Asymmetric Synthesis of $^{11}$C-Labelled L- and D-Amino Acids by Alkylation of Imidazolinedione Derivatives

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Alanine and phenylalanine have been labelled in the 3-position, and 2-aminoacidic acid in the 6-position, with the short-lived positron-emitting radionuclide $^{11}$C ($t_{1/2} = 20.3$ min). (R)- and (S)-2-tert-butyl-1-tert-butylxycarbonyl-3-methyl-4-imidazolinedione were alkylated with $[^{11}\text{C}]$methyl iodide, $[^2\text{H}]$benzyl iodide or 4-idoisobutyrof $[^{11}\text{C}]$nitric, prepared in multi-step syntheses starting from $[^{11}\text{C}]$carbon dioxide. 3-$^{11}$C-Labelled L- and D-alanine and phenylalanine were obtained after acidic hydrolysis in 75 and 30% radiochemical yields (decay-corrected) within 25 and 50 min, respectively. The radiochemical purities were higher than 98%. After a two-step hydrolysis procedure, L- and D-2-amino-6-$^{11}$C]adipic acid were obtained in 20–25% radiochemical yield (decay-corrected) within 45 min with a radiochemical purity of 85%. The enantiomeric purities were 98% for alanine and phenylalanine and >96% for 2-aminoacidic acid. In a typical synthesis, 385 MBq of [3-$^{11}$C]alanine were obtained, starting with 1.2 GBq [11C]carbon dioxide, with a synthesis time of 25 min.

The development of positron emission tomography (PET) has created a demand for biomolecules and pharmaceuticals labelled with short-lived positron-emitting radionuclides, such as $^{15}$N, $^{11}$C and $^{18}$F ($t_{1/2} = 10.0$, 20.3 and 110 min). PET is a non-invasive medical imaging technique, used for studies of biochemical and physiological processes in vivo.1–3 Amino acids labelled with these radionuclides have been successfully applied in PET studies of, for example, amino acid transport and protein synthesis, and have also been applied clinically in the diagnosis of tumours.4 In such studies, the use of both enantiomers of the labelled amino acid can be of advantage.5 The number of discovered naturally occurring non-proteinogenic amino acids is rapidly approaching 1000; many of these substances possess important biological functions. This, and the expanded use of the PET technique, have caused an increased interest in the development of general synthetic methods for the production of amino acids labelled with short-lived positron-emitting radionuclides.

$^{11}$C-Labelled amino acids have been synthesized using different approaches, and obtained in either racemic or enantiomerically enriched form.6–8 Methods for the fast resolution of racemates using enzymatic procedures or HPLC have been developed.9,10 However, a disadvantage is that half of the radioactivity is lost in the resolution procedure. Routes for asymmetric synthesis of $^{11}$C-labelled amino acids can thus be very useful, especially if the enantiomeric purity obtained is high enough to avoid the necessity of enantiomer resolution. Furthermore, the use of asymmetric synthesis makes it possible to prepare both enantiomers of the amino acid.

The asymmetric synthesis of L- and D-amino acids, $^{11}$C-labelled in the side chain is here presented. The $^{11}$C-labelled amino acids were synthesized via highly stereoselective alkylations of imidazolidinone derivatives with $[^{11}\text{C}]$labelled alkyl iodides (see Scheme 1).

Part of this work has previously been presented elsewhere.8,11 Since then, a similar approach has been presented for the asymmetric synthesis of some $^{11}$C- and $^{18}$F-labelled amino acids.12,13

Experimental

The $^{11}$C was produced by the $^{14}$N(p,x)$^{11}$C nuclear reaction using a nitrogen gas target bombarded with protons, produced by the Tandem van de Graaf accelerator (10 MeV protons) at The Svedberg Laboratory, Uppsala University, or lately by the Scanditronix MC-17 cyclotron (17 MeV protons) at the Uppsala University PET Centre. The $[^{11}\text{C}]$carbon dioxide formed was either used directly in the synthesis of $[^{11}\text{C}]$alkyl iodides or converted into hydrogen $[^{11}\text{C}]$cyanide using the Scanditronix

