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A simple, rapid procedure for nucleophilic radiosynthesis of aliphatic [^{18}F]trifluoromethyl groups†

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A procedure for the radiosynthesis of aliphatic [^{18}F]trifluoromethyl groups by reacting 1,1-difluorovinyl precursors with [^{18}F]fluoride ions, resulting in the equivalent of direct nucleophilic addition of H[^{18}F]F, has been developed. A variety of ^{18}F-labelled model compounds were then obtained and two potential [^{18}F]radiotracers were synthesised by a two step process starting from 1,1-difluorovin-2-yl 4-toluenesulfonate. The method is widely applicable for the synthesis of novel radiotracers in high radiochemical yields and good specific activity.

Quantitative imaging of radiotracer distribution with positron emission tomography (PET) provides invaluable information about molecular transactions in living subjects.

The true potential of PET, however, lies in the combination of a positron emitting radionuclide with a molecular entity that participates in a biochemical process of interest. With the increasing application of PET, spanning preclinical small animal imaging, applications in drug development, biomedical research and clinical diagnostic imaging there is a strong demand for novel radiotracers for a variety of biological targets.[1] Consequently, a wide portfolio of reliable chemical methodology is required allowing access to novel radiotracers suitable for increasingly complex imaging studies.[2]

^{18}F is the most frequently employed PET nuclide[3] providing a high positron yield and an almost exclusive decay *via* the β^+ decay branch (97%). This is paired with a low positron energy (638 keV) accounting for a high image resolution. Its profile is rounded by an expedient half-life (109.7 min) rendering ^{18}F suitable for multi-step reactions, of course based on the intrinsic requirement for rapid chemical procedures.[1,2]

Using a no-carrier-added (n.c.a.) [^{18}F]fluoride ion, high specific radioactivities *i.e.* a high ratio of radioactive to non-radioactive molecules (> 150 GBq μmol^{-1}) in the radiotracer formulation are easily feasible. This allows for PET-imaging of saturable biological systems under genuine tracer conditions.[1d,e]

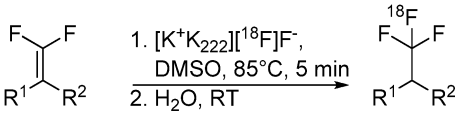
The trifluoromethyl (CF_3) group is a common motif in small molecule drugs. Trifluoromethylation of drug scaffolds is a common method of lead optimisation in drug development, thus a constant feed of novel bioactive compounds containing the group can be anticipated. The CF_3 group is a bioisostere for iodo, bromo, carbonyl, carboxyl, *sec*-propyl and *tert*-butyl groups[5] with a significantly higher stability. Due to electronegativity in the range between chlorine and fluorine it is a strong electron withdrawing group.

Few approaches for ^{18}F labelling of CF_3 groups have been reported to date. Their radiosynthesis has been further complicated by the low reactivity of leaving groups in difluoromethylene precursors, harsh reaction conditions, elaborate multi-step synthesis, and low specific activity in particular with carrier added [^{18}F]CF_3-labelled radiotracers obtained *via* electrophilic fluorination and isotopic exchange reactions.[4,6,10] This is a major limitation in ^{18}F radiochemistry.[1d,e] An efficient method to radiolabel the CF_3 group in high specific radioactivity would make accessible a wide range of compounds for PET-imaging in drug development and medical research.

To address this problem we have developed a novel one-step method for nucleophilic radiosynthesis of aliphatic [^{18}F]trifluoromethyl groups using a n.c.a. [^{18}F]fluoride ion under relatively mild conditions. Incorporation of the radiolabel is achieved *via* the equivalent nucleophilic addition of H[^{18}F]F to 2,2-difluorovinyl groups (Scheme 1).

To develop this method model substrate (**1a**) was used for the optimisation of the labelling conditions. Radiochemical yields (RCY) were determined by radio-HPLC.

Initial experiments were conducted in DMSO at 130 °C using a variety of common fluoride sources to elucidate the most viable form of activated [^{18}F]fluoride ion for this type of reaction (Table 1, entries 1 to 5). Notably, all of these can be readily prepared by SPE, elution in a small volume of MeCN containing a small amount of the appropriate metal salt and azeotropic removal of excess water. This is highly advantageous for general application of ^{18}F since this process can be automated on a variety of commercial synthesis modules.



