Routine Synthesis of Carbon-11-Carboxyl-Labeled L-Dopa

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Carbon-11-carboxyl-labeled L-dopa has been synthesized by the modified Bucherer-Strecker method. The reaction mixture was first purified by chiral HPLC followed by deprotection using hydriodic acid. The entire procedure was performed in a remotely operated system which gave the product in 28% radiochemical yield (decay corrected) in an overall synthesis time of 55–60 min.

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As part of our ongoing project (*1*) to characterize in vivo, cerebral dopaminergic neuronal dysfunction in Parkinson's disease and related movement disorders using positron emission tomography (PET) we desired a routine synthetic procedure for the preparation of L-1-^{11}C-dopa. We chose to use the modified Bucherer-Strecker amino acid synthesis developed by Washburn et al. (*2*) because this method is based on [^{11}C]cyanide, that was already available in our laboratory and because this chemistry should lend itself well to automation. Previously this method has been used to prepare a variety of ^{11}C-labeled amino acids but has not before been applied to the synthesis of ^{11}C-labeled dopa. The only reported syntheses of L-1-^{11}C-dopa are through the carbonation of an alpha-lithio-isonitrile precursor (*3*) or through the formation of the ^{11}C-alpha-aminonitrile (*4*).

In this paper we describe the synthesis of L-1-^{11}C-dopa starting from the bisulfite addition complex of 3,4-dimethoxyphenylacetaldehyde (*5*) (Fig. 1). The remotely operated system used to perform this synthesis, including chiral resolution and compound deprotection, will also be discussed.

MATERIALS AND METHODS

Eugenol (4-allyl-2-methoxyphenol) was used as purchased.* Hydriodic acid (55%)* was distilled from red phosphorous under H_2 and sealed in ampules in 3-ml portions containing 0.04% hypophosphorous acid as a stabilizer.

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Thin layer chromatography (TLC) was performed on aluminum backed plates.† Developed TLC plates were visualized by either exposure to ultraviolet light or spraying with an acidic solution (10% H_2SO_4) of ammonium molybdate (5% solution) followed by heating. Gas liquid chromatography (GLC) was performed using a 30 m × 0.75 mm ID wide bore capillary column.‡ Melting points are uncorrected and were determined with a capillary tube-oil bath apparatus. Boiling points are also uncorrected and refer to air-bath or Kugelrohr distillation temperatures. High performance liquid chromatography (HPLC) purification was carried out on a system consisting of an air actuated injector with a 5-ml sample loop,§ a single piston pump,¶ a radial compression module** containing an in-situ prepared chiral column (*6*), a uv detector and a NaI(Tl) scintillation detector. The HPLC eluant was 0.05*M* KH_2PO_4/1mm Cu^{2+} at pH 4.0 and was step flow programmed from 2–6 ml/min (2 ml/min for 2.5 min → 4 ml/min for 1 min followed by stepping to a constant flow setting at 6 ml/min). The desired product was collected at ~ 8–10 min. ^{1}H NMR spectra were recorded at 80 or 300 MHz with TMS as the external standard. Elemental analyses were performed by a commercial analytical laboratory.††

Production of [^{11}C]Cyanide

The $^{11}CO_2$ was produced on the TRIUMF CP-42 cyclotron using the $^{14}N(p,\alpha)^{11}C$ reaction at 15 MeV. The $^{11}CO_2$ in the N_2 target gas (300 ml/min) was converted, on line, to $^{11}CH_4$ by mixing the target gas with H_2 (200 ml/min) then passing the mixture over a Ni catalyst (20 g) at 450° C. Sequentially, the $^{11}CH_4$ was converted to H^{11}CN by combining the $^{11}CH_4$ with NH_3 (20 ml/min) and passing this mixture over Pt (5g) at 1,000° C (*7,8*). The H^{11}CN is trapped in distilled H_2O (1 ml) which has been equilibrated with NH_3 from the gas processing system.

