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Nuclear Medicine and Biology 30 (2003) 39–44

NUCLEAR MEDICINE — AND — BIOLOGY

www.elsevier.com/locate/nucmedbio

Synthesis of no-carrier-added C-11 labeled D- and L-enantiomers of phenylalanine and tyrosine for comparative PET Studies

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Received 25 February 2002; received in revised form 28 May 2002; accepted 10 June 2002

Abstract

The natural amino acids tyrosine (Tyr) and phenylalanine (Phe) were labeled with carbon-11 via a modified Bucherer-Strecker synthesis. A rapid reaction of the sodium bisulfite adduct of the aldehyde precursor with ammonia provided the precursor for radiosynthesis. [^{11}C]Cyanide displacement followed by base hydrolysis afforded the corresponding ^{11}C-labeled amino acids. The purification and chiral separation were simply achieved by using a combination of solid-phase extraction and chiral HPLC to afford individual enantiomers of each amino acid. The decay corrected radiochemical yields for each of the enantiomers were 12–16% with respect to the [^{11}C]cyanide after 40–45 min of radiosynthesis. Radiochemical purity of the products was >97% (typically >99%), enantiomeric excess was >98% with the specific radioactivity 2–3 Ci/μmol at the end of bombardment. Because of its simplicity and wide applicability, the described procedure could be the method of choice to produce [^{11}C]amino acids for PET studies.

Keywords: Tyrosine; Phenylalanine; Bucherer-Strecker synthesis; Chiral separation; C-11; Positron Emission Tomography

1. Introduction

α-Amino acids labeled with short-lived positron emitters carbon-11 ($t_{1/2}$ = 20 min) and fluorine-18 ($t_{1/2}$ = 110 min) have been extensively used for the visualization of tumors [31], measurements of protein synthesis rates [17] and amino acid decarboxylation rates [30] in humans using PET. Because of immense importance of amino acids in living systems and continuous development of positron imaging techniques such as small animal PET [23] and beta microprobes [32], it is likely that the application of ^{11}C-labled α-amino acids as the research tools will be expanded to other areas of life science. This will require a widely applicable, simple, cheap, robust and reliable synthetic method to produce these compounds. During the last three decades many synthetic schemes have been developed. However, they either required drastic reaction conditions such as high pressure and high temperature in corrosive environments [1,3,11,13]; or relatively complicated multistep syntheses with water-sensitive reagents involved [7,8, 22].

In an effort to develop a simple and facile radiosynthesis of aromatic ^{11}C-labeled amino acids for carrying out comparative studies with [^{11}C]boronophenylalanine ([^{11}C]BPA) [29], we further modified the previously described Bucherer-Strecker synthesis of [^{11}C]amino acids via aminonitriles [15,25]. We report here a simple and reliable procedure that affords both enatiomers of [^{11}C]Phe and [^{11}C]Tyr under mild conditions in high yields for PET studies. This described method should be applicable to the production of most ^{11}C-labeled amino acids, including our synthesis of [^{11}C]BPA that will be described elsewhere. A single chiral HPLC combined with simple solid-phase extraction achieved purification and chiral resolution at the same time to obtain the desired ^{11}C-labeled individual enantiomers.

2. Experimental

Chemicals were purchased from Aldrich Chemical Co. Gas phase [^{11}C]cyanide was produced from [^{11}C]CO_2 based on a procedure described previously [5]. It requires 15 min for the radiosynthesis of [^{11}C]cyanide after end of target bombardment. Reverse-phase cartridges from Waters (Milford, MA) and Varian (Palo Alto, CA) were activated by

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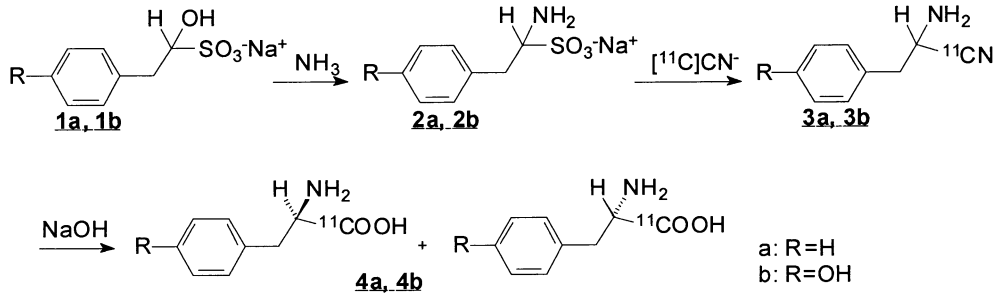
0969-8051/03/$ – see front matter
PII: S0969-8051(02)00349-9



Scheme 1. Radiosynthesis of [^{11}C]Phe and [^{11}C]Tyr.

eluting with 6 mL of ethanol followed by 6 mL of water; strong cation exchange (SCX) cartridges were used without any pretreatment. For TLC analysis, Macherey-Nagel polygram sil G/UV$_{254}$ plastic-back TLC plates (4 × 8 cm) were used. An automatic TLC scanner (Berthold Automatic TLC Linear Analyzer) was used as radioactivity detector. The unlabeled amino acids and aminonitriles were visualized by spraying the TLC plates with ninhydrin spray reagent (0.1%, E. Merk, Darmstadt, Germany) followed by heating or using iodine staining.

