

Multi-enzymatic Synthesis of β - ^{11}C -Labelled L-Tyrosine and L-DOPA

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The synthesis of L- $[\beta$ - $^{11}\text{C}]$ tyrosine (**3**) and L- $[\beta$ - $^{11}\text{C}]$ DOPA (**4**) from $[\text{C}^{11}]$ carbon dioxide via DL- $[\text{C}^{11}]$ alanine (**1**) using a combination of organic synthetic methods and a multi-enzymatic procedure are presented. The ^{11}C -labelled alanine was prepared by a methylation, and subsequent hydrolysis, of a glycine derivative, *N*-(diphenylmethylene)glycine *tert*-butyl ester (**8**), with $[\text{C}^{11}]$ methyl iodide (**7**) obtained from $[\text{C}^{11}]$ carbon dioxide. The enzymatic syntheses were performed using a *one-pot* reaction procedure with D-amino acid oxidase (D-AAO)/catalase, glutamic-pyruvic transaminase (GPT), and β -tyrosinase. The total synthesis time was 45–50 min, including HPLC purification, counted from the start of the $[\text{C}^{11}]$ methyl iodide synthesis. The decay corrected radiochemical yields were 25–30% of L- $[\beta$ - $^{11}\text{C}]$ tyrosine and L- $[\beta$ - $^{11}\text{C}]$ DOPA with radiochemical purities of >98%. The e.e. values were higher than 98% and the specific radioactivities were in the order of 2.5 GBq μmol^{-1} . In a typical run, 220 MBq of purified L- $[\beta$ - $^{11}\text{C}]$ DOPA was obtained from 4.4 GBq $[\text{C}^{11}]$ carbon dioxide within 50 min.

Molecules labelled with positron-emitting radionuclides, such as ^{11}C , ^{13}N and ^{18}F , with half-lives of 20.3, 9.97 and 109.7 min, respectively, have been used together with the positron emission tomography (PET) method for studying biological processes *in vivo*.^{1,2} The utilisation of labelled amino acids in PET investigations is of increasing interest due to their extensive use in the living system. Two of the most interesting amino acids are L-tyrosine and 3,4-dihydroxy-L-phenylalanine (L-DOPA) which play a significant role as intermediates in the naturally occurring biosynthesis of the neurotransmitter dopamine. In order to study dopamine synthesis and distribution *in vivo*, by means of PET, we have been looking for methods to synthesize tyrosine and DOPA specifically labelled with ^{11}C .^{3,4}

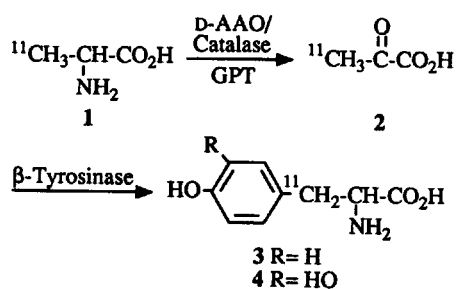
The position of the label in the amino acid is of great importance since the last step in the formation of dopamine *in vivo* is an enzymatic decarboxylation. If the labelling is in the carboxylic acid carbon, the radioactivity will be lost as carbon dioxide during the formation of dopamine. Any other position will, however, result in the labelled neurotransmitter. In the biosynthesis of dopamine only the L-enantiomer of the amino acid is used, consequently using a racemic mixture in a PET investigation will cause an increased background source of radioactivity from the D-enantiomer. There are methods available for the rapid resolution of ^{11}C -labelled racemic mixtures of amino acids,^{5–8} but half of the radioactivity is lost during the

resolution. Our attempt was therefore to find a method for the ^{11}C -labelling of the pure enantiomers of L-tyrosine and L-DOPA in a position different from the carboxylic acid carbon.

