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# Fully automated, high yielding production of *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB), and its use in microwave-enhanced radiochemical coupling reactions

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**A General Electric Medical Systems (GEMS) Tracerlab FX$_{FN}$ fluorine-18 synthesis module has been reconfigured to allow rapid (45 min), fully automated production of *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB) using the established three-step, one-pot synthesis procedure. Purification is by sep-pak only and [$^{18}$F]SFB is routinely obtained in 38% non-decay corrected yield, > 1 Ci/μmol specific activity, and > 95% radiochemical purity ($n = 20$). Moreover, this report includes our preliminary research efforts into improving peptide coupling reactions with [$^{18}$F]SFB using microwave-enhanced radiochemistry. Reaction times can be reduced by > 90%, when compared with traditional thermal reactions, with no significant effect on radiochemical reaction yield.**

**Keywords:** radiopharmaceutical chemistry; [$^{18}$F]SFB; microwave-promoted radiochemistry

## Introduction

The use of radiopharmaceuticals, in conjunction with positron emission tomography (PET) imaging, to non-invasively elucidate biochemical mechanisms, diagnose diseases, and monitor patient response to therapy, is becoming increasingly popular.[1] The radiopharmaceuticals are often tagged with fluorine-18 because the convenient half-life (110 min) allows for sophisticated synthetic manipulations and scope for distribution to satellite PET centers without a cyclotron. Small molecule radiopharmaceuticals are typically labeled with fluorine-18, by displacement of a leaving group with [$^{18}$F]fluoride, using well-established radiochemical reactions.[2–4] However, this strategy is not viable for radiolabeling larger biologically sensitive species (e.g. proteins). In such cases, fluorine-18 is typically introduced by reacting the bioactive molecule with a pre-formed, [$^{18}$F]labeled, bifunctional prosthetic group. Numerous prosthetic groups have been developed for such applications[2] and, to date, *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB, **4**) remains one of the most widely used.

Initially, the preparation of [$^{18}$F]SFB was hampered by lengthy syntheses requiring multiple reaction vessels, and was not trivial.[5] Typically, such syntheses were done manually[6] although the process has also been automated.[7] However, such automation requires access to multi-reaction vessel synthesis modules and so, in the last decade, we and others[8,9] have concentrated on developing improved methods for preparing [$^{18}$F]SFB. With this in mind, we adapted the three-step, one-pot method for preparing [$^{18}$F]SFB, reported by Kabalka,[8] for use with a General Electric Medical Systems (GEMS) Tracerlab FX$_{FN}$. This method eliminates the need for HPLC purification, using only solid-phase extraction (SPE) cartridges, and has proven extremely robust in our hands. In this study, we report modifications to a GEMS Tracerlab FX$_{FN}$ that enable the rapid (synthesis time = 45 min) and fully automated preparation of [$^{18}$F]SFB in high radiochemical yield (38% non-decay corrected, $n = 20$), > 1 Ci/μmol specific activity, and excellent radiochemical purity (> 95%).

With a reliable method for producing [$^{18}$F]SFB in hand, we use it routinely in our laboratory to radiolabel large bioactive molecules such as peptides. Typically, solutions of [$^{18}$F]SFB and peptide precursor are mixed in a vial and heated (heat gun) to promote reaction. Recently however, coupling of [$^{18}$F]SFB with less reactive precursors has been taking impractically long (> 40 min) to provide radiopharmaceuticals in high enough radiochemical yield for use in pre-clinical studies. Therefore, we have explored microwave-assisted radiochemistry, a promising technique for enhancing radiochemical reactions that has been recently reviewed,[10–13] as a means of improving coupling reactions with [$^{18}$F]SFB. In this report, we present preliminary results demonstrating that reaction times can be reduced by > 90%, when compared with traditional thermal reactions, with no significant effect on radiochemical reaction yield.

