Asymmetric Synthesis of L-[3-^{11}C]Alanine and L-[3-^{11}C]Phenylalanine by a Phase-Transfer Alkylation Reaction

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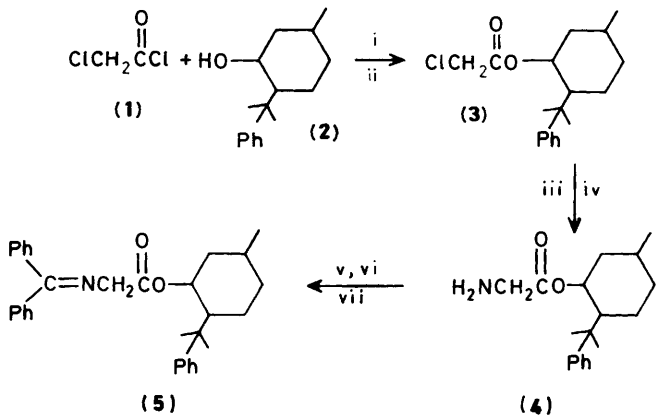
The asymmetric syntheses of enantiomerically enriched L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine ($t_{\frac{1}{2}}$ 20.4 min for ^{11}C) by phase-transfer alkylation of (−)-8-phenylmenthan-3-yl *N*-(diphenylmethylene)-glycenate with [^{11}C]methyl and [α-^{11}C]benzyl iodides, respectively, are presented. The total synthesis times were 35—55 min, from the start of synthesis of the [^{11}C]methyl and [α-^{11}C]benzyl iodides. The products were obtained in 15—40% (decay corrected) radiochemical yields and higher than 98% radiochemical purities. The enantiomeric purities of the amino acids were determined to be 52—55% enantiomeric excess (e.e.). In a typical run 600 MBq of L-[^{11}C]alanine was obtained, starting with 5.2 GBq of [^{11}C]carbon dioxide.

The use of specifically labelled short-lived positron-emitting radionuclides in biomolecules and pharmaceuticals is increasing due to their value in applications such as positron emission tomography (PET).[1] So far the impact of PET has mainly been in studies of energy metabolism using labelled glucose and glucose analogues[1c] or in studies of receptor binding in various neurological diseases using selective ligands[1d-g] labelled with radionuclides such as ^{11}C, ^{13}N, and ^{18}F with a $t_{\frac{1}{2}}$ of 20.4, 10.0, and 110 minutes respectively. There has also been interest in PET investigations using ^{11}C-labelled amino acids and results of clinical interest have been obtained using ^{11}C-labelled methionine in studies of tumours in the brain and the pituitary.[1h-j] Work on amino acid transport[1k] and protein synthesis using various ^{11}C- or ^{13}N-labelled amino acids is in progress. Furthermore amino acids such as tyrosine, DOPA, tryptophan, and 5-hydroxytryptophan are of special interest due to their role as neurotransmitter precursors. For these reasons we have been interested in developing fast and reliable synthetic methods for specifically labelling amino acids.

In such studies both enantiomeric forms are of interest. Methods for resolution of the enantiomers from a racemic mixture are available but have the disadvantage that the radiochemical yield will be low, since radioactivity is lost in the resolution.[2] Asymmetric synthesis is therefore an interesting approach, since in most cases, it will give the opportunity of preparing both enantiomers in high radiochemical yields.

