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Research Article

Synthesis of [4-^{11}C]amino acids via ring-opening of aziridine-2-carboxylates

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Summary

Ring-opening of *N*-(*tert*-butoxycarbonyl)aziridine-2-isopropyl carboxylate with no-carrier-added (n.c.a.) [^{11}C]cyanide is reported. Following purification by HPLC, the protected D,L-[4-^{11}C]β-cyanoalanine was subsequently hydrolysed, to yield D,L-[4-^{11}C]asparagine or D,L-[4-^{11}C]aspartic acid, or reduced followed by hydrolysis to give D,L-2,4-diamino[4-^{11}C]butyric acid. Typical syntheses starting with 10 GBq hydrogen [^{11}C]cyanide yielded 1–1.4 GBq of [4-^{11}C]amino acid within 30 min, giving the labelled amino acids in 30–40% decay corrected radiochemical yield (counted from [^{11}C]cyanide) with radiochemical purities of 95%, 98% and 60%, respectively. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: carbon-11; cyanide; aziridine; amino acid

Introduction

Positron emission tomography (PET) is a powerful technique for the *in vivo* study of biochemical processes and has become a valuable tool for both basic biomedical research and diagnostic imaging. This technique relies on the synthesis of tracer molecules labelled with short-lived positron emitting nuclides such as ^{11}C and ^{18}F. The short radioactive half-life of ^{11}C (20.4 min) imposes major constraints on

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the time available for synthesis of compounds labelled with this radionuclide. Carbon-11 chemistry requires rapid labelling techniques with the ^{11}C-label incorporated as late as possible in the synthetic pathway.

Amino acids labelled with short-lived positron emitting radionuclides are of particular interest when used in conjunction with PET for measurement of *in vivo* processes such as protein synthesis rates and amino acid transport[1] and neurotransmitter synthesis[2]. The use of ^{11}C-labelled methionine for the detection and delineation of malignant brain tumours has increased markedly over the last few years.[3] Other ^{11}C-labelled amino acids such as [^{11}C]DOPA,[4,5] [^{11}C]tyrosine[6,7] and [^{11}C]5-hydroxytryptophan[8–11] have been utilized in PET oncology studies in recent years. Furthermore, a recent paper reported a preliminary investigation of the uptake of ^{11}C-labelled glutamate, glutamine and aspartate in tumour cell aggregates and *in vivo* in both rats and humans.[12] Of these three amino acids, ^{11}C-aspartate appeared most promising, with a high uptake in neuroblastoma aggregates combined with relatively low organ uptake.

The results presented in this paper were obtained from a study into the application of aziridine ring-opening reactions in the synthesis of ^{11}C- and ^{18}F-labelled compounds.[13,14] Aziridine-2-carboxylates are known to undergo nucleophilic ring-opening reactions with a range of nucleophiles, leading to amino acid products.[15] Activation of the aziridine nitrogen has been shown to considerably increase reaction rates.[16] Ring-opening of *N*-activated aziridine-2-carboxylates with [^{11}C]cyanide could be useful for the synthesis of the amino acids [4-^{11}C]aspartic acid, [4-^{11}C]asparagine and 2,4-diamino[4-^{11}C]butyric acid via a β-[^{11}C]cyanoalanine ester. L-[4-^{11}C]aspartic acid and L-[4-^{11}C]asparagine have only previously been produced by enzymatic methods,[17–19] whereas D,L-2,4-diamino[4-^{11}C]butyric acid has also been produced using traditional synthetic methods.[20]

Results and discussion

2,3-Dibromo-propionic acid isopropyl ester was prepared in three steps from commercially available acrylic acid as described previously.[21] Reaction of this with liquid ammonia followed by reduced pressure distillation[22] gave racemic aziridine-2-isopropyl carboxylate (**1**). Reaction of **1** with di-*tert*-butyl dicarbonate in the presence of base gave