Purification and analyses of radioactive mixtures were performed using Knauer HPLC pumps, in-line variable wavelength UV detectors (set at 215 nm) and NaI crystal radioactivity detector. Peak areas were measured using two Hewlett-Packard 3390A recording integrators. A preparative chiral HPLC was done using Daicel Chemical, Crownpak CR(+), 150 × 10 mm column cooled in an ice-water bath with 15 mM H_2SO_4 eluent (prepared from sterile water) at flow rate 4 mL/min. The radiochemical purity was determined by radio TLC and analytical radio HPLC in the presence of unlabeled compound as a carrier. Analytical HPLC was done using Phenomenex, Ultremex-10, 250 × 4.6 mm column with 10 mM KH_2PO_4, pH 2.1–2% EtOH as an eluent at the flow rate 2 mL/min. Enantiomeric excess was determined by analytical chiral HPLC: Daicel Chemical, Crownpak CR(+), 150 × 4.6 mm column cooled in an ice-water bath; eluent: 15 mM H_2SO_4 at 1 mL/min. An analysis of the level of the UV adsorbing impurities of [^{11}C]amino acids was achieved using a gradient HPLC: Phenomenex, Ultremex-10, 250 × 4.6 mm column with 10 mM KH_2PO_4, pH 2.1–2% EtOH *vs.* CH_3CN linear gradient; 0–15 min, 0–100% CH_3CN at 2 mL/min; t_R: Phe 9.3 min and Tyr 7.8 min. For [^{11}C]Phe the specific radioactivity was estimated based on the UV peak of D-isomers and for [^{11}C]Tyr, UV peak of L-isomer on the preparative chiral HPLC.

3. Synthesis of [^{11}C]Phe (*4a*) (Scheme 1, R = H)

Sodium 1-hydroxy-2-phenylethanesulfonate (*1a*, phenylacetaldehyde sodium bisulfite adduct): A solution of $NaHSO_3$ (105 mg, 1.0 mmol) in 0.3 mL of water was slowly added to the solution of phenylacetaldehyde (120 μL, 1.0 mmol) in 10 mL of methanol. The mixture was left for 30 min at room temperature, and the solvent was rotary evaporated. Additional 5 mL of methanol was added and evaporated to eliminate water. The solid was washed with ether (8 mL) and dried. Yield was 182 mg (81%). ^{1}H NMR (D_2O, 400 MHz, δ) 7.50–7.36 (5H, m, -C_6H_5-), 4.69 (1H, dd, J = 10.8 Hz, J = 2.8 Hz, -CH_2CH), 3.42 (1H, dd, J = 14.4 Hz, J = 2.8 Hz, -CH_2CH =), 2.97 (1H, dd, J = 14.4 Hz, J = 10.8 Hz, -CH_2CH).

Synthesis and stability study of sodium 1-amino-2-phenylethanesulfonate (*2a*): An aqueous solution of NH_3 (28%, 3 μL, 54 μmol) was added to a solution of ***1a*** (6.5 mg, 29 μmol) in CD_3OD:D_2O 1:1 (0.8 mL), and the mixture was left at room temperature. Formation of ***2a*** after 1 h reaction time was indicated by ^{1}H NMR analysis. ^{1}H NMR (CD_3OD-D_2O, 400 MHz, δ) 7.49–7.33 (5H, m, -C_6H_5), 4.01 (1H, dd, J = 10.4 Hz, J = 3.6 Hz, -CH_2CH), 3.43 (1H, dd, J = 13.6 Hz, J = 3.6 Hz, -CH_2CH), 2.80 (1H, dd, J = 14.0 Hz, J = 10.4 Hz, -CH_2CH). However, ^{1}H NMR analysis taken the following day showed the disappearance of the specified aliphatic proton signals.

