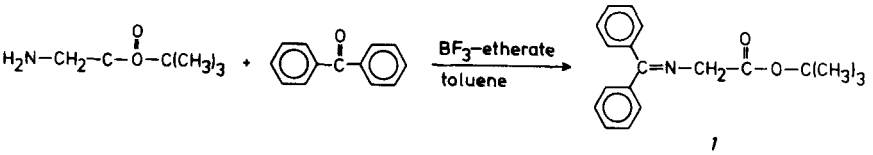
Scheme 1

The resolution of the corresponding racemic amino acids by D-amino acid oxidase (EC 1.4.3.3) immobilized on glutaraldehyde-activated glass beads[11] (CPG-500-80) yielded L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine in 99 % enantiomeric excess, as determined by enantiomeric analysis using GC (*N*-trifluoroacetylalanine methyl ester) or LC ([3-^{11}C]phenylalanine as free acid).

The L-^{11}C-amino acids produced will be used to study *amino acid transport and protein synthesis in the brain.*[12] Work is in progress to resolve the non-proteinogenic amino acids *3-5* by D-amino acid oxidase.

RESULT AND DISCUSSION

The protected glycine derivative, *N*-(diphenylmethylene)-glycine *tert*-butyl ester *1*, synthesized according to the route in Scheme 2, was obtained as colourless crystals in 85 % yield with a chemical purity, determined by GC, higher than 99 %.



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Scheme 2

This compound proved to be a useful substrate in the preparation of the racemic ^{11}C-amino acids *2-7* from the corresponding ^{11}C-alkyl iodides by the route shown in Scheme 1.

The radiochemical yield and purity of the ^{11}C-amino acids obtained in the crude products correlate strongly to the radiochemical yield and purity of the corresponding ^{11}C-alkyl iodide used. Since the main labelled impurities in the ^{11}C-alkyl iodide preparations were other ^{11}C-alkyl iodides, small amounts of the corresponding labelled amino acids were obtained in the crude products of *3-7*. [^{11}C]Methyl iodide, the major impurity in preparations of the alkyl iodides used in the syntheses of ^{11}C-amino acids *4-6*, was present in 0-25 % radiochemical yield. [1-^{11}C]Ethyl iodide used in the syn-

thesis of compound *3* had [2-^{11}C]isopropyl iodide as the most abundant labelled impurity. Thus, in the syntheses of *3*-*7*, 0-25 % of *2* was formed from [^{11}C]methyl iodide. Moreover, in the synthesis of *3*, [3-^{11}C]valine was obtained in 3-8 % radiochemical yield from the by-product [2-^{11}C]isopropyl iodide.

The crude products of compounds *2*, *4*, and *7* usually had radiochemical purities higher than 95 %. Here, the amounts of other labelled amino acids were usually less than 1 %. Compounds *3*, *5* and *6*, on the other hand, were obtained in 60-85 % radiochemical purity before purification. By preparative LC, the racemic ^{11}C-amino acids were obtained in 10-55 % radiochemical yield with 93-99 % radiochemical purity.

Instead of the rather time-consuming preparative LC procedure, a procedure utilizing a Sep-Pak® C_{18} column was used in the purification of *2*, *4* and *7*, resulting in radiochemical purities higher than 97 %. In those cases where the impurities mainly consisted of other ^{11}C-amino acids, i.e., in the synthesis of *3*, *5* and *6*, preparative LC was the preferred purification method.

The alkylation reactions were dependent on substrate, base and catalyst concentrations, reaction temperature, efficiency of phase mixing, and most important, the reactivity of the alkyl iodide.

On addition of the base solution containing the catalyst tetrabutylammonium hydrogen sulphate (TBAH) to the substrate in dichloromethane, a yellow colour immediately appeared, indicating the formation of the corresponding carbanion of *N*-(diphenylmethylene)glycine *tert*-butyl ester. In the synthesis of *7*, up to five equivalents of TBAH were occasionally required to produce the colour change. By performing "cold"

experiments, it was ascertained that alkylation of the substrate did not occur if one equivalent of iodide ions was added. A plausible explanation for the non appearance of colour on the addition of the base and catalyst may be that the iodide ions combine with TBA^+ to form a lipophilic ion-pair that is distributed mainly in the organic phase and reduce the efficiency of extraction of the hydroxide ions into the organic phase. The iodide ions in the dichloromethane that were causing this problem were obtained from hydriodic acid. This problem could be avoided by using freshly distilled hydriodic acid, and thus reducing the amount of iodine transferred to the organic phase.