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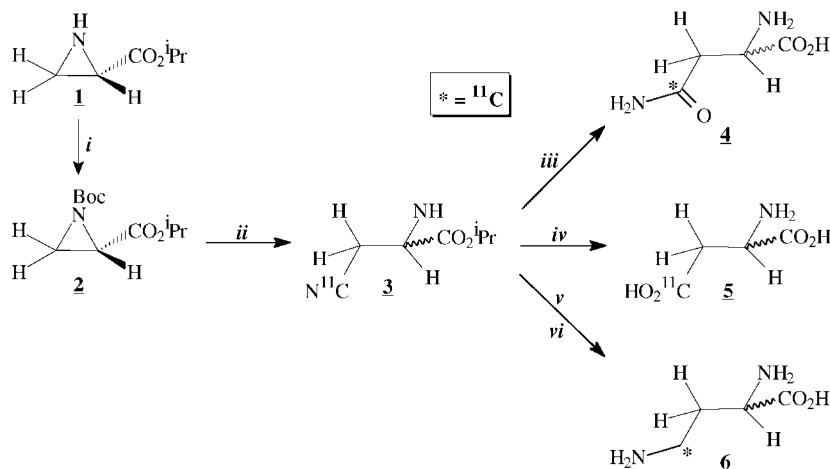
racemic *N*-(*tert*-butoxycarbonyl)aziridine-2-isopropyl carboxylate (**2**) (Scheme 1).



Scheme 1. *Reagents: i*, di-*tert*-butyl dicarbonate, triethylamine; *ii*, tetrabutylammonium [^{11}C]cyanide, DMF; *iii*, HCl (0.1 M); *iv*, HCl (4 M); *v*, $NaBH_4$/$CoCl_2$, MeOH; *vi*, HCl (conc.)

Reaction of **2** with tetrabutylammonium [^{11}C]cyanide was achieved by heating in DMF. In the absence of the tetrabutylammonium counter ion, 50% of the radioactivity remained unreacted after heating at 120°C for 60 min. This is attributed to the insolubility of hydrogen [^{11}C]cyanide in DMF. The rate of [^{11}C]cyanide incorporation was followed by HPLC analysis of samples taken from the reaction mixture at regular intervals. Total incorporation of [^{11}C]cyanide was achieved within 4 min by reaction with 5 mg of aziridine precursor (**2**) in 300 μl DMF. The reaction rate was dependent on both temperature and aziridine precursor concentration. Reducing the amount of precursor to 1 mg (in 300 μl solvent) halved the rate of reaction at 120°C with complete reaction of [^{11}C]cyanide in 8 min. Temperatures above 120°C did not lead to a significant reduction in reaction time but led to a reduction in the yield of the labelled intermediate, probably due to hydrolysis of the ester function. Experiments using the unactivated aziridine (**1**) demonstrated a marked reduction in reactivity, with 55% of the radioactivity remaining as [^{11}C]cyanide after 30 min heating at 120°C (Figure 1).

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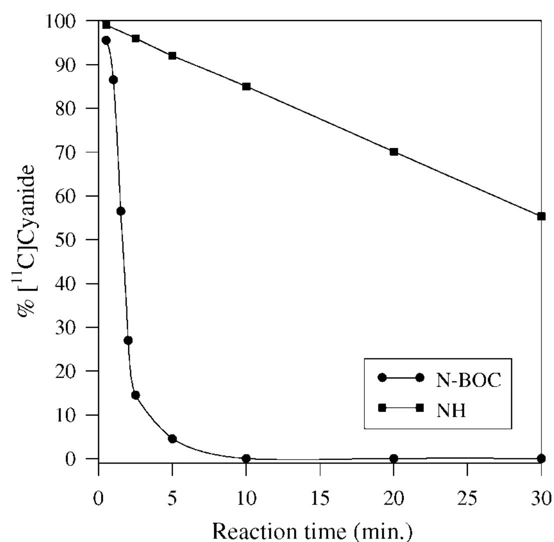

Figure 1. Time dependence of [^{11}C]cyanide consumption on reaction with activated and unactivated aziridine precursors

Purification of the crude reaction mixture was achieved using reversed phase HPLC (Figure 2). Following evaporation of the solvent *in vacuo*, the labelled intermediate (Figure 2, **b**) was reduced and/or hydrolysed to give the [4-^{11}C]amino acids (**4**−**6**) (Scheme 1). The identity of the labelled intermediate (D,L-[4-^{11}C]β-cyanoalanine isopropyl ester (**3**) was inferred based on the identity of the final products. There was no evidence for the isolation of the *N*-Boc intermediate of (**3**), which is assumed to be hydrolysed during the reaction. This intermediate would be expected to be more strongly retained on the preparative HPLC column. A ^{11}C-labelled by-product (ca. 25% of the total radioactivity, as determined by HPLC) which eluted after (**3**) was, however, consistently observed (Figure 2, **c**). Collection and hydrolysis of this fraction gave unidentified labelled products which did not correspond to α-amino acids. This by-product is proposed to be the product of [^{11}C]cyanide attack at the α-carbon of the aziridine ring to give 3-amino-3-cyanopropionic acid isopropyl ester. The relative amounts of **b** and **c** (Figure 2) decreased with prolonged reaction time or increased temperature, resulting in increased amounts of more polar products (Figure 2, **a**). Optimization of these factors gave a maximum yield (as a percentage of the total crude labelled product) of 48% of D,L-[4-^{11}C]β-cyanoalanine isopropyl ester (**3**).

