Practical Synthesis of 1-(2-Nitro-1*H*-imidazol-1-yl)-3-(tosyloxy) propan-2-yl acetate for the Radiosynthesis of [^{18}F]-FMISO

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Received September 16, 2014, Accepted November 18, 2014, Published online January 20, 2015

Hypoxia is related with many tumors due to a decreased oxygen condition. Novel synthesis for 1-(2-nitro-1*H*-imidazol-1-yl)-3-(tosyloxy)propan-2-yl acetate for the radiosynthesis of the [^{18}F]-Flouromisonidazole ([^{18}F]-FMISO), a hypoxia imaging marker used in positron emission tomography, from the readily available starting material is described. In this approach, one-pot two-step syntheses were used for the preparation of the [^{18}F]-FMISO precursors that contain acetyl group. The mild reaction conditions and easy treatments are attractive features in this synthesis. This new synthetic route provides alternative choices for the preparation of hypoxia imaging marker.

Keywords: [^{18}F]-FMISO, Positron emission tomography, Fluorine-18, Radiosynthesis, One-pot synthesis

Introduction

Hypoxia has been widely acknowledged as a feature of many tumors, particularly solid tumors, because of oxygen deficiency through a badly organize vasculature.[1] Hypoxic cells are related to malignant progression and the potential metastasis and, resultantly, prevent radiotherapy and chemotherapy.[2,3] Therefore, the verification and quantitative assessment of tumor hypoxia is a significant factor to establish proper therapeutic strategies for better clinical outputs.[3]

Hypoxia can be detected in hospitals using molecular imaging techniques such as positron emission tomography (PET). PET is commonly used in a noninvasive technique. Through 180° simultaneous detection of two gamma ray photons from positron–electron annihilation, distributed radiolabeled molecules provide crucial information about physiological and biological events.[4,5] Particularly, with physical properties: a half-life of 110 min and a low-energy positron of 649 KeV, ^{18}F is an ideal candidate for PET and produces molecular images with high resolution. In addition, a carbon—^{18}F covalent bond formed from the introduction of ^{18}F into organic molecules by either electrophilic or nucleophilic ways is very stable for the detection of biological events.[4,6]

In order to quantify the extent of hypoxia, PET radio tracers containing ^{18}F have been usually used in clinical study. One of the most developed PET tracers is nitroimidazole derivatives.[7]

The nitro group of nitroimidazoles undergoes an enzyme-mediated process that transfers single-electron to a free radical anion. This process is reversible in normoxic tissues, whereas generating reactive intermediates in hypoxic tissues covalently binds to cellular components. The trapping and accumulation of bio-reductive species in cells are associated with hypoxia and have been used as a hypoxia indicator, which suggests the presence of hypoxia in specific sites such as tumors and myocardium.[1,8]

Therefore, the various derivatives labeled with ^{18}F such as [^{18}F]-FMISO, [^{18}F]-FAZA, and [^{18}F]-FETNIM have been prepared.[9–12] Among them, one of the widely studied radiopharmaceuticals for PET imaging is [^{18}F]-FMISO. Therefore, [^{1} F]-FMISO is an attractive target molecule for imaging hypoxia conditions, and several research groups have devoted efforts to develop efficient synthesis of [^{18}F]-FMISO.[2,13,14]

The developed synthetic methods for [^{18}F]-FMISO consists of two types of reactions: general chemical synthesis of [^{18}F]-FMISO precursors and then radiochemical synthesis using radio isotopes. One of precursors reported for the synthesis of [^{18}F]-FMISO is a precursor containing acetyl group. In this study, we redesigned original synthetic method of [^{18}F]-FMISO and report an alternative synthetic route for new radio synthesis of [^{18}F]-FMISO (Figure 1).

Experimental

General. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded on a 600-MHz

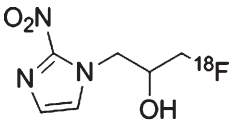

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Figure 1. The structure of [^{18}F]-FMISO.

spectrometer at room temperature. The chemical shifts are reported in δ units (ppm) relative to tetramethylsilane (TMS) and the coupling constants (*J*) quoted in Hz. Reaction progress was monitored by thin-layer chromatography (TLC) analysis. TLC analysis was performed using an aluminum plate with silica gel 60 F_{254} and TLC spots were visualized by exposure to UV light (254 nm). Flash chromatography was performed using 230–400 mesh silica gel and analytical grade solvent. Mass spectrometry was performed by Mass Spectrometry Service of Chonbuk National University.

