Syntheses of F-18 Labeled Fluoroalkyltyrosine Derivatives

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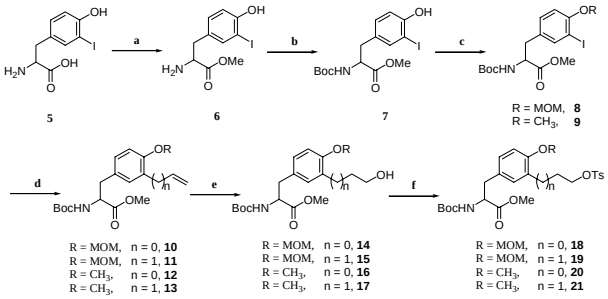
1. Introduction

Positron emission tomography (PET) offers the highest resolution of all nuclear medicine imaging modalities and allows quantitation of tracer concentration in tissues.[1] For more than 60 years, some of C-11 or F-18 labeled amino acids have been synthesized and evaluated for potential use in oncology, neurology and psychiatric disorders. Besides, a variety of radioisotope labeled amino acids have proven to be useful for imaging tumors, especially for brain tumor, lung tumor and breast tumor. These amino acids can be subdivided into two categories. The first category is represented by radiolabled naturally occurring amino acids and structurally similar analogues. Although these radiolabeled amino acids have proven useful in detecting brain and systemic tumors, it is susceptible to in vivo metabolism through multiple pathways that give rise to numerous radiolabled metabolites. On the other side, structurally similar amino acid analogues have some significant advantages over the natural amino acids. These nonnatural amino acids are not metabolized, which simplifieds the kinetic analysis of their uptake. On the basis of the promising results obtained with these nonnatural amino acids in preclinical studies, recent efforts have focused on the development of new F-18 labeled nonnatural amino acids.[2]

Recently, *O*-(2-[^{18}F]Fluoroethyl)-L-tyrosine (FET)[3,4], *O*-(3-[^{18}F]Fluoropropyl)-L-tyrosine (FPT)[5-7] were developed and evaluated among structurally similar to a new amino acid analogue. FET has shown high uptake in activated inflammatory cells using an experimental acute abscess model and in inflammation within lymph nodes. FPT was superior to FDG and had a slight advantage over FET in the differentiation of tumor from inflammation, and, like FET, it appeared to be a potential amino acid tracer for tumor imaging with PET. In this paper, we elected to introduce fluoroethyl and fluoropropyl groups at the R_1 positions and OCH_3 at R_2 position to the same effect of FET. Herein, we wish to report the synthesis and biological evaluation of four novel nonnatural fluorine-substituted tyrosine derivatives for brain tumor imaging, 3-(2-[^{18}F]fluoroethyl)tyrosine **1** (*ortho*-FET), 3-(3-[^{18}F]fluoropropyl)tyrosine **2** (*ortho*-FPT), *O*-methyl[3-(2-[^{18}F]fluoroethyl)tyrosine **3** (MFET), and *O*-methyl[3-(3-[^{18}F]fluoropropyl)tyrosine **4** (MFPT).

2. Methods and Results

The precursor for **1** (*ortho*-FET), **2** (*ortho*-FPT), **3** (MFET), and **4** (MFPT) were prepared in 6 steps from 3-iodotyrosine (**5**) as shown in scheme 1.



Scheme 1. a) TMSCl, MeOH, rt, 24 h, 75%; b) $(Boc)_2O$, TEA, MeOH, rt, 2 h, 70%; c) NaH, MOMCl, THF, 0 ^oC to 70 ^oC, 1 h, 65% for R= MOM; CH_3I, K_2CO_3, DMSO, rt, 2 h, 75% for R = CH_3; d) allyl(vinyl)tributylstannane, $Pd(PPh_3)_4$, 1,4-dioxane, 90 ^oC, 1 h, 50-60%; e) BH_3-THF complex, 4 N NaOH, 30% H_2O_2, THF, 0 ^oC, 2 h, 45-60%; f) TsCl, TEA, CH_2Cl_2, rt, 2 h, 75-85%.

3-Iodotyrosine methyl ester (**2**) was synthesized by the reaction of 3 equivalent of trimethylsilyl chloride (TMSCl) in MeOH at rt for 24 h, isolated by column chromatography after removal of TMSCl and MeOH by evaporation. *N*-(*tert*-butoxycarbonyl)-3-iodo-L-tyrosine methyl ester (**7**) was prepared by the reaction of $(Boc)_2O$ with TEA at rt for 2 h in yields of 70%. And preparation of MOM protected *N*-(*tert*-butoxycarbonyl)-3-iodo-L-tyrosine methyl ester (**8**) was synthesized with MOMCl, NaH in dried THF at 70 ^oC for 1 h in 65% yield. Inducing of methyl moiety (**9**) was obtained by CH_3I, K_2CO_3 in DMSO at rt for 2 h in 75% yield. Treatment of R group protected **8** or **9** with allyl(or vinyl)tributylstannane, $Pd(PPh_3)_4$ in anhydrous 1,4-dioxane at 90 ^oC for 1 h provided R group induced 3-allyl(or vinyl)tyrosine **10**, **11**, **12,** and**13** in 50-60% yield, respectively. Then the treatment of **10**, **11**, **12**, and **13** with borane-THF complex in THF at 0 ^oC for 2 h provided hydroborated compound. After the treatment of hydroborated compound with hydroperoxide and 4 N NaOH obtained the crude compound **14**, **15**, **16**, and **17** in 45-60% yield, respectively. Tosylation of alcohol afforded a tosylated **18**, **19**, **20**, and **21** in 75-85% yield. The authentic **1**,**2**,**3** and **4** were synthesized from tosylated compound using