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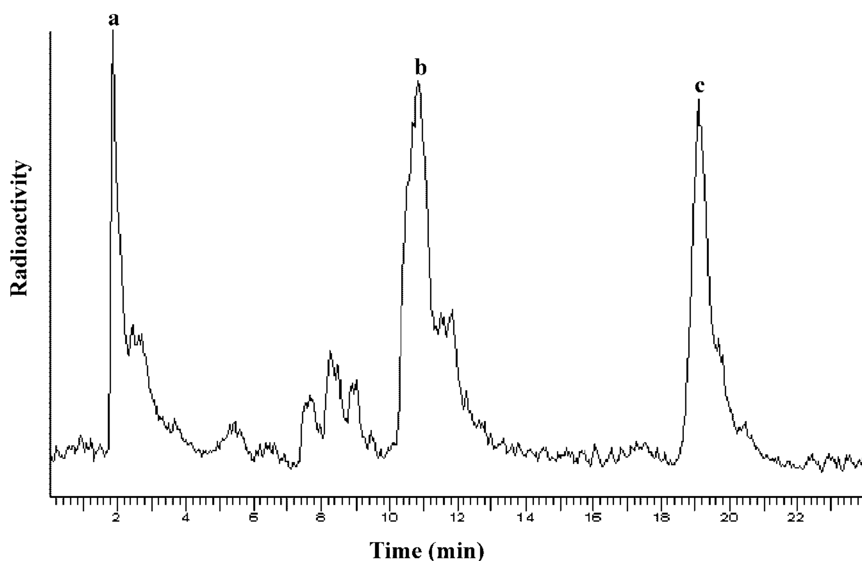

Figure 2. Preparative HPLC purification of crude product using column A (linear ordinates). a, hydrolysed products; b, D,L-[4-^{11}C]β-cyanoalanine isopropyl ester; c, unidentified ^{11}C-labelled product. Chromatographic conditions are described in the text

Typical syntheses starting with 10 GBq hydrogen [^{11}C]cyanide yielded 1–1.4 GBq of [4-^{11}C]amino acid within 30 min in 30–40% decay corrected radiochemical yield. The final products were analysed using chiral HPLC (Figure 3(a)–(c)). Product identities were confirmed by co-injection of authentic reference solutions. Radiochemical purities were 95% for **4**, 98% for **5** and 60% for **6**. The low radiochemical purity of **6** was due to unidentified ^{11}C-labelled products (possibly due to over-reduction to 2,4-diamino-butan-1-ol). Amino acid concentrations in the final products could not be measured due to their low UV absorbance. The specific activities of the final products were, however, estimated to be ca. 10–50 GBq/μmol at the end of synthesis, based on the specific activity of hydrogen [^{11}C]cyanide routinely produced in our laboratory.

Chiral aziridines have, in many cases, been shown to undergo ring-opening reactions without loss of stereochemical integrity.[23–25] In order to examine the stereochemistry of this reaction, the racemic aziridine precursor **2** was resolved by chiral HPLC. This gave a highly enantiomerically enriched sample (96.5% e.e.) which was labelled with [^{11}C]cyanide, purified and hydrolysed to [^{11}C]aspartate as

