Fully automated Click radiolabeling for [^{18}F]FLETT and the synthesis and coupling of [^{18}F]FBEM to glutathione using the iPHASE FlexLab module

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AIM

Despite the involvement of multi-step reactions and the need for purification of radioactive intermediates, Click radiolabeling and the radiolabeling of peptides or antibodies through thiol-reactive prosthetic groups, such as N-[2-(4-^{18}F-fluorobenzamido)ethyl]maleimide ([^{18}F]FBEM, **1**)[1], have become increasing important in radiopharmaceutical science. The aim of this project was to develop fully automated procedures for Click radiolabeling and the production and peptide/antibody conjugation of **1** using the commercially available dual reactor FlexLab module from iPHASE technologies (**Figure 1**).

BACKGROUND

FlexLab is a versatile ^{18}F synthesizer incorporating dual reactors, dual HPLC purification and dual product reformulation system that allows HPLC purification of the intermediate and final product. To the best of our knowledge, fully automated Click radiolabeling, as well as the synthesis and purification of **1** followed by the subsequent sulphydryl coupling has never been achieved in a single synthesis module. FlexLab offers the possibility to perform these multi-steps reactions in the same synthesis module, thus reducing radiation exposure to the operator.

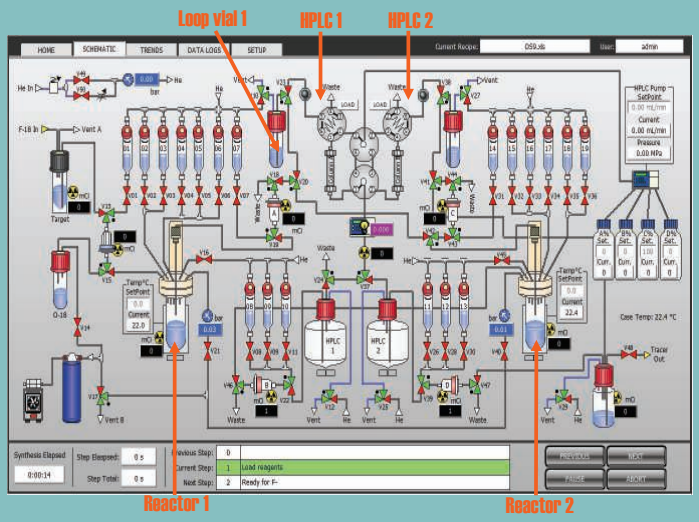


Figure 1. Schematic of the FlexLab module

METHOD

Click radiolabeling synthesis of [^{18}F]FLETT using FlexLab

2-[^{18}F]fluoroethylazide (**3**) was obtained from nucleophilic fluorination of 2-azidoethyl-4-toluene sulfonate (**4**) in reactor 1 and purified by distillation to reactor 2. Click reaction between **3** and ethynyldeoxyuridine (**5**)[2] was performed in reactor 2 in the presence of Cu(I) as catalyst followed by purification with HPLC 2 to give pure [^{18}F]fluoroethyltriazolylthymidine analog ([^{18}F]FLETT, **2**).

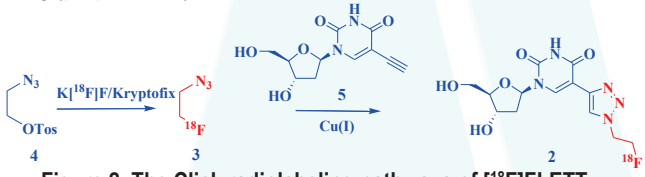
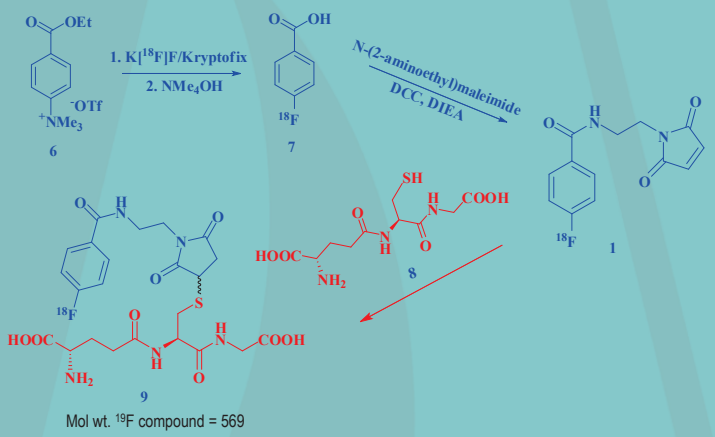


Figure 2. The Click radiolabeling pathways of [^{18}F]FLETT

Radiolabeling and coupling of [^{18}F]FBEM to glutathione using FlexLab

The (4-ethoxycarbonylphenyl)trimethyl ammonium triflate (**6**) was converted to 4-[^{18}F]fluorobenzoic acid (**7**) in reactor 1 and purified with C18 seppak. It was then transferred to the loop vial 1 and coupled with N-(2-aminoethyl)maleimide to form **1** followed by purification with HPLC 1. As a model system for future couplings to suitably modified single chain antibodies, **1** was coupled with glutathione (**8**) in reactor 2 and purified by HPLC 2.



Mol wt. ^{19}F compound = 569

Figure 3. The radiolabeling and coupling of [^{18}F]FBEM to glutathione

RESULTS

Click radiolabeling of [^{18}F]FLETT

Fully automated Click radiolabeling synthesis of [^{18}F]FLETT, **2** has been successfully carried out using an iPHASE FlexLab module with comparable yield to the previously developed manual method[3] (32.5%) and overall synthesis time reduced by 45 mins.

Radiolabeling and coupling of [^{18}F]FBEM to glutathione

The synthesis and purification of [^{18}F]FBEM, **1** have also been achieved in this fully automated synthesis module with radiochemical yield of 20%. The subsequent coupling to the sulfhydryl group of glutathione was found to be quantitative with a total synthesis time of 2 hours. **Figure 4** shows the Radio-HPLC and LCMS characterization of the coupled product **9**.

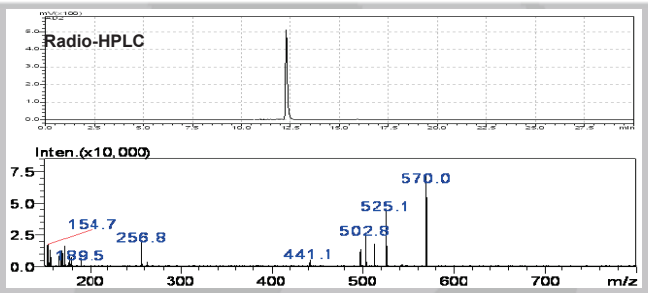


Figure 4. Characterisation of the [^{18}F]FBEM-Glutathione coupled product 9.

CONCLUSION

The FlexLab module allowed fully automated Click radiolabeling of [^{18}F]FLETT (**2**) and the preparation and coupling of [^{18}F]FBEM (**1**) to glutathione (**8**) in high radiochemical yields and shorter synthesis time.

REFERENCES

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[2] Ackermann U, *et al.* (2011) J. Label Compd. Radiopharm., 54, 260–266.