Note

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A simplified protocol for the automated production of succinimidyl 4-[^{18}F] fluorobenzoate on an IBA Synthera module

Uwe Ackermann,[a,b]* Shinn Dee Yeoh,[a] John I. Sachinidis,[a] Stan S. Poniger,[a] Andrew M. Scott,[a,b,c] and Henri J. Tochon-Danguy[a,b]

The important peptide labelling reagent succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) has been synthesised in 75–85% decay corrected radiochemical yield using the IBA Synthera platform (IBA Cyclotron Solutions, Louvain-la-neuve, Belgium) with the fluorodeoxyglucose-integrated fluidic processor nucleophilic and only four reagent vials in a single reactor. (4-ethoxycarbonylphenyl) trimethylammonium triflate was used as the labelling precursor and 1 M aqueous tetramethylammonium hydroxide for the hydrolysis of the intermediate ethyl 4-[^{18}F]fluorobenzoate. N,N,N′,N′-tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU) was then used to form [^{18}F]SFB from 4-[^{18}F]fluorobenzoate. By omitting the addition of acetic acid and introducing a combined hydrolysis/water removal step, the synthesis time was shortened to 58 minutes. After SepPak purification, the radiochemical purity of [^{18}F]SFB was 95.8–98.2%. These simplifications might be of significance to users of other automated synthesis modules.

Keywords: SFB; automated synthesis; protein labelling; nucleophilic aromatic substitution

Introduction

Succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) (1) is an important labelling agent for biomolecules such as proteins, peptides and antibodies.[1–3] [^{18}F]SFB does not require HPLC purification but is mostly used crude after removal of unreacted [^{18}F]KF kryptofix 2.2.2 complex by C-18 SepPak purification.[4,5]

In our laboratory, [^{18}F]SFB has been produced from (4-ethoxycarbonylphenyl)trimethylammonium triflate (2) using an old modified fluorodeoxyglucose (FDG) synthesiser with two reactor vials and nine reagent vials.[6]

However, low synthesis yields of 25 ± 15%, a long synthesis time of 90 minutes and an unreliable synthesis have prompted us to improve the [^{18}F]SFB synthesis.

Here, we report the synthesis of [^{18}F]SFB from the ester **2** using a single IBA Synthera module without any modifications with the standard, unmodified [^{18}F]fluorodeoxyglucose-integrated fluidic processor (FDG-IFP Nucleophilic) and only four reagent vials.

Experimental

General

No-carrier-added [^{18}F]fluoride was produced by the ^{18}O(p, n)^{18}F nuclear reaction with a 10 MeV proton beam generated by the IBA Cyclone 10/5 cyclotron (IBA Cyclotron Solutions, Louvain-la-neuve, Belgium) in a titanium target using recycled [^{18}O]H_2O at Austin Health, Centre for PET. Typical irradiation parameters were 20 µA for 30 minutes, which produced 5.4–8.1 GBq of [^{18}F]fluoride.

Solvents were purchased from MERCK and used as received. Reagents were purchased from Sigma–Aldrich and used without further purification. (4-Ethoxycarbonylphenyl)trimethylammonium triflate and [^{19}F]SFB were synthesised according to literature procedures.[7,5]

For quality control, a Shimadzu HPLC system equipped with a 5 µL injection loop, a SPD-20A UV-Vis detector (Shimadzu Scientific Instruments, Columbia, MD, USA) and two LC-20 AD solvent pumps (Shimadzu Scientific Instruments, Kyoto, Japan) for high pressure mixing of mobile phase was used. The stationary phase was a Phenomenex Gemini C-18 (Phenomenex, Torrance, CA, USA), 10 µ RP column, 150 × 2.3 mm. Acetonitrile (A) with 0.1% formic acid and water (B) with 0.1% formic acid were used as the mobile phase and a gradient elution technique was used for analysis: 0–18 minutes: 5–90% A, 18–30 minutes: isocratic 90% A. For the detection of radioactive compounds, an in-house built flow-through detector with a GM tube was used. Specific radioactivity was measured by HPLC using a mass standard curve of known concentrations of [^{19}F]**1**. FDG-IFP Nucleophilics were purchased from ABX advanced biochemical compounds, Germany and were used without any modifications.