Remotely Operated System

The remotely controlled apparatus (Fig. 2) consists of a stainless steel reaction (pressure) vessel (1.5 cm I.D. × 5.5 cm,

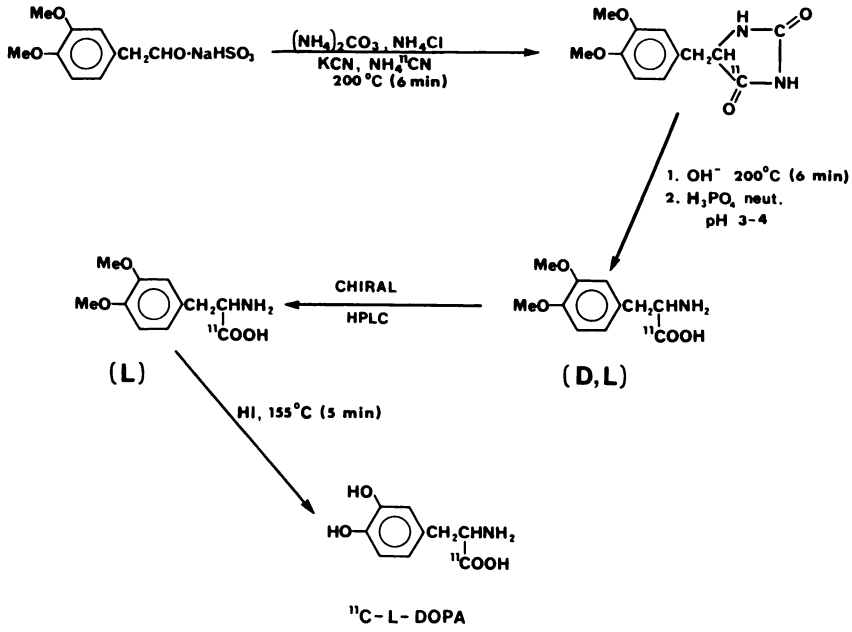

FIGURE 1
Flow scheme for the synthesis of L-1-^{11}C-Dopa.

~10 ml) held stationary above movable hot and cold baths. Mounted immediately adjacent to the top of the reaction vessel are two air actuated, teflon rotary valves.[¶] One valve has six positions. The purpose of this valve is to select one of several reactants to be drawn into the reaction vessel and to provide an outlet route for the product. The other valve is a two position, three-way valve which is connected directly to the reaction vessel. Its purpose is to seal the reaction vessel during heating, and to allow a syringe to inject reactants or extract product when the valve is open.

Below the reaction vessel, mounted on a turntable 120° apart, are a silicone oil bath for heating and an isopropanol bath for cooling the vessel. The turntable is directly driven by a 11 kg cm torque four phase stepping motor. The turntable and motor are mounted on a standard scissor type lab jack which has been converted to be driven by a second 11 kg cm torque four phase stepping motor in order to facilitate remotely raising and lowering of the turntable.

Lab jack movement, table rotation and bath temperature are all controlled by a single chip micro controller unit.[‡‡]

The HPLC system is as described above. Post HPLC steps are carried out with the use of teflon solenoid valves (1/16" id.), and a small rotary evaporator, with liquid transfers being facilitated with air pressure or vacuum.

Preparation of 3,4-Dimethoxyphenylacetaldehyde Sodium Bisulfite Complex

A rapidly stirred mixture of anhydrous potassium carbonate (40.0 g, 0.29 mol), methyl iodide (16 ml, 0.29 mol), eugenol (20.0 g, 0.12 mol) and tetra-*n*-butyl ammonium bromide (0.40

