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Fast and reliable method for the preparation of ortho- and para-[^{18}F]fluorobenzyl halide derivatives: Key intermediates for the preparation of no-carrier-added PET aromatic radiopharmaceuticals

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ABSTRACT

A fast and reliable method suitable for the automated preparation of (substituted) [^{18}F]fluorobenzyl halides from several [^{18}F]fluorobenzaldehydes was developed. Aromatic nucleophilic substitution of trimethylammonium benzaldehyde triflate and nitro precursors was realized with no-carrier-added [^{18}F]fluoride. After labeling, fluorine-18 containing aldehydes were trapped on a Solid Phase Extraction (SPE) cartridge and the subsequent conversion into benzyl halide was directly realized, on-line, on the support. Reduction of the aldehydes (>95%) was near-quantitative with an aqueous solution of $NaBH_4$. Halogenation was performed on the same support with different aqueous solutions of concentrated acid (HI, HBr, HCl). The conversion of benzyl alcohols into [^{18}F]fluorobenzyl halides (X = Cl, Br, I) usually proceeded within 2 min at high yields. The halogenation proceeded at room temperature, with the exception of the 2-[^{18}F]fluoro-3-methoxybenzyl, 2- and 4-[^{18}F]fluorobenzyl halides, which required the use of HBr/HOAc (33%). With this method, various [^{18}F]fluorobenzyl chloride, bromide and iodide compounds were obtained with high radiochemical purities (>90%) and with overall radiochemical yields of 15–70%. The radiosynthesis was completed in 30 or 45 min from EOB, starting from the ammonium or nitro precursor, respectively. By using the same solid support, 2- and 4-[^{18}F]fluorobenzyl bromide and iodide derivatives were near-quantitatively converted (>95%) into the corresponding azido compounds (60 °C, 5 min). As the reduction and halogenation steps were performed on solid supports, automation of the whole synthesis was straightforward.

1. Introduction

Fluorine-18 is the radionuclide of choice for the preparation of labeled radiopharmaceuticals for positron emission tomography (PET) studies. To date, a large number of aliphatic and aromatic organic compounds have been synthesized either from carrier-added (ca) electrophilic [^{18}F]fluorine ([^{18}F]F_2) generated by the ^{20}Ne(d,α)^{18}F reaction or from no-carrier-added (nca) nucleophilic [^{18}F]fluoride produced by the ^{18}O(p, n)^{18}F nuclear reaction. Compared to the electrophilic approach, the nca method has advantages such as high batch yields and high specific activity (i.e. high ratio of ^{18}F/^{19}F) of [^{18}F]fluoride [1,2].

PET probes with high specific activity allow injection of true tracer doses (i.e. very low actual injected mass of compound) which avoids saturation of the biological target with the [^{19}F] fluorine derivative. For example, brain receptors with limited expression may only be effectively visualized with high specific activity tracers. The risks of toxicological and pharmacological effects are also reduced at true tracer doses. Furthermore, in the study of biochemical processes with PET, high specific activity is desirable in order to avoid interference with the metabolic pathway of interest. Thus, in these situations, syntheses using electrophilic substitution with carrier-added ([^{18}F]F_2) are not recommended. Nucleophilic substitution with nca [^{18}F]fluoride has become the favoured method.

Nca nucleophilic [^{18}F]fluorination can be conducted both on aromatic and aliphatic compounds. Even in cases where the direct nucleophilic fluorination of complex molecules on an aliphatic carbon proceeds readily, the ability to label the aromatic part of such molecules with high specific activity remains a challenge. For

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the labeling of an aromatic moiety with [^{18}F]fluoride, strong electron withdrawing groups (NO_2, CN, CHO, RCO_2,...) in the ortho or para position of a good leaving substituent (NO_2, $N^+(Me)_3X^-$) are required. However using this approach, only a limited number of radiopharmaceuticals can be labeled in the last step of the process.

In some cases, late stage labeling of aryl rings in complex structures can be achieved via diverse aromatic diaryliodonium salts [3,4]. Alternatively, aromatic rings can also be labeled by ^{18}F–^{19}F isotopic exchange and the electron withdrawing group removed or subsequently converted into another organic function after the labeling step [5]. However, in most cases, the final product has to be built up in a multistep pathway from a previously synthesized, small, labeled aryl [^{18}F]fluoride (building block).

Until recently, one of the most versatile aromatic building blocks has been 4-[^{18}F]fluorobenzaldehyde. Its radiochemical synthesis was first reported from the corresponding nitrobenzaldehyde derivative in 1987 [6] and later from a 4-formyl-N,N,N-trimethyl-benzenaminium trifluoromethanesulfonate [7]. This [^{18}F]fluorinated building block is particularly useful in the introduction of an [^{18}F]aromatic moiety to complex molecules via multi-step approaches [8–24].

4-[^{18}F]fluorobenzaldehyde can also be converted into 4-[^{18}F]fluorobenzyl halide which can be used to alkylate various organic functions to generate new carbon–carbon, carbon–oxygen, carbon–nitrogen and carbon–sulphur bonds. Such a broad spectrum of uses makes this [^{18}F]fluorobenzylation approach very useful for the synthesis of radiopharmaceuticals containing a fluorobenzyl moiety. For example, by using [^{18}F]fluorobenzyl halides, a fluorobenzyl analog of the serotonin transporter DASB [25], a potential cannabinoid subtype-1 receptor ligand [^{18}F]PipISB [26], an imaging probe for prostate cancer [^{18}F]DCFBC [27], the PET imaging agent p-[^{18}F]BNTI for DOP receptors [28] and clickable peptides [29] have been prepared.

