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Synthesis of [^{18}F]xenon difluoride as a radiolabeling reagent from [^{18}F]fluoride ion in a micro-reactor and at production scale

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ABSTRACT

[^{18}F]Xenon difluoride ([^{18}F]XeF_2), was produced by treating xenon difluoride with cyclotron-produced [^{18}F]fluoride ion to provide a potentially useful agent for labeling novel radiotracers with fluorine-18 ($t_{1/2}$ = 109.7 min) for imaging applications with positron emission tomography. Firstly, the effects of various reaction parameters, for example, vessel material, solvent, cation and base on this process were studied at room temperature. Glass vials facilitated the reaction more readily than polypropylene vials. The reaction was less efficient in acetonitrile than in dichloromethane. Cs^+ or K^+ with or without the cryptand, K 2.2.2, was acceptable as counter cation. The production of [^{18}F]XeF_2 was retarded by K_2CO_3, suggesting that generation of hydrogen fluoride in the reaction milieu promoted the incorporation of fluorine-18 into xenon difluoride. Secondly, the effect of temperature was studied using a microfluidic platform in which [^{18}F]XeF_2 was produced in acetonitrile at elevated temperature (≥85 °C) over 94 s. These results enabled us to develop a method for obtaining [^{18}F]XeF_2 on a production scale (up to 25 mCi) through reaction of [^{18}F]fluoride ion with xenon difluoride in acetonitrile at 90 °C for 10 min. [^{18}F]XeF_2 was separated from the reaction mixture by distillation at 110 °C. Furthermore, [^{18}F]XeF_2 was shown to be reactive towards substrates, such as 1-((trimethylsilyl)oxy)cyclohexene and fluorene.

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1. Introduction

Positron emission tomography (PET) is increasingly applied to drug development [1] and clinical research [2] as a molecular imaging modality in animal and human subjects. PET can measure biochemical targets and processes provided that specific radiotracers labeled with a positron-emitting radionuclide are available. The development of such radiotracers is therefore an active field of research [3]. Fluorine-18 ($t_{1/2}$ = 109.7 min) is particularly attractive as a label because it can mimic hydrogen or hydroxyl in organic molecules. Furthermore, fluorine-18 can be produced in very high activities (multi-Curies) with moderate energy cyclotrons according to the ^{18}O(p,n)^{18}F nuclear reaction from ^{18}O-enriched water [4]. This process produces [^{18}F]fluoride ion, which can be used directly in nucleophilic substitution reactions to introduce fluorine-18 at aliphatic and aryl carbons bearing suitable leaving groups [3]. However, [^{18}F]fluoride ion cannot be used directly to replace hydrogen at aryl or alkyl C–H groups. Electrophilic reagents, such as [^{18}F]fluorine gas [5] and [^{18}F]acetyl hypofluorite [6], have been used for this purpose, but these labeling reagents cannot be prepared in a single step from cyclotron-produced [^{18}F]fluoride ion.

Xenon difluoride has a rich chemistry for fluorinating organic molecules, including arenes, alkenes, enols, thioethers, aryl aldehydes and aryl ketones under relatively mild conditions [7]. Xenon difluoride also performs fluorodeiodination [8], fluorodesilylation [9] and fluorodecarboxylation [10,11] reactions. Hence, ^{18}F-labeled xenon difluoride ([^{18}F]XeF_2) has long been recognized as a potentially useful 'electrophilic' radiofluorination agent. However, this potential has not been realized, mainly because of the lack of a practical means for producing [^{18}F]XeF_2 rapidly in useful activities (i.e., multi-mCi). In fact, only a few reports have described the use of [^{18}F]XeF_2 to prepare a PET radiotracer [12].

[^{18}F]XeF_2 was earlier prepared by the reaction of [^{18}F]fluorine with xenon [13], by the ^{19}F(p,pn)^{18}F nuclear reaction on XeF_2 [12b], and by 'fluorine exchange' of xenon difluoride with either [^{18}F]hydrogen fluoride [14] or [^{18}F]fluoride ion [15]. The exchange with [^{18}F]fluoride ion is attractive because of its direct use of readily available cyclotron-produced [^{18}F]fluoride ion. Thus, [^{18}F]XeF_2 was obtained from [^{18}F]fluoride ion (as [^{18}F]F^-–Cs^+–K 2.2.2) in dichloromethane in a 'glassy carbon' vessel at room temperature [15]. However, reaction of xenon difluoride with [^{18}F]F^-–K^+–K 2.2.2 in dichloromethane in either a glass or a Teflon vessel at room temperature did not produce [^{18}F]XeF_2 [16]. These seemingly dissonant results prompted us to pursue a more extensive investigation of the effects of reaction parameters, for example, vessel material, solvent, cation and base on the production of [^{18}F]XeF_2. Moreover, interest in applying micro-reactor technology

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to radiochemistry with fluorine-18 has recently increased, since it has been demonstrated that the use of this technology is advantageous for radiochemistry optimization and radiotracer production [17]. Here we also took advantage of a microfluidic device to study the synthesis of [^{18}F]XeF_2 from [^{18}F]fluoride ion at elevated temperatures. Finally, results from these studies in glass or plastic vials and in a micro-reactor facilitated our development of a new method for producing [^{18}F]XeF_2 on a multi-mCi scale.

2. Results and discussion

This study began with the replication of published results [15] and culminated in a new method for producing [^{18}F]XeF_2 on a multi-mCi scale from cyclotron-produced [^{18}F]fluoride ion. Essentially, experiments in closed reaction vials, at room temperature, showed that [^{18}F]XeF_2 production was low or slow in polypropylene vials and also in acetonitrile, relative to reactions in borosilicate glass vials or dichloromethane, respectively, whereas experiments in a silica glass micro-reactor showed that [^{18}F]XeF_2 could be produced in acetonitrile at elevated temperature. These key findings were exploited to devise the method for producing [^{18}F]XeF_2 on a multi-mCi production scale.

2.1. Synthesis of [^{18}F]xenon difluoride in conventional reaction vials at room temperature

Reactions of xenon difluoride are well-known to be influenced by the reaction vessel material [11,18,19]. Whether Pyrex (borosilicate) glass might be compatible with [^{18}F]XeF_2 production was a question of primary interest, since our original report had described [^{18}F]XeF_2 production in a 'glassy carbon' vessel [15]. Glassy carbon is not a glass, but a vitreous carbon [20]; its inert surface therefore clearly differs from that of borosilicate glass, which shows protolytic behaviour [21]. For comparison we also tested readily available plastic (polypropylene) vials.