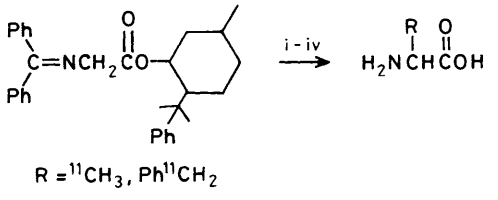
^{11}C-Labelled alkyl iodides are the most versatile and widely used precursors in the synthesis of positron-emitting radiotracers.[3] Among the different methods for the asymmetric synthesis of amino acids, the use of alkyl halides in alkylation reactions with glycine derivatives is a good choice for adaptation to ^{11}C-labelling. Since many alkylation methods for the asymmetric synthesis of amino acids are humidity and temperature-sensitive we needed to develop a more simple method with high reproducibility where the technical handling could be minimized. (−)-8-Phenylmenthol has been successfully used as a chiral auxiliary for asymmetric induction in many different applications.[4] A diastereoisomer of (−)-8-phenylmenthol has also been prepared which enables the induction of the opposite chirality.[4]

^{11}C-Alkyl iodides[3] can now be prepared and used in the synthesis of a number of racemic and enantiomerically enriched [3-^{11}C]amino acids by alkylation of chiral or non-chiral glycine derivatives.[5] [3-^{11}C]Amino acids can also be prepared by condensing [1-^{11}C]aldehydes with oxazolones.[6]



Scheme 1. *Reagents:* i, $PhNEt_2$; ii, C_6H_6; iii, NH_3; iv, DMSO; v, $Ph_2C{=}O$; vi, BF_3–diethyl ether; vii, xylene

In this paper, the synthesis of a new chiral glycine derivative, (−)-8-phenylmenthan-3-yl *N*-(diphenylmethylene)glycinate (**5**) is reported (Scheme 1). Compound (**5**) was employed in phase-transfer alkylation reactions (Scheme 2) with [^{11}C]methyl and [α-^{11}C]benzyl iodides to obtain L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine, respectively. The reactions were performed in a two-phase system with tetrabutylammonium hydroxide as a phase-transfer catalyst (Q^+OH^-).



Scheme 2. *Reagents:* i, Q^+QH^- aq./CH_2Cl_2; ii, RI; iii, NH_2OH; iv, OH^-

Results and Discussion

The short half-life of ^{11}C means that synthetic reactions and work-up procedures have to be fast. The labelled precursor, such as [^{11}C]methyl iodide or [α-^{11}C]benzyl iodide, should be added as late as possible in the synthetic scheme and it is important to select a substrate with appropriate protective

Table. Radiochemical yield and purity and diastereoisomeric and enantiomeric purity of L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine

Amino acids	Radiochemical yield (%)	Radiochemical purity (%)	Diastereoisomeric excess (%)	Enantiomeric excess (%)
L-[3-^{11}C]Alanine	40	>98	56	52
L-[3-^{11}C]Phenylalanine	15	>98	60	55

groups. In this paper the substrate has also been designed to create asymmetric induction.

The chiral handle used, (−)-8-phenylmenthol [(**2**), (1*R*,2*S*,5*R*)-(−)-2-(α,α-dimethylbenzyl)-5-methylcyclohexanol], was originally obtained according to a literature procedure[7] but has now become commercially available (Merck). Purification of (**5**) presented a problem. It was difficult to separate (**2**), formed by hydrolysis in the synthesis of (**4**), from (**4**) and (**5**). Some problems were also encountered in the separation of benzophenone from (**5**). However, the alkylation reactions seemed to be unaffected by the presence of minor amounts of (**2**) and benzophenone.

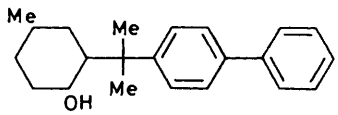
The ^{11}C-alkyl iodides were synthesized in one-pot systems.[3b] [^{11}C]Methyl iodide was obtained in 60—80% radiochemical yield within 5 min with a radiochemical purity higher than 98%.[8] The total synthesis time for [α-^{11}C]benzyl iodide, obtained in 4—7 ml dichloromethane, was 20—30 min, with the radiochemical yield and purity being 20—30% and 97—99%, respectively.[5c]