Glycerol-1,3-ditosylate (3): *p*-Toluenesulfonyl chloride (5.56 g, 29.2 mmol) was added dropwise to a stirred solution of glycerol (1.35 g, 14.6 mmol) in anhydrous pyridine (15 mL) at 0 °C. The mixture was stirred at 0 °C for 5 h and then at room temperature for 3 h. The reaction mixture was acidified by addition of concentrated HCl. The mixture was extracted with CH_2Cl_2 (100 mL), dried over with Na_2SO_4, and filtered. The filtrate was evaporated and the residual crude product was purified by flash column chromatography (EtOAc:Hex = 1:1.5) on silica gel to yield compound **3** (4.17 g, 71%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.4 Hz, 4H), 7.33 (d, *J* = 8.0 Hz, 4H), 4.02 (m, 4H), 3.35 (bs, 1H), 2.43 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.3, 130.2, 129.8, 127.9, 69.4, 67.2, 21.5.

Glycerol-1,3-ditosylate-2-O-acetylate (4): Acetyl chloride (0.25 g, 3.18 mmol) and triethylamine (0.45 g, 4.45 mmol) were added dropwise to a solution of compound **3** (0.85 g, 2.13 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was extracted with CH_2Cl_2 (30 mL) and dried over with Na_2SO_4. The residual was purified by flash column chromatography (EtOAc:Hex = 1:1.5) on silica gel to yield compound **4** (0.82 g, 87%) as a white solid. mp 79–82 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.4 Hz, 4H), 7.34 (d, *J* = 8.4 Hz, 4H), 5.05 (t, *J* = 4.8 Hz, 1H), 4.12 (s, *J* = 4.8 Hz, 4H), 2.43 (s, 6H), 1.91 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 145.3, 130.5, 129.9, 127.9, 68.1, 66.7, 21.6, 20.5.

Preparation of compound 4 from compound 3: *p*-Toluenesulfonyl chloride (2 g, 10.52 mmol) was added dropwise to a stirred solution of glycerol (0.53 g, 5.79 mmol) in anhydrous pyridine (6 mL) at 0 °C. The mixture was stirred at room temperature for 5 h. Then triethylamine (2.94 g, 29.05 mmol) in CH_2Cl_2 (15 mL) was added. After addition of acetyl chloride (1.82 g, 23.18 mmol) to CH_2Cl_2 (15 mL), the mixture was stirred at room temperature for 1 h and 30 min. The mixture was extracted with CH_2Cl_2 (50 mL), dried over with Na_2SO_4, and and filtered. The filtrate was evaporated and the residual crude product was purified by flash column chromatography (EtOAc:Hex = 1:1.5) on silica gel to yield compound **4** (1.21 g, 47%) as a white solid.

1-(2-Nitro-1*H*-imidazol-1-yl)-3-(tosyloxy)propan-2-yl acetate (5): 2-Nitroimidazole (0.23 g, 2.04 mmol) and cesium carbonate (0.66 g, 2.04 mmol) were added to compound **4** (1 g, 2.26 mmol) in anhydrous DMF (7 mL) solvent in a round bottom flask. The mixture was stirred at 110 °C for 1 h and then cooled. The mixture was extracted with ethyl acetate (20 mL) and dried over with Na_2SO_4. The mixture was filtered and evaporated. The residual was purified by flash column chromatography (EtOAc:Hex = 1:1) on silica gel to yield compound **5** (0.38 g, 48%) as a white solid. mp 100–103 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 9.0 Hz, 4H), 7.37 (d, *J* = 7.8 Hz, 4H), 7.10 (d, *J* = 2.8 Hz, 2H), 5.31 (m, 1H), 4.81 (t, *J* = 4.2 Hz, 1H), 4.50 (t, *J* = 4.2 Hz, 1H), 4.17 (s, *J* = 4.8 Hz, 2H), 2.45 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.3, 145.6, 131.8, 130.1, 129.9, 128.3, 127.8, 126.7, 68.7, 67.1, 49.3, 21.6, 20.3; ESI-MS *m/z* $(M + H)^+$ calcd for $C_{15}H_{17}N_3O_7S$ = 383.08, found 384.09.

