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# SYNTHESIS OF 3'-DEOXY-3'-[$^{18}$F]FLUORO-THYMIDINE WITH 2,3′-ANHYDRO-5′-*O*-(4,4′-DIMETHOXYTRITYL)-THYMIDINE

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## Summary

[$^{11}$C]Thymidine has been used as a proliferation marker in positron-emission-tomography (PET) studies of tumors. This compound showed metabolite related problems and the radiosynthesis proved to be difficult. Recently, the more stable 3'-deoxy-3'-[$^{18}$F]fluorothymidine ([$^{18}$F]FLT) has been suggested as an alternative. One advantage of [$^{18}$F]FLT is based on thymidine kinase-1 catalyzed phosphorylation of FLT and the intracellular accumulation of this metabolite without participation in DNA synthesis. The radiosynthesis of [$^{18}$F]FLT originally designed by Grierson et al. was found to be demanding especially regarding the workup of the [$^{18}$F]fluoride/1-(2-deoxy-3-*O*-nosyl-5-*O*-DMT-$\beta$-D-*threo*-pento-furanosyl)-3-DMBn-thymine reaction mixture. Instead, we used 2,3′-anhydro-5′-*O*-(4,4′-dimethoxytrityl)thymidine as a precursor for the synthesis of [$^{18}$F]FLT. In DMSO at 175 °C and in presence of Kryptofix® 2.2.2. we obtained 5.6±1,4 % [$^{18}$F]FLT (EOS).

**Keywords:** *Proliferation Marker, [$^{18}$F]FLT, PET imaging, 2,3′-anhydro-5′-O-(4,4′-dimethoxytrityl)-thymidine*

## Introduction

Thymidine is incorporated into DNA during the S-phase of the cell cycle and has, therefore, been used in $^{11}$C labeled form for the measurement of cell proliferation

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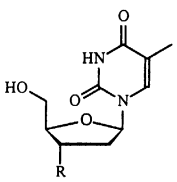
with positron-emission-tomography (PET). Although this radiopharmaceutical has proven useful as an imaging agent, its application is limited due to the rapid *in vivo* degradation and the short half-life of [$^{11}$C] ($t_{1/2}$ = 20 min) (1). These problems have been overcome by the thymidine analog 3′-deoxy-3′-[$^{18}$F]fluorothymidine [$^{18}$F]FLT (2). [$^{18}$F]FLT is more resistant than [$^{11}$C]thymidine against *in vivo* catabolism and its radioisotope has a longer half-life ($t_{1/2}$ = 110 min) (2). In addition, FLT was found to exhibit antiviral potency and was therefore suggested as a pharmaceutical for the treatment of HIV-1 positive patients (3). The initial metabolism of FLT is comparable with [$^{11}$C]thymidine. Phosphorylation at the 5'-position mediated by the thymidine kinase-1 causes intracellular trapping of the tracer. Although [$^{18}$F]FLT is not a substrate of DNA synthesis it mimics proliferative activity of the tissue. This is due to the upregulation of thymidine kinase-1 during the S-phase of the cell cycle (4). It could be shown that [$^{18}$F]FLT features an excellent proliferation marker for PET studies of tumors (2). In order to further improve the radiosynthesis of [$^{18}$F]FLT (5,6) we describe a simplified labeling method and a more convenient synthesis of the precursor 2,3′-anhydro-5′-*O*-(4,4′-dimethoxytrityl)thymidine 3.

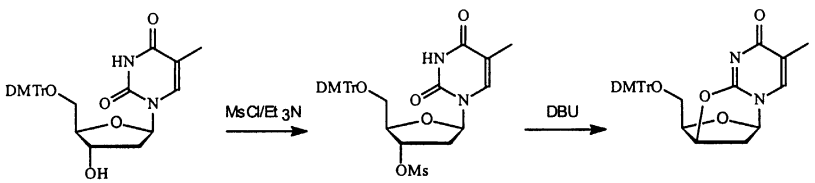



R = OH Thymidine
R = F FLT

## Results and Discussion

The synthesis of the precursor 2,3′-anhydro-5′-*O*-(4,4′-dimethoxytrityl)thymidine 3 has essentially been described by Matsuda et al. (7) and its route is outlined in below.