Recently there has been an increasing interest in applying enzymes as selective catalysts in organic chemistry.⁹ The advantages of using enzymes as catalysts in labelling molecules with a short-lived isotope are that they are often enantioselective and work quickly under mild conditions. Several ^{11}C -labelled amino acids have been synthesized as pure enantiomers by using enzymatic synthesis. L-Glutamic acid has, for example, been labelled with ^{11}C in both the α - and the γ -carboxylic positions utilizing enzymes.¹⁰

Another way of studying dopamine distribution is to use ^{13}N -labelled L-DOPA or analogues such as ^{18}F -labelled fluoro-DOPA in PET investigations. ^{13}N -Labelled L-DOPA has been synthesized by using the enzyme phenylalanine dehydrogenase in an exchange reaction with $[\text{C}^{13}]$ ammonia.¹¹ This method will naturally give a low specific radioactivity since L-DOPA itself is used as the substrate. ^{11}C - and ^{13}N -labelled amino acids have the advantage of differing only isotopically from the normal amino acid. This is not the case with ^{18}F -labelled fluoro-DOPA which has been used to visualize dopamine distribution in the human brain.^{12,13} Even if 6- $[\text{C}^{18}\text{F}]$ fluoro-L-DOPA, which is the actual species used, behaves qualitatively similar to L-DOPA there are differences in the metabolism. Another problem is that the methods which have been used for preparing 6- $[\text{C}^{18}\text{F}]$ fluoro-L-DOPA¹⁴ produce the radiotracer with low

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Scheme 1.

specific radioactivity. However, recently a no carrier-added synthesis has been reported.¹⁵

Tyrosine and DOPA have previously been labelled with ¹¹C by carboxylation of the appropriate isonitrile with [¹¹C]carbon dioxide,^{16,5} and by methods using [¹¹C]cyanide.^{17,18} All these methods produce the racemic carboxyl-labelled amino acids. With another approach racemic β-¹¹C-labelled tyrosine and DOPA have been produced utilising a condensation reaction with labelled *p*-anisaldehyde or veratraldehyde, respectively.^{3,19} We here report the synthesis of L-[β-¹¹C]tyrosine (3) and L-[β-¹¹C]DOPA (4) from [¹¹C]carbon dioxide using a combined organic synthetic and biosynthetic method. The multi-enzymatic synthesis was achieved in a *one-pot* reaction using D-amino acid oxidase (D-AAO)/catalase, glutamic-pyruvic transaminase (GPT), and β-tyrosinase, as shown in Scheme 1.

Results and discussion

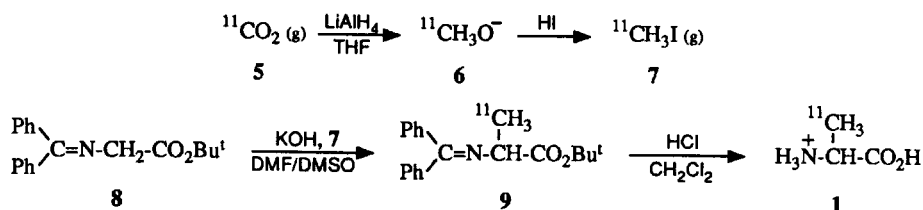
β-Tyrosinase (tyrosine phenol-lyase, EC 4.1.99.2) is a pyridoxal phosphate requiring enzyme which catalyses the stoichiometric conversion of L-tyrosine to phenol, pyruvate and ammonia.²⁰⁻²² It has been shown that the reverse of this α,β-elimination reaction can be used to synthesize L-tyrosine and L-DOPA using phenol and catechol as substrates.²³ Furthermore, a series of other phenol derivatives can be used as substrates to produce analogues of tyrosine.²⁴ Since [3-¹¹C]pyruvic acid can be obtained from racemic [3-¹¹C]alanine using an enzymatic method,²⁵ the combination with β-tyrosinase was a conceivable way to obtain β-¹¹C-labelled L-tyrosine and L-DOPA. In combination with ¹⁴C-labelled phenol and catechol, Ellis *et al.* used the catalytic ability of β-tyrosinase to prepare ring-¹⁴C-labelled tyrosine and DOPA.²⁶ β-Tyrosinase has also recently been utilised

to label L-tyrosine with the stable isotopes ²H, ¹³C, ¹⁸O and ¹⁵N using labelled phenol or ammonium sulfate in combination with whole cells of *Erwinia herbicola*.²⁷

In this method we have used a purified enzyme solution from *Citrobacter intermedius*. The purification was carried out according to a literature procedure²⁸ with some modifications. Some steps in the original procedure were omitted and a Phenyl Sepharose column was used instead of a hydroxylapatite column. In the Phenyl Sepharose chromatography step the yield of the enzyme activity in the collected fractions was over 80% with a tenfold purification. The final enzyme solution had more than 80% homogeneity analysed by SDS PAGE (sodium dodecyl sulfate polyacrylamide-gel electrophoresis). With this method a faster purification procedure has been achieved, which can be further shortened by using FPLC or HPLC systems, and with a higher total recovery of enzyme activity, ca. 35%. A detailed purification procedure will be published elsewhere.