2-amino-3-phenylpropanenitrile (*3a*): Compound ***3a*** was produced following the procedure described previously [20] from phenylacetaldehyde by the reaction with trimethylsilyl cyanide (TMSCN) in the presence of ZnI_2. ^{1}H NMR (1:1 CD_3CN: D_2O, 400 MHz, δ): 8.1–7.9 (5H, m, -C_6H_5-), 5.27 (1H, dd, J = 10.4 Hz, J = 5.6 Hz, -CH_2CH), 4.00 (1H, dd, J = 13.6, J = 5.6, -CH_2CH), 3.85 (1H, dd, J = 13.2, J = 10.0, -CH_2CH); mp 164–165°C dec. (lit. 167–168°C dec. [9], 173–175°C [20]); TLC, Silica, 1:2 hexane: ethyl acetate, Rf 0.44; analytical HPLC: t_R 9.7 min.

D- and L-[^{11}C]Phe ([^{11}C]*4a*): An aqueous solution of NH_3 (5 μL of 30%) was added to the solution of ***1a*** (5 mg, 22 μmol) in water (0.25 mL). The mixture was heated for 30 min at 60°C to produce ***2a.*** This reaction was performed prior to the radiosynthesis. [^{11}C]Cyanide, transferred using a stream of nitrogen, was bubbled through the cooled (ice-water bath) solution of ***2a.*** At the end of the transfer, the line introducing [^{11}C]cyanide to the reaction vessel was washed with 0.1 mL of water to ensure complete transfer of radioactivity. After 10 min of reaction at 60°C, the aminonitrile [^{11}C]***3a*** was generated, and it was then converted to the

amino acid using either acidic (initial attempt) or basic hydrolysis.

Acidic hydrolysis: Aqueous HCl (12 M, 0.4 mL) was added to the reaction vessel, and the mixture was heated for 10 min at 125–130°C to produce [^{11}C]***4a.*** The reaction vessel was briefly cooled (cold water), and the solution was neutralized by the addition of aqueous $(NH_4)_2HPO_4$ (1.05 mL of 0.36 g/mL solution). The mixture was filtered using Alltech Associates, 4 mL Extract-Clean, reservoir with frit and injected on Waters, Novapak 300 × 7.8 mm HPLC column run with 10 mM KH_2PO_4 pH 2.1–5% EtOH at 3 mL/min. A collected fraction of [^{11}C]***4a*** (t_R 10.9 min, fraction volume 2 mL) was reinjected onto a preparative chiral HPLC. The eluted fractions of D- and L-[^{11}C]Phe (t_R 6.1 and 8.0 min) were directly passed through 0.22 μm Millipore filters into the sterile vials containing 0.7 mL of 0.5 M $NaHCO_3$ and 0.5 mL of 14.6% NaCl (both sterile solutions) to yield a total volume of 10–12 mL of injectable solutions. A decay corrected yield with respect to [^{11}C]cyanide for each enantiomer was 8–10% after 55–60 min of the radiosynthesis.

Basic hydrolysis: NaOH (6.3 M, 0.2 mL) was added to the reaction vessel containing [^{11}C]***3a*** and the mixture was heated at 100–130°C (typically a linear increase of the temperature during 5 min followed by plateau) during 10 min. The reaction vessel was cooled in cold water, and the solution was acidified by the addition of 0.85 mL of 1 M H_2SO_4. The reaction mixture was transferred through Waters, CN cartridge (360 mg) that was further washed with water (1.0–1.2 mL). The combined solution (total volume 2 mL) was injected onto the preparative chiral HPLC. Collected fractions (t_R 7.8 and 10.7 min for D- and L-isomers) were passed (on-line) though the Varian SCX cartridges (100 mg). Each of the trapped enantiomers of Phe (5–8 mCi) was eluted with 5 mL of saline (0.9% NaCl) containing 0.3 mL of 0.5 M $NaHCO_3$ (both were commercial sterile solutions), passed through the 0.22 μm sterile Millipore filter and collected in sterile vials. The radiochemical and enantiomeric purities were determined by radio-TLC (Silica, 10 mM KH_2PO_4, pH 2.1: CH_3 CN 1:3, Rf 0.40), radio-analytical HPLC (t_R 11.0 min) and radio-analytical chiral HPLC (t_R for D- and L-[^{11}C]***4a***, 6.0 min and 8.0 min). A decay corrected yields with respect to [^{11}C]cyanide of individual enantiomers of [^{11}C]Phe were 12.3±2.6% (n = 9) with a synthesis time of 40–45 min and a specific radioactivity of 2–3 Ci/μmol.