Reactions of xenon difluoride may also depend on solvent [19]. In our original report [15], dichloromethane supported [^{18}F]XeF_2 production in glassy carbon but acetonitrile failed to do so. We therefore tested reactions in both solvents. It has been suggested that the production of [^{18}F]XeF_2 is promoted by hydrogen fluoride [16] that may be generated from the reaction of excess xenon difluoride or fluoride ion [22] with organic reaction components, such as the solvent (dichloromethane) or the kryptand (K 2.2.2). Reactions were therefore conducted with and without K 2.2.2, and also with different amounts of base (carbonate) that might be expected to consume any initially generated hydrogen fluoride. All reactions in this part of the study were carried out at room temperature.

2.1.1. In the presence of Cs^+–K 2.2.2 and NCA [^{18}F]fluoride ion

In glass vials, a reaction of no-carrier-added (NCA) [^{18}F]fluoride ion with a low amount of xenon difluoride (11 μmol) in the presence of Cs^+–K 2.2.2 in dichloromethane (300 μL) for 90 min produced no [^{18}F]XeF_2 but an increase in amount of xenon difluoride to 27 μmol increased RCY to 26% over 90 min. By use of an even higher amount of xenon difluoride (106 μmol), a much higher RCY (78%) was obtained after 90 min.

The presence of K 2.2.2 proved unnecessary, since comparable RCYs (76% vs. 78%) were obtained in its absence and somewhat more rapidly. This finding is consistent with the high reactivity of $Cs^{18}F$ in organic solvent [23], and also the weaker capacity of K 2.2.2 to bind the Cs^+ cation relative to its ability to bind the smaller K^+ cation [24]. However, the inclusion of K 2.2.2 was expedient, since it greatly reduced deposition of [^{18}F]fluoride ion onto glass vessel walls [25] and consequently improved the efficiency of radioactivity transfer from the glass vessel that was initially used for drying of the reagent to the vessel subsequently used for its reaction.

When the reaction vial material was changed from glass to polypropylene, with Cs^+–K 2.2.2 as cation and dichloromethane as solvent, the RCY of [^{18}F]xenon difluoride was very low (3%) when using a low amount (64 μmol) of xenon difluoride in a 90 min reaction, but became appreciable (31%) when the amount of xenon difluoride was nearly doubled.

In essence, the results obtained here in borosilicate glass vials in the presence of Cs^+–K 2.2.2 and NCA [^{18}F]fluoride ion accord with those obtained previously in glassy carbon reaction vials [15].

2.1.2. In the presence of K^+–K 2.2.2 and NCA [^{18}F]fluoride ion

The decay-corrected radiochemical yields (RCYs) of [^{18}F]XeF_2 from reactions of xenon difluoride with NCA [^{18}F]fluoride ion in the presence of K^+–K 2.2.2 under various conditions are listed in Table 1.

When K^+–K 2.2.2 was used as counter cation, the trend in RCYs was similar to that with Cs^+–K 2.2.2. A combination of low xenon difluoride amount (56 μmol), dichloromethane as solvent and glass as vial material gave [^{18}F]XeF_2 in 22% RCY after 120 min (Table 1, entries 1–3), while an approximate doubling of the amount of xenon difluoride resulted in 79% RCY after only 60 min (Table 1, entries 4 and 5). In contrast to the results obtained with Cs^+ as cation, omission of K 2.2.2 (Table 1, entries 6 and 7) almost halved the RCY (c.f. entries 7 vs. 5). This finding appears consistent with the shorter charge separation and therefore lower nucleophilicity of the $K^+/^{18}F^-$ ion pair compared to that of the K^+–K 2.2.2/$^{18}F^-$ ion pair. Only very low RCYs were obtained in a polypropylene vessel, even with a higher amount of xenon difluoride (Table 1, entries 8–11).

With the reaction still performed in a glass vial with K^+–K 2.2.2 as cation and with a low amount of xenon difluoride, change of solvent to acetonitrile resulted in no [^{18}F]XeF_2 after 120 min (Table 1, entry 12). However, when the amount of xenon difluoride was almost doubled, a high RCY of [^{18}F]XeF_2 was rapidly obtained (Table 1, entries 13 and 14). No yield was obtained in a polypropylene vial (Table 1, entries 15). By contrast, when K_2CO_3 was used in larger amounts than usual (Table 1, entries 16 and 17), production of [^{18}F]XeF_2 was almost completely inhibited.

2.1.3. In the presence of K^+–K 2.2.2 and CA [^{18}F]fluoride ion

The RCYs of [^{18}F]XeF_2 from reactions of xenon difluoride with carrier-added (CA) [^{18}F]fluoride ion in the presence of K^+–K 2.2.2 under various conditions are listed in Table 2. In glass vials, when the base, K_2CO_3, was replaced with KF (3 μmol), the RCYs followed the general trend observed in Table 1. The early rate of [^{18}F]XeF_2 production was however noticeably increased, since RCYs at 10 min were already substantial (Table 2, entries 1, 5 and 9). In polypropylene vials with KF replacing K_2CO_3, RCYs were generally low or very low (Table 2, entries 3, 7 and 11).

2.1.4. General trends and possible mechanisms

The data presented in Tables 1 and 2 revealed some strong general trends. First, reactions conducted in glass vessels occurred more readily than those in polypropylene vessels. Second, reactions conducted in dichloromethane gave higher RCYs than those in acetonitrile. Third, reactions conducted with low amounts of xenon difluoride (<70 μmol) gave low RCYs and those with high amounts (100–130 μmol) high RCYs. Fourth, the amount of added carbonate base (K_2CO_3) had a dramatic effect on reaction outcome; reactions proceeded readily in the absence of carbonate but were suppressed by higher amounts of carbonate. These observations with regard to the effect of various parameters on RCY are generally consistent with a major role for hydrogen fluoride in promoting the formation of [^{18}F]XeF_2, as proposed previously [16].

Hydrogen fluoride might be generated through reaction of xenon difluoride with the reaction vessel wall, trace water [26], solvent [27] or other susceptible material (e.g., K 2.2.2) [16] in the

Table 1
RCYs of [^{18}F]XeF_2 from the treatment of xenon difluoride with no-carrier-added (NCA) [^{18}F]fluoride ion in the presence of K^+ or K^+–K 2.2.2 as counter ion at RT, using different vial materials, amounts of xenon difluoride, solvents, and reaction times.