The inconvenience of working with enzymes, as well as other specific catalysts, is their sensitivity towards certain impurities. In the synthesis of racemic [3-¹¹C]alanine, when performed according to a previously presented procedure,²⁹ impurities arose which affected the reproducibility in the subsequent enzymatic reactions. However, by changing from a phase-transfer reaction, to perform the alkylation in *N,N*-dimethylformamide/dimethyl sulfoxide (DMF/DMSO) with potassium hydroxide as the base,²⁵ the problem was overcome (Scheme 2). Racemic ¹¹C-labelled alanine (1) was produced in 70% radiochemical yield, decay corrected, in 18 min with a radiochemical purity of over 99%.

High specific radioactivity is of great importance, since a high concentration of the substance injected might affect the biological system studied. To achieve as high a specific radioactivity of the labelled amino acids as possible the pyruvate concentration must be kept low. In pilot studies with ¹⁴C-labelled pyruvate we found that it was feasible to synthesize tyrosine utilizing β-tyrosinase even with low amounts of pyruvate (1 μmol). Other parameters which have to be specially considered in work with short-lived positron-emitting radionuclides are time and technical handling. In order to minimise synthesis time and radiation exposure of the chemist, a *one-pot* procedure for the biosynthetic steps was developed. Optimisation of pH and reaction temperature for the conversion of pyruvate to tyrosine were first performed using ¹⁴C-labelled pyruvate.



Scheme 2.

The final reaction conditions were however optimised with respect to the whole multi-enzymatic procedure by use of ^{11}C -labelled alanine. The reaction time was optimised to give as high total radioactivity as possible at the end of synthesis of the labelled amino acids. This means that even if a reaction is for example complete in 15 min, it may be more favorable to stop after 5 min since the decay of the radioactivity has to be considered. The multi-enzymatic synthesis was performed at 45 °C, pH 9.0, for 3 min during which about 55 % of the racemic alanine was converted into ^{11}C -labelled tyrosine or DOPA. A problem encountered in the utilisation of free enzymes is their low stability over long periods. This was avoided by freezing the enzymes (catalase, GPT and β -tyrosinase) in small portions and thawing them one at a time.

After completion of the enzymatic reactions the proteins were precipitated with hydrochloric acid. Filtration of the reaction mixture yielded a clear solution of the ^{11}C -labelled amino acid which then was purified by preparative HPLC. Since DOPA is easily oxidised, ascorbic acid was added to the mobile phase as an antioxidant. The β - ^{11}C -labelled L-tyrosine or L-DOPA was obtained in 25–30 % radiochemical yield, decay corrected, in approximately 6 ml buffer.

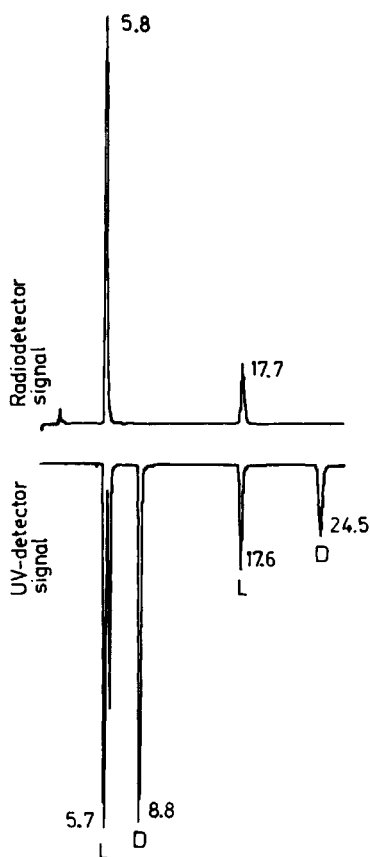


Fig. 1. LC-analysis of the enantiomeric purity of L-[β - ^{11}C]tyrosine with an added racemic reference, after derivatisation with Marfey's reagent using column C and the conditions described in the Experimental section. The retention times are given in minutes.