## Results and discussion

### Production of [$^{18}$F]SFB

In considering a method for production of [$^{18}$F]SFB to adapt for use with a Tracerlab FX$_{FN}$, we were particularly attracted by the

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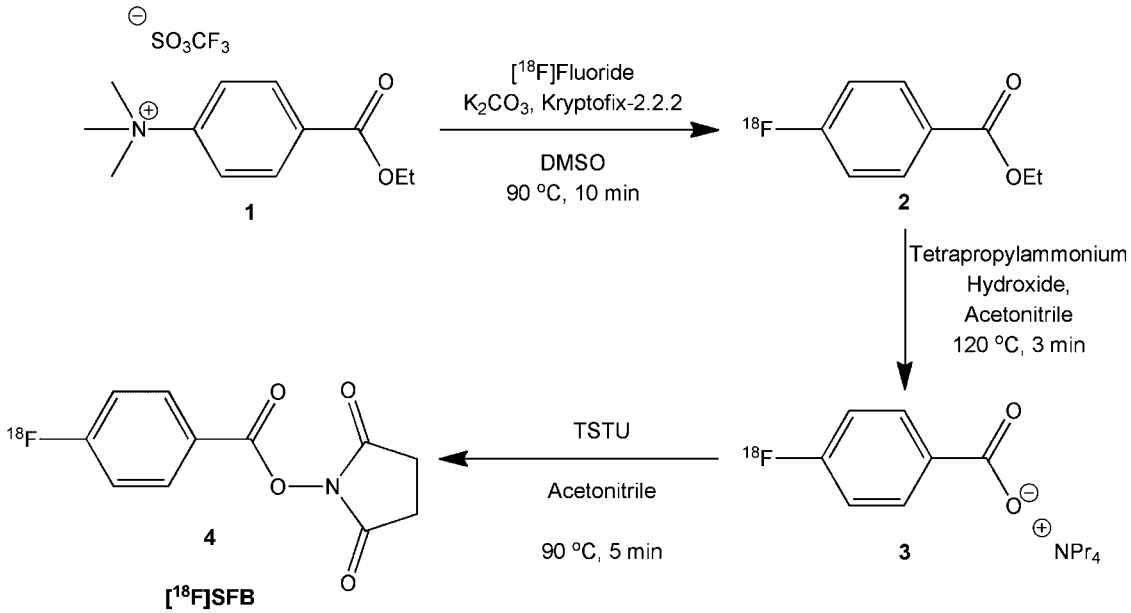
three-step, one-pot method for its production reported by Kabalka (Scheme 1).[8] This method is operationally simple and does not require HPLC purification.

To fully automate this synthesis of [$^{18}$F]SFB in our laboratory, simple modifications were made to a GEMS Tracerlab FX$_{FN}$ as shown in Figure 1. Luer lock fittings were incorporated into the line that normally connected valve-18 to the round-bottomed dilution flask. These fittings offered a straightforward means of connecting the line out from the reactor (through valve-14) directly to the round-bottomed dilution flask and bypassing the