4. Synthesis of [^{11}C]Tyr (*4b*) (Scheme 1, R = OH)

Sodium 1-hydroxy-2-(4-hydroxyphenyl)ethanesulfonate (*1b*, (4-hydroxyhenyl)acetaldehyde sodium bisulfite adduct): The precursor ***1b*** was prepared by following the procedure described previously [11,26] from synephrine by a pinacol-pinacolone type of rearrangement. ^{1}H NMR (D_2O, 400 MHz, δ) 7.24 (2H, d, J = 6.4 Hz, -C_6H_4-), 6.90 (2H, d, J = 5.2 Hz, -C_6H_4-), 4.57 (1H, dd, J = 10.8 Hz, J = 2.8 Hz, -CH_2CH), 3.60 (1H, dd, J = 14.4 Hz, J = 2.4 Hz, -CH_2CH), 2.83 (1H, dd, J = 14.4 Hz, J = 10.4 Hz, -CH_2CH).

Synthesis and stability of sodium 1-amino-2-(4-hydroxyphenyl)ethanesulfonate (*2b*): The synthesis was performed in the same fashion as the synthesis of ***2a*** except the precursor ***1b*** was used and D_2O was used as a solvent instead of the D_2O-CD_3OD mixture. ^{1}H NMR spectrum taken after 40 min of reaction indicated formation of ***2b.*** ^{1}H NMR (D_2O, 400 MHz, δ) 7.14 (2H, d, J = 8.4 Hz, -C_6H_4-), 6.72 (2H, d, J = 7.2 Hz, -C_6H_4-), 3.88 (1H, dd, J = 8.8 Hz, J = 5.6 Hz, -CH_2CH), 3.27 (1H, dd, J = 14.0 Hz, J = 3.2 Hz, -CH_2CH), 2.63 (1H, J = 14.0 Hz, J = 10.4 Hz, -CH_2CH). ^{1}H NMR taken the following day showed disappearance of the specified aliphatic proton signals.

2-amino-3-(4-hydroxyphenyl)propanenitrile (3b): Aqueous NH_3 (28%, 5 μL, 90 μmol) was added to the solution of ***1b*** (6 mg, 25 μmol) in D_2O (0.8 mL). After 70 min at room temperature, NaCN (1.5 mg, 31 μmol) was added to ***2b*** and the reaction was done for 3 h at room temperature. No work up was done. The solution containing >90% pure product was diluted with CH_3CN and used as a TLC and HPLC standard. ^{1}H NMR (D_2O, 400 MHz, δ) 7.13 (2H, d, J = 8.4 Hz, -C_6H_4-), 6.67 (2H, d, J = 8.4 Hz, -C_6H_4-), 4.07–4.02 (1H, m, -CH_2CH), 3.03–2.87 (2H, m, -CH_2CH). TLC, Silica, 1:2 hexane-ethyl acetate, Rf 0.30. Analytical HPLC: t_R 4.5 min.

[^{11}C]Tyrosine ([^{11}C]*4b*). The synthesis using acidic hydrolysis was unsuccessfully attempted in the same fashion as the synthesis of [^{11}C]Phe. During the synthesis an analysis of the reaction mixture, using TLC and analytical HPLC, was accomplished before and after the acidic hydrolysis. Basic hydrolysis was done as for [^{11}C]Phe, except the L-isomer of the product after the preparative chiral HPLC was filtered (sterile Millipore 0.22 μm filter) and directly collected into the sterile vial containing 0.7 mL of 0.5 M $NaHCO_3$ and 0.5 mL of 14.6% NaCl (both sterile solutions) yielding 10–12 mL of the injection solution. The retention times of the D- and L-[^{11}C]***4b*** on the preparative chiral HPLC were 8.3 and 10.8 min. The radiochemical and enantiomeric purities were determined by radio-TLC (Silica, 10 mM KH_2 PO_4, pH 2.1: CH_3 CN 1:3, Rf 0.50), radio-analytical HPLC (t_R 5.4 min) and radio-analytical chiral HPLC (t_R for D- and L-[^{11} C]***4b***, 9.0 min and 12.2 min. A decay corrected yields with respect to [^{11}C]cyanide of individual enantiomers of [^{11}C]Tyr were 15.0±4.0% (n = 9) with a synthesis time of 40–45 min and a specific radioactivity of 2–3 Ci/μmol.

5. Results and discussion

During the last three decades, many synthetic schemes for the production of ^{11}C-labeled Phe and Tyr have been developed [3,11,16,25], and recently, several enantioselec-

tive transformations leading to radiolabeled amino acids have also been reported [7,8,22]. Asymmetric radiosyntheses have apparent advantages such as potentially higher yield and easier purification of product. However, the reagents used are often complicated and not commercially available, and sometimes chiral HPLC is still needed if enantiomeric excess is not greater than 95%. Therefore we chose previously developed nonstereoselective modified Bucherer-Strecker synthesis [15,25] for the production of [^{11}C]Tyr and [^{11}C]Phe. This synthetic scheme had the advantages of simplicity and easy accessibility of the precursors. The most important goal was to directly apply the same synthetic procedure (with minor modifications) to the production of D- and L-isomers of various amino acids of interest.

