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Two-step radiosynthesis of [^{18}F]*N*-succinimidyl-4-fluorobenzoate ([^{18}F]SFB)

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The acylation reagent [^{18}F]*N*-succinimidyl-4-fluorobenzoate (^{18}F-SFB) has been prepared using a new two-step approach. The starting material *p*-[^{18}F]fluorobenzaldehyde (^{18}F-FBA) was obtained by an improved radiosynthesis with a decay-corrected radiochemical yield of 66 ± 6 % ($n = 3$). Reaction of ^{18}F-FBA with (diacetoxyiodine)benzene and *N*-hydroxysuccinimide and preparative HPLC purification furnished ^{18}F-SFB in an r.c.y. of 49 ± 6 % ($n = 3$), based on the starting radioactivity of ^{18}F-FBA. The radiochemical purity of ^{18}F-SFB was > 99%. Alternatively, purification by solid phase extraction gave ^{18}F-SFB with an r.c.y. of 77 ± 9% ($n = 4$) and a radiochemical purity of 89 ± 5% ($n = 4$). This radiochemical synthesis only used non-aqueous solvents, which simplifies the method and facilitates subsequent applications of ^{18}F-SFB.

Keywords: *N*-succinimidyl 4-fluorobenzoate; fluorine-18; fluorobenzaldehyde; PET; microwave

Introduction

Radiolabelling of peptides continues to attract the research interest in Nuclear Medicine. The positron-emitting fluorine-18 ($t_{1/2} = 110$ min) with its favourable decay characteristics plays an important role in positron emission tomography.[1–3] Specifically binding peptides are seen as attractive substrates for ^{18}F labelling.[4] In particular, the short biological half-life of many peptides matches the half-life of ^{18}F. Peptides can be easily synthesized and tailored for high biological activity and to facilitate radiolabelling. However, the starting fluorine-18 is usually only available as fluoride, which is a poor nucleophile in aqueous media. Consequently, many efforts have been made to synthesize fluorine-18 pre-labelled prosthetic reagents that can be conjugated to native and modified peptide functions.[4–6]

Undoubtedly the most popular approach to label native peptides is based on the active ester compound [^{18}F]*N*-succinimidyl-4-fluorobenzoate (^{18}F-SFB) for targeting lysine and *N*-terminal primary amino groups. Since the first report by Vaidyanathan and Zalutsky,[7] the methodology has been further improved and modified (Table 1).

All these currently known protocols isolate ^{18}F-SFB after a demanding three-step radiosynthesis. Nevertheless, the process has been established on automated platforms.[14–16] The reagent ^{18}F-SFB has been used to radiolabel numerous peptides such as human C-peptide,[17] neurotensin(8–13),[18] annexin-V,[19] insulin,[20] bombesin,[21] vasoactive intestinal peptide,[22] and RGD peptides,[23–24] as well as oligonucleotides.[25] So far, one-step approaches using *N*-succinimidyl benzoate precursors have been attempted but had no success.[7] However, there has been a recent report on the use of a 2-thienyl iodonium salt that gave ^{18}F-SFB in 8–23% non-isolated radiochemical yield.[26]

Further, ^{18}F-SFB was used as a building block for the radiosynthesis of 'secondary' peptide labelling precursors.[27,28] In addition, ^{18}F-SFB offers an interesting alternative for introducing the 4-[^{18}F]fluorobenzamide functionality into small molecules – avoiding the problems associated with the notoriously difficult substitution of poorly activated aromatic nitro groups.

Here we report a new two-step radiosynthesis for ^{18}F-SFB based on the readily accessible reagent *p*-[^{18}F]fluorobenzaldehyde (^{18}F-FBA).

Results and discussion

In 2006, Wang *et al*. published a method for the preparation of *N*-succinimidyl esters. Benzylic alcohols or aldehydes were oxidized using (diacetoxyiodo)benzene in the presence of *N*-hydroxysuccinimide. The reaction generated the corresponding active esters in high yields. The authors also postulated a possible reaction mechanism involving (*N*-succinimidyl acetoxyiodo)benzene species.[29] We found the methodology intriguing and applied it for the radiosynthesis of ^{18}F-SFB as shown in Figure 1.

