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Stereospecific, Semi-automated, N.C.A. Syntheses of *cis*-4-[^{18}F]fluoro-L-proline and *trans*-4-[^{18}F]fluoro-L-proline

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SUMMARY

Cis-4-[^{18}F]fluoro-L-proline **1** and *trans*-4-[^{18}F]fluoro-L-proline were prepared stereospecifically in a semi-automated, N.C.A. procedure using the General Electric FDG MicroLab, a system employing a quaternary 4-aminopyridinium resin to effect F-18 fluorination. Thus, in the MicroLab, the p-tosyloxy moiety of *trans*- or *cis*-N-t-butoxycarbonyl-4-p-tosyloxy-L-proline methyl ester (*trans* **2**) was displaced with [^{18}F]fluoride, and then the carbamate moiety was hydrolyzed with 0.1 N HCl. Next, by manual additions, the methyl ester was rapidly hydrolyzed with 1.0 N NaOH, and the pH was adjusted with phosphate buffer. The *cis* product was obtained in about 16% radiochemical yield 80 minutes from EOB, and the *trans* in about 18% yield. In both cases the radiochemical purity was greater than 99%, and no preparative HPLC was required. The procedure can be considered routine, and is virtually as simple as MicroLab production of FDG.

Key Words: PET, *cis*-[^{18}F]fluoroproline, *trans*-[^{18}F]fluoroproline, synthesis, stereospecific, fluorine-18

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INTRODUCTION

In connection with our efforts to image the early stages of diseases involving fibroses in the lung by positron emission tomography (PET), we focused our attention on *cis*-4-[^{18}F]fluoro-L-proline **1** (1). Since L-proline plays a much greater role in collagen biosynthesis than in the biosynthesis of most other proteins, a labelled L-proline, such as *cis*-4-[^{18}F]fluoro-L-proline, might act as a tracer of abnormal rates of collagen synthesis. The incorporation of 4-fluoro-L-prolines into collagen precursor was demonstrated previously by others (2). The *cis* isomer is of particular interest because of recent studies showing it to have better uptake characteristics than the *trans* isomer (3,4).

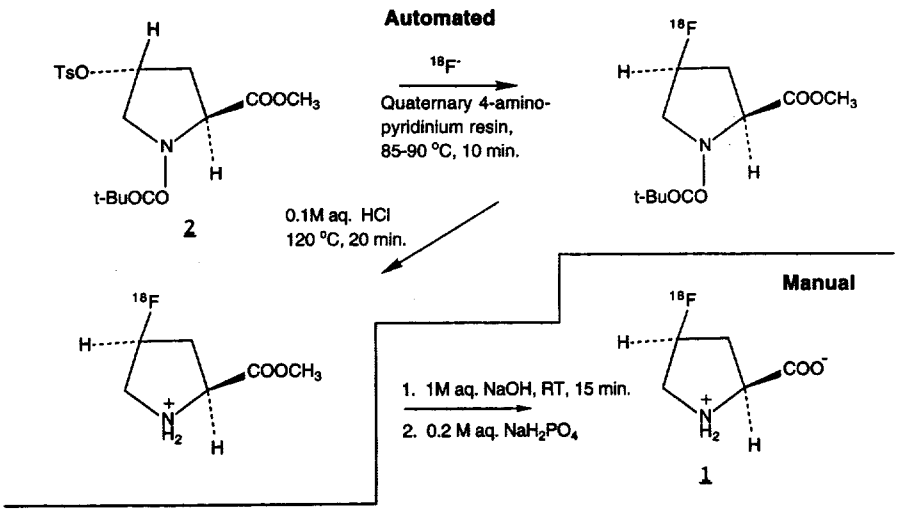
Unlabelled *cis*- and *trans*-4-fluoro-L-prolines have been synthesized by fluoride displacement of tosylate from the *trans* and *cis* isomers of N-carbobenzyloxy-4-p-tosyloxy-L-proline methyl ester, followed by deprotection (2). The *cis*-tosylate yielded *trans*-substituted product exclusively, but the *trans*-tosylate yielded an 83:17 *cis:trans* mixture. Syntheses of the pure isomers directly, *via* the action of DAST on 1,3-oxazolidinone derivatives of *cis*- and *trans*-4-hydroxy-L-proline, were recently reported (5).