**Scheme 1.**

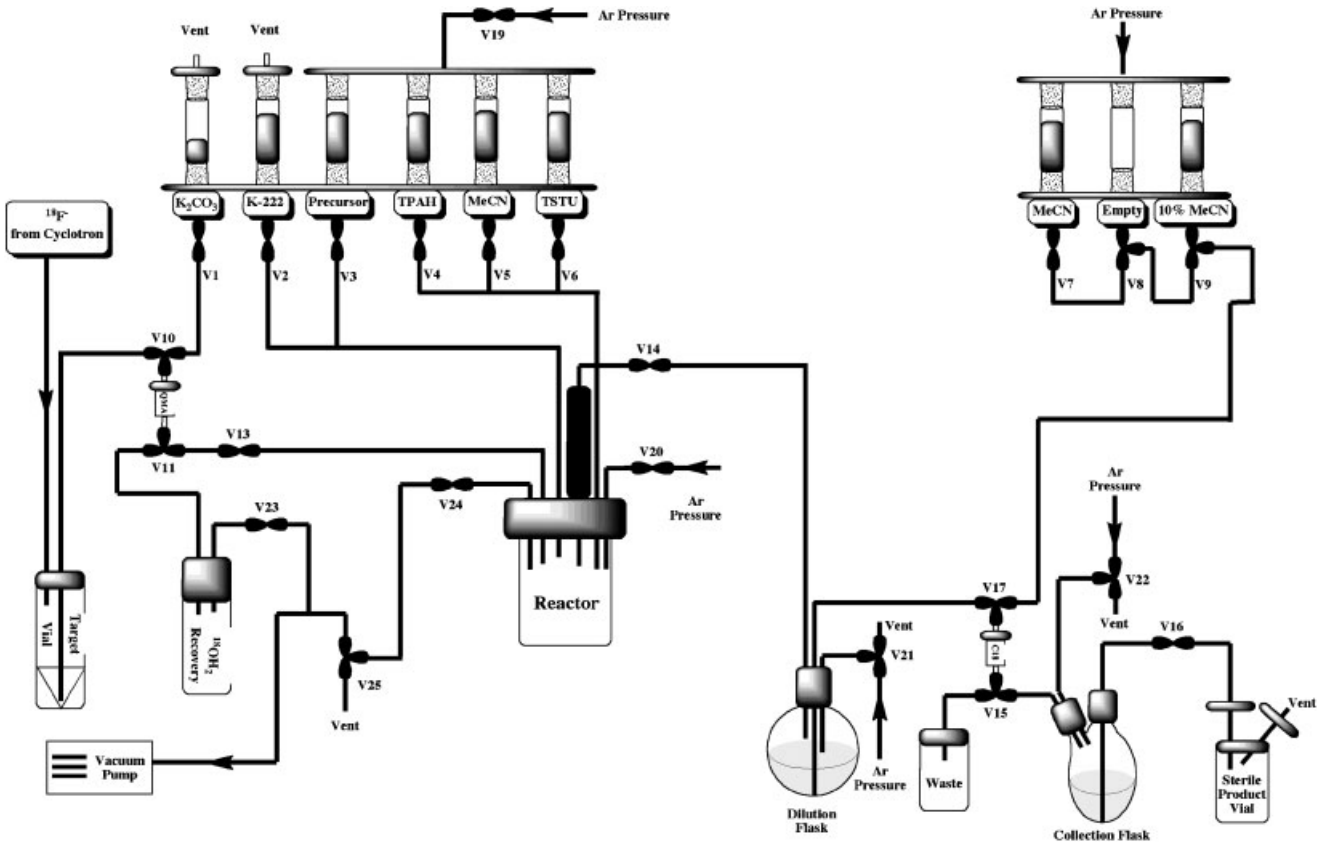



**Figure 1.** Modified Tracerlab FX$_{FN}$ configuration for production of [$^{18}$F]SFB.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HPLC system. Such modifications enabled straightforward switching between module configurations, allowing for easy alteration and access to radiopharamceuticals prepared using different radiochemical strategies.

Tracerlab $FX_{FN}$ Vials 1, 2, and 3 were loaded with potassium carbonate, kryptofix-2.2.2, and 4-(ethoxycarbonyl)-*N*,*N*,*N*-trimethylbenzenaminium triflate precursor (**1**), respectively. Fluoride-18 was delivered from the GEMS PETTrace cyclotron and trapped on a Waters QMA-light sep-pak to remove $[^{18}O]H_2O$. Fluoride-18 was then eluted into the reaction vessel using aqueous potassium carbonate (3.0 mg in 0.4 ml). A solution of kryptofix-2.2.2 (15 mg in 1 ml of MeCN) was added to the reaction vessel and the fluoride-18 was dried by azeotropic evaporation of the water/acetonitrile. Following drying, the trimethylbenzeneaminium triflate precursor (5 mg in 0.5 ml of DMSO) was added to the reaction vessel and the fluorination reaction was heated at 90°C for 10 min to provide [$^{18}$F]ethyl 4-fluorobenzoate (**2**). After this time, tetrapropylammonium hydroxide (TPAH, 1.0 M solution in water, 20 µl in 0.5 ml of MeCN) was added to saponify the ester. The saponification reaction was heated at 120°C for 3 min to yield [$^{18}$F]4-fluorobenzoic acid (**3**) as the corresponding tetrapropylammonium salt. On completion of the saponification, MeCN (1 ml) was added and evaporated to remove excess water from addition of the aqueous solution of TPAH. *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU, 10 mg in 0.6 ml of MeCN) was then added and the reaction vessel was heated at 90°C for 5 min to provide [$^{18}$F]SFB (**4**). The crude reaction mixture was cooled to 40°C and transferred from the reactor to the round-bottomed dilution flask (pre-charged with 20 ml of 1.5% acetic acid). The resulting solution was transferred through a Waters C18 Plus sep-pak to trap the crude reaction components. Following trapping, the C18 sep-pak was washed with 10% MeCN (10 ml), eluting unwanted hydrophilic impurities (including unreacted fluoride-18) to waste. Subsequently, [$^{18}$F]SFB was eluted off into the collection vial with neat MeCN (2 ml). Synthesis time was ~45 min and typical yields of [$^{18}$F]SFB using this method were 38% (non-decay corrected, $n = 20$), specific activity was >1 Ci/µmol, and radiochemical purity was always >95%. For the pre-clinical work described in this study, no efforts were made to optimize specific activity. However, there is scope for improving the specific activity (e.g. target rinsing prior to bombardment, etc.). The [$^{18}$F]SFB was either used as the obtained solution in MeCN or, alternatively, was evaporated to dryness (heat gun) and re-dissolved in a solvent more appropriate for the subsequent coupling reaction (e.g. DMSO).

