Radiofluorination

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Fast Production of Highly Reactive No-Carrier-Added [^{18}F]Fluoride for the Labeling of Radiopharmaceuticals**

Christian F. Lemaire, Joël J. Aerts, Samuel Voccia, Lionel C. Libert, Frédéric Mercier, David Goblet, Alain R. Plenevaux, and André J. Luxen*

Fluorine-18 has become the most widely used short-lived radioisotope for the labeling of radiopharmaceuticals for positron emission tomography (PET; ^{18}F, $t_{1/2} = 109.7$ min).[1] Depending on the mode of production, the no-carrier-added [^{18}F]fluoride ion can be obtained in aqueous solution. However, under these conditions it is strongly hydrated and therefore unreactive for nucleophilic substitution. Several methods have been developed to increase its reactivity.[2,3] Currently, the most well-established procedure requires trapping of the [^{18}F]fluoride ion on an anion-exchange resin and its subsequent elution with a small volume of an organic–aqueous solution (CH_3CN/H_2O, 50:50 v/v) of an inorganic weak base (potassium carbonate) and a cryptand (kryptofix K222).[4] After two or three azeotropic evaporation steps, the cryptand enables solubilization of the [^{18}F]fluoride ion in an active form in a polar aprotic solvent suitable for the subsequent labeling reaction (e.g. CH_3CN, dimethyl sulfoxide). However, this process, which requires several minutes (5–10 min) and complex automation, consumes radiochemical yield (3.1–6.1 %) and is not suited to the miniaturization of PET equipment.

As an example, the synthesis of [^{18}F]fluorodeoxyglucose (FDG), the most used PET metabolic tracer, with microreactors or microfluidic chips was recently reported.[5] The extremely high surface-area-to-volume ratio of these microreactors enables experiments to be performed on a far lower scale than is possible with conventional-scale reactors; lower starting amounts of very expensive precursors are required, the products are purer and obtained in higher yield and in shorter reaction times, and there is less waste. Moreover, hot-cell shielding is greatly facilitated. However, despite all these advantages, the [^{18}F]fluoride is always dried by the conventional method with K_2CO_3.[6] This unavoidable step can be considered as the main limitation to the size reduction of such systems.

Herein, we evaluate a new method for elution from the anion-exchange resin which would avoid the aforementioned azeotropic evaporation step with acetonitrile. This step is typically very difficult to implement on a microchip device. We examined a selection of organic bases as potential additives to replace the inorganic bases or salts classically used in the resin eluent.

A large variety of organic bases that differ, for example, in terms of strength, nucleophilicity, and steric hindrance, are commercially available (some are listed in Table 1 according to their pK_a value). These organic bases usually contain nitrogen atoms, the protonation of which can lead to highly reactive anions.[7]

Table 1: Effect of the nature of the base on the radiochemical yield of **2**.[a]

Entry	Base	pK_a[b]	RY[c] [%]	Water[d] [ppm]
1	none	–	15	4958
2	Et_3N	18.46	30	2392
3	sparteine	21.66	25	2912
4	TMG	23.3	27	1746
5	BTMG	23.56	95	3912
6	DBN	23.89	13	6641
7	DBU	24.3	47	3572
8	TMGN	25.1	87	2621
9	MTBD	25.44	93	4755
10	TBD	25.98	22	4899
11	P$_1$*t*Oct	26.5	81	3543
12	P$_1$*t*Bu	26.9	93	2748
13	BEMP	27.6	92	446
14	BTPP	28.4	76	3877
15	P$_2$Et	32.9	17	3536
16	Verkade *i*Pr	32.9	14	1960
17	P$_4$*t*Bu	41.9	6	2720