Two types of Bucherer-Strecker synthesis (transformation of an aldehyde into an amino acid via reaction with cyanide and ammonia) have been applied to the production of ^{11}C-labeled amino acids. The conventional type, which involved the formation of an intermediate [^{11}C]hydantoin, required heating of the aldehyde (or aldehyde sodium bisulfite adduct) in an aqueous solution of potassium cyanide, ammonium carbonate and ammonium chloride to 200°C in a high pressure reaction vessel. The intermediate hydantoin was then hydrolyzed at high temperature (200°C) in a concentrated solution of base [1,3,11,13]. In a modified Bucherer-Strecker synthesis, a [^{11}C]aminonitrile was synthesized via the substitution of the SO_3^--group of the aminosulfonic acid (produced by the reaction of the aldehyde sodium bisulfite adduct with ammonia or ammonium carbonate right before the radiosynthesis) by [^{11}C]cyanide. Hydrolysis of the generated aminonitrile lead to the formation of the desired amino acid [15,25].

[^{11}C]Phe and [^{11}C]Tyr have been prepared by conventional Bucherer-Strecker method; however, an addition of carrier cyanide was required. Moreover, the procedure required a reaction vessel that could withstand high temperature, high pressure and corrosive reactants [3,11]. This complicates a construction of the remote radiosynthesis set up and demands special safety precautions. We decided to develop a simple and universal radiosynthetic method that affords pure enantiomers of the desired amino acids via a modified Bucherer-Strecker synthesis. This method (Scheme 1) can be accomplished without a carrier cyanide, while the use of the lower temperature of the chemical reactions eliminates the need for a high pressure reaction vessel.

Although in a previously reported procedure as much as 400 mg of precursor has been used for each radiosynthesis [15], we found that 3–5 mg of the starting material was sufficient to achieve >70% incorporation of [^{11}C]CN^- into the [^{11}C]aminonitrile. (Unlabeled aminonitrile ***3a*** was synthesized as in [20], while ***3b*** was produced following the same procedure as for the labeled compound. Unlabeled aminonitriles were used as the standards to monitor the reactions with [^{11}C]cyanide.) The reaction conditions were not optimized; however, we found that the length of the reaction for the precursor formation is crucial for a successful radiosynthesis. A reaction time of 0.5 to 1 h in aqueous ammonia solution was necessary to produce aminosulfonates (***2a*** and ***2b***) [21,27]; however, it should be noted that after a prolonged (>15 h) storage at room temperature the generated aminosulfonates can be completely decomposed as evidenced by ^{1}H NMR.

In the previous reports, hydrolysis of [^{11}C]aminonitriles could be accomplished under either acidic [15,29] or basic [25] conditions. However, we found acid hydrolysis was not applicable to the synthesis of [^{11}C]Tyr. Most of the intermediate [^{11}C]aminonitrile [^{11}C]***3b*** decomposed under acidic conditions, while less than 5% was converted to [^{11}C]Tyr. When the hydrolysis was carried out under basic conditions [2,25], a quantitative conversion of [^{11}C]aminonitrile to [^{11}C]Tyr was achieved. Basic conditions also resulted in a complete conversion of intermediate [^{11}C]aminonitrile [^{11}C]***3a*** to [^{11}C]Phe within 7 minutes. The entire radiosynthesis could be conveniently carried out in one pot, and the intermediate purification was not required.

After removal of the hydrophobic impurities from the reaction mixture using Waters, CN cartridge, a single chiral HPLC achieved both purification and chiral resolution to obtain ^{11}C-labeled individual enantiomers of the amino acids. (A similar application of the chiral column in the synthesis of [^{11}C]L-DOPA has been reported [1].) The CN cartridge was used to remove hydrophobic impurities prior the HPLC because of its intermediate polarity (C18<C8<CN<diol cartridge). We found that C18 cartridge retained both Phe and Tyr, while the C8 cartridge trapped PheThe chiral column used for purification, Daicel, Crownpak CR(+), which also successfully separated enatiomers of 6-[^{18}F]fluoronorepinephrine [6], contains non-covalently bound crown ether complexes, and only aqueous solutions with <15% of the organic solvent can be used as the eluents [19]. Thus exclusion of highly hydrophobic impurities, which may permanently damage the column, prior to the HPLC purification is crucial. A C18 guard column (Securityguard, Phenomenex) was therefore used to protect the chiral column. A dilute solution of perchloric acid has been commonly used as an eluent for the chiral HPLC separations on the Crownpack CR(+) column [6,19]. However, the use of this acid as an eluent in the production of radiopharmaceuticals might be undesirable due to its relatively high toxicity, corrosiveness and a potential for forming explosive salts. We found that the use of sulfuric acid (15 mM aqueous solution) as an eluent provides slightly inferior but sufficient resolution of the enantiomers.

