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SYNTHESIS OF RADIOFLUORINATED O-TYROSINE AND PRELIMINARY EVALUATION AS POSSIBLE AROMATIC AMINO ACID DECARBOXYLASE TRACER

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Summary: [^{18}F]Fluoro-o-tyrosines (FOT) were prepared by the direct reaction of [^{18}F]acetylhypofluorite with L-o-tyrosine. Two isomers were formed in this reaction, 3-FOT and 5-FOT, with unoptimized decay-corrected isolated radiochemical yields of about 11% and 8%, respectively. Results of preliminary *in vitro* evaluation found 5-FOT to be the more promising isomer and suggest further evaluation of this compound as aromatic amino acid decarboxylase (AAAD) PET tracer should be undertaken.

Keywords: [^{18}F]Fluoro-o-tyrosine, AAAD, PET tracer, dopamine terminals

INTRODUCTION

We previously proposed non-catecholic L-DOPA analogs, o- and m-tyrosine, as tracers for dopamine nerve terminals since both compounds are not substrates of the enzyme catechol-O-methyltransferase (COMT) (1). COMT catalyzes the formation of 3-O-methyl-6-Fluoro-L-DOPA (3-OMe-6-FD) which has complicated PET studies using the current imaging agent for dopamine terminals, 6-Fluoro-L-DOPA (6-FD). 3-OMe-6-FD enters the brain, increases background noise in PET images and necessitates the correction of brain tracer input function for metabolite contribution to the PET data. Blocking the formation of this unwanted metabolite would then be advantageous. Several approaches have been proposed to remedy the undesirable effects of 3-OMe-6-FD in 6FD studies. These approaches include the blockade of COMT by the use of selective inhibitors (2) and a

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pharmacokinetic approach involving the blockade of 3-OMe-6-FD brain uptake by saturating the large neutral amino acid (LNAA) transporter (3). Both approaches have been shown to be effective in limiting the effect of 3-OMe-6-FD but both systemic pharmacologic approaches may be of limited use in routine human applications. Thus, a chemical approach of using non-COMT substrates as alternative PET dopamine tracers is reasonable. The lack of O-methyl metabolite formed by ^{18}F labelled m-tyrosine has indeed been demonstrated to result in PET images with improved contrast compared to those obtained with 6-FD (4, 5). Based on these results we suggested 6-fluoro-m-tyrosine (6FMT) to be the PET ligand of choice to visualize dopamine nerve terminals (5).

We have previously found decarboxylation by the enzyme AAAD to be a necessary step for the retention of a fluorinated m-tyrosine analog, [^{18}F]-6-F-(E)β-fluoromethylene-DL-m-tyrosine (6-F-FMMT), in dopamine terminals to form the PET image (6). This is similar to the finding that inhibition of central AAAD prevents the retention of 6-FD in monkey brain (7). Our interest in o-tyrosine analogs (8) as imaging agents is grounded on published data showing that o-tyrosine is as good or better AAAD substrate than both L-DOPA and m-tyrosine *in vitro* in three out of four studies (Table 1). In this study, we report the preparation of fluorinated o-tyrosine analogs and results of preliminary *in vitro* evaluation of these derivatives as potential AAAD PET imaging agent.

Table 1. Decarboxylation Rates of Tyrosines Compared to L-DOPA

SUBSTRATE	RELATIVE DECARBOXYLATION*			
	I	II	III	IV
L-DOPA	100	100	100	100
DL-o-TYROSINE	74	102	136	150
DL-m-TYROSINE	31	100	72	35
L-p-TYROSINE	0.5	-	-	-

* I: Ref. 16; II: Ref.17; III: Ref.18, IV: Ref.19

METHODS AND MATERIALS

DL-o-tyrosine was purchased from Sigma Chem. Co. (St. Louis, MO). Fluorine (1% in argon) was obtained from Cryogenic Rare Gas (Hanahan, SC), 2-methoxy-phenethylamine was purchased from Aldrich Chem. Co. (Milwaukee, WI). Several high performance liquid chromatographs were used in this study. A semi-preparative HPLC system consisted of a Rainin HPX pump, a Gilson