The original radiosynthesis of ^{18}F-FBA starting from 4-formyl-*N,N,N*-trimethyl benzeneaminium trifluoromethane sulphonate **1**[30] was adapted for microwave heating. Here, the combination of Kryptofix^{TM} and the mild base potassium hydrogen carbonate produced ^{18}F-FBA in near quantitative radiochemical yield in a time of only 10 s. The heating for a longer period of time already led to some degradation of the product. Figure 2 illustrates the fast reaction kinetics of the ^{18}F-fluorination. The average radiochemical yield as measured by analytical HPLC was 94 ± 3% ($n = 4$).

The solvent DMSO and other polar components were removed by passing the reaction mixture through a silica cartridge. This short silica column proved to be sufficient to separate ^{18}F-FBA from ^{18}F-fluoride and DMSO. ^{18}F-FBA was isolated in ethyl acetate with a decay-corrected radiochemical

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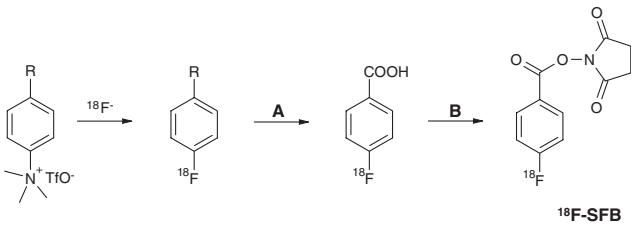
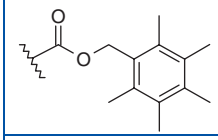
Table 1. Three-step radiosyntheses of ^{18}F-SFB in the literature



R	A	B	RCY[a]	Reference
-CHO	$KMnO_4$/NaOH/HCl	NHS/DCC	25%	Vaidyanathan and Zalutsky[7]
-CHO	$KMnO_4$/NaOH/HCl	DSC[b]	51%	Vaidyanathan and Zalutsky[8,9]
-COOEt	NaOH/HCl	TSTU[c]	50–60%	Wester *et al.*[10]
-COOEt	Pr_4NOH	HSTU[d]	43.8 ± 4.6%	Tang *et al.*[11]
-COO^{t}Bu	TFA	TSTU	44–53%	Wüst *et al.*[12]
	TFA	DSC/DMAP	44%	Azarian *et al.*[13]

[a]Total radiochemical yield, decay-corrected.
[b]*N,N′*-discuccinimidyl carbonate.
[c]*O*-(*N*-succinimidyl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate.
[d]*O*-(*N*-Succinimidyl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate.

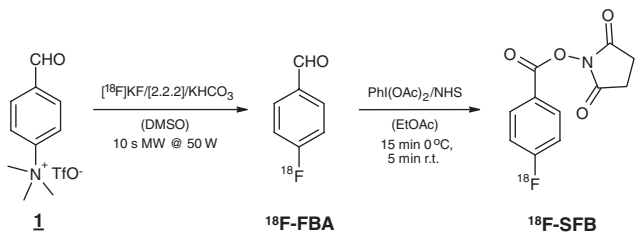


Figure 1. Preparation of ^{18}F-SFB via ^{18}F-FBA.