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (8): (2,2-Dimethyl-1,3-dioxolan-4-yl)methanol (0.2 g, 1.97 mmol) and triethyl amine (0.6 g, 5.93 mmol) were dissolved in anhydrous CH_2Cl_2 (2 mL). *p*-Methanesulfonyl chloride (0.34 g, 2.96 mmol) was added dropwise to the mixture. After the mixture was stirred at room temperature for 8 h, 2-nitroimidazole (0.35 g, 3.09 mmol), cesium carbonate (1 g, 3.08 mmol), and anhydrous DMF (2 mL) were added to the reaction mixture. The reaction mixture was stirred at 110 °C for 12 h. The crude was extracted with CH_2Cl_2 (20 mL) and dried over with Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (EtOAc:Hex = 1:1.5) on silica gel to yield compound **8** (0.29 g, 65%) as a yellowish oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27 (s, 1H), 7.18 (s, 1H), 4.78 (dd, *J* = 11.4, 2.4 Hz, 1H), 4.62–4.51 (m, 1H), 4.44 (dd, *J* = 7.8, 5.4 Hz, 1H), 4.22 (dd, *J* = 7.2, 6.6 Hz, 1H), 3.73 (dd, *J* = 10.2, 5.4 Hz, 1H), 1.43 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 144.9, 128.2, 127.3, 110.4, 74.0, 66.4, 52.1, 26.6, 25.3; ESI-MS *m/z* $(M + H)^+$ calcd for $C_9H_{13}N_3O_4$ = 227.09, found 228.25.

3-(2-Nitro-1*H*-imidazol-1-yl)propane-1,2-diol (9): Trifluoroacetic acid (10 g, 87.7 mmol) was added to a solution of **8** (1.99 g, 8.76 mmol) in anhydrous MeOH (20 mL) and stirred at room temperature for 7 h. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2:MeOH = 10:1) on silica gel to give compound **9** (1.49 g, 91%) as a white solid. mp 110–112 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.15 (s, 1H), 4.77 (dd, *J* = 10.2, 3.6 Hz, 1H), 4.37 (dd, *J* = 10.2, 4.2 Hz, 1H), 4.01–3.95 (m, 1H), 3.61–3.55 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.1, 128.0, 126.8, 70.3, 63.4, 52.3; ESI-MS *m/z* $(M + H)^+$ calcd for $C_6H_9N_3O_4$ = 187.06, found 188.10.

2-Hydroxy-3-(2-nitro-1*H*-imidazol-1-yl)propyl 4-methylbenzenesulfonate (10): *p*-Toluenesulfonyl chloride (0.37 g, 1.92 mmol) was added to a solution of compound **9** (0.37 g,

1.96 mmol) in anhydrous pyridine (3 mL) and stirred at room temperature for 23 h. The mixture was washed with brine and extracted with ethyl acetate (20 mL). The extracted was dried over with Na_2SO_4 and filtered. The filtrate was evaporated and then purified by flash column chromatography (EtOAc:Hex = 1.5:1) on silica gel to yield compound **10** (0.43 g, 65%) as a yellowish solid. mp 141–143 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, J = 7.8 Hz, 2H), 7.45 (d, J = 7.2 Hz, 2H), 7.36 (s, 1H), 7.08 (s, 1H), 4.6 (dd, J = 7.8, 3.6 Hz, 1H), 4.32 (dd, J = 5.4, 2.4 Hz, 1H), 4.11–4.00 (m, 3H), 2.45 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.5, 132.6, 129.8, 127.8, 126.8, 70.8, 67.3, 20.2; ESI-MS (ESI) m/z $(M + H)^+$ calcd for $C_{13}H_{15}N_3O_6S$ = 341.07, found 342.10.