	Vessel	XeF_2 (μmol)[a]	K_2CO_3 (μmol)	K 2.2.2 (μmol)	Solvent	Time (min)	RCY of [^{18}F]XeF_2 (%)[b]
1	Glass	56	3.6	11	CH_2Cl_2[c]	10	n.d.
2	Glass	56	3.6	11	CH_2Cl_2	60	8
3	Glass	56	3.6	11	CH_2Cl_2	120	22
4	Glass	122	3.6	11	CH_2Cl_2	10	66
5	Glass	122	3.6	11	CH_2Cl_2	60	79
6	Glass	125	3.0	0	CH_2Cl_2	10	8
7	Glass	125	3.0	0	CH_2Cl_2	60	40
8	Polypropylene	62	3.6	11	CH_2Cl_2	10	n.d.
9	Polypropylene	62	3.6	11	CH_2Cl_2	60	n.d.
10	Polypropylene	121	3.6	11	CH_2Cl_2	50	1
11	Polypropylene	121	3.6	11	CH_2Cl_2	90	3
12	Glass	58	3.6	11	MeCN[d]	120	n.d.
13	Glass	118	3.6	11	MeCN	10	67
14	Glass	118	3.6	11	MeCN	60	67
15	Polypropylene	125	3.6	11	MeCN	120	n.d.
16	Glass	95	13.9	41.7	MeCN	10–180	2–3
17	Glass	122	23.1	69.3	MeCN	10–180	n.d.

Data within horizontal lines are from the time-course of a single reaction mixture.
n.d. = not detected (<1%).
[a] Reaction volume was 300 μL.
[b] Calculated as % peak area in the HPLC radio-chromatogram of the reaction mixture.
[c] Efficiency of [^{18}F]F^-–K^+–K 2.2.2 transfer from preparation vessel to reaction vessel was ~20%.
[d] Efficiency of [^{18}F]F^-–K^+–K 2.2.2 transfer from preparation vessel to reaction vessel was ~80%.

reaction medium. Thus, xenon difluoride is less stable in Pyrex glass vessels than in Teflon-FEP vessels [19]. The instability in glass is attributed to the acidity of the surface, which may therefore generate some hydrogen fluoride from fluoride ion. This may explain why [^{18}F]XeF_2 production was generally greater in glass vessels. Xenon difluoride is reduced slowly with water at room temperature and produces hydrogen fluoride [26]. Xenon difluoride is known to react readily with dichloromethane at RT, but more stubbornly with acetonitrile, in each case to generate the HF_2^- anion, presumably via the generation of hydrogen fluoride [26]. In turn, this may explain why the RCYs of [^{18}F]XeF_2 were generally lower in acetonitrile than in dichloromethane. Xenon difluoride has been shown to react slowly with K 2.2.2, also with the likely production of hydrogen fluoride [16]. It seems likely that incorporation of fluorine-18 into xenon difluoride only occurs readily once any generated hydrogen fluoride has been produced in excess of the available base (CO_3^{2-}).

Originally, it was proposed that [^{18}F]XeF_2 production was catalyzed in glassy carbon vessels by Cs^+–K 2.2.2 complex [15], and subsequently this proposal was questioned [16]. However 'naked' fluoride ion does exchange with xenon difluoride under rigorously anhydrous and HF-free conditions [16]. Also, remarkably, aqueous hydrogen/potassium [^{18}F]fluoride slowly exchanges with xenon difluoride at low temperature (~0.8% after 2 h at 0 °C) [26]. The [^{18}F]fluoride ion used in our reactions was 'dried' by azeotropic evaporation of the ^{18}O-water with acetonitrile, and would therefore not be completely 'naked' but hydrated to a low and unknown extent [28]. Nevertheless, the reactivity of this reagent would be much greater than that of aqueous fluoride ion. Therefore, it is conceivable that a direct exchange mechanism

Table 2
RCYs of [^{18}F]xenon difluoride from the treatment of xenon difluoride with carrier-added (CA) [^{18}F]fluoride ion in the presence of KF or KF–K 2.2.2 at RT.

	Vessel	XeF_2 (μmol)	KF (μmol)	K 2.2.2 (μmol)	Solvent	Time (min)	RCY of [^{18}F]XeF_2 (%)[a]
1	Glass	56	3	0	MeCN[b]	10	20
2	Glass	56	3	0	MeCN	40	19
3	Polypropylene	60	3	0	MeCN	10	15
4	Polypropylene	60	3	0	MeCN	40	21
5	Glass	90	3	0	CH_2Cl_2[c]	10	72
6	Glass	90	3	0	CH_2Cl_2	40	72
7	Polypropylene	102	3	0	CH_2Cl_2	10	n.d.
8	Polypropylene	102	3	0	CH_2Cl_2	40	2
9	Glass	118	3	3.3	MeCN	10	43
10	Glass	118	3	3.3	MeCN	40	71
11	Polypropylene	107	3	3.3	MeCN	10	n.d.
12	Polypropylene	107	3	3.3	MeCN	40	n.d.

Data within horizontal lines were obtained by following the time-course of a single reaction mixture.
n.d. = not detected (<1%).
[a] Calculated as % peak area of the HPLC radio-chromatogram of the reaction mixture.
[b] Efficiency of K^+[^{18}F]F^- transfer from preparation vessel to reaction vessel was ~80% in the presence of K 2.2.2 and ~20% in its absence.
[c] Efficiency of K^+[^{18}F]F^- transfer from preparation vessel to reaction vessel was ~20%.

may be operational under some circumstances, such as in the reaction conducted in a glass vessel with $Cs^{18}F$ in dichloromethane, which rapidly gave high RCYs of [^{18}F]XeF_2.

2.2. Synthesis of [^{18}F]xenon difluoride in a micro-reactor

In experiments in glass or polymer vials, acetonitrile provided several clear advantages over dichloromethane as reaction solvent. First, it was the solvent of choice for obtaining dry [^{18}F]F^-–K^+–K 2.2.2 from cyclotron-irradiated ^{18}O-enriched water. Treatment of aqueous [^{18}F]fluoride ion in the presence of potassium carbonate and K 2.2.2 to cycles of acetonitrile addition and evaporation rapidly provided this reagent. Moreover this radiofluorination reagent was more soluble in acetonitrile than in dichloromethane. Thus, typically, only about 20% of the dried [^{18}F]F^-–K^+–K 2.2.2 could be transferred efficiently out of a glass preparation vessel in dichloromethane solution, while 80% or more could be transferred in acetonitrile solution. Furthermore, [^{18}F]XeF_2 generated in acetonitrile solution could be stored in a polypropylene vial for several hours without decomposition. Finally, any unreacted [^{18}F]fluoride ion could be readily removed from acetonitrile solutions of [^{18}F]XeF_2 by filtration of the reaction mixture through a small plug of silica gel. Thus, we were interested to test whether [^{18}F]XeF_2 could be produced in acetonitrile at elevated temperature, and proposed to test this on a small reaction scale in a commercially available microfluidic device.

