Asymmetric Synthesis of L-[3-^{11}C]Alanine

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 synthesis of L-[3-^{11}C]alanine from [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester and [^{11}C]methyl iodide is reported. The alkylation was carried out using anhydrous conditions in THF with 2,2,6,6-tetramethylpiperidyl-lithium as base. Following a rapid two-step hydrolysis with hydroxylamine acetate and hydrochloric acid, respectively, the product was purified by preparative liquid chromatography, which gave L-[3-^{11}C]alanine, in 12–28 % radiochemical yield, with a radiochemical purity higher than 95 %. The optical purity was determined to be 89 % e.e. by gas chromatography.

The possibility of studying human metabolism *in vivo* by means of positron-emission tomography has stimulated the development of synthetic methods for many compounds labelled with short-lived positron emitters, such as ^{11}C($t_{\frac{1}{2}}$ = 20.4 min).[1]

In our laboratory the synthesis of ^{11}C-labelled aliphatic and aromatic amino acids has been of particular interest. We have focused on asymmetric synthesis even if other resolving methods, such as liquid chromatography[2] and treatment by D-amino acid oxidase[3] have been used. So far, the only ^{11}C-labelled amino acid obtained directly in either D- or L-form by organic synthesis is methionine[4]. In this case, the stereochemistry had already been established prior to labelling with [^{11}C]methyl iodide. Asymmetric synthesis was first applied in ^{11}C-synthesis in the alkylation of a chiral isonitrile with [^{11}C]methyl iodide using an extractive ion-pair technique which yielded L-[3-^{11}C]alanine in 48 % e.e.[5]

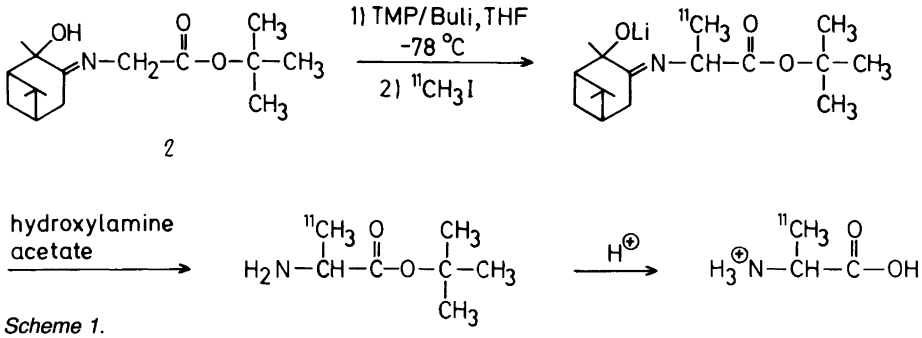
The use of a chiral rhodium complex in the asymmetric hydrogenation of the condensation product obtained from ^{11}C-labelled benzaldehyde[6] and oxazolones gave L-[3-^{11}C]phenylalanine in 60 to 80 % e.e.[7] Enzymatic methods have also been used in the syntheses of ^{11}C-labelled amino acids. L-[4-^{11}C]aspartic acid was synthesized from [^{11}C]carbon dioxide by the use of phosphoenolpyruvate carboxylase(EC 4.1.1.31) and glutamic/oxaloacetic acid transaminase(EC 2.6.1.1) immobilized on sepharose supports.[8] Recently, L-[3-^{11}C]serine was prepared from [^{11}C]methanol, using the enzymes alcohol oxidase (EC 1.1.3.13), catalase and serinehydroxymethyl transferase (EC 2.1.2.1) immobilized on glutaraldehyde activated glass beads.[9]

The asymmetric synthesis of L-[3-^{11}C]alanine from [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester[10] and [^{11}C]methyl iodide[11], according to Scheme 1, is reported in this paper.

