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Efficient Radiosynthesis of 2-[^{18}F]Fluoroadenosine: A New Route to 2-[^{18}F]Fluoropurine Nucleosides

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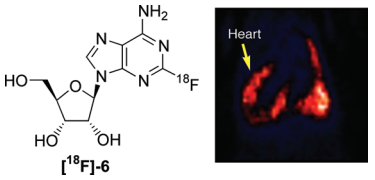
ABSTRACT An efficient method to incorporate the fluorine-18 radionuclide in 2-nitropurine-based nucleosides was developed. The nucleophilic radiofluorination of the labeling precursor with [^{18}F]KF under aminopolyether-mediated conditions (Kryptofix 2.2.2/K_2CO_3) followed by deprotection was straightforward and, after formulation, gave 2-[^{18}F]fluoroadenosine, ready for injection with a radiochemical yield of 45 ± 5%, a radiochemical purity of >98%, and a specific radioactivity up to 148 GBq/μmol. A micropositron emission tomography imaging and biodistribution study on rodents was reported.

KEYWORDS [^{18}F]Fluorine, nucleoside, purine, 2-[^{18}F]fluoroadenosine, PET



[^{18}F]-6

Positron emission tomography (PET) is based on the use of β^+-emitting radionuclides and is now a widely used technique for noninvasive medical imaging at clinical or preclinical stages.[1−3] From the chemistry point of view, the synthesis, purification, and formulation of the radiopharmaceutical should be efficient within a short time and a limited number of steps, high yielding, and reproducible to ensure the feasibility of PET studies.

Many fluorinated nucleosides have been synthesized and studied as potential antitumor and antiviral agents.[4] The development of labeling methods for introducing a fluorine-18 atom provides the possibility of access to novel PET probes for in vivo imaging.

Most of the strategies used for the [^{18}F] radiolabeling of nucleosides and purine derivatives are based on a fluorination of the sugar moiety at the 2′-, 3′-, or even 5′-position or on a side chain.[5,6] In contrast, the direct [^{18}F] labeling of the purine ring has been scarcely described. The [^{18}F] labeling at the 8-position was reported using [^{18}F]F_2 but in low yield (less than 2% decay-corrected).[7] The 6-position was efficiently labeled with [^{18}F]F^- via substitution of the trimethylammonium salt on various substrates,[8,9] while for the 2-position radiolabeling was reported only in low yield (5% yield with low specific radioactivity).[10,11]

Introduction of a fluorine atom at the 2-position on the purine ring often results in beneficial effects from metabolic stability due to enzymatic resistance, especially toward adenosine deaminase, and therefore has a longer in vivo lifetime.[12,13] Thus, an efficient and direct [^{18}F] labeling of purine at the 2-position might provide access to highly valuable tools for PET imaging.

Among the potent targets, 2-[^{18}F]fluoroadenosine ([^{18}F]-**6**) is an attractive candidate as 2-fluoroadenine-based nucleosides are potential tracers in cardiology and oncology.[11,14,15] Moreover, when considering the radiochemistry of [^{18}F]-**6**, only limited methods have been so far described: The Schiemann reaction widely used for the classical fluorination of purines[16] was proven to be inapplicable to radiochemistry, and the conventional methods using substitution of iodine or fluorine by [^{18}F]KF or [^{18}F]AgF were of low efficiency (low yields and low specific radioactivities were obtained).[10,11]

Our strategy to introduce a fluorine-18 atom is illustrated in Figure 1. This approach is based on the use of a nitro group at the 2-position of purines to activate the purine ring and to act as a leaving group for the [^{18}F] nucleophilic aromatic substitution. The radical nitration step necessitates the full protection of the starting material as the reaction does not tolerate any labile protons.

Previously reported methods proved the benzoyl group to be the most suitable for the full protection of adenosine and subsequent nitration.[17] Radical nitration was carried out according to the described procedure using TBAN/TFAA to afford 2-nitro-pentabenzoyl adenosine **3** as the labeling precursor.[17,18] In radiochemistry, for a complete analysis of radiochemical reactions and identification of radiofluorinated products, nonradioactive fluorinated compounds were needed as standard references. Substitution of the nitro group of **3** by an excess of Bu_4NF in dimethyl formamide was previously reported, but the resulting 2-fluoroadenosine derivatives were not isolated.[18] Modification of this protocol, using **3** in acetonitrile with only 1.3 equiv of Bu_4NF, enabled us to isolate the fluorinated derivatives **4** and **5** in 35 and 58%, respectively, after purification (Scheme 1). Under

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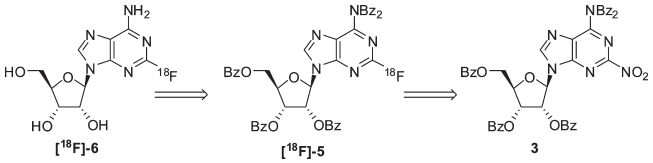


Figure 1. Retrosynthesis of [^{18}F]-**6**.