The microfluidic platform used in this study allowed radiofluorination reactions to be performed under controlled conditions of reaction time, temperature, reagent concentrations and reagent stoichiometry, as has been previously described [29]. The micro-reactor itself is composed of narrow-bore (100 μm i.d.) fused-silica glass tubing. We established that the silica glass micro-reactor was resistant to dilute xenon difluoride solutions and could be exposed repetitively without obvious damage or incident, even at raised temperatures.

Reaction (or residence) times in the micro-reactor were determined by the total rate of influx of reagents. In initial experiments, the total rate of influx was 20 μL/min, with xenon difluoride solution and [^{18}F]F^-–K^+–K 2.2.2 solution each infused at 10 μL/min, giving a reaction time of about 94 s. For reactions conducted with [^{18}F]fluoride ion in the presence of K^+–K 2.2.2 in acetonitrile, no reaction occurred below 65 °C. At 85 °C, [^{18}F]XeF_2 was detected (Fig. 1). At 125 °C, nearly half the radioactivity was incorporated into [^{18}F]XeF_2. When the counter ion was changed to Cs^+ (K 2.2.2 present), exchange was clearly observable, even below 65 °C (chromatogram not shown). Cs^+ was not however the cation of choice because the efficiency for transfer of $^{18}F^-$–Cs^+–K 2.2.2 from the drying vessel into the storage loop of the micro-reactor system was low (19%).

The concentration of xenon difluoride within the micro-reactor was 135 mM. At any instant the amount of xenon difluoride present in the micro-reactor (internal volume 31.4 μL) would have been about 700 μg (~4 μmol). Therefore, the micro-reactor enables low amounts of xenon difluoride to be used relative to that used in a sealed reactor. Thus, simultaneous use of a high activity of [^{18}F]fluoride ion (e.g., 1 Ci in the storage loop) would have potential to produce a specific radioactivity greater than 30 mCi/μmol, which is comparable to or better than that normally obtained for other electrophilic radiofluorination agents. For example, the specific radioactivity of cyclotron-produced [^{18}F]fluorine gas is typically in the 1–10 mCi/μmol range [30].

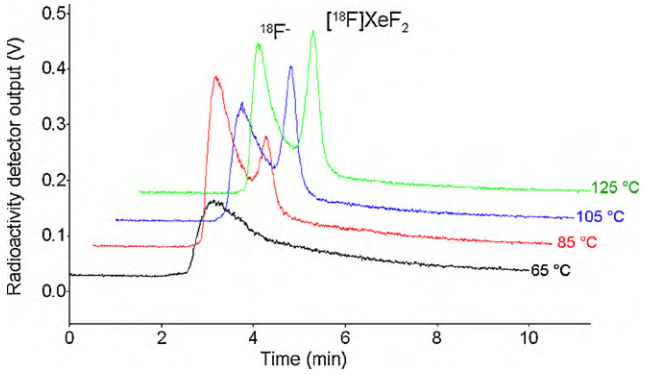


Fig. 1. Stacked plot of radio-HPLC chromatograms showing synthesis of [^{18}F]xenon difluoride in a micro-reactor using [^{18}F]F^-–K^+–K 2.2.2 and xenon difluoride as reagents in acetonitrile at different temperatures.

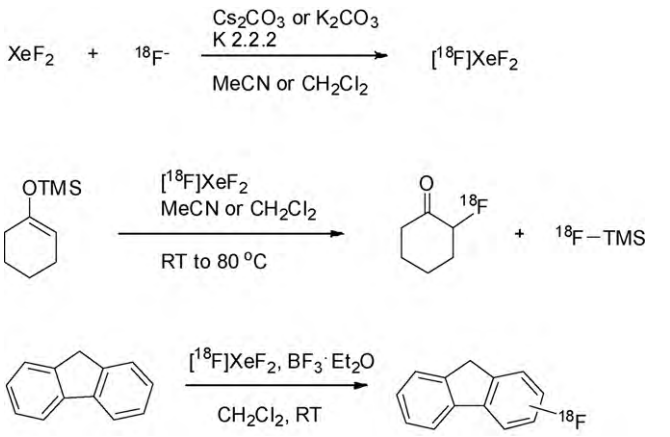
2.3. Large-scale production of [^{18}F]xenon difluoride

The micro-reactor experiments showed that a high RCY of [^{18}F]XeF_2 could be produced rapidly in acetonitrile by heating the reaction mixture to around 125 °C. In view of this finding, the reaction was carried out at production scale in conventional Pyrex glass apparatus (Table 3). Reaction was performed at 90 °C, because in a crimp-sealed vial it was found difficult to contain xenon difluoride in acetonitrile above this temperature. No more than 115 mCi of [^{18}F]fluoride ion were used in order to avoid excessive personal radiation burden in the absence of fully automated equipment. However, the results are expected to be fully scalable to the highest activities of [^{18}F]fluoride ion that can be produced from a single cyclotron irradiation (multi-Ci). It was found that [^{18}F]XeF_2 could be separated from inorganic material and K 2.2.2 in the reaction mixture by filtration through a plug of silica gel or by distillation at 110 °C through a double-tipped needle into a collection vial. The overall RCY of [^{18}F]XeF_2 varied between 13 and 43%, but was highest when a high amount of xenon difluoride was used (Table 3).

Table 3
Multi-mCi production of [^{18}F]xenon difluoride from [^{18}F]fluoride ion.

	XeF_2 (μmol)[a]	[^{18}F]F^- ion reagent (mCi)	Activity collected (mCi)	Decay-correction of collected activity (mCi)	[^{18}F]XeF_2 as % of collected activity	RCY of [^{18}F]XeF_2 (%)[b]
1	34	19.7	1.74	2.01	0	0
2	56	16.8	6.30	7.38	44	19
3	102	25.0	7.20	8.33	55	18
4	109	115	28.7	33.4	45	13
5	114	31.3	5.85	12.0	58	22
6	137	26.8	9.88	11.6	71	31
7	143	109	43.1	51.8	58	28
8	172	15.5	5.40	6.24	40	16
9	179	17.4	5.00	6.77	52	20
10	253	23.4	7.89	13.3	76	43

[a] Reaction volume, 300 μL.
[b] Decay-corrected to the start of reaction from [^{18}F]fluoride ion reagent.