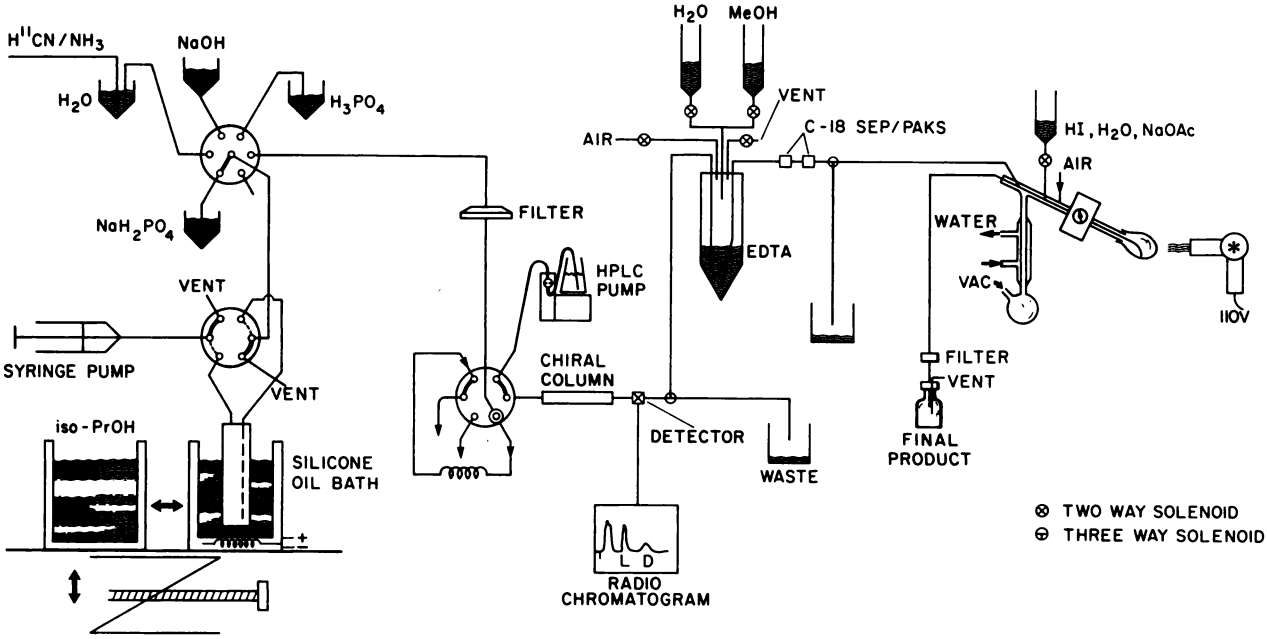

FIGURE 2
Schematic for remotely operated system for the preparation of L-1-^{11}C-Dopa.

g, 1.2 mmol) in methyl ethyl ketone (200 ml) was refluxed for 12 hr with the exclusion of moisture. The cooled mixture was filtered and the collected solids were washed thoroughly with EtOAc. The combined filtrates were concentrated and the residual light brown mobile oil was taken up into EtOAc. The solution was washed successively with water and brine followed by drying and concentration. The residue was distilled (bp 99–100°C/0.0025 mm) to afford 21.0 g (97%) of a colorless oil. Analysis of the distillate by GLC (SPB-1, 200°C isothermal, 2.0 ml/min) revealed it to be homogeneous. ^{1}H NMR ($CDCl_3$) δ:3.30 (broadened d, 2H, J = 6.5 Hz, —$\underline{CH_2}$—CH—CH_2), 3.85 and 3.87 (s, s, 3H, 3H, —$OC\underline{H_3}$), 4.95 (m, 1H, —CH_2—CH = $C\underline{H_2}$), 5.12 (M, 1H, —$CH_2\underline{CH}$—$C\underline{H_2}$), 5.95 (m, 1H, —CH_2—$\underline{CH}$—CH_2), 6.65–6.88 (m, 3H, aromatic protons). Anal. Calcd. for $C_{11}H_{14}O_2$: C 74.13; H 7.92. Found: C 74.17; H 7.93.

