The Synthesis of α-([^{11}C]Methyl)phenylalanine and α-([^{11}C]Methyl)tyrosine from ^{11}C-Labelled Malonic Esters

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The synthesis of α-([^{11}C]methyl)tyrosine **4a** and α-([^{11}C]methyl)phenylalanine **4b** from dimethyl 2-(4-methoxybenzyl)-2-([^{11}C]methyl)malonate **1a** and dimethyl 2-benzyl-2-([^{11}C]methyl)malonate **1b**, respectively is reported. After the alkylation of dimethyl 2-(4-methoxybenzyl)malonate or dimethyl 2-benzylmalonate with [^{11}C]methyl iodide, a selective enzymatic hydrolysis to monomethyl 2-(4-methoxybenzyl)-2-([^{11}C]methyl)malonic acid **2a** or monomethyl 2-benzyl-2-([^{11}C]methyl)malonic acid **2b** was carried out using pig liver esterase (EC 3.1.1.1). Conversion of the carboxylic acid moiety of **2a**, **b** into the corresponding isocyanate **3a**, **b** was achieved by a modified Curtius rearrangement using diphenylphosphoryl azide (DPPA). The simultaneous hydrolysis of the isocyanate and ester moieties of **3a**, **b** using conc. hydrochloric acid produced the desired amino acids **4a**, **b**. In the case of α-([^{11}C]methyl)tyrosine, the methyl ether protecting group was removed using trimethylsilyl chloride–sodium iodide prior to acidic hydrolysis. The amino acids were obtained in 12–20% decay corrected radiochemical yields in a synthesis time of 45–50 min, counted from the start of [^{11}C]methyl iodide synthesis, and with a radiochemical purity greater than 98% after preparative HPLC purification. Enantiomeric purities of **4a**, **b** were determined by the HPLC separation of the respective 1-fluoro-5-(2,4-dinitrophenyl)-L-alanine amide (FDAA) diastereoisomers, which showed an enantiomeric excess of 62% for **4a** but no observable enantiomeric induction for **4b**. To confirm the position of labelling, a ^{13}C synthesis was carried out using (^{13}C)methyl iodide in the same reaction sequence, and the product analysed by ^{13}C NMR spectroscopy.

The use of short-lived radiochemicals in *in vivo* tracer studies using positron emission tomography (PET) has proved to be a valuable tool for studying a variety of biological processes in living animals and especially humans.[1a–g] Among the short-lived positron emitting radionuclides, ^{11}C, ^{13}N and ^{18}F ($t_{\frac{1}{2}}$ 20.3, 10.0 and 110 min, respectively) are most commonly used for labelling. Because of the decay characteristics, rapid incorporation of the label into the molecule of interest is essential if PET investigations are to be made possible. Despite this limitation, a wide variety of tracers have been synthesised and used in *in vivo* tracer studies using PET.[2a–d] [2-^{18}F]Fluoro-2-deoxy-D-glucose has shown to be a useful tool in the study of glucose metabolism in both normal and diseased states.[1a] L-Methionine has been labelled with ^{11}C ($t_{\frac{1}{2}}$ = 20.3 min), and is now used routinely in the visualisation and delineation of brain tumours.[1b–d] Other endogenous amino acids have been used to study *in vivo* metabolic pathways in normal and diseased states. Metabolic abnormalities have been visualised, using for example [^{11}C]DOPA[2b] and [^{11}C]-5-hydroxytryptophan,[2c] in individuals suffering from Parkinson's disease[1e] and depression.[1f] Another approach to obtain *in vivo* information is to use labelled ligands which can selectively bind to, and specifically inhibit the function of, various proteins. This can give quantitative information about the function and localisation of these proteins in normal and diseased states. The 'suicide inhibitor' [^{11}C]-L-deprenyl[2d] has been used in this way to study monoamine oxidase (MAO) activity in the human brain by irreversible covalent binding with the enzyme itself.[1g–i] Substrates exhibiting reversible protein binding can also be used to obtain information about enzyme activity.

The exogenous α-alkyl analogues of phenylalanine, tyrosine and DOPA have shown to induce a variety of biological effects. α-Methylphenylalanine has been used as an inhibitor of liver phenylalanine hydroxylase to produce a model for hyperphenylalaninemia.[3] α-Methyltyrosine and α-methylDOPA are known to inhibit brain tyrosine hydroxylase[4] and DOPA decarboxylase,[5] respectively, causing decreased dopamine synthesis from phenylalanine, tyrosine and DOPA in the dopamine neurone. As such, these α-methyl amino acids are potentially interesting tracers for use in the quantification and localisation of these enzymes in normal and diseased states *in vivo* using PET.