The alkylation products were obtained in higher than 90% radiochemical yields within 5 minutes at 45 °C, with ultrasonic mixing of the phases. On addition of the base and catalyst, the reaction mixture became yellow, indicating the formation of the corresponding carbanion of the substrate. Occasionally, in reactions with [α-^{11}C]benzyl iodide, no colour change was observed and the product was obtained in very low radiochemical yields. This problem was solved by adding more catalyst until the solution became yellow.[5c]

Removal of the protecting groups was performed in two steps. The amino-protecting group was removed within 3 min using hydroxylamine. The hydrolysis was most efficient at about pH 4—6; at higher or lower pH the deprotection reaction was found to be very slow. Hydrolysis of the ester to the free amino acid with aqueous sodium hydroxide was complete within 5 min at 130 °C. Following hydrolysis, the solution was passed through a Sep-Pak® C-18 column. The total synthesis times, calculated from release of [^{11}C]carbon dioxide from the molecular sieves to the purified products, were between 35 and 55 min* (see Table). A discrepancy between the diastereoisomeric excess determinations of the diastereoisomeric alkylation products and the enantiomeric excess values of the free amino acids, in the order of 7—8%, may be explained either by racemisation or kinetic resolution during the hydrolysis. According to previous studies, alkaline hydrolysis using these conditions causes negligible racemisation.[9]

The phase-transfer reaction has thus been shown to be a convenient and practical method for the synthesis of L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine. The use of another alcohol such as 8-(biphenyl-4-yl)menthol (**6**)† as a chiral handle may increase the enantiomeric purity of the products. Such studies are now in progress.

Using column D (see Experimental section) it was possible to separate the diastereoisomers of the alkylation products, enabling the amount of asymmetric induction in the alkylation step to be determined. The two diastereoisomers were collected and hydrolysed separately giving in both cases the ^{11}C-labelled amino acid. The determination of the enantiomeric purities of [3-^{11}C]alanine and [3-^{11}C]phenylalanine were performed by three methods adapted to the analysis of compounds labelled with short-lived radionuclides, using either g.c. (Method *a*) or l.c. (Method *b* and *c*).



(**6**)

In the first method, the [3-^{11}C]amino acids were converted into the *N*-trifluoroacetyl methyl ester derivatives[10] and analysed by g.c. (column M).[11] This method afforded good separations of the L- and D-derivatives of [3-^{11}C]alanine, with retention times of about 10 and 12 min respectively. The enantiomers of phenylalanine were not fully resolved and the retention times were about 40—55 min, leading to difficulties in determining the enantiomeric purity. The second method, employing column C, was applied to the separation of the enantiomers of [3-^{11}C]phenylalanine, by l.c., as free amino acids.[12] This system was very sensitive to pH and salts and inadequate separation of the two enantiomers was occasionally found; D- and L-alanine could not be separated by this method. Another, more reliable method for the determination of the enantiomeric purity was based on separation of diastereoisomeric derivatives of the amino acids by a C-18 column (B). The [3-^{11}C]amino acids were converted into diastereoisomeric derivatives in a reaction with *N*-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (Marfey's reagent).[13] To ensure that no kinetic resolution occurred, all the [3-^{11}C]amino acid had to be converted into the diastereomeric product. This method, used for both [3-^{11}C]alanine and [3-^{11}C]phenylalanine, was found to be practical and reliable.

The results in the Table are taken from method *a*, the results from the other methods being of the same order of magnitude.

Experimental

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

Analytical l.c. was performed on a Hewlett-Packard 1090 liquid chromatograph equipped with a u.v. diode-array detector or a Waters system (pump M501, u.v. absorbance detector M440, data and control system M840) in series with a β-flow detector[14] and any of the following columns: (A) Supelco, 250 × 4.6 mm (i.d.) LC-NH_2 5 μ column, (B) Alltech, 250 × 4.6 mm (i.d.) C-18 10 μ column, (C) Daicel Chem. Ind. Ltd., CHIRALPACK WH, 250 × 9 mm (i.d.) 10 μ column, or (D) Nucleosil 50, 250 × 4.6 mm (i.d.) 5 μ column. Ammonium formate (0.05M, pH 3.5) (E), methanol (F), potassium

