Automated Radiosynthesis of cis- and trans-4-[18F]Fluoro-L-proline Using [18F]Fluoride

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ABSTRACT: The positron emission tomography imaging agents cis- and trans-4-[18F]fluoro-L-proline are used for the detection of numerous diseases such as pulmonary fibrosis and various carcinomas. These imaging agents are typically prepared by nucleophilic fluorination of 4-hydroxy-L-proline derivatives, with [18F]fluoride, followed by deprotection. Although effective radiofluorination reactions have been developed, the overall radiosynthesis process is suboptimal due to deprotection methods that are performed manually, require multiple steps, or involve harsh conditions. Here we describe the development of two synthetic routes that allow access to precursors, which undergo highly selective radiofluorination reactions and rapid deprotection, under mild acidic conditions. These methods were found to be compatible with automation, avoiding manual handling of radioactive intermediates.

INTRODUCTION

α-Amino acids are the key building blocks of life, acting as structural components of peptides and proteins.1 They also play an important role in biochemical and physiological processes, including energy metabolism, and in the formation of neurotransmitters and hormones. Due to the varied and important roles of α-amino acids in nature, their structural analogues have often been used to study biological processes and mechanisms.2,3 Positron emission tomography (PET) in combination with 18F-labeled α-amino acids (Figure 1) has been used for the non-invasive generation of molecular, functional, and metabolic information for a wide range of diseases.4 Although most applications have focused on imaging various forms of cancer, compounds such as 6-[18F]fluoro-L-DOPA have been used to investigate neurodegenerative disorders, including Parkinson’s disease.5 The cis and trans isomers of 4-[18F]fluoro-L-proline, [18F]1 and [18F]2, respectively, have also been used to image a number of disease conditions. Proline and 4-hydroxyproline are major structural components of collagen (15–30%), and therefore, [18F]1 and [18F]2 have been used to investigate abnormal collagen biosynthesis in diseases such as liver cirrhosis, lung fibrosis, and various carcinomas.4,6,7

Due to the importance of cis- and trans-4-[18F]fluoro-L-proline ([18F]1 and [18F]2, respectively), several methods for the radiosynthesis of these compounds have been developed.4c The most common approach involves the reaction of 4-sulfonyloxy-L-proline derivatives with [18F]fluoride, leading to fluorination with inversion of configuration (Scheme 1a). Development of the fluorination step by automation has resulted in fast and efficient reactions, while formation of the undesired diastereomer (usually as a minor product) can be controlled by reaction temperature and removed by HPLC at the end of the process.6 The limitations of these approaches occur during the deprotection stage, which due to harsh conditions is performed manually. For example, removal of the Boc-protecting group and hydrolysis of the ester were done as
with a single-step deprotection (Scheme 1b).

Combines a highly effective nucleophilic radiochemistry studies. Due to the limitations of previous side products, resulting in a decrease in the radiochemical yield of deprotection during the preparation of the labeled targets.

Our synthesis of cis-4-fluoro-L-proline (1) began with the esterification of commercially available (2S,4R)-N-Boc-4-hydroxy-L-proline (4) with O-tert-butyl-N,N-diisopropylsulfonamide (Scheme 2). This gave tert-butyl ester 5 in 68% yield. A

**Scheme 1. Synthesis of cis-4-[18F]Fluoro-L-proline**

a) Previous syntheses of cis-4-[18F]fluoro-L-proline

b) This work: Two-step automated synthesis of cis-4-[18F]fluoro-L-proline

For one of our imaging programs, we required access to cis- and trans-4-[18F]fluoro-L-proline ([18F]1 and [18F]2, respectively) as well as the nonradioactive analogues as standards for radiochemistry studies. Due to the limitations of previous approaches, we sought to develop a fully automated synthesis involving both a nucleophilic radiofluorination reaction and a single-step deprotection process. We now report the nonradioactive synthesis of both cis- and trans-4-fluoro-L-proline (1 and 2, respectively) from readily available (2S,4R)-N-Boc-4-hydroxy-L-proline, using a deoxyfluorination reaction with morpholinosulfur trifluoride as the key step. Also described is a fully automated synthesis of [18F]1 and [18F]2, which combines a highly effective nucleophilic radiofluorination with a single-step deprotection (Scheme 1b).