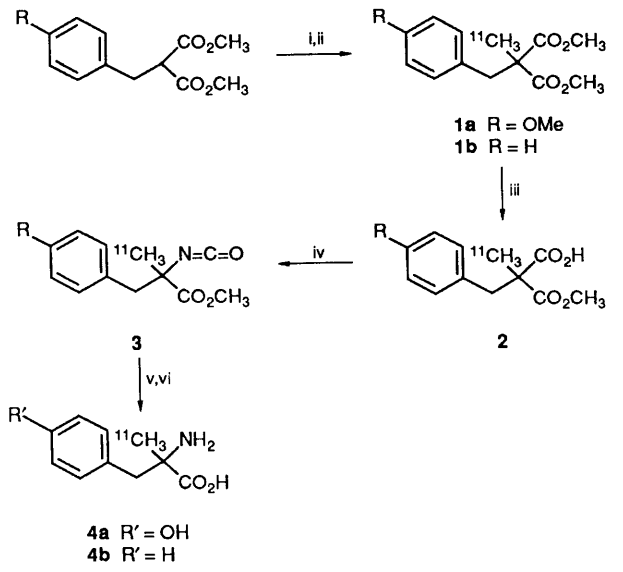
Table 1 Radiochemical yields/purities, synthesis times and enantiomeric purities of **4a** and **4b**

Compound	Radiochemical yield (%)	Radiochemical purity (%)	Synthesis time (min)[a]	ee (%L)
4a	12–16	>98	50	62
4b	15–20	>98	45	racemic

[a] Counted from start of ^{11}C-methyl iodide synthesis.

Results and Discussion

α-([^{11}C]Methyl)tyrosine **4a** and α-([^{11}C]methyl)phenylalanine **4b** were synthesised from dimethyl 2-(4-methoxybenzyl)-2-([^{11}C]methyl)malonate **1a** and dimethyl 2-benzyl-2-([^{11}C]-methyl)malonate **1b**, respectively, by the route shown in Scheme 1. In a typical synthesis starting from 3 GBq (81 mCi) [^{11}C]carbon dioxide, 75 MBq (2 mCi) **4a**, or 115 MBq (3.1 mCi) **4b** was produced in 50 or 45 min, respectively (see Table 1 for radiochemical yields *etc.*). Alkylation of the respective substrates with [^{11}C]methyl iodide, prepared by an 'on-line' system[6] was achieved in *ca.* 76% radiochemical yields counted from [^{11}C]carbon dioxide. The subsequent pig liver esterase [EC 3.1.1.1 (PLE)] catalysed hydrolysis[7] of **1** to the monoester **2** was also carried out in good radiochemical yields (*ca.* 90%) in



Scheme 1 *Reagents:* i, NaH, DMSO; ii, $^{11}CH_3I$, DMSO; iii, PLE, DMSO–H_2O; iv, DPPA, TEA, toluene; v, trimethylsilyl chloride, NaI, toluene; vi, HCl

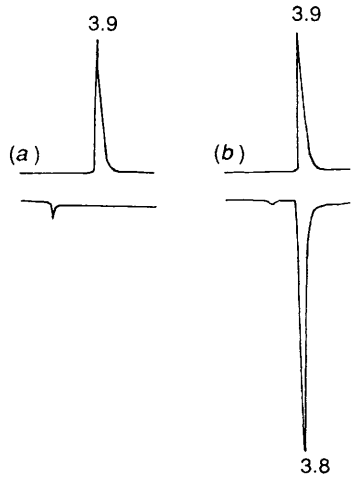


Fig. 1 (*a*) Analytical HPLC chromatogram of purified **4a** and (*b*) with added reference (t_R 3.9 min). Top trace, radioactivity; bottom trace, UV.

Fig. 2 HPLC enantiomeric analysis of **4b** by derivatisation with FDAA (reference D,L-α-methylphenylalanine added). Top trace, radioactivity; bottom trace, UV.