variable wavelength UV detector, a pair of NaI detectors for coincidence counting, a 10μ Econosil C-18 column (250mm x 10mm) purchased from Alltech Associates Inc. (Deerfield, IL) and 0.02M NaOAc pH 3.5 as mobile phase flowing at 5ml/min. Two analytical HPLC systems each with a Gilson pump were utilized. For radioactive samples, a Rainin Microsorb MV 3μ C-18 (80mm x 4.9mm) and a sensitive flowthrough βγγ triple coincidence detector (9) was used while for non-radioactive L-DOPA samples, a Rainin Microsorb MV 3μ C-18 (100mm x 4.9mm) and an BAS LC-4C electrochemical (EC) detector set at +0.9V was used. For both analytical systems an ion pairing mobile phase used consisted of 50mM chloroacetic acid, 62.5mM NaOH, 20mM citric acid, 2mM n-butylamine, 1.5mM Na_2EDTA and 1mM octylsulfonate at pH 3.5 and 5% absolute methanol flowing at a rate of 1.5 ml/min. Retention times were L-DOPA= 2.3 min, 5FOT, 3FOT=3 min, enzyme products=8.3 min.

Synthesis of Fluorinated o-Tyrosine

Direct fluorination of D,L-o-tyrosine (18.2mg, 100 μmol) in 3 ml 1:1 trifluoroacetic acid and glacial acetic acid was accomplished by bubbling 150 μmol acetylhypofluorite (AcOF) prepared by the method of Jewett et al. (10). After evaporation of solvent, the residue was dissolved in mobile phase and injected into preparative HPLC described above. HPLC peaks corresponding to three products were collected separately, dried and dissolved in D_20-DCl with TMS. These samples were then analyzed by ^1H and ^{19}F NMR spectroscopy where spectra were recorded at 400 MHz and 376.5 MHz, respectively, on a Brucker AM-400 WB instrument. The ^{19}F NMR spectra of o-, m-, and p-fluorophenol standards were also taken to assist in identifying the products. Results are summarized in Tables 2 and 3. Preparative HPLC was used to obtain another batch of monofluorinated products which was analysed using Kratos MS-80RFA EI mass spectrometer.

Synthesis of Radiofluorinated o-Tyrosine Derivatives

L-o-Tyrosine (LOT) was prepared from commercial racemic mixture using an enzymatic reaction described by Tong et al. (11). Chiral HPLC using the method of Grierson and Adam (12) showed that the LOT isolated was enantiomerically pure. Radiofluorination of LOT was then done following essentially the same method used in the preparation of cold FOT. Into a solution of LOT (20mg, 110 μmol) in 5ml trifluoroacetic acid - glacial acetic acid (1:1) was bubbled 20-30 mCi [^{18}F]-AcOF (80 μmol) prepared as previously described (13). After the cyclotron gas target was emptied into the reaction vessel, the solvent was evaporated with He gas flow and the residue was dissolved in mobile

phase and directly injected into pre-calibrated preparative HPLC. Radioactive peaks shown in Figure 1 corresponding to 3-[^{18}F]FOT (T_R=17.9 min) and 5-[^{18}F]FOT (T_R=20 min) were collected separately. Total radiosynthesis time was about one hour from the end of bombardment.

In Vitro Evaluation of Fluorinated o-Tyrosines as Aromatic L-Amino Acid Decarboxylase Substrates