[a]*Centre for Nuclear Medicine and PET, Austin Health, Vic., Australia*

[b]*School of Medicine, Dentistry and Health Sciences, The University of Melbourne, Vic., Australia*

[c]*Ludwig Institute for Cancer Research, Melbourne-Austin Branch, Vic., Australia*

**Correspondence to: Uwe Ackermann, Centre for PET, Austin Health. E-mail: ackerman@petnm.unimelb.edu.au*

Synthesis of [^{18}F]SFB

Preparation of the Synthera module:

Vial 1: Eluent (20 mg kryptofix 2.2.2 (53 µmol) and 3.5 mg K_2CO_3 (25 µmol)) in 0.4 mL of acetonitrile plus 0.2 mL of water
Vial 2: 5 mg (4-ethoxycarbonylphenyl)trimethylammonium triflate (20 µmol) in 1 mL dimethyl sulfoxide (DMSO)
Vial 3: 20 mg TSTU (66 µmol) in 1 mL acetonitrile
Vial 4: 20 µL of a 1 M aqueous tetramethylammonium hydroxide (20 µmol) in 4 mL acetonitrile

After loading the reagents, [^{18}F]fluoride from the target was trapped on a QMA ion exchange cartridge (Waters Corporation, Milford, MA, USA) and eluted using eluent from vial 1. The kryptofix complex was dried at 110 °C for 5 minutes and 95 °C for 3 minutes under vacuum and argon flow. After drying, the precursor was added from vial 2 and labelling was achieved by heating to 110 °C for 15 minutes. Base solution was then added from vial 4 and the solution was heated to 90 °C for 15 minutes under vacuum and argon flow. This achieved hydrolysis of ethyl 4-[^{18}F]fluorobenzoate to 4-[^{18}F]fluorobenzoate during the evaporation step as well as removal of the water that was added as part of the 1 M aqueous tetramethylammonium hydroxide solution. N,N,N′,N′-tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU) in 1 mL of acetonitrile was then added from vial 3 and 4-[^{18}F]fluorobenzoate converted to [^{18}F]SFB at 110 °C for 5 minutes. The [^{18}F]SFB solution was then transferred into an in-house built reformulation module where it was diluted in 60 mL of water and subsequently trapped on a Waters C-18 Plus SepPak (Waters Corporation, Milford, MA, USA). After washing of the SepPak with 5 mL of water and drying with nitrogen, [^{18}F]SFB was eluted with 1 mL of acetonitrile.

[^{18}F]SFB was obtained in decay corrected radiochemical yields of 80 ± 5% (n = 22) and the radiochemical purity was 97 ± 1.2%. Specific radioactivity was 80.1–149.1 GBq/µmol at the end of synthesis and the total synthesis time was 58 minutes.