5 min. Since the radiochemical yield was found to vary with different batches of PLE, on purchase of a new enzyme preparation, the amount of protein required for effective hydrolysis had to be adjusted accordingly. As the conversion of **2** into the corresponding iscocyanate **3** required anhydrous conditions, a solid phase extraction using a disposable C-18 column (Sep Pak) was utilised. Elution with tetrahydrofuran (THF) and subsequent evaporation left a dry product; typically 50–60% radiochemical yield was obtained. Initial investigations using sodium azide[8] in the Curtius rearrangement were found to be problematical due to the formation of side-products and decomposition of the sodium azide at higher temperatures. A more reliable procedure for the azide formation was achieved by using DPPA[9] and triethylamine in toluene with radiochemical yields of 95%. This reaction is known to proceed with retention of configuration. Cleavage of the phenolic methyl ether protecting group of the α-methyltyrosine precursor was found to be most efficient (85% radiochemical yield) using trimethylsilyl chloride–sodium azide,[10] and was performed before the final hydrolysis step. Attempts to use 57% hydriodic acid in the removal of the protecting group gave lower yields and caused difficulties in subsequent work-up procedures. The hydrolysis of **3** was achieved using conc. hydrochloric acid at 150 °C yielding **4**, which was adjusted to pH 6.5–7, and purified by preparative HPLC. An analytical chromatogram of purified **4b** is shown in Fig. 1. Data showing typical synthesis times, radiochemical yields, purities, *etc.* is presented in Table 1.

Enantiomeric purities of the products were established by derivatisation with 1-fluoro-5-(2,4-dinitrophenyl)-L-alanine amide (FDAA)[11a,b] yielding diastereoisomeric products which were separated by reversed phase HPLC. These results showed that L-**4a** was obtained in 62% ee, whereas **4b** was found to be racemic. A typical chromatogram is shown in Fig. 2. Although **4a** and **4b** were only partially converted into diastereoisomers, studies showed that no significant kinetic resolution took place.

The enantioselectivity of PLE has been shown to be dependent on the length of the substituent in the 2-position. For small substituents (≤4 carbon atoms) the monoester is produced in the *S*-form, whereas for larger groups (≥5 carbon atoms) the enantioselectivity is reversed to give the *R*-monoester.[12] This has been attributed to the presence of a hydrophobic pocket at the active site of the enzyme, which is only large enough to accommodate side-chains of up to *ca.* 4 carbon atoms. Substrates with longer side-chains are unable to fit into this pocket, giving rise to a reversal in the stereochemical outcome of the hydrolysis. In the case of **1b**, the benzyl group is between 4 and 5 carbon chain-lengths long and gave rise to a racemic product, whereas with **1a**, the asymmetric induction was increased to 62% ee as a result of the longer side-chain (between 6 and 7 carbons). The enantiomeric purities reported here are somewhat lower than those found in the literature, where unlabelled **2a** and **2b** have been produced with ee values of 82 and 45% respectively.[7] A number of factors can be responsible for these differences: (*a*) It is known that for certain substrates the enantioselectivity of PLE can be increased by a lowering of the incubation temperature[13] at the cost of the reaction rate. Indeed a decrease of the reaction temperature

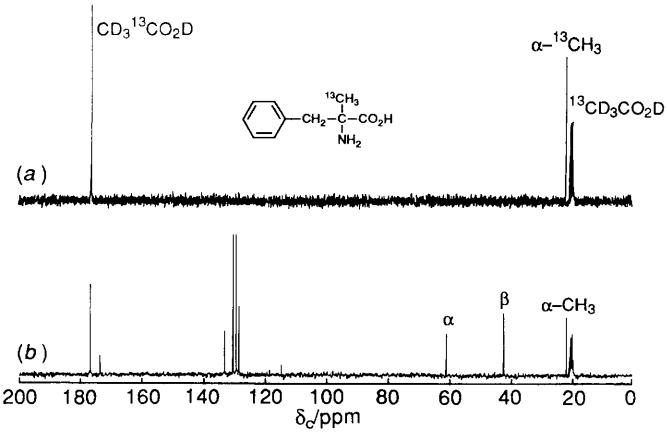


Fig. 3 ^{13}C NMR spectra of (*a*) α-([^{11}C]methyl)phenylalanine and (*b*) reference α-methylphenylalanine

from 37 to 25 °C (as in ref. 7) produced L-**4a** with an ee of 70%. However, considering the 20.3 min half-life of ^{11}C, the increased incubation times were found to be impractical. (*b*) In this work it was found that the rate of the enzyme-catalysed hydrolyses were increased when aqueous TRIS buffer (0.5 mol dm^{-3}) was used instead of aqueous potassium phosphate (0.095 mol dm^{-3}) as found in ref. 7. (*c*) Commercial preparations of PLE are mixtures of several isoenzymes, and due to individual variations in this composition, it is not yet clear as to whether different preparations give identical results. (*d*) It is also possible that crystallisation effects during 'normal' scale work-up procedures (*cf.* tracer work up procedures, where the intermediates are not usually isolated) can be a contributary factor.