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

† See W. G. Dauben and R. A. Bunce, *Tetrahedron Lett.*, **23**, 4875.

dihydrogen phosphate (0.01M, pH 4.6) (G), acetonitrile (H), acetonitrile–water (500:70, v/v) (I), triethylamine phosphate (0.05M) (J), 0.05 mM $CuSO_4$ (K), and hexane–propan-2-ol (100:0.2, v/v) (L) were used as mobile phases.

Analytical g.c. was carried out on a Hewlett-Packard 5880A gas chromatograph (FID detector) equipped with either a 1.75 m 5% SP-300 100/120 Supelcoport column (M) or a 70 cm 3% PS-400/chrom W HP 80/100 column (N), in series with a β-gasflow detector.[15] Dichloromethane (CH_2Cl_2), used as solvent in the alkylation reaction, was purified by passing through an aluminium oxide column (basic grade 1). Tetrahydrofuran (THF) was dried by distillation over sodium/benzophenone into a glass vessel containing activated 4 Å molecular sieves. N.m.r. spectra were performed on a Jeol FX-60 or a Varian XL-300 n.m.r. spectrometer. For the ^{13}C n.m.r. assignments the APT and DEPT techniques in the Varian XL-300 system were used. I.r. spectra were obtained using a Perkin-Elmer 177 grating infrared spectrometer.

(−)-8-*Phenylmenthan*-3-*yl Chloroacetate* (**3**).—(−)-8-Phenylmenthol (**2**) (2.8 g, 12 mmol) and *N*,*N*-diethylaniline (2.3 g, 15 mmol) were dissolved in benzene (15 ml). The solution was heated to 35 °C, chloroacetyl chloride (1.7 g, 15 mmol) in diethyl ether (3 ml) was added and the mixture was heated to 60—70 °C for 6 h, after which water (20 ml) was added to dissolve the salt. Following extraction with diethyl ether (30 ml), the combined organic phases were washed successively with 0.5M sulphuric acid (10 ml), water (10 ml), 10% aqueous sodium hydrogen carbonate (10 ml), and brine (10 ml) and then dried ($MgSO_4$). Evaporation and two recrystallizations from ethanol gave 8-phenylmenthan-3-yl chloroacetate (**3**) (2.4 g, 65%), m.p. 82—83 °C, $[\alpha]_D^{20.00}$ 11.1° ± 0.1° (*c* 1.07 in EtOH). The chemical purity was determined to be higher than 99% by g.c. [column N, flow 30 ml/min^{-1}, linear temperature gradient (5 °C min^{-1}) from 180 °C to 210 °C, N_2, R_t 6.5 min]. The identity of the product was confirmed by n.m.r. spectroscopy: δ_C(300 MHz; $CDCl_3$; standard $CDCl_3$) 21.8, 22.8, and 29.7 (Me), 26.2, 34.5, and 41.5 (CH_2), 40.8 ($ClCH_2$), 31.3 and 50.3 (CH), 75.8 (OCH), 39.5 (C), 125.1, 125.3, and 128.0 (ArCH), 151.6 (ArC), and 166.4 (CO).

(−)-8-*Phenylmenthan*-3-*yl Glycinate* (**4**).—Compound (**3**) (6.0 g, 19 mmol) was dissolved in a mixture of dimethyl sulphoxide (DMSO) (150 ml) and distilled water (5 ml), saturated with NH_3(g), and the reaction mixture was stirred for 6 h at room temperature. Distilled water (150 ml) was added, the mixture was extracted with diethyl ether (4 × 50 ml), and the combined organic phases were washed with water and brine, dried ($MgSO_4$) and evaporated. (−)-8-Phenylmenthan-3-yl glycinate (**4**) was obtained as an oil (5.0 g, 90%). The chemical purity was determined by g.c., under the same conditions as described above, and found to be 95% (R_t 6.7 min). N.m.r. and i.r. spectroscopy confirmed that the desired product had been obtained: δ_C(60 MHz; $CDCl_3$; standard $CDCl_3$) 21.8, 23.0, and 29.6 (Me), 26.2, 34.5, and 41.7 (CH_2), 43.7 (NCH_2), 31.2 and 50.2 (CH), 74.3 (OCH), 39.4 (C), 125.2 and 127.7 (ArCH), 151.8 (ArC), and 172.9 (CO).