For more than twenty years there has been a need for a reliable nca nucleophilic synthesis for preparation of aromatic amino acids such as 6-[^{18}F]fluoro-L-dopa [30] and 2-[^{18}F]fluoro-L-tyrosine [31]. As nucleophilic substitution with fluoride is not directly applicable to these electron rich compounds, their routine production is based on the synthesis of a [^{18}F]fluorobenzyl halide derivative, followed by subsequent addition of the amino acid function. In our laboratory, the synthesis strategy for [^{18}F]fluorobenzyl bromide includes three major steps:

- labeling of a nitro or ammonium precursor with nca [^{18}F]fluoride by aromatic nucleophilic substitution using the aldehyde function as the withdrawing group,
- trapping of the [^{18}F]fluoro labeled aldehyde on a solid support and its subsequent reduction into the respective alcohol with an aqueous solution of $NaBH_4$,
- halogenation of the [^{18}F]fluorobenzyl alcohol on the same solid support with gaseous HBr.

However, the highly corrosive nature of this hydrogen bromide gas and the difficulty in its manipulation, have impeded attempts to use this synthesis method. Moreover, serious difficulties may become apparent in synthesis modules, resulting in technical failures and jeopardizing the reliability of the synthesis procedure. Furthermore, this approach is not well suited to the preparation of the 2- and 4-[^{18}F]fluorobenzyl bromides. According to our experience, most of the radioactivity trapped on the tC18 Sep-Pak® cartridge during the halogenation step is lost as volatile species and only very low radiochemical yields of 2- and 4-[^{18}F]fluorobenzyl bromides can be obtained (5–20%).

In order to improve access to these [^{18}F]fluoro amino acids and to other [^{18}F]labeled aromatic radiopharmaceuticals bearing a fluorobenzyl moiety, the chemistry previously developed in our laboratory for nca preparation of aromatic fluorobenzyl bromides required modification. These optimizations realized in order to simplify and facilitate the automation of the process are described below. The method can now be applied at high activities (>37 GBq) and it has been extended to the preparation of other chlorido, bromido or iodido [^{18}F]fluorobenzyl derivatives at high specific activities.

2. Results and discussion

2.1. Labeling of aromatic aldehydes

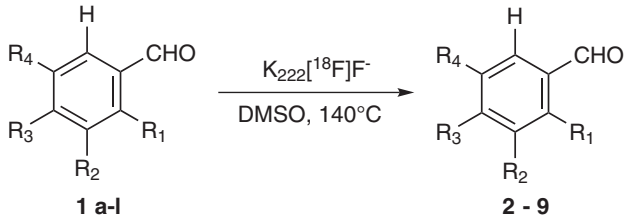
The first step of the synthesis consisted of nucleophilic substitution of an aryltrimethylammoniumtriflate or nitro compound in Me_2SO (Scheme 1). A labeling temperature of 140 °C for 2.5 min was found to be optimal for all tested ammonium salts (Table 1). Under these conditions, compound **1d** afforded **5** with a radiochemical yield (RCY) of only 50% (Scheme 1 and Table 1). However, radiolabeling yields close to 70% were obtained from all the other ammonium salts investigated (**1a–1c**). Generally, similar results were achieved from the corresponding nitro precursors (**1e–1h**), although heating at 140 °C for at least 15 min was required. With the exception of compound **9** (Table 1), which affords a radiochemical yield of only 4% in Me_2SO, yields for compounds **6**–**8** are also in accordance with those reported in the recent literature [8,32,33].

After labeling, [^{18}F]aldehydes were diluted with water and then trapped on either a 1 g environmental Sep-Pak® cartridge (compounds **2**–**3**, **7**–**9**) or on a tC18 Sep-Pak® cartridge from Waters containing 400 mg of phase (**4**–**6**). During this process, impurities such as K_2CO_3, K_{222}, Me_2SO, [^{18}F]F$^-$ are removed. Moreover, the cationic nature of the trimethylammonium precursor greatly facilitates the purification of the [^{18}F]fluorinated aldehyde which, unlike the nitro derivative, can easily be separated from the starting compound using this procedure.

2.2. Reduction step

Sodium borohydride is one of the most widely used reagents for the reduction of aldehydes into alcohols [26,30,31,34–39]. As previously demonstrated [30], this reduction of [^{18}F]fluorobenzaldehyde into the corresponding alcohol requires direct addition of an aqueous solution of $NaBH_4$ to the tC18 Sep-Pak® cartridge. As the aldehydes are trapped on a solid support, the reduction step was very easy to automate. Indeed, after reaction, the large excess of reagent is easily eliminated by flushing the cartridge with nitrogen and/or washing with water while the [^{18}F]fluorobenzyl alcohol is retained on the support.

For all compounds investigated, the reduction step was fast (<2 min) at RT. Moreover, the reductive agent is still active after several hours and the reduction yield reliably reaches 95% with only a small volume (2–3 mL) of dilute $NaBH_4$ solution (5–6 mg/mL) at a flow rate of 5 mL/min. For the next halogenation step, the alcohol derivative was kept on the solid support.