[a] Reaction conditions: **1a** (40 mg), CH_3CN (1 mL), solution of $^{18}F^-$ (20 μL), base (50 μmol), 100 °C, 5 min. [b] pK_a value of the conjugate acid in CH_3CN. [c] Radiochemical yield (decay-corrected). [d] Water content after labeling. BEMP = 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine, BTMG = 2-tert-butyl-1,1,3,3-tetramethylguanidine, BTPP = tert-butylimino-tri(pyrrolidino)phosphorane, DBN = 1,5-diazabicyclo[4.3.0]non-5-ene, DBU = 1,8-diazabicyclo-[5.4.0]undec-7-ene, TMG = 1,1,3,3-tetramethylguanidine, MTBD = 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene, P$_1$*t*Bu = *tert*-butylimino-tris-(dimethylamino)phosphorane, P$_1$tOct = *tert*-octylimino-tris(dimethylamino)phosphorane, P$_1$tBu = *tert*-butylimino-tris(dimethylamino)phosphorane, P$_2$Et = *N*′′′-ethyl-*N*,*N*,*N*′,*N*′-tetramethyl-*N*′′-[tris(dimethylamino)phosphoranylidene]phosphorimidic triamide, P$_4$tBu = *N*-[[tert-butylimino-bis[tris(dimethylamino)phosphoranylideneamino]phosphoranyl]-imino-bis(dimethylamino)phosphoranyl]-*N*-methylmethanamine, TBD = 1,5,7-triazabicyclo[4.4.0]dec-5-ene, TMGN = 1,8-bis(tetramethylguanidino)naphtalene, Verkade *i*Pr = 2,8,9-triisopropyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane.

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[*] Dr. C. F. Lemaire, Dr. J. J. Aerts, L. C. Libert, Dr. F. Mercier, D. Goblet, Dr. A. R. Plenevaux, Prof. A. J. Luxen
University of Liège, B30—Cyclotron Research Center
Sart Tilman, 4000 Liège (Belgium)
Fax: (+32) 4-366-2946
E-mail: christian.lemaire@ulg.ac.be

Dr. S. Voccia
Trasis sa
Voie de Liège, 2, 4053 Embourg (Belgium)

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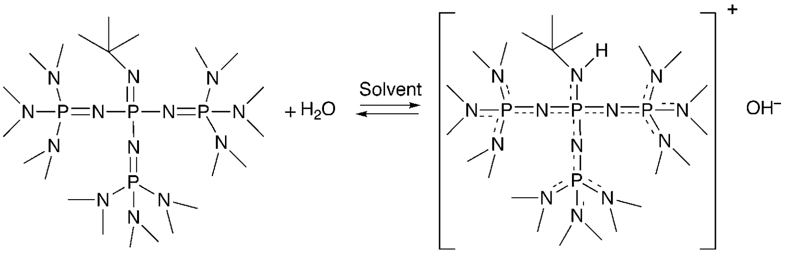
Acetonitrile is the solvent of choice for the [^{18}F]radiolabeling of aliphatic compounds. Generally, this polar aprotic solvent contains a small amount of water (0.01–0.5%). In the presence of a Schwesinger base, such as P$_4$*t*Bu (pK_a = 41.9) or P$_4$*t*Oct (pK_a = 42.7), we believed that the deprotonation of water should occur and that a sufficient amount of hydroxide anion should be present at equilibrium (Scheme 1).[8] In this case, the elution of [^{18}F]fluoride trapped on the Sep-Pak QMA (silica-based hydrophilic strong anion exchange surface, Waters) support should be induced by this hydroxide generated in solution.



Scheme 1. Possible pathway for the generation of hydroxide anions with the strong organic base P$_4$*t*Bu.

The elution of [^{18}F]fluoride was initially attempted with acetonitrile containing different amounts of water (ppm) and the less strong organic base P$_2$Et (pK_a = 32.9), which was chosen over the P$_4$*t*Bu base for ease of handling (Figure 1).