### Microwave-accelerated coupling reactions with [$^{18}$F]SFB

Typically, solutions of [$^{18}$F]SFB, obtained in acetonitrile, were concentrated using a heat gun at 60–70°C under a stream of nitrogen gas (15 ml/min). *Note*: Temperatures higher than 70°C can decompose [$^{18}$F]SFB so this step should be carefully monitored. Following evaporation, [$^{18}$F]SFB was re-dissolved in a smaller volume of DMSO (0.5 ml) and this solution was then divided between several reaction vials, allowing for multiple coupling reactions to be carried out in parallel. Until recently, we carried out coupling reactions using a manual setup. Solvent evaporation and reaction heating were simply achieved by suspending a vial above a heat gun and temperature was controlled by varying power to the heat gun using a temperature controller. In a typical reaction, the peptide precursor (0.2–0.5 mg) and diisopropylethylamine (DIPEA, 10 µl, *Note*: In our experience, addition of DIPEA is essential for the reaction to proceed) were dissolved in DMSO (0.1 ml). [$^{18}$F]SFB in DMSO (0.1 ml) was then added and the reaction vial was heated at 60°C for 30–40 min (*Note:* Prolonged heating at this stage can lead to decomposition of sensitive precursors and products). After this time, reactions had typically reached their endpoint (determined from the analytical radio-HPLC trace) and crude reaction mixtures were then purified by semi-preparative HPLC to provide radiolabeled peptides. If necessary, collected HPLC fractions were reformulated into isotonic solutions suitable for injection using standard SPE techniques.

In connection with a project developing novel oncology PET biomarkers to quantify over-expression of neuropilins, receptors of angiogenic vascular endothelial growth factor, we were recently attempting to label a number of novel peptides with [$^{18}$F]SFB. In most cases, the thermal set-up discussed above was adequate for preparing pre-clinical doses of our experimental new biomarkers. However, with certain peptide precursors, which were found to be less reactive and somewhat thermally unstable, longer reaction times resulted in decomposition leading to low yields, and we were struggling to obtain sufficient amounts of biomarker for pre-clinical evaluation. Therefore, alternative strategies for improving [$^{18}$F]SFB coupling reactions were considered and attention was turned to microwave-promoted reactions. Microwave-accelerated radiochemical reactions have been shown to be more efficient than their thermal counterparts,[10–13] and we were curious about what, if any, enhancement might be achieved by carrying out SFB coupling reactions in a microwave reactor.

Our laboratory is equipped with a Resonance Instruments Model 521 Microwave Power Generator. The single vial microwave reactor is attached to the control unit and the 521 microwave is capable of providing variable microwave power from 10 to 150 W. Moreover, reactions can be heated from 30 to 180°C. In order to gauge the impact of the microwave reactor on coupling reactions with [$^{18}$F]SFB, two model test compounds ([$^{18}$F]A7R (**5**) and [$^{18}$F]RGD (**6**), Figure 2) were selected. RGD peptides such as **6** are known biomarkers for angiogenesis,[14–16] while radiolabeled heptapeptide ATWLPPR (A7R, **5**) is a scaffold of interest in our efforts to quantify over-expression of neuropilins.[17]

Initially, A7R was chosen for preliminary experiments to compare thermal heating to microwave irradiation. For the microwave reactions, A7R precursor (0.2–0.5 mg) and DIPEA (10 µl) were dissolved in DMSO (0.1 ml) in a small glass vial. [$^{18}$F]SFB (2–10 mCi) in DMSO (0.1 ml) was added, and the vial was placed in the microwave reaction cavity. The microwave power was set to 100 W and microwave radiation was then applied for different time points. Note that unlike thermal reactions, in which multiple samples were removed from a single reaction vial over time for analysis, a fresh reaction was prepared in a new glass vial for each individual microwave time point tested. Reaction temperature was measured using a digital thermometer and was found to be <120°C under these conditions. While this temperature is higher than the thermal reaction, reaction time is significantly reduced and yield of product is acceptable for pre-clinical use. Samples were removed from the vial, on completion of the irradiation, for HPLC analysis (Figure 3, Table 1). After 3 min of microwave irradiation (>90% reduction in reaction time compared to 40 min thermal reaction),


