AUTOMATED SYNTHESES OF *N*-SUCCINIMIDYL 4-[^{18}F]FLUOROBENZOATE ([^{18}F]SFB) AND *N*-[2-(4-^{18}F-FLUOROBENZAMIDO)ETHYL]MALEIMIDE ([^{18}F]FBEM) USING A CUSTOMISED IBA SYNTHESISER.

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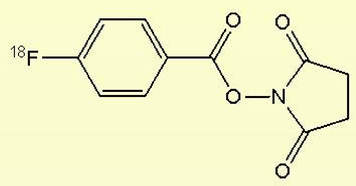
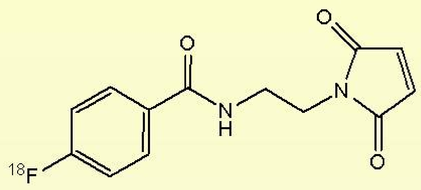
OBJECTIVES

The objectives of this project were to make modifications to an IBA synthesis module, to fully automate the [^{18}F]SFB and [^{18}F]FBEM syntheses, and to improve the synthesis and radiochemical yield of [^{18}F]SFB in a one-pot reaction.



1 ([^{18}F]SFB)



2 [^{18}F]FBEM

INTRODUCTION

The application of biomolecules such as proteins, peptides and antibodies labeled with positron emitting radioisotopes has rapidly developed to becoming an invaluable field in targeted molecular imaging for *in vivo* studies of many physiological and pathological processes.[1,2] The radionuclide ^{18}F (β+, $t_{1/2}$ = 110 min) can easily be incorporated into the molecule using a prosthetic group. Although a large range of prosthetic groups have been developed, [^{18}F]SFB is undoubtedly one of the most versatile ^{18}F-labelling methods being used due to its *in vivo* stability and high radiochemical yield.[3,4]

This frequent utilisation of [^{18}F]SFB requires full automation of the radiosynthesis using a reliable, remotely controlled operating system. In recent years, modifications to commercial modules have been made in an attempt to improve the synthesis of [^{18}F]SFB for routine application.[3,5] However, these can be complicated, requiring multiple intermediate cleaning and purification steps.[6]

Here the syntheses of [^{18}F]SFB and [^{18}F]FBEM are presented on the IBA synthesis module originally designed in 1991 for the routine production of 2-[^{18}F]fluorodeoxyglucose ([^{18}F]FDG). This module has 2 reactor vials but was limited by only 5 reagents additions. The incorporation of 3 more reagent vials (Vials 6, 7, & 8) significantly expanded the synthetic capabilities of the IBA chemistry module (**Figure 1**).

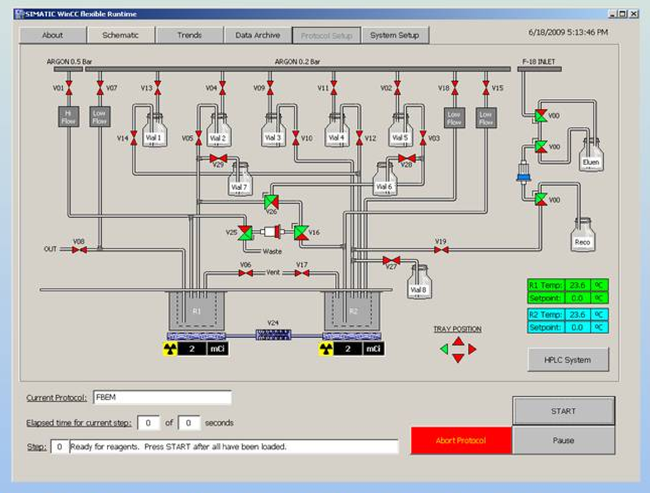


Figure 1. Scheme of the module of the modified IBA module.

EXPERIMENTAL

Materials and methods

All solvents were of analytical grade and chemicals were used as received from the Aldrich Chemical Company Inc. unless otherwise indicated. The precursor, ethyl 4-(trimethylammoniumtriflate)benzoate was prepared according to previously published procedures.[7] ^{18}F was produced using the IBA Cyclone 10/5, negative ion cyclotron (Belgium) via the ^{18}O(p, n)^{18}F nuclear reaction on 97% enrich [^{18}O]H_2O. Sep-Pak QMA and Sep-Pak tC18 Plus cartridges were purchased from Waters (Milford, MA).