Regarding F-18 analogs, a 1983 paper reports the synthesis of carrier-added 4-[^{18}F]fluoroproline by [^{18}F]fluoride displacement of triflate from N-tosyl-O-trifluoromethane-sulfonyl-L-proline methyl ester using [^{18}F]tetraethyl-ammonium fluoride, however analysis was by paper chromatography, and there was no confirmation of the stereochemistry of the product (6). Subsequently, *cis*- and *trans*-4-[^{18}F]fluoro-L-prolines were reported synthesized by Hamacher *et al. via* Kryptofix-222 mediated [^{18}F]fluoride displacement of tosylate from the *trans* and *cis* isomers of N-t-butoxycarbonyl-4-p-tosyloxy-L-proline methyl ester (*trans* **2**), followed in turn by strong acid hydrolysis of the protecting groups, ion exchange pH adjustment, preparative HPLC purification, and solid phase extraction of product from the chromatography medium (3,5,7). Interestingly, under these conditions, the *trans*-tosylate yielded an 82:18 *cis:trans* product mixture, while the *cis*-tosylate yielded a similar 83:17 *trans:cis* mixture (*cf.* 2).

Initially, we decided on the Hamacher route for our attempted production of *cis*-4-[^{18}F]fluoro-L-proline. However, noting the general similarity between this scheme and those that have been automated for the routine synthesis of [^{18}F]FDG, *viz.* [^{18}F]fluoride displacement followed by hydrolytic deprotection, we decided to attempt to adapt our General Electric FDG MicroLab system to the production of *cis*-4-[^{18}F]fluoro-L-proline (8). Use of the MicroLab for the synthesis of products other than [^{18}F]FDG has been reported for only three other fluorine-18 tracers (9). Because of the deliberately closed design of the MicroLab, the only variables readily manipulable are the contents of the four reagent vials feeding the system, and the make-up of the final train of purification media. The MicroLab features a single-use, disposable cassette (Fig. 1) in which the substance to be radiofluorinated, normally mannose triflate in acetonitrile solution, is slowly passed through a small heated column of a quaternary 4-aminopyridinium resin on which has been trapped [^{18}F]fluoride ion (10,11). As the [^{18}F]fluorinated product is formed at the resin, it is carried in the acetonitrile stream to another vessel, where in the subsequent step, the hydrolytic deprotection takes place. Finally, the pH is adjusted with buffer, and the whole mixture is filtered, in succession, through a C18 cartridge, an alumina N cartridge, and a 0.22 micron filter into a sterile receiving vial.

RESULTS AND DISCUSSION

The adaptation of the MicroLab to the syntheses of *cis*- and *trans*-4-[^{18}F]fluoro-L-prolines is simple (Scheme 1 and Fig. 1). Without any changes in programmed timing or temperatures, it is only necessary to substitute different chemicals in three of the system's four reagent vials. Aside from the different starting precursors (*e.g.* 2), 0.1 N aqueous HCl was substituted for 1.0 N aqueous HCl in the hydrolysis step, and water was substituted for buffer in the neutralization step. In our earlier studies of the synthesis of the *cis* isomer, only the precursor was substituted, but 1.0 N HCl, while effective at removing the carbamate, was not giving complete hydrolysis of the methyl ester within the MicroLab's programmed time. Adjusting the strength of the acid to 6N still did not yield sufficiently complete removal. Then it was observed that the methyl ester was rapidly hydrolyzed with



Scheme 1. Semi-automated synthesis of *cis*-4-[^{18}F]fluoro-L-proline.

aqueous NaOH at room temperature. Substituting NaOH in vial #4 yielded complete methyl group removal, but also caused partial dissolution of Sep-Pak alumina, thereby rendering the Sep-Pak ineffective at retaining unreacted [^{18}F]fluoride and causing cloudy precipitates in the final filtered product. These results necessitated carrying out the ester hydrolysis and subsequent neutralization by hand.