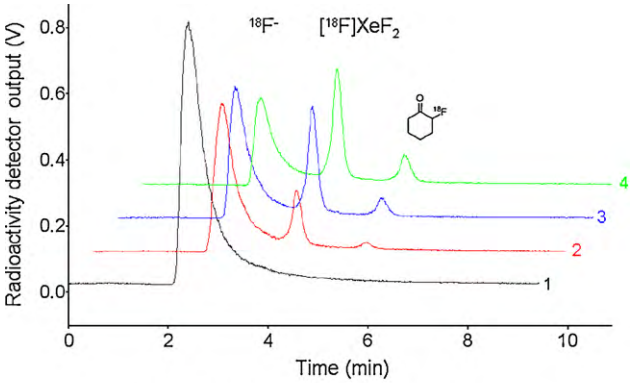


Scheme 1. Synthesis of [^{18}F]xenon difluoride from [^{18}F]fluoride ion and use of [^{18}F]xenon difluoride as a radiofluorination agent.

2.4. Reactivity of [^{18}F]xenon difluoride

The reactivity of [^{18}F]xenon difluoride, produced in either the micro-reactor or on a larger scale in a glass vial, was tested with the known substrates 1-((trimethylsilyl)oxy))cyclohexene [19] and fluorene [31] (Scheme 1).

The synthesis of [^{18}F]XeF_2 and its subsequent reaction with 1-((trimethylsilyl)oxy))cyclohexene in acetonitrile were achieved at micro-reactor scale by utilizing the two micro-reactors and three available delivery pumps of the apparatus. The first micro-reactor was used for [^{18}F]XeF_2 production and the second for its reaction with a substrate (Fig. 2). When the solvent, xenon difluoride concentration and infusion speeds were kept constant, the RCY of [^{18}F]XeF_2 increased with reaction temperature, and consequently the RCY of the product [^{18}F]2-fluorocyclohexanone also increased. When both micro-reactors were maintained at room temperature no product was observed. When reactor 1 was heated to 100 °C, [^{18}F]XeF_2 was produced. The resulting [^{18}F]XeF_2 reacted with 1-((trimethylsilyl)oxy))-cyclohexene at room temperature in micro-reactor 2 to give [^{18}F]2-fluorocyclohexanone. When the temperature of reactor 2 was increased, [^{18}F]2-fluorocyclohexanone production also increased, albeit still to a low RCY (Fig. 3). The identity of the [^{18}F]2-fluorocyclohexanone was confirmed by GC analysis.



HPLC trace	*T* of reactor 1 (°C)	*T* of reactor 2 (°C)
1	RT	RT
2	100	RT
3	100	60
4	100	80

Fig. 3. Stacked plot of radio-HPLC chromatograms showing reaction of [^{18}F]xenon difluoride with 1-((trimethylsilyl)oxy))cyclohexene in a micro-reactor at different temperatures. Note [^{18}F]fluorotrimethylsilane (not shown) eluted at 20.8 min.

When the radiofluorination of 1-((trimethylsilyl)oxy))cyclohexene was carried out in acetonitrile in a glass vessel with [^{18}F]XeF_2 that had been distilled out of a Pyrex glass reaction vial, the RCY of [^{18}F]2-fluorocyclohexanone was 38%. The remainder of the radioactivity was comprised of [^{18}F]fluorotrimethylsilane (40%) and unreacted [^{18}F]fluoride ion (22%). Hence, the reaction

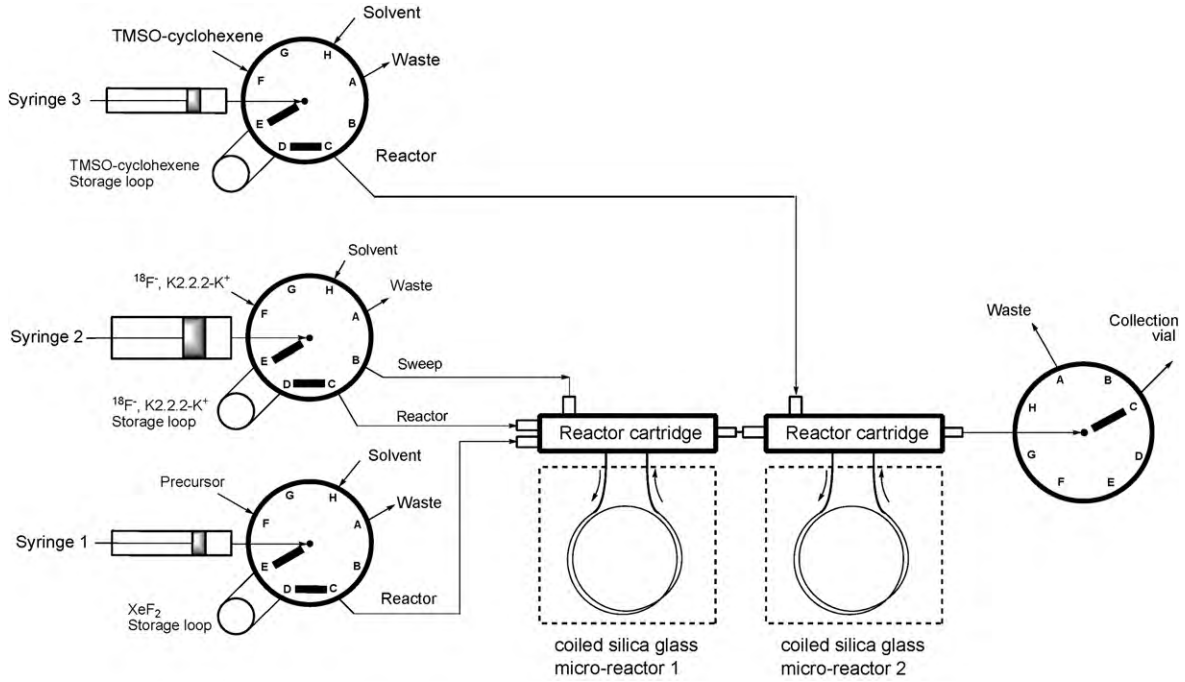


Fig. 2. A schematic illustration of micro-reactors, reagent storage and delivery, product collection valves, and flow directions for reaction of [^{18}F]xenon diluoride (produced in micro-reactor 1) with TMSO-cyclohexene in micro-reactor 2.

had proceeded to 78% of the theoretical RCY. In this type of procedure, acetonitrile distils over with the [^{18}F]XeF_2 reagent, restricting the use of the reagents to reactions in acetonitrile or acetonitrile mixtures.