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RNP-17 gas processing system according to a general procedure described previously. Analytical HPLC was performed on a Hewlett-Packard 1090 liquid chromatograph equipped with a UV-diode array detector in series with a β⁺-flow detector. The analytical columns used were a Nucleosil C-18 250 × 4.6 mm (i.d.) 10 μm column (A), a Bondapak C-18 300 × 4.6 mm (i.d.) 10 μm (B), a Nucleosil NH₂ 250 × 4.6 mm (i.d.) 10 μm column (C), a Beckman Ultrasil AX 250 × 4.6 mm (i.d.) 5 μm column (D), or a Crownpak CR(+ 150 × 4 mm (i.d.) column (E). The mobile phases were used were 0.05 M ammonium formate (pH 3.5) (F), 0.01 M potassium dihydrogen phosphate (pH 4.6) (G), 0.1 M potassium dihydrogen phosphate (pH 4.4), aqueous perchloric acid (pH 2.0) (H), saline (K), acetonitrile (MeCN)–water (50:7, v/v) (L) and methanol (M). Preparative LC was carried out using either of two systems: (1) Waters M-45 pump and a Spherisorb ODS-1 250 × 10 mm (i.d.) 10 μm column in series with a Pharmacia dual path monitor UV-2 and a GM tube connected to a rate meter, (2) Beckman 126 pump and a Spherisorb ODS-1 250 × 10 mm (i.d.) 10 μm column connected to a Beckman 166 UV-detector in series with β⁺-flow detector. A modified Gilson 231 autosampler was used for injection and fraction collection.

The identity of the [1⁴C]alkyl halides and the [1⁴C]amino acids was confirmed using LC-analysis and addition of reference substances. In all cases, the response from the UV-detector was simultaneous with that from the radiodetector, corrected for the delay between the detectors.

Solid-phase extraction C-18 EC Chromabond (100 mg) columns were preconditioned with 3 ml dichloromethane, 5 ml methanol and 10 ml distilled water, prior to use. The Sep-Pak C-18 columns were preconditioned with 10 ml of ethanol and 10 ml of distilled water. Tetrahydrofuran (THF) was dried by distillation over sodium–benzophenone under a nitrogen atmosphere. Hydridic acid (HI; 57°c) was distilled and stored in the freezer. The imidazolidinone derivatives, (R)- and (S)-2-tert-butyl-1-tert-butoxyacarbonyl-3-methyl-4-imidazolidinone [(R)- and (S)-Boc-BMI] were synthesized according to a previously described procedure, or purchased from Aldrich.

/¹⁴C/Methyl iodide. The [¹⁴C]methyl iodide was prepared by reduction of [¹⁴C]CO₂ with lithium aluminium hydride (LAH) (ca. 1 M) and treatment with HI, followed by distillation in a stream of nitrogen gas into the reaction vessel, using a one-pot reactor as described in detail elsewhere.

/¹⁴C/Benzyl iodide. The [¹⁴C]benzyl iodide was produced by trapping the [¹⁴C]CO₂ in a solution of phenylmagnesium bromide in THF, followed by reduction with LAH and treatment with HI. The product was isolated using a liquid–liquid extraction work-up procedure with light petroleum ether as the organic phase, as described in detail elsewhere. The petroleum ether was evaporated off and the [¹⁴C]benzyl iodide redissolved in THF for use in the alkylation reaction.

4-Iodobutyro[¹⁴C]nitrile. Hydrogen [¹⁴C]cyanide was trapped in THF containing Kryptofix® (222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) and 0.5 M aqueous potassium hydroxide. Addition of 1,3-diodopropane and heating yielded 4-iodobutyro[¹⁴C]nitrile, as described in detail elsewhere.

/³⁷C/Amino acids. The imidazolidinone [(R)- or (S)-Boc-BMI] (10 mg, 39 μmol) was dissolved in THF (300 μl) and 1.2–1.3 equivalents (60–65 μl, 46–50 μmol) of a mixture of 750 μl butyllithium (1.5 M), 195 μl TMP (2,3,6,6-tetramethylpiperidine) and 525 μl THF were
added at −72°C. The [11C]alkyl halides were added either in a stream of nitrogen gas or as a THF solution, and the cooling bath was then removed. After 5–8 min at ambient temperature, the alklylation products were hydrolysed by addition of 9 M hydrochloric acid (1.5 ml), and heated at 200°C for 8–10 min. The resulting solution was diluted with 4 ml of water and passed through a Sep-Pak C-18 column. The eluent was evaporated off to dryness and the residue containing the [3-13C]amino acids was redissolved in saline. The alklylation reactions and the hydrolysis were followed by LC (column A, solvents F-M 30:70 v/v, flow 2 ml min⁻¹, column temp. 40°C, wavelength 254 nm). The identity and radiochemical purity were determined by LC using column C (solvents G-L 5:95 v/v linear gradient to 40:60 over 0–8 min, flow 2 ml min⁻¹, column temp. 40°C, wavelength 254 nm). The retention times were 6.2 and 4.5 min for the 3-13C-labelled alanine and phenylalanine, respectively. For the preparative purification of [3-13C]phenylalanine, the semipreparative LC system (1) was used (solvent K, flow 4 ml min⁻¹, wavelength 254 nm), the retention time for [3-13C]phenylalanine was 4.5 min.