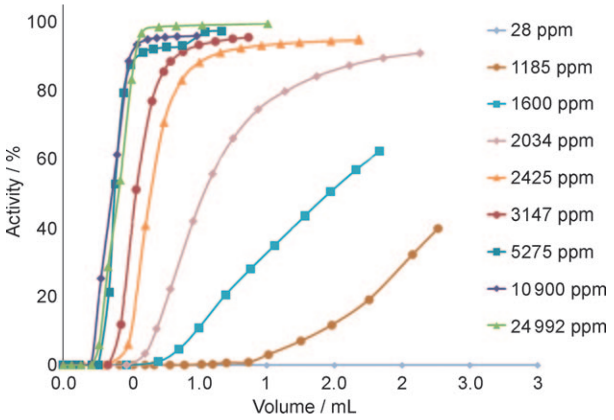


Figure 1. Elution profile for the elution of [^{18}F]fluoride from a QMA cartridge with CH$_3$CN containing the P$_2$Et base (90 µmol) and variable amounts of water (20–25 000 ppm).

From this data it appears that dry acetonitrile does not enable elution of the radioactive fluoride. With amounts of water between 3100 and 25 000 ppm, [^{18}F]fluoride was eluted nearly quantitatively, and the activity on the support was decreased to less than 3%. However, the elution volume was smaller with eluents containing greater amounts of water.

We also evaluated the influence of the strength of several organic bases. Matching amounts of bases with pK_a values between 18 and 42 were used.[9–13] The percentage of elution with a small volume (0.5–1 mL) increased as the pK_a value of the base increased from the low pK_a value of TMG to the high pK_a value of P$_4$*t*Bu (Figure 2). However, pK_a values of around 30 were required for quantitative elution of the [^{18}F]fluoride trapped on the cartridge. Thus, Verkade superbases can also be used for elution.

Similar effects were observed when water was replaced with other dry compounds containing an acidic hydrogen atom, such as alcohols (e.g. methanol, 2-propanol; Figure 3). In this case, the [^{18}F]fluoride is displaced from the cartridge by the alkoxide anions generated by deprotonation of the alcohol, as is well-illustrated by curve A (Figure 3), which shows that no elution occurs until the addition of a small amount of dry methanol to the eluent.

To evaluate the reactivity of the eluted [^{18}F]fluoride, we selected the labeling of the mannose triflate precursor to FDG as a model reaction (Scheme 2, Table 1). Low radiochemical yields were observed when either the P$_2$Et organic base was used or when no organic bases were used (Table 1, entries 1 and 15). However,

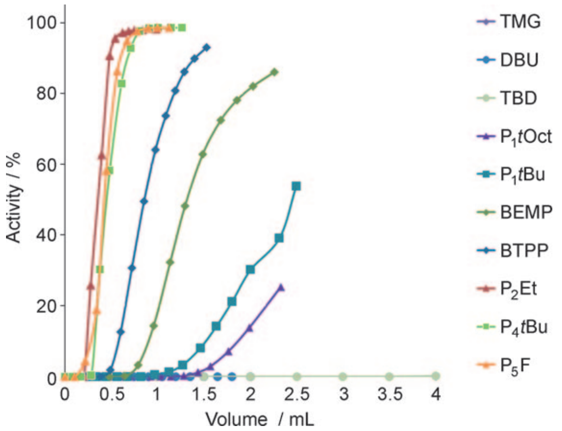
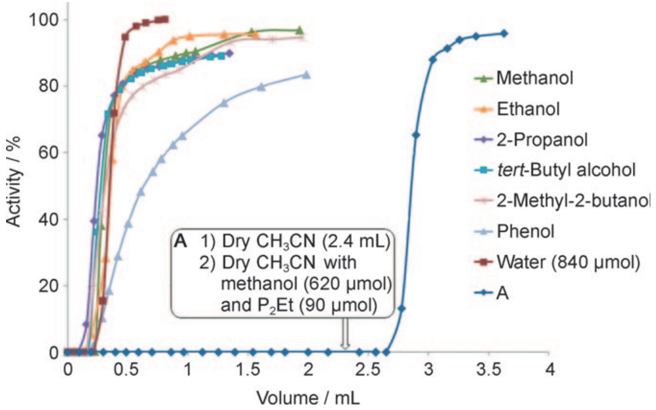
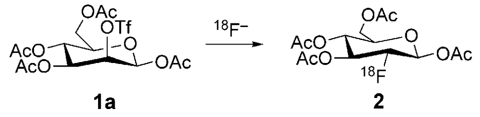


Figure 2. Elution profile for the elution of [^{18}F]fluoride from a QMA cartridge with CH$_3$CN containing water (10 900 ppm) and an organic base (90 µmol). P$_5$F = tetrakis[tris(dimethylamino)phosphoranylidenamino]phosphonium fluoride.



