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High radiochemical yield synthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine using (5′-*O*-dimethoxytrityl-2′-deoxy-3′-*O*-nosyl-β-D-*threo* pentofuranosyl)thymine and its 3-*N*-BOC-protected analogue as a labeling precursor

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Abstract

We prepared 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT) from 3′-*O*-nosyl thymidine derivative **3** or its pyrimidine ring *N*-BOC-protected analogue **5** and optimized [^{18}F]fluorination condition for a high radiochemical yield. The optimal condition for [^{18}F]fluorination with precursor **3** was 30 mg (41.1 μmol)/300 μl CH_3CN at 130°C for 5 min, while precursor **5** required 34 mg (40 μmol)/300 μl CH_3CN at 110°C for 5 min. After HPLC purification at neutral pH, we achieved high radiochemical yields of 40 ± 5.2% and 42 ± 5.4% (decay-corrected) within 60 min of preparation time with radiochemical purities of >97%.

Keywords: [^{18}F]FLT; DNA synthesis; Thymidine; Cell proliferation; PET imaging; [^{18}F]fluoride

1. Introduction

For tumor imaging with positron emission tomography (PET), a glucose derivative, [^{18}F]fluorodeoxy glucose ([^{18}F]FDG), is widely used. However, [^{18}F]FDG is not specific for tumor imaging because glucose is also utilized by other benign cells such as activated macrophage [8,14].

Radioisotope-labeled thymidine derivatives have been developed to overcome the limitations of [^{18}F]FDG and to image cellular proliferation by PET [3,4,10,13]. [^{18}F]Fluorine-labeled thymidine (FLT) appears to be the most promising radiopharmaceutical because of the lack of in vivo degradation, metabolic trapping in proliferating cells, and the favorable half life for PET imaging [13]. However, the low radiochemical yield has been a major obstacle for routine clinical use of [^{18}F]FLT. Use of a commercially available anhydrothymidine derivative as a precursor enabled the preparation of [^{18}F]FLT with short reaction steps [5,9]. However, it's radiochemical yield still remained as low as 1.7–13.8%. The another trial, a nosylate precursor was used, but similar results were obtained [6]. Moreover, precursor synthesis required seven long chemical reaction steps and three more steps for [^{18}F]fluorine labeling. In addition, ceric ammonium nitrate used for the deprotection formed a precipitate during the synthesis, which is not suitable for the automatic synthesis to obtain high radioactivity [6]. Recently, Martin et al. reported a new method for synthesis of [^{18}F]FLT using a nosylate precursor with an *N*-BOC-protecting group on a pyrimidine. They reported that the radiochemical yield was 19.8% (decay corrected) within 85 min. However, their radiolabeling conditions were not optimized for high radiochemical yield [11].

In this study, we synthesized (5′-*O*-dimethoxytrityl-2′-deoxy-3′-*O*-nosyl-β-D-*threo* pentofuranosyl)thymine and an *N*-BOC-protected analogue as precursors for [^{18}F]FLT synthesis, and evaluated various [^{18}F]fluorination and purification conditions to achieve high radiochemical yield.

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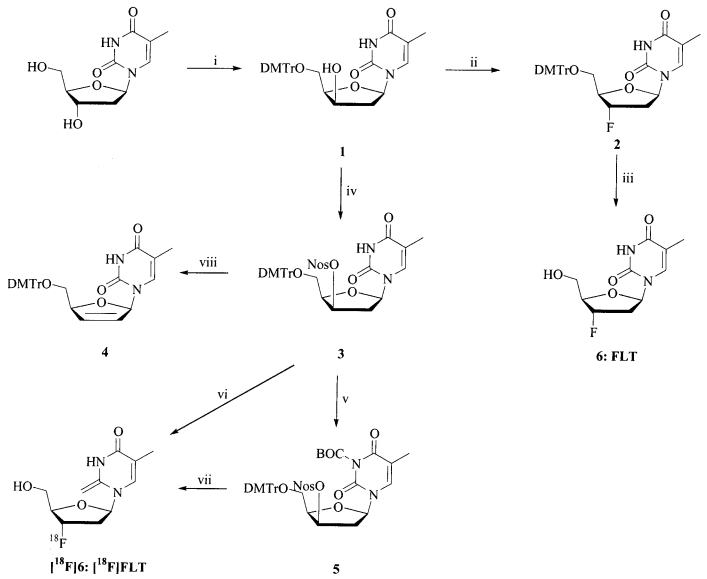

i: 1. DMTrCl, pyridin, 2. MsCl, 3. 10N NaOH, EtOH; ii: DAST, CH_2Cl_2; iii: 1N HCl; iv: Nosyl chloride, AgOTf; v: $(t\text{-BOC})_2O$, THF; vi: 1. $[^{18}F]F^-$, K_{222}, CH_3CN, 2. 1H HCl / TFA; vii: $[^{18}F]F^-$, K_{222}, CH_3CN, 2. 1N HCl; viii: TBAF; (DMTrCl = dimethoxytrityl chloride; MsCl = methansulfonyl chloride; Nosyl chloride = 4-nitrobenzenesulfonyl chloride, DAST = (diethylamino)sulfur trifluoride; TFA = trifluoroacetic acid; TBAF: tetrabuylammonium flouride); *t*-BOC = *tert*-butoxycarbonyl; AgOTf = silver trifluoromethanesulfonate

Fig. 1. Scheme for the chemical and radiochemical synthesis of $[^{18}F]$FLT.