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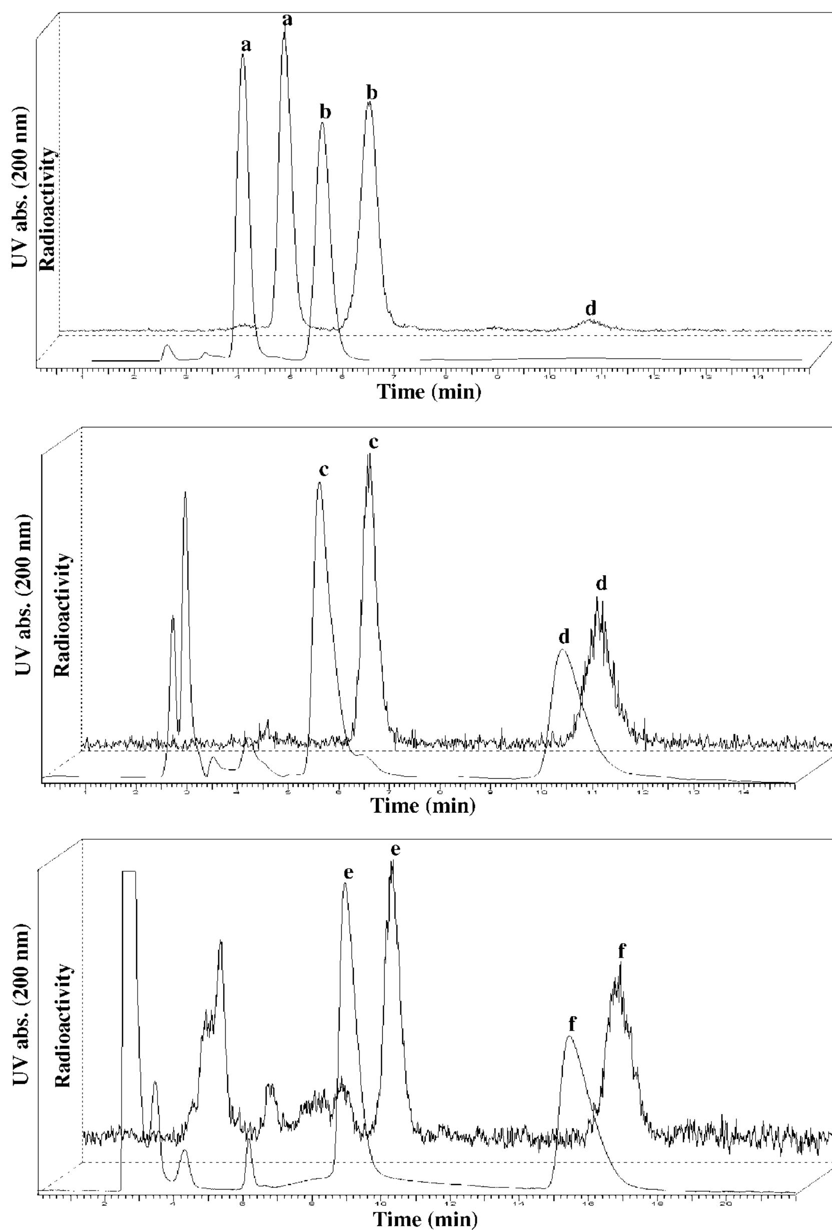

Figure 3. Analytical chromatograms of product solutions of 4, 5 and 6 with added reference. a – D-[4-^{11}C]asparagine, b – L-[4-^{11}C]asparagine, c – D-[4-^{11}C]aspartic acid, d – L-[4-^{11}C]aspartic acid, e – D-[4-^{11}C]-2,4-diaminobutyric acid, f – L-[4-^{11}C]-2,4-diaminobutyric acid (radioactivity is decay corrected and on a linear scale). Chromatographic conditions are described in the text

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before. Analysis by chiral HPLC, however, revealed a racemic product. Chiral HPLC analysis of the initially formed ^{11}C-labelled product (3) before hydrolysis suggested that racemization occurred during the ring-opening reaction. This racemization has been noted previously[25] and is attributed to the acidity of the β-hydrogen. Attempts to avoid racemization by using the less polar solvent, dibutyl ether, again produced a racemic product. There exist, however, a number of chiral HPLC columns (see for example Diacel Chiral HPLC catalogue) which could be utilized for resolution of the produced racemic amino acids prior to *in vivo* PET experiments.

Experimental

Materials and methods

All chemicals were obtained from Aldrich or Sigma Chemical Companies unless otherwise stated. Anhydrous solvents for labelling and other reactions (where anhydrous is stated) were supplied by Aldrich in Sure/Seal™ containers. HPLC grade solvents were supplied by Aldrich Chemical Co. Ltd. or Merck. References samples of aspartate, asparagine and 2,4-diaminobutyric acid (both L and D,L) were obtained from Sigma. Waters™ Sep-Pak solid phase extraction cartridges were obtained from Millipore Corporation. NMR data were obtained using a Bruker 300 spectrometer (^{1}H : 300.075 MHz; ^{13}C : 75.462 MHz). Chemical shifts are reported in ppm (δ) relative to TMS internal standard ($\delta = 0.00$ ppm) for ^{1}H and ^{13}C. Mass spectra were recorded using a Micromass 7070F spectrometer (EI + ve mode). Preparative HPLC was performed using a Perkin-Elmer model 200 isocratic pump with a 1 ml injection loop connected in series with a Phenomenex Nucleosil 5 C18, 250×10 mm column (column A), an Applied Biosystems 759A variable UV detector and a GM tube radiodetector of in-house design. Analytical HPLC was performed using a Perkin-Elmer model 250 pump with a 20 µl injection loop connected in series with either a Phenomenex Selectosil SCX, 250×4.6 mm column (column B), a Daicel–Crownpak Cr^{+}, 150×4 mm chiral column (column C) or a Daicel OD-H 250×4.6 mm chiral column (column D), a Perkin-Elmer LC295 variable wavelength UV detector and a sodium iodide radiodetector of in-house design.