1a-10a **[^{18}F]1b-10b**

Scheme 1 Addition of H[^{18}F]F.

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Table 1 Optimisation of reaction conditions for the formation of [^{18}F]**1b**

Entry	M[^{18}F]F	Solvent	T/°C	Time/min	RCY/%
1	K[K222][^{18}F]F	DMSO	130	6	62 ± 0.5
2	Cs[^{18}F]F	DMSO	130	6	60 ± 1
3	TBA[^{18}F]F	DMSO	130	6	54 ± 1
4	Ag[^{18}F]F	DMSO	130	6	59 ± 2
5	K[K222][^{18}F]F + CuOTf	DMSO	130	6	48 ± 0.5
6	K[K222][^{18}F]F	DMSO	110	3	78 ± 3
7	K[K222][^{18}F]F	DMSO	90	3	91 ± 1
8	K[K222][^{18}F]F	DMSO	85	3	92 ± 1
9	K[K222][^{18}F]F	DMSO	70	3	25 ± 4
10	K[K222][^{18}F]F	DMF	90	3	54 ± 3
11	K[K222][^{18}F]F	MeCN	82	3	63 ± 3
12	K[K222][^{18}F]F	THF	66	3	77 ± 1
13[a]	K[K222][^{18}F]F	DMSO	90	3	72 ± 4
14[b]	K[K222][^{18}F]F	DMSO	90	3	61 ± 10

[a] 2.5 mg of the precursor. [b] 1 mg of the precursor.

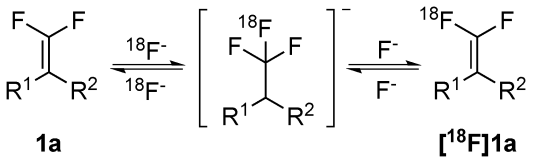
Moderate to good yields were obtained for all [^{18}F]fluoride sources with no pronounced preference (Table 1). It was, therefore, decided to optimise the reaction using the potassium 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan (K222) cryptate [^{18}F]fluoride ion complex, the most commonly used form of highly reactive "naked" [^{18}F]fluoride ion. This would enable a more straightforward transfer of the method into general application.

The time dependency of the RCY as a function of reaction temperature was then investigated (Table 1, entries 6–8) and an increase was observed with a decrease in temperature with the best results being obtained at 85 °C for 3 minutes. Further reduction of the reaction temperature or substitution of the solvent did not prove to be beneficial (Table 1, entries 9–12). Reduction of the amount of the labelling precursor below 5 mg also led to a reduction of the RCY (Table 1, entries 13 and 14).[12]

^{19}F model studies in order to optimise the conditions were omitted since it has been claimed that under stoichiometric conditions synthetically useful yields can only be achieved using "wet" solvents[7] by providing water molecules to promote the reaction. Contrary to stoichiometric conditions the presence of water has been shown to lead to deactivation of the substoichiometric [^{18}F]fluoride ion in n.c.a. radiochemistry,[1c] due to extensive hydratisation of the nanomolar quantities of [^{18}F]fluoride ion. Careful azeotropic removal of excess water is necessary, otherwise significantly diminished yields[9] are the result. Moreover, most n.c.a. labelling reactions display pseudo-first order kinetic profiles, which cannot be compared to typical second order reaction kinetics obtained for nucleophilic addition and substitution under stoichiometric conditions. In our case, addition of stoichiometric amounts of potassium fluoride to the reaction mixture led to a significant reduction in the reaction rate.

We have investigated the effect of the concentration of water in order to gain insights into the reaction mechanism using a tracer technique. In some cases (Table 1, entries 1–6 and 10–12), the "precursor" radiolabelled with ^{18}F [^{18}F]**1a** was observed as a by-product of the reaction.