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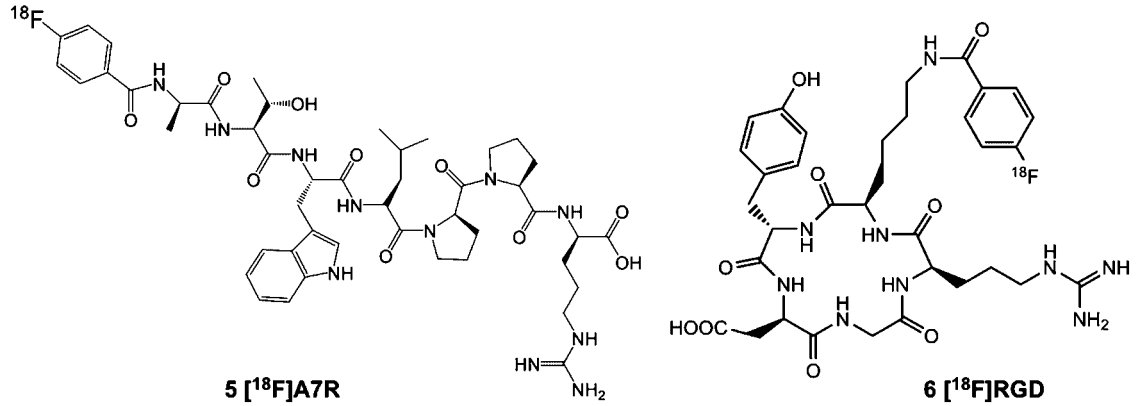


**Figure 2.** Peptides labeled with [$^{18}$F]SFB.

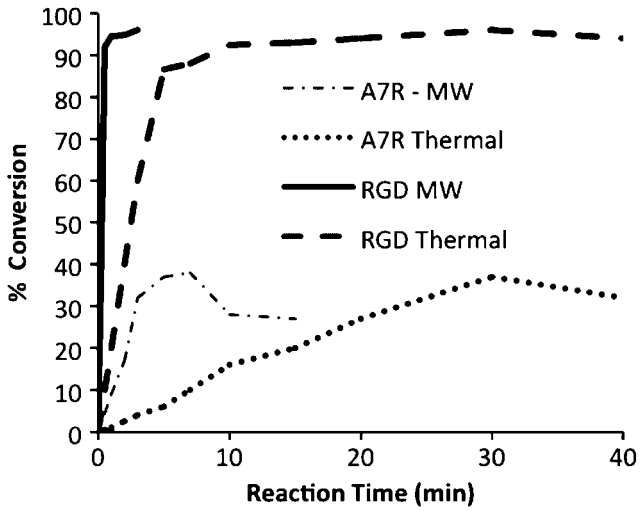


**Figure 3.** Percentage conversion of [$^{18}$F]SFB coupling reactions.

**Table 1.** Percentage conversion in microwave vs thermal coupling reactions using [$^{18}$F]SFB

| A7R | | | | RGD | | | |
|---|---|---|---|---|---|---|---|
| MW[a] | | Thermal[b] | | MW[a] | | Thermal[b] | |
| Time (min) | % | Time (min) | % | Time (min) | % | Time (min) | % |
| 1 | 9 | 5 | 6 | 0.5 | 92 | 5 | 87 |
| 2 | 17 | 10 | 16 | 1 | 94 | 10 | 92 |
| 3 | 32 | 20 | 27 | 2 | 95 | 20 | 94 |
| 7 | 38 | 30 | 37 | 3 | 96 | 30 | 96 |
| 10 | 28 | 40 | 32 | | | 40 | 94 |
| 15 | 27 | | | | | | |

[a]Microwave reactions carried out at 100 W power level, ([$^{18}$F]RGD $n=3$; [$^{18}$F]A7R $n=3$).
[b]Thermal reactions carried using a heat gun out at 60°C, ([$^{18}$F]RGD $n=3$; [$^{18}$F]A7R $n=20$)

high enough conversion (32%, $n=3$) had occurred to allow purification and subsequent use in pre-clinical experiments although this could be increased to 38% by microwaving for 7 min. However, extending the reaction time much beyond 7 min led to charring in the reaction vial and a drop off in yield, both attributable to decomposition of the product. Reflecting our experience with thermally mediated [$^{18}$F]SFB coupling reactions, addition of DIPEA was also essential for the microwave-promoted reactions to proceed and eliminating it resulted in no product formation. In contrast to the microwave-mediated reactions, it took 30 min to get comparable (37%, $n=20$) conversion to [$^{18}$F]A7R using the conventional thermal reaction set-up.