Scheme 1. [^{18}F]Labeling of various benzaldehyde derivatives.

Table 1
Radiochemical yields (RCY) for the [^{18}F]labeling of trimethylammonium and nitrobenzaldehyde derivatives (d.c., decay corrected).

Starting compound (Scheme 1)					[^{18}F]Fluoro benzaldehyde		RCY (d.c., %)
	$N^+(Me)_3$	NO_2	OCH_3	H		^{18}F	
1a	R_1	–	–	R_2, R_3, R_4	**2**	R_1	69 ± 3 (n = 5)
1e	–	R_1					73 (n = 2)
1b	R_3	–	–	R_1, R_2, R_4	**3**	R_3	73 ± 4 (n = 15)
1f	–	R_3					71 ± 5 (n = 25)
1c	R_1	–	R_3	R_2, R_4	**4**	R_1	74 ± 5 (n = 50)
1g	–	R_1					69 ± 2 (n = 3)
1d	R_1	–	R_3, R_4	R_2	**5**	R_1	50 ± 5 (n = 10)
1h	–	R_1					61 ± 4 (n = 5)
1i	–	R_1	OCH_2O (R_3,R_4)	R_2	**6**	R_1	70 ± 3 (n = 3)
1j	–	R_3	R_1	R_2, R_4	**7**	R_3	83 ± 3 (n = 3)
1k	–	R_1	R_2	R_3, R_4	**8**	R_1	34 (n = 2)
1l	–	R_1	R_4	R_2, R_3	**9**	R_1	4 ± 1 (n = 5)

2.3. Halogenation step

According to the literature, halogenation and particularly bromination reactions are feasible with hydrobromic acid either in an organic medium (i.e.: HBr in ether) [39], in gaseous form [30] or in aqueous solution [40]. The first approach, with (e.g.) an ethereal solution of HBr, must be avoided as the alcohol trapped on the support will be eluted during the process. For the reasons described above, the use of gaseous hydrobromic acid on the solid support (tC18) must also be avoided [30]. In this paper, a concentrated aqueous solution of hydrobromic acid was passed through the solid support to circumvent these problems and optimize the process, thus facilitating the S_N1 reaction of primary alcohols (Scheme 2, HX = HBr). This halogenation step was then further investigated, starting from either ammonium salt or a nitroprecursor. In both cases, the same [^{18}F]fluorobenzyl halide is obtained. To differentiate between the products synthesized via the ammonium salt or nitro routes, the same chemical numbering has been used, with either the "@" or "#"-sign added, respectively.

2.3.1. From ammonium precursors

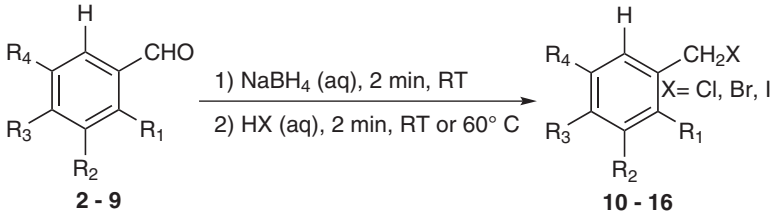
The synthesis of 2-[^{18}F]fluoro-4-methoxybenzyl bromide (compound **13$^{@}$**, Table 2, Scheme 2) was first investigated from the [^{18}F]fluorobenzaldehyde **4** obtained from an ammonium precursor. The bromination reaction was realized at room temperature by slow passage of 0.5–1 mL of HBr (48%) through the support. After 10 min, most of the acid present on the cartridge was removed using a gentle flow of nitrogen (2 min). Thereafter, for analytical purposes, almost all of the activity trapped on the SPE tC18 was eluted with 2 mL of acetonitrile. Using HPLC and TLC analytical methods, the bromination step appeared near-quantitative at room temperature (≥85%) after only 1–2 min. This general strategy and the typical TLC profiles observed during the synthesis of 2-[^{18}F]fluoro-4-methoxybenzyl bromide (**13$^{@}$**) are summarized in Scheme 3.

These conditions (HBr (48%), 2 min, RT) were then selected for synthesis of the 2- and 4-[^{18}F]fluorobenzyl bromides (**10$^{@}$**, **11$^{@}$**) and the 2-[^{18}F]fluoro-4,5-dimethoxybenzyl bromide (**12$^{@}$**) (Scheme 2 and Table 2). Even where the conversion yield of **5**–**12$^{@}$** was greater than 80%, compounds **10$^{@}$** and **11$^{@}$** (X = Br) were only obtained from **2** and **3** with very low yields, even after long halogenation reaction times (>30 min) at RT (Scheme 2 and Table 2).

In contrast, heating of the wetted support for 2 min at 60 °C allowed a high yield conversion of the benzylic alcohols into the corresponding benzyl bromide derivatives **10$^{@}$**and **11$^{@}$** (Table 2, 60 °C). However, for all investigated compounds, attempts to reduce the concentration of the hydrobromic acid solution from 48% to 24% resulted in low halogenation yields (<10%).

