Synthesis and application of 4-[^{18}F]fluorobenzylamine: A versatile building block for the preparation of PET radiotracers†

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A novel synthesis of 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) by means of transition metal-assisted sodium borohydride reduction of 4-[^{18}F]fluorobenzonitrile ([^{18}F]FBN) is described. This approach could successfully be extended to borohydride exchange resin (BER) enabling a viable option for use in automated syntheses. [^{18}F]FBA was used for the synthesis of 4-[^{18}F]fluorobenzylamine-based thiol group-reactive prosthetic groups 4-[^{18}F]fluorobenzyl-2-bromoacetamide ([^{18}F]FBBA) and 4-[^{18}F]fluorobenzylamidopropionyl maleimide ([^{18}F]FBAPM). [^{18}F]FBBA and [^{18}F]FBAPM were obtained in radiochemical yields of 75% and 55%, respectively. Feasibility of using [^{18}F]FBAPM as novel prosthetic group for peptide and protein labelling was demonstrated with cysteine-containing tripeptide glutathione (GSH). [^{18}F]FBBA was used for labelling of a fully phosphorothioated 20mer oligodesoxynucleotide (ODN).

Introduction

Positron emission tomography (PET) has become a powerful non-invasive molecular imaging technique which provides functional information on physiological, biochemical and pharmacological processes in living subjects.[1] Recent advances in probe development and imaging technology have placed PET in a unique position among other molecular imaging methodologies as a truly translational research approach. In combination with suitable radiolabeled probes, also referred to as radiotracers, PET offers exceptional possibilities to study pharmacokinetics, including metabolism, and modes of action of novel and established drugs *in vivo*.[2]

Among the available PET radionuclides, the short-lived positron emitter fluorine-18 (^{18}F, $t_{1/2}$ = 109.8 min) is particularly useful due to its favorable nuclear and chemical properties.[3] Organic radiochemistry with ^{18}F differs significantly from conventional chemistry. Radiosyntheses involving the short-lived positron emitter ^{18}F require fast and efficient reactions while considering the extraordinary stoichiometry resulting from the typically produced submicromolar amounts of radiolabelled compounds.

^{18}F has found numerous applications for the labelling of both, small molecules and compounds of high molecular weight like peptides, proteins, and oligonucleotides. However, incorporation of ^{18}F into high molecular weight compounds represents a special challenge. Peptides, proteins and oligonucleotides can usually not be labelled with ^{18}F at high specific activity directly due to the required strongly basic reaction conditions at elevated temperatures. Over the last decades, various bifunctional labelling precursors, also referred to as prosthetic groups, have been developed allowing ^{18}F labelling under mild conditions.[4] Prominent examples include the amine group reactive acylating agent *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and thiol group-reactive prosthetic group 4-[^{18}F]fluorobenzyl-2-bromo-acetamide ([^{18}F]FBBA).

[^{18}F]FBBA has been shown to successfully radiolabel DNA-type probes such as modified oligonucleotides and peptide nucleic acids.[5] An automated synthesis of this compound employing a Zymate robot system has also been reported, however with relatively low radiochemical yields of 3 to 18%.[6]

4-[^{18}F]Fluorobenzylamine ([^{18}F]FBA) is the key intermediate in the synthesis of prominent oligonucleotide labelling prosthetic group [^{18}F]FBBA. However, compared to the dominant role of other ^{18}F-labelled aryl fluorides, such as [^{18}F]fluorobenz-aldehydes, [^{18}F]fluorobenzyl halides, and [^{18}F]fluorohalo-benzenes for the synthesis of ^{18}F-labelled radiotracers, only very few examples are known where [^{18}F]FBA was utilized. Besides the frequent use of [^{18}F]FBA for the preparation of [^{18}F]FBBA, [^{18}F]FBA was also used for the synthesis of ^{18}F-labelled prostaglandins, folic acid and epidermal growth factor receptor (EGFR) ligand.[7]

Various approaches for the synthesis of [^{18}F]FBA have been reported. All syntheses rely on the formation of 4-[^{18}F]fluorobenzonitrile ([^{18}F]FBN) and subsequent conversion into [^{18}F]FBA by means of various reducing agents. Haradahira *et al.* synthesized [^{18}F]FBA by the reduction of [^{18}F]FBN with borane-dimethylsulfide as the reducing agent.[7b] The reported radiochemical yield was 39 to 49%. Borane-mediated reduction of a [^{18}F]FBN derivative was also reported by Vaidyanathan *et al.* employing $NaBH_4/I_2$ as the BH_3 source for reducing 3-iodo-4-[^{18}F]fluorobenzonitrile in the synthesis of 4-[^{18}F]fluoro-3-iodobenzyl)-guanidine.[8] However, most reports describe synthesis of [^{18}F]FBA *via* reduction of [^{18}F]FBN with $LiAlH_4$ under anhydrous conditions.