**RESULTS AND DISCUSSION**

Our primary aim during this project was the design and synthesis of proline derivatives that would undergo clean and efficient nucleophilic fluorination reactions and that could be deprotected in a single step, under mild conditions. Previous syntheses of 4-fluoroprolines have generally used an N-protected derivative of 4-hydroxyproline methyl ester as the starting material. However, issues were reported during the nucleophilic fluorination step involving intramolecular participation of the ester carbonyl, which led to the formation of a fluoroproline byproduct (17%) with retention of configuration. In this project, it was proposed that the use of a more bulky proline derivative, such as N-Boc-L-proline tert-butyl ester S, would minimize any intramolecular reactions during the fluorination step. Furthermore, the use of two acid-sensitive protecting groups would allow rapid and mild deprotection during the preparation of the labeled targets.

Our synthesis of cis-4-fluoro-L-proline (1) began with the esterification of commercially available (2S,4R)-N-Boc-4-hydroxy-L-proline (4) with O-tert-butyl-N,N-diisopropylsulfonamide (Scheme 2). This gave tert-butyl ester 5 in 68% yield. A

**Scheme 2. Synthesis of Precursor 6 and cis-4-Fluoro-L-proline (1)**

The precursor for radiofluorination studies and the synthesis of cis-4-[18F]fluoro-L-proline ([18F]1 was prepared by tosylation of 5 under standard conditions. An initial attempt to complete the synthesis of cis-4-fluoro-L-proline (1) investigated the nucleophilic fluorination of tosyl derivative 6 using TBAF. However, this led to elimination of tosic acid and the isolation of dehydroproline derivatives. Instead, (2S,4R)-hydroxy-L-proline 5 was treated with morpholinosulfur trifluoride, and this allowed the single-step synthesis of 7 in 63% yield. Analysis of the 1H NMR spectrum of the crude reaction material showed the absence of only the cis diastereomer, confirming complete inversion of configuration. This result suggests that the sterically encumbered tert-butyl ester prevents any intramolecular participation of the carbonyl and the formation of the undesired fluorinated trans diastereomer. Acid-mediated deprotection of 7 using 2 M hydrochloric acid at room temperature gave after recrystallization cis-4-fluoro-L-proline (1) in 64% yield.

To access trans-4-fluoro-L-proline (2) using the same approach, we synthesized (2S,4R)-N-Boc-4-hydroxy-L-proline (9). As (2S,4R)-N-Boc-4-hydroxy-L-proline (4) is readily available and inexpensive, we investigated the strategy for inversion of configuration of the 4-hydroxy group. Previous methods have activated the 4R-hydroxy group of (2S,4R)-4-hydroxy-L-proline ester derivatives by mesylation or using a Mitsunobu reaction, followed by inversion with benzoic acid and then hydrolysis of the resulting ester. Raines and co-workers described a three-step approach involving hydroxy group mesylation, inversion by intramolecular lactonization with the α-carboxylic acid, and then lactone hydrolysis. Inspired by this, we developed a two-step approach in which lactone 8 was initially prepared by an intramolecular Mitsunobu reaction of (2S,4R)-N-Boc-4-hydroxy-L-proline (4) (Scheme 3). Lactone 8 was then hydrolyzed at room temperature with lithium hydroxide to give (2S,4S)-N-Boc-4-hydroxy-L-proline (9) in 71% yield over the two steps. This approach was scalable, allowing the multigram synthesis of 9. With the (2S,4S)-diastereomer 9 in hand, the
same series of steps (tert-butyl esterification and tosylation) was used to access precursor 11. Similarly, reaction of 10 with morpholinosulfur trifluoride gave 4-fluoroproline 12 as a single diastereomer, and deprotection under mild acidic conditions gave trans-4-fluoro-L-proline (2) in good overall yield.

