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Efficient synthesis of (2S)-*tert*-butyl 2-(2-bromopropanamido)-5-oxo-5-(tritylamino)pentanoate as a precursor of PET radiotracer [^{18}F]FPGLN

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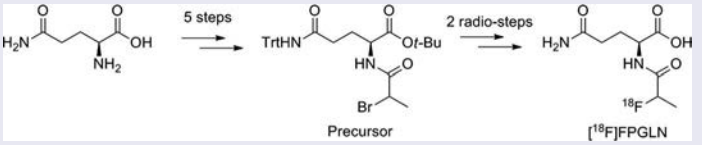
ABSTRACT

This study describes a convenient protocol for the synthesis of (2*S*)-*tert*-butyl 2-(2-bromopropanamido)-5-oxo-5-(tritylamino)pentanoate, which can serve as an appropriate precursor of (2*S*)-5-amino-2-(2-[^{18}F]fluoropropanamido)-5-oxopentanoic acid (*N*-(2-[^{18}F]fluoropropionyl)-L-glutamine, [^{18}F]FPGLN) for tumor positron emission tomography imaging. Five-step synthesis starting from L-glutamine provided the desired precursor with high yields. In addition, a simple method for the preparation of [^{18}F]FPGLN from this easily available precursor was developed using a two-step ^{18}F-labeling strategy.

GRAPHICAL ABSTRACT



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KEYWORDS
Glutamine derivatives; new precursor; positron emission tomography; radiotracer

Introduction

^{18}F-labeled amino acid and its derivatives have received much attention due to the potential value for clinical application in positron emission tomography (PET) tumor imaging.[1,2] As a part of our broad interest in radiolabeled amino acid tracers, a series of *N*-substituted ^{18}F-labeled amino acid derivatives containing 2-[^{18}F]fluoropropionyl group have been developed for PET tumor imaging in our research group, this class of the radiotracers demonstrated high tumor to background contrast and favorable pharmacokinetics in vivo and was not incorporated into protein.[3–6] (2*S*)-5-Amino-2-(2-[^{18}F]fluoropropanamido)-5-oxopentanoic acid (*N*-(2-[^{18}F]fluoropropionyl)-L-glutamine, [^{18}F]FPGLN), a typical *N*-^{18}F-labeled amino acid derivative, has been successfully synthesized using a multistep ^{18}F-incorporation method in our previous study (Scheme 1).[5] In vivo

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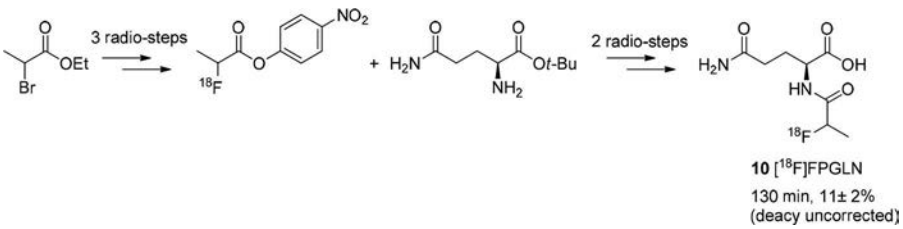
Supplemental data (full experimental detail, ^{1}H NMR and ^{13}C NMR spectra, HPLC traces) can be accessed on the publisher's website.



Scheme 1. Five-step radiosynthesis of [^{18}F]FPGLN using early-step ^{18}F-incorporation strategy. *Note*: [^{18}F]FPGLN, *N*-(2-[^{18}F]fluoropropionyl)-L-glutamine.

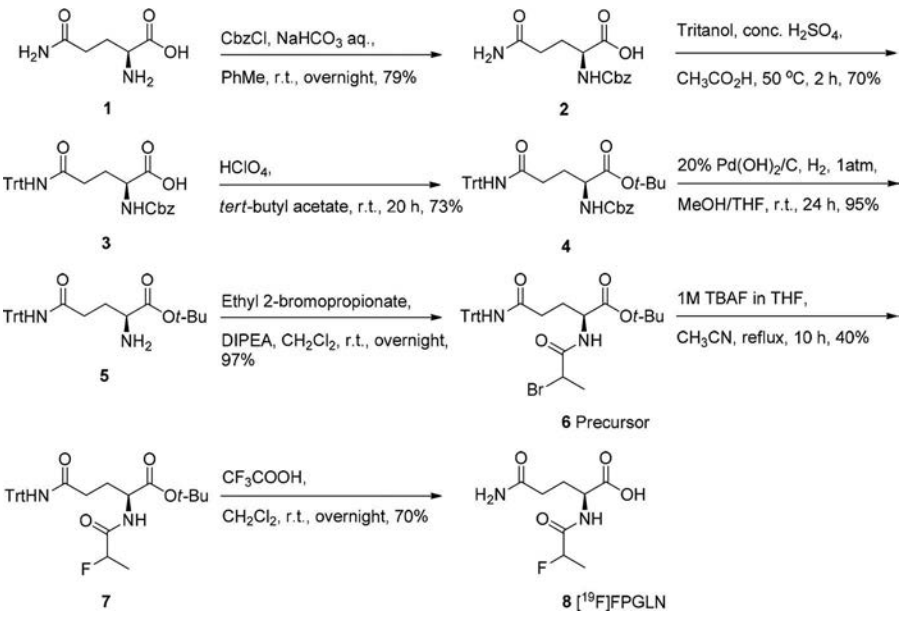
PET studies showed high [^{18}F]FPGLN uptake in SPC-A-1 lung adenocarcinoma and PC-3 prostate cancer-bearing mouse models. The encouraging preclinical results suggest that [^{18}F]FPGLN should deserve further investigation as an amino acid metabolic PET radiotracer for tumor imaging.