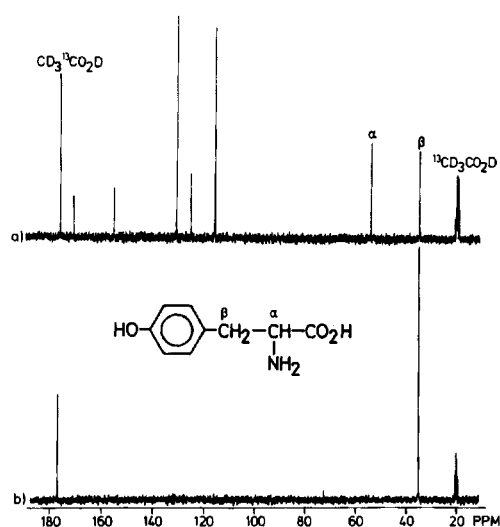


Fig. 2. ^{13}C NMR spectra of (a) L-tyrosine and (b) L-[β - ^{13}C]tyrosine in $\text{D}_2\text{O}/\text{CD}_3\text{CO}_2\text{D}$ (2:1) with 3 drops of D_2SO_4 added.

After pH-adjustment to ca. 6 for tyrosine and 4.5 for DOPA (since DOPA is sensitive to high pH) the solutions were sterile-filtered before use in PET investigations. The radiochemical purities were analysed by HPLC and were found to be over 98 %. The total synthesis time was 45–50 min counted from release of [^{11}C]carbon dioxide.

The enantiomeric purities were assessed by HPLC after derivatisation with *N*-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (Marfey's reagent) to yield the diastereomers of the respective amino acids.³⁰ A racemic sample of the appropriate amino acid was always added to the labelled product before the derivatisation to insure that no kinetic resolution occurred. Tyrosine gave two pairs of diastereomers, Fig. 1, which is presumably due to the nitrogen and phenolic oxygen derivatives of the amino acid. In the case of DOPA, the situation was even more complex. However, no radioactive components corresponding to the D-enantiomers were detected. The solutions containing the ^{11}C -labelled amino acids have been examined regarding sterility and pyrogenicity and were found to be suitable for use in human studies. Determination of the specific radioactivities were performed by comparison with standards and found to be about 2.5 GBq μmol^{-1} .

To confirm the position of the label, by NMR, a ^{13}C -synthesis was carried out. The same procedure as described for the synthesis of L-[β - ^{11}C]tyrosine was repeated but (^{13}C)-methyl iodide was used in the alkylation. After purification by HPLC the collected fraction was evaporated to dryness and the residue was dissolved in 0.75 ml of $\text{D}_2\text{O}/\text{CD}_3\text{CO}_2\text{D}$ (2:1) with 3 drops of D_2SO_4 . The ^{13}C NMR spectrum was compared with a spectrum of original L-tyrosine (Fig. 2).

In conclusion, the multi-enzymatic method described above for the synthesis of enantiomerically pure β - ^{11}C -labelled L-tyrosine and L-DOPA is applicable to a series of tyrosine analogues by the use of other phenol derivatives. Furthermore, applying this method in combination with

$1\text{-}^{11}\text{C}$ -labelled alanine or pyruvate is a conceivable way of preparing the carboxy-labelled enantiomerically pure *L*-tyrosine and *L*-DOPA. It may also be possible to use [^{13}N]ammonia as a substrate for β -tyrosinase to produce the ^{13}N -labelled amino acids. Work in this area is in progress.

