Automated synthesis of 5-[^{18}F]fluoro-α-methyl tryptophan (5-[^{18}F]F-AMT) to image breast cancer brain metastases in rodent

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Objectives:
Tryptophan is an essential amino acid that is metabolized in cancer by indoleamine 2,3-dioxygenase 1 (IDO1) to L-kynureine, promoting tumor growth and immune system suppression. (1) Carbon-11-α-methyl tryptophan was identified as an inhibitor of IDO1 and not a substrate for protein synthesis. (2) Recently, a fluorine-18 version, 5-[^{18}F]F-AMT (Figure), has been shown to be taken up in melanoma xenografts. (3) The inspiration behind the current project was to image brain metastatic cancer using 5-[^{18}F]F-AMT. Based on the foundational synthetic effort work (3), we sought to automate the production of 5-[^{18}F]F-AMT on the Sofie Biosciences ELIXYS FLEX/ CHEM module to prepare the tracer preclinical imaging studies and future human translational research studies.

Methods:
The BPin precursor was prepared in 5 steps from methyl tryptophan as described in Giglio et. al. (3). The original labeling procedure utilized tetrabutyl ammonium [^{18}F]fluoride, however in our hands the labeling was inconsistent. We employed a labeling technique published by Mossine et. al. (4, 5) to achieve consistent labeling. Labeling using the KOTf/ K_2CO_3 system allowed us to concentrate the [^{18}F]fluoride ion on a QMA sep pak without adding large amounts of K_2CO_3 into the BPin/ copper catalyst reaction. Larger amounts of DMF (350 μL: 50 μL) was needed for the [^{18}F]fluorination step, thus a C18 sep pak was required to lower the total reaction volume for the TFA decyclization step. DMF (50 μL) was back into the 500 μL of TFA to keep the hydrophobic compound soluble for the reaction. The TFA was evaporated and NaOH was added to the reaction for deprotection. The reaction mixture was neutralized and injected for purification onto an analytical HPLC. The product was collected, diluted up, and loaded onto a C18 light sep pak. The final purified product was eluted with ethanol and taken to dryness. The final formulation of 0.9% saline was added and imaging studies were conducted.

Results:
Incorporation yields (analyzed by radioTLC, non-decay corrected) were sufficient and consistent (25% $\pm$ 5%, n = 5). The automation on the ELIXYS required volume and reaction condition adjustments to achieve suitable synthetic yields. The decyclization

and deprotection scheme was one of the most challenging parts of the automation with various partially protected species being present in the HPLC purification. All of the possible species formed from various partial reactions are all separable on the HPLC using the gradient solvent system of 5: 95% acetonitrile with 0.1% TFA over 30 mins. The addition of the 50 μL of DMF to the TFA led to more efficient decyclization. NaOH was substituted for KOH in the deprotection reaction as older KOH did not accomplish complete deprotection.

Conclusions:
Automation of 5-[^{18}F]F-AMT was completed on the Sofie Biosciences ELIXYS FLEX/ CHEM module and the synthesis takes 120 ± 10 minutes, with a yield of 1.52 ± 1.04% (n = 3, decay corrected). Adequate amounts of 5-[^{18}F]F-AMT (37- 185 MBq) were formulated and injected into the metastatic tumor model in mice to perform imaging studies. The purification of [^{18}F]F-AMT is still under examination, when larger volumes of the reaction mixture are loaded onto the HPLC the yield is lower. These lower amounts of purified product injections led directly to the low yields presented.

Acknowledgements:
The work was supported by grant number W81XWH-17-1-0033 from the US Department of Defense Breast Cancer Research Program (BF).

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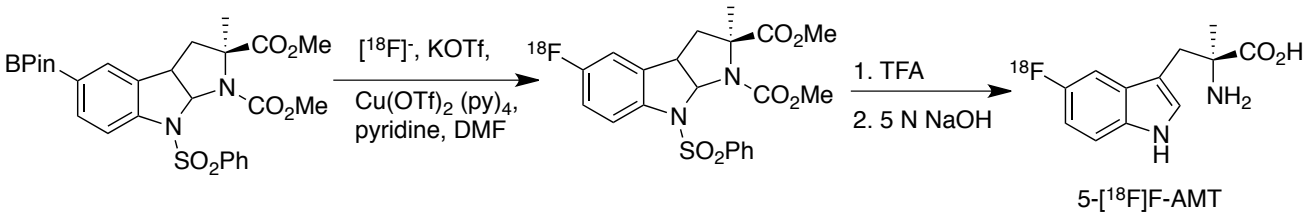
Figure:



5-[^{18}F]F-AMT

Keywords: 5-[^{18}F]F-AMT, boron pinacol ester, ELIXYS, copper- mediated labeling