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Research Article

Preparation of 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT) in ionic liquid, [bmim][OTf]

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Summary

Although 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT) is a prospective radiopharmaceutical for the imaging of proliferating tumor cell, it is difficult to prepare large amount of [^{18}F]FLT. We herein describe the preparation of [^{18}F]FLT in an ionic liquid, [bmim][OTf] (1-butyl-3-methyl-imidazolium trifluoromethanesulfonate). At optimized condition, [^{18}F]fluorinationin ionic liquid with 5 μl of 1 M $KHCO_3$ and 5 mg of the precursor yielded 61.5 ± 4.3% ($n = 10$). Total elapsed time was about 70 min including HPLC purification. The rapid synthesis of [^{18}F]FLT can be achieved by removing all evaporation steps. Overall radiochemical yield and radiochemical purity were 30 ± 5% and >95%, respectively. This method can use a small amount of a nitrobenzenesulfonate precursor and can be adapted for automated production. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: [^{18}F]-3′-Deoxy-3′-fluorothymidine ([^{18}F]FLT); ionic liquids; tumor imaging; PET

Introduction

[^{18}F]Fluorodeoxyglucose ([^{18}F]FDG) has been the most widely used in positron emission tomography (PET) for tumor imaging. As glucose is utilized by benign cells such as activated macrophage, [^{18}F]FDG gives little non-specific results for tumor imaging. [^{11}C]Thymidine has been used for the

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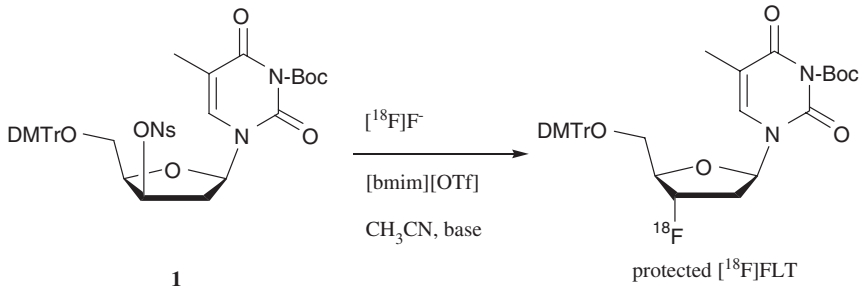
diagnosis of tumor or measurement of tumor's multiplication rate in PET. Because [^{11}C]thymidine has some limitations to track metabolism due to its rapid *in vivo* degradation and short half-life of C-11, 3′-[^{18}F]fluoro-3′-deoxythymidine ([^{18}F]FLT) has recently been developed.[1–4] The [^{18}F]FLT promises to be one of the most promising radiopharmaceuticals due to lack of *in vivo* degradation, metabolic trapping in proliferating cells, and a favorable half life for PET imaging.[3] Radiochemical yield of [^{18}F]FLT synthesis was less than 10% when anhydrothymidine derivative was used as a precursor.[5] In addition, Oh *et al.* recently reported that [^{18}F]FLT was obtained in 40–60% radiochemical yield using over 30 mg of *p*-nitrobenzenesulfonate (nosylate) precursor.[6–7] These drawbacks have been the major obstacles for routine clinical use of [^{18}F]FLT. Recently, ionic liquids have been reported to be excellent reaction media for many reactions,[8–10] and their usefulness for fluorination has also been published.[11,12] Interestingly, it was found that a nucleophilic fluorination of precursor of [^{18}F]FDG could be carried out with high efficiency in the presence of water.[13] Recently, we have developed a new method for fluorine-18 labeling using an ionic liquid as a reaction medium. In the present study, we eliminated the azeotropic drying procedure, the most time wasting step, of the [^{18}F]FLT synthesis by using a solvent containing an ionic liquid ([bmim][OTf], 1-butyl-3-methyl-imidazolium trifluoromethanesulfonate) as a reaction medium. For the synthesis of [^{18}F]FLT, *N*-Boc-protected analogue was used as a precursor and the amount of precursor, the kind and equivalent of base, and the amount of ionic liquid ([bmim][OTf]) were optimized.

