Asymmetric Synthesis of L-2-Amino[3-^{11}C]butyric Acid, L-[3-^{11}C]Norvaline and L-[3-^{11}C]Valine

Gunnar Antoni and Bengt Långström*

Department of Organic Chemistry, Institute of Chemistry, University of Uppsala, Box 531, S-751 21 Uppsala, Sweden

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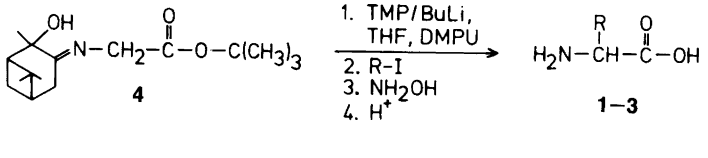
The short-lived radionuclide ^{11}C ($t_{1/2}$ = 20.4 min) has been used in the asymmetric synthesis of L-2-amino[3-^{11}C]butyric acid, L-[3-^{11}C]-norvaline and L-[3-^{11}C]valine. The syntheses were performed by alkylation of [(+)-2-hydroxypinanyl-3-idene]-glycine *tert*-butyl ester under anhydrous conditions in tetrahydrofuran/1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidinone with lithiated 2,2,6,6-tetramethylpiperidine as base, using the appropriate ^{11}C-alkyl iodides prepared in a one-pot reactor from [^{11}C]carbon dioxide. Following removal of the protecting groups, the –[3-^{11}C]amino acids were obtained in 80–82 % enantiomeric excess and in 9–25 % radiochemical yields, decay corrected and calculated on the basis of the amount of [^{11}C]carbon dioxide at the start of the syntheses within 50–55 min.

^{11}C-Labelled enantiomerically pure amino acids, in particular the L-forms, are of great interest in biomedical research applying the positron-emission tomography (PET) technique.[1a,b] Methods for obtaining enantiomerically enriched or pure L-^{11}C-amino acids are available, such as oxidative deamination,[2] resolution by liquid chromatography,[3,4] enzymatic synthesis[5,6,7] and asymmetric organic synthesis.[8,9,10]

The previously presented synthesis of L-[3-^{11}C]alanine[10] has now, with minor modifications, been extended to the synthesis of 3-^{11}C-labelled L-2-aminobutyric acid (**1**), L-norvaline (**2**) and L-valine (**3**), using the corresponding ^{11}C-alkyl iodides synthesized according to a recently reported procedure.[11,12] The syntheses were performed by asymmetric alkylation of [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester[13] (**4**) in a mixture of tetrahydrofuran (THF)/1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidinone (DMPU) with lithiated 2,2,6,6-tetramethylpiperidine (TMP) as base, using [1-^{11}C]ethyl, [1-^{11}C]propyl and [2-^{11}C]isopropyl iodides. Following removal of the protecting groups, **1**, **2** and **3** were obtained (Scheme 1).

The ^{11}C-alkyl iodides were synthesized in the one-pot reactor system shown in Fig. 1, which is described in detail elsewhere.[11]



R= $CH_3{}^{11}CH_2$, $CH_3CH_2{}^{11}CH_2$, $H_3C{}^{11}CH$ (H_3C)

Scheme 1.

*To whom correspondence should be addressed.

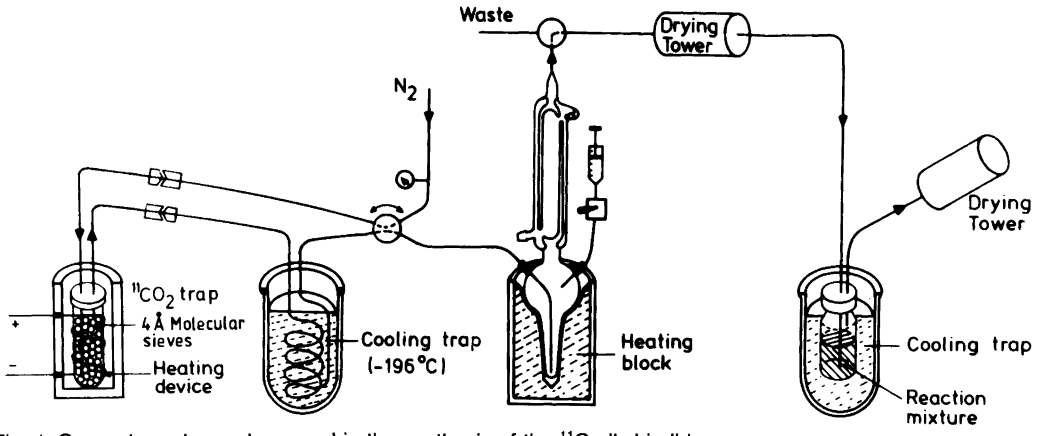


Fig. 1. One-pot reactor system used in the synthesis of the ^{11}C-alkyl iodides.