With the use of "cold" substrates (stable nuclides), L-alanine was obtained in an enantiomeric excess of 87 %, determined by capillary GC.[12] When the synthesis was carried out using [^{11}C]methyl iodide and a cold substrate, the L-[3-^{11}C]alanine was obtained in 89 % e.e., determined by enantiomeric analysis using GC.

One important reason why asymmetric synthesis is preferable to enzymatic synthesis in the preparation of ^{11}C-labelled amino acids is that both labelled enantiomers can be obtained, and in this specific case it is possible to prepare D- or L-^{11}C-amino acids labelled in the 3-position. The method is also of great interest in the preparation of other amino acids by the use of other labelled alkyl halides.[13]

*To whom correspondence should be addressed.



Scheme 1.

Discussion and results

L-[3-^{11}C]alanine was synthesized according to Scheme 1. [^{11}C]methyl iodide was prepared in 3–5 min by the routine procedure, starting with [^{11}C]carbon dioxide, described elsewhere.[11]

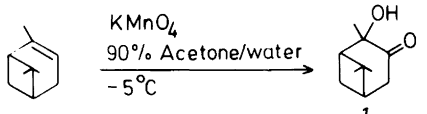
The alkylation reaction proceeded with an enantiomeric excess between 85 and 95 %, determined from the diastereomeric ratio of the alkylation product by analytical HPLC. The yields depended on the conditions of preparation of the base used, 2,2,6,6-tetramethylpiperidyl-lithium, and on complications occurring in the synthesis of [^{11}C]methyl iodide, *i.e.*, small amounts of hydriodic acid or iodine distilling over to the reaction flask. Since the imine group makes the alkylation product sensitive to racemization, the hydrolysis was carried out in two steps. The first one, using 0.6 M hydroxylamine acetate in 70 % aqueous ethanol at room temperature, removed the imine group. Hydrolysis of the *tert*-butyl ester group was then performed with 6 M hydrochloric acid at 110 °C, with little or no racemization. Purification by preparative LC gave L-[3-^{11}C]alanine with a radiochemical purity higher than 95 % in 12–28 % radiochemical yield, time-corrected, and based on the amount [^{11}C]carbon dioxide released from the molecular sieves.

In a typical production run, about 5–10 mCi L-[3-^{11}C]alanine was isolated, starting with 125–175 mCi [^{11}C]carbon dioxide. The total time for synthesis was 30–45 min.

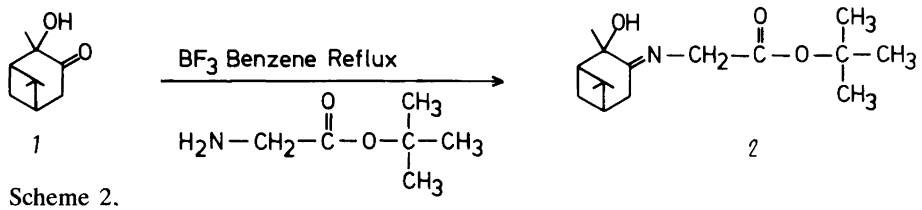
The chiral substrate, [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester, used to induce chirality in the alkylation reaction, was prepared from (+)-2-hydroxypinan-3-one[10] and glycine *tert*-butyl ester according to Scheme 2, and obtained in a purity of 95 % by column chromatography.

The product, obtained as an yellow oil, gave colourless crystals in hexane with a purity higher than 99 %, but the yield was too low to afford a preparative method for purification. The synthesis of (+)-2-hydroxypinan-3-one was performed according to Scheme 3, and the product was obtained in a purity higher than 98.5 %, according to GC analysis.

The optical purity of (+)-2-hydroxypinan-3-one was determined by polarimetric measurement to be 97 % e.e.



Scheme 3.



Scheme 2.