However, the complicated multistep and time-consuming protocol makes it difficult to meet the demands for further preclinical and clinical research. Although a straightforward two-step ^{18}F-labeling method for [^{18}F]FPGLN can help us to overcome multistep protocol's limitations, the prerequisite of two-step method is how to easily obtain the labeling precursors. This prompted us to develop an easy strategy to prepare a new precursor, which is suitable for the radiosynthesis of [^{18}F]FPGLN through a two-step ^{18}F-labeling method.

In this work, a novel labeling precursor **6**, (2*S*)-*tert*-butyl 2-(2-bromopropanamido)-5-oxo-5-(tritylamino)pentanoate, was readily synthesized from inexpensive materials through five-step reactions. In addition, a simple method for the preparation of [^{18}F]FPGLN was also developed through two-step reactions, including ^{18}F-fluorination reaction of the readily available precursor with complex substance cryptand $K_{2.2.2}/K^{18}F$ and deprotection reaction with trifluoroacetic acid.

Results and discussion

The purpose of this study is to synthesize an appropriate precursor which can easily be prepared, fluorinated, and deprotected. The brominated precursor **6** was synthesized by multistep reactions (Scheme 2), with a modification of the reported procedure.[7,8] As shown in Scheme 2, at first, the amino protection reaction of L-glutamine with benzyl chloroformate in the presence of $NaHCO_3$ gave 79% yield of compound **2**. Subsequently, the amide group was protected by the reaction of compound **2** with triphenylmethanol and acetic anhydride in glacial acetic acid under the catalysis of concentrated sulfuric acid. Next, transesterification of the carboxylic acid **3** with *tert*-butyl acetate in the presence of perchloric acid as a catalyst provided *tert*-butyl ester **4**. Then, deprotection of carboxybenzyl group (Cbz) in compound **4** by catalytic hydrogenation with 20% $Pb(OH)_2/C$ in a mixed solution of MeOH and THF (v/v = 1:1) gave compound **5**. According to the procedures reported in the literature,[7,8] the hydrogenolysis reaction was catalyzed by 10% Pb/C in dioxane. However, we found that the reported reaction would not complete even in high pressure and heating conditions but could proceed smoothly to afford compound **5** in good yields (95%) using 20% $Pb(OH)_2/C$ as a catalyst under very mild conditions (room temperature and atmosphere pressure). Finally, the precursor **6** was synthesized by amidation reaction of compound **5** with 2-bromopropionyl bromide.

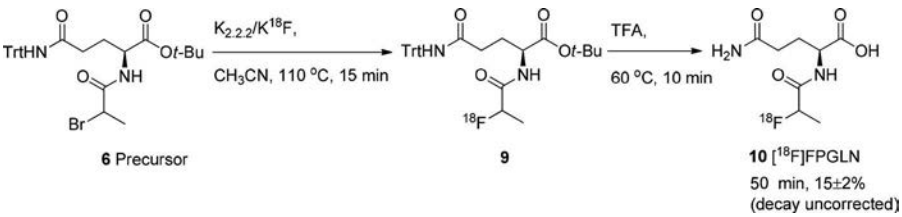


Scheme 2. Synthesis of precursor **6** and standard compound **8**. *Note*: [^{18}F]FPGLN, *N*-(2-[^{18}F]fluoropropionyl)-L-glutamine.

The precursor **6** was synthesized from L-glutamine as starting material through five-step reactions, including benzyloxycarbonylation, tritylation, transesterification, catalytic hydrogenation, and amidation, in the total yield of 37%. As described above, the five-step synthetic route suitable for mass production has advantages of readily available cheap materials, mild reaction condition, convenient operation, and simple post-treatment process.