Using analogous conditions, the coupling of RGD with [$^{18}$F]SFB was compared under both thermal and microwave-mediated conditions, although for this particular peptide both were found to be extremely efficient reactions. Microwave-enhanced reactions were carried out in an analogous fashion to that described above: RGD precursor (0.2–0.5 mg) and DIPEA (10 μl) were dissolved in DMSO (0.1 ml) in a glass reaction vial. [$^{18}$F]SFB (2–10 mCi) in DMSO (0.1 ml) was then added and the reaction was irradiated at 100 W for different time points. Samples were removed from the vial, on completion of the reaction, to monitor progress by HPLC analysis (Figure 3, Table 1). Remarkably, >90% conversion occurred after 30 s of microwave radiation ($n=3$), while heating at 60°C for 10 min with a heat gun ($n=3$) was required to achieve equivalent conversions (Figure 3, Table 1). As with the A7R reactions described above, yields began to drop off after prolonged heating (30–40 min for thermal reactions). The crude microwave reaction mixtures for [$^{18}$F]labeled A7R and RGD were then purified by semi-preparative HPLC, and reformulated using standard C18 sep-pak techniques, as required.

## Experimental

### General considerations

Reagents and solvents were all commercially available and used, as received, without further purification: Sterile Water for Injection, USP was purchased from Hospira; acetic acid, anhydrous acetonitrile, DMSO, DIPEA, potassium carbonate, tetrapropylammonium hydroxide, and TSTU were purchased from Sigma Aldrich; kryptofix-2.2.2 was purchased from Acros Organics; SFB reference standard and precursor were purchased from ABX Advanced Biochemicals; A7R and RGD precursors, and unlabeled reference standards, were purchased from the University of Michigan Protein Structure Facility. QMA and C18 Sep-pak cartridges were purchased from Waters, Inc. and conditioned with ethanol (10 ml) and sterile water (10 ml) prior to use.


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### Production of [$^{18}$F]SFB

To prepare [$^{18}$F]SFB, the Tracerlab was configured as shown in Figure 1 and vials were loaded as follows: Vial 1: potassium carbonate (3.0 mg in 0.4 ml of water); Vial 2: kryptofix-2.2.2 (15 mg in 1 ml of MeCN); Vial 3: 4-(ethoxycarbonyl)-*N*,*N*,*N*-trimethylbenzenaminium triflate precursor (5 mg in 0.5 ml of DMSO); Vial 4: TPAH (20 μl in 0.5 ml of MeCN); Vial 5: acetonitrile (1 ml); Vial 6: TSTU (10 mg in 0.6 ml of MeCN); Vial 7: MeCN (2 ml); Vial 9: 10% MeCN in water (10 ml); dilution flask: 20 ml of 1.5% acetic acid.

Fluoride-18 was produced via the $^{18}O(p,n)^{18}F$ nuclear reaction using a GEMS PETTrace cyclotron equipped with a high yield fluorine-18 target. Fluoride-18 was delivered from the cyclotron (in a 2-ml bolus of [$^{18}$O]$H_2O$) and trapped on a QMA-light sep-pak to remove [$^{18}$O]$H_2O$. Fluoride-18 was then eluted into the reaction vessel using aqueous potassium carbonate (3.0 mg in 0.4 ml of water). A solution of kryptofix-2.2.2 (15 mg in 1 ml of acetonitrile) was then added to the reaction vessel and the fluoride-18 was dried by evaporating the water–acetonitrile azeotrope. Evaporation of the azeotrope was achieved by heating the reaction vessel to 80°C and drawing full vacuum for 4 min. After this time, the reaction vessel was cooled to 60°C and subjected to both an argon stream and vacuum draw simultaneously for another 4 min.