To a cooled (0°C bath) and stirred mixture of *o*-methyleugenol (10.0 g, 56.1 mmol) in 1:1 THF/water (100 ml) was added a warm solution of $KMnO_4$ (8.77 g, 55.4 mmol) in water (80 ml) over 20 min. The addition of the oxidant was sufficiently slow to maintain a reaction temperature below 20–25°C. After the addition was complete the mixture was stirred for a further 5 min followed by the successive addition of $NaHSO_3$ (12 g, ~0.1 mol) and 12 N HCl (20 ml). Upon the addition of the acid the reaction mixture cleared (temp <35°C). The resultant yellow two phase liquid-liquid mixture was diluted with brine to a total volume of 400 ml. The aqueous phase was extracted with EtOAc (4×75 ml). The combined organic solutions were washed with brine and dried followed by concentration to a viscous green oil. This material was taken up into 20 ml of 99:1 $CHCl_3$/MeOH and the solution was loaded onto a column of silica gel (400-230 mesh, 5×18 cm). The column was first rapidly eluted with 99:1 $CHCl_3$/MeOH to a point where the bulk of a rapidly migrating yellow band had been washed from the column (~350 ml). The column was next eluted with 4:1 $CHCl_3$/MeOH with fractionation (25 ml) of the eluate. Fractions containing mostly pure diol were combined and concentrated. The residue was dried at room temperature under vacuum for 18 hr to afford 6.89 g of a yellow and white solid which exhibited mp 59–64°C. This material was twice distilled (bp 135–150°C/0.002 mm) to afford 6.57 g of a straw colored viscous oil. This material was dissolved in 50 ml of boiling 44% EtOAc/Et_2O. Upon cooling to room temperature for 5 hr a layer of fine white crystals had deposited. Further cooling to −15°C for 10 hr followed by collection of the crystals with ether washing afforded 5.70 g (48%) of vacuum dried material which exhibited mp 66–69°C. This material was homogeneous by TLC (SiO_2, 95:5 $CHCl_3$/MeOH, R_f = 0.18). Anal. Calcd. for $C_{11}H_{16}O_4$: C 62.25; H 7.60. Found: C 62.26; H 7.46.

A solution of the recrystallized diol (2.51 g, 11.8 mmol) in ether (100 ml) was prepared by refluxing the mixture until the solid had dissolved. To the cooled (0°C) solution was added a solution of $NaIO_4$ (2.52 g, 11.8 mmol) in water (50 ml) in one portion. A faint brown two phase mixture was obtained. The progress of the reaction was monitored by TLC (SiO_2, 95:5 $CHCl_3$/MeOH) which revealed extensive reaction after 5 min and complete reaction after 10 min. After this time some white precipitate had formed. Water was added until the precipitate had dissolved and the mixture was extracted with EtOAc. The extract was washed with water containing a trace of $Na_2S_2O_3$ then brine followed by drying and concentration to afford a faint green oil. This material was distilled (bp 110–120°C/0.0035 mm) to afford 1.52 g (71%) of a colourless oil. This material was homogenous by TLC (SiO_2, 30% EtOAc/Hex, R_f = 0.29). ^{1}H NMR ($CDCl_3$) δ:3.60 (d, 2H, J = 3Hz, —$C\underline{H_2}CHO$), 3.88 (s, 6H, —$OC\underline{H_3}$), 6.65–6.95 (m, 3H, aromatic protons), 9.75 (t, 1H, J = 3Hz, —$CH_2C\underline{H}O$). Anal. Calcd. for $C_{10}H_{12}O_3$: C 66.65; H 6.71. Found: C 66.49; H 6.77.

The distilled aldehyde (1.52 g) was taken up into ether (20 ml) and the solution was added to a rapidly stirred solution of $NaHSO_3$ (10 g) in water (20 ml). After 1 min the precipitation of a flocculent white material had begun. After 5 min the mixture had become so thick that stirring was difficult. The mixture was filtered over a fine porosity frit and the moist solids were washed liberally with THF. The product was vacuum dried at room temperature to afford 3.20 g of a white powder. The excessive yield clearly indicated the isolation of additional $NaHSO_3$ in combination with the desired complex. Elemental analysis of the powder revealed: 29.74% C; 3.64% H; 15.26% S suggesting an empirical formula of $C_{10}H_{12}O_3$ (1.9) $NaHSO_3$ for the product.

Preparation of [^{11}C]Dihydroxyphenylalanine ([^{11}C]Dopa)