2. Results and discussion

2.1. Chemistry

[5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-2′-deoxy-3′-*O*-(4-nitrobenzenesulfonyl)-*β*-D-*threo* pentofuranosyl]thymine **3** and its *N*-BOC-protected analogue **5** were prepared as shown in Fig. 1. Starting from thymidine, the overall chemical yields were 54% and 28% for **3** and **5**, respectively. After protecting the 5′-OH with a 4,4′-dimethoxytrityl group, the configuration of the 3′-OH was epimerized as described in the literature [1]. This three-step synthesis with one final purification gave compound **1** with 77% overall yield from thymidine. Treatment of compound **1** with DAST[(Diethylamino)sulfur trifluoride] in a mixture of CH_2Cl_2:THF (9:1, v:v) gave the cold standard 3′-deoxy-3′-fluoro-5′-*O*-DMTr-thymine **2** with 28% yield. The epimerized 3′-OH in compound **1** was nosylated with a combination of *p*-nitrobenzenesulfonyl chloride and silver triflate in pyridine with 70% yield [6]. This nosylated precursor **3** was isolated as a brown solid and used for $[^{18}F]$fluorination. It was stable in a refrigerator over 6 months. After hydrolysis of the 5′-*O*-dimethoxytrityl group with 1 N HCl, we obtained FLT with 97% yield as a final cold standard that showed the same NMR spectrum as commercially available FLT. An amine group of pyrimidine was protected with t-BOC anhydride. Due to the high steric hindrance between the *N*-BOC group and nosylate group, the protection required a long reaction time of 5 hrs. After purification, we obtained compound **5** as a yellow solid with 53% yield. With DAST, we obtained FLT **6** as in previous reports [1,7], and there were no side products. Fluorination of nosylate compound **3** with 1 M tetrabutylammonium fluoride in THF gave different results compared with DAST. With this reagent, elimination product 3′-deoxy-5′-*O*-DMTr-thymidinene **4** was obtained as a major product (38%) and 3′-fluorinated compound, 3′-deoxy-3′-fluoro-5′-*O*-DMTr-thymine **2** was obtained as a minor product (5%) according to the NMR spectrum along with unidentified compounds (Fig. 1).

To optimize the conditions for the purification of FLT during HPLC purification, we injected FLT as cold standard in CH_3CN and under different pH conditions using 1 N HCl and 2 M sodium acetate (Fig. 2). When we injected cold standard FLT in CH_3CN solution, the retention time was 11 min with only one peak under our HPLC conditions. However, when we injected FLT with acidic or basic conditions, FLT showed different HPLC chromatograms and retention times. These findings suggest that neutralization after hydrolysis is essential.

2.2. Radiochemistry

$[^{18}F]$FLT was prepared in two steps, one-pot reaction from precursor as shown in Fig. 1. With varing concentration of precursor **3**, temperature and reaction time, the optimal condition was assessed. The radiochemical yield of $[^{18}F]$fluorination with this precursor was highly dependent on the precursor concentration and reaction temperature. The best radiochemical yield of 85.0 ± 5.4% for $[^{18}F]$fluorination was obtained in reaction with 30 mg/300 μl

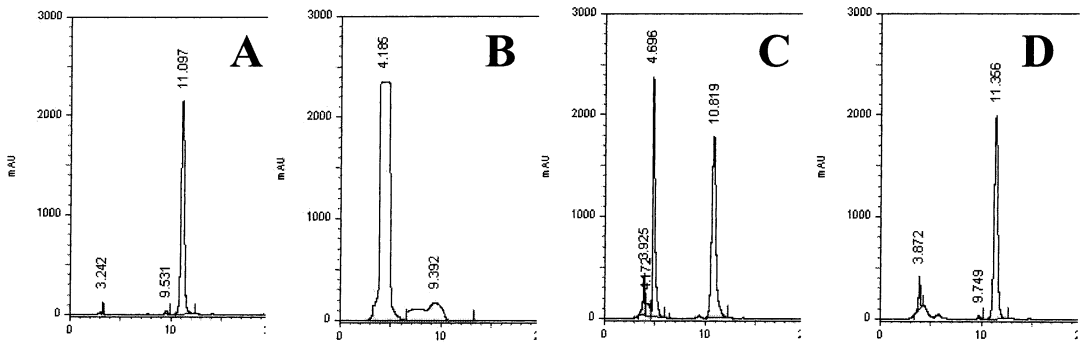

Fig. 2. pH-dependence of the HPLC chromatogram of FLT. The resolution of the HPLC chromatogram of FLT was highly dependent on the pH of the injected solution. The best result was obtained at neutral pH (D). A: 20 μg of FLT/20 μl of CH_3CN; B: 20 μg of FLT/20 μl of CH_3CN and 250 μl of 1 N HCl (pH = 3); C: 20 μg of FLT/20 μl of CH_3CN, 250 μl of 1 N HCl and 1.25 ml of 0.5 M sodium acetate (pH = 5); D: 20 μg of FLT/20 μl of CH_3CN, 250 μl of 1 N HCl and 1.5 ml of 2 M sodium acetate (pH = 7).