The likely mechanism for its formation under these conditions is an addition–elimination mechanism (Scheme 2), rather nucleophilic substitution of ^{19}F for ^{18}F. A nucleophilic attack of fluoride ions onto the *gem*-difluorosubstituted, yet electrophilic carbon centre of the double bond would be the initial



Scheme 2 Hypothetical nucleophilic addition–elimination mechanism.

step, followed by equilibration of the anionic intermediate with intact olefin competing with protonation to form the stable 1,1,1-trifluoroalkyl group. Since this mechanism could also result in an undesired dilution of the final radioactive product with unlabelled **1b** due to the incorporation of ^{19}F$^-$ into **1a**, we determined the specific radioactivity of [^{18}F]**1a** obtained under these conditions.

For this experiment, a batch of about 5 GBq [^{18}F]fluoride ions was produced. The resultant specific activity was 86 MBq nmol^{-1}, which is in good accordance with the typical values obtained in low activity ^{18}F-production batches. It can, therefore, be concluded that there is no significant deteriorating effect by equilibration of trifluoro-carbanion and difluorovinyl species.

In order to elucidate potential ^{18}F/^{19}F isotopic exchange with fluorine atoms in the product, a control experiment was conducted. Non-radioactive **1b** was reacted with [^{18}F]fluoride ions for 15 minutes and the formation of [^{18}F]**1b** was monitored.

Only trace amounts of approximately 1 percent were formed after 5 minutes and even after 15 minutes at 110 °C less than 3% of [^{18}F]**1b** were found. These findings indicate a low susceptibility of aliphatic CF_3 groups to isotopic exchange under our reaction conditions.

Nevertheless, in our assumption a small amount of water would benefit 'trapping' of the anionic intermediate in the course of the reaction by protonation, thus avoiding excessive equilibration of ^{18}F and ^{19}F addition–elimination products, thereby providing insights into the reaction mechanism. For this reason, we investigated the dependency of the radiochemical yield of [^{18}F]**1a** on the water content of the reaction medium.

When fresh, anhydrous DMSO[9] was used, labelled precursor **1a** was formed as a byproduct at 130 °C. Contrarily, the ratio between [^{18}F]**1b** and [^{18}F]**1a** gradually improved in favour of the desired product [^{18}F]**1b** when the water content in DMSO was increased. Maximum yield was obtained with about 5 ppm of water being added to the reaction mixture with only trace amounts of [^{18}F]**1a** being formed. We surmised that increasing solvatisation then impedes the formation of both products at higher water concentrations with [^{18}F]**1b** being the almost exclusive product at 10 ppm. Only traces of [^{18}F]**1b** were formed at 100 ppm and no labelled product was found at 1000 ppm (see Fig. S1).[12] Hence we reasoned that our assumption that the addition reaction might proceed *via* an intermediate equilibrium where the final labelling product is only obtained in good yields when the intermediate carbanion is rapidly protonated, *i.e.* by trace amounts of water in the reaction mixture, would be strongly supported by these findings. Conversely, at very low water concentrations, an addition–elimination mechanism as outlined in Scheme 2 dominates and leads to increasing amounts of [^{18}F]**1a** being formed. However, our optimised reaction conditions (Table 1) furnished excellent radiochemical yields for [^{18}F]**1b**.

Table 2 Substrate scope of the reaction at 75 °C in DMSO

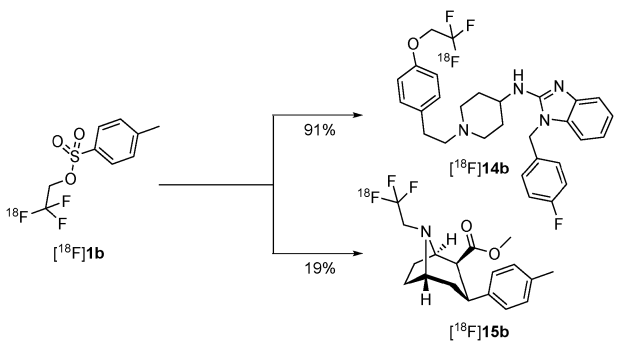
Precursor	R^1	R^2	Product	RCY/%
1a	OTs	H	[^{18}F]**1b**	93
2a	OTs	CH_3	[^{18}F]**2b**	52
3a	OBn	H	[^{18}F]**3b**	82
4a	NBn_2	H	[^{18}F]**4b**	79
5a	4-MeOPh	H	[^{18}F]**5b**	69
6a	4-Me_2NPh	H	[^{18}F]**6b**	67
7a	4-O_2NPh	H	[^{18}F]**7b**	89(81)[4]
8a	4-FPh	H	[^{18}F]**8b**	79
9a	$PhCH_2$	H	[^{18}F]**9b**	65
10a	6-$MeOC_{10}H_6$	CH_3	[^{18}F]**10b**	63