Radiochemical yields for the *cis* isomer averaged 15.8% (14.9-16.8%, n=4) and for the *trans* isomer 17.6% (14.2-19.8%, n=5), all normalized to 100% RCP. Although they are modest, the employment of an automated system allows the use of large amounts of starting [^{18}F]fluoride ion activity, and thus the procedure is capable of producing thousands of MBq (>100 mCi) of either product. Also, radiochemical yield is dependent on the amount of precursor. Use of approximately 20 mg of either product precursor gave the average yields reported above, while using 29.8 mg and 40.3 mg of *cis*-product precursor gave yields of 23.6% and 26.4%, respectively. These particular precursor amounts apply specifically to the use of the MicroLab, since only two-thirds of the precursor (*i.e.* 2/3 of the solution of the precursor in 1.5 mL acetonitrile) loaded into the synthesis module is actually delivered across the resin. Thus, lower amounts of precursor might suffice in non-MicroLab set-ups to give similar yields. In this regard, since most of the starting activity remains on the

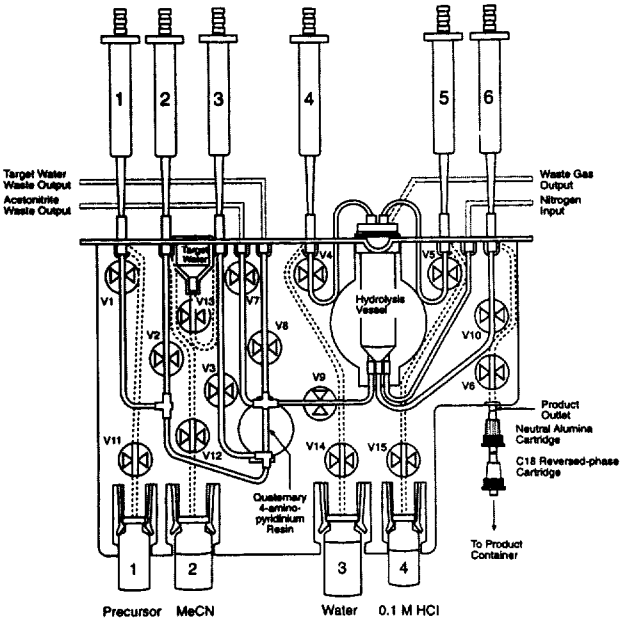


Fig. 1. Single-use FDG MicroLab cassette outfitted for 4-[^{18}F]fluoroproline production (14). Operation of syringes and valves are computer program controlled.

resin at EOS (as unreacted fluoride, *vide infra*), and since the precursor is in contact with the resin-held-[^{18}F]fluoride for only a short period (because only a small fraction of the dilute precursor-acetonitrile solution is in contact with the resin at any given time), lengthening the reaction time, in either a MicroLab setting or a non-MicroLab setting, might well lead to increased radiochemical yields.

Balancing the moderate radiochemical yield in the automated MicroLab is the striking stereospecificity and overall cleanness of the procedure. The syntheses reported by Hamacher, which employ Kryptofix-222 to effect fluorination, require preparative HPLC for the separation of the isomeric mixtures produced. In the studies reported here, there was little or no undesired isomer produced. Using authentic samples of *cis* and *trans* isomers, co-injections were made with our product preparations, and it was shown unequivocally that the isomer ratios were always greater than 99:1 (Fig. 2). Indeed, overall, the unchromatographed products were never less than 99% radiochemically pure (Fig. 3, top). This high level of radiochemical purity was achieved with set-ups in which the alumina N SepPak