Problematically, all these reaction conditions are particularly difficult to maintain in automated synthesis units where the transfer of reagents through common lines compromises the anhydrous conditions required to ensure reduction of the nitrile group. The use of molecular sieves and sodium sulfate drying cartridges, and flushing the lines with drying solvents, have been attempted to improve the radiochemical yield.[6,9] Moreover, the formation of aluminium salts during the reduction can also create

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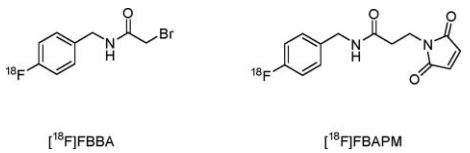
problems in a synthesis unit as transfer of the [^{18}F]FBA reaction mixture can block transfer lines in the synthesis unit. Therefore, an alternative method for the reduction step which tolerates the presence of water would be a welcome improvement in the synthesis of [^{18}F]FBA.

In this work we report on the improved synthesis of [^{18}F]FBA utilizing the borohydride/transition metal catalyst method for nitrile reduction. We also report on a solid-phase approach for nitrile group reduction using a $NaBH_4$ impregnated resin which, upon activation with a metal catalyst, rapidly and quantitatively reduces [^{18}F]FBN to [^{18}F]FBA. Elution from an activated borohydride exchange cartridge (BER) eliminates precipitates and provides a more viable option for use in automated synthesis units.

The feasibility of the proposed approach was demonstrated by the efficient synthesis of [^{18}F]FBA-based thiol group-reactive prosthetic groups [^{18}F]FBBA and 4-[^{18}F]fluorobenzylamido-propionyl maleimide ([^{18}F]FBAPM), respectively (Scheme 1).



[^{18}F]FBBA [^{18}F]FBAPM

Scheme 1 Thiol group-reactive prosthetic groups [^{18}F]FBBA and [^{18}F]FBAPM.

[^{18}F]FBBA and [^{18}F]FBAPM were used for the radiolabelling of a fully phosphorothioated 20mer ODN and cysteine-containing tripeptide glutathione (GSH), respectively.

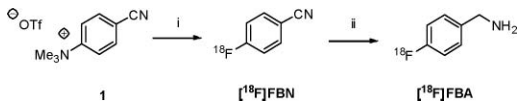
Results and discussion

Synthesis of 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) *via* transition metal-assisted $NaBH_4$ reduction

Unlike $LiAlH_4$, $NaBH_4$ is a rather mild reducing agent. $NaBH_4$ readily reduces aldehydes, ketones, and acyl halides, but it is virtually inert to many other functional groups, including nitrile groups. However, the reducing potential of $NaBH_4$ is significantly enhanced in the presence of various transition metal salts like $NiCl_2$, $Co(OAc)_2$ and $CuCl_2$. In the presence of aqueous solutions of transition metal salts $NaBH_4$ readily reduces nitrile groups through the formation of metal borides *in situ* followed by the release of hydrogen. Using $CoCl_2$, and $NaBH_4$, Osby *et al.* reported that benzylamine could be produced in high yield from benzonitrile.[10] More recently, Khurana *et al.* demonstrated that high yields of benzylamine could be obtained in 5 min when incubated with $NiCl_2$ and $NaBH_4$ in anhydrous ethanol at ambient temperature.[11]

The favorable reducing characteristics of $NaBH_4$ in the presence of transition metal salts for nitrile groups prompted us to set up the radiosynthesis of [^{18}F]FBA exploiting transition metal-assisted $NaBH_4$ reduction of [^{18}F]FBN. The synthesis of [^{18}F]FBA is depicted in Scheme 2.