Following drying of the fluoride, a solution of trimethylbenzeneaminium triflate precursor (5 mg in 0.5 ml of DMSO) was added and the reaction vessel was heated to 90°C for 10 min to provide [$^{18}$F]ethyl 4-fluorobenzoate. After this time, tetrapropylammonium hydroxide (TPAH, 20 μl in 0.5 ml of MeCN) was added to saponify the ester group. Heating at 120°C for 3 min provided [$^{18}$F]4-fluorobenzoic acid as the corresponding TPA salt. After saponification, 1 ml of acetonitrile was added and evaporated (70°C, 5 min) with a stream of argon to remove any residual water left over from the saponification. *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU, 10 mg in 0.6 ml of MeCN) was added and the reaction vessel was heated at 90°C for 5 min to provide [$^{18}$F]SFB. The crude reaction mixture was cooled down (40°C) and transferred to the dilution flask (pre-charged with 5 ml of 5% acetic acid and 15 ml of water). The resulting solution was transferred through a Waters C18 Plus sep-pak and the C18 sep-pak was then washed with 10% MeCN (10 ml). Following washing, elution into the collection vial with neat MeCN (2 ml) gave [$^{18}$F]SFB in 38% radiochemical yield (non-decay corrected, $n = 20$), $>1.0$ Ci/μmol specific activity, and $>95\%$ radiochemical purity. The [$^{18}$F]SFB could be used as the obtained solution in MeCN or, alternatively, could be evaporated to dryness (heat gun) and re-dissolved in DMSO.

### Quality control of [$^{18}$F]SFB

Radiochemical purity and identity were analyzed using a Shimadzu LC-2010 HPLC equipped with a Bioscan FC3300 radioactivity detector and UV detector. Column: Chromolith RP18, 100 × 4.6 mm (Merck, Germany); Mobile Phase: 0.1% TFA in 50% MeOH, flow rate: 0.8 ml/min; UV: 254 nm, RT: ~3.0 min. Non-radioactive [$^{19}$F]SFB was used as the reference standard.

### Reaction of peptides with [$^{18}$F]SFB

*Thermal reactions*

Peptide precursor (0.2–0.5 mg) and DIPEA (10 μl) were dissolved in DMSO (0.1 ml) in a small glass vial. [$^{18}$F]SFB (2–10 mCi) in DMSO (0.1 ml) was added and the vial placed in our custom-built heat gun apparatus. The heat gun was set to 60°C and the reaction was maintained at this temperature for up to 40 min (Table 1). Samples were removed periodically for HPLC analysis (as described in the section Quality control of [$^{18}$F]SFB-labeled peptides).

*Microwave-enhanced reactions*

Peptide precursor (0.2–0.5 mg) and DIPEA (10 μl) were dissolved in DMSO (0.1 ml) in a small glass vial. [$^{18}$F]SFB (2–10 mCi) in DMSO (0.1 ml) was added, and the vial was placed in the reaction cavity of a Resonance Instruments Model 521 Microwave Power Generator. Microwave radiation at 100 W was applied for different lengths of time (Table 1). A new reaction was prepared for each time point and samples were removed from the reaction vial, on completion of microwave irradiation, for HPLC analysis (as described in the section Quality control of [$^{18}$F]SFB-labeled peptides).

### Quality control of [$^{18}$F]SFB-labeled peptides

Radiochemical purity and identity were analyzed using a Shimadzu LC-2010 HPLC equipped with a Bioscan FC3300 radioactivity detector and UV detector. Column: Chromolith RP18, 100 × 4.6 mm (Merck, Germany); Mobile Phase: 0.1% TFA in 35% MeOH, flow rate: 1.0 ml/min; UV: 254 nm, RT: ~6.5 min for [$^{18}$F]SFB, 6.0 min for [$^{18}$F]RGD, and 20 min for [$^{18}$F]A7R.

## Conclusions

In conclusion, a GEMS Tracerlab $FX_{FN}$ synthesis module has been modified to enable rapid, fully automated, preparation of [$^{18}$F]SFB. Synthesis time is 45 min and this method is routinely employed in our laboratory to provide [$^{18}$F]SFB in 38% yield (non-decay corrected), $>1.0$ Ci/μmol specific activity, and $>95\%$ radiochemical purity. Moreover, we have demonstrated that microwave-accelerated peptide coupling reactions using [$^{18}$F]SFB are far more efficient than their conventional thermal counterparts. Reaction times can be reduced by more than 90% without significant impact on radiochemical yield and, in light of the positive results disclosed in this study, we anticipate microwave-accelerated reactions to play an increasingly significant role in our radiochemical laboratory in the future.

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