A 1) Dry CH$_3$CN (2.4 mL)
2) Dry CH$_3$CN with methanol (620 µmol) and P$_2$Et (90 µmol)

Figure 3. Elution profile for the elution of [^{18}F]fluoride from a QMA cartridge with dry CH$_3$CN containing a dry alcohol (840 µmol) and P$_2$Et (90 µmol).

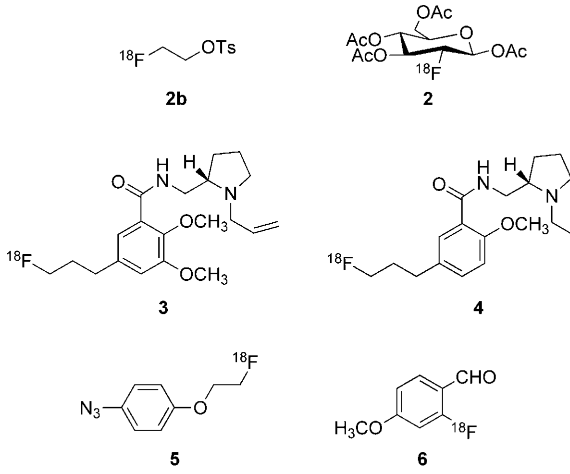


Scheme 2. Labeling step in the synthesis of [^{18}F]FDG (Tf = trifluoromethanesulfonyl).

high (> 85 %) and reproducible radiochemical yields were only observed upon the addition of an organic base (Table 1, entries 5, 8, 9, 12, and 13). These high yields were obtained only with bases having either a guanidine or phosphazene structure, no labile hydrogen atoms, and a pK_a value between 23 and 28. Although aliphatic nucleophilic substitutions are usually performed under basic conditions, the reason for such prerequisite features of these organic bases remains unclear.

Some applications of this new strategy to the synthesis of ^{18}F-labeled aliphatic and aromatic compounds are summarized in Table 2. All precursors investigated were converted into the corresponding ^{18}F-fluorinated compounds (Scheme 3) in the presence of BTMG in high radiochemical yields. In contrast to the conventional K222/K_2CO_3 drying procedure, during which 10–30 % of the activity can be lost by adsorption on the glass reactor surfaces, this method enables the retention in solution of more than 97 % of the activity of the [^{18}F]fluoride eluted from the cartridge until the end of the process. In microsystems, the retention of this activity in solution will positively impact both the radiochemical yield and the radioprotection problems resulting from the residual reactor activity.

The high reactivity of [^{18}F]fluoride is well-demonstrated in the case of **2**, for which a radiochemical yield of 60 % was observed at room temperature (Table 2). Besides acetonitrile/water, classical solvents, such as toluene, with small amounts of an alcohol can be used to elute the fluoride (e.g. toluene/CH_3OH). The use of nonpolar and aprotic solvents, such as toluene, in this context has seldom been described. This modification could aid the development of new applications in [^{18}F]fluorine radiochemistry. Radiochemical TLC purities of the different labeled products (Table 2) were confirmed by HPLC analysis.



Scheme 3. Structures of the labeled compounds (Ts = *p*-toluenesulfonyl).

Finally, in another set of experiments, we demonstrated that this approach can be used for the synthesis of various PET compounds, **1b**–**6** (Table 2). The syntheses were carried out either with 20 mg of the precursor or on a scale more adapted to the potential use of microreactors (0.5–4 mg). Even with these low amounts of precursors, good radiochemical yields were observed for all the compounds investigated. Moreover, in these experiments, compounds **2**, **3**, and **4** were obtained with at least 600 MBq after purification, with both high chemical and high radiochemical purities.