2-Amino[6-13C]adipic acid. The alklylation was carried out as described above for the [3-13C]amino acids. The 4-iodobutyro[13C]nitrile was added as a THF solution at −72°C to the anion of (R)- or (S)-Boc-BMI and the reaction mixture was kept at ambient temp. for 10 min. The alklylation product was purified, from unchanged [13C]cyanide and 4-iodobutyro[13C]nitrile, by adding 4 ml of water and then passing the mixture through a C18-SPE column. The impurities were washed out with 3 × 2 ml water and the alklylation product was eluted with 2 ml dichloromethane. The dichloromethane was removed by shaking the reaction vessel at 70°C for 5 min. Hydrolysis was then carried out in two steps. Firstly, 0.6 ml 10 M aqueous sodium hydroxide solution was added, then the vessel was sealed and heated at 150°C for 10 min. Secondly, 1.5 ml 9 M HCl was added and the heating was continued for another 5 min. The 2-amino[6-13C]adipic acid was purified using semipreparative HPLC using system (2) (solvent K, flow 6 ml min⁻¹, wavelength 254 nm). The reaction mixture was neutralized with sodium hydroxide before injection. The retention time for 2-amino[13C]adipic acid was 3.1 min. The alklylation reaction was followed by LC (column B, solvents F-L 40:60 v/v, flow 2 ml min⁻¹, column temp. 40°C, wavelengths 254 nm). The retention time for the alkylated imidazolidinone was 3.2 min. The hydrolysis was followed using column D (solvent H isocratic, flow 0.7 ml min⁻¹, column temp. 40°C, wavelength 230 nm). The retention time for 2-amino[13C]adipic acid was 6.9 min.

[6-13C]Lysine. The anion of (R)- or (S)-Boc-BMI was alklylated with 4-iodobutyro[13C]nitrile and the formed alklylation product was purified with the SPE-procedure as described above for 2-amino[6-13C]adipic acid. The dichloromethane was distilled off by shaking the reaction vessel at 70°C for 5 min. Dry methanol (400 μl) was added to redissolve the alklylation product which then was transferred to a septum-equipped vial containing cobalt chloride (1.5 mg), sodium borohydride (1 mg) and tert-butyllamine–borane (1.5 mg) in methanol (200 μl). The reaction mixture was heated at 90°C for 15 min, HCl (1.5 ml 9 M) was added and the vial was heated at 150°C for 15 min. The resulting solution was diluted with water (2 ml) and passed through an NH₂-SPE. The hydrolysis was followed using column D (solvent H isocratic, flow 0.7 ml min⁻¹, column temp. 40°C, wavelength 230 nm). The retention time for [6-13C]lysine was 6.4 min.

Analysis of enantiomeric excess. For the determination of enantiomeric purity, the 3-13C-labelled amino acids were converted into diastereomeric derivatives by reaction with N-(5-fluro-2,4-dinitrophenyl)-L-alanine amide (Marfey’s reagent) as described in detail elsewhere.21,22 The derivatives were separated by LC (column A, solvents F-M 70:30 linear gradient to 50:50 over 0–10 min for alanine and 60:40 to 35:65 over 0–15 min for phenylalanine, flow 2 ml min⁻¹, column temp. 40°C, wavelength 340 nm). The retention times were 6.0 and 9.0 min for the diastereoisomers of L- and D-[3-13C]alanine and 8.2 and 12.2 min for the diastereoisomers of L- and D-[3-13C]phenylalanine, respectively. The enantiomeric purity of the 2-amino[6-13C]adipic acid was determined using a chiral HPLC-column (Column E, solvent J, flow 0.4 ml min⁻¹, column temp. 25°C, wavelength 200 nm). The retention times were 4.34 min for the D-form and 6.3 min for the L-form of the 2-amino[6-13C]adipic acid.