Results and discussion

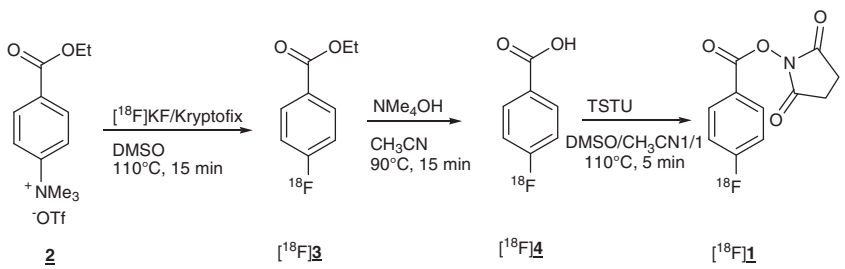
The radiolabelling of ethyl 4-[^{18}F]fluorobenzoate (3) was achieved by reacting the (4-ethoxycarbonylphenyl)trimethylammonium triflate (2) precursor with dried [^{18}F]KF kryptofix 2.2.2 complex in DMSO at 110 °C for 15 minutes. A 1 M aqueous solution of tetramethylammonium hydroxide in 4 mL of dry acetonitrile was then added and the mixture heated to 90 °C for 15 minutes under vacuum and argon flow. This achieved hydrolysis of ethyl 4-[^{18}F]fluorobenzoate (3) to 4-[^{18}F]fluorobenzoic acid (4) during the evaporation process as well as removal of water through azeotropic distillation with acetonitrile. The resulting 4-[^{18}F]fluorobenzoate (4) solution in the remaining 1 mL of DMSO was reacted with TSTU at 110 °C for 5 minutes to form [^{18}F]SFB (1) (Figure 1). The crude [^{18}F]SFB solution was then transferred to an in-house built reformulation unit where it was diluted with 60 mL of water, trapped on a C-18 SepPak and eluted with 1 mL of acetonitrile.

[^{18}F]SFB was synthesised in 58 minutes with decay corrected radiochemical yields of 80 ± 5% (n = 22) and a specific activity of 80.1–149.1 GBq/µmol at the end of synthesis. After simple SepPak purification, [^{18}F]SFB had a radiochemical purity of 97 ± 1.2% and the product is obtained in 1 mL of acetonitrile. Figure 2 shows a typical HPLC trace where the retention time of [^{18}F]SFB is 18.5 minutes. The retention times for compounds **3** and **4** are 21.6 and 16.8 minutes, respectively, thus showing that the conversions in each step are almost quantitative.

The shorter synthesis time was achieved because our method does not require acidification of the crude reaction mixture before trapping on a C-18 SepPak and it also combines the hydrolysis of 4-[^{18}F]fluorobenzoate (3) and the removal of water, which was added as part of the 1 M aqueous tetramethylammonium hydroxide solution, in a single step. From our experience with the previously used, modified FDG synthesiser, we believe that hydrolysis takes place at the end of the acetonitrile evaporation. It is also important to note that with the exception of the [^{18}F]KF kryptofix 2.2.2 complex, evaporation to dryness is not achieved at any point of this synthesis protocol because of the presence of high boiling DMSO. Because of the protocol modifications described, it has been possible to perform the [^{18}F]SFB (1) synthesis using an unmodified FDG-IFP Nucleophilic with only four reagent vials. The FDG-IFP Nucleophilic is a disposable kit that clips onto the Synthera module and can be used to perform nucleophilic substitution reactions followed by base or acid hydrolysis plus cartridge purification. This kit is commonly used for the synthesis of [^{18}F]FDG, [^{18}F]FLT, [^{18}F]FAZA or [^{18}F]FMISO. The IFP can be ejected at the end of synthesis and because all radioactivity is contained within the IFP, the Synthera module itself can be used for subsequent radiosyntheses on the same day with a new IFP. The use of IFPs avoids cross contamination and minimises the radiation exposure of the operator.

In summary, using the IBA Synthera module, we were able to increase the yields of [^{18}F]SFB (1) from 25 ± 15% to 80 ± 5% (n = 22), shorten the synthesis time and significantly improve the reliability of the synthesis compared with the old modified FDG synthesiser method we have used previously. Compared with the recently published [^{18}F]SFB synthesis methods, our synthesis does not require modifications to commercially available modules and has the highest yields of all procedures published.[4,8,9] Furthermore, our method delivers [^{18}F]SFB in a final volume of 1 mL of acetonitrile, which will make evaporation to dryness quicker, if dry [^{18}F]SFB is required for the subsequent peptide labelling step.

The method of Tang *et al.* is a manual synthesis that requires C-18, alumina and SCX cartridges for [^{18}F]SFB purification and produces



2 [^{18}F]3 [^{18}F]4 [^{18}F]1

Figure 1. Synthesis of succinimidyl 4-[^{18}F]fluorobenzoate (1).

