

Synthesis of 3'-deoxy-3'-fluorothymidine (FLT) 5'-O-glucuronide: a reference standard for imaging studies with [^{18}F]FLT \dagger

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Reaction of methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetimidoyl)- α -D-glucopyranuronate with 3'-deoxy-3'-fluorothymidine in the presence of trimethylsilyl trifluoromethanesulfonate gave (2*R*,3*R*,4*S*,5*S*,6*S*)-2-(((2*R*,3*S*,5*R*)-3-fluoro-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)-methoxy)-6-(methoxycarbonyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate, which was hydrolysed to 3'-deoxy-3'-fluorothymidine-5'-glucuronide. Analysis of this reference standard by high performance liquid chromatography enabled the identification of [^{18}F]3'-deoxy-3'-fluorothymidine-5'-glucuronide in blood samples from six human patients who had been administered [^{18}F]3'-deoxy-3'-fluorothymidine.

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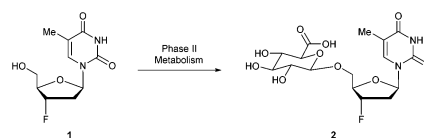
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Introduction

Positron emission tomography (PET) is a key and rapidly expanding clinical imaging technology, which involves the systemic administration of a tracer or ligand labelled with a positron-emitting isotope. Within the body, the positrons released are annihilated on collision with electrons releasing two photons of γ -radiation, which are detected by placing the patient in a PET camera. Signal processing allows construction of tomographic images that are valuable in the diagnosis and treatment of a wide range of diseases, including cancer and neurological disorders. The nucleoside analogue 3'-deoxy-3'-fluorothymidine (FLT, **1**), in its ^{18}F -labelled form, [^{18}F]FLT, has proven to be a valuable biomarker for imaging tumour proliferation by PET. In interpreting PET data, it is important to know the extent of systemic tracer metabolism, as the metabolites will contribute to the PET signal, but may not measure the same process as the parent tracer. Formation of 3'-deoxy-3'-fluorothymidine-5'-glucuronide (FLT-5'-glucuronide **2**, Scheme 1) was proposed as a major route of metabolism for nucleoside **1**, based on the observation that β -glucuronidase destroyed the metabolite.¹ In 5 patients, based on sensitivity to

β -glucuronidase treatment, 5–15% of plasma ^{18}F was present as the metabolite at 60 min, and metabolite correction was performed to generate accurate PET image data.¹ As part of a continuing programme to define the metabolism and distribution of FLT in the body, we have synthesised the 5'-glucuronide **2** to provide a definitive metabolic standard.

The glucuronidation of drugs and other xenobiotics is a crucial detoxification process during phase II metabolism.^{2,3} Thus, glucuronidation is used by humans and animals to assist the excretion of toxic substances and drugs *via* the kidneys or in the bile by the liver. Numerous papers^{4–9} have described the synthesis of glucuronides of alcohols and phenols, but the literature provides limited guidance on which of many published procedures is guaranteed to afford success when applied to a new target. The chemical synthesis of glucuronide **2** has not been reported. Our objective was to develop an efficient synthetic route for the preparation of **2**, for use as an HPLC standard in the analysis of plasma samples from patients undergoing [^{18}F]FLT PET scanning. We report a reliable procedure for the synthesis of glucuronide **2**, which should be applicable to a range of alcohols and phenols. We also report a potential trap in the use of one published method, which when applied to FLT, gave primarily an orthoester, isomeric with the desired protected glucuronide. The application of the synthetic FLT-5'-glucuronide **2** in PET is also described.



Scheme 1 Glucuronidation of 3'-deoxy-3'-fluorothymidine (FLT).

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\dagger DRN conceived this study; BTG, SJH and DRN wrote the manuscript; SJH and TR performed the chemical synthesis supervised by CC, BTG and RJG; MG performed the HPLC analysis.

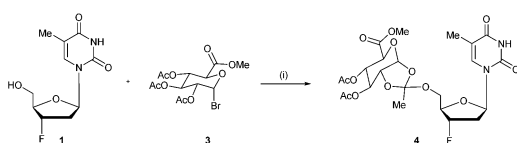
Results and discussion

A synthesis envisaged for FLT-5'-glucuronide **2** used commercially available fluorothymidine **1** and the popular glucuronidating agent acetobromo- α -D-glucuronic acid methyl ester **3**. Several attempts to synthesise the desired glucuronide were made using different reaction conditions with **3**. Reaction of ester **3** with Ag_2CO_3 and quinoline, in the presence of freshly activated 4 Å molecular sieves (MS) [Koenigs–Knorr method¹⁰] gave no reaction. A second attempt employed NaH as the base, as well as Ag_2CO_3 , with the addition of 4 Å MS. Due to the poor solubility of the starting materials, the reaction was carried out in THF at 0 °C, with slow warming to ambient temperature. Again, no reaction occurred, which was also the case when NIS and 4 Å MS in 1,2-dichloroethane were used.¹¹