The radiosynthesis of [18F]1 and [18F]2 using a TRACERlab FX90 automated synthesizer and precursors 6 and 11 was next investigated. During these experiments, no-carrier-added [18F]fluoride from the cyclotron was trapped on a carbonate-precotioned quaternary methylammonium (QMA) cartridge, eluted into the reactor with a solution containing K222/K2CO3, and then azeotropically dried. To compare the effectiveness of precursors 6 and 11 with previous methods, [18F]fluoride was reacted initially with commercially available (2S,4R)-proline methyl ester derivative 3 under literature conditions (Scheme 4). This involved reaction with [18F]-fluoride at 110 °C for 10 min, followed by deprotection with 2 M hydrochloric acid during the deprotection stage. Following a total reaction time of 74 min, this gave [18F]1 in a decay-corrected RCY of 42%. A benefit of a slightly longer radiofluorination reaction time was that less precursor was required for complete conversion of [18F]fluoride. With precursor 6, the amount for each run could be reduced from 16 to 5 mg. The study next investigated the use of milder conditions to remove the acid-labile protecting groups. Radiofluorination of 6, followed by deprotection with 2 M hydrochloric acid at 60 °C, gave [18F]1 in 19% RCY (entry 2). It was proposed that the lower RCY for this production was partly due to the use of a strong cation exchange (SCX) cartridge during the final formulation stage. Therefore, the two-step process was repeated using both a shorter reaction time (5 min) for the deprotection step and a mixed-mode cation exchange (MCX) cartridge during the formulation (entry 3). This gave a 42% RCY of [18F]1 after a total reaction time of 71 min. Further optimization was achieved by avoiding an evaporation stage after initial radiofluorination (entry 4). This resulted in a shorter overall reaction time of 63 min and gave [18F]1 in 36% RCY. The corresponding radio-HPLC chromatogram under these optimized conditions showed clean synthesis of [18F]1 (Figure 2). The reaction mixture was found to contain 98.8% [18F]1, with <0.4% trans isomer [18F]2.15,16

The optimized conditions were then used for the automated production, isolation, and purification of [18F]1 (Scheme 5). After a total synthesis time of 59 min, this gave [18F]1 in 41 ± 3.6% RCY (n = 9) with a >99% radiochemical purity. The molar activity of [18F]1 was found to be >0.641 GBq μmol−1.17 The optimized conditions were then used for the automated synthesis of [18F]2 using precursor 11. In a similar manner, the two-step process was found to be highly selective for the preparation of [18F]2, generating the trans isomer in 97.7% yield, with 2.2% of the cis isomer also observed.13 Use of this method for the automated production and purification of [18F]2 gave the target after a total synthesis time of 75 min, in 34 ± 4.3% RCY (n = 11) with a >99% radiochemical purity. The molar activity of [18F]2 was found to be >0.320 GBq μmol−1.17 The stability of formulated products [18F]1 and [18F]2 using radio-HPLC was analyzed at 2 and 11 h points from the end of synthesis.15 For both isomers, there was no observed radiochemical byproduct after 11 h, which confirmed that these imaging agents are stable within this time frame to decomposition pathways, such as epimerization or radiolysis.
CONCLUSIONS

In summary, a new approach for the preparation of cis-4-fluoro-L-proline (1) has been developed from (2S,4R)-N-Boc-4-hydroxy-L-proline (4). The use of a sterically hindered tert-butyldi (2S,4R)-N-Boc-4-hydroxy-L-proline (9) from commercially available (2S,4R)-diastereomer 4 via an intramolecular Mitsunobu reaction and lactone hydrolysis, a similar approach was developed for the preparation of trans-4-fluoro-L-proline (2). The tosylated derivatives were then investigated as substrates for a fully automated synthesis of cis- and trans-4-[^18]F]-fluoro-L-proline ([^18]F]1 and [^18]F]2, respectively). The bulky precursors underwent clean and reproducible radiofluorination, and the use of two acid-sensitive protecting groups allowed deprotection under mild conditions. It should be noted that both steps are highly amenable to automation when using a synthesizer and, thus, avoid typically harsh conditions and manual handling of radioactive intermediates.