When elution of the SPE cartridge was performed with a small volume of an organic solvent such as toluene or dichloromethane (<3 mL), a second phase appeared in the recovery vial. This phase contained the residual acid which was otherwise still present on the cartridge after flushing with nitrogen. For compounds **12$^{@}$** and **13$^{@}$**, this unwanted acid was eliminated by inclusion of an on-line cartridge containing 1–1.5 g of K_2CO_3 after the tC18. Unfortunately, a degradation of the [^{18}F]fluorobenzyl bromides **10$^{@}$** and **11$^{@}$** occurs on the potassium carbonate cartridge and at the end of the elution, the [^{18}F]fluorocompounds are mainly recovered in the form of alcohols. Obviously, this problem can be circumvented by replacing the carbonate cartridge with a sodium formate and a Na_2SO_4 cartridge after the tC18 Sep-Pak® cartridge. Alternative cartridges containing materials such as KH_2PO_4 and$NaHCO_3$ were also evaluated, but without success.

For compounds **10$^{@}$** and **11$^{@}$**, heating of the cartridge can be avoided if the aqueous concentrated hydrobromic acid is substituted with a solution of HBr in HOAc (33%). However, in this case, it was necessary to trap the previously labeled aldehydes on a tC18 environmental Sep-Pak® cartridge connected to a second oasis HLB cartridge (400 mg). After reduction, as described above,

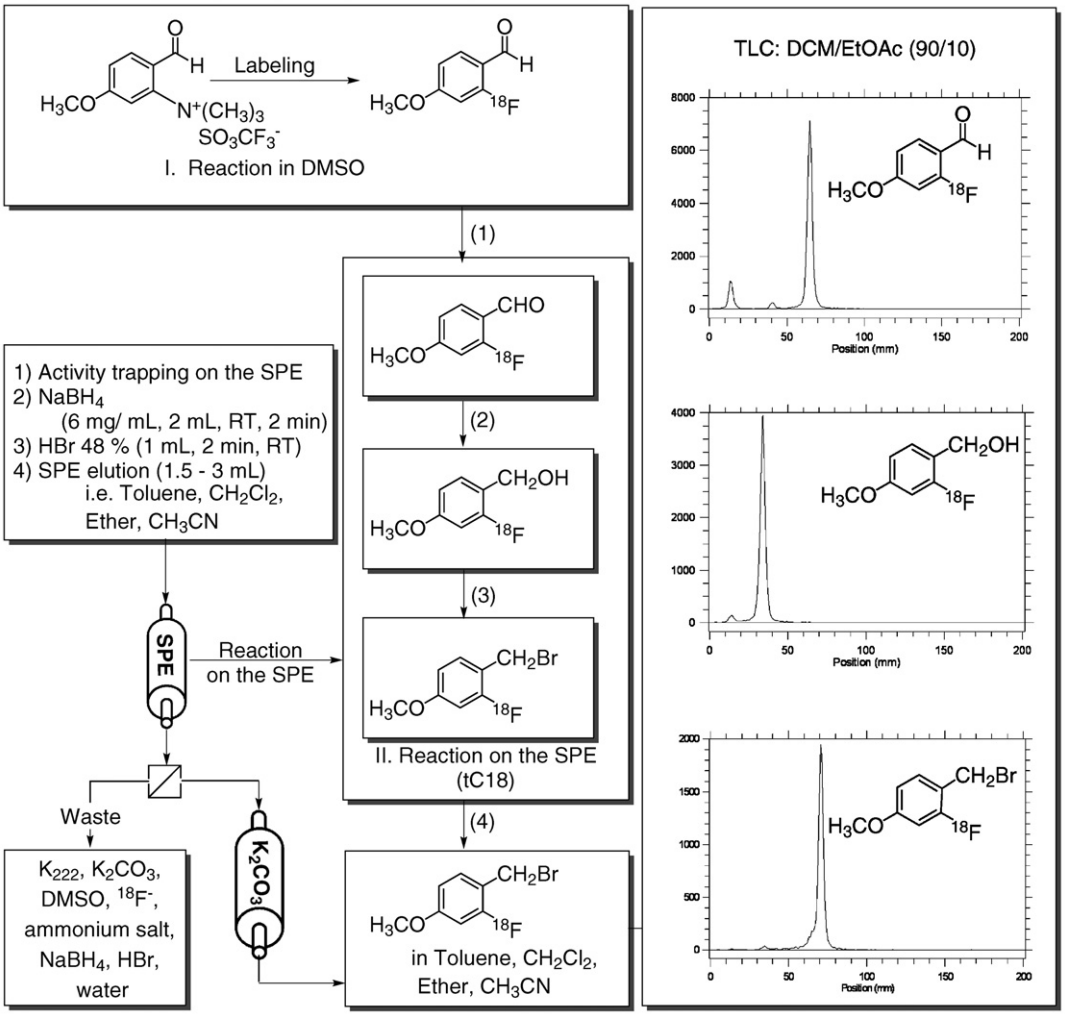


Scheme 2. Synthesis of [^{18}F]fluorobenzyl halide derivatives on a SPE cartridge.

Table 2
Radiochemical yields (RCY) in various [^{18}F]fluorobenzyl halides synthesized, according to Scheme 2, from the corresponding ammonium (@) and nitro (#) precursors ([^{18}F]F trapped on QMA = 100%; *tC18 (400 mg): ** HBr/HOAc [33%]).