All high-performance liquid chromatography (HPLC) were performed using a UFLC-HPLC Shimadzu system and a radioflow detector to analyse the products. The semi-preparative HPLC column used was a Phenomenex Gemini 10μ C18 110A (250 x 10 mm, 10μm), isocratic flow 25% MeCN-water, flow rate 4 mL/min. Independent quality control was performed on a liquid chromatography mass spectrometer (LCMS 2010 EV, Shimadzu). The column used was a Phenomenex Gemini 5μ C18 110A (150 x 4.6 mm, 5μm) isocratic flow 35% ACN-water, flow rate 0.5 mL/min.

Nuclear magnetic resonance (NMR) spectra for ^{1}H nuclei were recorded on a Varian Unity 500 NMR spectrometer operating at 500 MHz and the chemical shifts were reported relative to the deuterochloroform solution ($CDCl_3$).

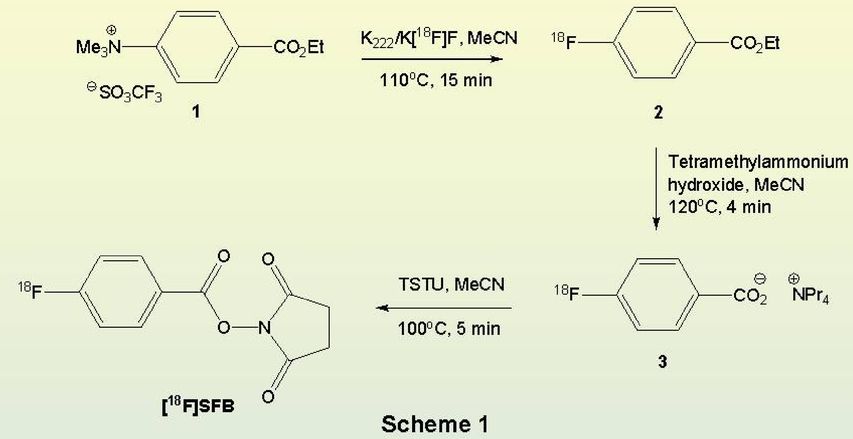
The two-pot chemistry module was controlled by a Siemens PLC and the process can be visualised by the in-house developed software based on WinCC flexible by Siemens.

K_{222}/K[^{18}F]F complex

After the [^{18}F]fluoride from the cyclotron was delivered to the synthesiser module, the radioactivity was passed through a Sep-Pak QMA cartridge to trap [^{18}F]F whilst the [^{18}O]water was collected for recycling. The [^{18}F]F was eluted with 0.6 ml of the solution of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane [Kryptofix 222 (K_{222})] (20 mg) and K_2CO_3 (3.5 mg) in MeCN-Water into reaction vial 2 (R2). The tray for R2 was lifted up during solvent evaporation where a constant flow of argon blew into R2. Azeotropic drying was repeated twice with 1ml of portions of MeCN to generate the anhydrous K_{222}/K[^{18}F]F complex.

Radiosynthesis of N-succinimidyl 4-[^{18}F]fluorobenzoate

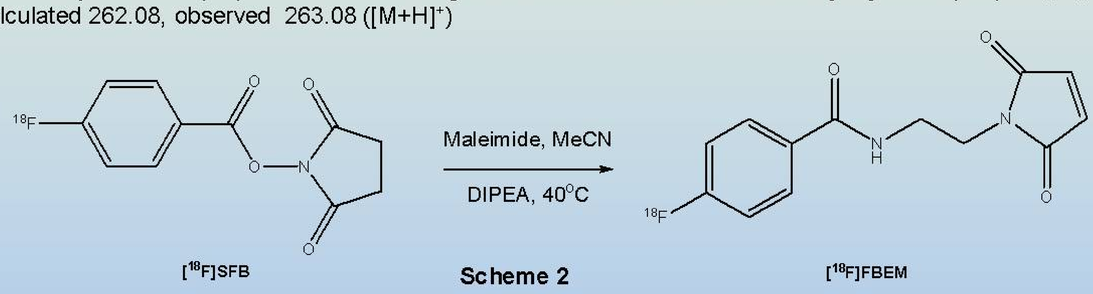
Ethyl 4-(trimethylammoniumtriflate)benzoate (**1**) (5.0 mg, 20 μmol) in anhydrous DMSO (1 ml) from vial 1 was added to the dried K_{222}/K[^{18}F]F complex in R2. The mixture was then heated at 110°C for 15 min to produce ethyl 4-[^{18}F]fluorobenzoate (**2**). The ethyl ester was hydrolysed with 20 μL of tetramethylammonium hydroxide (1.0 M in water) and 1 ml MeCN from vial 8 at 120°C for 4 min to form compound **3**. A 2ml aliquot of MeCN was added and the mixture was azeotropically dried, then another 2ml aliquot of MeCN was added and the drying process was repeated. A solution of *N,N,N,N'*-tetramethyl-O-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) (15 mg, 50 μmol) in MeCN (1 mL) from vial 3 was added and the solution heated at 100°C for 5 min. After cooling, 5% aqueous acetic acid (3 mL) from vial 2 and water (4 mL) from vial 5 were added. The reaction mixture was passed through a C18 Sep-Pak cartridge, where [^{18}F]SFB was trapped. The cartridge was washed with water (4 mL) from vial 5 and [^{18}F]SFB was eluted with MeCN (1 mL) from vial 6 into R1. Independent quality control was performed on the product. [^{18}F]SFB has a retention time of 17.3 min. [^{18}F]SFB (ESI): $C_{11}H_8FNO_4$ calculated 237.04, observed 409.1 ([M+NaCH$_3$CN(H$_2$O)n)]).



