Automation of radiolabeling two-step one-vessel ditosylate synthon using Trasis AllinOne

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Introduction

Direct fluorination of tosylate or mesylate precursor has been a wide-spread and reliable way for radio-fluorination. This approach can be difficult to achieve when a tosylate or mesylate precursor cannot be easily obtained or is unstable. A possible alternative is to radiolabel ethylene 1,2-ditosylate or 1,3-propanediol di-p-tosylate to form a fluorinated synthon[1]. We have previously reported the feasibility of eliminating high performance liquid chromatography (HPLC) purification after the formation of the synthon to shorten the radiolabeling time and demand on the module configuration. We built upon this success and investigated the possibility to further eliminate the need of a second reaction vessel. Here, we present an alternate approach, using [^{18}F]FP-TMP, an analog of the antibacterial agent trimethoprime[2], as an example, to demonstrate the feasibility of purifying the fluorinated synthon via filtration, and concentrating and trapping the synthon on a Sep-pak. The reaction vessel was then cleaned and dried, which allowed the reaction vessel to be ready for the second step and eliminate the need of second reaction vessel. The syntheses of other similar compounds are also discussed here. We take advantages of the Trasis 30-valve AllinOne (AIO) module[3] to perform the two-step and one-vessel automated synthesis. This process has been fully automated and has demonstrated great use for tracer synthesis in pre-clinical studies.

Objective

We illustrated the automation of a two-step, one-vessel synthesis by fluorination of a F-18 labeled synthon, followed by o-alkylation on the Trasis AIO module. We took advantage of the poor solubility of ditosylate in water to introduce a simple filtration step in replacement of HPLC purification[4]. Further we reused the reaction vessel after cleaning and drying for the second step.

The developed automated synthesis should have the following characteristics:

- Fully automated to reduce personnel radiation exposure
- High radiochemical purity
- Reliable synthesis with sufficient yields
- Overall synthesis time compatible with the half-life of radionuclide
- Convenient module set-up to minimize preparation time and compatible with commercially available parts
- USP or GMP compliant

Methods and Material

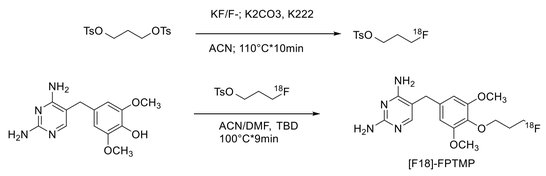
The radiolabeling steps can be divided into the following:

1. [^{18}F]F$^-$ transferring and drying
2. Radiolabeling of synthon, [^{18}F]fluorpropyl tosylate ([^{18}F]FPTos) or [^{18}F]fluorethyl tosylate ([^{18}F]FETos)
3. Synthon purification via 0.45 µm filter and SPE concentration
4. Cleaning and drying reaction vessel
5. Drying of synthon
6. O-alkylation
7. HPLC purification
8. Formulation

The radiolabeling and purification of the synthon were optimized first. Afterward, the O-alkylation was incorporated into the synthesis sequence. Several runs were performed to ensure the method yielded consistent results. The resulting synthesis method was robust and gave high yield of target product.

A 30-valve Trasis AllinOne module was used. A compound specific synthesis script was developed for synthesis control. The graphic interface of the software, shown in Figure 1, allowed us to monitor synthesis progress, including movement of valves, status of the heater and monitoring of the pressure throughout the process.

[^{18}F]FP-TMP



The original [^{18}F]FP-TMP radiolabeling method was developed on AllinOne as a two-pot, two-step synthesis. The purification of the radiolabeled synthon involved filtration of the unlabeled tosylate and a Sep-pak cartridge to concentrate the synthon. Afterwards, the hydroxyl precursor was incorporated into the synthon. The overall process gave an average of 18% decay-corrected yield.

The formation of the synthon, [^{18}F]FPTos, was carried out by heating 5 mg 1,3-propanediol di-*p*-tosylate in 1 mL acetonitrile at 110 ° C for 7 min, at the presence of cryptofix/potassium carbonate. After the reaction was completed, the mixture was cooled down to 60 ° C and quenched with 20 mL water. The resulting mixture was then passed through a 0.45µm nylon filter and a pretreated OASIS HLB cartridge. The excess tosylate was filtered as precipitate and the [^{18}F]FPTos was trapped on the HLB cartridge.