For the most reactive ^{11}C-alkyl iodides, [^{11}C]methyl iodide and [1-^{11}C]benzyl iodide, the alkylation reaction time needed to reach quantitative yield of the product under the described reaction conditions was 3-5 minutes. The alkylation reactions with [1-^{11}C]ethyl iodide and [1-^{11}C]-propyl iodide were quantitative within 10 min at 40-45 °C whereas [1-^{11}C]butyl iodide and [1-^{11}C]isobutyl iodide required about 15 min at the same temperature for conversion to the wanted product in 75 % radiochemical yield (counted on the amount of ^{11}C-alkyl iodide).

Removal of the protecting groups to liberate the ^{11}C-amino acids was performed quantitatively by hydrolysis with 6 M hydrochloric acid within 5 min, at 130 °C, for all the ^{11}C-amino acids. In Table 1, the radiochemical yields and purities of the ^{11}C-amino acids are listed together with the experimental conditions used.

Several authors have shown the usefulness of amino acid oxidases (D or L) in the preparation of L and D ^{11}C-amino acids.[8d,e,j] In this paper, the D-amino acid oxidase was used to prepare L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine

Table 1. Radiochemical yield and purity of the DL-[3-^{11}C]-amino acids and experimental conditions used in the alkylation reaction.

Amino acid	Conc. 1 (M)	Alkylation reaction time (min)	Radiochemical yield (%)*	Radiochemical purity (%)**
[3-^{11}C]Alanine	0.14	5	55	99
2-Amino-[3-^{11}C]-butyric acid	0.23	10	37	93
[3-^{11}C]Norvaline	0.62	10	45	98
[3-^{11}C]Norleucine	0.62	15	30	94
[3-^{11}C]Leucine	0.62	15	10	97
[3-^{11}C]Phenylalanine	0.23	5	25	99

* Counted on the decay corrected amount of ^{11}C-amino acid in per cent of [^{11}C]carbon dioxide released from the molecular sieves.

** Determined by LC of the preparative LC-purified product.

from the corresponding racemic ^{11}C-amino acids prepared by the methods described above. The enzymatic reaction was completed within 10-20 min. Separation of the ^{11}C-amino acid from the corresponding ^{11}C-α-keto acid was carried out by means of a cation exchange resin, giving the labelled product in 99 % radiochemical purity and 99 % enantiomeric excess (e.e.), as determined by LC and GC analyses.

Figure 1 shows the chromatograms obtained from the enantiomeric analysis of [3-^{11}C]phenylalanine. An estimate of the e.e. was obtained by comparing the ^{11}C-α-keto acid formed with the remaining amount of ^{11}C-amino acid, using analytical LC, and is exemplified by the resolution of DL-[3-^{11}C]alanine in Figure 2.

The [3-^{11}C]-α-keto acids decomposed slowly during the reaction probably as a result of the formation of peroxides.

The compound eluting at 4.9 min in Figure 2 is probably formed in that way. Decomposition seems to be avoided by the use of an enzyme preparation in which catalase (EC 1.11.1.6) is co-immobilized with D-amino acid oxidase. The resolution of DL-[3-^{11}C]phenylalanine using 150 units of *D-amino* acid oxidase (determined before immobilization) required 15-20 min to reach an e.e. of 99 %, whereas in the resolution of [3-^{11}C]alanine, 99 % e.e. was attained within 10 min.