EXPERIMENTAL SECTION

All reagents and starting materials were obtained from commercial sources and used as received unless otherwise stated. Dry solvents were purified using a solvent purification system. Brine refers to a saturated solution of sodium chloride. All reactions were performed in oven-dried glassware under an atmosphere of argon unless otherwise stated. All mixtures for reactions performed at increased temperatures were heated using an oil bath. Flash column chromatography was carried out using silica gel (40−63 μm). Aluminum-backed plates precoated with silica gel 60 (UV 254) were used for thin layer chromatography and visualized under ultraviolet light and by staining with KMnO₄, ninhydrin, or vanillin. ¹H NMR spectra were recorded on an NMR spectrometer at 400 or 500 MHz, and data are reported as follows: chemical shift in parts per million relative to tetramethylsilane or the solvent as the internal standard (CDCl₃, δ 7.26), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet or overlap of nonequivalent resonances, integration). ¹³C{¹H} NMR spectra were recorded on an NMR spectrometer at 101 or 126 MHz, and data are reported as follows: chemical shift in parts per million relative to tetramethylsilane or the solvent as the internal standard (CDCl₃, δ 77.0), multiplicity with respect to hydrogen (deduced from DEPT experiments, C, CH, CH₂, or CH₃). IR spectra were recorded on a FTIR spectrometer; wavenumbers are indicated in inverse centimeters. Mass spectra were recorded using electron impact or electrospray ionization techniques. HRMS spectra were recorded using a dual-focusing magnetic analyzer mass spectrometer. Melting points are uncorrected. Optical rotations were determined as solutions irradiating with the sodium D line (λ = 589 nm) using a polarimeter. [α]D values are given in units of 10⁻¹ deg cm² g⁻¹.

Di-tert-butyld (2S,4R)-4-Hydroxypyrrolidine-1,2-dicarboxylate (5). To a solution of N-(tert-butoxycarbonyl)-(2S,4R)-4-
hydroxypropylidine-2-carboxylic acid (4) (0.500 g, 2.16 mmol) in dry tetrahydrofuran (2.5 mL), under argon at 0 °C was added tert-butyl N,N'-disopropylcarbamidaminate (0.500 mL, 2.16 mmol). The reaction mixture was heated to 70 °C for 3 h, followed by further addition of tert-butyl N,N'-disopropylcarbamidaminate (0.500 mL, 2.16 mmol). The reaction mixture was heated for a further 18 h. The reaction mixture was filtered through Celite and then concentrated in vacuo. Purification by flash column chromatography eluting with 50% ethyl acetate in hexane gave di-tert-butyl (25,4R)-4-hydroxypropylidine-1,2-dicarboxylate (5) as a white solid (0.420 g, 68%): [α]D19 = +53.5 (c 0.2, CHCl3). [lit.20 [α]D = 65.3 (c 1.0, CHCl3)]. Spectroscopic data matched the literature.20

**Di-tert-butyl (25,4R)-4-(Tosloxy)pyrrolidine-1,2-dicarboxylate (6).** To a solution of di-tert-butyl (25,4R)-4-hydroxypropylidine-1,2-dicarboxylate (5) (1.00 g, 4.35 mmol) in dry tetrahydrofuran (200 mL) under argon at 0 °C was added triphenylphosphine (0.700 g, 2.63 mmol), followed by dropwise addition of diisopropylcarbamimidate (0.965 mL, 4.35 mmol), followed by further addition of tert-butyl N,N'-disopropylcarbamidaminate (0.965 mL, 4.33 mmol) after 3 h. Purification by column chromatography eluting with 40% ethyl acetate in hexane gave di-tert-butyl (25,4S)-4-hydroxypropylidine-1,2-dicarboxylate (10) (0.500 g, 1.74 mmol), dry dichloromethane (10 mL), pyridine (0.280 mL, 3.48 mmol), 4-dimethylaminopyridine (0.0210 g, 0.174 mmol), and ethyl 3-chloropropionate (4.00 g, 18.8 mmol) in a mixture of water (60 mL), tetrahydrofuran (40 mL), and methanol (40 mL) was added lithium hydroxide monohydrate (2.36 g, 56.3 mmol). The reaction mixture was stirred at room temperature for 18 h and concentrated in vacuo, and ethyl acetate (100 mL) was added to the oily residue. The solution was acidified using a saturated aqueous solution of potassium hydrogen sulfate, and the aqueous layer was extracted with ethyl acetate (3 × 150 mL). The combined extracts were dried over MgSO4, and concentrated in vacuo to give N-(ter-butoxycarbonyl)-(25,4S)-4-hydroxypropylidine-2-carboxylic acid (9) as a white solid (4.00 g, 92%): [α]D19 = −38.5 (c 0.3, MeOH). [lit.21 [α]D = −39.0 (c 0.7, MeOH)]. Spectroscopic data matched the literature.21