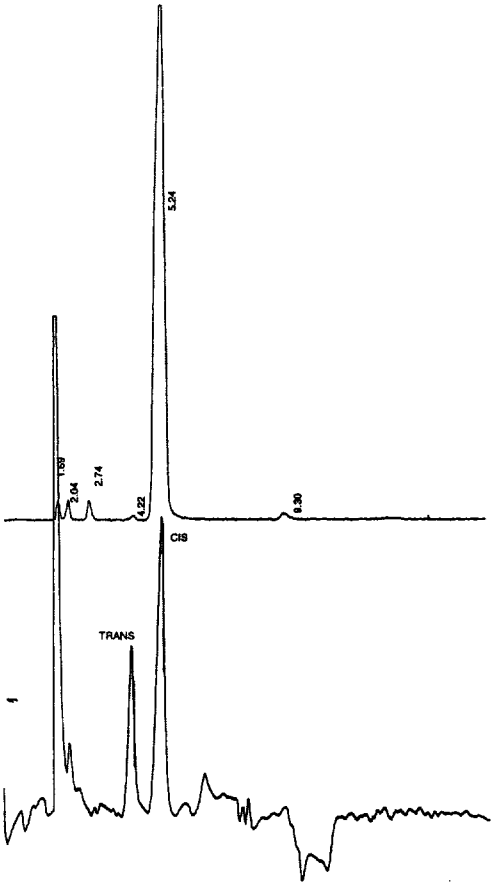


Fig. 2. Chromatograms of co-injection of authentic *cis*- and *trans*-4-fluoro-L-proline and *cis*-product preparation (Top: radioactivity; Bottom: differential refractometric). Performed on the Astec polymer-NH_2 column.

cartridge preceded the C18. With the cartridges reversed, the radiochemical purity dropped to an average of 95.7% (*e.g.* Fig. 2, top). With the alumina N SepPak placed first, a partial neutralization occurs resulting in a reduction of protonated species. This allows trapping on the C18 to be more effective.

In order to demonstrate that the F-18 fluorinations were truly stereospecific (and not the result of some selective retention effect), *cis* product was prepared without the benefit of Sep-Paks and final filter. The radiochromatograms of these preparations still showed little (<1%) or no isomeric impurity, and radiochemical purity was still greater than 90%. There was, however, more chemical impurity in

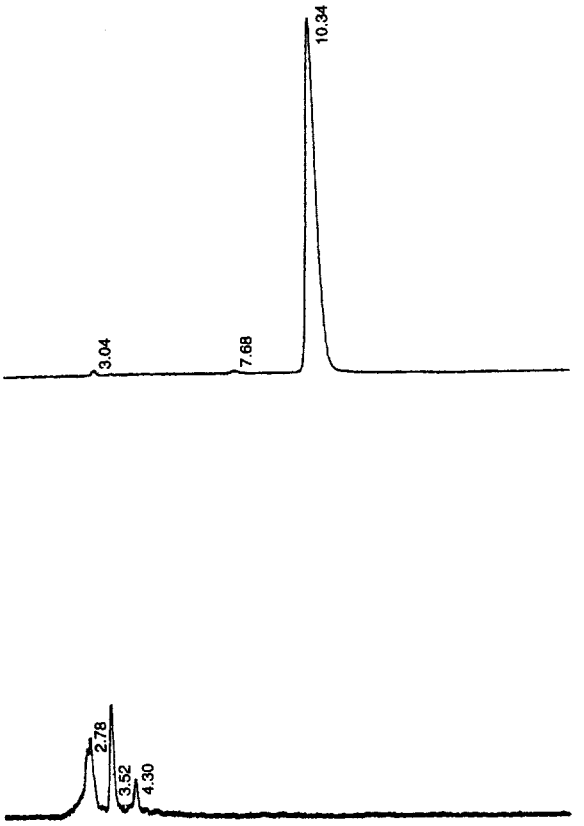


Fig. 3. Typical chromatograms of *cis*-4-[^{18}F]fluoro-L-proline preparation (Top: radioactivity; Bottom: UV absorbance at 254 nm and low attenuation). Performed on the Waters μ-Bondapak NH_2 column.