**Scheme 1**




1 2 3

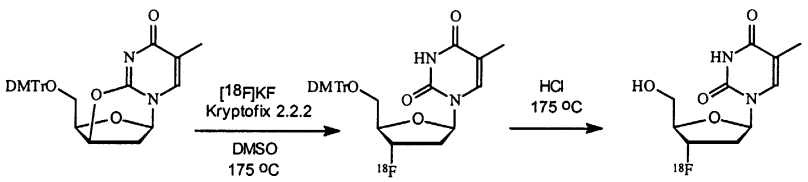
The following variations were introduced which simplified the synthesis of this compound: a. commercially available 5′-*O*-(4,4′-dimethoxytrityl)thymidine 1 was used as

a starting material and b. the two synthesis steps leading to precursor 3 were performed subsequently in the same reaction solution without separating the mesylated intermediate 2. The overall yield amounted to 93% compared to 71% reported in the literature (7).

Precursor 3 is susceptible to nucleophilic attack of a series of nucleophiles leading to various 3'-deoxy-3'-substituted thymidines. The reactions leading to anhydro-ring opening needed, however, high thermal energy to form products with substituents at the 3'-exo-sugar ring position (7).

**Scheme 2**




3 4 [$^{18}$F]FLT

According to Scheme 2 the synthesis of [$^{18}$F]FLT was accomplished by using a mixture of precursor 3, Kryptofix® 2.2.2. and potassium [$^{18}$F]fluoride at molar ratios of 1/1.5/nca. High temperature was achieved by performing the reaction in dry DMSO. The optimal temperature was found at 175 °C. The influence of the reaction time on the formation of [$^{18}$F]FLT is illustrated in Figure 1. Maximum yields were obtained at 1 hour reaction time. The subsequently performed deprotection of the 4,4'-dimethoxytritylated intermediate 4 using HCl afforded [$^{18}$F]FLT in a radiochemical yield of 5.6±1,4 % (EOS; n = 5). Starting with 7.5 GBq [$^{18}$F]fluoride we obtained 420 MBq [$^{18}$F]FLT. This result ranges little below the radiochemical yield reported for the original [$^{18}$F]FLT synthesis 7,6% (EOS) (5,6). Other aprotic solvents usually applied for nucleophilic substitution with [$^{18}$F]fluoride failed. Performing the reaction in boiling acetonitrile or 1,4-dioxane for up to 60 minutes did not lead to significant amounts of

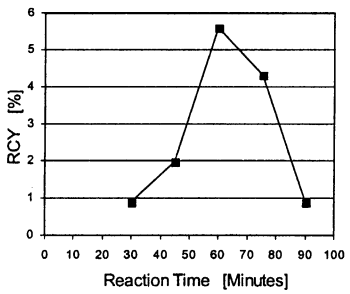



**Figure 1.** The influence of the reaction time on [$^{18}$F]FLT formation

[$^{18}$F]FLT. Only in DMF a small portion of the labeled precursor could be detected. A similar drawback was observed by replacing Kryptofix® 2.2.2. with tetrabutylammonium carbonate as phase transfer catalyst.

After $^{18}$F-labeling the dimethoxytrityl group of the FLT precursor was cleaved by the addition of HCl. Heating at 175 °C for 1 minute proved sufficient for complete deprotection. Extension of the reaction time and deviation of the indicated temperatur or HCl concentration lead to incomplete deprotection or product decomposition.

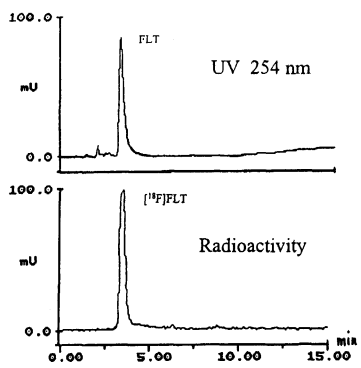



**Figure 2.** HPLC of purified [$^{18}$F]FLT (Radioactivity) spiked with unlabeled FLT (UV)

Work up of the [$^{18}$F]FLT-reaction mixture included first filtration over a neutral aluminium oxide cartridge followed by RP-HPLC separation. The aluminium oxide cartridge proved to be helpful because it retained a great percentage of [$^{18}$F]fluoride and Kryptofix® [2.2.2.] (6). A typical analytical HPLC run of the purified [$^{18}$F]FLT (lower HPLC) is shown in Figure 2. Coelution of admixed unlabeled FLT (upper HPLC) indicated the identity of the product. In addition, the synthesis of unlabeled FLT was performed using the method described here. HPLC analysis and ESI-MS of the product proved the formation of FLT.