TBAF·3H_2O and deprotected with 4 N HCl in yields of 30-45%.

Radiochemical syntheses of *ortho*-[^{18}F]FET, *ortho*-[^{18}F]FPT, [^{18}F]MFET, and [^{18}F]MFPT The preparation of F-18 labeled tyrosine derivatives have been achieved by the nucleophilic substitution of precursors (**18**, **19**, **20**, and **21**) with [^{18}F]fluoride ion in acetonitrile at 90 ^{o}C for 20 min followed by hydrolysis with 4 N HCl at 110 ^{o}C for 40 min in open condition. The isolation of the labeled compounds was performed by HPLC using a semi-preparative column [Hibar® pre-Packed RT 250-10 (Lichrosorb RP-18, 5 μm), EtOH/H_2O/AcOH=100:875:25, 2.5 g NH_4OAc/L, flow rate: 2 mL for **1** (*ortho*-FET), **2** (*ortho*-FPT); EtOH/H_2O/AcOH=200:775:25, 2.5 g NH_4OAc/L, flow rate: 3 mL for **3** (MFET), and **4** (MFPT)]. The further purification was done by loading the purified F-18 labeled tyrosine derivatives through the strong cation exchange resin and collected pure **1**, **2**, **3**, and **4** with the phosphate buffered solution (9 mL, pH = 7.4) and sodium bicarbonate solution (2.5 mL, 8.4%) mixture. Quality control was performed by HPLC [LiChrosorb RP-18, 250 x 4 mm, chiral column (Crownpak CR (+), 150 x 4.0 mm)]. The radiochemical yield was about 15% for **1**, 45% for **2**, 24% for **3,** and 48% for **4** after HPLC purification, respectively. Radiochemical purity was more than 95%. The specific activity after HPLC purification was > 37.0 GBq/μmol for **2**, **3**, **4** and 12.0 ± 3.4 GBq/μmol. The total elapsed time for the preparation of [^{18}F]fluorotyrosine derivatives from EOB to EOS (End-of-synthesis) was 110 min. The quality control of the final product was conducted by HPLC using an analytical column.

Ratio of D- and L-form [^{18}F]fluoroalkyltyrosine derivatives. The ratio of D- and L-form [^{18}F]fluoroalkyltyrosine derivatives were checked by use chiral column (Crownpak CR (+), 150×4.0 mm, 5% MeOH:H_2O (pH = 2.0, $HClO_4$), flow rate: 0.8 mL/min) HPLC system as D-[^{18}F]Fluoroalkyltyrosine: 17-23% and L-[^{18}F]Fluoroalkyltyrosine 77-83% (n=3).

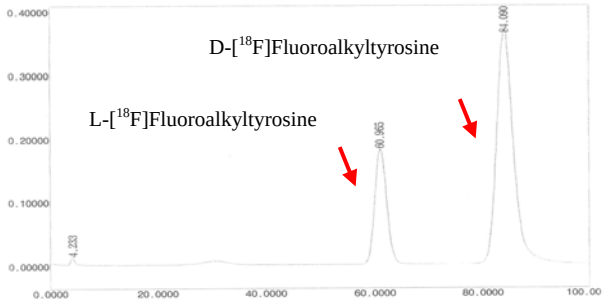


Figure 1. The ratio of D- and L-form M[^{18}F]FPT

Measurement of Partition Coefficient. The partition coefficient of [^{18}F]fluoroalkyltyrosine were measured using 2.0 mL of 1-octanol as the organic phase and 2.0 mL water phase. 20 μL of the radioactive sample in HPLC eluent solution were added and mixed for 2 min at room temperature. The radioactivity of 300 μL of each phase was measured.

3. Conclusion

F-18 labeled tyrosine derivatives was directly prepared from each precursor, and the radiochemical yield and radiochemical purity were 15-48% and 95%, respectively. We will evaluate through in vivo uptake assays using 9L glioma cell in tumor-bearing rats after intravenous injection and confirmed using PET image of rat with 9L as an attractive candidates for tumor imaging agents. Consequently, F-18 labeled tyrosine derivatives could expect to use as a new amino acid tracer for brain tumors imaging with PET.

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