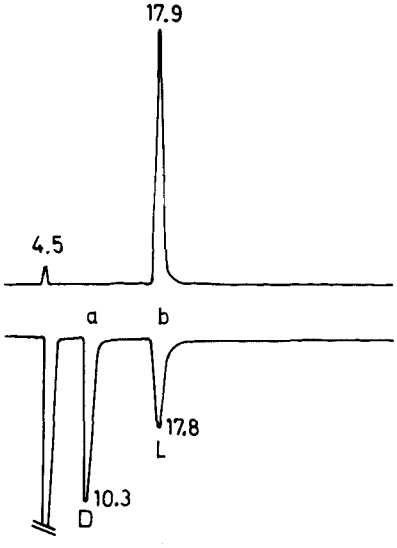

Figure 1. Enantiomer analysis of L-[3-^{11}C]phenylalanine by LC. Upper row: signal from radiodetector; lower row, UV detector; with retention times in min (reference: a = D-phenylalanine, b = L-phenylalanine).

Starting with 100-150 mCi [^{11}C]carbon dioxide, 3-30 mCi of the racemic ^{11}C-amino acids were obtained with total reaction times in the order of 30-50 min. The yields of the L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine were 1-2 mCi and 0.2-0.4 mCi, respectively, with a total synthesis time of 70-100 minutes.

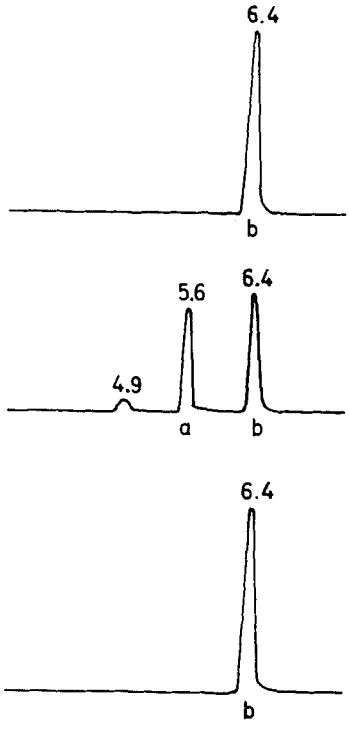

Figure 2. Radiodetector signals: (a) separation of [3-^{11}C]-pyruvic acid, and (b) separation of [3-^{11}C]alanine. Upper row: purified DL-[3-^{11}C]alanine; middle row: after treatment with D-amino acid oxidase; lower row: purified L-3-^{11}C]-alanine.

The radiochemical yields were determined on the decay-corrected, purified product and related to the amount of [^{11}C]carbon dioxide released from the molecular sieves. The identity of the labelled products was confirmed by LC analysis. In all cases the radioactive signal was simultaneous with the UV signal from added reference compound (corrected for time delay between UV and β-flow detector).

The results presented show that the phase-transfer alkylation method is a reliable and practical synthetic pathway to the [3-^{11}C]-labelled racemic amino acids 2-7. It is possible to further extend this method to any ^{11}C-alkyl

iodide, and thus provide access to other [3-^{11}C]-labelled amino acids. However, the reaction times for the less reactive ^{11}C-alkyl iodides will be longer. [3-^{11}C]Valine has been synthesized by the phase-transfer alkylation reaction described here using *1* and [2-^{11}C]isopropyl iodide and will be presented elsewhere.[13]

Exchanging the achiral substrate *N*-(diphenylmethylene)-glycine *tert*-butyl ester used for a chiral one is an interesting approach for obtaining the enantiomerically enriched or pure ^{11}C-amino acids directly. This work is in progress using *N*-(diphenylmethylene)glycine (-)-8-phenylmenthol ester in a phase-transfer alkylation reaction.

EXPERIMENTAL

General

The ^{11}C was prepared by the ^{14}N(p ,α)^{11}C reaction on a nitrogen gas target at the tandem Van de Graaff accelerator at the University of Uppsala and obtained as [^{11}C]carbon dioxide. The [^{11}C]carbon dioxide formed was trapped in lead-shielded 4 Å molecular sieves and transported to the chemistry laboratory.