## Conclusion

Summarizing the data from the literature and resuming the experiences of our experiments we conclude that the cyclic precursor 2,3′-anhydro-5′-*O*-(4,4′-dimethoxytrityl)-thymidine 3 features an electrophilc center at the exo-3'-position suitable for nucleophilic substitution with [$^{18}$F]fluoride. However, the high temperatures necessary for the substitution and the anhydro-ring opening indicates well known intrinsic disadvantages of the tracer [$^{18}$F]fluoride, namely, its low nucleophilicity and the non-carrier added concentration. In addition the reduced nucleofuge

characteristics of the 2,3′-anhydro group necessitates harsh thermal reaction conditions. All these points may speak against this approach, however, the simplified protection strategy of precursor 3, where the anhydro structure acts simultaneously as a leaving and protection group of the 3-*N*-position at the pyrimidine ring, represents an attractive simplification for the precursor synthesis, labeling and workup of [$^{18}$F]FLT. Several reaction parameters used for the radiosynthesis, like choice of solvents and catalysts are currently tested to further improve the RCY.

Recently, Machulla et al. reported also about the application of 2,3′-anhydro-5′-*O*-(4,4′-dimethoxytrityl)thymidine 3 as a precursor for [$^{18}$F]FLT synthesis (8). With the exception of reaction time and reaction temperature the results they obtained were comparable with our findings. While in our experiments a reaction temperature of 175 °C for 60 minutes was necessary to attain optimal yields (Fig. 1) Machulla et al. achieved best results heating at 160 °C for 10 minutes (8). The other parameters like the amount of precursor (10 mg) and the choice of solvent (DMSO) were comparable leading to [$^{18}$F]FLT yields of 5.6% (EOS) in this work vs. 6.5% (EOS) reported by Machulla et al. (8).

## Experimental

### Materials and Methods

5′-*O*-(4,4′-dimethoxytrityl)thymidine, methanesulfonyl chloride, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 3'-deoxy-3'-fluorothymidine (FLT) (all Aldrich, Deisenhofen; Germany) and Kryptofix® 2.2.2. (Merck, Darmstadt; Germany) were purchased from indicated sources. All solvents were of analytical grade and used without further purification. Only DMSO was distilled and stored over molecular sieves. Neutral aluminum oxide cartridges were obtained from Waters Corp. (Milford, Massachusetts, USA). $^{1}$H-NMR spectra were measured on a Bruker AC-500 spectrometer and referenced to tetramethylsilane. Electrospray ionisation (ESI) mass spectra were carried out with a Finnigan TSQ 7000 triplequadrupole system. HPLC was carried out on Merck-Hitachi system equipped with UV (254 nm) and a γ radiation detector (GABI, Raytest Isotopenmeßgeräte GmbH, Straubenhardt; Germany). Reverse phase HPLC columns (analytical:250x4 mm; semi preparative 250x25) were

filled with Nucleosil 120-10 C18 (Ziemer, Mannheim; Germany). The solvent mixture used throughout isocratic HPLC measurements consisted of 3% EtOH in $H_2O$. The flow rates were adjusted to 1 ml/min and 7 ml/min for analytical and semi preparative runs, respectively. Column chromatography was performed with silica gel 60 (40-63 μm, Merck, Darmstadt; Germany), at a flow rate of 5 ml/min. Analytical TLC was performed using Polygram Sil G/UV 254 plates (Macherey&Nagel, Düren; Germany). Melting points were determined with Büchi 535 apparatus and were not corrected. [$^{18}$F]fluoride was obtained from a Scanditronix MC 32 NI cyclotron at the German Cancer Research Center (DKFZ) using $^{18}$O-enriched water and the $^{18}O(p,n)^{18}F$ reaction. Separation of $[^{18}F]F^-$ from $[^{18}O]H_2O$ was achieved with an anion exchange cartrige (SPE cartridge Chromafix 30-PS-$HCO_3$; Macherey and Nagel, Düren; Germany). Elution with aqueous potassium carbonate (0.4 ml, 75 μM) yielded a solution of potassium [$^{18}$F]fluoride. Doses of about 7.5 GBq were used throughout the experiments.