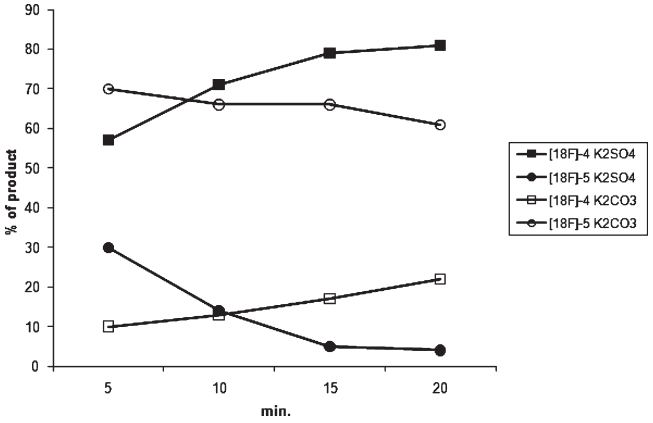
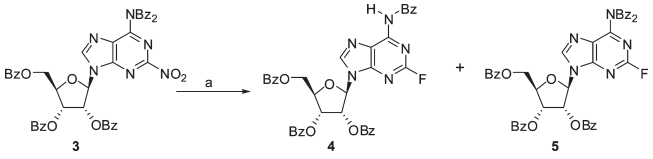


Figure 2. Influence of potassium salts on the fluorination reaction.

Scheme 1. Compounds **4** and **5**[a]

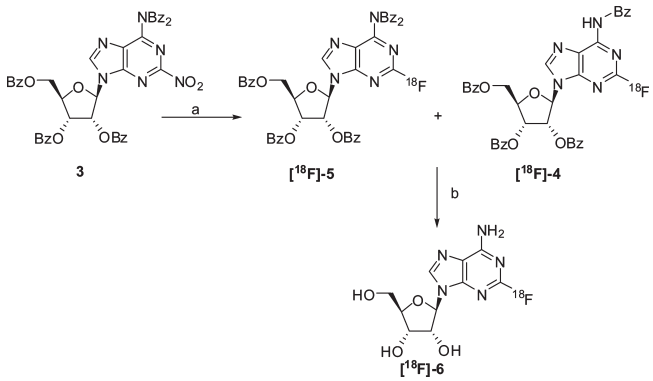


[a] Reagents and conditions: (a) Bu_4NF, CH_3CN, 0 °C.

the reaction conditions, **4** was formed by a selective monodeprotection at N-6 of the purine ring.

First attempts to synthesize [^{18}F]-**5** using [^{18}F]KF under classical conditions with K_2CO_3[19] and **3** in dimethyl sulfoxide (DMSO) at 140 °C proved to be inefficient, and no labeled compound was observed. Further attempts and modifications of the reaction conditions demonstrated that the amount and the nature of the potassium salt used to generate [^{18}F]KF were crucial for the success of the fluorination step. When using a nonbasic potassium salt such as K_2SO_4 (even in excess), the reaction led to the formation of the fluorinated products in high yield (up to 80%). On the other hand, the use of K_2CO_3 was found to be efficient only when used in low quantities (excess of precursor **3** as compared to K_2CO_3). Surprisingly, the composition of the products was found to be dependent on the nature of the potassium salt, and the selectivity of the reaction toward [^{18}F]-**4** or [^{18}F]-**5** could be shifted when using K_2SO_4 instead of K_2CO_3 (Figure 2). Optimization of the reaction conditions (molar ratio **3**/K_2CO_3 = 1.6) and the use of CH_3CN instead of DMSO enabled a very efficient radiolabeling of [^{18}F]-**5** in 92−98% in 8 min with less than 5% of [^{18}F]-**4** (Scheme 2).

Scheme 2. Radiosynthesis of [^{18}F]-**6**[a]



[a] Reagents and conditions: (a) Compound **3**, [^{18}F]KF, K_2CO_3, K_{222}, CH_3CN, 55−60 °C, 8 min. (b) MeOH/$NH_3 \cdot H_2O$ (28%), 70 °C, 20 min.