Analytical LC was performed on a Hewlett-Packard 1090 or a 1084 liquid chromatograph equipped with any of the following columns: (A) 250x4.6 mm (i.d.) Supelco LC-NH_2 5 µm column, (B) Altech 250x4.6 mm (i.d.) C_{18} 10 µm column or (C) CHIRALPAK WH (Daicel Chem. Ind. Ltd) 250x4.6 mm (i.d.) 5 µm column in series with a β-flow detector.
Ammonium formate (0.05 M, pH 3.50) (D), methanol (E), potassium dihydrogen phosphate (0.01 M, pH 4.6) (F) and acetonitrile/water (500:70, v:v) (G), copper sulphate (0.25 mM) (H) were used as mobile phases.
Preparative LC was carried out on a Waters system equipped

with a M-441 UV detector in series with a GM tube and a 250x10 mm C_{18} Nucleosil 30 µm column (I).
Analytical GC was carried out on a Hewlett-Packard 5880 A gas chromatograph equipped with either a 175x0.3 cm, 5 % SP-300 100/120 Supelcoport glass column (J) or a 70x0.3 cm 3 % PS-400/chrom W HP 80/100 glass column (K), in series with a β-gasflow detector.[14]
Tetrahydrofuran (THF) used as solvent in the Grignard reactions was dried by standard procedures prior to use. Dichloromethane (CH_2Cl_2) used as solvent in the alkylation reaction was purified by passing through an aluminium oxide column (basic grade 1). - NMR spectra were performed on a Jeol FX-60 NMR-spectrometer.

SYNTHETIC PROCEDURE

N-(Diphenylmethylene)glycine *tert*-butyl ester (*1*)

Glycine *tert*-butyl ester was prepared according to the procedure used to synthesize proline *tert*-butyl ester, as described in reference 15. Benzophenone (3g, 16.5 mmol) and glycine *tert*-butyl ester (2g, 15.2 mmol) were dissolved in 25 ml toluene and 50 µl of boron trifluoride etherate was added as catalyst. The reaction mixture was refluxed for 8 hours while the water formed was removed by azeotropic distillation. The reaction mixture was washed with 5 ml of 5 % aqueous citric acid followed by 2x5 ml of brine. The organic phase was separated and dried over magnesium sulphate. The solid material obtained after evaporation to dryness was crystallized twice in hexane/diethyl ether (20:1, v:v), giving the product in 85 % yield (m.p. 115-117 °C). The product was identified as *N*-(diphenylmethylene)-glycine *tert*-butyl ester by the use of ^{1}H and ^{13}C NMR spectroscopy. Analytical GC with column K under the follow-

ing conditions: flow 30 ml/min (N_2), oven temperature 150 °C, showed a purity higher than 99 %.

[1-^{11}C]-Labelled alkyl iodides[1,10]

The [1-^{11}C]-labelled alkyl iodides were prepared in one-pot reaction systems from [^{11}C]carbon dioxide and the corresponding alkylmagnesium bromides, followed by a lithium aluminium hydride (LAH) reduction and conversion to the ^{11}C-alkyl iodide by refluxing with 54 % hydriodic acid as reported in detail elsewhere.[10] [^{11}C]Methyl iodide was obtained by trapping [^{11}C]carbon dioxide directly in a solution of LAH in THF. The ^{11}C-labelled alkyl iodides; [^{11}C]-methyl, [1-^{11}C]ethyl, [1-^{11}C]propyl, [1-^{11}C]isobutyl and [1-^{11}C]butyl iodide, were transferred in a stream of nitrogen gas from the reaction flask through a drying tower (sodium hydroxide/phosphorus pentoxide) and trapped in dichloromethane cooled at -78 °C.

[1-^{11}C]Benzyl iodide was extracted from the hydriodic acid solution with dichloromethane. The organic phase was washed once with water and then passed through a 5x0.5 cm column filled with sodium disulphite/aluminium oxide (basic grade 1), approximately 30/70 by volume.

The radiochemical purity was determined by analytical LC under the following conditions: column B, solvents D/E, 50/50 (v/v) 0-5 min, gradient 5-7 min D/E, 20/80 (v/v), flow 2 ml/min, column temperature 40 °C, wavelength 254 nm.