To the stainless steel Strecker bomb was added the bisulfite addition complex (28 mg, 74 μmol), ammonium carbonate (57 mg, 600 μmol), and solutions of ammonium chloride (200 μl, 93 μmol) and KCN (50 μl, 25 μmol). After target irradiation the H^{11}CN was bubbled through water (≤1 ml) and this solution ($NH_4{}^{11}CN$) was drawn into the bomb. The bomb was then sealed and heated to 200°C for 6 min. The bomb was cooled (1 min) in an isopropanol bath, NaOH added (1 ml, 6.25*M*) to the bomb and the sealed bomb heated again to 200°C for 6 min. The bomb was cooled, phosphoric acid (1 ml, 6.25*M*) was added and the entire neutralized contents were transferred from the bomb through a 0.8 μm filter[48] directly into the 5 ml HPLC injection loop. The injection loop had previously been filled with 0.05*M* KH_2PO_4 (pH 4.0) containing no copper. This was necessary to avoid the precipitation of copper salts within the loop. Finally potassium dihydrogen phosphate (1 ml, 3.25*M*) was added to the bomb and this wash solution was also transferred through the filter and into the injection loop. The sample was injected and the flow rate step programmed from 2–6 ml/min (see Materials and Methods). The fraction containing the L-1-^{11}C-Di-*O*-Me-dopa was collected (15–20 ml) in a vessel containing aqueous Na_2EDTA (5 ml, 0.2*M*). The solution was then passed through two C-18 SEP PAKs connected in series. The SEP PAKs were washed with water (5 ml) followed by the elution of the adsorbed L-Di-*O*-Me-Dopa with ethanol (10 ml). The ethanol eluate was transferred to the rotary evaporator and the solution was concentrated to dryness. Hydriodic acid (3 ml, see above) was added to the residue and the solution was heated for 5 min (hot air gun, 150°–260°C). The HI was evaporated and two portions of H_2O (5 ml) were added and successively evaporated. The residue was dissolved in USP H_2O (3–5 ml) containing NaOAc (150 mg) and the solution was transferred from the evaporator through a membrane filter into a multi-injection vial to give L-1-^{11}C-dopa in 28% radiochemical yield (decay corrected) in 55–60 min. The specific activity was determined to be ~400 mCi/mmol by calculation from the known amount of added carrier KCN.

The final product was >98% radiochemically pure as determined by C-18 and chiral HPLC. The final product was also chemically and enantiomerically pure as determined by ^{1}H NMR, HPLC (C-18, uv detection), and chiral HPLC.

Final product Dopa ^{1}H NMR (300 MHz-D_2O): δ 3.10 (dd, 1H, —C<u>H</u>$_2$—CH—), 3.23 (dd, 1H, —C<u>H</u>$_2$—CH—), 4.25 (dd, 1H, —CH$_2$—C<u>H</u>—), 6.73 (m, 1H, aromatic H-5 or H-6), 6.83 (s, 1H, aromatic H-2), 6.88 (m, 1H, aromatic H-5 or H-6).

RESULTS AND DISCUSSION

The synthetic procedure described here for the preparation of L-1-^{11}C-dopa is based on the modified Bucherer-Strecker amino acid synthesis developed as a general route to ^{11}C amino acids by Washburn et al. (*2*) (Fig. 1). In order to use this procedure the aldehyde precursor had to be synthesized since it was not commercially available. The aldehyde was prepared in a three-step synthesis starting from eugenol and was used as its bisulfite addition complex for ease of handling. The decay corrected radiochemical yield for the final product was 28% and the overall synthesis time was 55–60 min. Starting from 200 mCi of $NH_4{}^{11}CN$ ~7 mCi of injectable final product is obtained at the end of synthesis. The specific activity was determined to be ~400 mCi/mmol (EOS) by calculation from the known amount of added carrier KCN. The synthesis has been carried out at least ten times with the system described here. We have prepared routine samples of [^{11}C]dopa for sterility and pyrogenicity testing and are presently preparing samples for animal brain imaging.