[^{18}F]FBN was easily prepared by the reaction of 4-cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethansulfonate **1** with the powerful nucleophilic radiofluorination agent [^{18}F]KF (generated by treatment of cyclotron-produced [^{18}F]fluoride with Kryptofix (K_{222})/K_2CO_3) in dry DMSO at elevated temperature (95 °C) in a



1 [^{18}F]FBN [^{18}F]FBA

Scheme 2 *Reaction conditions*: (i) [^{18}F]KF, K_{222}, K_2CO_3, DMSO, 95 °C, 15 min; (ii) $NaBH_4/Co(OAc)_2$, THF–H_2O, room temperature.

Table 1 Reduction of [^{18}F]FBN with $NaBH_4/Co(OAc)_2$[a]

Entry	$NaBH_4$/mg	$Co(OAc)_2$/mg	Radiochemical yield [%][b]
1	10	5	75
2	15	5	49
3	25	10	61
4	30	10	50
5	40	20	58
6[c]	10	5	>95

[a] All reactions (except for entry 6) were carried out in THF at room temperature for 15 min. [b] Radiochemical yield determined by radio-TLC representing the percentage of [^{18}F]FBA present in the reaction mixture. [c] Reaction was performed in THF–H_2O (2 : 1) at room temperature for 5 min.

sealed reaction vial. After purification *via* solid-phase extraction (SPE), [^{18}F]FBN was obtained in high radiochemical yields of >80% and high radiochemical purity greater 95%. Sodium borohydride in combination with $Co(OAc)_2$ as transition metal salt was used to reduce the nitrile group of [^{18}F]FBN. For this purpose, [^{18}F]FBN was eluted with 1 mL of THF from the SPE-cartridge into a reaction vial containing different amounts of $NaBH_4$ and $Co(OAc)_2$. The reaction was performed at room temperature for 5–15 min.

The results of the reduction of [^{18}F]FBN with $NaBH_4/Co(OAc)_2$ are summarized in Table 1.

The results in Table 1 clearly show that the reduction is not quantitative (in the case of entries 1–5), and that the reaction depends on the $NaBH_4/Co(OAc)_2$ ratio and the solvent. Best results for the reduction in pure THF as the solvent were obtained with 10 mg of $NaBH_4$ and 5 mg of $Co(OAc)_2$ (entry 1). Extension of the reaction time to 30 min did not improve the radiochemical yield. Reduction of the amount of $Co(OAc)_2$ (less than 5 mg) resulted in a significant drop in radiochemical yield (<25%). A plausible explanation for the incomplete reaction at larger amounts of $NaBH_4$ and $Co(OAc)_2$ (entries 2–5) is the fixation of large amounts of formed Co_2B at the glass walls which is then not available for the reduction. Moreover, larger amounts of Co_2B also favor absorption of substantial amounts of [^{18}F]FBA. However, significant higher radiochemical yields of >95% were achieved when pure THF as the solvent was replaced with 2 : 1 mixture of THF and water (entry 6). Using THF–H_2O (2 : 1) as the solvent accelerates the reaction, and complete conversion of [^{18}F]FBN is accomplished at room temperature after 5 min. The formed Co_2B appears as a fine crystalline precipitate in THF–H_2O without the tendency to stick on the glass walls. Comparable results were achieved with $CoCl_2$ as transition metal salt instead of using $Co(OAc)_2$.

Synthesis of 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) using borohydride exchange resin (BER)-transition metals

All metal catalysts used in the BER study ($NiCl_2$, $Co(OAc)_2$ and to a lesser extent $CuSO_4$) were able to activate the BER as seen by

Table 2 Reduction of [^{18}F]FBN with BER[a]

Time after addition of [^{18}F]FBN to BER	Radiochemical yield [%][b] 1 min	3 min	5 min
$NiCl_2$	94	95	93
$Co(OAc)_2$	74	93	95
$CuSO_4$	<1	4	10

[a] All reactions were carried out in EtOH–H_2O at room temperature. [b] Radiochemical yield determined by radio-TLC representing the percentage of [^{18}F]FBA present in the reaction mixture.

the change in colour of the beads from tan to black. Activation of BER was only observed with $NaBH_4$ embedded resin prepared in-house. Commercially available borohydride-containing resin was not reactive regardless of the metal catalyst or solvents used (data not shown). The addition of $NiCl_2$ to the BER showed the most rapid activation (within 10 s), followed by $Co(OAc)_2$ (1 min). Activation of the resin with $CuSO_4$ was observed to occur only after a prolonged incubation time of 3–5 min. The rate of conversion of [^{18}F]FBN to [^{18}F]FBA also varied with the catalyst used. $CuSO_4$ demonstrated the slowest rate of nitrile reduction with 10% conversion at 5 min. In contrast, 74% of [^{18}F]FBN had been converted into [^{18}F]FBA with $Co(OAc)_2$ by 1 min, with 93% and 95% reduction by 3 min and 5 min, respectively. $NiCl_2$ demonstrated the most rapid rate of reduction with 94% of the nitrile converted to [^{18}F]FBA after 1 min of incubation with the BER-transition metal catalyst mixture. The results are summarized in Table 2.