In conclusion, this new elution strategy appears promising when compared to the classical K222/K_2CO_3 method. When one of a variety of organic bases is used, the azeotropic evaporation step is no longer required, and the automation

Table 2: Radiochemical applications.[a]

Labeled compound	Precursor[b] (mg)	Water in the eluent [ppm][c]	QMA elution yield [mL][d]	$^{18}F^-$ solution used for labeling [μL]	BTMG added [μmol]	*t* [min], *T* [°]	TLC purity [%]	RLY [%][e]	Water content [ppm][f]
1b	OTs (20)	5200	0.8 (97.2)	500	73	5, 100	65	63.4[g]	3700
	OTs (4)	6300	0.9 (97)	200	25	5, 120	85	83.3[g]	3200
3	OTs (20)	6300	0.7 (97.9)	700	83	5, 110	90	86.8[h]	3333
	OTs (0.5)	6300	0.9 (98.3)	50	15	5, 120	88	86.4[g]	3150
4	OTs (20)	10900	0.7 (99)	700	98	5, 110	89	86.1[h]	4002
	OTs (1)	6300	0.9 (98.3)	50	15	4,120	86	83.5[g]	2750
5	OMs (20)	10900	1 (99)	500	73	5, 100	75	73.4[g]	4800
	OMs (3.5)	6300[i]	0.9 (91)	500	25	4, 130	78	75.7[g]	–
2	OTf (20)	5275	1 (98.7)	1000	49	5, 100	91	88.9[h]	2442
	OTf (1.8)	6300	0.9 (98.3)	50	15	3, 120	74	71.2[g]	–
2	OTf (20)	6300	1 (98.2)	100	98	5, RT	62	60.8[g]	2100
2	OTf (20)	[j]	1 (93)	300	98	10, 100	93	90.4[g]	–
6	Me_3N^+ TfO^- (20)	6300	0.8 (98.2)	800	98	5, 120	65	61.9[h]	2641
	Me_3N^+ TfO^- (2.5)	6300	0.9(94)	100	15	3, 140	71	68.9[g]	3235

[a] Reaction conditions: precursor (0.5–20 mg), CH_3CN. [b] Leaving group in the precursor to the labeled compound. [c] Eluent: CH_3CN/P_2Et (45 μmol)/water. [d] The yield is given as a percentage in parentheses. [e] Radiolabeling yield (decay-corrected). [f] Amount of water at the end of the labeling process. [g] RLY = activity (%) in solution after labeling × TLC purity. [h] RLY = QMA elution yield (%) × activity (%) in solution after labeling × TLC purity). [i] P_2Et: 30 μmol. [j] Toluene/CH_3OH (6000 ppm). Ms = methanesulfonyl.

steps are greatly facilitated. An appropriate selection of solvent, protic additive, organic base, and temperature should enable optimization of the reactivity of [^{18}F]fluoride. This fast approach opens the way to a new general strategy for the recovery of highly reactive [^{18}F]fluoride in low solvent volumes and is well-suited to the labeling of various starting substrates (aliphatic and aromatic) without the need for the classical water-evaporation step. This approach should be useful for both the direct labeling of sensitive compounds, such as protein and peptides, and the miniaturization of PET equipment.

Experimental Section

Typical procedure: [^{18}F]fluoride was trapped on a QMA cartridge (CO_3^{2-}, Waters). The cartridge was then washed with dry CH_3CN (2–5 mL), and the [^{18}F]fluoride was eluted with a freshly prepared CH_3CN solution (1 mL) containing water (20–25000 ppm) and a strong organic base (45 μmol). ^{18}F fluorination was performed directly with a precursor (0.5–40 mg) previously solubilized in dry CH_3CN (0.5–1 mL), BTMG base (15–98 μmol), and the solution of [^{18}F]fluoride (10–1000 μL). The mixture was heated at 100 °C for 5 min and then cooled to room temperature. The radiochemical yield was determined by TLC and HPLC.

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