Results and discussion

Generally, mesyl-, tosyl-, and triflate (trifluoromethanesulfonyloxy) are typical leaving groups in the nucleophilic [^{18}F]fluorination reactions using fluoride-18 fluoride obtained from an ^{18}O(p,n)^{18}F reaction, providing the corresponding [^{18}F]fluoroalkanes in excellent yields. In [^{18}F]fluorination process using [^{18}F]fluoride, an anion exchange resin (QMA) is frequently used to recovery O-18 water and a small amount of water is also essential to elute [^{18}F]fluoride. In addition, water has to be removed by azeotropic distillation with acetonitrile. Although this drying step is necessitated, over-drying or not enough drying of water makes failure of reaction or decrease of yield sometimes. It was reported that the labeling process using ionic liquid ([bmim][OTf]) showed tolerance for a small amount of water.[12]

In the present study, therefore, the [^{18}F]fluorination was evaluated using a minimum amount volume of water. Furthermore, the selection of base appeared to be very important during the reaction. Some metal bases such as potassium bicarbonate, potassium carbonate, or cesium carbonate have been shown to give high fluorination yields. However, the metal bases have low



1 protected [18F]FLT

Scheme 1. Synthesis of protected [18F]FLT

Table 1. Labeling yield with various amounts of carbonates

	Radiolabeling yield (%),[a] detected by TLC ($n = 3$)		
Base (μl)	1 M $KHCO_3$	1 M Cs_2CO_3	1 M K_2CO_3
2.5	20.4 ± 2.7	34.1 ± 4.2	17.7 ± 1.9
5.0	52.4 ± 3.4	43.0 ± 3.4	33.1 ± 2.7
10.0	46.6 ± 2.9	18.6 ± 2.8	34.4 ± 3.7
15.0	34.4 ± 2.0	13.4 ± 2.3	40.1 ± 3.3
20.0	NA[b]	NA	51.4 ± 4.2
25.0	NA	NA	38.4 ± 3.0

[a] Reaction condition: 10 mg of nosylate precursor **1**, 200 μl of acetonitrile, 200 μl of [bmim][OTf], x μl of [18F]fluoride in $H_2O + y$ μl of base = 70 μl for 15 min at 120°C.
[b] NA = not attempted.

solubility in water containing acetonitrile without Kryptofix[2.2.2].[12] Therefore, optimization of the metal bases was carried out (Scheme 1).

As shown in Table 1, the amounts of metal bases were optimized with fixed 1 M concentration of metal bases such as potassium bicarbonate, potassium carbonate, or cesium carbonate and a fixed amount of the precursor (10 mg). The highest yield was obtained when 5 μl of potassium bicarbonate or 20 μl of potassium carbonate were used (Table 1).

Under the most optimal condition of potassium bicarbonate (1 M, 5 μl) and potassium carbonate (1 M, 20 μl), as shown at Table 1, 5 mg of the precursor and 5 μl of potassium bicarbonate gave the best yield (61.5 ± 4.3%) (Table 2).

Finally, as shown in Table 3, the best yield was obtained when 200 μl of ionic liquid was used. The optimized labeling conditions were as follows: 5 mg of nosylate precursor **1**, 200 μl of acetonitrile, 200 μl of [bmim][OTf], 65 μl of [18F]fluoride in H_2O and 5 μl of 1 M $KHCO_3$ for 15 min at 120°C. The reaction solvent – ionic liquid – was removed with silica gel cartridge and the fluorine-18 labeled product was deprotected by the treatment with 0.5 N HCl (300 μl) for 5 min at 110°C. The synthesis of [18F]FLT was confirmed by radio-TLC (Figure 1) and separated using HPLC (Figure 2).

Total elapsed time was about 70 min including deprotections and HPLC purification step. Overall radiochemical yield and purity were 30 ± 5% (decay

Table 2. Labeling yield with various amounts of 1

	Radiolabeling yield (%),[a] detected by TLC ($n = 3$)	
1 (mg)	1 M $KHCO_3$ (5 µl)	1 M K_2CO_3 (20 µl)
5	61.5 ± 4.3[b]	29.2 ± 3.6
10	52.4 ± 3.4	51.4 ± 4.2
15	24.1 ± 1.2	53.0 ± 2.8
20	20.8 ± 5.1	25.6 ± 6.5

[a] Reaction condition: In 200 µl of acetonitrile, 200 µl of [bmim][OTf], and 65 or 50 µl of [^{18}F]fluoride in H_2O + 5 µl of 1 M $KHCO_3$ or 20 µl of 1 M $KHCO_3$, respectively, for 15 min at 120°C.
[b] $n = 10$.