**Di-tert-butyl (25,4S)-4-Hydroxypropylidine-1,2-dicarboxylate (10).** Di-tert-butyl (25,4S)-4-hydroxypropylidine-1,2-dicarboxylate (10) was prepared as described for di-tert-butyl (25,4R)-4-hydroxypropylidine-1,2-dicarboxylate (5) using N-(ter-butoxycarbonyl)-(25,4S)-2-oxo-3-oxo-5-azabicyclo[2.2.1]heptane-5-carboxylic acid (8) (2.36 g, 56.3 mmol). The reaction mixture was stirred at room temperature for 18 h and concentrated in vacuo, and ethyl acetate (100 mL) was added to the oily residue. The solution was acidified using a saturated aqueous solution of potassium hydrogen sulfate, and the aqueous layer was extracted with ethyl acetate (3 × 150 mL). The combined extracts were dried over MgSO4, and concentrated in vacuo to give N-(ter-butoxycarbonyl)-(25,4S)-4-hydroxypropylidine-2-carboxylic acid (9) as a white solid (4.00 g, 92%): [α]D19 = −38.5 (c 0.3, MeOH). [lit.21 [α]D = −39.0 (c 0.7, MeOH)]. Spectroscopic data matched the literature.21

**Di-tert-butyl (25,4S)-4-Fluoropyrrolidine-2-carboxylic acid (4).** Di-tert-butyl (25,4S)-4-fluoropyrrolidine-2-carboxylate (4) (4.000 g, 2.600 mmol) in dry tetrahydrofuran (200 mL) under argon at 0 °C was added triphenylphosphine (8.17 g, 31.1 mmol), followed by dropwise addition of diisopropyl azodicarboxylate (6.13 mL, 31.1 mmol). The reaction mixture was stirred at room temperature for 18 h and concentrated in vacuo. Purification by column chromatography eluting with 80% diethyl ether in hexane gave tert-butyl (15,4S)-2-oza-3-oxo-3-oxo-5-azabicyclo[2.2.1]heptane-5-carboxylate (9) (2.000 g, 70%): [α]D19 = +43.8 (c 1.0, CHCl3). [lit.20 [α]D = +64.3 (c 1.0, CHCl3)]. Spectroscopic data matched the literature.20

**Di-tert-butyl (25,4R)-4-Fluoropyrrolidine-1,2-dicarboxylate (7).** To a solution of di-tert-butyl (25,4R)-4-fluoropyrrolidine-2-carboxylate (4) (6.000 g, 26.00 mmol) in dry tetrahydrofuran (200 mL) under argon at 0 °C was added triphenylphosphine (8.17 g, 31.1 mmol), followed by dropwise addition of diisopropyl azodicarboxylate (6.13 mL, 31.1 mmol). The reaction mixture was stirred at room temperature for 18 h and concentrated in vacuo. Purification by column chromatography eluting with 80% diethyl ether in hexane gave tert-butyl (15,4S)-2-oza-3-oxo-3-oxo-5-azabicyclo[2.2.1]heptane-5-carboxylate (9) (2.000 g, 70%): [α]D19 = +43.8 (c 1.0, CHCl3). [lit.20 [α]D = +64.3 (c 1.0, CHCl3)]. Spectroscopic data matched the literature.20
fluopyrrolidine-1,2-dicarboxylate (12) (0.0800 g, 0.280 mmol), acetonitrile (0.25 mL), and 2 M hydrochloric acid (2.5 mL). This gave (25S,4S)-4-fluopyrrolidine-2-carboxylic acid hydrochloride (2) as an off-white solid (0.0331 g, 70%); mp 148–152 °C dec; IR ( neat) 3672, 2987, 1738, 1682, 1406, 1242, 1220, 1067, 1051 cm⁻¹; [α]D²⁰ -6.5 (c 0.1, MeOH); 1H NMR (400 MHz, CD3OD) δ 5.47 (dt, J = 5.1, 3.6 Hz, 1H), 4.61 (dd, J = 10.5, 7.9 Hz, 1H), 3.73–3.52 (m, 2H), 2.84–2.27 (m, 1H), 2.39 (dddd, J = 38.5, 14.8, 10.5, 3.6 Hz, 1H); 13C(CH3) NMR (101 MHz, CD3OD) δ 169.3 (C), 92.0 (d, Jc–c = 177.0 Hz, CH3), 58.0 (CH), 51.7 (d, Jc–c = 24.0 Hz, CH3), 35.4 (d, Jc–c = 22.1 Hz, CH3); MS (ESI) m/z 134 (M + H+, 100); HRMS (ESI) m/z [M + H]+ calcd for C5H9FNO2 134.0612, found 134.0613.