CH_3CN of precursor **3** at 130°C for 5 min (n = 3) (Table 1). A radioTLC chromatogram showed only two peaks of unbound [^{18}F]fluoride and [^{18}F]fluorinated-precursor. With higher precursor concentration of 40 mg/300 μl CH_3CN, we obtained a similar radiochemical yield of 86.1 ± 1.8%.

The radiochemical yield was also dependent on the reaction time (Table 2). Unlike in a normal radiochemical reaction, the radiochemical yield in the [^{18}F]fluorination of precursor **3** decreased with increasing reaction time. Reaction at 130°C for 5 min was sufficient for obtaining the high radiochemical yield and we obtained 85.0 ± 5.4% of [^{18}F]fluorination yield. With an increase in the reaction time, while no radiochemical impurities were seen, the proportion of unbound [^{18}F]fluoride increased on the radioTLC chromatogram. Changing the conditions for developing radioTLC to separate this portion was not successful (data not shown). The amount of [^{18}F]fluorinated compound may decrease at 130°C with time, or elimination products may increase at 130°C due to basic reaction conditions resulting from the initial potassium carbonate. [^{18}F]Fluorination yield in the reaction for 30 min was 77.9 ± 6.8% and it was lower than the reaction for 5 min.

The synthesis of 3′-deoxy-3′-[^{18}F]fluoro-5′-*O*-(4,4′-dimethoxytriphenylmethyl) thymine was confirmed by the co-injection with cold standard **2** in radioactive HPLC chromatogram and they showed the same retention time peaks on UV and radioactive chromatograms.

The reaction using *N*-BOC-protected precursor **5** showed similar radiochemical yield of 82 ± 5.4% under optimal [^{18}F]fluorination condition with 34 mg (40 μmol) of precursor/300 μl CH_3CN at 110°C for 5 min (Table 3 and 4). Thus, precursor **5** required slightly milder reaction condition (110°C) than precursor **3** to give a similar radiochemical yield. As with precursor **3**, increasing reaction time of more than 5 min resulted in a decrease in radiochemical yield. Use of higher precursor concentration of 46 mg did not increase a radiochemical yield.

Previous studies have shown that masking of the pyrimidine-NH acidic proton with protecting groups such as BOC or 2,4-dimethoxybenzyl group affords a 10–20 fold increase in radiochemical yield for [^{18}F]fluorination compared with unprotected precursors [5,6,11]. However, in this study, the radiochemical yield with *N*-BOC protected precursor was only slightly higher than that with unprotected precursor **3**. Our study is different from previous ones in terms of temperature and ligand concentration. In previous studies, reaction conditions with 10–28 μmol of ligand concentration and 100–120°C of reaction temperature for [^{18}F]fluorination resulted in final radiochemical yields of 13–20% after HPLC purification [5,6,11]. Differently, with 40.1–41.1 μmol of ligand concentration (precursor **3** and **5**) and 110–130°C of reaction temperature, we obtained 40–42% of final radiochemical yield after HPLC purification. In

Table 1
Effect of temperature and the concentration of precursor **3** on [^{18}F]fluorination yield for the preparation of [^{18}F]FLT

Radiochemical yield* (n = 3)				
Temperature (°C)	Concentration of precursor (mg/300 μl CH_3CN)			
	10	20	30	40
100	9.1 ± 2.9%	21.4 ± 2.9%	60.2 ± 2.8%	61.3 ± 1.9%
110	10.5 ± 3.5%	54.2 ± 2.8%	77.5 ± 4.2%	78.2 ± 3.4%
120	16.9 ± 2.9%	65.4 ± 9.2%	78.5 ± 2.9%	77.8 ± 2.7%
130	25.4 ± 2.5%	75.4 ± 2.0%	85.0 ± 5.4%	83.9 ± 2.6%

* Reaction time: 5 min; radioactivity of [^{18}F]fluoride: 370 MBq.

Table 2
Effect of reaction time on [^{18}F]fluorination yield for the preparation of [^{18}F]FLT using precursor **3**

Radiochemical yield* (n = 3)				
Time (min)	Concentration of precursor (mg/300 μl CH_3CN)			
	10	20	30	40
5	25.4 ± 2.5%	75.4 ± 2.0%	85.0 ± 5.4%	83.9 ± 2.6%
10	24.9 ± 6.5%	72.9 ± 6.5%	85.1 ± 2.5%	83.0 ± 1.5%
20	20.4 ± 2.9%	68.4 ± 2.9%	80.5 ± 2.9%	83.2 ± 2.7%
30	15.9 ± 6.2%	67.4 ± 1.2%	77.9 ± 6.8%	80.0 ± 5.1%

* Reaction temperature: 130°C; radioactivity of [^{18}F]fluoride: 370 MBq.