DL-[^{11}C]Alanine (2) Table 1, Scheme 1

In a 2-ml conical glass flask equipped with a rubber septum, 20 mg (0.068 mmol) of *1* was dissolved in 0.5 ml dichloromethane and cooled to -78 °C. [^{11}C]Methyl iodide, prepared by the routine procedure, was transferred by a stream of nitrogen gas to the reaction vessel. A solution of 10 mg

tetrabutylammonium hydrogen sulphate (TBAH, 0.037 mmol) in 0.35 ml 10 % aqueous sodium hydroxide solution (w:v) was added and the alkylation reaction was performed in an ultrasonic bath at 45 °C for 5 min. The organic phase was separated and transferred to a 7-ml glass vessel containing 0.8 ml 6 M hydrochloric acid and heated at 130 °C for 5 min, allowing the dichloromethane to slowly distil off.

The DL-[3-^{11}C]alanine obtained was purified either by preparative LC using column I and solvent D (room temperature, flow 4 ml/min), or by passing the solution obtained after hydrolysis through a Sep-Pak® C_{18} column, which retained lipophilic impurities (labelled or otherwise).

The purity of the alkylation product was controlled by the use of analytical LC employing column B, under the following conditions: flow 1.5 ml/min, solvents D/E, 15/85, (v/v), column temperature 40 °C, wavelength 254 nm. The purified DL-[3-^{11}C]alanine identified with the use of authentic material as reference, was analysed by means of column A and solvents F and G, under the following conditions: flow 2 ml/min, F/G, 5/95 (v/v) gradient 0-6 min F/G, 40/60 (v/v), column temperature 40 °C, wavelength 230 nm.

2-Amino[3-^{11}C]butyric acid (*3*), [3-^{11}C]norvaline (*4*), [3-^{11}C]norleucine (*5*) and [3-^{11}C]leucine (*6*), Table 1, Scheme 1

Compounds *3-6* were synthesized from 1 and the corresponding ^{11}C-alkyl iodides: [1-^{11}C]ethyl, [1-^{11}C]propyl, [1-^{11}C]butyl, and [1-^{11}C]isobutyl iodides. The ^{11}C-alkyl iodides, prepared according to the description above, were transferred in a stream of nitrogen gas to a conical 2-ml glass vessel

equipped with a rubber septum and trapped in 0.4 ml dichloromethane at -78 °C. Compound *1* and one equivalent of TBAH (counted on the molar amounts of *1*), dissolved in 0.35 ml dichloromethane and 0.40 ml 20 % aqueous sodium hydroxide (w:v) solution, respectively, were added. The alkylation reaction was performed in an ultrasonic bath at 45 °C for 10-15 min. The organic phase was separated and transferred to a 7-ml glass vessel containing 0.8 ml of 6 M hydrochloric acid and heated at 130 °C for 5 min.

The alkylation reaction was followed by means of analytical LC using column B with solvents D and E (same conditions as for compound *2*). Preparative LC (column I and solvent D, flow 4 ml/min at room temperature) or a C_{18} Sep-Pak® column were used to purify the products. With the analytical LC conditions described in the synthesis of *2*, and adding reference compounds, it was possible to determine the radiochemical purity of compounds *3*-*6*.

DL-[3-^{11}C]Phenylalanine (*7*), Scheme 1, Table 1

In a 7-ml flask equipped with a screw-cap, 30-50 mg of *1* (0.1-0.17 mmol) was placed. [1-^{11}C]Benzyl iodide, prepared according to the procedure described above, in 2-5 ml dichloromethane was added. A solution of 50-300 mg TBAH (0.15-0.9 mmol) in 1-2 ml 20 % aqueous sodium hydroxide solution (w:v) was introduced and the alkylation reaction was performed using an ultrasonic bath at 45 °C for 5 min. After addition of 2 ml water, the organic phase was separated and transferred to another 7-ml flask containing 0.8 ml 6 M hydrochloric acid. The mixture was heated at 130 °C for 5 min, allowing the dichloromethane to distil off during the hydrolysis. The product was purified by the Sep-Pak® procedure or by preparative LC. Analytical and preparative

LC conditions were as mentioned in the synthesis of 2.