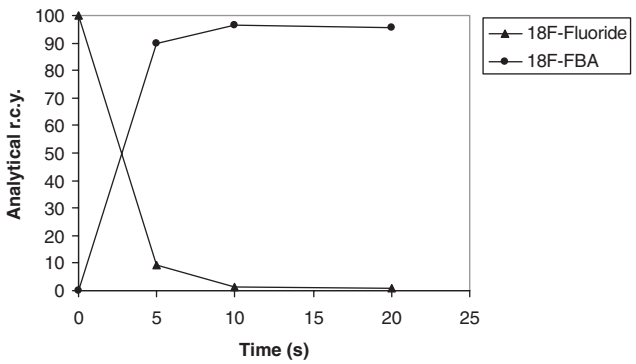


Figure 2. Analytical radiochemical yield of ^{18}F-FBA by microwave heating (50 W).

yield of 66 ± 6% ($n = 3$) and a radiochemical purity >99%. The major stable impurity of ^{18}F-FBA was 4-dimethylaminobenzaldehyde. Traces of other UV-active by-products with similar polarity as ^{18}F-FBA have been observed as well.

The radiolabelled active ester ^{18}F-SFB was prepared by adding *N*-hydroxysuccinimide and (diacetoxyiodo)benzene. It was found that a low reaction temperature of 0°C was essential for this step. This observation is also reflected in the original non-radioactive synthesis for similar *N*-succinimidyl esters.[29] Preparative HPLC gave ^{18}F-SFB with a decay-corrected radiochemical yield of 49 ± 6% ($n = 3$), based on the starting radioactivity of ^{18}F-FBA. The radiochemical purity of ^{18}F-SFB was >99 % (Figure 3). ^{18}F-FBA was found to be quantitatively consumed in the reaction. The radioactive by-products have not been identified. The specific radioactivity of ^{18}F-SFB as obtained from this manual radiosynthesis was estimated to be >0.43 GBq/μmol (11.7 mCi/μmol), based on the UV detection limit. All of the earlier-mentioned stable by-products of ^{18}F-FBA were effectively removed by the HPLC purification of ^{18}F-SFB. The total synthesis time was 2.75 h.

In an alternative approach, ^{18}F-SFB was purified by solid-phase extraction (SPE) using a silica SepPak cartridge. This protocol resulted in a shorter overall processing time (1.67 h) and a significantly higher radiochemical yield of 77 ± 9% ($n = 4$). However, the radiochemical purity was somewhat reduced with 89 ± 5% ($n = 4$). Thus, if subsequent conjugation reactions with ^{18}F-SFB can tolerate a certain level radiochemical impurity, the SPE method would be able to produce sufficient labelling reagent.

The new radiosynthesis of ^{18}F-SFB should be easily adapted to an automated platform, reducing the required synthesis time. Guo *et al.* have recently demonstrated that a commercial radiosynthesis module can be linked with a mono-modal microwave device.[31]

Attempts to prepare ^{18}F-SFB without purification of the ^{18}F-FBA intermediate were not successful.

Experimental

General

Chemicals including anhydrous solvents were purchased from Sigma-Aldrich (Gillingham, UK). HPLC solvents were obtained from Fisher Scientific (Loughborough, UK). No-carrier-added aqueous [^{18}F]fluoride was produced from the ^{18}O(p,n)^{18}F