We further improved the process by simplifying the reaction to a one-vessel process. After cooling the reaction vessel to 80 ° C , the reaction vessel was rinsed with 4 mL acetonitrile twice and dried under nitrogen flow at 110 ° C and 125 ° C for 3 min respectively. The trapped [^{18}F]FPTos was then eluted with 2.5 mL of acetonitrile through a Dry Cartridge and loaded into a syringe before the content was transferred to the original reaction vessel. The hydroxyl precursor (4 mg) and 10 mg TBD in 0.8 mL DMF were added. The mixture was heated at 100 ° C for 9 min and cooled down to 40 ° C. After mixing with 6 mL mobile phase, it was purified by semi-preparative HPLC with an Agilent SB-C18 column (5 µm, 100 × 9.4 mm). The mobile phase was 32 % MeOH in 0.1M NH_4HCO_2 buffer and the flow rate was 5 mL/min. The desired product was eluted at approximately 15 min and diluted with water, which was loaded on a ^{t}C18 Plus Light cartridge. The final formulation was 1 mL ethanol with 10 mL saline.

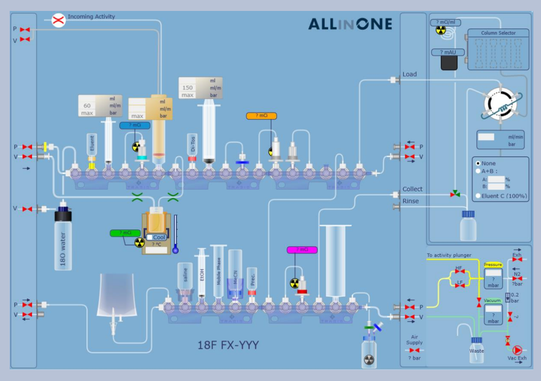


Figure 1. AllinOne software user interface of [^{18}F]FP-TMP

Figure 2. AIO module with loaded cassettes and reagents

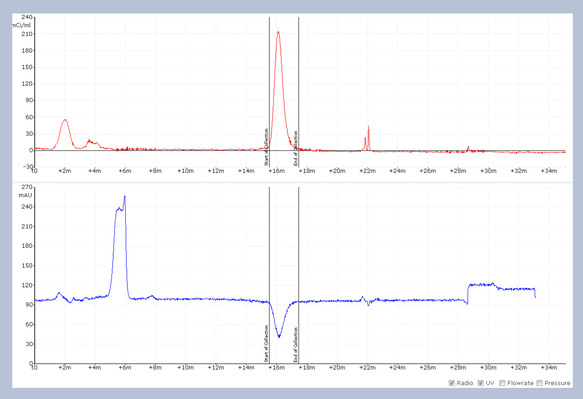


Figure 3. Chromatogram of semi-prep HPLC purification for [^{18}F] FP-TMP. Top: Radioactivity; Bottom: UV

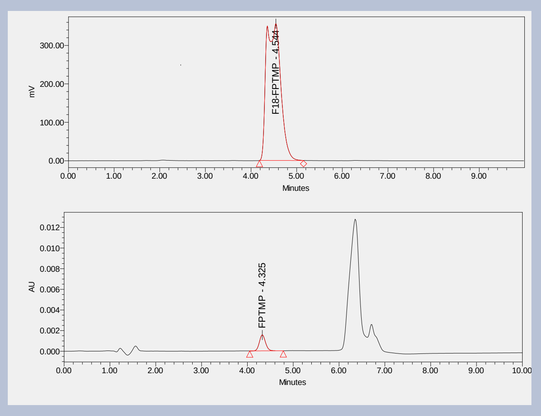
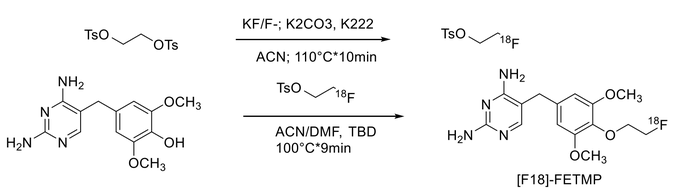


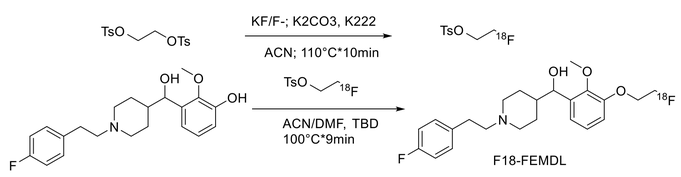
Figure 4. Chromatography of QC HPLC for [^{18}F] FP-TMP with gradient method. Top: Radioactivity; Bottom: UV

[^{18}F]FE-TMP



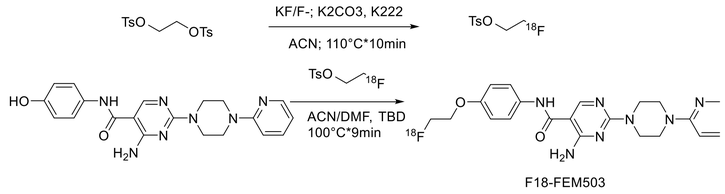
The synthon, [^{18}F]fluoroethyl tosylate, was obtained from incubating ethylene di(*p*-toluenesulfonate) with [^{18}F]F$^-$ following the same procedures to make [^{18}F]FPTos. The condition for the O-alkylation is the same as the one used in [^{18}F]FP-TMP. The overall yield was around 13%.