Once the beads were activated with $NiCl_2$, the activity of the transition metal/borohydride as reducing agent was rapidly expended. Conversion was quantitative if the [^{18}F]FBN/ethanol mixture was added within 1 min after the activation of the BER resin. Longer wait times prior to the addition of the nitrile decreased the extent of reduction. Thus, 78% conversion to [^{18}F]FBA was observed if [^{18}F]FBN was added 3 min after bead activation. This effect was even more pronounced at later time points reaching only 16% conversion from [^{18}F]FBN to [^{18}F]FBA after a wait time of 5 min and less than 1% after a wait time of 15 min.

Synthesis of prosthetic groups 4-[^{18}F]fluorobenzyl-2-bromoacetamide ([^{18}F]FBBA) and 4-[^{18}F]fluorobenzyl-amidopropionyl maleimide ([^{18}F]FBAPM)

Synthesis of novel thiol-reactive prosthetic group [^{18}F]FBAPM commenced with the synthesis of [^{18}F]FBA according to the described $Co(OAc)_2$-assisted $NaBH_4$ reduction of [^{18}F]FBN. The synthesis of [^{18}F]FBAPM is given in Scheme 3.

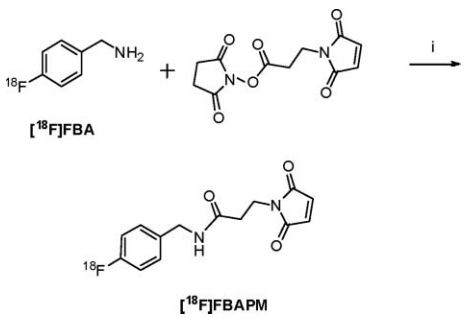
After Sep-Pak purification, [^{18}F]FBA was treated with *N*-succinimidyl-3-maleimide-propionate in the presence of *N*-methyl-morpholine. Without the use of *N*-methyl-morpholine as auxiliary base, no product formation was observed. The effect of *N*-methyl-morpholine upon the acylation reaction between [^{18}F]FBA and *N*-succinimidyl-3-maleimide-propionate is summarized in Table 3.

The use of 50 μL of *N*-methyl-morpholine gave the best results, and a radiochemical yield of 83% based upon [^{18}F]FBA was achieved (entry 3). Increase of the amount of *N*-methyl-morpholine to 100 μL and 200 μL did not improve the radiochemical yield (entries 4 and 5). Lower amounts of *N*-methyl-morpholine (25 μL) gave only moderate radiochemical yields of 30% (entry 2).

Table 3 Effect of *N*-methyl-morpholine on radiochemical yield for coupling of [^{18}F]FBA with *N*-succinimidyl-3-maleimide-propionate[a]

Entry	*N*-methyl-morpholine/μL	Radiochemical yield [%][b]
1	0	0
2	25	30
3	50	83
4	100	77
5	200	71

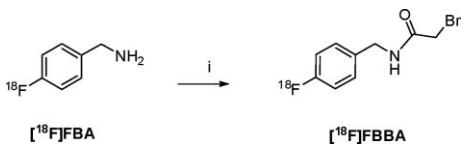
[a] All reactions were carried out in THF (200 μL) at room temperature for 10 min. [b] Radiochemical yield determined by radio-TLC representing the percentage of [^{18}F]FBAPM present in the reaction mixture.



Scheme 3 *Reaction conditions*: (i) *N*-Methylmorpholine, THF, 10 min, 60 °C.

Thus, optimized reaction conditions for the synthesis of [^{18}F]FBAPM included the use of 2–3 mg of *N*-succinimidyl-3-maleimide-propionate and 50 μL of *N*-methyl-morpholine in THF (200 μL) at room temperature. The reaction mixture was purified by means of semi-preparative HPLC to afford [^{18}F]FBAPM in a radiochemical yield of 55% (decay-corrected based upon [^{18}F]fluoride) within a total synthesis time of 60–70 min. The specific activity was determined to be 50–70 GBq/μmol, and the radiochemical purity exceeded 95%.