Table 3
Effect of temperature and the concentration of precursor **5** on [^{18}F]fluorination yield for the preparation of [^{18}F]FLT

Radiochemical yield* (n = 3)				
Temperature (°C)	Concentration of precursor (mg/300 μl CH_3CN)			
	11	23	34	46
100	6.6 ± 3.5%	32.4 ± 2.9%	54.7 ± 9.1%	70.0 ± 1.8%
110	8.1 ± 2.9%	54.5 ± 6.2%	82.0 ± 5.4%	83.9 ± 5.4%
120	18.4 ± 2.9%	68.5 ± 4.5%	81.2 ± 2.4%	83.9 ± 2.5%
130	12.1 ± 3.8%	69.2 ± 1.9%	81.0 ± 5.4%	84.0 ± 3.6%

* Reaction time: 5 min; radioactivity of [^{18}F]fluoride: 370 MBq.

accordance with previous studies, we also had low radiochemical yield with 12.9–13.7 μmol of ligand concentration. These results indicate the importance of optimization of precursor concentration and reaction temperature for high radiochemical yield. With concentrations of 40 mg (54.8 μmol) precursor **3** and 46 mg (54.3 μmol) precursor **5**, the radiochemical yield did not increase further, and we had problems during HPLC purification due to very high amount of unreacted precursor.

Hydrolysis of the dimethoxytrityl group of 3′-deoxy-3′-[^{18}F]fluoro-5′-*O*-(4,4′-dimethoxy triphenylmethyl)thymine was performed using 1 N HCl or trifluoroacetic acid [12]. To complete the hydrolysis reaction, either 250 μl of 1 N HCl or 60 μl of trifluoroacetic acid was used. Compared to hydrolysis with 1 N HCl solution, hydrolysis with trifluoroacetic acid was advantages in that complete hydrolysis could be achieved at room temperature with a smaller reaction volume that would make automatic synthesis possible. After hydrolysis, the final mixture was neutralized with 2 M sodium acetate solution and the pH was adjusted to 6–7. The best resolution in HPLC purification procedure was obtained under neutral pH, while poor results were obtained under acidic conditions.

Alumina N Sep-Pak cartridge purification of the reaction mixture before HPLC application, increased the radiochemical yield. Without Alumina N Sep-Pak cartridge purification, [^{18}F]FLT was not completely separated from the reaction mixture after HPLC purification resulting in the

Table 4
Effect of reaction time on [^{18}F]fluorination yield for the preparation of [^{18}F]FLT using precursor **5**

Radiochemical yield* (n = 3)				
Time (min)	Concentration of precursor (mg/300 μl CH_3CN)			
	11	23	34	46
5	8.1 ± 2.9%	54.5 ± 6.2%	82.0 ± 5.4%	83.9 ± 5.4%
10	11.5 ± 2.9%	69.1 ± 2.5%	81.9 ± 2.6%	82.5 ± 2.6%
20	8.7 ± 2.4%	60.2 ± 2.3%	80.2 ± 2.9%	80.5 ± 3.7%
30	6.9 ± 3.5%	58.1 ± 3.9%	75.4 ± 2.1%	79.4 ± 1.6%

* Reaction temperature was 110°C; radioactivity of [^{18}F]fluoride: 370 MBq.

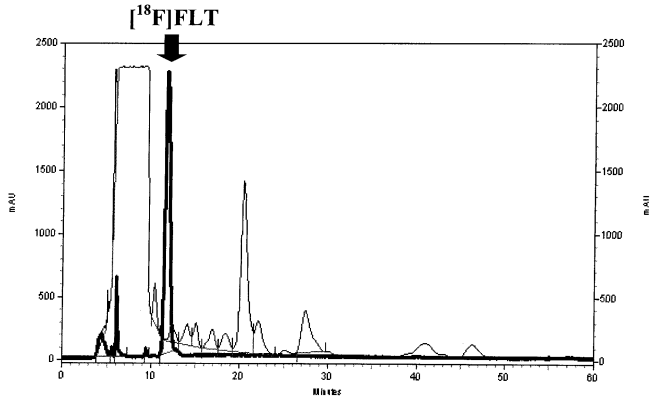

Fig. 3. HPLC chromatogram of the [^{18}F]FLT reaction mixture. The peak at a retention time of 11.67 min is [^{18}F]FLT. The bold line is the radioactive chromatogram and the dashed line is the UV chromatogram at 267 nm.

radiochemical yields of only 20 ± 1.9%. In contrast, the radiochemical yield doubled after Alumina N Sep-Pak treatment and no other [^{18}F]fluoride-labeled compounds were found on the HPLC chromatogram [6]. When we injected cold FLT and purified [^{18}F]FLT, they showed the same retention time. The HPLC chromatogram of the crude product is shown in Fig. 3 after adjusting the pH and Alumina N Sep-Pak cartridge purification, and the HPLC chromatogram of HPLC-purified [^{18}F]FLT is shown in Fig. 4. With the *N*-BOC-protected precursor, hydrolysis proceeded with only 1 N HCl. For complete hydrolysis of the dimethoxytrityl group and the BOC protecting group in precursor **5**, 500 μl of 1 N HCl was needed.