To confirm the position of labelling, a ^{13}C synthesis was carried out using (^{13}C)methyl iodide in the same reaction sequence. The ^{13}C NMR spectrum showed a single peak at δ 21.8 corresponding to the α-methyl signal from a reference spectrum (see Fig. 3).

The synthetic versatility of malonic esters in organic chemistry has been known for decades. With the help of functional group transformations (*e.g.* decarboxylation, PLE catalysed hydrolysis), we are now extending the range and versatility of chemistry using short-lived radionuclides at a μmole level by taking advantage of this flexible precursor. The use of labelled malonic ester precursors in the rapid synthesis of ^{11}C-labelled fatty acids, β-keto esters, barbiturates and amines is now being investigated.[14] Preliminary experiments indicate that this approach is suitable for use in rapid-labelling synthesis, where the time aspect of the chemistry is of overriding importance. Further applications of malonic esters in rapid labelling syntheses will be presented elsewhere.

Experimental

General.—The ^{11}C was produced at the tandem van de Graff accelerator at the University of Uppsala by the $^{14}N(p,\alpha)^{11}C$ reaction using a nitrogen gas target. The [^{11}C]carbon dioxide formed was trapped in a lead shielded oven containing activated 4 Å molecular sieves and transported to the chemistry laboratory. [^{11}C]Methyl iodide was produced according to the standard procedure adopted in our laboratory.[6]

Analytical HPLC was carried out on a Hewlett Packard 1090 liquid chromatograph equipped with a diode-array UV detector (254 nm) in series with a β^+-flow detector[15] using the following columns: (A), a 250 × 4.6 mm Nucleosil 10 μm, or (B), a 250 × 4.6 mm Supelco LC-NH_2 5 μm. Preparative HPLC was performed on a Waters M-6000 pump with a Waters M-45 fixed wavelength detector (254 nm) in series with (C), a 250 × 10 mm C-18 column (Nucleosil 30 μ) and a β^+-flow detector. The following mobile phases were used: (D), ammonium formate (0.05 mol dm^{-3}, pH 3.5), (E), methanol; (F), potassium dihydrogenphosphate (0.01 mol dm^{-3}; (G), acetonitrile–water (50:7 v/v), and (H) ethanol. NMR spectra were recorded using a Varian XL-300 NMR spectrometer.

Pig liver esterase [PLE (EC 3.1.1.1)], 200 units mg $protein^{-1}$, suspension in $(NH_4)_2SO_4$ (3.2 mol dm^{-3}, pH 8) was purchased from Sigma. Before each synthesis, 300 or 100 μl protein suspension (1200 or 400 units) for respective substates **1** and **1b** was centrifuged, the buffer decanted and the centrifugate stored at 4 °C until required. Tris(hydroxymethyl)aminomethane buffer (TRIS, 0.5 mol dm^{-3}, pH 7) was passed through a 0.22 μm pore filter and stored for up to two weeks at 4 °C.

[^{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 specially designed one-pot reaction vessel[6] containing a solution of lithium aluminium hydride in THF (0.8 mol dm^{-3}; 0.5 ml). After evaporation of the THF, hydriodic acid (57%; 2 ml) was added and the reaction mixture heated at reflux during which the [^{11}C]methyl iodide formed was distilled over to a reaction vial in a stream of nitrogen gas.

Dimethyl 2-(4-*Methoxybenzyl*-2-([^{11}C]*methyl*)*malonate* **1a**.—Dimethyl 2-(4-methoxybenzyl)malonate (2 mg, 8 μmol) was dissolved in DMSO (100 μl) together with one equivalent of NaH (50% oil dispersion; 0.4 mg, 8 μmol) in a 1 ml microvial equipped with a septum. After being heated at 80 °C for *ca.* 15 min, the substrate was alkylated at ambient temperature by passage over the freshly prepared [^{11}C]methyl iodide in a stream of nitrogen gas. Methylation proceeded almost instantaneously to yield **1a**. Analytical HPLC conditions: column A, mobile phase D–E (30:70) isocratic, flow 2 ml min^{-1}; t_R 3.0 min.