[^{18}F]FBA was further used for the synthesis of the prominent oligonucleotide labelling agent [^{18}F]FBBA (Scheme 4).

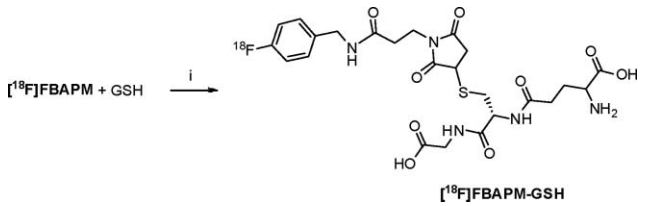


Scheme 4 *Reaction conditions*: (i) 2-bromoacetyl bromide, CH_2Cl_2, room temperature.

[^{18}F]FBBA was rapidly formed upon addition of 2-bromoacetyl bromide/CH_2Cl_2, 1/10 (v/v) to [^{18}F]FBA, with near quantitative conversion (97%) of [^{18}F]FBA to [^{18}F]FBBA within 5 min. A decay-corrected radiochemical yield of 75% before HPLC purification was achieved within 60 min which represents a considerable improvement to the previously reported 18% yield using $LiAlH_4$ as the reducing agent.[6]

^{18}F-labelling of glutathione (GSH) with [^{18}F]FBAPM and fully phosphorothioated 20mer oligodeoxynucleotide (ODN) with [^{18}F]FBBA

The labelling properties of [^{18}F]FBAPM as a novel thiol group-reactive prosthetic group for peptide and protein labelling was assessed by the reaction with cysteine-containing tripeptide glutathione (GSH) (Scheme 5).



Scheme 5 *Reaction conditions*: (i) phosphate buffer, pH 7.2, room temperature.

A solution of [^{18}F]FBAPM in EtOH was added to GSH solutions at various concentrations (1.0 mg mL^{-1}, 100 μg mL^{-1}, 10 μg mL^{-1}, 1 μg mL^{-1}) in phosphate buffer (pH 7.2) at room temperature. The conversion of maleimide [^{18}F]FBAPM into [^{18}F]FBAPM-GSH was monitored by radio-TLC reflecting the percentage of radioactivity area of conjugate [^{18}F]FBAPM-GSH relative to the total radioactivity area. The results are summarized in Table 4.

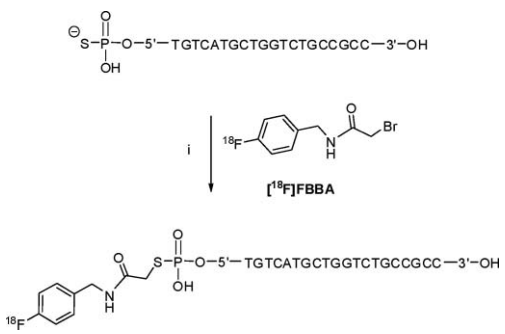
Table 4 Radiolabelling of GSH with [^{18}F]FBAPM[a]

GSH concentration	1 mg mL^{-1}	100 μg mL^{-1}	10 μg mL^{-1}	1 μg mL^{-1}
% [^{18}F]FBAPM-GSH	>95	>95	>95	65

[a] All reactions were carried out at room temperature for 20 min.

For GSH concentrations >10 μg mL^{-1}, conversion of [^{18}F]FBAPM was greater 95%. At a lower GSH concentration of 1 μg mL^{-1}, conversion was incomplete reaching 65%. These findings are consistent with our previous results dealing with the labelling of cysteine-containing peptides by means of other maleimide-containing ^{18}F-labelled prosthetic groups ([^{18}F]FBAM, [^{18}F]FBOM and [^{18}F]FDG-MHO). This makes [^{18}F]FBAPM an attractive prosthetic group for the mild and efficient labelling of cysteine-containing peptides.

Alkylation of a fully phosphorothioated 20mer ODN was performed with purified [^{18}F]FBBA as shown in Scheme 6.



Scheme 6 *Reaction conditions*: (i) phosphate buffered saline (0.1 M, pH 8, methanol, 1/1 (v/v), 120 °C, 30 min.