Experimental

Apparatus and materials. The ^{11}C was produced by the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ nuclear reaction using a nitrogen gas target and bombardment with a 10 MeV proton beam produced by a tandem Van der Graaff accelerator at the Svedberg Laboratory, University of Uppsala. The [^{11}C]carbon dioxide formed was trapped in lead-shielded 4 Å molecular sieves and transported to the chemistry laboratory. [^{11}C]Methyl iodide was synthesized by a general method used in our laboratory described previously.³¹ *N*-(Diphenylmethylene)-glycine *tert*-butyl ester was prepared according to a literature procedure.²⁹

LC-analyses were performed on a Hewlett-Packard 1090 liquid chromatograph fitted with a UV diode array detector in series with a β^+ -flow detector³² and any of the following columns: (A) 250×4.6 mm LC-NH₂ (Nucleosil) 10 μm column, (B) 250×4.6 mm C-18 (Spherisorb ODS2) 5 μm column, and (C) 250×4.6 mm C-18 (Nucleosil) 10 μm column. The following mobile phases were used: (D) 0.01 M potassium dihydrogen phosphate pH 4.6, (E) 500:70 (v:v) acetonitrile/water, (F) 17 mM acetic acid, (G) 0.05 M ammonium formate pH 3.5, and (H) methanol. Preparative LC was carried out using a Waters M-6000A pump and a 250×10 mm C-18 (Nucleosil) 10 μm column in series with a Waters M 441 UV detector and a tubing coiled around a GM tube. ^{13}C NMR spectra were recorded on a Varian XL-300 NMR spectrometer. For the NMR assignments of the methine and methylene carbons in *L*-tyrosine the APT technique in the Varian XL-300 system was used.

Solid phase extraction C-18 (Supelco) columns were pre-conditioned with 3 ml dichloromethane, 10 ml ethanol, and 20 ml distilled water prior to use. Tetrahydrofuran (THF) was dried by distillation from sodium-benzophenone. All other chemicals were used without further purification.

All enzymes except β -tyrosinase were purchased from Sigma. D-Amino acid oxidase, D-AAO (EC 1.4.3.3), from porcine kidney, crystalline suspension in 3.6 M ammonium sulfate, pH 6.5, was used without further handling. Glutamic-pyruvic transaminase, GPT (EC 2.6.1.2), from porcine heart, lyophilized powder, was dissolved in 50 mM potassium phosphate buffer pH 7.5 containing 0.2 mM pyridoxal 5-phosphate. Catalase (EC 1.11.1.6) from bovine liver, crystalline suspension in water containing 0.1% thymol, was dialysed against 50 mM potassium phosphate buffer, pH 7.5. In order to maintain the catalytic activity of the enzymes, the prepared enzyme solutions of GPT, catalase, and β -tyrosinase were stored in the freezer in small portions. After being thawed they could be kept in the

refrigerator for approximately one month before the enzyme activity started to decrease.

Enzyme assay. β -Tyrosinase activity was assayed by determining the amount of pyruvate produced from *L*-tyrosine in potassium phosphate buffer, pH 8.0, at 30°C.²⁸ One unit was defined as the amount of enzyme which catalysed the formation of 1 μmol pyruvate per min under the same conditions.

Purification of β -tyrosinase. β -Tyrosinase, tyrosine phenolase (EC 4.1.99.2), was purified from cell extract of *Citrobacter intermedius*, grown in a medium supplemented with *L*-tyrosine, according to Kumagai *et al.*²⁸ with some modifications. Briefly: after the cells (200 g cell pellet) had been sonicated, the supernatant was collected by centrifugation and precipitated with 30–70% saturated ammonium sulfate (AmS). The precipitates were dissolved in and dialysed against 0.1 M potassium phosphate buffer, pH 7.0, containing 0.5 mM dithiothreitol (DTT) and 0.1 mM pyridoxal 5-phosphate (PLP), and then applied to a column (5×40 cm) of DEAE-Toyopearl equilibrated with 10 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM DTT and 0.1 mM PLP (buffer A). The column was washed with buffer A and then eluted with a linear gradient of 0–1.0 M sodium chloride in buffer A. The active fractions were combined, added by AmS to 10% saturation and applied to a column (2.5×50 cm) of Phenyl-Sepharose equilibrated with 10% AmS saturated buffer A. The column was washed with 10% AmS saturated buffer A and then eluted with a linear gradient of 10 to 0% saturation of AmS in buffer A. The active fractions were collected and concentrated by 70% saturation of AmS. The final preparation after dialysis against 10% AmS saturated buffer A had a specific activity of 1.2 μmol min⁻¹ mg⁻¹ protein, and a protein concentration of 56.7 mg ml⁻¹ as determined by the method of Lowry *et al.*,³³ using bovine serum albumin as the standard.