The deprotection of the intermediate [^{18}F]-**5** was found to be a critical step. Removal of K_2CO_3/K_{222} with a Sep-Pak silica gel cartridge was found to be mandatory for subsequent deprotection, as the use of a crude solution led mainly to defluorination. The use of the NH_3/MeOH system was found to be the most efficient method[20] for the deprotection reaction, and [^{18}F]-**5** was converted into the desired product [^{18}F]-**6** in 70−80% after heating the solution at 70 °C for 20 min. A higher temperature was found to be unfavorable as defluorination increased rapidly.

After neutralization, the crude solution was purified by semipreparative high-performance liquid chromatography, and the collected fraction [^{18}F]-**6** was adsorbed on a C18 Sep-Pak. After the cartridge was dried, [^{18}F]-**6** was eluted with CH_3CN, evaporated, and formulated in a sterile saline solution.

From this route, [^{18}F]-**6** was obtained in 45 ± 5% overall radiochemical yield, with a radiochemical purity of >98% and a specific radioactivity up to 148 GBq/μmol.[21] This high-yielding radiosynthesis enabled us to evaluate in vivo [^{18}F]-**6** under baseline conditions.

2-Fluoroadenosine is known to be a ligand of adenosine receptors (A_1AR, A_{2A}AR, A_{2B}AR, and A_3AR), a subclass of the super family of G-protein-coupled receptors involved in various pathologies.[22] A recent review highlighted the interest of PET imaging agents for mapping of adenosine receptors in CNS.[23] The adenosine receptors also mediate a wide range of responses in the heart,[24] and the use of labeled adenosine with β^+-emitting atoms for PET studies was envisaged as an attractive candidate for local blood flow measurement.[15]

A preliminary study was to evaluate [^{18}F]-**6** in male Sprague−Dawley rats. After iv injection, the in vivo [^{18}F]-**6** biodistribution was visualized by microPET scans over a 60 min period. A high uptake in lung, heart, spleen and kidneys was observed (Figure 3). These images were in agreement with the post-mortem biodistribution study performed at 60 min after microPET imaging. The measurement of the radioactivity in selected organs showed the highest values in the lung ≫ kidney > heart > spleen along with an important urinary elimination (Table 1). The high level of radioactivity

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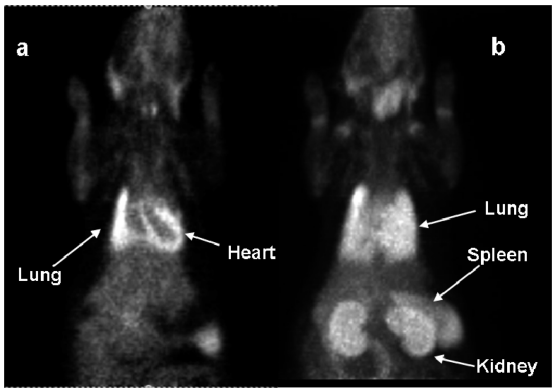

Figure 3. MicroPET images in rat obtained 60 min after intravenous injection of [^{18}F]-**6**: (a) coronal image and (b) maximum intensity projection image.

Table 1. Biodistribution of [^{18}F]-**6** in Selected Organs 60 Min Postinjection (n = 2)

organs	[^{18}F]-**6** (% ID/g ± SD)
lung	9.15 ± 0.15
heart	3.22 ± 0.20
kidney	3.75 ± 0.15
spleen	2.85 ± 0.35
intestine	1.45 ± 0.35
liver	0.72 ± 0.01
thymus	0.65 ± 0.15
blood	0.47 ± 0.09
urine	6.70 ± 0.35
brain	0.06 ± 0.01

observed in the lung suggests that this target may have some specific receptors for [^{18}F]-**6** compound. Further studies will be necessary to confirm this hypothesis. Interestingly, the high accumulation in the spleen has been previously described with [^{18}F]fluoroadenosine derivatives.[25] However, this study in which interesting preliminary data have been recorded will be supplemented in the future to try to demonstrate specificity of this radiotracer.

In conclusion, the most important result was the development of an efficient method to incorporate fluorine-18 radionuclide with a 2-nitropurine-based nucleoside. This [^{18}F]fluorination approach provides a high reproducibility with a fast reaction time and was applied successfully to the radiosynthesis of [^{18}F]-**6** with a higher isolated radiochemical yield and a higher specific activity than previously achieved.

SUPPORTING INFORMATION AVAILABLE

Experimental procedures and characterization for compounds **2**−**6**, [^{18}F]-**6**, and biological protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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