Treatment of ODN with [^{18}F]FBBA at 120 °C in phosphate buffered saline/methanol resulted in the formation of ^{18}F-labelled ODN. The isolated radiochemical yield after purification with NAP™-10 columns was about 30% based upon [^{18}F]FBBA. The radiochemical purity was greater than 95%, and the specific activity was determined to be 3.2 GBq/μmol (n = 14).

Conclusions

We have developed a convenient and facile method for the conversion of readily available [^{18}F]FBN into [^{18}F]FBA. The application of Co(II)/$NaBH_4$ as reducing agent can be extended to the use of borohydride-exchange resins (BERs) as the hydride source, which will further facilitate automation of [^{18}F]FBA and its broader use as a versatile ^{18}F-labelling building block.

Based on the novel synthesis of [^{18}F]FBA we have prepared [^{18}F]FBAPM as a novel prosthetic group for labelling of cysteine-containing peptides as exemplified by the reaction with GSH. [^{18}F]FBA was also used for the preparation of established oligonucleotide labelling agent, [^{18}F]FBBA, for subsequent conjugation with 5′-thiol-functionalized oligonucleotides.

Experimental

General

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova-400 at 400 MHz and 100 MHz, respectively. Chemical shifts (δ) were determined relative to the solvent and converted to the TMS scale. Mass spectra were obtained on a Agilent Technologies 6220 oaTOF by electrospray ionisation. Flash chromatography was conducted using MERCK silica gel (mesh size 230–400 ASTM). Thin-layer chromatography (TLC) was performed on Merck silica gel F-254 aluminium plates, with visualization under UV light (254 nm). All chemicals were obtained from commercial suppliers (reagent grade) and used without further purification.

Chemical syntheses

4-Fluorobenzylamidopropionyl maleimide (FBAPM). Fluorobenzylamine (85 μL, 750 μmol) in DMF (1 mL) was added dropwise over 2 h to a solution of *N*-succinimidyl-3-maleimidepropionate (200 mg, 750 μmol) and *N*-methyl-morpholine (50 μL) in DMF (500 μL). The reaction mixture was stirred at room temperature for an additional hour. The solvent was evaporated under reduced pressure and the residue was washed with 1 N HCl (2 × 5 mL) followed by water (2 × 5 ml). The residue was dissolved in dichloromethane and purified by column chromatography (dichloromethane–MeOH 40/1) to afford 116.5 mg (56%) of the desired product. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.57 (t, J = 7.1 Hz, 2 H), 3.86 (t, J = 7.1 Hz, 2 H), 4.38 (d, J = 5.8 Hz, 2 H), 5.86 (s, 1H), 6.71 (s, 2H), 7.00 (m, 2H) 7.24 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 34.3, 34.7, 43.0, 115.6 (d, J = 21.6 Hz), 129.7 (d, J = 7.6 Hz), 133.9 (d, J = 33.0 Hz), 134.2, 169.4, 170.5. HRMS (ESI, positive): calcd. for $C_{14}H_{13}O_3N_2FNa$ [M+Na]$^+$ 299.0802, found 299.0802.

Coupling of FBAPM with glutathione (GSH). Glutathione (GSH) (22.3 mg, 72.3 μmol) dissolved in phosphate buffer (300 μL, pH 7.1) was added to [^{19}F]FBAPM (20 mg, 72.3 μmol) in ethanol

(200μL). The mixture was stirred at room temperature for 60 min. The product FBAPM-GSH was isolated with preparative TCL (10% NH_4OAc (w/w) in MeOH–CH_2Cl_2 (3 : 1)) to give 24.0 mg (57%) of the desired compound. HRMS (ESI, negative): calcd. for $C_{24}H_{29}O_9N_5FS$ $[M - H]^-$ 582.1675, found 582.1678.