Reactions of xenon difluoride are commonly performed in other solvents, besides acetonitrile, such as dichloromethane [7]. We therefore tested whether [^{18}F]XeF_2 produced in dichloromethane could be used as a labeling agent in the same solvent. [^{18}F]XeF_2 produced in dichloromethane in a glass vessel was treated with fluorene in the same pot. [^{18}F]Fluorofluorenes were obtained in low RCYs (5–10%). Only one radioactive peak was observed in the HPLC analysis of product. Further analysis of this product by GC however showed that two radiofluorinated products had been formed, consistent with the previously observed production of 2-fluoro- and 4-fluoro-fluorene in 23% yield from the reaction of xenon difluoride with fluorene in dichloromethane for 3 h at room temperature [31].

3. Conclusion

In conclusion, [^{18}F]XeF_2 was produced by treating xenon difluoride with cyclotron-produced [^{18}F]fluoride ion in dichloromethane at room temperature or in acetonitrile at elevated temperature. [^{18}F]XeF_2 was obtained on a multi-mCi scale through reaction of [^{18}F]fluoride ion with xenon difluoride in acetonitrile at 90 °C for 10 min followed by separation from reaction mixture by distillation at 110 °C. Studies are now in progress to explore further the utility of this labeling agent and to improve the specific radioactivity of this labeling agent over that currently attainable.

4. Experimental

4.1. General procedures

Xenon difluoride (99.99%), kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, K 2.2.2; 98%), ammonium formate (99.995%), potassium carbonate (99%), cesium carbonate (99%), potassium fluoride (99.99%), dichloromethane (anhydrous, ≥ 99.8%), acetonitrile (anhydrous, 99.8%), 1-((trimethylsilyl)oxy)cyclohexene (99%), and fluorene (98%) were purchased from Sigma–Aldrich (Milwaukee, WI) and used as received. Acetonitrile (high purity solvent, Burdick & Jackson; Morristown, NJ) for HPLC mobile phase was also used without further treatment. NCA [^{18}F]fluoride ion was obtained through the ^{18}O(p,n)^{18}F nuclear reaction by irradiating [^{18}O]water (95 atom%) for 90–120 min with a proton beam (14.1 MeV; 20 μA) produced by a PETrace cyclotron (GE, Milwaukee, WI). Cyclotron-produced aqueous [^{18}F]fluoride ion was dried azeotropically in the presence of appropriate base (Cs_2CO_3, K_2CO_3 or KF) with or without K 2.2.2 (in 1.5 molar excess of metal ion if used), under microwave-enhanced conditions [32].

Radioactivity was measured with a calibrated dose calibrator (Atomlab 300; Biodex Medical Systems Inc., Shirley, NY). Radiosyntheses were performed in lead-shielded hot-cells. RCYs were estimated by integrating the radio-peaks on HPLC chromatograms or by measuring isolated product. GC was performed on a 6850 Network GC system (Agilent Technologies, Foster City, CA) using a DB-WAX capillary column (30 m length, 0.25 mm ID).

The production of [^{18}F]XeF_2 was monitored by radio-HPLC on a system comprising a solvent module (System Gold 126; Beckman Coulter, CA) coupled to a UV absorbance detector (λ = 250 nm [33]; Model 168; Beckman Coulter) and a radioactivity detector (PMT, Flow-count; Bioscan, Washington, DC). Xenon difluoride is quite stable in aqueous media [34] and is also stable for hours in acetonitrile. A mixture of acetonitrile and 25 mM aqueous ammonium formate solution was therefore used as mobile phase for the HPLC of xenon difluoride on a reversed phase column (C18, 5 μm, 250 mm × 4.6 mm; Phenomenex). This method separated [^{18}F]XeF_2 (t_R = 3.9–4.1 min) from [^{18}F]fluoride ion (t_R = 2.6–2.7 min), although not with complete resolution, since no-carrier-added [^{18}F]fluoride ion tends to exhibit broad tailing. By this HPLC method, we verified that an acetonitrile solution of [^{18}F]XeF_2 was stable for up to 3 h at room temperature in a polypropylene vial. Hence, all analytes were injected onto HPLC columns in solvent composed predominantly of acetonitrile. Column and elution conditions are described later for each individual procedure.

4.2. Synthesis of [^{18}F]xenon difluoride in Pyrex glass or polypropylene vials

Anhydrous [^{18}F]F^-–K^+–K 2.2.2 solution (2–10 mCi) in acetonitrile (~300 μL) was prepared from cyclotron-produced [^{18}F]fluoride ion, potassium carbonate (3.6 μmol) and K 2.2.2 (11 μmol) and added to a clear Pyrex glass vial (2.0 mL, Waters) containing xenon difluoride (2–30 mg; 11.8–177 μmol). The reaction mixture was allowed to stand at room temperature for a set period. An aliquot of the reaction mixture (10 μL) was taken into a plastic vial (PP LV, 500 μL, Grace) and quenched with acetonitrile (300 μL). A sample (10–30 μL) was analyzed on a reverse phase radio-HPLC column (C18, 5 μm, 250 mm × 4.6 mm, Phenomenex) eluted at 1 mL/min with aqueous $HCOONH_4$ (25 mM)-MeCN at 55: 45 (v/v) (HPLC method 1). The retention time of [^{18}F]XeF_2 was 3.9–4.1 min.

Reactions under modified conditions (e.g. Cs^+, K^+ or Cs^+–K 2.2.2 as the cation, dichloromethane as solvent and PP LV vial as reaction vessel) were performed similarly.

4.3. Synthesis of [^{18}F]xenon difluoride in a micro-reactor

For this experiment two syringe pumps and one micro-reactor of the micro-fluidic apparatus (Nanotek; Advion; cf. Fig. 2) were used. Acetonitrile solutions of [^{18}F]F^-–K^+–K 2.2.2 (10–50 mCi; K^+–K 2.2.2; 26 mM) and xenon difluoride (270 mM), were loaded into separate storage loops (capacity 255 μL). Solutions (20 μL) from each loop were infused simultaneously at 10 μL/min into the coiled silica glass micro-reactor (100 μm i.d., 4-m long; internal volume 31.4 μL). Reactions were quenched by diluting the reactor effluent with acetonitrile (400 μL) in a plastic vial. An aliquot (20 μL) of the reaction mixture was analyzed with HPLC method 1. The procedure was repeated with the micro-reactor at different set temperatures.