[^{18}F]Fluorobenzyl halide [RCY (%), decay corrected]									
	^{18}F	OCH_3	H	X = Cl		X = Br		X = I	
				RT	60 °C	RT	60 °C	RT	60 °C
10$^{@}$	R_1	–	R_2, R_3, R_4		12 ± 2 (n = 3)	<2–3	40 (n = 2)		52 (n = 2)
10$^{\#}$					14		59		62
11$^{@}$	R_3	–	R_1, R_2, R_4		41	<2–3	64 ± 4 (n = 9)		63 ± 5 (n = 6)
11$^{\#}$					36		59		58
12$^{@}$	R_1	R_3, R_4	R_2	40*		40 ± 3* (n = 3)		45 ± 5* (n = 6)	
12$^{\#}$						51*		52*	
13$^{@}$	R_1	R_3	R_2, R_4	60*		63 ± 3* (n = 23)		65 ± 5* (n = 26)	
13$^{\#}$				35*		44*		53*	
14$^{\#}$	R_1	OCH_2O (R_3,R_4)	R_2			48*		65*	
15$^{\#}$	R_3	R_1	R_2, R_4			71		72	
16$^{\#}$	R_1	R_2	R_3, R_4			15			30
						19**			

the support was wetted and kept wet using the HBr in HOAc solution for 2–3 min. Under these conditions, the bromination reaction proceeded at RT in less than 3 min with 0.75 mL of this solution. More than 95% of the activity remained trapped on the tC18 cartridge. Before subsequent elution, the residual HBr/HOAc on the SPE support was neutralized with a sodium formate solution at pH 4. An ammonium formate solution at pH 4 can also be used [29]. During this process, up to 50% of the activity trapped on the tC18 cartridge can be transferred to the oasis HLB support. After toluene elution (3 mL), 2-[^{18}F]fluorobenzyl bromide (**10**$^{@}$) and 4-[^{18}F]fluorobenzyl bromide (**11**$^{@}$) were obtained with a radiochemical yield of 32% and 58 ± 9% (n = 4) respectively. The large



Scheme 3. General strategy and typical TLC profiles observed during the synthesis of 2-[^{18}F]fluoro-4-methoxybenzyl bromide (**13**).

variation in yield observed during this HBr/HOAc halogenation step resulted in the formation of a second unidentified radioactive peak (as observed by radio TLC). At present, no attempts to optimize this step have been made.

In a second set of experiments, HBr was substituted with a concentrated aqueous solution of HCl and HI (Scheme 2, X = Cl and I). The hydroiodic acid (57%) used was commercially available and was not stabilized with hypophosphorous acid. However, it was previously decolorized with red phosphorus. Synthesis of the chloride and iodide derivatives was realized using HCl and HI as described above for the bromide compounds. The main results obtained after 2 min of reaction either at RT or 60 °C are summarized in Table 2. Radiochemical yields of the benzyl halides **10**$^{@}$–**13**$^{@}$ (X = Cl, Br, I) were calculated from the activity trapped on the QMA cartridge (100%) at the start of the synthesis. At the end of the process, the activity eluted from the tC18 cartridge was measured and the radiochemical purity of the various benzyl halides was determined by TLC and HPLC analyses. From this data, radiochemical yields of the [^{18}F]fluorobenzyl halides were calculated.

Considering the radiochemical yields of [^{18}F]fluorobenzaldehyde and [^{18}F]fluorobenzyl halide obtained from ammonium precursors, the conversion rate of [^{18}F]aldehyde to [^{18}F]halide (X = Cl, I) for compounds **11**$^{@}$–**13**$^{@}$ was measured at 56–90%. For compound **10**$^{@}$, this conversion was only 17% for the chlorinated derivative and 75% for the iodide derivatives.

During TLC and HPLC analysis, some decomposition of the benzyl bromide and iodide derivatives can be observed on the TLC.

2.3.2. From nitro precursors

Radiochemical synthesis of these [^{18}F]fluorobenzyl chloro, bromo and iodo derivatives (**10**$^{\#}$–**13**$^{\#}$) was also realized from selected nitro precursors. The radiochemical yields of the final benzyl halide derivatives (X = Cl, Br, I) obtained are also summarized in Table 2. These yields were calculated from the initial activity trapped on the QMA cartridge and corrected for the TLC purity of different synthesized compounds (**10**$^{\#}$–**16**$^{\#}$).

From the data shown in this table, it appears that the halogenation reaction is also feasible from the nitro starting precursor. In this case, high conversion yields of the aldehyde into the corresponding halogenated compound (X = Br, I) were obtained either at RT (compounds **12**$^{\#}$–**15**$^{\#}$) or at 60 °C (compounds **10**$^{\#}$, **11**$^{\#}$, **16**$^{\#}$). Yields were very low for chlorinated compounds **10**$^{\#}$ and **11**$^{\#}$. Moreover, when the reaction was conducted from the 6-nitropiperonal (**1i**, Table 1) the methylenedioxy group protecting the catechols was stable under the conditions developed for halogenation. The same amount of reagents as for the syntheses starting from the ammonium precursor were used.

2.4. Application

One major advantage of the strategy presented in this paper is that the elution of the [^{18}F]fluorobenzyl halide from the C18 support can be realized with a large variety of organic solvents. Toluene, diethyl ether, *tert*-butyl methyl ether, cyclopentyl methyl ether, CH_2Cl_2, $CHCl_3$, DMF, CH_3CN have been successfully used for elution.