The use of the crown ether-based chiral column is advantageous as compared to the L-proline-copper chiral column previously used for the enantiomeric separation of [^{11}C]Tyr [18,25]. The efficiency of the enantiomeric separation on the L-proline-copper chiral column gradually decreases thus requiring routine column checks and periodic column regeneration. A chiral column used in our syntheses

required only eluting with water at the end of the radiosynthesis. The column was stored in the refrigerator. After more than 50 injections performed, no deterioration in the column performance has been noticed.

Fractions collected from the chiral column that corresponded to the D- and L-enantiomers of [^{11}C]Phe and the D-enantiomer of [^{11}C]Tyr contained non radioactive impurities based on the high UV-adsorption at 215 nm. The presence of impurities was also observed by the reverse-phase HPLC. These collected fractions were therefore further purified by trapping the [^{11}C]amino acids on a cation exchange resin (Varian, BondElute SCX) followed by elution with the solution of sodium bicarbonate in saline. This method, as shown by the reverse-phase HPLC with UV-detection, completely eliminated impurities coeluted with the products on the chiral column. The decay corrected radiochemical yields for each of the enantiomers with respect to the [^{11}C]cyanide after 40–45 min of the synthesis were 12.3±2.6% (n = 9) for [^{11}C]Phe and 15.0±4.0% (n = 9) for [^{11}C]Tyr (the error is a standard deviation). The radiochemical purity was >97% (usually 99%), and enantiomeric excess was >98%. The specific radioactivity of the products was 2–3 Ci/μmol. A general comparison indicates that our estimated uncorrected yield of the individual enantiomers with respect to the [^{11}C]CO_2 1.5% lies within the reported range of 0.4–5.3% reported yields of the individual enantiomers of [^{11}C]Phe and [^{11}C]Tyr [3,7,10,11,18]. We expect that an optimization of our method can significantly increase the yield.

In principle, our synthesis can be further modified to include a chiral induction to asymmetrically incorporate [^{11}C]CN-group based on reports on the asymmetric Bucherer-Strecker syntheses [4,14,28]. However, the racemic approach used in ours and a number of other recent radiosyntheses [12,18] is significantly more simple and straightforward. The described procedure has several advantages: [1] the precursors were synthesized in one step from the commercially available compounds, while [^{11}C]cyanide can be readily produced by automatic synthesizer; [2] during the radiosynthesis, all of the reactions were carried out in a single vessel using water as the solvent; [3] the reaction temperature did not exceed 130°C and no special reaction vessel was necessary; [4] the same synthetic procedure, which included the use of single chiral HPLC column for purification and chiral resolution, was applied for the synthesis of individual enantiomers of all three amino acids of interest: [^{11}C]Phe, [^{11}C]Tyr and [^{11}C]BPA (the synthesis of [^{11}C]BPA will be described elsewhere). The only difference was that a fraction of L-[^{11}C]Tyr did not require a post column purification; and [5] the final collected fractions could be directly (after pH and salinity adjustment) used for injection in PET studies.

As a final note regarding environmental protection issues, the HPLC purification and resolution of enantiomers was accomplished using an aqueous solution of sulfuric acid as an eluent, and the system can be washed with ethanol and water. Accordingly, the liquid waste after pH adjustment contained only minor (<5 mg) quantities of relatively non-toxic organic material and *ca.* 200 mg of Na_2SO_4 in *ca.* 20% ethanol-water (200–250 mL total volume) which is 1–2 orders of magnitude below general limits for the disposal of waste into laboratory drain [24]. This eliminates expensive, time- and space consuming processing of the waste liquids generated during the radiosynthesis as hazardous waste. All of these factors make our procedure widely applicable, simple, reliable, robust, quick to set up, relatively easy to automate and producing minimal quantities of the hazardous waste; and make it advantageous for the developing stage of research.

6. Conclusion

No-carrier-added [1-^{11}C]α-Amino acids D- and L-[^{11}C]phenylalanine and [^{11}C]tyrosine were synthesized from [^{11}C]CN^- using a simple one-pot two-stage procedure, a modified Bucherer-Strecker synthesis. The products were purified using Sep-Pak cartridges along with chiral HPLC. Our procedure provides a simple, reliable and widely applicable method for the synthesis of the [1-^{11}C]α-amino acids. In the cases when several ^{11}C-labeled amino acids must be prepared for comparative studies, or when a specific amino acid is needed for short-term study this procedure has clear advantages over the asymmetric radiosyntheses.