evidence in the UV-absorbance chromatograms. In other experiments, the resin-containing reaction column was recovered hot and extracted of its residual radioactivity (98.3% of the residual for *cis* and 98.4% for *trans* preparations), using in turn, acetonitrile, 0.1M HCl, and acetonitrile. Analysis of the extracts showed only [^{18}F]fluoride ion. Additionally, almost no activity eluted with the initial acetonitrile extraction, thus demonstrating, incidentally, the unlikeliness of fluoride leakage during production. Even so, co-injections with [^{18}F]fluoride were performed, and the products were shown to contain little or no fluoride. Finally, to check for false radiochromatograms, dose calibrator assaying of the HPLC column, after

chromatography of batches of both *cis* and *trans* products, showed not more than 1% (decay-corrected) of the injected radioactivity remaining on the column.

Gottlieb suggested that a *cis-trans* mixture results from participation by the carboxylate ester moiety during nucleophilic substitution on *trans*-tosylate (2). His *cis*-tosylate gave only *trans* product. It could be, that with our method, participation by the ester is inhibited by competition from the positive charge spread over the 4-(4-methylpiperidino)pyridinium ion of the resin. However, this does not explain how Hamacher also observed an 83:17 *tran:cis* mixture of 4-[^{18}F]fluoro-L-prolines starting from the *cis*-tosylate. In order to determine if the apparent stereospecificity of our syntheses was related to the hydrolysis conditions, *cis*- and *trans*-tosylate precursors were subjected to Hamacher fluorination conditions, but de-protected under the conditions reported here. In both cases isomeric mixtures were obtained which fell within the ranges Hamacher reported. This argues against both preferential stereoisomerization, and preferential hydroxide displacement of [^{18}F]fluoride in one of the isomers. Additional evidence against the latter possibility is the presence of little (<1%) or no [^{18}F]fluoride ion in the radiochromatograms of product preparations.

Finally, regarding chemical purity, the HPLC UV-absorbance chromatogram (detection at 254 nm) of the *cis* and *trans* product preparations showed a group of three or four small peaks with the integrator attenuator set as low as 4 (Fig. 3, bottom). No UV-active compounds were conclusively identified.

EXPERIMENTAL

Materials and Methods

[^{18}F]fluoride was produced with a GE PETtrace cyclotron using 16 MeV proton bombardment of [^{18}O]water from Isotec Inc.

Precursor material for the radiosynthesis of *cis*-4-[^{18}F]fluoro-L-proline **1**, *i.e.* *trans*-N-t-butoxycarbonyl-4-p-tosyloxy-L-proline methyl ester **2**, was prepared straightforwardly from *trans*-4-hydroxy-L-proline (Acros) by modifying the procedure for the synthesis of *trans*-N-carbobenzyloxy-4-p-tosyloxy-L-proline methyl ester (12). The identity of the compound was confirmed by ^{1}H NMR ($CDCl_3$

at 300 MHz), ^{13}C NMR ($CDCl_3$ at 75 MHz) and mass spectroscopy. The precursor material showed no sign of deterioration at room temperature after 12 months. Precursor material for the radiosynthesis of *trans*-4-[^{18}F]fluoro-L-proline, *i.e. cis*-N-t-butoxycarbonyl-4-p-tosyloxy-L-proline methyl ester, was prepared by tosylation of *cis*-N-t-butoxycarbonyl-4-hydroxy-L-proline methyl ester (Bachem). Authentic samples of *cis*- and *trans*-4-fluoroprolines, whose ^{1}H NMRs matched those reported in the literature (13) were synthesized in-house and also obtained from H. H. Coenen for comparison studies.

Standard MicroLab supplies from GE were used, including the cassettes, accessory syringes and reagent vials. The MicroLab's programmed timings and temperatures were not changed. During the 10 minute fluorination step, the quaternary 4-aminopyridinium resin column was heated at 90 deg. C., and during the 20 minute hydrolysis step, the temperature was 120 deg. C.