Monomethyl 2-(4-*Methoxybenzyl*)-2-([^{11}C]*methyl*)*malonic Acid* **2a**.—Compound **1a** was added to the previously prepared PLE centrifugate, followed by TRIS buffer (100 μl) which was used to rinse the reaction vessel. After incubation at 37 °C for 5 min, the resulting compound **2a** was purified using a disposable C-18 Sep Pak cartridge pre-conditioned with THF (2 ml) and water (10 ml). After diluting **2a** to 10 ml in water, the solution was passed through the Sep Pak, washed with water (2 × 2 ml) and the product subsequently eluted with THF (4 × 200 μl) into a 7 ml screwcap vessel (the first fraction was discarded). The THF was removed by heating and shaking at 150 °C. Analytical HPLC conditions: column A, mobile phase D–E (30:70) isocratic, flow 2 ml min^{-1}; t_R 2.3 min.

α-([^{11}C]*Methyl*)*tyrosine* **4a**.—Toluene (400 μl), triethylamine (100 μl) and DPPA (200 μl) were added and shaken in the sealed vessel at 150 °C for 5 min to produce the methyl 2-(4-methoxybenzyl)-2-isocyanato[3-^{11}C]propionate **3a**. The septum was removed and toluene (500 μl), trimethylsilyl chloride (500 μl) and NaI (10 mg) was added. After re-sealing, the vessel was heated and shaken at 150 °C for 5 min. Hydrolysis to **4a** was achieved with conc. HCl (1 ml, 150 °C, 10 min) in the sealed vessel. After adjusting the pH to 6.5–7 the product was passed through a 0.22 μm pore filter and purified by preparative HPLC using the following conditions: column C, mobile phase D–H (90:10) isocratic, flow 6 ml min^{-1}; t_R 5.2 min.

Authentication of the product was achieved by co-chromatographing with an unlabelled reference sample using the following analytical HPLC conditions: column B, mobile phase F–G (5:95 to 40:60 in 0–8 min), flow 2 ml min^{-1}; t_R 3.9 min.

α-([^{11}C]*Methyl*)*phenylalanine* **4b**.—As above, except that

100 μl (400 units) of pig liver esterase was used for the selective methyl ester hydrolysis and that the ether cleavage step using trimethylsilyl chloride was omitted. Analytical conditions as for **4a**; t_R **4b** 3.6 min.

α-([^{13}C]*Methyl*)*phenylalanine*.—Dimethyl 2-benzylmalonate (10 mg, 45 μmol) was added to NaH [2 mg, 42 μmol (50% dispersion)] in DMSO (100 μl). The synthesis was carried out with ^{13}C-methyl iodide (7 mg, 49 μmol) as described above but with increased reaction times: alkylation (15 min, 80 °C), PLE hydrolysis (20 min, 37 °C), Curtius rearrangement (DPPA–TEA–toluene; 15 min, 150 °C) and hydrolysis (conc. HCl; 10 min, 150 °C). After preparative HPLC purification, the relevant fraction was evaporated to dryness and the product re-dissolved in D_2O–CD_3CO_2D (2:1) with 3 drops of D_2SO_4 and subsequently analysed by ^{13}C NMR. The spectrum shows a single peak at δ 21.8 (Fig. 3) corresponding to the α-methyl peak from reference α-methylphenylalanine [δ_C(300 MHz; D_2O; standard CD_3CO_2D) 21.8 (CH_3), 42.5 (NCH), 61.1 (CH_2), 128.5, 129.4, 130.4 (ArCH), 133.2 (ArC) and 173.6 (CO)].

Determination of Enantiomeric Purity.—After preparative HPLC purification, the relevant fraction was evaporated and the residue dissolved in water (100 μl) and DFAA (1% acetone solution; 200 μl). The solution was transferred to a 1 ml reaction vial containing non-labelled **4a** or **4b** (5 μmol) and sodium hydrogen carbonate (1.0 mol dm^{-3}; 40 μl). The reaction vessel was sealed, heated at 60 °C for 15 min, and then cooled and treated with hydrochloric acid (2 mol dm^{-3}; 20 μl). The resulting solution was analysed by HPLC using column A and the following conditions: **4a**, flow 2 ml min^{-1}, solvents D–G, gradient 0–10 min 45–50% G, wavelength 340 nm; **4b**, as above except for, solvents D–E, gradient 0–10 min 45–55% E. Retention times for the DFAA–derivatives of the amino acids were: 6.2, 6.9, 6.8 and 7.7 min for L-**4a**, D-**4a**, L-**4b** and D-**4b**, respectively.

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