Preparation of compound 5 from compound 10: Acetyl chloride (0.066 g, 0.84 mmol) was added dropwise to a solution of compound **10** (0.14 g, 0.41 mmol) and triethylamine (0.13 g, 1.27 mmol) in anhydrous CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 1 h and 30 min. The reaction mixture was extracted with CH_2Cl_2 (20 mL), dried over with Na_2SO_4, and filtered. The filtrate was evaporated and then purified by flash column chromatography (EtOAc: Hex = 1:1) on silica gel to yield compound **5** (0.14 g, 90%) as a white solid.

Preparation of compound 5 from compound 9: *p*-Toluenesulfonyl chloride (0.29 g, 1.56 mmol) was added dropwise to a stirred solution of compound **9** (0.31 g, 1.63 mmol) in anhydrous pyridine (4 mL) at 0 °C. The mixture was stirred at room temperature for 6 h. Then triethylamine (0.5 g, 4.94 mmol) in CH_2Cl_2 (10 mL) was added. After addition of acetyl chloride (0.38 g, 4.89 mmol) to CH_2Cl_2 (10 mL), the mixture was stirred at room temperature for 1 h and 30 min. The mixture was extracted with CH_2Cl_2 (20 mL), dried over with Na_2SO_4, and filtered. The filtrate was evaporated and the residual crude product was purified by flash column chromatography (EtOAc:Hex = 1:1.1) on silica gel to yield compound **5** (0.34 g, 57%) as a white solid.

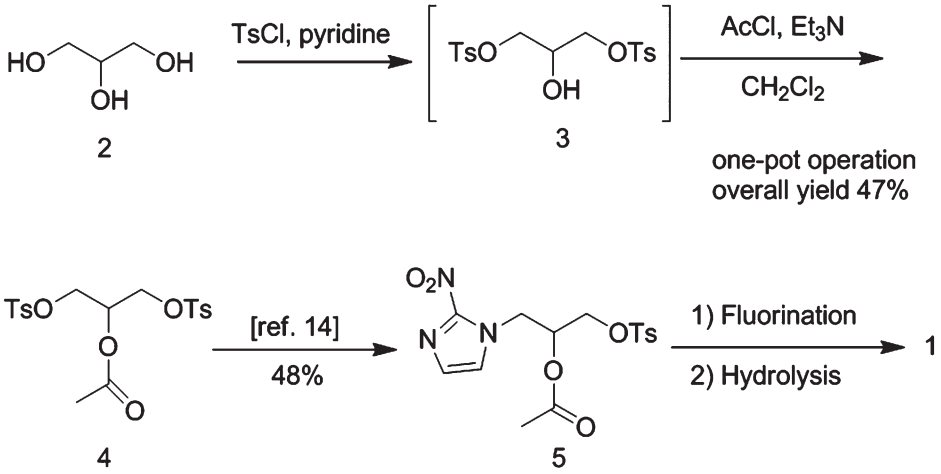
Results and Discussion

In this study, [^{18}F]-FMISO precursor with acetyl group, compound **5**, was prepared by two synthetic methods.