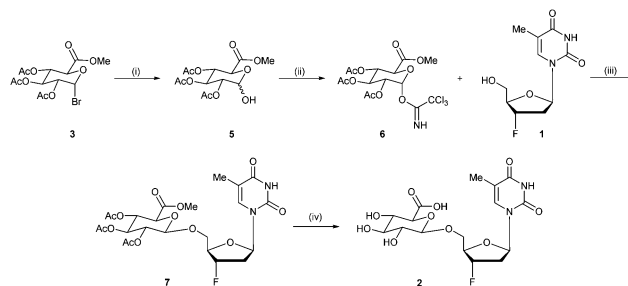
Partial success was achieved when silver trifluoromethanesulfonate (AgOTf) was employed,¹² along with 2,6-lutidine and 4 Å MS in toluene/ CH_2Cl_2 .^{8,13,14} The triflate was added at –36 °C and then the reaction mixture was allowed to warm to ambient temperature. The desired product was apparently detected by LC-MS, but the reaction gave several by-products and no desired product could be isolated. The solvent was changed from toluene/ CH_2Cl_2 to THF and the reaction was carried out at low temperature (–36 °C) with exclusion of moisture, air and light. The reaction progressed, albeit slowly, to give 50% of isolated product following column purification. Although the desired mass was detected by LC-MS, the ^1H NMR spectrum lacked one expected acetate resonance around δ 2.0, which was replaced by a three-proton singlet at δ 1.7. With this observation, and taking into account the detected correct mass, this product was identified as orthoester **4** (Scheme 2).

In an alternative approach, acetobromo- α -D-glucuronic acid methyl ester **3** was converted to the hemiacetal **5** [84% yield of a 3 : 1 (α : β) mixture (NMR analysis)] using Ag_2CO_3 catalysed hydrolysis.¹⁵ Although hemiacetal **5** is itself a useful glycosyl donor, the compound was reacted with CCl_3CN using Cs_2CO_3 as base to give the α -trichloroacetimidate **6** (Scheme 3).^{6,16}

The trichloroacetimidate was used as an alternative glucuronidation reagent for **1**, with TMSOTf as catalyst, at –30 °C to room temperature, to generate the desired protected [^{19}F]FLT-glucuronide **7** (23% after semi-preparative HPLC purification) with no anomer or orthoester formation, although there was significant recovered fluorothymidine **1**. It was found that unreacted FLT co-eluted with the product upon 'flash' column purification, so it was necessary to perform semi-preparative HPLC in order to obtain the pure product. Global deprotection was achieved, as before, using 2 M KOH (aq.) in THF– H_2O ,



Scheme 2 Formation of the orthoester. Reagents and conditions: (i) AgOTf , 2,6-lutidine, 4 Å MS, THF, –36 °C–room temperature, 20 h, 50%.



Scheme 3 Synthesis of FLT-5'-glucuronide. Reagents and conditions: (i) Ag_2CO_3 , H_2O , acetone–water (4 : 1), room temperature, 24 h, 84%; (ii) CCl_3CN , Cs_2CO_3 , CH_2Cl_2 , room temperature, 2 h, 82%; (iii) TMSOTf, 4 Å MS, CH_2Cl_2 , –30 °C–room temperature, 22 h, 23%; (iv) 2 M KOH (aq.), THF– H_2O (4 : 1), 0 °C–room temperature, 2 h, 97%.

giving 3'-deoxy-3'-fluorothymidine-5'-glucuronide **2** in 97% yield after semi-preparative HPLC. The protocol described is also a reliable method for the synthesis of glucuronides of a variety of phenolic drugs (B. T. Golding, unpublished results).

To illustrate the utility of the synthetic [^{19}F]FLT-glucuronide, the material was used to establish HPLC conditions for the separation of [^{19}F]FLT and [^{19}F]FLT-glucuronide (see Fig. 1, Chromatogram A). Crucially, the retention times were very different: 2.0 minutes for [^{19}F]FLT and 1.1 minutes for [^{19}F]FLT-glucuronide (see below). The method was then applied to the analysis of levels of [^{18}F]FLT and [^{18}F]FLT-glucuronide in an extract of blood from a patient given FLT as part of a study to evaluate the utility of FLT PET as a surrogate response biomarker in pancreatic cancer. Following the administration of a single injection dose of 196 MBq of [^{18}F]FLT to a patient, a blood sample was taken 30 minutes post injection and analysed using HPLC with radiochemical detection. Chromatogram B clearly shows [^{18}F]FLT and [^{18}F]FLT-glucuronide in the clinical sample.