L-DOPA and fluorinated o-tyrosines were evaluated as AAAD substrates using the method described by Rahman et al. (14). Briefly, male HSD rats (250 g) from Harlan Sprague Dawley (Madison, WI) were anesthetized with ether and sacrificed by cervical dislocation. Kidneys were quickly removed, minced and homogenised in 10 times its volume of cold 0.32M sucrose using a glass homogenizer. The homogenate was centrifuged at 1000xg (4 °C) for 10 min to remove cell debris. The supernatant was mixed with an equal volume of a 30mM phosphate buffer solution (pH 7.2) containing 0.3mM EDTA, 0.17mM ascorbic acid, 0.1mM pargyline HCL, 0.01mM pyridoxal phosphate and 0.5mM Fe^{++}. Before addition of substrates, the homogenate was pre-incubated at 37 °C for 1 min. Each reaction mixture consisted of 100 µL kidney homogenate and 100µl substrate, 3-[^{18}F]FOT (10µCi), 5-[^{18}F]FOT (10µCi) or L-DOPA (125 µM), in 0.02M NaOAc. Mixtures were incubated at 37 °C for 7 min and reactions were stopped by the addition of 75 µl 5N perchloric acid to precipitate proteins. Centrifugation at 3000xg at 4 °C was done to isolate the supernatants which were analyzed in precalibrated analytical HPLC systems as described above. The amount of corresponding product or residual substrate were quantified from the chromatograms. Samples without the kidney homogenate were also analyzed for each substrate to correct for non-enzymatic decarboxylation. To obtain % conversion of 3-[^{18}F]FOT and 5-[^{18}F]FOT to the corresponding products, counts under the peak of the respective products were divided by the sum of the counts under the substrate and product peaks. The product peaks were assumed to correspond to the respective amines since these peaks were not observed when the enzyme homogenate was absent and also these products had the same retention times as putative fluorinated o-tyramines. For L-DOPA, % conversion was calculated by comparing the peak area of the residual L-DOPA peak in reaction mixtures to those of mixtures without the enzyme homogenate. Triplicate determinations per substrate were done.

RESULTS AND DISCUSSION

Fluorination of DL-o-tyrosine using acetylhypofluorite was rapid as seen in the results of the radiofluorination reaction. The chromatogram of the cold reaction described above showed three

products were formed but more than half of o-tyrosine remained unreacted even after 1.5 molar excess AcOF was used. This is similar to that observed in the fluorination of FMMT with AcOF wherein more than 3-fold molar excess AcOF was needed to completely use up the starting material (15). Mass spectrometric analysis of the HPLC purified major product showed parent m/e peak at 199 corresponding to FOT ($C_9H_{10}NO_3F$). Summaries of the results of ^1H and ^{19}F NMR spectroscopic analyses of the products are shown in Tables 2 and 3.

Table 2. Proton NMR Analysis

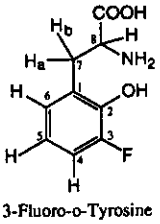
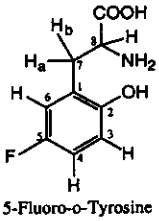
	Chem. Shift, ppm	Multiplicity	*J* value, Hz	Assignment
 3-Fluoro-o-Tyrosine	5.55	dd	13.9; 7.7	H7a
	5.77	dd	13.9; 5.7	H7b
	6.81	dd	7.0; 5.5	H8
	9.20	m	-	H5
	9.31	d	7.49	H6
	9.43	t	9.46	H4
 5-Fluoro-o-Tyrosine	5.55	dd	13.9; 7.7	H7a
	5.77	dd	13.9; 5.7	H7b
	6.81	dd	7.0; 5.5	H8
	9.37	m	-	H3
	9.46	m	-	H4, H6

Table 3. ^{19}F NMR Data#

Sample	Chemical Shift, ppm
o-fluorophenol	- 60.3
m-fluorophenol	- 34.8
p-fluorophenol	- 47.4
3-fluoro-o-tyrosine	- 58.8
5-fluoro-o-tyrosine	- 46.4
3,5-difluoro-o-tyrosine	- 43.3, - 54.1

#Previously reported at the 11th Int'l. Symp. Radiopharm. Chem., Vancouver, Canada (Ref. 8).

Results of these spectroscopic analyses identified the product peaks in the order of elution in HPLC (Figure 1) to be 3-FOT, 5-FOT and 3,5-diFOT, respectively. This product distribution, which is

similar to the previously observed product distribution in the reaction of AcOF with FMMT (15), is not unexpected since the hydroxy group is ortho-, para- directing in electrophilic fluorination reactions.

Figure 1 is a sample radiochromatogram of the preparative radioHPLC of the [^{18}F]-AcOF-LOT reaction. Although non-volatile radioactivity measured after this reaction was about 30% (29.8 % ± 3.4%, n=3), the average recovered HPLC decay-corrected radiochemical yields for 3-FOT and 5-FOT were 10.9% and 7.8%, respectively. End of synthesis specific radioactivities were in the order of 300 mCi/mmol. Although relatively low, these specific radioactivities can be raised 10-15-fold with the corresponding increase in starting [^{18}F]-F_2. Nonetheless, these unoptimized values were sufficient for the preliminary evaluation of both tracers as AAAD substrates.