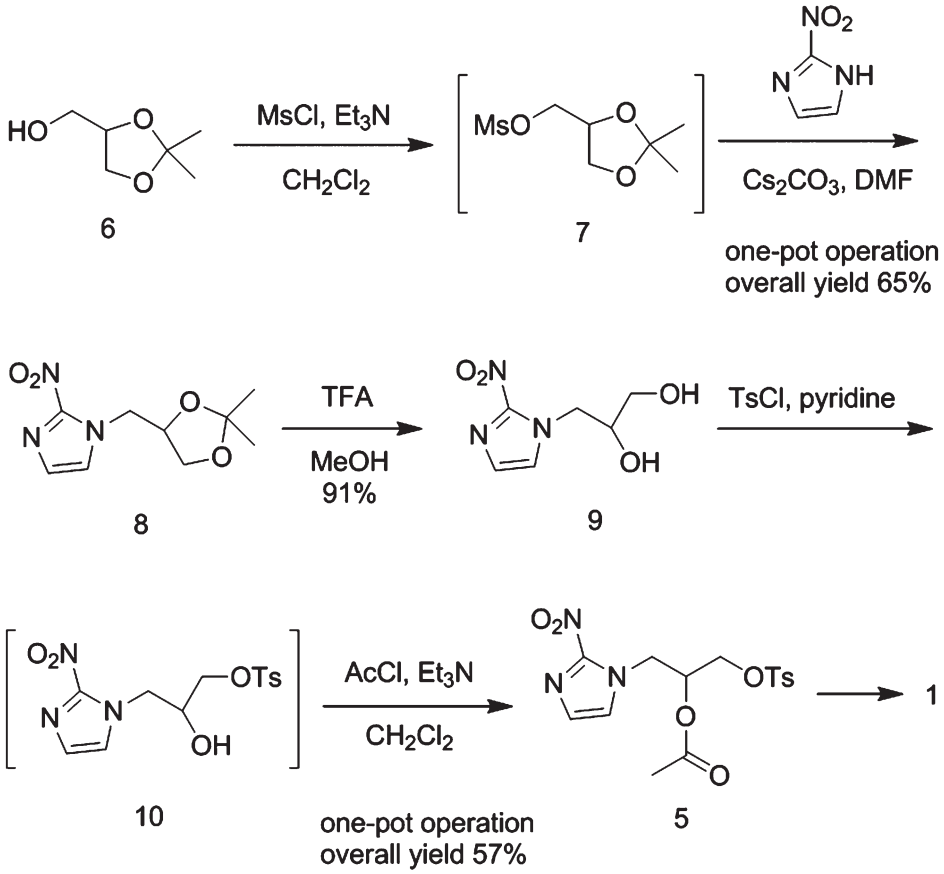
It is well known that the syntheses of the precursor for [^{18}F]-FMISO from glycerol have been widely accepted and studied its radiochemical synthesis to [^{18}F]-FMISO, even though the reported overall yield is low due to the limiting step to substitute 2-nitroimidazole for tosylate in the ditosylate intermediate.[2,13,14] Therefore, we utilized glycerol as a starting material for our first synthetic approach of the precursor for [^{18}F]-FMISO (Scheme 1). Glycerol was treated with 2 equiv of tosyl chloride in pyridine for 5 h to provide glycerol-1,3-ditosylate, compound **3**, in 71% yield which was a previously reported procedure by Oh et al.[13] A secondary alcohol of compound **3** was protected by the treatment with acetyl chloride and triethylamine in CH_2Cl_2. After checking each reaction step separately, we introduced one-pot synthesis in the first synthetic step because one-pot operation is a useful technique for the performance of multiple transformations in a single reaction vessel and is one of the most efficient methods for the reduction of purification procedures and prevention of chemical waste production. One-pot operation for the preparation of compound **4** was performed from compound **2** via ditosylation of primary alcohols and acetylation of secondary alcohol, and showed a 47% overall yield from compound **2**.

And then one of the tosyl moieties of primary alcohol was converted into 2-nitroimidazole group in the presence of Cs_2CO_3 and 2-nitroimidazole in DMF according to the previously reported method.[14]

Even though compound **5** was successfully prepared from glycerol in the first synthetic method, the yield in substitution reaction of 2-nitroimidazole group led overall yield to be lower like the other studies using glycerol. Thus, we considered another synthetic method using a five-membered ring structure as a starting material. The second synthesis of [^{18}F]-



Scheme 1. Synthesis of [^{18}F]-FMISO precursor from glycerol (method A).



Scheme 2. Synthesis of [^{18}F]-FMISO precursor from (2,2-dimethyl-1,3-dioxolan-4-yl)methanol (method B).

FMISO shown in Scheme 2 started with 2,2-dimethyl-1,3-dioxolane-4-methanol, an inexpensive commercially available compound.

Initially, the treatment of 2,2-dimethyl-1,3-dioxolane-4-methanol with methanesulfonyl chloride and triethylamine in CH_2Cl_2 successfully resulted in the production of compound **7**, and then the SN_2 reaction with 2-nitroimidazole at 110 °C for 7 h was carried out to provide compound **8** in 74% yield. For these reaction steps, one-pot synthesis consisting of mesylation of primary alcohol and nucleophilic reaction of the mesylates with 2-nitroimidazole was performed for the incorporation step of 2-nitroimidazole into the five-membered ring structure. After one-pot operation, compound **8** was successfully obtained in 65% yield.