The separation of the optical isomers was achieved using an in situ modified HPLC column which has been described before (*6*). Briefly, the column consists of an analytical silica column (0.8 cm × 10 cm, 10 μm) which has D-proline groups covalently bound to the surface of the silica. Having the D-enantiomer of proline attached to the column allows the L-enantiomer of Di-*O*-Me-dopa to elute before the D-isomer. Surprisingly, the entire contents of the Strecker bomb (~5 ml), after filtration, can be injected in one portion onto this chiral column without a deleterious effect on the resolution (Fig. 3). To achieve this excellent resolution it was necessary to flow program the eluant in a stepwise fashion with the initial injection flow rate starting at 2 ml/min and progressing to 6 ml/min within 3.5 min. All of the inorganic salts elute within 5 min (~25% of total activity) and L-^{11}C-Di-*O*-Me-dopa elutes in 8–10 min (~37% of total activity). Note that the area of the first component appears comparatively large due to the slower flow rate for the first 3.5 min. Because of the ability to inject large volumes of crude reaction mixture onto the chiral column, we have chosen to separate the enantiomers at this stage rather than after deprotection. It should also be noted that too much ammonia from the $^{11}CN^-$ production can strip the Cu^{2+} from the column and destroy the resolution. To prevent this we limit the volume of trapping H_2O to ≤1 ml = <10 mmol NH_4OH. The chiral column also serves to chemically purify the intermediate before the deprotection step. Because of this the deprotection step proceeds very cleanly. The Cu^{2+} in the collected fraction was removed by addition of EDTA followed by selective extraction of the ^{11}C-Di-*O*-Me-dopa onto two C-18 SEP PAKs. After the SEP PAKs are washed with water the product is eluted with ethanol directly into the rotary evaporator. This product was analyzed (polarography) and found to contain <2 ppm Cu^{2+}. Deprotection with HI is rapid (5 min) and the HI was evaporated readily. After addition of water and re-evaporation the product is colorless and is dissolved in USP H_2O (3–5 ml) containing NaOAc (150 mg) and filtered, prior to radiopharamceutical use.

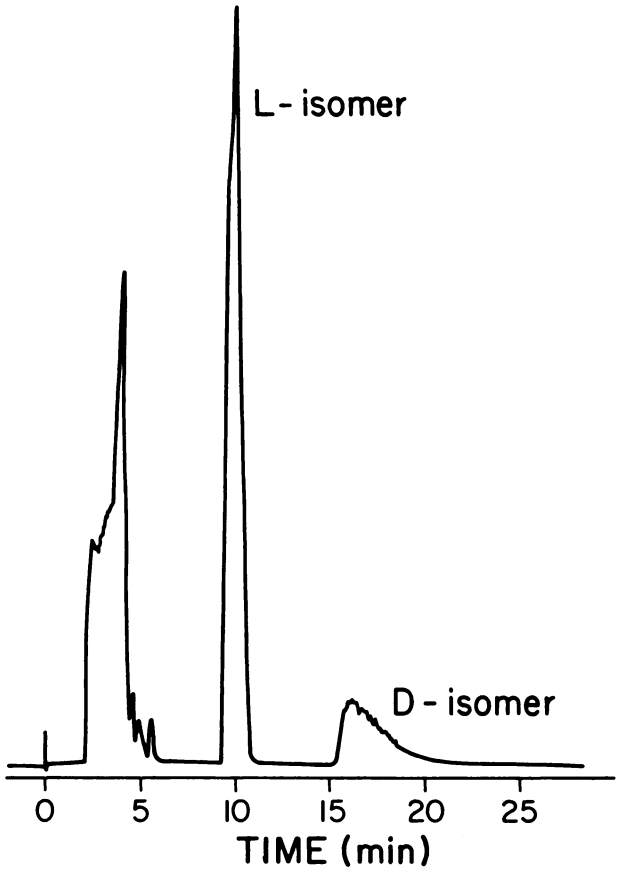

FIGURE 3
Chiral HPLC radiochromatogram of the bulk (5 ml) crude Strecker reaction mixture. The initial fraction contains unincorporated ^{11}C activity (~25%), while the fractions of L- and D-di-*O*-methyldopa each contain (~37%) of the total initial activity (decay corrected). Visually the apparently larger area for the unincorporated activity is a result of using slower flow rates during this time and the small area of the D-isomer relative to the L-isomer reflects the decay of ^{11}C during purification.

NOTES

* Aldrich Chemical Co., Milwaukee, WI.

† Merck No. 5534, Darmstadt, West Germany.

‡ (Supelco SPB-1) Supelco Canada Ltd., Ontario.

§ Rheodyne 7126 (5 ml loop), Rheodyne Inc., Cotati, CA.

¶ Eldex B-100-S, Eldex Laboratories Inc., San Carlos, CA.

** Waters RCM-100, Millipore Waters, Mississauga, Ontario.

†† Canadian Microanalytical Services Ltd., New Westminster, B.C., Canada.

‡‡ Motorola 68(7)05 R3, Motorola, Austin, TX.

§§ Millipore SLAAV255F, Millipore Corp., Bedford, MA.

¶¶ Rheodyne 5032 & 5012, Rheodyne Inc., Cotati, CA.

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