For the investigation of the substrate scope of our reaction, several labelling precursors[7,8] were synthesized and reacted with [K^+K222][^{18}F]F^- cryptate under optimized conditions (Table 2).

Difluorovinyl-functionalised labelling precursors are easily accessible from a variety of established methods. In our case, the model compounds were obtained in good yields using known transformations by either elimination or Wittig–Horner reactions.[8] Interestingly, all model compounds were labelled in good radiochemical yields except the tetra-substituted olefin **2a** which was obtained in considerably lower yield. These findings suggest that the direct addition of [^{18}F]fluoride ions to 2,2-difluorovinyl groups is a practicable way for the synthesis of diversely functionalised ^{18}F-labeled compounds.

As a prosthetic group, the 2-[^{18}F]trifluoroethyl group provides great potential as a metabolically insensitive, readily available supplement to PET chemistry. With n.c.a [^{18}F]**1b** in hand we briefly examined the reactivity of this alkylating agent towards different nucleophiles. For this reason, [^{18}F]**1b** was reacted with the N and O nucleophiles **11a–13a** in DMF using Cs_2CO_3 as a base. Moderate to good yields were obtained within 10 minutes for **11b–13b** (Table S1)[12]. [^{18}F]**1b** was also used to synthesise radiotracer candidates **14b** and **15b** as a proof of concept for radiotracer synthesis.[10]

Compound **14b**, an alleged imaging agent for neurofibrillar tangles formed by hyperphosphorylated human τ-protein in Alzheimer's disease, was obtained in a radiochemical yield of 91% (Scheme 3). Compound **15b** was formed in relatively low yield, however this is a common finding in N-alkylation of tropanes. Nevertheless, these findings show that [^{18}F]**1b** is well suited as a labelling agent.



Scheme 3 Radiotracer synthesis.

We have demonstrated a novel, versatile methodology for the nucleophilic radiosynthesis of [^{18}F]trifluoromethyl-labelled compounds which have not been accessible so far. Direct nucleophilic ^{18}F-fluorination of CF_3 groups in high specific activity opens up a wide range of potential candidates for radiotracer studies. Furthermore, accessing such "native" functionalities in biologically well characterised molecules will be highly favourable for the invasive introduction of additional fluorine atoms or fluorinated prosthetic groups into radiotracer candidates.

In addition to direct labelling under mild conditions, secondary labelling agents such as [^{18}F]**1b** provide a novel route for the introduction of metabolically insensitive ^{18}F-labels in heteroatom bound prosthetic groups.[6d] Due to the high stability of C–F bonds in CF_3-groups, these are less susceptible to oxidative defluorination than aliphatic ^{18}F fluorides. This is to the best advantage of brain imaging studies, where uptake of [^{18}F]fluoride ions into the skull will seriously confound PET data. We conclude that our novel approach is an advancement of utmost utility in the field of PET radiochemistry.

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9 Sigma-Aldrich 41647 DMSO puriss., absolute, over molecular sieve ($H_2O \leq 0.005\%$), $\geq 99.5\%$ (GC) was used. Higher water concentrations were prepared by adding water. See ESI†.
10 Carrier added [^{18}F]**7a** has been synthesised *via* ^{18}F/^{19}F isotopic exchange and used to prepare compound **6** in low specific activity. See: (**6d**).
11 Characterisation of **19b** and **20b** as radiotracers for brain imaging studies will be reported elsewhere.
12 See ESI† for detailed experimental procedures and analytical data.