After HPLC purification, we obtained 107.6 ± 6.1 MBq of [^{18}F]FLT from 370 MBq of [^{18}F]fluoride (decay corrected radiochemical yield: 40.0 ± 5.2%) with a radiochemical purity of 98.0 ± 1.8% using precursor **3**. With precursor **5**, the decay corrected yield was 42.0 ± 5.4% (127.8 ± 3.6 MBq from 370 MBq of [^{18}F]fluoride), and the radiochemical purity was 97.0 ± 2.1%. The specific activity after HPLC purification was 1.2 and 1.4 Ci/μmol for precursor **3** and **5**, respectively. The total synthesis time, including HPLC purification, was 60 min for both precursors. There

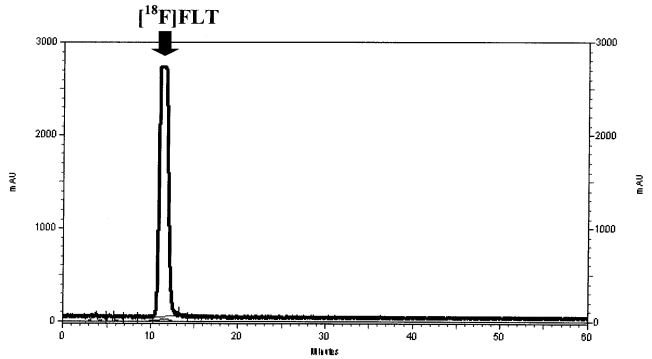

Fig. 4. HPLC chromatogram of purified [^{18}F]FLT. The peak at a retention time of 11.02 min is [^{18}F]FLT. The bold line is the radioactive chromatogram and the dashed line is the UV chromatogram at 267 nm. After purification, only one radioactive peak was found.

were no apparent differences in radiochemical yields, radiochemical purity or specific activity between the two precursors, while *N*-BOC-protected precursor **5** required milder reaction conditions than precursor **3**.

With high [^{18}F]fluoride radioactivities, we obtained similar radiochemical yields. With precursor **3**, decay corrected radiochemical yields were 43.8%, 53.6% and 45.0% with 1110, 1850 and 3700 MBq of [^{18}F]fluoride, respectively. Likewise, decay corrected radiochemical yields of 53.6%, 52.5%, and 47.5% were obtained with 1110, 1850 and 3700 MBq of [^{18}F]fluoride, respectively, using precursor **5**. Radiochemical purity after HPLC purification was 97.4 ± 2.1%.

In the presence of phase-transfer catalysts such as tetrabutylammoniun carbonate or tetrabutylammonium hydroxide, we obtained unidentified [^{18}F]fluorine labeled compounds. In the chemical synthesis of cold standard compounds with tetrabutylammonium, elimination compound **4** was obtained as described in previous reports [2,7]. However, we only obtained one unknown compound during the reaction of [^{18}F]fluoride ion and these phase-transfer catalysts. The radiochemical yield of [^{18}F]fluorination was also very high (92 ± 2.4%) with only one [^{18}F]fluorinated product. Although there was only one [^{18}F]fluorine-labeled compound, we did not think it was an elimination product, since cold elimination compound did not contain a fluorine atom and the HPLC chromatogram was different from that of compound **4**.

3. Conclusion

[^{18}F]FLT was synthesized from a new nosylate and its *N*-BOC-protected analogue (**3** and **5**), which gave radiochemical yields of 40 ± 5.2 and 42 ± 5.4% and radiochemical purities of 98 ± 1.8% and 97 ± 2.1%, respectively. For total synthesis of [^{18}F]FLT, 60 min were required using these precursors. Pyrimidine ring *N*-BOC-protected precursor **5** required a lower reaction temperature than unprotected precursor **3**. Alumina N Sep-Pak cartridge purification of the reaction mixture before HPLC application significantly increased the radiochemical yield. With these precursors, [^{18}F]FLT can be prepared reliably without lengthy precursor synthesis or a long reaction time, which should facilitate the clinical application of [^{18}F]FLT.

4. Experimental

4.1. General procedure

FLT standard, chemicals and all solvents were purchased from Sigma-Aldrich Korea (Seoul, Korea). Concentration was performed under reduced pressure at a water bath temperature of below 40°C. Thin layer chromatography (TLC) was performed with Merck Silica Gel 60 F_{254} and chemical spots were checked by UV light or H_2SO_4 charring. Flash column chromatography was performed with silica gel Merck 60 (Art 9385, 230–400 mesh). NMR spectra were recorded on a Bruker instrument at 400 MHz. Coupling constants (*J*) are reported in Hz and chemical shifts are in ppm (δ) relative to a residual solvent peak or internal standard. Assignments were based on 2D Cosy and 2D Heterocosy experiments. FAB-MS were recorded on a JEOL JMX-AX 505WA mass spectrometer (Tokyo, Japan) by adding either 3-NBA or glycerol. LC-MS was performed with a JEOL JMX-LC in APCI-positive mode (Tokyo, Japan).