The direct synthesis of these benzyl halides (X = Cl, Br, I) **10–16** on the tC18 solid support avoids complex purification and helps to shorten the duration of all processes. Consequently, automation of the synthesis is greatly simplified.

Moreover, since the total eluted volume does not exceed 2–2.5 mL, the [^{18}F]fluorobenzyl halides can be used directly in subsequent reactions without additional evaporation steps.

10: o-^{18}F
11: p-^{18}F
17: o-^{18}F
18: p-^{18}F

Scheme 4. Synthesis of 1-(azidomethyl)-[^{18}F]fluorobenzenes (**17**) and (**18**).

2.4.1. Amino acid synthesis

Starting from an ammonium salt and with an automated system it is now possible to prepare various nca amino acids. For example, with this approach, 2-[^{18}F]fluoro-4-methoxybenzyl halide (**13**) (X = Br, I) was prepared with high radiochemical purity and with high radiochemical yield in less than 25 min. This key intermediate **13** reacts with a glycine derivative under PTC to produce a protected [^{18}F]labeled tyrosine at 90% yields [41]. After hydrolysis and HPLC purification, 2-[^{18}F]fluoro-L-tyrosine was obtained with a radiochemical yield of 45% (d.c.).

2.4.2. Azide synthesis

This solid support strategy was also used to convert the 2 and 4-[^{18}F]fluorobenzyl bromide and iodide derivatives **10** and **11** into the corresponding azide derivatives:1-(azidomethyl)-4-[^{18}F]fluorobenzene (**18**) and 1-(azidomethyl)-2-[^{18}F]fluorobenzene(**17**) (Scheme 4).

In this case, **10** and **11** were synthesized as described above, but before subsequent chemistry, excess acid on the SPE support (HBr 48%, HI 57% or HBr/HOAc 33%) was neutralized with a few mL of a sodium formate solution at pH 4 (see Section 4). The conversion of the benzyl halides **10** and **11** into the corresponding azide derivatives **18** and **17** was then realized on the same cartridge by wetting the support with a saturated aqueous solution of sodium azide. After heating of the cartridges for 5 min at 60 °C, the excess of azide reagent was removed by flushing the SPE support with nitrogen for 2 min. Finally, **17** or **18** were eluted with 2 mL of CH_3CN. These compounds were identified by comparison of their retention times with previously synthesized reference samples [27]. HPLC and TLC analysis showed that the halide conversion into the azide derivative was greater than 95%. According to this procedure, 1-(azidomethyl)-4-[^{18}F]fluorobenzene (**18**) and 1-(azidomethyl)-2-[^{18}F]fluorobenzene (**17**) were obtained in less than 40 min with a decay-corrected radiochemical yield of between 30% and 60% depending of the method used (see Section 4).

3. Conclusions

The nca synthesis of various [^{18}F]fluorobenzyl halides (X = Cl, Br, I) has been greatly simplified by using a solid support strategy and a concentrated solution of acid. By simply changing the nature of the acid (HCl (37%), HBr (48%), HI (57%) or HBr/HOAc (33%)), various (substituted) [^{18}F]fluorobenzyl chloride, bromide and iodide derivatives were easily prepared. Depending on the substrate, all bromination reactions proceeded at RT with either hydrobromic acid in water (HBr 48%) or hydrobromic acid in acetic acid (33%).

Generally, this approach proceeded with reproducible yields and without further modification (except the labeling duration) from either ammonium salts or nitro starting precursors. Starting from the bromide or iodide derivative, 1-(azidomethyl)-4-[^{18}F]fluorobenzene (**18**) and 1-(azidomethyl)-2-[^{18}F]fluorobenzene (**17**) were directly synthesized on the same support with good radiochemical yield and purity.

As the whole process (reduction, halogenation and purification steps) occurs on solid supports, this synthesis was readily

Table 3
Main retention factors [R_f: CH_2Cl_2/EtOAc (90/10)] and retention times [R_t, min.; CH_3CN/water (70/30)] of the labeled [^{18}F]fluorobenzaldehydes and [^{18}F]fluorobenzyl halides (X = Cl, Br, I).

Precursor	[^{18}F]Fluorobenzaldehyde		[^{18}F]Fluorobenzyl halide					
	R_f	R_t (min)	X = Cl		X = Br		X = I	
			R_f	R_t (min)	R_f	R_t (min)	R_f	R_t (min)
1a **1e**	0.87	3.6	0.91	4.42	0.88	4.6	0.89	5.2
1b **1f**	0.82	3.3	0.89	4.4	0.93	4.7	0.90	4.9
1c **1g**	0.77	3.1	0.95	4.1	0.92	4.4	0.92	4.8
1d **1h**	0.65	2.8	0.72	3.3	0.88	3.4	0.74	3.8
1i	0.7	3.5			0.85	4.3	0.86	4.8
1j	0.91	3.4			0.97	4.7	0.92	5.4
1k	0.68	3.6			0.84	4.3	0.84	4.7
1l	0.73	3.6						

implemented in an automated system. Compound **12** (X = Br, I) is of particular interest as it mimics DOPA. Nca preparation of **12** from the ammonium precursor is now possible in less than 25 min with high radiochemical purity and high radiochemical yield [35].