[^{11}C]Methyl iodide. The [^{11}C]carbon dioxide was released from the lead-shielded molecular sieves upon heating and transferred in a stream of nitrogen gas to a solution of 0.5 ml 0.8 M lithium aluminium hydride in THF in a specially designed *one-pot* reaction flask.³¹ After evaporation of the THF the residue was treated with 2 ml 57% hydriodic acid and the reaction mixture was heated to reflux during which the [^{11}C]methyl iodide formed was distilled off and transferred with nitrogen gas to the reaction vessel.

***DL*-[β - ^{11}C]Alanine.** 3 mg (10 μmol) *N*-(diphenylmethylene)-glycine *tert*-butyl ester were dissolved in 0.5 ml DMF/DMSO (9:1) in a 1.5 ml vial equipped with a septum. 2 μl 5 M potassium hydroxide were added to the solution before the [^{11}C]methyl iodide was transferred to the reaction vessel in a stream of nitrogen gas. The reaction solution was heated at 80°C for 2 min and then transferred to a syringe, containing 20 ml distilled water, connected to a

solid phase extraction C-18 column. The solution was applied to the column which was then washed with 3 ml water before the radioactivity trapped in the column was eluted with 2 ml dichloromethane into a 7 ml open glass vessel containing 0.8 ml 6 M hydrochloric acid. The two-phase reaction mixture was heated and shaken at 130°C for 5 min while the dichloromethane distilled off. The racemic [3- ^{11}C]alanine solution was diluted with 1–2 ml water before the radiochemical purity was analysed by HPLC using column A and the following conditions: flow 2 ml min⁻¹, solvents D/E, gradient 0–8 min 95–60 % E, column temperature 40°C, wavelength 230 nm. The retention time was 6.1 min for [3- ^{11}C]alanine.

L-[β - ^{11}C]Tyrosine and *L*-[β - ^{11}C]DOPA. The solution containing the racemic [3- ^{11}C]alanine was evaporated to dryness, and the residue was redissolved in 0.6 ml 0.1 M tris (hydroxymethyl)aminomethane/hydrochloric acid (TRIS/HCl) buffer, pH 9.0. The solution was added to a mixture of AmS, TRIS/HCl, pH 9.0, α -ketoglutarate (α -KG), flavin adenine dinucleotide (FAD), and PLP, which was adjusted to pH 9.0 with 1 M potassium hydroxide and then the enzymes were added. The final solution (1 ml) contained 3.2 units D-AAO, 20 units GPT, 3600 units catalase, 3.4 units β -tyrosinase, 0.1 M TRIS/HCl, pH 9.0, 0.15 M AmS, 10 mM α -KG, 0.1 mM PLP, and 17 μM FAD. The reaction mixture was thermostatted at 45°C for 15 s after which 10 μl of a 2.5 M ethanolic solution of phenol for tyrosine or catechol for DOPA were added. After another 3.0 min at 45°C the reaction was stopped by adding 0.1 ml conc. hydrochloric acid. The denatured proteins were removed by filtration through a 0.22 μm pore filter and the resulting clear solution was purified using the previously described preparative LC system. The mobile phases used were 17 mM acetic acid/ethanol 95:5 (v:v), for tyrosine, and 17 mM acetic acid containing 1 mM ascorbic acid, for DOPA, flow 4.0 ml min⁻¹. The radioactive fractions containing the appropriate compound were collected and in the case of tyrosine evaporated, to remove the ethanol, and redissolved in 5 ml saline and 1 ml phosphate buffer, pH 7.4. After pH adjustment to approximately 6 for tyrosine and 4.5 for DOPA, the solution was sterilised by passage through a 0.22 μm pore filter into a sterile vial and was then used for human and animal applications. The radiochemical purity was analysed by HPLC: (1) Column A, flow 2.0 ml min⁻¹, solvents D/E, gradient 0–8 min 95–60 % E, column temperature 40°C, wavelength 230 nm. Retention times were 4.9 min for *L*-[β - ^{11}C]tyrosine and 4.7 min for *L*-[β - ^{11}C]DOPA; (2) column B, flow 2.0 ml min⁻¹, solvents F/H, isocratic 15 % H (for tyrosine) and 0 % H (for DOPA), column temperature 60°C, wavelength 278 nm. The retention times were 3.9 min for *L*-[β - ^{11}C]tyrosine and 5.3 min for *L*-[β - ^{11}C]DOPA.