Radiosyntheses

No-carrier added aqueous [^{18}F]fluoride ion was produced on a TR-19/9 cyclotron (Advanced Cyclotron Systems, Burnaby, Canada) by irradiation of [^{18}O]H_2O *via* the $^{18}O(p,n)^{18}F$ nuclear reaction. Resolubilization of the aqueous [^{18}F]fluoride was accomplished with Kryptofix® 2.2.2 (K_{222}) and K_2CO_3. High performance liquid chromatography (HPLC) analyses were carried out with a SUPELCOSIL LC-18S column (4.6 × 250 mm, 5 mm) using an indicated isocratic eluent from a gradient pump L2500 (Merck Hitachi) with a flow rate of 1 mL min^{-1}. The products were monitored by a UV detector L4500 (Merck Hitachi) at 254 nm and by γ-ray detection with a scintillation detector GABI (X-RAYTEST). Semi-preparative HPLC was carried out on a Knaur chromatography system (Berlin, Germany) equipped with a Phenomenex Nucleosil C18 column (10 × 250 mm, 10 μm), integrated radioactivity detection and UV detection at 254 nm, using gradient elution with 0.2% TFA in water (solvent A) and methanol (solvent B): 0 min 70% A, 30% B; 10–22 min 20% A, 80% B. The flow rate was 2 mL min^{-1}. Radio-TLC was performed on Merck silica gel F-254 aluminium plates.

Optimization of 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) synthesis *via* $Co(OAc)_2$-assisted $NaBH_4$ reduction of 4-[^{18}F]fluorobenzonitrile ([^{18}F]FBN). 4-Cyano-*N*,*N*,*N*-trimethyl-anilinium trifluoromethansulfonate **1** (1 mg) was dissolved in dry DMSO (300 μL) and added to a vial containing dried [^{18}F]fluoride (50–150 MBq). The vial was sealed and the mixture was heated at 95 °C for 15 min. Water (10 mL) was added, and the mixture was passed through a Sep-Pak cartridge (Sep-Pak Plus tC18, Waters). The cartridge was washed with water (5 mL) and [^{18}F]FBN was eluted with THF (1 mL). Radio-TLC analysis (SiO_2, petroleum ether/ethyl acetate (1 : 1), R_f 0.7) of the eluent indicated high radiochemical purity (>95%) of [^{18}F]FBN. [^{18}F]FBN was eluted into a second reaction vial containing various amounts of $Co(OAc)_2$ and $NaBH_4$. Aliquots were taken and the progress of the reduction reaction was monitored by radio-TLC (SiO_2, *n*-BuOH/HOAc–H_2O (4 : 1 : 1)). The R_f value of [^{18}F]FBA was 0.4 under these conditions.

Synthesis of 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) using borohydride exchange resin (BER)-transition metal reduction. BER was prepared as reported by Sim *et al.*[12] Briefly, to 20 g of Amberlite IRA-400 anion exchange resin was added 50 mL of 1M $NaBH_4$ in THF. The mixture was stirred for 15 min at ambient temperature. The BER was washed thoroughly with distilled water and dried under vacuum at 60 °C for 5 h. The dried resin was stored under nitrogen atmosphere at 4 °C.

The C18 Sep-pak cartridge containing [^{18}F]FBN was eluted with 1 mL of anhydrous ethanol. 200 mg of BER (*ca.* 3 mmol BH_4^-/g resin) was combined with 0.06 mmols of $Co(OAc)_2$, $NiCl_2$, or $CuSO_4$ in 0.5 mL distilled, deionized water. Upon activation of the bead (evident by black discolouration of the resin), 0.5 mL of [^{18}F]FBN was added. Radio-TLC was performed at 1, 3, and 5 min after the addition of [^{18}F]FBN. Alternately, [^{18}F]FBN was added 1, 3, 5, or 15 min after the addition of the transition metal salt to the resin followed by radio-TLC analysis (SiO_2, *n*-BuOH/HOAc–H_2O (4 : 1 : 1), R_f ([^{18}F]FBA) = 0.4).

Radiosynthesis of [^{18}F]FBAPM. 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) was prepared *via* reduction of [^{18}F]FBN with $NaBH_4/Co(OAc)_2$ as described (*vide supra*). After reduction of [^{18}F]FBN, the reaction mixture was filtered through a Millipore filter and diluted with 0.01 N NaOH (15 mL). The solution was passed through a SepPak cartridge (SepPak Plus tC18 (Waters) to retard approximately 60 to 70% of [^{18}F]FBA at the cartridge. Other SepPak cartridges (*e.g.* Oasis (Waters), Chromafix HR-P (Macherey-Nagel) and Chromafix C18 ec (Macherey-Nagel) were less effective. The cartridge was dried in a stream of nitrogen and eluted with THF (2 mL). The volume of the solvent was reduced to 0.5 mL through gentle heating and a stream of nitrogen. *N*-Methylmorpholine (50 μL) and *N*-succinimidyl-3-maleimide-propionate (2–3 mg) were added, and the reaction mixture was heated at 60 °C for 10 min. The reaction mixture was purified by means of semi-preparative HPLC using gradient elution as indicated in the general section for radiosynthesis. Product fraction (13–15 min) was collected, diluted with water (15 mL) and passed through a LiChrolut RP18 cartridge (500 mg). The cartridge was eluted with ethanol (1.5 mL) to afford [^{18}F]FBAPM. In a typical experiment, starting from 750 MBq of [^{18}F]fluoride, 275 MBq of [^{18}F]FBAPM (55%, decay-corrected) could be prepared within a total synthesis time of 60–70 min. The specific activity was determined to be 50–70 GBq/μmol, and the radiochemical purity exceeded 95%. Radio-HPLC analysis: CH_3CN/0.1 M triethylammonium chloride (40/60), t_R = 4.4 min.