Radiochemistry: General Experimental.

No-carrier-added aqueous [18F]fluoride was produced via the 18O(p,n)18F nuclear reaction by irradiation of 18O-enriched water by a GE PET/CT 8 cyclotron. All radiofluorination reactions were carried out on a GE TRACERlab FXQ automated synthesizer. Sep-Pak QMA Carbonate Plus Light cartridges (Waters) were preconditioned with water (10 mL) prior to use, Oasis MCO Plus Short (Waters) and Bond Elut SCX 1 g (Agilent) cartridges were preconditioned with ethanol (5 mL) and then with water (10 mL) prior to use. The starting activity for calculating the radiochemical yield was determined from the GM reading taken immediately following delivery of [18F]fluoride to the synthesizer. The final activity readings were recorded using a Capintec CRC-35 PET dose calibrator.

Analytical HPLC Method.

Analytical HPLC was carried out on a Thermo Dionex Ultimate system equipped with a Berthold FlowStar LB 513 radio flow detector and a DAD-3000 UV detector. An isocratic mobile phase of 60% acetonitrile in water was used with a Phenomenex Luna 5 μm NH2 100 Å, 250 mm × 4.6 mm column at a flow rate of 1 mL min⁻¹. The nonradioactive standards were detected using a UV wavelength of 210 nm.

cis-4-[18F]Fluoro-L-proline [18F]1. Cyclotron target water containing [18F]fluoride was transferred to and trapped on a Sep-Pak QMA Carbonate Plus Light cartridge. The activity was eluted into a reaction vessel using a solution of Kryptofix 222 (15 mg) and potassium carbonate (2.4 mg) in acetonitrile (0.80 mL) and water (0.40 mL). This solution was dried by being stirred at 100 °C under vacuum and a stream of helium gas for 2 min. This process was repeated twice using acetonitrile (2 × 1 mL). The [18F]fluoride was then completely dried by applying full vacuum for 1 min. Di-tert-butyl (2S,4R)-4-(tosyloxy)pyrrolidine-1,2-dicarboxylate (5) (5.0 mg) in acetonitrile (1.0 mL) was added to the reaction vessel, which was sealed, and the mixture heated to 110 °C for 15 min while being stirred. The reaction mixture was then cooled to 60 °C, and a 4 M aqueous solution of hydrochloric acid (1.0 mL) was added (resulting in a 2 M concentration of hydrochloric acid). The reaction mixture was stirred at this temperature for 5 min and then concentrated by applying vacuum under a stream of helium gas. The resultant residue was then cooled to 30 °C and diluted with a 50% aqueous solution of acetonitrile (2.0 mL). The reaction mixture was then transferred into the HPLC injector loop for purification. Purification was performed by semipreparative HPLC with a SYKMN S1122 solvent delivery system using a Phenomenex Luna 5 μm NH2 100 Å, 250 mm × 10 mm column and eluted using a 60% aqueous solution of acetonitrile at a flow rate of 4 mL min⁻¹. The product fraction was identified using a gamma detector at a retention time of approximately 7 min. trans-4-[18F]Fluoro-L-proline [18F]2 was isolated in 34 ± 4.3% radiochemical yield with a radiochemical purity of >99% (n = 11). The total synthesis time from delivery of [18F]fluoride to extraction of the product was 57 ± 1.2 min.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00755.

HPLC chromatograms and 1H and 13C(1H) NMR spectra of all compounds (PDF).

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Notes

The authors declare no competing financial interest.

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