4.4. Synthesis of [^{18}F]xenon difluoride in glass vials at a multi-mCi level

Anhydrous [^{18}F]F^-–K^+–K 2.2.2 solution (15–115 mCi) in acetonitrile (~300 μL) was added to a clear Pyrex glass V-vial (1.0 mL, Alltech) containing xenon difluoride (5.7–42.8 mg; 34–253 μmol). The vial was closed with a crimp seal having a PTFE-coated septum, and then heated at 90 °C for 10 min. The vial was connected via a double-tipped needle to another similarly sealed empty V-vial sitting on dry ice. The reaction vessel was heated at 110 °C so that any volatile [^{18}F]XeF_2, together with acetonitrile, was transferred to the collection vial. An aliquot of the collected product (10 μL) was quenched with acetonitrile (300 μL) in a plastic vial (PP LV, 500 μL, Grace) and a sample (10–20 μL) analyzed with HPLC method 1.

4.5. Reaction of [^{18}F]XeF_2 with 1-((trimethylsilyl)oxy)cyclohexene in a micro-reactor

Acetonitrile solutions of [^{18}F]F^-–K^+–K 2.2.2 (40 mCi; K^+–K 2.2.2; 26 mM), xenon difluoride (370 mM) and 1-((trimethylsilyl)oxy)cyclohexene (223 mM), were separately loaded into designated storage loop (255 μL capacity) of the microfluidic apparatus

(Fig. 2). [^{18}F]Fluoride ion solution (20 μL) and xenon difluoride solution were infused simultaneously, each at 5 μL/min, into coiled silica glass micro-reactor 1 (100 μm i.d., 4-m long; internal volume 31.4 μL). The reaction mixture from this reactor was then infused (10 μL/min) simultaneously with the 1-((trimethylsilyl)oxy)cyclohexene solution at a rate of 5 μL/min into the second micro-reactor (100 μm i.d., 4-m long; internal volume 31.4 μL). The effluent from this reactor was quenched by dilution with acetonitrile (400 μL) in a plastic vial. An aliquot (20 μL) of the reaction mixture was analyzed with HPLC method 1 ([^{18}F]2-fluorocyclohexanone, t_R = 5.3 min; [^{18}F]Me_3SiF t_R = 20.8 min). The radioactive fraction was also analyzed by GC (2-fluorocyclohexanone t_R = 6.18 min). The procedure was repeated with the micro-reactors set at different temperatures.

4.6. Reaction of distilled [^{18}F]xenon difluoride with 1-((trimethylsilyl)oxy)cyclohexene

Anhydrous [^{18}F]F^-–K^+–K 2.2.2 solution (5–20 mCi) in acetonitrile (~300 μL) was added to a clear Pyrex glass V-vial (1.0 mL, Alltech) containing xenon difluoride (2–30 mg; 11.8–177 μmol). The reaction mixture was kept at 90 °C for 10 min. [^{18}F]XeF_2 together with acetonitrile was distilled over 10 min into another V-vial containing 1-((trimethylsilyl)oxy)cyclohexene (2–10 μL). The reaction was allowed to stand at room temperature for 20 min or heated to 80 °C for 5–10 min. An aliquot of the reaction mixture (10 μL) was quenched with acetonitrile (400 μL) in a plastic vial (PP LV, 500 μL, Grace). A sample (20 μL) was then analyzed with HPLC method 1.

4.7. Reaction of [^{18}F]xenon difluoride with fluorene in a glass vial in situ

Anhydrous [^{18}F]F^-–Cs^+–K 2.2.2 solution (5–10 mCi) in dichloromethane (200 μL) was added to a clear Pyrex glass vial (1.0 mL, Alltech) containing xenon difluoride (10–20 mg, 59–118 μmol). The reaction mixture was kept at room temperature for 30 min. Fluorene (1.6 mg, 10 μmol) in dichloromethane (50 μL) and $BF_3.Et_2O$ (1 M, 3 μL) were added to the vial. The reaction mixture immediately turned from blue to brown and was left standing at room temperature for 30–60 min. An aliquot of the reaction mixture (10 μL) was quenched with acetonitrile (300 μL) in a plastic vial (PP LV, 500 μL, Grace) and a sample (20 μL) was then analyzed on a reverse phase HPLC column (Phenomenex, C18, 5 μ, 250 mm × 4.6 mm) eluted at 1 mL/min with aqueous $HCOONH_4$ (25 mM)-MeCN at 35:65 (v/v). The retention time of [^{18}F]fluorofluorene(s) was 15.1 min. The radioactive product fraction was collected and analyzed by GC [fluorofluorene(s) t_R = 10.4 min].