Results and discussion

The imidazolidinone derivatives, (R)- and (S)-Boc-BMI, were synthesized according to a previously described procedure,16 or more recently purchased from Aldrich. The [13C]alkyl iodides and 4-iodobutyro[13C]nitrile were prepared according to procedures described in detail elsewhere.17–20 The lithium enolate of the Boc-BMI’s were generated by treatment with TMP–lithium in THF at −72°C. The [13C]alkyl iodides were added, the cooling bath was removed and the reaction vessel was then allowed to stand at room temperature for 5–10 min. For the [13C]methyl and [α-13C]benzyl iodides, the alklylation reaction was almost complete within 5 min. The labelled alklylation product using 4-iodobutyro[13C]nitrile was obtained in 62–94% radiochemical yield in 10 min reaction time. After acidic hydrolysis the [3-13C]amino acids were purified by use of a Sep-Pak C-18 cartridge to give the [3-13C]alanine in 75% and the [3-13C]phenylalanine in 30% decay-corrected radiochemical yields, both in >98% radiochemical purity within 25 and 50 min synthesis time, respectively. The hydrolysis to the 2-amino[6-13C]adipic acid was carried out in two steps, first with sodium hydroxide and then hydrochloric acid. The radiochemical yield was 20–25% decay-corrected and
counted from $^{13}\text{C}$ cyanide with a 45 min synthesis time. The radiochemical purity was 85\%.

The enantiomeric purities for the [3-$^{13}\text{C}$] amino acids were determined by derivatization of the amino acids with Marfey’s reagent followed by LC analysis,\textsuperscript{21,22} and for the 2-amino[6-$^{13}\text{C}$] adipic acid by the use of a chiral HPLC-column, Crownpak CR(+). Both enantiomeric forms of the [3-$^{13}\text{C}$] amino acids were obtained in approximately 98\% e.e. D- and L-2-amino[6-$^{13}\text{C}$] adipic acid were obtained in >96\% e.e. The enantiomeric purities of the commercially available (R)- and (S)-Boc-BMI\textsuperscript{15}’s, used in the synthesis of the amino acids, can vary a few percent, according to measurements done by Lindström et al.,\textsuperscript{23} which may explain the variation in e.e. of the 13C-labelled amino acids.

In the synthesis of 2-amino[6-$^{13}\text{C}$] adipic acid, we found that the two-step procedure for hydrolysing both the imidazolidinone and nitrile moieties was more effective than using solely acidic or basic conditions. In the hydrolysis of the nitrile functionality, aqueous sodium hydroxide containing ca. 15\% hydrogen peroxide was also investigated, without any increase of the yield. In the cases of [3-$^{13}\text{C}$] phenylalanine and 2-amino[6-$^{13}\text{C}$] adipic acid, the acidic hydrolysis was sometimes incomplete. Purification was then carried out using semipreparative HPLC. A promising solution to the hydrolysis problem with the Boc-BMI’s is to use a new pair of chiral glycin derivatives, (R)- and (S)-4-alloxy-2-tert-butyl-2,5-dihydroimidazole-1-carboxylate, recently presented by Blank and Seebach.\textsuperscript{24} Racemization during hydrolysis at high temperature in 13C-labelled synthesis of amino acids has been reported.\textsuperscript{25,26} Though the conditions of the hydrolysis in this case were drastic, the high enantiomeric excesses obtained indicate that little or no racemization occurred. [6-$^{13}\text{C}$] Lysine was obtained in preliminary experiments from alkylation with 4-iodobutyryl[13C] nitrite on (S)-Boc-BMI, followed by reduction (cobalt chloride, sodium borohydride and tert-butylamine-borane)\textsuperscript{27} and subsequent hydrolysis. Attempts to synthesise [3-$^{13}\text{C}$] valine using [2-$^{13}\text{C}$] isopropyl iodide in the alkylation reaction were unsuccessful. The use of multifunctional electrophilic [13C] precursores opens up the possibility of synthesizing more complex 13C-labelled amino acids using this method. In an alternative approach, amino acids are labelled via [13C] cyanide substitution on 5-(bromoalkyl)-Boc-BMI’s which give, for example, [5-$^{13}\text{C}$] glutamic acid. Work in this area is in progress.

The excellent stereoselectivity and the availability of both enantiomeric forms of the imidazolidinones as well as other 13C-labelled electrophilic precursors, can make this synthetic route suitable as a general method for the preparation of D- and L-amino acids labelled with [13C] in the side chain. Alkylation of the imidazolidinone derivatives with first an unlabelled and then a [13C]-labelled alkyl halide has been shown to be a possible approach for obtaining 13C-labelled $\alpha$-methyl amino acids,\textsuperscript{12} which are of special interest because of their role as enzyme-inhibitors in the biosynthesis of certain neurotransmitters.

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References

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