Results and discussion

An important factor determining the degree of asymmetric induction in the synthesis of the [3-^{11}C]amino acids is the enantiomeric purity of the cold, i.e. unlabelled, substrate [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester (**4**). The optical purities of **4** and (+)-2-hydroxypinan-3-one (**5**) used to synthesize **4**, as determined by polarimetry, were in accordance with those reported in the literature (97 % e.e.).[13]

In the preceding paper (cf. Ref. 10), the purification of **4** presented a problem. Using the "dry column" flash chromatography[14] procedure, **4** was obtained with a purity higher than 99.5 %. Although the product gradually decomposed, the purity was still higher than 96 % after storing at 4 °C, under N_2, for several months. The yield of the alkylation reactions seemed unaffected by the side-products formed as a result of decomposition.

Factors determining the results of the alkylation reactions were reaction temperature, concentration of **4**, and the solvent mixture used, as well as the reactivity of the ^{11}C-alkyl iodides. Occasionally, on distillation of the ^{11}C-alkyl iodides from the hydriodic acid, small amounts of water co-distilled, which partially inhibited the alkylation reaction.

The alkylations were found to proceed slowly when employing reaction conditions similar to those used in the synthesis of [3-^{11}C]alanine.[10] To increase the radiochemical yield of the alkylation products, the more polar solvent DMPU was added. In the reactions with [1-^{11}C]ethyl and [1-^{11}C]propyl iodides the amount of DMPU was not critical. Approximately 5–10 % (*v/v*) was enough to speed up the alkylation reaction to give the labelled products in radiochemical yields higher than 90 %, calculated on the basis of the amount of ^{11}C-alkyl iodide, at −72 °C with a reaction time of 3–7 min. Under these reaction conditions, [2-^{11}C]isopropyl iodide gave only a small amount of product after 15 min. A steady increase in radiochemical yield of the alkylation products from [2-^{11}C]isopropyl iodide was observed, however, as a function of the concentration of DMPU, and increasing the temperature to −42 °C also raised the radiochemical yield considerably.

In work with short-lived radionuclides, apart from the time needed for the work-up procedure, the reaction time must be taken into account. Since rather a long time was needed for the alkylation reaction with [2-^{11}C]isopropyl iodide – about 12–16 min to obtain 90 % radiochemical yield of the product – the optimal reaction time was determined by plotting the amount of radioactivity of the alkylation product against the reaction time (Fig. 2) by the procedure described elsewhere.[15]

According to Fig. 2, on increasing the concentration of DMPU in the reaction mixture from 36 % to 44 %, the optimal reaction time was 4 min instead of 8 min and resulted in an increase in the radiochemical yield of the alkylation product from 53 % to 76 %. Increasing the concen-

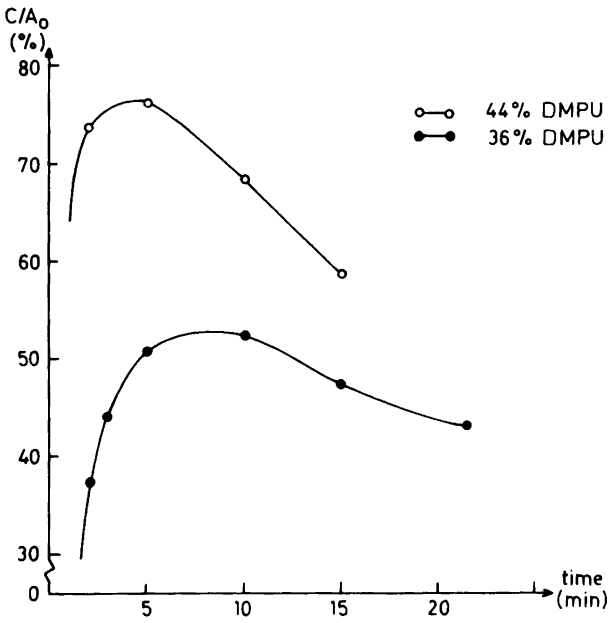


Fig. 2. Alkylation product (C) normalized to the amount of radioactivity in the form of [2-^{11}C]isopropyl iodide at the start of the synthesis (A_0), versus the reaction time. Two experiments with different concentrations of DMPU (36 % or 44 %) performed at −42 °C, (0.17 M **4**) are shown.

trations of DMPU and **4** further was impractical, since the solutions became very viscous and lithiated **4** occasionally precipitated. The asymmetric induction in the alkylation reaction may be affected by the concentration of DMPU. However, the inaccuracy in the determinations of the enantiomeric purities was greater than any such solvent effect.