[^{18}F]-FEMDL



The synthesis of [^{18}F]FE-MDL followed the same procedure of [^{18}F]FE-TMP but replacing 2 mg TMP-OH precursor with MDL105725. The reaction purification was performed using a Luna C18 (2) 5µm 250 X 10 mm column. The mobile phase was 32% acetonitrile in 68% water with 0.1%TFA at 5 ml/min flow rate.

[^{18}F] FEM503



After obtaining [^{18}F]FETos, [^{18}F]FE-M503 was made substituting the precursor desmethyl M503 at the second step. HPLC purification used a Agilent SB-C18 column. The mobile phase was 53% methanol in 20mM ammonium bicarbonate solution on Agilent SB-C18 5µm 100 x 9.4 mm column at 5 ml/min flow rate.

Discussion

- We have developed a two-step, one-vessel, one-HPLC method for the radiolabeling using the synthon. In order to obtain the synthon with sufficient purity for the following step, the excess di-tosylate was removed by filtration of the precipitate after the adding of water to reaction mixture. A nylon-membrane filter was used. The filtrate was concentrated using an HLB cartridge before elution with acetonitrile.

The reaction vessel still had solvent, impurities and unreacted [^{18}F]F$^-$ after the radioactive synthon was loaded to the OASIS HLB Light cartridge. The reaction vessel was cleaned by adding acetonitrile twice followed by drying, similar to the drying of the fluoride. The resulting cleaned reaction vessel was now suitable for enabling the O-alkylation, the second step. Four compounds were readily radiolabeled.

For the second step, the amount of base affected the yields. For example, 4.5 eq. of TBD vs 1 eq. TMP-OH gave high yield of [^{18}F]FP-TMP while this ratio gave low yield for other phenol precursors, such as MDL105725 and desmethyl M503, due to excess of byproducts. The ratio of base and precursor was further investigated. Low ratio of base,1.2 eq., was required for the coupling of the MDL precursor. The resulting product had less byproducts and higher yields.

This approach circumvented the problem of unstable tosylated precursor. For instance, the direct labeling of [^{18}F]FE-M503 gave high labeling yield when the precursor was freshly prepared. However, the yield decreased from 35% to less than 10% in two weeks. Although this two-step labeling did not gave the highest yield, it gave consistent labeling yields over a longer period of time.

Conclusions

Most commercially available synthesis modules have a one-reaction-vessel design that limits the application of synthon labeling followed by alkylation. We aimed to resolve this problem by investigating the possibility of carrying out this two-step labeling reaction using only one reaction vessel. Four compounds were successfully radiolabeled using this one-vessel process. Previously, radiolabeling of [^{18}F]FP-TMP was accomplished by two-vessel, one HPLC method. This new one-vessel process was tested several times and gave decay-corrected yields between 11-21%, comparable with the two-vessel reaction. The resulting product had similar radiochemical and chemical purities with both greater than 95%. The molar activities are 18.9 ± 9.9 GBq/µmol. The lower specific activity is likely due to low starting radioactivity.

In addition to the fluoropropyl synthon, we also investigated the feasibility of using the same procedure but replacing the floropropyl with a fluoroehtyl synthon. Two different compounds, [^{18}F]FE-MDL, [^{18}F]FE-M503 and [^{18}F]FE-TMP, were tested. The radiolabeling yields for [^{18}F]FE-M503 were consistent and ranged from 13.7-16.1% with molar activities of 94.1 ± 24.9 GBq/µmol. [^{18}F]FE-TMP gave yields of 11-14% and the molar activities were 182.3 ± 112.6 GBq/µmol. [^{18}F]FE-MDL gave yields of 14-20% and the molar activities were 148.5 ± 32.3 GBq/µmol.

In conclusion, the two-step, one-vessel process of fluorinating ditosylate synthon had been successfully developed on Trasis AllinOne module with comparable results from direct radiolabeling or two-vessel method. With this simpler radiolabeling configuration, this automated method can be adopted to wide-range of synthesis modules.

References

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