**Synthesis of 2,3′-anhydro-5′-*O*-(4,4′-dimethoxytrityl)thymidine**

250 mg 5′-*O*-(4,4′-dimethoxytrityl)thymidine 1 (0.46 mmol), dissolved in 30 ml dry dichloromethane, was reacted with 80 μl methanesulfonyl chloride (1.08 mmol) in the presence of 0.75 ml triethylamine (5.34 mmol). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 3 h. Therafter, without isolating the resulting mesylate 2, this reaction mixture was used to continue the reaction sequence outlined in Scheme 1 by adding 0.337 ml 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) (2.25 mmol) and refluxing for 2 h. Finally, the reaction mixture was washed with water, the organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was redissolved in 2 ml dichloromethane/methanol/triethylamine (95/5/0.05; v/v) and purified by column chromatography (25x3.5 cm) using the same solvent mixture. Evaporation of the product fractions afforded 0.225 g compound 3 (0.427 mmol; 93%). Mp 120-125 °C. TLC $R_f$ 0.78 (silica gel; $CH_2Cl_2$/MeOH 9/1 v/v). Positive FAB-MS of the precursor revealed an exact mass of *m/z* 527.2204 ($[M+H]^+$ theor. 527.2182 for $C_{31}H_{31}N_2O_6$). $^1$H-NMR ($CDCl_3$, 500 MHz): δ=7.42-7.38 [2H, m, DMTr-H], 7.31-7.23 [6H, m, DMTr-H], 7.20-7.15 [1H,

m, DMTr-H], 6.92 [1H, q, H6], 6.82-6.77 [4H, m, DMTr-H], 5.46 [1H, dd, H1'], 5.13-5.10 [1H, m, H3'], 4.24 [1H, ddd, H4'], 3.77 [6H, s, $OCH_3$], 3.34 [1H, dd, H5'], 3.31 [1H, dd, H5'], 2.64 [1H, ddd, H2'], 2.36 [1H, ddd, H2'], 1.89 [3H, dd, 5-$CH_3$].

### Synthesis of 3'-deoxy-3'-fluorothymidine (FLT)

Kryptofix® 2.2.2. (46,9 mg, 0.124 mmol) dissolved in 0.4 ml acetonitrile, potassium carbonate (10 mg, 0.07 mmol) dissolved in 0.1 ml water and potassium fluoride (50 µl of an aqueous 1.72 M solution, 0.09 mmol) were dried under a stream of helium gas. To the residue 2,3′-anhydro-5′-*O*-(4,4′-dimethoxytrityl) thymidine dissolved in dry DMSO (0.4 ml) was added and the mixture was heated to 175 °C. After 1 h the protecting group was removed with 1 M aqueous HCl by heating at 175 °C for 1 min. The reaction mixture was analyzed by HPLC and compared with an FLT standard. Positive FAB-MS of the product isolated by HPLC revealed an exact mass of *m/z* 245.0941 ($[M+H]^+$ theor. 245.0937 for $C_{10}H_{14}FN_2O_4$).

### Radiosynthesis of [$^{18}$F]FLT

[$^{18}$F]$F^-$ solution (7.5 GBq) was added to a mixture of Kryptofix® 2.2.2. (43.5 mg, 0.115 mmol) dissolved in 0.4 ml acetonitrile and potassium carbonate (23 mg, 0.167 mmol) dissolved in 0.1 ml water. The solution was dried by heating under a stream of helium. 10 mg 2,3′-Anhydro-5′-*O*-(4,4′-dimethoxytrityl) thymidine (0.019 mmol) dissolved in 0.4 ml dry DMSO was added to the residue and the mixture was heated to 175 °C. One hour later the protecting groups were removed by adding 0.4 ml 1 M aqueous HCl and heating for 1 min at 175 °C. The reaction solution was prepurified by passage through a neutral aluminum oxide SepPak cartridge and then separated by HPLC yielding 5.6±1,4 % [$^{18}$F]FLT (n = 5).

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