Acknowledgments

This research was carried out at Brookhaven National Laboratory under contract DEAC02-98CH 10886 with the U.S. Department of Energy and Office of Biological and Environmental Research. We are grateful for the assistance of V. Garza, C. Shea, Y. Xu and R. Carciello. The authors are indebted to R. Ferrieri for fruitful discussions as well as help with the operation of the radiosynthesis setup. We are also thankful to J. Fowler for reviewing this manuscript.

References

[1] M.J. Adam, J.R. Grierson, T.J. Ruth, K. Pedersen, B.D. Pate, Routine synthesis of carbon-11 carboxyl-labeled L-DOPA, J. Nucl. Med. 28 (1987) 1599–1603.

[2] G. Antoni, H. Omura, M. Ikemoto, R. Moulder, Y. Watanabe, B. Langstrom, Enzyme catalyzed synthesis of L-[4-^{11}C]aspartate and L-[5-^{11}C]glutamate, J. Labelled Cpd. Radiopharm. 44 (2001) 287–294.

[3] D.L. Casey, G.A. Digenis, D.A. Wesner, L.C. Washburn, J.E. Chaney, R.L. Hayes, A.P. Callahan, Preparation and preliminary tissue studies of optically active D-[^{11}C]phenylalanine, Int. J. Appl. Radiat. Isot. 32 (1980) 325–330.

[4] T.K. Chakraborty, K.A. Hussain, G.V. Reddy, α-Phenylglycinol as chiral auxiliary in diastereoselective Strecker synthesis of α−amino acids, Tetrahedron, 51 (1995) 9179–9190.