(−)-8-*Phenylmenthan*-3-*yl* N-(*Diphenylmethylene*)*glycinate* (**5**).—Compound (**4**) was used without further purification in the synthesis of (**5**). To compound (**4**) (1.26g, 4.4 mmol) and benzophenone (0.73 g, 4 mmol) in xylene (10 ml), boron trifluoride–diethyl ether (50 μl) was added. The reaction mixture was heated to reflux over a period of 14 h, while the water formed was removed by azeotropic distillation. The reaction mixture was diluted with diethyl ether (50 ml) and washed with an aqueous solution of 10% citric acid (2 × 5 ml), 5% aqueous sodium hydrogen carbonate (5 ml) and brine (5 ml). The organic phase was dried ($MgSO_4$) and the solvents evaporated. The product was purified by dry column flash chromatography.[16] Analysis by g.c. showed a purity of 90—95% (column N, flow 30 ml min^{-1}, linear oven temperature gradient, 10 °C min^{-1}, from 210 °C to 280 °C, N_2, R_t 16 min). N.m.r. spectroscopy confirmed that the desired product had been obtained: δ_C(300 MHz; $CDCl_3$; standard Me_4Si) 21.8, 24.8, and 28.1 (Me), 26.6, 34.6, and 41.7 (CH_2), 55.3 (NCH_2), 31.3 and 50.4 (CH), 74.7 (OCH), 39.7 (C), 124—131 (ArCH, m), 136.2, 139.3, and 151.5 (ArC), 169.9 (CN), and 171.4 (CO).

[^{11}C]*Methyl Iodide*.—[^{11}C]Carbon dioxide was released from the molecular sieves by heating and transferred in a stream of nitrogen gas (150—250 ml min^{-1}) to the one-pot system described previously[8] and trapped in a solution of lithium aluminium hydride (*ca.* 0.5M; 0.5 ml) in THF. The solvent was evaporated off and 57% hydriodic acid (1.5 ml) was added. The [^{11}C]methyl iodide formed was distilled off and transferred in the nitrogen gas stream through a drying tower (sodium hydroxide–phosphorus pentaoxide) to the reaction vessel. Analysis by l.c. (column B, solvents E–F 50:50 v/v, wavelength 254 nm, column temperature 40 °C, flow 2 ml min^{-1}, R_t 2.5 min) confirmed the identity of the product.

[α-^{11}C]*Benzyl Iodide*.—[^{11}C]Carbon dioxide was transferred, as above, to the one-pot system and trapped in a solution of phenylmagnesium bromide (0.8M, 0.6 ml) in THF. After a reaction time of 4 min at room temperature, a solution of lithium aluminium hydride (*ca.* 1M; 1.2 ml) in THF was added and the solvents were evaporated off. 57% Hydriodic acid (1.5 ml) was added and the solution was diluted with water (10 ml) and then passed through a solid phase extraction (SPE) C-18 column. After washing with water (10 ml) the column was flushed dry using a nitrogen gas stream. The [α-^{11}C]benzyl iodide was eluted with dichloromethane (2—3 ml) and the solution was then passed through a small column (10 cm, sodium hydrogen sulphite–aluminium oxide basic grade 1, 3:7 v/v). Analysis by l.c. (column B, solvents E–F 20:80 v/v, wavelength 254 nm, column temperature 40 °C, flow 2 ml min^{-1}, R_t 3.3 min) confirmed that the desired product had been obtained.

