Automated radiosynthesis of 1-(2-[^{18}F]fluoroethyl)-tryptophan, a potential substrate for indoleamine 2,3-dioxygenase PET imaging

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Introduction

Indoleamine 2,3-dioxygenase (*h*-IDO) is an enzyme catalyzing the initial and rate-limiting step in the catabolism of tryptophan along the kynurenine pathway (Figures 1 & 2). This enzyme is located in brain and has been detected in high concentration in human tumor cells. Furthermore this enzyme could be responsible for the suppression of immune responses by blocking locally T-lymphocyte proliferation.[1,2]

Therefore, a radiotracer based on tryptophan structure seems to be well adapted to bring out the presence of *h*-IDO, and thus, of tumors.

Herein, an automated radiosynthesis of the 1-(2-[^{18}F]fluoroethyl)-tryptophan (^{18}FETrp), a radiotracer previously described,[3] is reported.

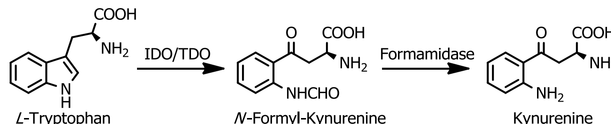


Figure 1 | The two first steps in the catabolism of *L*-tryptophan along the kynurenine pathway

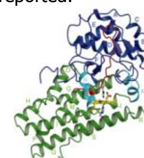


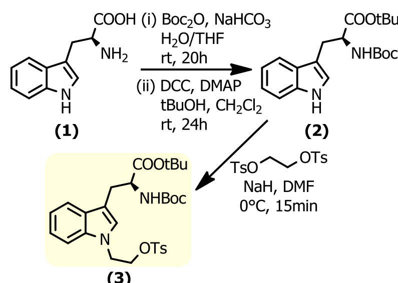
Figure 2 | Structure of *h*-IDO

Results

The automated radiosynthesis of ^{18}FETrp **5** needs the prior synthesis of a precursor owing a tosyl moiety which allows the easy introduction of the radioactive [^{18}F]fluoride ($t_{½}$ = 109,7min) by a classical method with potassium carbonate and kryptofix[4,5].

1. Precursor Synthesis

The tosylate precursor **3** was synthesized in three steps starting from *L*-tryptophan **1** (Scheme 1).



Scheme 1 | Synthesis of 1-(2-tosyloxyethyl)-tryptophan **3** (precursor)

This amino acid was firstly protected with two acidic leaving groups, with a global yield of 38% for two steps.

Then the intermediate **2** was alkylated with ethylene glycol ditosylate, to afford the tosylate precursor **3** (yield: 49%).

2. Automated Radiosynthesis on FASTlab™

To optimize radioprotection, the whole radiosynthesis of 1-(2-[^{18}F]fluoroethyl)-tryptophan was carried out on a *GE Healthcare FASTlab™* automated system (Figure 2).

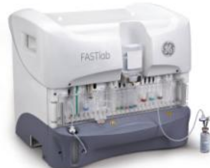
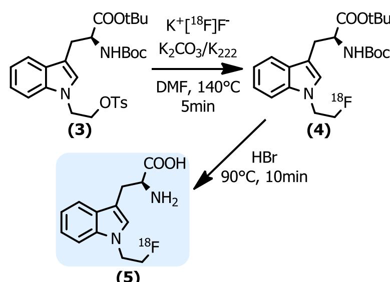


Figure 2 | GE Healthcare FASTlab™ synthesizer

The tosylate precursor **3** was labeled[4,5] (Scheme 2) under different conditions (Table 1). The best radiochemical decay-corrected yield (RCY) (57%) was obtained when the labeling was carried out in DMF, for 5 minutes at 140°C (n = 3).