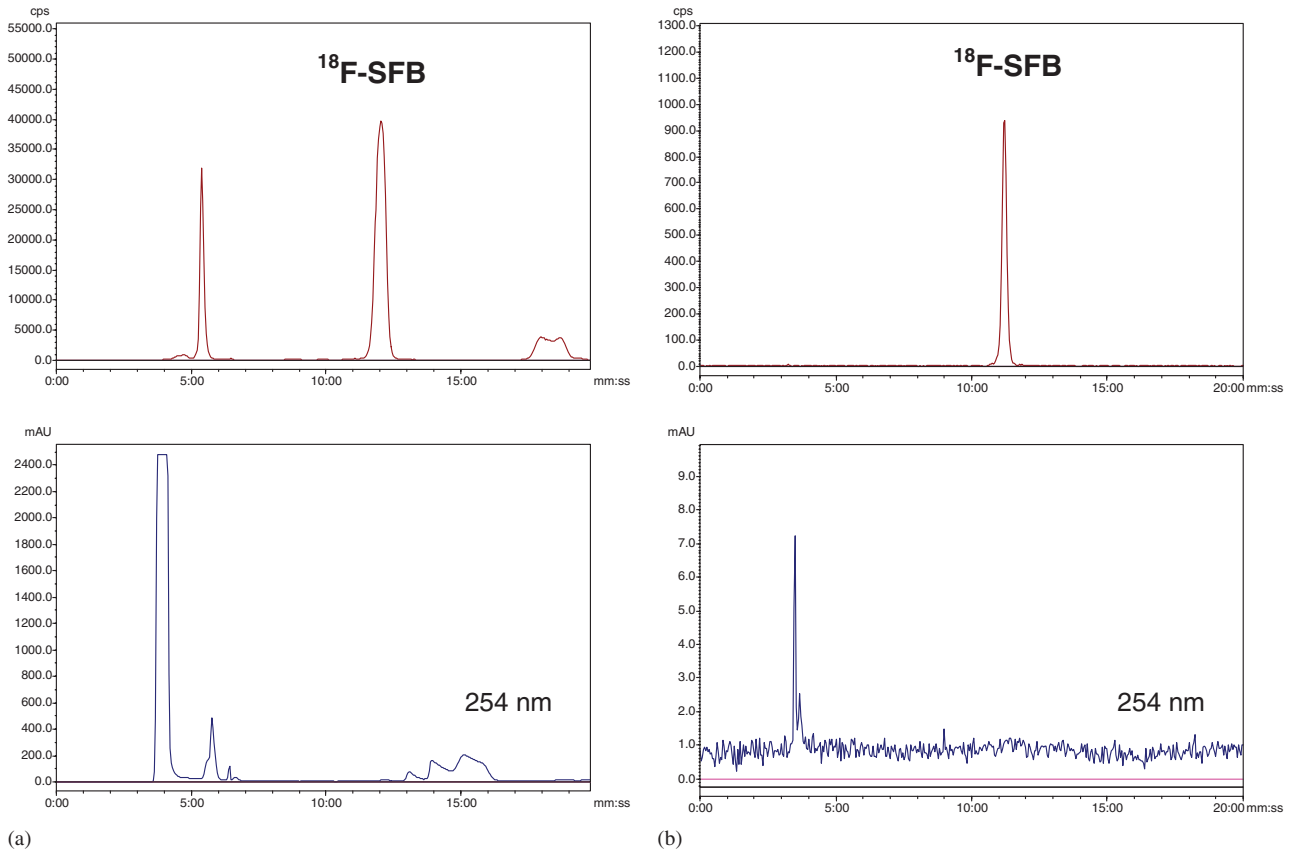


Figure 3. Preparative normal-phase HPLC showing (a) an ^{18}F-SFB preparation using ^{18}F-FBA, and (b) isolated and re-injected ^{18}F-SFB (Top: radioactivity channel, bottom: UV channel).

reaction (PETTrace cyclotron, GE Medical Systems) by the irradiation of an isotopically enriched [^{18}O]H_2O target using a 16.4 MeV proton beam. 4-*N,N,N*-trimethylaniliniumphenylmethanone trifluoromethanesulphonate (**1**) was prepared following a published method.[30] [^{19}F]*N*-succinimidyl-*p*-fluorobenzoate reference compound was also synthesized according to the literature.[10] Microwave heating was accomplished using a Resonance Instruments device (Model 521).

The radio-HPLC system consisted of a Beckman System Gold® instrument equipped with a gamma detector (Bioscan Flow-count, FC3300 NaI PMT or FC3400 PIN Diode) and a UV detector (Linear UVIS 200) set at 254 nm. A Phenomenex Luna C18(2) column (50 × 4.6 mm i.d., 3 μm; flow rate 1 mL/min) was used for analytical reverse-phase HPLC (solvent A: water, solvent B: acetonitrile, gradient 5% to 8% B in 15 min). The analytical normal-phase HPLC was done using a Luna Silica(2) column, 150 × 4.6 mm i.d., 3 μm, (Phenomenex) and a mobile phase consisting of *n*-hexane/50% ethyl acetate (flow rate 1 mL/min). The preparative normal-phase HPLC used a Luna Silica(2) column, 250 × 10 mm i.d., 5 μm, (Phenomenex), equipped with a silica SecurityGuard SemiPrep cartridge 10 × 10 mm (Phenomenex). The mobile phase was *n*-hexane/50 % ethyl acetate eluting with 4 mL/min.