Before the preparation of [^{18}F]FPGLN, nonradiolabeled standard compound **8** was also synthesized and used for identification and characterization of the target radiotracer [^{18}F]FPGLN **10**. A two-step reaction process was used as follows. Fluorination reaction of the precursor **6** in the TBAF–THF–CH_3CN mixture was performed to give fluorinated compound **7** under anhydrous conditions. Cleavage of the *tert*-butyl and trityl protecting groups was performed in a mixture of TFA and CH_2Cl_2 (v/v = 10:1) at room temperature to afford compound **8**, which was a nonradioactive standard of [^{18}F]FPGLN **10**. It is worth noting that the trityl group of glutamine derivative is stable to strong mineral acids but can be cleavable by TFA.[9]

N-(2-[^{18}F]fluoropropionyl)-L-glutamine was synthesized by the modification of procedures reported in the literature.[10,11] The radiosynthesis of [^{18}F]FPGLN staring from the readily available precursor **6** was developed through a two-step ^{18}F-labeling reaction procedure (Scheme 3). [^{18}F]FPGLN **10** was synthesized by ^{18}F-fluorination reaction of **6** with $K_{2.2.2}/K^{18}F$, followed by an acid deprotection reaction with TFA in $15 \pm 2\%$ ($n = 5$) of decay uncorrected radiochemical yield within the whole radiosynthesis time of 50 min. The specific activity was 60 ± 12 GBq/μmol. Compared with the previous study,[5] two-step radiosynthesis of [^{18}F]FPGLN with Sep-Pak plus C18 cartridge purification in this study had obvious advantages of short radiosynthesis time.



Scheme 3. Two-step radiosynthesis of [^{18}F]FPGLN from precursor **6** using a late-step ^{18}F-introduction strategy. *Note*: [^{18}F]FPGLN, *N*-(2-[^{18}F]fluoropropionyl)-L-glutamine.

Conclusion

In this study, (2*S*)-*tert*-butyl 2-(2-bromopropanamido)-5-oxo-5-(tritylamino)pentanoate as a novel precursor of [^{18}F]FPGLN has been readily synthesized in high yields from glutamine as starting materials through five-step reactions. In addition, [^{18}F]FPGLN has been successfully synthesized from this readily available precursor through two-step radiosynthesis. The new preparation method of [^{18}F]FPGLN has the advantages of simple two-step reactions, short radiosynthesis time, and relatively high yield. Studies on the automated synthesis and further in vivo evaluation of this radiotracer are in progress and will be reported in due course.

Experimental

All commercial reagents and solvents used in the reaction were obtained from commercial sources and used without any further purification. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR were recorded on a Bruker 400 MHz or 600 MHz spectrometer. All chemical shifts are reported in ppm relative to tetramethylsilane and peak multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Sep-Pak cartridges (light QMA cartridges, and C18 plus cartridges) were purchased from Waters. Before they were used, light QMA cartridges were preconditioned with sodium bicarbonate ($NaHCO_3$) aqueous and water (H_2O), C18 plus cartridges were preconditioned with ethanol (EtOH) and H_2O, respectively. [^{18}F]Fluoride was acquired through the nuclear reaction ^{18}O(p, n)^{18}F by irradiation of more than 95% ^{18}O-enriched water target with 10-MeVproton beam on the cyclone 10/5 cyclotron.

(2S)-tert-Butyl 2-(2-bromopropanamido)-5-oxo-5-(tritylamino)pentanoate 6

2-Bromopropionyl bromide (0.98 g, 4.53 mmol) was added dropwise to a solution of (S)-*tert*-butyl 2-amino-5-oxo-5-(tritylamino)pentanoate **5** (4.00 g, 4.53 mmol) and DIPEA (0.59 g, 4.53 mmol) in dry dichloromethane (50 mL) with stirring at about 0 °C. Afterward, the solution was warmed to room temperature and stirred overnight. Finally, diluted with water, extracted with dichloromethane (3 × 50 mL), combined the organic phase, washed with 10% citrate solution 100 mL, water 75 mL, brine 75 mL, dried over Na_2SO_4, filtered, and concentrated the solvent to afford as white solid **6** (5.05 g, 97%), mp 202 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.17 (m, 16H), 7.10 (dd, $J = 41.6$, 7.4 Hz, 1H), 6.99 (d, $J = 21.7$ Hz, 1H), 4.44–4.24 (m, 2H), 2.50–2.16 (m, 3H), 2.00–1.86 (m, 1H), 1.82 (d, $J = 7.0$ Hz, 3H),

1.46 (d, $J = 3.0$ Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.08, [170.63, 170.50], [169.67, 169.58], 144.60, 128.72, 127.97, 127.05, [82.70, 82.66], 70.68, [53.28, 52.97], 44.05, [33.56, 33.45], [28.57, 28.18], 28.01, 22.62. HRMS (ESI): *m*/*z* [M+H] calcd. for $C_{31}H_{35}BrN_2O_4$: 578.1780. Found: 578.1783.