Scheme 2 | Radiochemical synthesis of 1-(2-[^{18}F]fluoroethyl)-tryptophan **5** (^{18}FETrp)

Table 1 | Influence of solvent and temperature on the radiochemical decay-corrected yield (RCY) of **3**

Solvent	Temperature	Time	RCY
ACN	90 °C	10 min	28%
	120 °C	3 min	25%
DMF	140 °C	3 min	51%
	140 °C	5 min	57%

The intermediate **4** was purified on a $^{t}C_{18}$ solid phase extraction cartridge (*Sep-Pak®*) and hydrolyzed at 90°C for 10 minutes, to give the ^{18}FETrp **5** (Scheme 2).

Finally the labeled compound **5** was purified on semi-preparative HPLC and formulated.

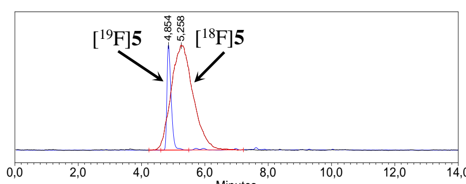


Figure 3 | HPLC chromatogram of purified and formulated nca ^{18}FETrp (in red) and cold reference ^{19}FETrp (in blue)

The fully automated process takes around 40 minutes and the ^{18}FETrp **5** was obtained, after purification on semi-preparative HPLC, with global radiochemical decay-corrected yield of 30% (n = 5). The radiochemical purity was >98%.

3. Enzymatic Tests

In vitro enzymatic tests with recombinant *h*-IDO[6] were carried out with cold reference ^{19}FETrp, in presence of methylene blue [100µM], ascorbic acid [200mM] and sodium phosphate buffer [50mM, pH 6.5] at 37°C. Figure 4 shows the decrease of the fluorescence signal of ^{19}FETrp, studied by HPLC, according to the time of incubation. This decrease is due to the opening of the indole ring of the substrate. For a concentration of substrates smaller than K_m, the k_{cat}/K_m – values (Table 2) were determined from curves (Figure 4). Furthermore, [^{19}F]**5** is not a substrate of recombinant *h*-TDO[7], an enzyme expressed in liver (Table 2). Thus ^{19}FETrp is a specific substrate of *h*-IDO.

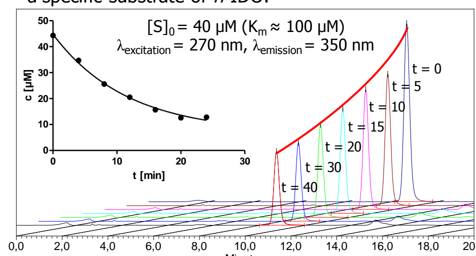


Figure 4 | HPLC chromatogram superposition showing the decrease of the fluorescence signal of ^{19}FETrp in function of incubation time

Table 2 | *In vitro* enzymatic tests realized with [^{19}F]**5** and some other substrates known for *h*-IDO and *h*-TDO (37°C, pH 6.5)

Tested Substrates	Percentage of substrate consumed after incubation with[6,7]: *h*-IDO 0.5µM 1 h	*h*-IDO 0.5µM 4 h	*h*-IDO k_{cat}/K_m [$10^2 M^{-1}s^{-1}$]	*h*-TDO 1µM 1h	*h*-TDO 10µM 1h
L-Trp **1**	> 97	100	1700±400	67	100
N-Me-Trp	100	100	38±17	0	3
5-HO-Trp	100	100	21±4	0	14
[^{19}F]**5**	84	100	5.8±0.7	0	2

Conclusion

Herein, an automated synthesis of 1-(2-[^{18}F]fluoroethyl)-tryptophan, with good radiochemical yields, has been developed. *In vitro* studies with cold reference ^{19}FETrp show that [^{19}F]**5** is a good and specific substrate of *h*-IDO. Moreover, some studies with this new radiochemical compound, still under progress, could confirm that ^{18}FETrp ([^{18}F]**5**) is a molecule of choice to bring out the presence of *h*-IDO, an enzyme which is located in brain and tumor cells.

References

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Acknowledgements

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