Table 3. Labeling yield with various amounts of [bmim] [OTf]

	Radiolabeling yield (%),[a] detected by TLC ($n = 3$)
[bmim][OTf] (µl)	1 M $KHCO_3$ (5 µl)
100	49.5 ± 5.4
200	61.5 ± 4.3[b]
300	40.3 ± 3.6
400	37.3 ± 4.5

[a] Reaction condition: 5 mg of nosylate precursor **1**, 200 µl of acetonitrile, amount of [bmim][OTf], 65 µl of [^{18}F]fluoride in H_2O + 5 µl of 1 M $KHCO_3$ for 15 min at 120°C.
[b] $n = 10$.

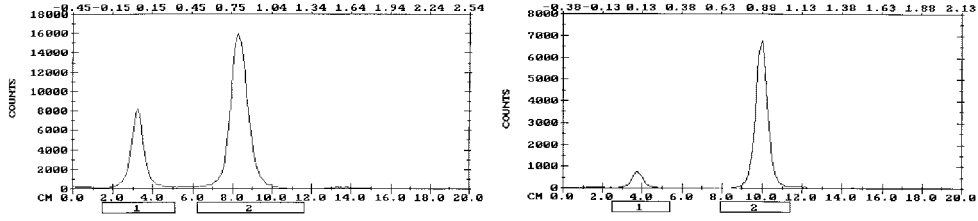


Figure 1. Monitoring of reaction by radio-TLC [left: fluorination (MeOH/$CHCl_3$/hexane = 1:4:5) right. Hydrolysis (5% water: acetonitrile)]

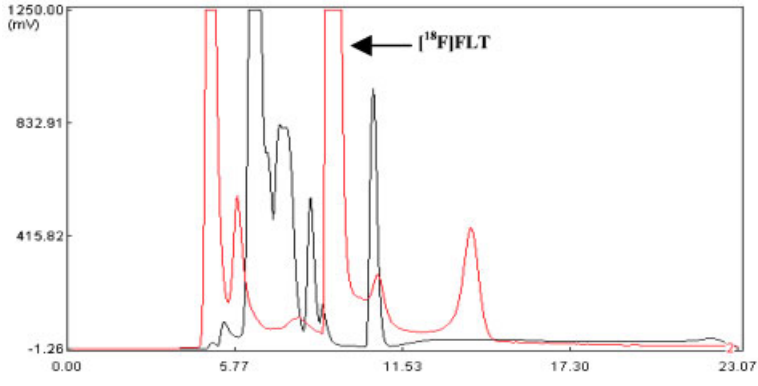


Figure 2. [^{18}F]FLT purification using HPLC (7.8 mm × 300 mm, µBondapak RP-18, 10 µ, EtOH/H_2O = 10/90 [v/v], flow rate: 2.5 ml/min)

corrected) and >95%, respectively. Furthermore, we effectively decreased the amount of nosylated precursor and the method can be adapted to routine production of [^{18}F]FLT.

The above optimized condition was satisfactory when a small amount of water – 65 μl of [^{18}F]fluoride in O-18 water and 5 μl of 1 M $KHCO_3$ was used. However, when this condition was applied to a large amount of water such as over 100 μl, the labeling yield was dramatically dropped. Therefore, to reduce total volume of [^{18}F]fluoride in O-18 water, QMA (SPE cartridge Chromafix 30-PS-HCO_3) cartridge was used. When we extracted the activity from QMA cartridge using the best conditions described in Tables 1 and 2, about 15% of the total activity loaded was eluted with 80 μl of $KHCO_3$ (5 μl of 1 M $KHCO_3$ +75 μl of H_2O) and 250 μl of acetonitrile. However, when 80 μl of K_2CO_3 (20 μl of 1 M K_2CO_3 + 60 μl of H_2O) were used, over 90% of the activity was extracted. Therefore, we adopted 1 M K_2CO_3 as the eluting base for a large scale of activity (over 37.0 GBq); the optimized labeling conditions would be 10 mg of the precursor, 80 μl of K_2CO_3 (20 μl of 1 M K_2CO_3 + 60 μl of H_2O), 250 μl of CH_3CN and 200 μl of ionic liquid. [^{18}F]Fluorination yield was 48 ± 6.0% ($n = 3$) and overall radiochemical yield and purity were 25 ± 5% and >95%, respectively.