[3-^{11}C]*Amino Acids*.—Compound (**5**) (10—30 mg) was dissolved in dichloromethane (0.7 ml) in a vial equipped with a septum. To this solution were respectively added [α-^{11}C]benzyl and [^{11}C]methyl iodides, the former in dichloromethane solution and the latter in a stream of nitrogen gas. A solution of tetrabutylammonium hydrogen sulphate (20—50 mg) in aqueous sodium hydroxide (2.5M, 0.7 ml) was added and the alkylation reaction was performed in an ultrasonic bath at 45 °C for 5 min. The organic phase was separated, a 0.5M solution of hydroxylamine in 80% ethanol was added, and the solution was heated at 45 °C for 5 min. Aqueous sodium hydroxide (5M, 2 ml) was added and the solution heated at 130 °C for 5 min. The alkylation reaction and hydrolysis were followed by l.c. using column B (solvents E–F 10:90 v/v, flow 2 ml min^{-1}, column temperature 40 °C, wavelength 254 nm). The [^{11}C]amino acids were purified using a Sep-Pak® C-18 column. Analysis of the radiochemical purity was performed by l.c. (column A, solvents G–I 5:95 v/v linear gradient to 40:60 during 0–8 min, wavelength 254 nm, column temperature 40 °C, flow 2 ml min^{-1}, R_t, alanine 5.9 min and phenylalanine 3.9 min).

Separation of the Diastereoisomers of the Alkylation Products.—A small amount of the reaction mixture from the alkylation step, was diluted with the mobile phase (L) and analysed using column D (solvent L, wavelength 254 nm, column temperature 25 °C, flow 2 ml min^{-1}, R_t alanine 3.0 and 3.3 min and phenylalanine 4.5 and 5.0 min).

Determination of Enantiomeric Purity.—Method a, *by g.c.* For determination of the enantiomeric purity by g.c., the [3-^{11}C]-alanine was converted into the *N*-trifluoroacetyl methyl ester derivative by the following procedure. The solution containing [3-^{11}C]alanine was evaporated to dryness. The solid residue was dissolved in a 3M solution of dry hydrogen chloride in methanol (5 ml) and the mixture was heated at 100 °C for 10 min in a sealed flask. The excess of methanol and hydrogen chloride were evaporated. Dichloromethane (3 ml) and trifluoroacetic anhydride (3 ml) was added and the mixture was heated at 100 °C for 8 min in a sealed flask. The solvent was evaporated off at room temperature and the product was dissolved in 50—200 μl of dichloromethane and analysed using column M (flow 30 ml min^{-1}, oven temperature 90 °C, N_2, R_t D-form 10.5 min and L-form 12.5 min).

Method b, *by l.c.* [3-^{11}C]Phenylalanine was injected as the free amino acid in water (column C, solvent K, wavelength 254 nm, column temperature 55 °C, flow 1.5 ml min^{-1}, R_t D-form 7.5 min and L-form 10 min).

Method c, *by l.c.* The [3-^{11}C]amino acids were converted into the diastereoisomeric derivatives according to the following procedure.[13] To the amino acid (5 μmol) in water (100 μl), a 1% solution of *N*-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide in acetone (200 μl) and aqueous sodium hydrogen carbonate (1.0M; 40 μl) were added. The mixture was heated at 60 °C for 15 min then cooled and hydrochloric acid (2M; 20 μl) added. The diastereoisomeric derivatives were analysed by l.c. (column B, solvents J–H and a linear gradient from 20% to 50% of H in 15 min for alanine and in 8 min for phenylalanine, wavelength 313 nm, column temperature 25 °C, flow 2 ml min^{-1}, R_t alanine: L-form 9.3 min, and D-form 10.8 min; R_t phenylalanine: L-form 10 min and D-form 11 min).

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