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Chiral analytical HPLC using the Crownpak column was performed at a temperature of approximately 0°C with the aid of a column cooler/heater of in-house design. Column D was also used for preparative chiral HPLC of the aziridine precursor. Labelling reactions were performed using a fully automated synthesis unit described elsewhere.[26]

N-(tert-butoxycarbonyl)aziridine-2-isopropyl carboxylate (2)

To a solution of di-*tert*-butyl dicarbonate (1.69 g, 7.75 mmol) and triethylamine (0.78 g, 7.75 mmol) in anhydrous DMF (5 ml) a solution of aziridine-2-isopropyl carboxylate (1.0 g, 7.75 mmol) in anhydrous DMF (2 ml) was added, dropwise with stirring. The resulting solution was stirred at room temperature for 48 h. After addition of dichloromethane (25 ml), the mixture was extracted with saturated aqueous sodium hydrogen carbonate (2×25 ml), saturated aqueous sodium chloride (25 ml) and distilled water (25 ml). The remaining organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give *N*-(*tert*-butoxycarbonyl)aziridine-2-isopropyl carboxylate as a clear oil (600 mg, 34%). δ ^{1}H ($CDCl_3$) 1.28 (t, $J = 6.0$ Hz, 6H, $(CH_3)_2$), 1.45 (s, 9H, $(CH_3)_3$), 2.37 (dd, $J = 1.6$, 5.5 Hz, 1H, CH), 2.50 (dd, $J = 1.6$, 3.3 Hz, 1H, CH), 2.97 (dd, $J = 3.3$, 5.5 Hz, 1H, CH), 5.07 (sept, $J = 6.0$ Hz, 1H, CH); δ ^{13}C ($CDCl_3$) 21.7, 21.8 ($2 \times$ CH), 27.9 ($C(\underline{C}H_3)_3$), 31.2 (CH), 35.1 (CH), 69.5 (CH_2), 82.0 ($\underline{C}(CH_3)_3$), 159.6 (C=O), 168.0 (C=O); *m*/*z* (super saturated) 230 (M+1) 186 (M-$CH(CH_3)_2$), 174, 170 (M-$OCH(CH_3)_2$), 142 (M-$CO_2CH(CH_3)_2$), 130 (M-Boc).

Production of hydrogen [^{11}C]cyanide

[^{11}C]carbon dioxide was produced via the ^{14}N(p,α)^{11}C reaction in a nitrogen gas target (2% oxygen) with 16.5 MeV proton bombardment, using a General Electric Medical Systems PETtrace 200 cyclotron. On-line conversion to hydrogen [^{11}C]cyanide was achieved according to previously reported procedures.[27,28] Briefly, this process involves reduction of [^{11}C]carbon dioxide with hydrogen gas over a nickel catalyst at 400°C to produce [^{11}C]CH_4, followed by reaction with ammonia over a platinum catalyst at 1000°C to produce hydrogen [^{11}C]cyanide which is delivered in a mixture of hydrogen and ammonia at 50 ml/min.

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Synthesis of D,L-*[4-^{11}C]β-cyanoalanine isopropyl ester (3)*

No-carrier-added (n.c.a.) hydrogen [^{11}C]cyanide, produced as described above, was bubbled through a solution of *N*-(*tert*-butoxycarbonyl)aziridine-2-isopropyl carboxylate (5 mg, 21.9 nmol) in a 100 mM solution of tetrabutylammonium hydrogen carbonate in anhydrous DMF (300 μl) in a septum sealed 0.9 ml pear-shaped vial at room temperature. The reaction vial was then placed in a heated aluminium block (120°C) for 4 min. The solution was subsequently diluted with 50 mM phosphoric acid (500 μl) and the mixture injected onto a HPLC column (column A) eluted with water:acetonitrile (85 : 15) at 8 ml/min. The fraction corresponding to the radioactive product was collected (rt = 11 min) and transferred to a rotary evaporator, where the solvent was removed *in vacuo*.