L-[β - ^{13}C]Tyrosine. 20.1 mg (68 μmol) *N*-(diphenylmethylene)glycine *tert*-butyl ester were dissolved in 0.5 ml DMF/DMSO (9:1) in a 1.5 ml vial equipped with a septum.

13.6 μl 5 M potassium hydroxide and 3.75 μl (60.2 μmol) (^{13}C)methyl iodide were added to the solution which was then heated at 80°C for 10 min. The reaction mixture was then treated as above for [3- ^{11}C]alanine except that 1.5 ml hydrochloric acid was used for the hydrolysis. After evaporation of the alanine solution, the residue was dissolved in 1.4 ml 0.1 M TRIS/HCl and transferred to a tube containing 160 μl 0.5 M TRIS/HCl, pH 9.0, 300 μl 1.5 M AmS, 100 μl 2 M α -KG, 25 μl 1.7 mM FAD and 25 μl 10 mM PLP. The pH was adjusted to 9.0 with 1 M potassium hydroxide and then 6.4 units D-AAO, 7200 units catalase, 40 units GPT, 4.1 units β -tyrosinase, and 40 μl of a 2.5 M ethanolic solution of phenol were added. The reaction mixture was thermostatted at 45°C for 20 min after which 0.2 ml conc. hydrochloric acid was added. After filtration, the crude product was purified by HPLC using the same system as described above. The collected fraction was evaporated to dryness and redissolved in 0.75 ml D₂O/CD₃CO₂D (2:1) to which 3 drops of D₂SO₄ had been added. The ^{13}C NMR spectrum shows a single peak at δ 35.06 (CD₃CO₂D used as the reference, δ 20.00) which is consistent with the methylene carbon in *L*-tyrosine.

Enantiomeric purity. The collected fractions of the ^{11}C -labelled amino acids from the preparative HPLC were evaporated to dryness and the residue was dissolved in 0.1 ml water. The solution was transferred to a 1.5 ml glass vial containing a mixture of 0.2 ml 1 % *N*-(5-fluoro-2,4-dinitrophenyl)-*L*-alaninamide in acetone, 5 μmol DL-tyrosine or DL-DOPA, and 40 μl 1 M sodium hydrogen carbonate. The vial was sealed with a septum and the reaction was left to proceed at 60°C for 15 min after which 20 μl 2 M hydrochloric acid was added. The resulting solution was analysed by HPLC using column C and the following conditions: flow 2.0 ml min⁻¹, solvents G/H, gradient 0–20 min 35–60 % H, column temperature 50°C, wavelength 340 nm. Retention times for the *N*-derivatised amino acids were 5.7, 8.8, 4.5 and 6.4 min for *L*-tyrosine, D-tyrosine, *L*-DOPA and D-DOPA, respectively.

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References

- Greitz, T., Ingvar, D. H. and Widén, L. *The Metabolism of the Human Brain Studied with Positron Emission Tomography*, Raven Press, New York 1985.
- Phelps, M., Mazziotta, J. and Schelbert, H. *Positron Emission Tomography and Autoradiography: Principles and Applications for the Brain and Heart*, Raven Press, New York 1986.
- Halldin, C., Bergson, G., Malmberg, P. and Långström, B. J. *Labelled Compd. Radiopharm.* 21 (1984) 1178.
- Fasth, K. J., Antoni, G., Malmberg, P. and Långström, B. J. *Labelled Compd. Radiopharm.* 26 (1989) 88.