Radiosynthesis of (^{18}F-FBA)

A Wheaton vial (3 mL) was charged with Kryptofix^{TM} (5 mg, 13.3 μmol), potassium hydrogen carbonate (0.7 mg, 7.2 μmol) dissolved in water (50 μL), acetonitrile (1.0 mL), and ^{18}F–water (0.5 mL, 270 MBq, 7.3 mCi). The water was removed azeotropically at 100°C using a nitrogen stream (50 mL/min). Anhydrous acetonitrile (3 × 0.5 mL) was added and sequentially evaporated under previous conditions. The vial was cooled to room temperature and a solution of **1** (2 mg, 6.4 μmol) in anhydrous DMSO (0.2 mL) added. After microwave heating (2 × 5 s, 50 W, set temperature = 80°C), the reaction mixture was loaded onto a silica SepPak-plus cartridge (Waters). The cartridge was flushed with nitrogen (1 min, 50 mL/min) to remove most of the DMSO. ^{18}F-FBA was then eluted using anhydrous ethyl acetate (3 radioactive fractions of 0.5 mL). The radiochemical yield of ^{18}F-FBA was 78 MBq (2.1 mCi). The average decay-corrected radiochemical yield of ^{18}F-FBA was 66 ± 6% (n = 3).

Radiosynthesis of [^{18}F]*N*-succinimidyl-*p*-benzoate with HPLC purification

In a Wheaton vial, *N*-hydroxysuccinimide (50 mg, 430 μmol) was mixed with ^{18}F-FBA (46 MBq, 1.2 mCi) in ethyl acetate (0.5 mL) and cooled to 0°C. After addition of (diacetoxyiodo)benzene (28 mg, 86 μmol), the reaction vial was first kept for 15 min at 0°C and then allowed to warm up to room temperature for 5 min. The decanted reaction mixture was injected onto preparative HPLC. The radiochemical yield of isolated ^{18}F-SFB was 19 MBq (0.5 mCi). The average decay-corrected radiochemical yield of ^{18}F-SFB was 49 ± 6% (n = 3).

Radiosynthesis of [^{18}F]*N*-succinimidyl-*p*-benzoate with SPE purification

The radiosynthesis was carried out as described above except with magnetic stirring in both reaction steps and the use of DMF

vs DMSO for the preparation of ^{18}F-FBA. The crude reaction mixture of ^{18}F-SFB was transferred onto an SPE cartridge (SepPak 'Plus Silica', Waters WAT020520) that had been conditioned with ethyl acetate (10 mL) and *n*-hexane (10 mL) and dried with a stream of nitrogen (100 mL/min) for 2 min. The loaded cartridge was flushed with nitrogen (100 mL/min) for 1 min and eluted with *n*-hexane (3 mL). The ^{18}F-SFB was collected by elution with ethyl acetate (4 fractions of 1 mL). The average decay-corrected radiochemical yield was $77 \pm 9\%$ ($n = 4$) and the radiochemical purity $89 \pm 5\%$ ($n = 4$).

Conclusions

In summary, a novel two-step radiosynthesis of the acylation reagent ^{18}F-SFB has been established. The intermediate ^{18}F-FBA was produced in high radiochemical yields by an improved procedure using microwave heating. In contrast to previously published protocols, the new synthesis of ^{18}F-SFB is purely based on non-aqueous chemistry. This is potentially beneficial in terms of synthesis simplification and time saving for conjugation reactions. The reaction volume of ^{18}F-SFB can readily be adjusted by evaporation, both for subsequent aqueous or non-aqueous coupling reactions. The new method has also the potential to become automated.

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