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References

[1] M. Rudin, R. Weissleder, Nat. Rev. Drug Discov. 2 (2003) 123–131.
[2] (a) G. Sedvall, Triangle 30 (1991) 11–20;
(b) T. Jones, Eur. J. Nucl. Med. 23 (1996) 807–813;
(c) J.M. Miller, D. Kumar, J.J. Mann, R.V. Parsey, Curr. Radiopharm. 1 (2008) 12–16.
[3] (a) L. Cai, S.Y. Lu, V.W. Pike, Eur. J. Org. Chem. 17 (2008) 2853–2873;
(b) P.W. Miller, N.J. Long, R. Vilar, A.D. Gee, Angew. Chem. Int. Ed. 47 (2008) 8998–9033;
(c) S.M. Ametamey, M. Honer, P.A. Schubiger, Chem. Rev. 108 (2008) 1501–1506.
[4] (a) T.J. Ruth, A.P. Wolf, Radiochim. Acta 26 (1979) 21–24;
(b) M. Guillaume, A. Luxen, B. Nebeling, M. Argentini, J.C. Clark, V.W. Pike, Appl. Radiat. Isot. 42 (1991) 749–762.
[5] V. Casella, T. Ido, A.P. Wolf, J.S. Fowler, R.R. Macgregor, T.J. Ruth, J. Nucl. Med. 21 (1980) 750–757.
[6] (a) D.M. Jewett, J.F. Potocki, R.E. Ehrenkaufer, J. Fluorine Chem. 24 (1984) 477–484;
(b) R. Chirakal, G. Firnau, E.S. Garnett, Appl. Radiat. Isot. 39 (1988) 1099–1101.
[7] (a) R. Filler, Isr. J. Chem. 17 (1977) 71–79;
(b) M. Zupan, Xenon halide halogenations, in: S. Patai, Z. Rappaport (Eds.), The Chemistry of Functional Groups, Suppl. D, John Wiley and Sons Ltd., Chichester, 1983, pp. 657–679;
(c) M.A. Tius, Tetrahedron 51 (1995) 6605–6634;
(d) M. Tramšek, B. Žemva, Acta Chim. Slov. 53 (2006) 105–116.
[8] E.W. Della, N.J. Head, J. Org. Chem. 57 (1992) 2850–2855.
[9] A.P. Lothian, C.A. Ramsden, Synlett 10 (1993) 753–755.
[10] (a) T.B. Patrick, K.K. Johri, D.H. White, W.S. Betrand, R. Mokhtar, M.R. Kilbourn, M.J. Welch, Can. J. Chem. 64 (1986) 138–141;
(b) T.B. Patrick, S. Khazaeli, S. Nadji, K. Hering-Smith, D. Reif, J. Org. Chem. 58 (1993) 705–708.
[11] C.A. Ramsden, M.M. Shaw, Tetrahedron Lett. 50 (2009) 3321–3324.
[12] (a) S. Sood, G. Firnau, E.S. Garnett, Int. J. Appl. Radiat. Isot. 34 (1983) 743–745;
(b) C.-Y. Shiue, K.-C. To, A.P. Wolf, J. Labelled Compd. Radiopharm. 20 (1983) 157–162.
[13] R. Chirakal, G. Firnau, G.J. Schrobilgen, J. Mckay, E.S. Garnett, Int. J. Appl. Radiat. Isot. 35 (1984) 401–404.
[14] G. Schrobilgen, G. Firnau, R. Chirakal, E.S. Garnett, J. Chem. Soc. Chem. Commun. (1981) 198–199.
[15] H. Constantinou, F.I. Aigbirhio, R.G. Smith, C.A. Ramsden, V.W. Pike, J. Am. Chem. Soc. 123 (2001) 1780–1781.
[16] N. Vasdev, B.E. Pointner, R. Chirakal, G.J. Schrobilgen, J. Am. Chem. Soc. 124 (2002) 12863–12868.
[17] (a) S.Y. Lu, V.W. Pike, in: P.A. Schubiger, L. Lehmann, M. Friebe (Eds.), PET Chemistry—The Driving Force in Molecular Imaging, Springer-Verlag, Heidelberg, 2007, pp. 271–287;
(b) H. Audrain, Angew. Chem. Int. Ed. 46 (2007) 1772–1775;
(c) A.M. Elizarov, Lab Chip 9 (2009) 1326–1333;
(d) P.W. Miller, J. Chem. Technol. Biotechnol. 84 (2009) 309–315.
[18] (a) P. Nongkunsarn, C.A. Ramsden, J. Chem. Soc. Perkin Trans. 1 (2) (1996) 121–122;
(b) C.A. Ramsden, R.G. Smith, Org. Lett. 1 (1999) 1591–1594;
(c) M.M. Shaw, R.G. Smith, C.A. Ramsden, J. Fluorine Chem. 116 (2002) 71–73.
[19] C.A. Ramsden, R.G. Smith, J. Am. Chem. Soc. 120 (1998) 6842–6843.
[20] (a) F.C. Cowlard, J.C. Lewis, J. Mater. Sci. 2 (1967) 507–512;
(b) P.J.F. Harris, Philos. Mag. 84 (2004) 3159–3167.
[21] M. Iuliano, L. Ciavatta, G. De Tomasso, J. Colloid Interface Sci. 310 (2007) 402–410.
[22] K.O. Christe, W.W. Wilson, J. Fluorine Chem. 47 (1990) 117–120.
[23] J.R. Ballinger, B.M. Bowen, G. Firnau, E.S. Garnett, F.W. Teare, Int. J. Appl. Radiat. Isot. 35 (1984) 1125–1128.
[24] J.M. Lehn, J.P. Sauvage, J. Am. Chem. Soc. 97 (1975) 6700–6707.
[25] (a) J.W. Brodack, M.R. Kilbourn, M.J. Welch, J.A. Katzenellenbogen, Appl. Radiat. Isot. 37 (1986) 217–221;
(b) R.J. Nickles, S.J. Gatley, J.R. Votaw, M.L. Kornguth, Appl. Radiat. Isot. 37 (1986) 649–650.
[26] E.H. Appelman, Inorg. Chem. 6 (1967) 1268–1269.
[27] (a) W.W. Dukat, J.H. Holloway, E.G. Hope, P.J. Townson, R.L. Powell, J. Fluorine Chem. 62 (1993) 293–296;
(b) H. Meinert, S.Z. Rüdiger, Z. Chem. 7 (1967) 239–1239;
(c) L.P. Ingman, J. Jokisaari, K. Oikarinen, R. Seydoux, J. Magn. Reson. 111 (1994) 155–160.
[28] M.L. Korguth, T.R. DeGrado, J.E. Holden, S.J. Gatley, J. Labelled Compd. Radiopharm. 25 (1988) 369–381.
[29] (a) S. Lu, A.M. Giamis, V.W. Pike, Curr. Radiopharm. 2 (2009) 49–55;
(b) J.H. Chun, S. Lu, Y.S. Lee, V.W. Pike, J. Org. Chem. 75 (2010) 3332–3338.
[30] S.M. Qaim, J.C. Clark, C. Crouzel, M. Guillaume, H.J. Helmeke, B. Nebeling, V.W. Pike, G. Stöcklin, in: G. Stöcklin, V.W. Pike (Eds.), Radiopharmaceuticals for Positron Emission Tomography, Kluwer Academic Publishers, Dordrect/London, Dev. Nucl. Med., vol. 24, 1993, pp. 1–33.
[31] (a) M. Zupan, J. Iskra, S. Stavber, J. Org. Chem. 63 (1998) 878–880;
(b) J. Iskra, S. Stavber, M. Zupan, Collect. Czech. Chem. Commun. 73 (2008) 1671–1680.
[32] N. Lazarova, F.G. Siméon, J.L. Musachio, S.Y. Lu, V.W. Pike, J. Labelled Compd. Radiopharm. 50 (2007) 463–465.
[33] (a) E.H. Appelman, J.G. Malm, J. Am. Chem. Soc. 86 (1964) 2297–2298;
(b) H. Kunkely, A. Vogler, Inorg. Chim. Acta 357 (2004) 2407–2409.
[34] J.L. Weeks, M.S. Matheson, Inorg. Syn. 8 (1966) 260–264.