5. Bolster, J. M., Vaalburg, W., Van Veen, W., Van Dijk, T., Van der Molen, H. D., Wynberg, H. and Woldring, M. G. *Int. J. Appl. Radiat. Isot.* 34 (1983) 1650.
6. Casey, D. L., Digenis, G. A., Wesner, D. A., Washburn, L. C., Chaney, J. E., Hayes, R. L. and Callahan, A. P. *Int. J. Appl. Radiat. Isot.* 32 (1981) 325.
7. Washburn, L. C., Sun, T. T., Byrd, B. L. and Callahan, A. P. *J. Nucl. Med.* 23 (1982) 29.
8. Wu, J., Harper, P. V. and Lathrop, K. A. *J. Nucl. Med.* 22 (1981) 74.
9. Akiyama, A., Bednarski, M., Kim, M. J., Simon, E. S., Waldmann, H. and Whitesides, G. M. *Chem. Br.* 23 (1987) 645 and references therein.
10. Cohen, M. B., Spolter, L., Chang, C. C., Behrendt, D., Cook, J. and MacDonald, N. S. *Int. J. Appl. Radiat. Isot.* 33 (1982) 613.
11. Gelhard, A. S., Cooper, A. J. L., Asano, Y. and Filc-Derico, S. *J. Nucl. Med.* 29 (1988) 931.
12. Garnett, E. S., Firna, G. and Nahmias, C. *Nature (London)* 305 (1983) 137.
13. Nahmias, C., Garnett, E. S., Firna, G. and Lang, A. *J. Neurol. Neurosurg. Psychiatry* 69 (1986) 223.
14. Chaly, T. and Diksic, M. *J. Nucl. Med.* 27 (1986) 1896.
15. Lemaire, C., Guillaume, M. and Christiaens, L. *J. Nucl. Med.* 30 (1989) 752.
16. Bolster, J. M., Vaalburg, W., Paans, A. M. J., Van Dijk, T. H., Elsinga, P. H., Zijlstra, J. B., Piers, D. A., Mulder, N. H., Woldring, M. G. and Wynberg, H. *Eur. J. Nucl. Med.* 12 (1986) 321.
17. Halldin, C., Schoeps, K.-O., Stone-Elander, S. and Wiesel, F.-A. *Eur. J. Nucl. Med.* 13 (1987) 288.
18. Hwang, D.-R., Kilbourn, M. and Welch, M. *J. Labelled Compd. Radiopharm.* 23 (1986) 1064.
19. Halldin, C. and Långström, B. *Int. J. Appl. Radiat. Isot.* 35 (1984) 779.
20. Kakihara, Y. and Ichihara, K. *Med. J. Osaka Univ. (Osaka)* 3 (1953) 497.
21. Uchida, M., Takemoto, Y., Kakihara, Y. and Ichihara, K. *Med. J. Osaka Univ. (Osaka)* 3 (1953) 509.
22. Yoshimatsu, H. *Med. J. Osaka Univ. (Osaka)* 9 (1957) 727.
23. Yamada, H., Kumagai, H., Kashima, N., Torii, H., Enei, H. and Okumura, S. *Biochem. Biophys. Res. Commun.* 46 (1972) 370.
24. Nagasawa, T., Utagawa, T., Goto, J., Kim, C.-J., Tani, Y., Kumagai, H. and Yamada, H. *Eur. J. Biochem.* 117 (1981) 33.
25. Bjurling, P., Watanabe, Y. and Långström, B. *Appl. Radiat. Isot.* 39 (1988) 627.
26. Ellis, B. E., Major, G. and Zenk, M. H. *Anal. Biochem.* 53 (1973) 470.
27. Walker, T. E., Matheny, C., Storm, C. B. and Hayden, H. *J. Org. Chem.* 51 (1986) 1175.
28. Kumagai, H., Yamada, H., Matsui, H., Ohkishi, H. and Ogata, K. *J. Biol. Chem.* 245 (1970) 1767.
29. Antoni, G. and Långström, B. *J. Labelled Compd. Radiopharm.* 24 (1987) 125.
30. Marfey, P. *Carlsberg Res. Commun.* 49 (1984) 591.
31. Långström, B., Antoni, G., Gullberg, P., Halldin, C., Malmberg, P., Nägren, K., Rimland, A. and Svård, H. *J. Nucl. Med.* 28 (1987) 1037.
32. Långström, B. and Lundqvist, H. *Radiochem. Radioanal. Lett.* 41 (1979) 375.
33. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* 193 (1951) 265.

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