Synthesis of D,L-*[4-^{11}C]asparagine (4)*

D,L-[4-^{11}C]β-cyanoalanine isopropyl ester **(3)** was dissolved in 100 mM HCl (3 ml) and transferred to a glass tube, which was subsequently heated at 110°C for 8 min. The solution was neutralized by passage through a column of Dowex AG11 A8 ion exchange resin (5 ml), which was then flushed with a further 7 ml of distilled water to give a 10 ml solution of D,L-[4-^{11}C]asparagine **(4)**. HPLC analysis was performed on column C at ca. 0°C eluting with perchloric acid (113.6 mM, pH 1.0) at 0.4 ml/min with on-line UV absorbance and radioactivity detection. The two enantiomers (D and L) eluted at 4.3 and 6.1 min, respectively ($k'_D = 0.66$; $k'_L = 1.30$; $\alpha = 1.97$; Rs = 2.78), and the two radioactive peaks were found to be of equal magnitude after decay correction of the data. The radiochemical purity was found to be 95%. Product identities were confirmed by co-injection of reference samples of racemic and L-asparagine.

Synthesis of D,L-*[4-^{11}C]aspartic acid (5)*

D,L-[4-^{11}C]β-cyanoalanine isopropyl ester **(3)** was dissolved in 4 M HCl (3 ml) and transferred to a glass tube, which was subsequently heated at 110°C for 10 min. The solution was neutralised by passage through a column of Dowex AG11 A8 ion exchange resin (15 ml), which was then flushed with a further 7 ml of distilled water to give a 10 ml solution of D,L-[4-^{11}C]aspartic acid **(5)**. HPLC analysis was performed on column C

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at ca. 0°C eluting with perchloric acid (113.6 mM, pH 1.0) at 0.4 ml/min. The two enantiomers (D and L) eluted at 5.6 and 10.4 min, respectively ($k'_D = 1.19$; $k'_L = 2.91$; $\alpha = 2.44$; Rs = 5.11), and the two radioactive peaks were found to be of equal magnitude after decay correction of the data. The radiochemical purity was found to be 98%. Product identities were confirmed by co-injection of reference samples of racemic and L-aspartic acid.

Synthesis of D,L-*2,4-diamino-[4-^{11}C]butyric acid (**6**)*

D,L-[4-^{11}C]β-cyanoalanine isopropyl ester (**3**) was dissolved in methanol (2 ml) and transferred to a pressure tube containing cobalt chloride (10 mg, 0.077 mmol) and sodium borohydride (30 mg, 0.793 mmol). The mixture was shaken, then heated at 90°C for 5 min. The mixture was subsequently quenched with concentrated HCl (1 ml) followed by passage through a C18 Waters™ Sep-Pak (solid phase extraction cartridge) to remove particulates and lipophilic impurities. The product solution was diluted with distilled water and analysed without further purification. HPLC analysis was performed on column C at ca. 0°C eluting with perchloric acid (113.6 mM, pH 1.0) at 0.4 ml/min. The two enantiomers (D and L) eluted at 9.5 and 16.3 min, respectively ($k'_D = 2.31$; $k'_L = 4.75$; $\alpha = 2.04$; Rs = 3.71), and the two radioactive peaks were found to be of equal magnitude after decay correction of the data. The radiochemical purity was ca. 60%. Product identities were confirmed by co-injection of reference samples of racemic and L-2,4-diaminobutyric acid.

Chiral HPLC purification

A solution of the aziridine carboxylate (ca. 50 mg/ml) was dissolved in hexane:IPA (98 : 2) and injected onto column D, eluted with hexane : IPA (98 : 2) at 0.75 ml/min. The two enantiomers were well separated (baseline separation, UV absorption, 200 nm) and fractions were collected using a Gilson FC 203B fraction collector. This procedure was repeated until sufficient quantities of enantiomerically enriched aziridine were collected. Enantiomeric purity was determined on dilute samples of the aziridine using the same HPLC column and conditions.

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Conclusion

We have reported here in full the first application of aziridine ring-opening for the synthesis of ^{11}C-labelled compounds. This method was successful for the synthesis of three [4-^{11}C]amino acids. Stereospecific synthesis using a highly enantiomerically enriched aziridine-2-carboxylate precursor was not achieved and only racemic amino acid products were obtained.

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