*4.1.1. [5′-O-(4,4′-Dimethoxytriphenylmethyl)-2′-deoxy-β-D-threopentofuranosyl]thymine (**1**)*

A solution of thymidine (1.5 g, 6 mmol) and dimethoxytrityl chloride (DMTrCl; 2.54 g, 7.5 mmol) was stirred in pyridine (30 ml) for 3 hrs at room temperature. The solution was cooled to 0° and methanesulfonyl chloride (MsCl; 1.16 ml, 15 mmol) was added. After 3 hrs, the mixture was concentrated and the reaction solvent was co-evaporated with toluene. The mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution and H_2O. After the organic phase was dried with anhydrous Na_2SO_4, the concentrated organic residue was dissolved in EtOH (120 ml) and 3 ml of 10 N NaOH solution was added. After the solution was heated for 1.5 hrs at 80°C, it was neutralized with acetic acid and concentrated with toluene. The residue was purified by column chromatography on silica gel with n-hexane:ethyl acetate (1:3, v/v) to give **1** as a brown solid (2.52 g, 77%). R_f = 0.48 (Hexane:ethyl acetate = 1:3, v/v); mp: 77°C; FAB-MS (glycerol) *m/e* 545 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.29–6.84 (m, 14H, Ar-H & H-6), 6.12 (dd, 1H, $J_{1',2'}$ = 6.0 Hz, H-1′), 4.27 (t, 1H, H-3′), 3.85 (m, 1H, H-4′), 3.73 (s, 6H, OCH_3), 3.42 (m, 2H, H-5), 2.51 (m, 2H, H-2), 1.79 (s, 3H, 5-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): 85.2 (C-1′), 83.7 (CPh_3), 80.3 (C-4′), 69.0 (C-3′), 60.1 (C-5′), 55.3 (OCH_3), 41.4 (C-2′), 12.8 (CH_3).

*4.1.2. 3′-Deoxy-3′-fluoro-5′-O-(4,4′-dimethoxytriphenylmethyl) thymine (**2**)*

The solid **1** (0.55 g, 1.01 mmol) was dissolved in 20 ml of CH_2Cl_2:THF (9:1, v/v). The solution was cooled to 0°C, (diethylamino)sulfur trifluoride (DAST; 0.25 ml, 1.92 mmol) was added, and the mixture was stirred for 2 hrs at room temperature. Then the mixture was diluted with CH_2Cl_2 and washed with aq. $NaHCO_3$ solution. The organic layer was dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica with n-hexane:ethyl acetate (2:1, v/v) to give **2** as a white solid (0.16 g, 28%). R_f = 0.50 (Hexane:ethyl acetate = 1:1, v/v); mp: 88°C; FAB-MS (3-NBA) *m/e* 547 $[M+H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.47 (s, 1H, NH), 7.61–6.83 (m, 14H, DMTr-H and H-6), 6.49 (dd, 1H, $J_{1',2'}$ = 5.6 Hz, $J_{1'F}$ = 9.4 Hz, H-1′), 5.30 (dd, 1H, $J_{3',4'}$ = 4.8 Hz, $J_{3'F}$ = 54 Hz, H-3′), 4.33 (d, 1H, $J_{4'F}$ = 28.8 Hz, H-4′), 3.79 (s, 6H, OCH_3), 3.53 (dd, 1H, $J_{5a',5b'}$ = 2.0 Hz, $J_{5a'F}$ =

10.8 Hz, H-5a′), 3.38 (dd, 1H, $J_{5a',5b'}$ = 2.4 Hz, $J_{5b'F}$ = 10.4 Hz, H-5b′), 2.72–2.62 (m, 1H, H-2a′), 2.42–2.29 (dd, 1H, H-2b′), 1.43 (s, 3H, 5-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): 164.4 (C-4), 151.0 (C-2), 135.7 (C-5), 112.1 (C-6), 95.0 ($J_{3',F}$ = 176.62 Hz, C-3′), 87.7 (CPh_3), 85.0 (C-1′), 84.6 ($J_{4',F}$ = 25.43 Hz, C-4′), 63.8 ($J_{5,F}$ = 10.88 Hz, C-5′), 55.7 (OCH_3), 39.2 ($J_{2',F}$ = 21.19 Hz, C-2′), 12.1 (CH_3).

*4.1.3. [5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-2′-deoxy-3′-*O*-(4-nitrobenzesulfonyl)-β-D-*threo *pentofuranosyl]thymine (**3**)*

The solid **1** (0.80 g, 1.48 mmol) was dissolved in pyridine (10 ml) at 0°C and 4-nitrobenzenesulfonyl chloride (Nosyl chloride; 0.92 g, 2.91 mmol) and silver trifluoromethanesulfonate (AgOTf; 1.1 g, 2.91 mmol) were added [6]. The reaction mixture was stirred for 50 min at 0°C. Then the reaction temperature was increased to room temperature for 40 min. After the reaction mixture was diluted with EtOAc, it was filtered, and the filtrate was washed with H_2O and brine. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography on silica gel with hexane:ethyl acetate (2:1, v/v) to give **3** as a yellow solid (0.75 g, 70%). R_f = 0.80 (Hexane:ethyl acetate = 1:3, v/v); mp: 122°C; FAB-MS (3-NBA) *m/e* 730 $[M+H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.83 (s, 1H, NH), 8.22–6.80 (m, 18H, Ar-H & H-6), 6.17 (dd, 1H, $J_{1',2a'}$ = 7.6 Hz, $J_{1',2b'}$ = 2.8 Hz, H-1′), 5.21 (t, $J_{3',4'}$ = 4.0 Hz, 1H, H-3′), 4.21 (dd, 1H, $J_{3',4'}$ = 5.6 Hz, $J_{4',5'}$ = 9.2 Hz, H-4′), 3.79 (s, 6H, OCH_3), 3.53 (dd, 1H, $J_{5a',5b'}$ = 6.4 Hz, $J_{4',5'}$ = 10 Hz, H-5a′), 3.21 (dd, 1H, $J_{5a',5b'}$ = 5.2 Hz, $J_{4',5'}$ = 10.2 Hz, H-5b′), 2.77–2.72 (m, 1H, H-2a′), 2.47–2.42 (dd, 1H, $J_{1',2'}$ = 2 Hz, $J_{2a',2b'}$ = 15.8 Hz, H-2b′), 1.73 (s, 3H, 5-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): 86.8 (C-1′), 84.1 (CPh_3), 81.5 (C-4′), 80.3 (C-3′), 60.3 (C-5′), 55.1 (OCH_3), 39.1 (C-2′), 12.3 (CH_3).