Hydrolysis of compound **8** (Table 1) was carried out by the treatment of acid. In order to find the optimal condition of ring-opening step, several acids were treated. The HCl treatment in MeOH at 45 °C for 5 h gave lower yield of diol compound **9**. Hydrolysis using of Dower 50WX4 for 4 h led to produce compound **9** in 74% yield. When trifluoroacetic acid was utilized in MeOH, the yield of product was increased to 91%.

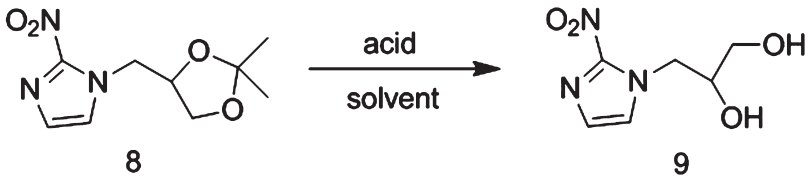
Next, tosylated compound **10** was successfully prepared in the present of *p*-toluenesulfonyl chloride and anhydrous pyridine at room temperature for 23 h. Then the treatment with acetyl chloride and triethylamine at room temperature for 8 h gave compound **5**, FMISO precursor, in 90% yield. In these step, we also tried to perform one-pot synthesis of compound **5** from compound **9**, which consist of tosylation and protection of secondary alcohol with acetyl group. This one-pot sequent operation afforded compound **5** in 57% yield, and the second synthesis of compound **5** from compound **6** was proceeded with 34% yield.

The utilization of compound **5** prepared in this study was investigated through performing radiosynthesis of [^{18}F]-FMISO (Scheme 3). The [^{18}F]fluorination substitution reaction was carried out with [^{18}F]-fluoride and K_2CO_3/Kryptofix$_{222}$ in MeCN at 120 °C for 10 min, and deprotection reaction of acetyl group by treatment with 1 M HCl in MeCN was performed at 100 °C for 5 min. After neutralization with 2 M NaOH, purified [^{18}F]-FMISO was isolated using a reverse-phase HPLC column. The final product was identified by commercially available reference standard of [^{18}F]-FMISO.

Conclusion

In summary, new facile processes for the synthesis of 1-(2-nitro-1*H*-imidazol-1-yl)-3-(tosyloxy)propan-2-yl acetate, the precursor of hypoxia maker [^{18}F]-FMISO, from two different starting materials were developed. These synthetic routes started with commercially available inexpensive materials

Table 1. Hydrolysis of compound **8**.



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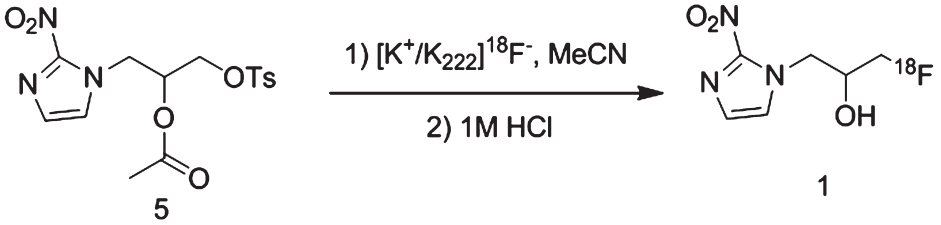
Entry	Acid	Solvent	Temp. (°C)	Isolation yield (%)
1[a]	HCl	MeOH	45	13
2[b]	Dower 50WX4	H_2O	85	74
3[c]	TFA	MeOH	22	91

[a] Reaction runs for 5 h.
[b] Reaction runs for 4 h.
[c] Reaction runs for 7 h.



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Scheme 3. Radiosynthesis of [^{18}F]-FMISO from 1-(2-nitro-1*H*-imidazol-1-yl)-3-(tosyloxy)propan-2-yl acetate.

such as glycerol and (2,2-dimethyl-1,3-dioxolan-4-yl)methanol, and involved one-pot operations that gave shorter and efficient methods for the completion of preparation of desired compound. These efficient syntheses can be amenable to the synthesis of [^{18}F]-FMISO for the PET imaging process.

Acknowledgment. The work was supported by a grant [2014] from Chonbuk National University Medical School Fund.

Supporting Information. HPLC data are available in the online version of this article.

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