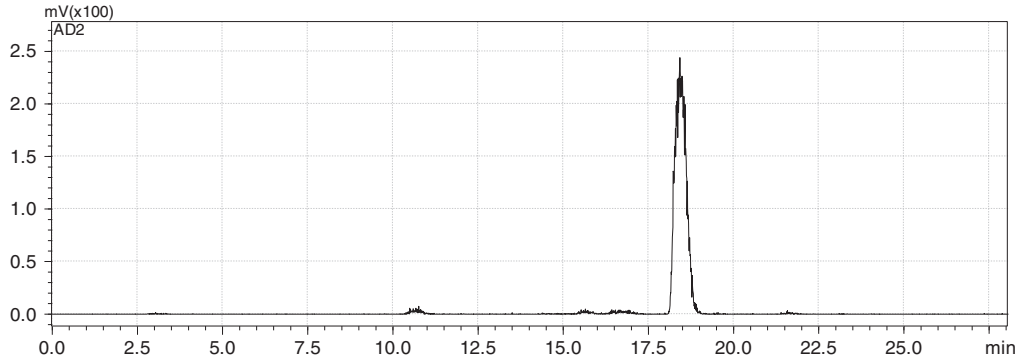


Figure 2. Radio-HPLC trace of succinimidyl 4-[^{18}F]fluorobenzoate after C-18 SepPak purification.

[^{18}F]SFB in 43.8 ± 4.6% yields.[4] The synthesis time is given as less than 60 minutes, which is comparable with our synthesis time of 58 minutes, and [^{18}F]SFB is obtained in 2 mL of acetonitrile. Glaser *et al.* have published a manual [^{18}F]SFB synthesis using microwave assisted labelling of a 4-N,N,N-trimethylaniliniumphenylmethanone trifluoromethanesulphonate precursor.[8] This method requires SepPak purification of the intermediate *p*-[^{18}F]fluorobenzaldehyde, which is then oxidised with (diacetoxyiodo)benzene in the presence of *N*-hydroxysuccinimide to form [^{18}F]SFB. The synthesis times were 2.75 hours for HPLC purification and 1.67 hours for a SepPak method that gave [^{18}F]SFB in only 89% radiochemical purity. Although this synthesis protocol seems less attractive than our method, we believe that the chemistry approach described by Glaser *et al.* is extremely innovative and has significance beyond the [^{18}F]SFB production. The method published by Scott *et al.* uses a Tracerlab FX_{FN} module with very minor modifications and the synthesis as well as the SepPak purification is fully automated.[9] Overall, this method produces [^{18}F]SFB in a shorter time frame (~45 minutes) but with lower radiochemical yields (38% nondecay corrected) and in a larger volume of acetonitrile (2 mL) than our method. The Tracerlab FX_{FN} method uses only 10 mg of TSTU, an amount we found was too low to achieve complete conversion of 4-[^{18}F]fluorobenzoate into [^{18}F]SFB with the Synthera module. The high radiochemical yields and excellent radiochemical purity of our synthesis may be due to a longer, 15 minutes fluorination reaction and the subsequent 15 minutes combined hydrolysis/water removal step. If implemented on the Tracerlab FX_{FN} module, our method may further improve the [^{18}F]SFB production on this system. A key advantage of using the Synthera module over the Tracerlab FX_{FN} module is the use of disposable IFPs. The IFPs can be ejected at the end of synthesis, thus allowing the operator to use the Synthera module for subsequent radiotracer productions on the same day without any cross contamination and minimal radiation exposure. This may be important for busy PET centres with limited radiochemistry resources.

Conclusion

Using the IBA Synthera module as well as a modified synthesis procedure, the decay corrected radiochemical yields of [^{18}F]SFB have been increased from 25 ± 15% to 80 ± 5% and the synthesis time has been shortened by 32 minutes. This setup is now routinely used in our laboratory for the [^{18}F]SFB production. This simplified protocol can potentially be implemented in other automated synthesis modules.

Acknowledgements

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