*4.1.4. 3′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl) thymidinene (**4**)*

To a solution of **3** (0.137 g, 0.188 mmol) in 7 ml of CH_3CN was added 500 μl 1 M tetrabutylammonium fluoride (TBAF)/THF. The reaction mixture was stirred for 5 min at room temperature and then heated for 10 min at 90°C. TLC of the reaction mixture revealed one major compound and two minor compounds. One of the latter was the F-compound **2** (5%) while the other (28%) was unknown. After the evaporation of the solvent, the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography on silica gel with hexane:ethyl acetate (1:1 v/v) to give **4** as a yellow oil (0.0392 g, 38%). R_f = 0.20 (Hexane:ethyl acetate = 1:1, v/v); FAB-MS (3-NBA) *m/e* 527 $[M+H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.45–6.81 (m, 14H, DMTr-H & H-6), 7.05 (m, 1H, H-1′), 6.36 (d, 1H, $J_{2',3'}$ = 6.08 Hz, H-3′), 5.89 (d, 1H, $J_{2',3'}$ = 5.84 Hz, H-2′), 4.97 (s, 1H, H-4′), 3.78 (s, 6H, OCH_3), 3.44 (dd, 1H, $J_{5a',5b'}$ = 2.68 Hz, $J_{4',5'}$ = 10.5 Hz, H-5a′), 3.35 (dd, 1H, $J_{5a',5b'}$ = 3.68 Hz, $J_{4',5'}$ = 10.4 Hz, H-5b′), 1.24 (s, 3H, 5-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): 89.6, 86.4, 85.8, 64.6 (C-5′), 55.2 (OCH_3), 11.2 (CH_3).

*4.1.5. 3-N-t-Butoxycarbonyl-(5′-*O*-(4,4′-dimethoxytriphenylmethyl)-2′-deoxy-3′-*O*-(4-nitrobenzenesulfonyl)-β-D-*threo *pentofuranosyl)thymine (**5**)*

The solid **3** (1.41 g, 1.93 mmol) was dissolved in THF (20 ml) and *t*-butoxycarbonyl anhydride (*t*-BOC_2O; 0.49 ml, 2.12 mmol) was added. The reaction mixture was stirred for 80 min at room temperature a stoichiometric amount of dimethylaminopyridine (DMAP) was added, and stirring was continued at room temperature for 4 hrs. The reaction mixture was concentrated and diluted with ethyl acetate. The mixture was then washed with H_2O, 1N HCl, and aqueous $NaHCO_3$. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography on silica gel with hexane:ethyl acetate (3:1, v/v) to give **5** as a yellow solid (0.86 g, 53%). R_f = 0.60 (Hexane:ethyl acetate = 1:1, v/v); mp: 106°C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.25–6.80 (m, 18H, Ar-H & H-6), 6.14 (dd, 1H, $J_{1',2a'}$ = 7.4 Hz, $J_{1',2b'}$ = 2.8 Hz, H-1′), 5.19 (t, $J_{3',4'}$ = 4.0 Hz, 1H, H-3′), 4.21 (dd, 1H, $J_{3',4'}$ = 5.6 Hz, $J_{4',5'}$ = 9.0 Hz, H-4′), 3.80 (s, 6H, OCH_3), 3.53 (dd, 1H, $J_{5a',5b'}$ = 6.4 Hz, $J_{4',5'}$ = 10.2 Hz, H-5a′), 3.23 (dd, 1H, $J_{5a',5b'}$ = 5.2 Hz, $J_{4',5'}$ = 10.2 Hz, H-5b′), 2.76–2.69 (m, 1H, H-2a′), 2.44 (dd, 1H, $J_{1',2'}$ = 2 Hz, $J_{2a',2b'}$ = 15.8 Hz, H-2b′), 1.72 (s, 3H, 5-CH_3), 1.60 (s, 9H, $(CH_3)_3C$); ^{13}C-NMR (100 MHz, $CDCl_3$): 86.9 (C-1′), 84.4 (CPh_3), 81.7 (C-4′), 80.0 (C-3′), 60.3 (C-5′), 55.1 (OCH_3), 39.4 (C-2′), 27.3 ($(CH_3)_3C$), 12.4 (CH_3).