(2S)-tert-Butyl 2-(2-fluoropropanamido)-5-oxo-5-(tritylamino)pentanoate 7

The 1M TBAF in THF (4 mL) was added dropwise to a solution of (2*S*)-*tert*-butyl 2-(2-bromopropanamido)-5-oxo-5-(tritylamino)pentanoate **6** (1.16 g, 2 mmol) in dry CH_3CN, left to stir at 90 °C for 10 h, then the removal of solvent to afford an oil crude. The crude product was purified by column chromatography on silica gel (petroleum ether/EtOAc = 2:5) to afford the title compounds as white solid **7** (0.40 g, 40%), mp 153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.18 (m, 16H), 7.06 (dd, $J = 14.3$, 8.0 Hz, 2H), 4.96 (dp, $J = 49.2$, 6.7 Hz, 1H), 4.45 (qd, $J = 9.1$, 3.8 Hz, 1H), 2.47–2.17 (m, 3H), 1.98–1.84 (m, 1H), 1.56 (ddd, $J = 24.5$, 6.7, 5.1 Hz, 3H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.21, [171.08, 171.01], [170.88, 170.85, 170.59, 170.54], 144.69, 128.75, 127.95, 127.03, [89.41, 89.37, 87.58, 87.55], [82.72, 82.68], 70.67, [52.18, 52.06], [33.71, 33.63], [28.99, 28.84], 28.02, [18.77, 18.55, 18.44, 18.23]. ^{19}F NMR (376 MHz, $CDCl_3$) δ −182.18 to −182.58 (m). HRMS (ESI): *m*/*z* [M+H] calcd. for $C_{31}H_{35}FN_2O_4$: 518.2581. Found: 518.2575.

(2S)-5-Amino-2-(2-fluoropropanamido)-5-oxopentanoic acid 8

(2*S*)-*tert*-Butyl-2-(2-fluoropropanamido)-5-oxo-5-(tritylamino)pentanoate **7** (320 mg, 0.62 mmol) was added to a mixed solution of trifluoroacetic acid (20 mL) and dichloromethane (2 mL). The resulting mixture was stirred overnight at room temperature. After the removal of solvent under vacuum to give the residue, 1 M lithium hydroxide solution (30 mL) was added and extracted with diethylether (3 × 20 mL). The aqueous phase was made acidic with 1N hydrochloric acid to pH = 4, extracted with ethyl acetate (3 × 40 mL), combined the organic phase, and washed with water 40 mL, brine 40 mL, dried over Na_2SO_4, evaporated the solvent to afford yellow oil **8** (96 mg, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.21–7.06 (m, 1H), 5.04–4.93 (m, 1H), 4.41–4.28 (m, 1H), 2.27 (dt, $J = 30.7$, 7.2 Hz, 2H), 2.19–2.07 (m, 1H), 1.99–1.86 (m, 1H), 1.49–1.38 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 176.43, 174.57, [173.65, 173.60, 173.44, 173.40], [90.17, 88.36], [52.80, 52.77], [31.26, 31.23], [27.62, 27.56], [19.01, 18.91, 18.79, 18.69]. ^{19}F NMR (565 MHz, CD_3OD) δ −184.36 to −184.63 (m). HRMS (ESI): *m*/*z* [M+H] calcd. for $C_8H_{13}FN_2O_4$: 220.0859. Found: 220.0865.

(2S)-5-Amino-2-(2-[^{18}F]fluoropropanamido)-5-oxopentanoic acid 10

[^{18}F]Fluoride was trapped on QMA cartridge and eluted by K_2CO_3 (3 mg in 0.1 mL H_2O) and $K_{2.2.2}$ (13 mg in 0.9 mL CH_3CN) complex solution. $[K/K_{2.2.2}]^{+18}F^-$ elution was evaporated under a nitrogen flow and completely remove the solvent at 95 °C under vacuum. A mixture of precursor **6** (5–10 mg) in 1 mL anhydrous CH_3CN was added to the reactor. ^{18}F-fluorination reaction was performed at 110 °C for 15 min. The reaction mixture was cooled, diluted with water (10 mL), passed through a preconditioned a Sep-Pak C18 plus cartridge, and washed with water (5 mL). The ^{18}F-labeled intermediate **9** was eluted with

1 mL of ethanol. The solvent was removed by evaporation under high vacuum. TFA (0.50 mL) was added to the residue and heated for 10 min at 60 °C. Then TFA solution was blown to dryness under nitrogen flow. Residue was diluted by water (0.5 mL) and filtered to give [^{18}F]FPGLN **10**. The decay uncorrected radiochemical yield of [^{18}F]FPGLN **10** was 15 ± 2% ($n = 5$) from [^{18}F]fluoride with the total synthesis time of 50 min and the specific activity was 60 ± 12 GBq/μmol.

Acknowledgments

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