Radiolabelling with GSH. [^{18}F]FBAPM in ethanol (25 μL, 5–7 MBq) was added to 500 μL of GSH solution (1.0 μg mL^{-1} to 1.0 mg mL^{-1}) in phosphate buffer (pH 7.2) and was incubated at ambient temperature. Conversion was monitored by radio-HPLC after 20 min. Radio-HPLC analysis: CH_3CN/0.1 M triethylammonium chloride (40/60), t_R = 2.3 min for [^{18}F]FBAPM-GSH.

Synthesis of [^{18}F]FBBA and radiolabelling of ODN. Synthesis of [^{18}F]FBBA was performed in a GE TRACERlab FX_{FDG} automated synthesis unit as previously described[6] starting from [^{18}F]FBA prepared *via* $Co(OAc)_2$-assisted $NaBH_4$ reduction of 4-[^{18}F]fluorobenzonitrile ([^{18}F]FBN).

[^{18}F]FBBA was purified by normal phase HPLC using a Knaur chromatography system (Berlin, Germany), equipped with a Waters Prep Nova-Pak® HR Silica column (300 × 7.8 mm; porosity 6 μm), integrated radioactivity detection and UV detection at 254 nm (fixed wavelength detector K-200, Knaur, Berlin, Germany). The radioactive peak corresponding to [^{18}F]FBBA (t_R = 8 min) was collected by isocratic elution with dichloromethane–ethyl acetate (95/5, v/v) at a flow rate of 3 mL min^{-1}. The collected fraction was concentrated to dryness under a gentle stream of nitrogen. Radio-TLC demonstrated that semi-preparative HPLC produced pure ^{18}F]FBBA (determined by co-spotting ^{18}F]FBBA with the non-radioactive *N*-(4-fluorobenzyl)-2-bromoacetamide standard (50% ethyl acetate/heptane; [^{18}F]FBBA R_f 0.4).

Typically, 150 to 460 MBq of purified ^{18}F]FBBA was collected for radiolabeling ODNs.

Lyophilized ODN, 0.25 mg, was reconstituted with 0.5 mL of purified [^{18}F]FBBA (150 to 460 MBq) in phosphate buffered saline (PBS) 0.1 M, pH 8:methanol, 1 : 1 (v/v). The mixture was heated in a vented vial at 120 °C for 30 min. Methanol 50%, 0.5 mL, was added midway through the reaction to maintain volume. Unreacted [^{18}F]FBBA was separated from ^{18}F-labelled ODN using a NAP™-10 column (GE Healthcare, UK) by eluting [^{18}F]ODN with 1.5 mL PBS 0.01 M, pH 7.2. No further purification was performed.

Analysis of the radiolabelled ODN was performed with reverse phase HPLC using a Beckman Coulter Inc system consisting of a Model 168 Diode Array u.v. module, 260 nm; a Model 126 analytical dual pump; a radioactivity detector (Ortec, TN): ACE *Mate*™ Single Channel Analyzer; and a Phenomenex Luna, 10 μm, 10 × 250 mm, C18 column (Torrance, CA, USA) with guard column. A gradient mobile phase was employed: triethylammonium acetate 100 mM, pH 7.0 (*aq*)/acetonitrile 90/10 to 60/40 over 30 min, followed by a washout phase of 30/70 for 5 min at a flow rate of 3 mL min^{-1}. The concentration of ^{18}F-labelled ODNs was determined by UV spectroscopy at 260 nm using a Beckman DU 7400 spectrophotometer.

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