This procedure represents a substantial improvement for nca preparation of [^{18}F]fluoro aromatic benzyl halides (X = Br, I). These are key substrates for the preparation of amino acids and other nca aromatic radiopharmaceuticals and can be synthesized at high levels of radioactivity (>37 GBq) under good radioprotection conditions.

4. Experimental

4.1. General

Nitro precursors, fluoro compounds and other chemicals were purchased from standard commercial sources (ABCR, Acros, Apollo Scientific, Aldrich, HE Chemicals) and were used without further purification. Kryptofix 222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan), K_2CO_3, hydrochloric acid (37%), hydrobromic acid (48%) and hydroiodic acid (57%) were obtained from Merck. Before use, the hydroiodic acid was decolorized by reflux on red phosphorus and after filtration stored at 0 °C. Oxygen-18 enriched water ($H_2{}^{18}O$ > 95%) was obtained from Rotem industry. SPE cartridges tC18 (400 mg), QMA Sep-Pak® (130 mg), Sep-Pak® plus tC18 environmental cartridges (1 g) or HLB cartridge (400 mg) were obtained from Waters (Milford, MA, USA). Sep-Pak® dry cartridge (Na_2SO_4, 2.85 g) were also from Waters. The trifluoromethanesulfonate salts **1a–1d** were synthesized according to procedures reported in the literature [42] and characterized by standard methods. The [^{19}F]fluoro compounds **10**, **11**(X = I); **12** (X = Br and I); **14** (X = Br) were also synthesized according to reported methods [30,35,43,44].

Thin layer chromatography (TLC) was carried out on Merck silicagel 60 F254 silica plates and the radioactive spots were quantitatively analyzed on a Berthold TLC scanner. TLC eluents were similar for all compounds synthesized (CH_2Cl_2/EtOAc, 90/10). Radioactivity was measured in a dose calibrator and all radiochemical yields were decay-corrected. The non-radioactive fluoro compounds were used as reference on the same TLC plates and the UV spot detected at 254 nm.

To confirm the TLC radiochemical purity values, HPLC analyses of the reaction mixture were also performed. The identity of the [^{18}F]labeled compounds was established after injection and coinjection of the corresponding [^{19}F]fluorinated compounds on the same analytical HPLC system. Analytical HPLC was performed with a Waters 600E pump, a manual rheodyne injector (20 μL loop), a 996 Waters PDA detector and the Empower chromatography manager software from Waters. For radioactivity detection, an NaI detector from Eberline was used. A symmetry X-TERRA RP-C18 column (5 μm, 150 mm × 3.9 mm) was eluted with a mixture consisting of CH_3CN/water (70/30) at a flow rate of 1 mL/min. Retention factors (R_f) and retention times (R_t, min) of the different compounds are presented in Table 3.

First experiments were conducted with a low level of activity (100 MBq). For syntheses at a higher level of activity (200 MBq–110 GBq) an automated module was used [41].

4.2. Nca [^{18}F]fluoride production

No-carrier-added [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction by bombardment of enriched ^{18}O–water (>95%) with 18 MeV protons using a negative ion cyclotron (See Ref. [30] for a better description).

4.3. Radiochemical synthesis

All benzyl halides were synthesized in three steps from either trimethyl ammonium or nitro precursors, according to the following methods:

4.3.1. Labeling step

600–800 μL of the ammonium substrate or nitro precursor in Me_2SO (water content 3000 ppm, 15–20 mg/mL) were added to the dry KryptofixK_{222}/[^{18}F]fluoride complex (111–370 MBq), prepared as previously described [30]. The mixture was then allowed to react at 140 °C for 2.5 min. The nitro starting precursor was labeled at the same temperature but for 15 min. After labeling, the Me_2SO solution was diluted with water (20–30 mL) and the whole solution passed through a previously activated tC18 Sep-Pak® cartridge. An environmental tC18 cartridge (1 g) was used for the trapping of aldehydes **2**, **3**, **7–9** (Table 1). For compounds **4–6** a cartridge containing 400 mg of phase material was more suitable. The [^{18}F]fluorobenzaldehyde trapped on the support was then washed with 10 mL of water. For analytical purposes, the activity retained on the cartridge was eluted with 2 mL of CH_3CN and was analyzed by TLC and HPLC (see above). Radiolabeling yields are summarized in Table 1.

4.3.2. Reduction step

Reduction of the aldehyde was realized directly on the column by passing 2–3 mL of an aqueous solution of $NaBH_4$ (6 mg/mL)

through the cartridge. For the subsequent halogenation step, the activity was kept on the support. For analytical purposes, the support was washed with water (5 mL) and the benzyl alcohol eluted from the tC18 cartridge with 2 mL of CH_3CN. TLC and HPLC analyses indicated that the reduction was near quantitative (>95%) for all compounds.