Removal of the protecting groups was performed in two steps. First, the amino protecting group was removed by treatment with hydroxylamine and then the *tert*-butyl ester by acid hydrolysis. It was found essential to remove the amino protecting group before carrying out the acid hydrolysis. Direct acid hydrolysis of the crude alkylation product resulted in the formation of several unidentified labelled products and only 30–50 % free amino acid. Removal of the chiral handle was thus performed by reaction with hydroxylamine in an ethanolic solution at pH 4–5, and the reaction was complete in 1 min at 50 °C. The subsequent acid hydrolysis of the amino acid ester was accomplished within 5 min.

The radiochemical purity of the crude ^{11}C-amino acids depends mainly on the radiochemical purity of the ^{11}C-alkyl iodide produced. In the synthesis of **2**, ^{11}C-labelled alanine and valine were obtained in small amounts from [^{11}C]methyl and [2-^{11}C]isopropyl iodides formed as by-products in the synthesis of the [1-^{11}C]ethyl iodide.[11] [3-^{11}C]Valine occasionally contained small amounts of ^{11}C-labelled alanine and 2-aminobutyric acid, whereas L-[3-^{11}C]norvaline contained only minor amounts of [3-^{11}C]alanine. However,

Table 1. Radiochemical yields, radiochemical purities and enantiomeric purities of the [3-^{11}C]amino acids.

Amino acid	Radiochemical yield/%	Radiochemical purity/%	Enantiomeric excess/%
L-2-Amino[3-^{11}C]butyric acid	25	98	82
L-[3-^{11}C]Norvaline	25	99	80
L-[3-^{11}C]Valine	9	99	80

Table 2. Radiochemical yields and purities of the ^{11}C-alkyl iodides.

Alkyl iodide	Radiochemical yield/%	Radiochemical purity/%
[1-^{11}C]Ethyl iodide	46	85
[1-^{11}C]Propyl iodide	55	95
[2-^{11}C]Isopropyl iodide	40	90

it was possible to separate all the amino acids by preparative LC, with retention times of 6–11 min; to avoid elution of the amino acids with the solvent front, the water content of the solution injected into the preparative LC column should be low. The total synthesis times, including preparative LC purification, were 50–55 min, counted from release of [^{11}C]carbon dioxide from the molecular sieves. The results of the syntheses are presented in Table 1.

The radiochemical yields were calculated for the decay-corrected purified product and were based on the amount of [^{11}C]carbon dioxide released from the molecular sieves.

The radiochemical yields and purities of the ^{11}C-alkyl iodides,[11,12] presented in Table 2, influenced the results of the [3-^{11}C]amino acids obtained. The extent of alkylation and the efficiency of removal of the protecting groups are also important.

The identity of the [3-^{11}C]amino acids was verified by LC analyses. Simultaneous appearance of signals from the radio detector and the mass detector (from added authentic material) confirmed that the desired products had been obtained.

Determination of the enantiomeric excess of the products was performed by GLC[16], using the *N*-trifluoroacetyl methyl ester derivatives (Fig. 3).[17]

Experimental

General. The ^{11}C was produced by the ^{14}N (p,α)^{11}C nuclear reaction in a target with nitrogen gas, using 10 MeV protons produced by the Tandem Van de Graaff accelerator at the University of Uppsala. The ^{11}C was obtained as [^{11}C]carbon dioxide, which was trapped in lead-shielded 4Å molecular sieves and transported to our laboratory.

Analytical LC was carried out on a Hewlett-Packard 1090 liquid chromatograph equipped with either an Alltech 250×4.6 (i.d.) mm C-18 10-μm column (A), or a Supelco 250×3.9 (i.d.) mm LC-NH_2 5 μm column (B) in series with a β-flow detector.[18]

Preparative LC was performed on a Waters system (UV detector M441, pump A 6000) equipped with a 150×10 mm (i.d.) LC-NH_2 5-μm column (C) in series with tubing coiled around a GM tube. Ammonium formate (0.05 M, pH 3.5) (D), methanol (E), potassium dihydrogen phosphate (0.01 M, pH 4.6) (F), acetonitrile/water (500:70, *v/v*) (G), and acetonitrile/0.1 % phosphoric acid (92:8 *v/v*) (H) were used as mobile phases.