*4.1.6. 3′-Deoxy-3′-fluorothymidine (**6**)*

After dissolving solid **2** (0.0245 g, 0.0448 mmol) in CH_3CN (4 ml), hydrolysis of the dimethoxytrityl group was performed using 0.5 ml of 1 N HCl. The reaction proceeded at 42°C for 5 min. FLT was extracted with water and the water was co-evaporated with toluene. The residue was purified by column chromatography to give **6** as a yellow solid (11 mg, 97%). mp: 176°C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.4 (s, 1H, NH), 7.70 (s, 1H, H-6), 6.22 (dd, 1H, $J_{1',2'}$ = 5.6 Hz, $J_{1',F}$ = 9.2 Hz, H-1′), 5.29 (dm, 1H, $J_{3',F}$ = 59.8 Hz, H-3′), 4.14 (dt, 1H, $J_{4',F}$ = 27.8 Hz, H-4′), 3.62 (m, 2H, H-5′), 2.37 (m, 2H, H-2′), 1.78 (s, 3H, 5-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): 163.7 (C-4), 150.5 (C-2), 135.8 (C-5), 109.8 (C-6), 94.9 ($J_{3',F}$ = 172.8 Hz, C-3′), 84.8 ($J_{4',F}$ = 22.7 Hz, C-4′), 83.7 (C-1′), 60.9 ($J_{5',F}$ = 10.9 Hz, C-5′), 36.9 ($J_{2',F}$ = 20.2 Hz, C-2′), 12.3 (CH_3).

*4.1.7. Radiosynthesis of [^{18}F]FLT ([^{18}F]**6**)*

[^{18}F]Fluoride in 95%-enriched ^{18}O water was produced by an ^{18}O(p,n)^{18}F nuclear reaction using an 18 MeV IBA cyclotron (Cyclone 18/9, Leuven, Belgium). [^{18}F]Fluoride ion (370 MBq) in 0.5 ml of water was trapped on a QMA cartridge and eluted with a mixture of Kryptofix [2.2.2]

(28.0 mg, 74.3 μmol) and K_2CO_3 (5.5 mg, 39.8 μmol) dissolved in 1 ml of CH_3CN/H_2O (7/3 v/v). The solution was dried completely by azeotropic distillation with CH_3CN (2 × 0.5 ml) under a gentle N_2 gas stream at 100°C. The final dried residue was dissolved in 300 μl CH_3CN. Then, 10, 20, 30 or 40 mg (13.7, 27.4, 41.1, or 54.8 μmol) of nosylate compound **3** or 11, 23, 34, or 46 mg (12.9, 27.2, 40.1, or 54.3 μmol) of *N*-BOC protected precursor **5** was added. The reaction mixture was heated at 100–130° for 5–30 min and developed a brown color. The dimethoxytrityl protecting group was removed by adding 250 μl 1 N HCl or 60 μl of trifluoroacetic acid. With 1 N HCl, the reaction mixture was heated for 5 min at 50°C. After addition of the hydrolysis reagent, the reaction mixture changed to orange color. However, with trifluoroacetic acid, it needed to stand at room temperature for 5 min. After neutralization with 1.5 ml of 2 M sodium acetate, the mixture was applied to an Alumina N cartridge (Waters, USA) and the eluate was collected in a glass test tube. The cartridge was washed with 1–1.5 ml of water, which was collected in the same glass test tube [6]. The collected solution was applied to HPLC for purification. For each precursor concentration, we performed three experiments with 370 MBq/0.5 ml of [^{18}F]fluoride.

With the *N*-BOC-protected precursor **5** (11–34 mg, 13–40 μmol), we only used 500 μl of 1 N HCl for hydrolysis of the dimethoxytrityl and BOC groups.

We also synthesized [^{18}F]FLT with a high [^{18}F]fluoride radioactivity of 1110, 1850 and 3700 MBq. Synthetic condition with precursor **3** was heating at 130°C for 5 min with 30 mg of precursor. As for precurosr **5**, 34 mg of precursor was heated at 110°C for 5 min.

4.1.8. Analysis and HPLC purification of [^{18}F]FLT

Reactions were analyzed by thin layer chromatography using a silica gel plate (Merck, Darmstadt, Germany) and eluted with CH_2Cl_2:CH_3OH = 9:1 (1:1 v/v). The R_f value on silica gel was checked by a TLC scanner (Bioscan, Washington DC, USA). The R_f values of [^{18}F]fluoride, [^{18}F]FLT and [^{18}F]DMT-FLT on radioTLC were 0.0, 0.5 and 0.9, respectively.

In addition, HPLC (Thermo Separation Products, CA, USA) equipped with a NaI(Tl) detector (Bioscan, Washington, DC, USA) and a UV detector (267 nm) was used for the identification and purification of [^{18}F]fluorine-labeled product. [^{18}F]FLT ([^{18}F]**6**) was separated from the reaction mixture on an Alltech Econosil C_{18} column (250 × 10 mm, 10 μ) eluted with water:ethanol (85:15, v/v) at a flow rate of 3 ml/min.

To optimize the conditions for HPLC purification of [^{18}F]FLT, we tested four reagent conditions. They were A [20 μg of unlabeled FLT in 20 μl of acetonitrile (base solution)]; B [the base solution along with 250 μl of 1N HCl (pH 3)]; C [the base solution along with 250 μl of 1N HCl and 1.25 ml of 0.5 M sodium acetate (pH 5)]; and D [the base solution along with 250 μl of 1N HCl and 1 ml of 1 M sodium acetate (pH 7)] and these conditions were applied to HPLC, respectively. All samples were injected in a volume of 2 ml and dilution was performed with deionized water.

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