Scheme 1

Radiosynthesis of N-[2-(4-^{18}F-Fluorobenzamido)ethyl]maleimide

The [^{18}F]FBEM was synthesized by coupling [^{18}F]SFB with 2 mg of *N*-(2-aminoethyl)maleimide in 500 μL MeCN and 20 μL *N*-Ethyl-diisopropylamine (DIPEA) from vial 7 and heated at 40°C in R1. The crude reaction was purified by the semi-preparative column to give a retention time of 14.5 min. [^{18}F]FBEM (ESI): $C_{13}H_{11}FN_2O_3$ calculated 262.08, observed 263.08 ([M+H]$^+$)



[^{18}F]SFB **Scheme 2** [^{18}F]FBEM

RESULTS AND DISCUSSION

The facile synthesis of [^{18}F]SFB, which consisted of a simplified three steps in a one-pot procedure in R2, was based on a recent publication by Tang and co-workers.[8] The preparation of [^{18}F]SFB involved: 1) [^{18}F]fluorination of an aromatic precursor, ethyl 4-(trimethylammoniumtriflate)benzoate at 110°C, 2) saponification to generate 4-[^{18}F]fluorobenzoate salt ([^{18}F]FBA), and 3) activation of the salt with TSTU to form [^{18}F]SFB. The crude reaction mixture was trapped on a C18 Sep-Pak cartridge (omitting the use of other cartridges used in the literature) and eluted with acetonitrile, to afford [^{18}F]SFB that can then be used for labeling of free amino groups.

[^{18}F]FBEM was synthesized by coupling the [^{18}F]SFB with *N*-(2-aminoethyl)maleimide in R1. [^{18}F]FBEM was HPLC purified on a semi-preparative column. The product can be employed to react with a thiol containing peptide and hence providing a labeling agent for Positron Emission Tomography (PET) tracers.

In this study, DMSO replaced acetonitrile as solvent for the [^{18}F]fluorination reaction. Radiofluorination was variable when acetonitrile was used due to erractic solvent evaporation at 110°C. The experimental failure rate was lower when DMSO was used as a solvent. The radiochemical yield of [^{18}F]SFB in DMSO was up to 65 ± 5% (mean ± std; n = 8) whilst the radiochemical yield of [^{18}F]FBEM was 50 ± 5% (n = 4).

The identities of [^{18}F]SFB and [^{18}F]FBEM were confirmed by comparison with non-radioactive reference compounds through HPLC, MS and ^{1}H NMR. ^{1}H NMR of SFB ($CDCl_3$) δ 2.78 (s, 4H), 7.12-7.16 (m, 2H), 8.11-8.14 (m, 2H). ^{1}H NMR of FBEM ($CDCl_3$) δ 3.08 (dd, 2H), 3.60-3.67 (m, 2H), 6.69 (s, 2H), 7.11-7.01 (m, 2H), 7.73-7.76 (m, 2H).

CONCLUSION

The customised IBA synthesis module was successfully modified for the preparation of both the [^{18}F]SFB and [^{18}F]FBEM tracers. Modification to the synthesis increased the [^{18}F]SFB radiochemical yield from 43.8 $\pm$ 4.6%[8] to 65 $\pm$ 5%.

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