4.3.3. Halogenation step

4.3.3.1. Procedure A. For the on-column halogenation of the benzyl alcohol to the benzyl halides, 0.5–0.8 mL of an aqueous solution of HCl (37%), HBr (48%) or HI (57%) were slowly passed through the support (1–2 min). For compounds **12**–**15** (X = Cl, Br, I), the reaction was conducted at RT and for compounds **10**–**11** and **16** the cartridge was heated by hot air stream from an air dryer at a controlled temperature of 60–65 °C. After reaction, the Sep-Pak® was flushed with nitrogen for 1 min and the [^{18}F]fluorobenzyl halide derivatives were directly eluted with an organic solvent such as CH_3CN, dichloromethane or toluene (3 mL). For compounds **12**–**15**, the residual inorganic acid was removed by passing the solution through a small "home-made" potassium carbonate column (1.5 g). For compounds **10**–**11** and **16**, this cartridge was substituted with a sodium formate cartridge (1.5 g) and an additional commercial Na_2SO_4 cartridge. The [^{18}F]fluorobenzyl halides **10**–**16** were then eluted from the SPE and recovered in about 2.5 mL of an organic solvent (i.e. toluene, DCM, ether). The radiochemical yield and purity of the different products were determined by radio TLC and HPLC as described above (Table 2).

4.3.3.2. Procedure B (For compounds 10, 11 and 16). For the RT on-column bromination of the benzyl alcohol to the benzyl bromide **10**–**11** and **16**, the previously labeled aldehyde was trapped on a tC18 cartridge (1 g) connected to an oasis HLB cartridge (400 mg). After reduction, as described above, 0.5–0.8 mL of hydrobromic acid in acetic acid (33%) was slowly passed through the two SPE cartridges. After 2–3 min of reaction at room temperature (RT), the support was slowly washed with 2 mL of an aqueous solution of sodium formate at pH 4 and then flushed with nitrogen for 1 min. During this washing, part of the activity trapped on the tC18 cartridge was eluted and trapped on the oasis HLB support. The [^{18}F]fluorobenzyl bromide derivatives **10**, **11** and **16** were then directly eluted with an organic solvent such as dichloromethane or toluene (3 mL) through three additional commercial Na_2SO_4 drying cartridges.

The 2 and 4-[^{18}F]fluorobenzyl bromides (**10**–**11**) and **16** were then recovered in 2–2.5 mL of toluene or dichloromethane. The radiochemical yield and purity of these compounds was determined by radio-TLC and radio-HPLC analyses as described above.

The sodium formate solution was prepared by dissolving 13.6 g of this salt in 50 mL of water. The pH of the solution was then adjusted to 4 with HCl (37%).

4.3.4. Synthesis of 1-(azidomethyl)[^{18}F]fluorobenzenes (17) and (18)

4.3.4.1. Via HBr 48% or HI 57%. Synthesis of 2 and 4-[^{18}F]fluorobenzyl halides (**10**–**11**, X = Br, I) was realized with HBr (48%) or HI (57%) as described in procedure A (Section 4.3.3.1). After reaction, the Sep-Pak® was washed with an aqueous solution of sodium formate at pH 4 and the azide synthesis realized on the same cartridge as described above (Section 4.3.4.1). Via the HBr and the HI pathway, the radiochemical yield of 2-[^{18}F]fluorobenzyl azide (**17**) was 56% and 57%, respectively. Via these two different acids, radiochemical yield of 4-[^{18}F]fluorobenzyl azide (**18**) was around 60%.

4.3.4.2. Via HBr/HOAc 33%. Synthesis of 2 and 4-[^{18}F]fluorobenzyl bromides (**10** and **11**) was realized as described in procedure B (Section 4.3.3.2). After reaction, the Sep-Pak® was washed with an aqueous solution of sodium formate at pH 4 (about 2 mL, the pH of the last drop should be around 4) and then flushed with nitrogen for 1 min. Azide synthesis on the cartridge was then realized by passing a saturated aqueous solution of sodium azide (2 mL) through the tC18 support. After heating for 5 min at 60 °C, excess reagent was removed by flushing the SPE support with nitrogen for 2 min. Finally, the 2 and 4-[^{18}F]fluorobenzyl azides (**17**) and (**18**) were eluted with 2.5 mL of CH_3CN and analysed by HPLC on a X-TERRA RP-C18 column (5 μm, 150 mm × 3.9 mm) with a mixture consisting of CH_3CN water (50/50) at a flow rate of 1 mL/min (R_t **17** = 8.8 min.; R_t **11** = 10.1 min., R_t **18** = 8.7 min). Radiochemical purity, determined by TLC analysis (R_f **17** = 0.92; R_f **18** = 0.9 (CH_2Cl_2/AcOEt 90/10)) was above 65%. Radiochemical yields for the 2- and 4-[^{18}F]fluorobenzyl azide compounds (**17** and **18**) were 30 and 55%, respectively.

4.4. Determination of radiochemical labeling yield

After labeling, the Me_2SO solution was withdrawn from the reaction vessel and transferred into a second glass vial. Residual activity in the reaction vessel and the activity transferred (Activity in solution) were counted and corrected for decay. For the determination of the radiochemical yield, TLC and HPLC samples (1–2 μL) withdrawn from the vial containing the activity in solution, were diluted with a small amount of CH_3CN. Radiochemical purities were then determined by TLC analysis. Decay corrected (d.c.) radiochemical yields (RCY) were obtained from the activity trapped on the QMA support according to the following equation:

$$\mathrm{RCY\,(d.c.)} = \frac{[\text{Activity in solution (d.c.)} \times \text{TLC radiochemical purity (\%)}]}{\text{Activity on QMA (d.c.)}}$$

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