Conclusion

Based on a recent report of nucleophilic [^{18}F]fluorination of some halo- and mesyloxyalkanes to corresponding [^{18}F]fluoroalkanes using [^{18}F]fluoride ion and metal carbonate in ionic liquid [bmim][OTf] in the presence of some water, we prepared [^{18}F]FLT and optimized synthetic condition in ionic liquid. This method not only improved radiochemical yield of [^{18}F]FLT, but also reduced the amount of precursor. Besides this method using ionic liquid is easy to synthesis, it has shorter synthetic time and less eliminated by-product formation relative to the previous literature. But, this method is slightly decreased radiochemical yield in scale-up condition as well as difficult in separation of polar compounds. The optimized labeling conditions were 5 mg of precursor, 5 μl of 1 M $KHCO_3$ (for a small amount [^{18}F]fluoride in O-18 water) or 20 μl of 1 M K_2CO_3 (for a large amount [^{18}F]fluoride in O-18 water), 250 μl of CH_3CN, and 200 μl of ionic liquid. When used 5 μl of 1 M $KHCO_3$, the labeling yield was 61.5 ± 4.3% ($n = 10$) and overall radiochemical yield and radiochemical purity were 30 ± 5% and >95%, respectively. In case of using 20 μl of 1 M K_2CO_3, [^{18}F]fluorination yield was 48 ± 6.0% ($n = 3$) and overall radiochemical yield and purity were 25 ± 5% and >95%, respectively. The specific activity after HPLC purification was >37.0 GBq/μmol in a large scale activity condition. We effectively reduced the amount of nosylated precursor and this method could be used to routine production of [^{18}F]FLT.

Experimental

3-N-tert-Butoxycarbonyl-(5′-O-(4,4′-dimethoxytriphenylmethyl)-2′-deoxy-3′-O-(4-nitrobenzenesulfonyl)-β-D-threopentofuranosyl)thymine

This was synthesized by the modified method of known procedures.[6] Sep-Pak Silica light cartridge was purchased from Waters (USA), QMA cartridge (SPE cartridge Chromafix 30-PS-HCO_3) was from Macherey-Nagel Ins. (USA) and [bmim][OTf] was from Chem Tech Research Incorporation (C-TRI Co. Korea). Thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica plates and radio-TLC was monitored on a Bioscan AC-3000 scanner (Washington DC, USA). High Performance Liquid Chromatography (HPLC) was carried out on a Young-Lin System (Young-Lin Instrument, Korea) with a semi preparative column (μBondapak RP-18, 10 μ, 7.8 × 300 mm) and simultaneously monitored by a Young-Lin UV instrument (267 nm) and Raytest GABI γ-detector. F-18 was produced with MC-50 cyclotron by irradiation of $H_2^{18}O$ at *Korea Institute of Radiological and Medical Sciences* (*KIRAMS*).

Typical procedure

F-18 containing water (about 55.0 GBq, 1.0–1.5 ml) was extracted from QMA cartridge using 80 μl of K_2CO_3 (20 μl of 1 M K_2CO_3 + 60 μl of H_2O and 250 μl of acetonitrile. This solution was added to the mixture of nosylate precursor **1** (10 mg) in [bmim][OTf] (200 μl). The mixture was stirred at 120°C for 15 min under open system. After 2 ml of dichloromethane was added, the reaction mixture was loaded on two silica Sep-Pak, and then eluted with MeOH:CH_2Cl_2 (1:9). Collected eluate was evaporated with N_2 and diluted with 1 ml of acetonitrille and 0.5 N HCl (300 μl). The mixture was stirred at 110°C for 5 min. After cooling to room temperature, the mixture was purified by HPLC using a semi-preparative column (μBondapak RP-18, 5 μ, EtOH/H_2O = 10/90 [v/v], flow rate: 2.5 ml/min). Collected activity of pure [^{18}F]FLT was 8–10 GBq. Total elapsed time was about 70 min including HPLC purification. Overall radiochemical yield and purity were 25 ± 5% (decay corrected) and > 95%, respectively. The specific activity after HPLC purification was > 37.0 GBq/μmol.

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