Analytical GLC was performed on a Hewlett-

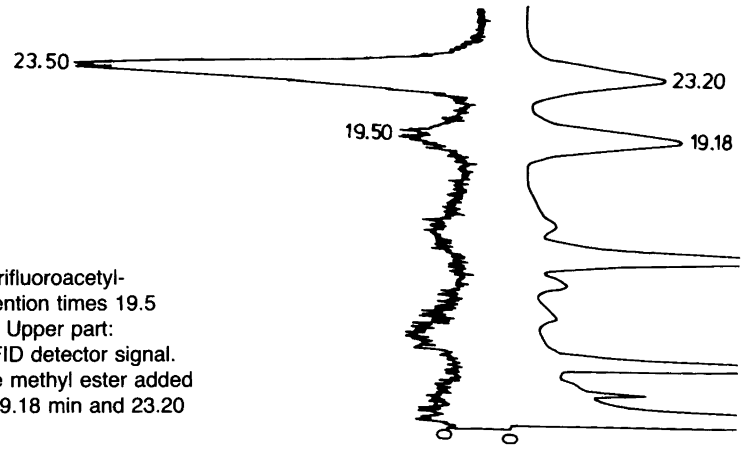


Fig. 3. Separation of D- and L-*N*-trifluoroacetyl-[3-^{11}C]norvaline methyl ester (retention times 19.5 min and 23.50 min, respectively). Upper part: radiodetector signal; lower part: FID detector signal. D- and L-*N*-trifluoroacetylnorvaline methyl ester added as reference (retention times of 19.18 min and 23.20 min, respectively).

Packard 5880 A gas chromatograph (FID detector) equipped either with a 200×0.3 cm glass column of Supelco 3 % SP-300 on Supelcoport (I), or a 70×0.3 cm glass column of 5 % PS-400/chrom W HP 80/120 (J) in series with a β-gasflow detector.[19]

Synthetic procedure. [(+)-2-Hydroxypinanyl-3-idene]glycine tert-butyl ester (**4**). The synthesis of **4** was performed according to the description in Refs. 10 and 13. The crude product was purified by the "dry column" flash chromatography procedure, as follows: A sintered-glass filter (3.5×3 cm) with silica gel S (0.032–0.063 mm) was fitted to a glass vessel connected to a water aspirator. A hexane/diethyl ether mixture (50:50 *v/v*) was poured on top of the silica and aspirated through it. The crude **4** (600 mg) was dissolved in 10 ml of the same hexane/diethyl ether mixture and placed on the silica surface which was again aspirated to dryness. The components in the sample were then eluted successively by applying 10 ml portions of hexane/diethyl ether mixtures with increasing concentrations of ether. The silica was aspirated to dryness between additions of eluent and the eluate was collected. The hexane/diethyl ether mixtures used, together with the percentage of **4** in the collected fractions after evaporation, are presented in Table 3. The combined fractions 5–7 were evaporated and 290 mg (48 % yield) of **4** was obtained with a chemical purity higher than 99.5 %, determined by GLC (column J, oven temperature 150 °C, N_2 flow 30 ml min^{-1}).

^{11}C-Alkyl iodides. The syntheses of the ^{11}C-alkyl iodides were performed in the one-pot reactor system (Fig. 1) described in detail elsewhere.[11] The [^{11}C]carbon dioxide was transferred from the 4Å molecular sieves in a stream of nitrogen gas to the one-pot reactor and trapped in the appropriate reagent solution. In the synthesis of ethyl, propyl and isopropyl iodides, 0.7 ml of 0.25 M methylmagnesium bromide in THF, 0.7 ml of 0.8 M ethylmagnesium bromide in THF and 0.7 ml of 0.8 M methyllithium in THF/diethyl ether (50:50 *v/v*) were used, respectively. After a reaction time of 2–4 min, 1–1.5 ml of LAH (1 M) in THF was added and the solvents were removed. 2 ml of 54 % hydriodic acid were then added, and the product was distilled off and transferred in a stream of nitrogen gas through a drying tower (sodium hydroxide/phosphorus pentoxide) to the reaction vessel. The entire procedure took 10–15 min, counted from the release of the [^{11}C]carbon dioxide from the molecular sieves to completion of the trapping of the ^{11}C-alkyl iodide in the reaction vessel.