The optical purity of [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester was assumed to be the same as that of the ketol. Polarimetric determination gave a specific rotation in agreement with the value reported in literature.[10]

In the cold synthesis, the optical purity of the L-form of alanine was determined by chiral capillary GC to be 87% e.e. Four methods were tested to determine the optical purity of L-[3-^{11}C]alanine: capillary GC[12], transfer RNA[14], enantiomer separation on HPLC[2] or GC using chiral stationary phases (column C and M respectively). The amount of product formed after synthesis and derivatization was too low to give reliable results with the capillary GC system used. The β-gas flow[15] detector is only compatible with gas chromatographic systems that give higher gas flow than can be achieved with capillary GC systems. Moreover, the maximum injection volume in the capillary GC system is too low to give reliable results with the β-gas flow detector used. However, the results indicate an excess of L-form over D-form. It was not possible to separate the enantiomers of alanine on the LC-column.

The coupling between transer RNA and L-[3-^{11}C]alanine which is necessary for the determination of optical purity by the t-RNA method depends on the use of the proper buffer system. So far, no such system has been found. However, with the use of the above mentioned GC column (M) in series with a β-gas flow detector,[15] it was possible to determine the enantiomeric excess of the labelled amino acid as the *N*-trifluoroacetylalanine methyl ester to be 89% e.e. The derivatization procedure took about 45 min.

The total time for synthesis, counted from the release of [^{11}C]carbon dioxide up to the end of the preparative LC purification of the product, was 30–45 min. The product was identified by means of three LC systems. In all three cases the radioactive signal was simultaneous (time-corrected for time delay between mass detector and β-flow detector) with the UV or fluorescence signal from added alanine or its isoindol derivative (OPA-derivative) Ref. 16.

The availability of other ^{11}C-alkyl halides[13], as well as the other optical form of the starting material, indicates that this synthetic route might be a general method for the preparation of D- or L-amino acids labelled in the 3-position with ^{11}C.

Experimental

General

Analytical LC was carried out on a Hewlett-Packard 1084B or 1090 liquid chromatograph equipped with one of the following columns: A) 250×4.6 mm C-18 Nucleosil 10 μm, B) 200×4.6 mm Lichrosorb-NH_2 5 μm, C) 120×4.6 mm Lichrosorb 100 10 μm with 3-glycidoxypropyltrimethoxysilanyl-L-proline, Cu^{2+} (Ref. 2), D) 150×3.9 mm Nova-Pak C-18 5 μm in series with a variable wavelength detector or a Waters fluorescence detector 420 in series with a β-flow detector.

Preparative LC was performed on a Waters system equipped with a M-441 UV detector in series with a GM tube and a 250×10 mm C-18 Nucleosil 30 μm column, E). The solvents used in LC were F) aqueous 0.1 M ammonium formate, pH 3.5, G) methanol, H) methanol/tetrahydrofuran/0.05 M sodium acetate, 0.05 M disodium hydrogen phosphate, pH 7.5, (20/20/960, v/v), I) methanol water (65/35 v/v), J) 0.01 M potassium dihydrogen phosphate, pH 4.6, and K) acetonitrile/water (50/7, v/v).

Analytical gas chromatography was carried out on a Hewlett-Packard 5880A GC equipped with a β-gas flow detector and the following columns were used: L) 70×0.3 cm glass column with 3% PS-400/chrom W HP 80/100, M) 200×0.32 cm column with 5% SP-300, 100/120 Supelcoport, N) 175×0.3 cm glass column with the same packing material as column M.

Capillary GC was performed on a Hewlett-Packard 5830A GC equipped with an 18835 capillary inlet system. The column used was Chrompack 50 m×0.23 mm, fused silica WCOT, XE-60-2-Valine-S-Pea (0.11 μm), O).

The ^{11}C was produced 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. [^{11}C]carbon dioxide was trapped in 4 Å molecular sieves in a lead shield and transported to the chemistry laboratory.

The solvent used in the alkylation reaction (THF) was carefully dried by distillation over calcium hydride and stored over 4 Å activated molecular sieves.

NMR spectra were recorded on a JEOL FX-60 or JEOL FX-100 NMR spectrometer.