Immobilization of D-amino acid oxidase (EC 1.4.3.3)[11]

Controlled porous glass beads (CPG-500-80, 20-80 mesh, 1 g) were washed with 20 ml 1 M hydrochloric acid for 20 min at room temperature. The acid was removed, 10 ml of 5 % nitric acid was added and the mixture was heated to reflux for 10 min. After washing with water, the glass beads were silanized with 16 ml hydrochloric acid (pH 3.5) and 4 ml of Silan A 1100 (Union Carbide) by shaking at 75 °C for 3.5 h. The glass beads were then washed with sterile phosphate buffer (0.05 M, pH 7) and activated with 5 ml 25 % glutaraldehyde solution (aq.) and 19 ml phosphate buffer for 3.5 h at room temperature. The beads were transferred to another glass vessel and washed with sterile 0.1 M pH 8.3 tetrasodium pyrophosphate buffer. A solution of 8.5 mg D-amino acid oxidase (150 units) dissolved in 12 ml of the same buffer was then added. The immobilization was then performed by shaking at 0 °C for 3.5 h. The remaining glutaraldehyde-activated sites were blocked by shaking with 10 ml of a 0.1 M solution of glycine in 0.1 M tetrasodium pyrophosphate, pH 8.3, at room temperature for 30 min. The incorporation of D-amino acid oxidase was determined to higher than 97 % by the use of Bio-Rad assay.[16] The immobilized enzymes were stored at 4 °C in 0.1 M tetrasodium pyrophosphate buffer pH 8.3.

L-[3-^{11}C]Alanine and L-[3-^{11}C]phenylalanine

The solution containing the ^{11}C-amino acid, purified either by preparative LC or by the Sep-Pak® C_{18} procedure, was evaporated to dryness. The residue was redissolved in 2-3 ml 0.1 M tetrasodium pyrophosphate buffer pH 8.3 (with di-

sodium flavine adenosine dinucleotide 20-50 µg/ml). The solution was adjusted to pH 8.3 and transferred to a two-necked 25-ml conical glass vessel, containing 8.5 mg D-amino acid oxidase, immobilized on 1 g (CPG-500-80) glass beads in 1 ml of the tetrasodium pyrophosphate buffer, (150-200 µg FAD/ml). The enzyme reactor, equipped with rubber septa and an inlet and outlet needle for oxygen was thermostated at 37 °C. Oxygen was passed through the reaction mixture (>500 ml/min) for 10-17 min. The solution was removed from the glass beads and acidified with 1 ml 2 M hydrochloric acid and passed through a cation exchange resin (Dowex 50 W-X4, 100-200 mesh, hydrogen form, 3x0.7 cm). The column was washed with distilled water until the level of radioactivity detected in the eluted fractions was insignificant. The ^{11}C-amino acid was then eluted by the use of 0.2 M aqueous sodium hydroxide solution. To the collected fraction, 2 ml of 0.1 M phosphate buffer (pH 7.4) was added and the pH of the solution adjusted to 7.4. After sterile filtration through a 0.22 µm filter, the solution was ready for biomedical investigations - in this case, the mechanism of amino acid transport in monkey brain. The results from these experiments will be presented elsewhere.[12] Analytical LC conditions were as already described.

The optical purity of [3-^{11}C]alanine was determined by converting the [3-^{11}C]alanine to *N*-trifluoroacetylalanine methyl ester by the following procedure.[4a] The amino acid solution was evaporated to dryness and the residue dissolved in 5 ml methanol/dry hydrogen chloride (1-2.5 M) and the solution was heated at 100 °C for 5-10 min. After evaporation of the solvent, 1.5 ml trifluoroacetic anhydride in 2 ml dichloromethane was added and the solution heated for 5 min at 100 °C. The excess reagents were removed by eva-

poration at room temperature and the residue was dissolved in 0.1-0.2 ml dichloromethane. The procedure took less than 30 minutes. The enantiomeric analysis was then performed by GC under the following conditions: column J, flow 30 ml/min, oven temperature, 90 °C. Optical purity of L-[3-^{11}C]phenylalanine was determined by use of analytical LC under the following conditions: column C, with solvent H, flow 1.5 ml/min, temperature 55 °C, wavelength 254 nm (Fig. 1).

ACKNOWLEDGEMENT

This work forms part of a project financially supported by the Swedish Natural Science Research Council by grants K-KU 3464 and K-KU 2446. We are indebted to Dr Petter Malmborg for assistance in radionuclide production.

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