Alumina N Plus and C18 Plus Sep-Paks were from Waters Corp., and the vented 0.22 micron filters were from Millipore Corp. Just prior to installation, the alumina N Plus Sep-Pak was rinsed with approximately 10 mL deionized water, and the C18 Plus Sep-Pak was rinsed with 5 mL 95% ethanol, followed by 10 mL deionized water. Anhydrous acetonitrile was obtained from Aldrich Chemical Corp. in SureSeal bottles.

Analytical high pressure liquid chromatography (HPLC) was carried out on an Astec (Advanced Separation Technologies, Inc.) polymer-NH_2 column, 5 micron, 4.6 mm x 250 mm or on a Waters μ-Bondapak NH_2 column, 10 micron, 3.9 mm x 300 mm, using 70:30 acetonitrile-0.01 M sodium phosphate buffer at pH 7 with a flow rate of 1.0 mL/minute. Radiation detection was with an EG&G 3 inch x 3 inch NaI(Tl) crystal and 3 inch photomultiplier tube, and mass detection was with a Knauer Model A0298 differential refractometer situated close to the NaI(Tl) detector. Additional HPLC work was carried out using UV absorbance detection at 220 nm with a Spectra-Physics Spectra 100 detector and at 254 nm with a Waters Model 440 detector. NMR spectra were obtained on a Varian Gemini 300 Broadband.

Tracer Production

A typical production run of *cis*-4-[^{18}F]fluoro-L-proline involved preparing the Sep-Paks (Alumina N Plus and C18 Plus); then loading vial #1 with approximately 20 mg of *trans*-N-t-butoxycarbonyl-4-p-tosyloxy-L-proline methyl ester, vial #2 with acetonitrile (ca. 5 mL), vial #3 with distilled water (ca. 10 mL), and vial #4 with 0.1N aqueous HCl (ca. 2 mL); and then assembling the components onto the cassette and installing it into the MicroLab, being sure that the Alumina N Plus Sep-Pak preceded the C18 Plus (Fig. 1). After installation, 10 microliters of 0.84% aqueous sodium bicarbonate was added to the [^{18}F]fluoride receiver in the cassette, for pH adjustment. This part of the production took approximately 20-30 minutes and was begun approximately 30 minutes prior to EOB. After delivery of the [^{18}F]fluoride (4 minutes), the automatic synthesis protocol lasts 50 minutes. Following this, the receiving vial was disconnected and removed to the hot cell. Immediately 1.0 mL 1M aqueous NaOH was added to the vial, and after 15 minutes 5.0 mL of sterile 0.2 M NaH_2PO_4 was added. An approximately 1 mL aliquot was taken for testing of appearance, pH, radiochemical purity (HPLC), radionuclidic purity, sterility, and apyrogenicity. From EOB, the product was ready for use in 80 minutes, this including pH testing and HPLC analysis. In this way radiochemical yields were about 16%, and radiochemical purity was greater than 99%. All batches passed apyrogenicity testing (LAL reagent) and sterility testing (tryptic soy and fluid thioglycolate).

In a fashion identical to the above procedure, *trans*-4-[^{18}F]fluoro-L-proline was prepared from *cis*-N-t-butoxycarbonyl-4-p-tosyloxy-L-proline methyl ester. EOS radiochemical yields were about 18%, and radiochemical purity was greater than 99%. All batches passed apyrogenicity testing (LAL reagent) and sterility testing (tryptic soy and fluid thioglycolate).

CONCLUSIONS

This semi-automated procedure is a convenient method for the routine production of significant amounts of *cis*-4-[^{18}F]fluoro-L-proline **1** or *trans*-4-[^{18}F]fluoro-L-proline without the need for preparative HPLC, and we reasonably

expect that the chemistry can be transferred to other systems, automated and manual, which can make use of the quaternary 4-aminopyridinium resin.

DEDICATION

This paper is dedicated to the memory of Mr. Lucian J. Ciletti, Washington, Pennsylvania, an influential mentor and teacher of the author and many others, who died on March 5, 1999 during the course of this work.

ACKNOWLEDGEMENTS

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