Synthetic procedure

(+)-2-Hydroxypinan-3-one (1) and [(+)-2-hydroxypinanyl-3-idene]glycine tert-butyl ester (2), (Schemes 3 and 2).

Compound (*1*)and (*2*)were synthesized according to Ref. 10. The latter compound was purified by column chromatography [aluminium oxide basic grade I 150×20 mm, eluted with hexane/methanol (97.5/1.5, v/v)]. It was possible to obtain a small amount as colourless crystals, with a melting point of 101.5–102.5 °C, from hexane. ^{1}H NMR and ^{13}C NMR spectroscopy were used to identify the product.

The products were analysed by analytical GC (column L) using the following conditions. Flow 25 ml/min, N_2, time 0–3 min at 80 °C then 10 °C/min to 160 °C. Retention times for glycine *tert*-butyl ester, compound (*1*)and (*2*) were 1.15, 7.0 and 16.4 min, respectively.

L-Alanine

L-Alanine was synthesized according to Ref. 10. The enantiomeric purity was determined by GC using columns N and O.

Retention times for D- and L-*N*-trifluoroacetylalanine methyl ester were 4.15 and 4.85 min (100 °C flow 25 ml/min, N_2, column N) and for D- and L-*N*-trifluoroacetylalanine isopropyl ester 7.9 and 8.1 min (135 °C, flow 0.95 ml/min, N_2, column O), respectively.

L-[3-^{11}C]alanine (Scheme I)

[(+)-2-Hydroxypinanyl-3-idene]glycine *tert*-butyl ester (4–10 mg, 1.4×10^{-5}–3.6×10^{-5} mol) and 2,2,6,6-tetramethylpiperidyl-lithium (3.9×10^{-5}–9.7×10^{-5} mol) in THF, total volume 0.35–0.45 ml, was cooled to −78 °C with dry ice/ethanol.

[^{11}C]Methyl iodide was then prepared by the routine procedure in 3–5 min (Ref. 11) and was transferred through a drying tower to the reaction vessel by a stream of nitrogen gas. The alkylation reaction was concluded in 5–7 min. The reaction was quenched by the addition of 0.6 M hydroxylamine acetate in 70 % aqueous ethanol and the mixture was stirred for 7–9 min at room temperature. Hydrogen chloride (6 M, 1.5 ml) was added and the solution heated in a sealed vessel for 5 min at 100 °C. Purification by preparative LC gave L-[3-^{11}C]alanine.

The amino acid was identified as the OPA derivative[16] by the use of column D and solvents H and I (gradient, time 0–10 min., I=30 to %I=50, flow 1.5 ml/min, wavelength *ex.* 395 nm, *em.* 405 nm, retention time 6.85 min) or as free acid using column A, with F and G as solvents (%G=10, flow 3 ml/min, retention time 1.28 min, wavelength 254 nm) and column B with solvents J and K (gradient time 0–4 min %K=95 to %K=60, time 4–8 min, %=60 to %K=45, flow 2 ml/min, wavelength 220 nm, retention time 4.3 min).

Determination of the optical purity of L-[3-^{11}C]alanine

[3-^{11}C]Alanine was converted to the *N*-trifluoroacetylalanine methyl ester by the following procedure. The amino acid was evaporated to dryness and dissolved in 5 ml of a 6.1 M solution of dry hydrogen chloride in methanol. The mixture was heated to 110 °C for 15 min in a sealed flask. The excess methanol and hydrogen chloride were evaporated. The solid residue was cooled and mixed with 2 ml of dichloromethane and 2.5 ml of trifluoroacetic anhydride and heated to reflux for 15 min. in a sealed flask. The solvents were then evaporated at room temperature and the liquid residue dissolved in 0.5 ml of dichloromethane. The enantiomeric analysis was performed on GC using a column packed with 5 % SP-300, 100/120 Supelcoport as the chiral medium.

Retention times and conditions were as already described.

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