Determination of the radiochemical purity and the identity of the products were performed in independent experiments by LC analysis [column A, solvents D/E (50:50) for 5 min, then gradient elution for 5–10 min with D/E to 15:85 *v/v*, flow 2 ml min^{-1}, wavelength 254 nm, oven temperature 40 °C].

^{11}C-Amino acids **1–3**. A base solution was prepared by adding 1 ml of butyllithium (1.5 M in hexane) to a solution of 260 µl of TMP in 700 µl of THF cooled to −72 °C (base solution A), or 333 µl of DMPU and 366 µl of THF (base solution B). From A (synthesis of **1** and **2**) or B (synthesis of **3**) 2.2 equiv., calculated on the basis of the molar amount of **4**, were taken and added to **4** dissolved in a THF/DMPU mixture kept at −72 °C. The solution immediately became red-brown on the addition of the base solution, indicating the formation of the lithiated dianion of the substrate. The ^{11}C-alkyl iodides were trapped in the reaction mixture and the alkylation reaction was performed at −72 °C, except in the synthesis of [3-^{11}C]valine, where the temperature was −42 °C; the reaction conditions used in the alkylation reaction are summarised in Table 4. The reaction was quenched after 5–8 min by introducing 0.8 ml of a 1.25 M hydroxylamine solution, prepared by dissolving 0.7 g of hydroxylamine hydrochloride in 1.92 ml of (glacial)

Table 3. Hexane/diethyl ether mixtures used in the purification of **4** by "dry column flash chromatography", and percentage of **4** in the collected fractions after evaporation.

Fraction No.	Hexane/diethyl ether (*v/v*)	Amount of **4**/%
1	50:50	0
2	40:60	0
3	30:70	60
4	25:75	92
5	20:80	99.5
6	15:85	99.5
7	10:90	99.5

Table 4. Reaction conditions used in the synthesis of the [3-^{11}C]amino acids.

^{11}C-Alkyl iodide	4/mg	THF/ml	DMPU/ml	Base solution/ ml	Alkylation reaction time/min
[1-^{11}C]Ethyl iodide	25	0.2	0.1	0.265	5
[1-^{11}C]Propyl iodide	30	0.1	0.2	0.330	5
[2-^{11}C]Isopropyl iodide	40	0	0.3	0.425	8

acetic acid and 4 ml of ethanol and adjusting the volume to 8 ml with water. The reaction mixture was heated at 45–50 °C for 2–4 min with ultrasonic mixing. 2 ml of 6 M hydrochloric acid were then added and the reaction vessel heated at 130 °C for 5 min, followed by passage through a Sep-Pak® C-18 column and evaporation to dryness. To the solid residue obtained after evaporation, 3 ml of acetonitrile was added and the solution was decanted from the precipitated salts and injected into the preparative LC column (column C, flow 4 ml min^{-1}, solvent H, room temperature, wavelength 254 nm). The collected fractions were evaporated to dryness and 5 ml of 0.1 M phosphate buffer (pH 7.4) was added and the pH of the solution adjusted to 7.4. The identity and radiochemical purity of the products were determined by LC [column A, solvents D/E (15:85 *v/v*), flow 2 ml min^{-1}, wavelength 254 nm, column temperature 40 °C; column B, solvents F/G (5:95 *v/v*), gradient elution to F/G 40:60 0–6 min, flow 2 ml min^{-1}, column temperature 40 °C, wavelength 230 nm].

N-*Trifluoroacetyl methyl ester derivatives.* The *N*-trifluoroacetyl methyl ester derivatives of the amino acids were obtained by a modification of the procedure presented in Ref. 17. The solution containing the ^{11}C-amino acid was evaporated to dryness, and 5 ml of 1.5–3 M dry hydrogen chloride in methanol was added and the reaction mixture heated at 100 °C for 10 min in a sealed vessel. The reaction mixture was evaporated to dryness, and 3 ml of dichloromethane and 2 ml of trifluoroacetic anhydride were added. The solution was heated in a sealed vessel at 100 °C for 10 min, and the excess solvents and reagents were then removed by evaporation at room temperature and 100 µl of dichloromethane was added. The enantiomeric excess determinations were performed by GLC (column I, flow 45 ml min^{-1} N_2, FID detector, column temperatures 77, 85 and 80 °C for the derivatives of **1**, **2** and **3**, respectively).

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*International Communication University of Uppsala.