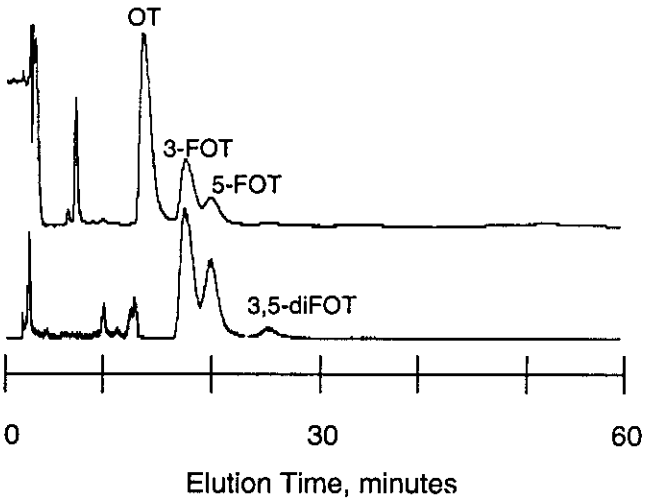


Figure 1. Radio-HPLC of the reaction of [^{18}F]-AcOF with L-o-tyrosine. The top trace is the UV (254nm) mass trace while the bottom trace is the coincidence radioactivity detector trace.

Mammalian kidneys, which are rich sources of aromatic L-amino acid decarboxylase (AAAD) enzyme (16-19), were used in this study. Decarboxylated fluoro-o-tyrosine products were prepared from 2-methoxyphenylethylamine which was first demethylated with 48% HBr and then reacted with AcOF and finally purified by preparative HPLC. Although these fluorinated products were not characterized any further, their retention times in analytical HPLC were the same as those of the enzyme products of 3-FOT and 5-FOT, thus, are very likely the fluorinated o-tyramines.

As seen in Table 4, under the enzyme conditions used in this study, 5-FOT is the better AAAD substrate compared to 3-FOT but L-DOPA is a better AAAD substrate than either fluorinated o-tyrosine. Although no comparison of these FOTs to 6-FDOPA and 6-FMT were made, two goals of this experiment were (1) to determine whether these fluorinated o-tyrosines were AAAD substrates and (2) to determine which isomer is the better AAAD substrate and thus the more promising tracer. Based on these results, 5-FOT was selected for further evaluation in studies using PET and non-human primates currently underway. It is noteworthy that 5-FOT is a positional isomer of 6-FMT wherein the fluoro- and hydroxy- substituents are transposed.

Table 4. Comparison of L-DOPA and Fluoro-L-o-Tyrosines as AAAD Substrates

Substrate	% Converted to Amine*
L-DOPA	72.6 ± 0.6
3-fluoro-o-tyrosine	11.1 ± 0.6
5-fluoro-o-tyrosine	58.1 ± 1.5

*Mean of 3 determinations ± S.E.

If PET studies show 5-FOT to be better than 6-FMT as AAAD imaging agent, the direct radiofluorination reaction done here will not be suitable for routine production since the 5-FOT product is a minor product and most likely to be contaminated with the earlier eluting major product 3-FOT. We have found that the formation of 5-FOT can be enhanced by using O-, N-bis-trifluoroacetyl-o-tyrosine as starting material wherein a reversal of the isomer product distribution found in the reaction using the unprotected o-tyrosine was observed (Murali and DeJesus, unpublished). However, for complete regioselective preparation of 5-FOT, a fluorodestannylation reaction using a stannylated protected starting material similar to that developed for 6-FMT (20) would be preferred. Preparation of this starting material should be straightforward since the bromination of o-tyrosine gives solely the 5-bromo-o-tyrosine (8) which can then be converted to the 5-trialkyltin-o-tyrosine derivative.

In summary, we have prepared [^{18}F]-labelled o-tyrosines by the direct reaction of [^{18}F]-AcOF with L-o-tyrosine producing two isomers, 3-FOT and 5-FOT, with decay corrected isolated yields of about 11% and 8%, respectively. *In vitro* evaluation found 5-FOT to be the more promising isomer suitable for further evaluation as AAAD PET tracer. If shown to be a better AAAD PET imaging agent than current tracers, 5-FOT can be prepared regioselectively via a radiofluorodestannylation reaction.

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