[5] D.R. Christman, R.D. Finn, K.I. Karlstrom, A.P. Wolf, The production of ultra high activity ^{11}C-labeled hydrogen cyanide, carbon dioxide, carbon monoxide and methane via the $^{14}N(p,\alpha)^{11}C$ reaction (XV), Int. J. Appl. Radiat. Isot. 26 (1975) 435–442.
[6] Y.-S. Ding, J.S. Fowler, S.J. Gatley, S.L. Dewey, A.P. Wolf, Synthesis of high specific activity (+)- and (-)-6-[^{18}F]fluoronorepinephrine via the nucleophilic aromatic substitution reaction, J. Med. Chem. 34 (1991) 767–771.
[7] K.J. Fasth, G. Antoni, B. Langstrom, Asymmetric-synthesis of L-[3-^{11}C] alanine and L-[3-^{11}C] phenylalanine by a phase-transfer alkylation reaction, J. Chem. Soc. Perk. Trans. 12 (1988) 3081–3084.
[8] K.J. Fasth, K. Hornfeldt, B. Langstrom, Asymmetric-synthesis of ^{11}C-labeled L-amino and D-amino acids by alkylation of imidazolidinone derivatives, Acta. Chem. Scand. 49 (1995) 301–304.
[9] M. Freifelder, R.B. Hasbrouck, Synthesis of primary 1,2-diamines by hydrogenation of α-aminonitriles, J. Am. Chem. Soc. 82 (1960) 696–698.
[10] C. Halldin, B. Langstrom, Asymmetric synthesis of L-[3-^{11}C]phenylalanine using chiral hydrogenation catalyst, Appl. Rad. Isotopes. 35 (1984) 945–948.
[11] C. Halldin, K.-O. Schoeps, S. Stone-Elander, F. A. Wiesel, The Bucherer-Strecker synthesis of D- and L-[1-^{11}C]tyrosine and the *in vivo* study of L-[1-^{11}C]tyrosine in human brain using positron emission tomography, Eur. J. Nucl. Med. 13 (1987) 288–291.
[12] T. Haradahira, O. Inoue, K. Kobayashi, K. Suzuki, Synthesis and evaluation of ^{11}C-labeled nonpeptide antagonists for cholecystokinin receptors: [^{11}C]L-365,260 and [^{11}C]L-365,346, Nucl. Med. Biol. 25 (1998) 203–208.
[13] R.L. Hayes, L.C. Washburn, B.W. Wieland, T.T. Sun, R.R. Turtle, T.A. Butler, Carboxyl-labeled [1-^{11}C]aminocyclopentanecarboxylic acid, a potential agent for cancer detection, J. Nucl. Med. 17 (1976) 748–751.
[14] H. Ishitani, S. Komiyama, Y. Hasegawa, S. Kobayashi, Catalytic assymetric Strecker synthesis. Preparation of enantiomerically pure α-amino acid derivatives from aldimines and tributyltin cyanide or achiral aldehydes, amines, and hydrogen cyanide using a chiral zirconium catalyst, J. Am. Chem. Soc. 122 (2000) 762–766.
[15] R. Iwata, T. Ido, T. Takahashi, H. Nakanishi, S. Iida, Optimization of [^{11}C]HCN production and no-carrier-added [1-^{11}C]amino acid synthesis, Appl. Rad. Isotopes. 38 (1986) 97–102.
[16] M.R. Kilbourn, Synthesis of carbon-11 labeled amino acids, Int. J. Nucl. Med. 12 (1985) 345–348.
[17] A.C. Kole, J. Pruim, O.E. Nieweg, R.J. van Ginkel, H.J. Hoekstra, H. Schraffordt Koops, W. Vaalburg, PET with L-[1-^{11}C]tyrosine to visualize tumors and measure protein synthesis rates, J. Nucl. Med. 38 (1997) 191–195.
[18] G. Luurtsema, J. Medema, P.H. Elsinga, G.M. Visser, W. Vaalburg, Robotic synthesis of L-[1-^{11}C]tyrosine, Appl. Rad. Isotopes. 45 (1994) 821–828.
[19] Y. Machida, H. Nishi, K. Nakamura, Enantiomer separation of hydrophobic amino compounds by high-performance liquid chromatography using crown ether dynamically coated chiral stationary phase, J. Chrom. A. 830 (1999) 311–320.
[20] K. Mai, G. Patil, Facile synthesis of α-aminonitriles, Tett. Lett. 25 (1984) 4583–4586.
[21] H. McIlwain, Amino-sulphonic acid analogues of natural amino-carboxylic acids, J. Chem. Soc. 7 (1941) 75–77.
[22] I.K. Mosevich, O.F. Kuznetsova, D.A. Vasil'ev, A.A. Anichkov, M.V. Korsakov, Automated synthesis of [3-^{11}C]-L-alanine involving asymmetric alkylation with [^{11}C]CH_3I of the nickel complex of the Schiff base derived from glycine and (S)-2-N-(N '-benzylprolyl)aminobenzophenone, Radiochemistry, 41 (1999) 273–280.
[23] R. Myers, The biological application of small animal PET imaging, Nucl. Med. Biol. 28 (2001) 585–593.
[24] M.J. Pitt, E. Pitt, Disposal to Drains, In: *Handbook of Laboratory Waste Disposal*. J Wiley & Sons, New York, pp, 137–161, 1985.
[25] J. Pruim, A.T. Willemsen, W.M. Molenaar, A. van Waarde, A.M. Paans, M.A. Heesters, K.G. Go, G.M. Visser, E.J. Franssen, W. Vaalburg, Brain tumors: L-[1-^{11}C]tyrosine PET for visualization and quantification of protein synthesis rate, Radiology, 197 (1995) 221–226.
[26] J.H. Robbins, Preparation and properties of p-hydroxyphenylacetaldehyde and 3-methoxy-4-hydroxyphenylacetaldehyde, Arch. Biochem. Byophys. 114 (1966) 576–584.
[27] T. Shiba, K. Miyoshi, S. Kusumoto, Synthesis of alanyl-1-aminoethanesulfonic acid, Bull. Chem. Soc. Jap. 50 (1977) 254–257.
[28] D.M. Stout, L.A. Black, W.L. Matier, Assymetric Strecker synthesis: Isolation of pure enantiomers and mechanistic implications, J. Org. Chem. 48 (1983) 5369–5373.
[29] A. Studenov, Y.-S. Ding, R. Ferrieri, M. Miura, J. Coderre, J.S. Fowler, Synthesis and PET studies of [^{11}C]D- and [^{11}C]L-boronophenylalanine for improving BNCT in brain tumors, J. Labelled Cpd. Radiopharm. 44 (2001) S345–S347.
[30] A. Sundin, B. Eriksson, M. Bergstrom, P. Bjurling, K.J. Lindner, K. Oberg, B. Langstrom, Demonstration of [^{11}C]5-hydroxy-L-tryptophan uptake and decarboxylation in carcinoid tumors by specific positioning labeling in positron emission tomography, Nucl. Med. Biol. 27 (2000) 33–41.
[31] W.A. Weber, H.J. Wester, A.L. Grosu, M. Herz, B. Dzewas, H.J. Feldmann, M. Molls, G. Stocklin, M. Schwaiger, o-(2-[^{18}F]Fluoroethyl)-L-tyrosine and L-[methyl-^{11}C]methionine uptake in brain tumours: initial results of a comparative study, Eur. J. Nucl. Med. 27 (2000) 542–9.
[32] C.L. Woody, S. Stoll, D.J. Schlyer, S. Shokouhi, M. Gerasimov, N. Volkow, J. Fowler, S. Dewey, An intracerebral beta microprobe for studying radiotracer kinetics in freely moving animals, J. Nucl. Med. 42 (2001) 377.