[^{18}F]FLT and [^{18}F]FLT-glucuronide concentrations were analysed in the blood plasma of six patients on 10 occasions, four patients being studied after two separate [^{18}F]FLT PET scans. The HPLC retention time for [^{18}F]FLT was within the range 1.6–2.2 minutes, and the retention time for [^{18}F]FLT-glucuronide was within the range 1.05–1.12 minutes. The HPLC retention times for the analysis of non-radioactive FLT and

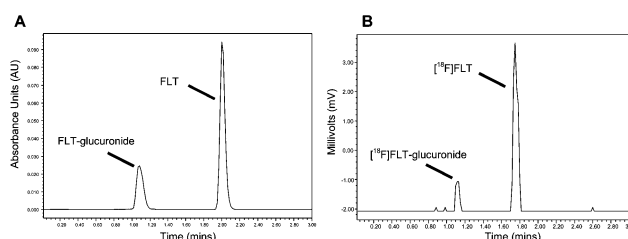


Fig. 1 HPLC analysis of FLT and FLT-glucuronide. (A) Chromatogram identifying the retention times of FLT and FLT-glucuronide. (B) Chromatogram showing the detection of [^{18}F]FLT and [^{18}F]FLT-glucuronide in a plasma sample prepared 30 min after an [^{18}F]FLT dose of 196 MBq [the glucuronide peak represents 13.6% of the total activity recorded for FLT and FLT-glucuronide (FLT accounting for 86.4%)].

FLT-glucuronide standards were in the range 1.99–2.01 and 1.07–1.09 minutes, respectively, and hence the retention times of the FLT and FLT-glucuronide standards and the radioactive peaks detected in the blood plasma of patients are consistent, although in the case of [^{18}F]FLT in blood plasma the HPLC retention time was more variable.

Conclusions

The *de novo* synthesis of [^{19}F]FLT-glucuronide **2** was successfully achieved in four steps, through glycosylation of FLT **1** with the trichloroacetimidate of a suitably protected glucuronic acid. The synthetic FLT-glucuronide was used to define unequivocally the HPLC characteristics of the compound and thereby demonstrate the presence of the metabolite in blood samples from patients administered [^{18}F]FLT.

Experimental

Chemistry materials and methods

Chemicals and solvents were obtained from reputable suppliers. Acetobromo- α -D-glucuronic acid methyl ester **3** was purchased from Apollo Scientific (95+%). Solvents were either dried by standard techniques or purchased as anhydrous. Petrol was reagent grade (bp range 40–60 °C). All reactions that required inert or dry atmosphere were carried out under dry nitrogen. Glassware was dried in an oven prior to use. Column chromatography was carried out either using 40–60 μm mesh silica in glass columns under medium pressure (MPC) or with a Biotage SP4 flash purification system using KP-SilTM silica. Thin layer chromatography (TLC) was performed on 20 mm pre-coated plates of silica gel (Merck, silica gel 60F254); visualisation was made using ultraviolet light (254 nm) or by staining with anisaldehyde. Semi-preparative HPLC was performed with an Agilent 1200 Preparative HPLC machine using a Waters XTerra RP18 column (5 μm , 19 \times 150 mm) eluting with 0.1% formic acid in water–acetonitrile gradient. All melting points were measured using a Stuart Scientific SMP3 apparatus or Stuart automatic melting point apparatus (SMP40) and are uncorrected. NMR spectra were recorded on a Bruker BioSpin Ultrashield Plus (500 MHz for ^1H ; 125 MHz for ^{13}C) using deuterated solvent as lock. IR spectra were recorded on an Agilent Cary 630 FTIR Spectrometer. UV analysis was performed using a Hitachi U-2900 spectrophotometer, with the samples dissolved in ethanol. LCMS was carried out on a Waters Acquity UPLC system with PDA and ELSD operating in positive and negative ion electrospray mode, employing an Acquity UPLC BEH C18, 1.7 μm , 2.1 \times 50 mm column with 0.1% formic acid and water–acetonitrile (5–95%) for gradient elution. HRMS were measured using a Finnigan MAT 95 XP or a Finnigan MAT 900 XLT.

Methyl 2,3,4-tri-O-acetyl- α , β -glucopyranuronate 5. Methyl 2,3,4-triacetyl- α , β -glucopyranuronate **5** was prepared from acetobromo- α -D-glucuronic acid methyl ester **3**, according to the literature¹⁵ using silver carbonate in acetone–water. After passing the reaction mixture through a pad of Celite® the solvent was removed *in vacuo* to yield a dark orange gum. MPC

[petrol–ethyl acetate (100 : 0) \rightarrow (80 : 20) \rightarrow (50 : 50)] of the crude residue afforded methyl 2,3,4-tri-O-acetyl- α , β -glucopyranuronate **5** as a white solid (84%), ratio of α to β anomers (by ^1H NMR) = 75 : 25 (α : β); mp 89.2–90.5 °C, lit.¹⁵ 91–92 °C; IR (cm^{-1}) 1713, 1734 (C=O), 3317 (O–H); m/z (ES): 357 ($\text{M}^+ + \text{Na}$); ^1H NMR (CDCl_3) (α -anomer) δ 2.05 (3H, s, OAc), 2.06 (3H, s, OAc), 2.11 (3H, s, OAc), 3.36 (1H, d, J = 4.0 Hz, OH), 3.77 (3H, s, CO_2CH_3), 4.61 (1H, d, J = 10.1 Hz, H-5), 4.94 (1H, dd, J = 3.6 and 10.1 Hz, H-2), 5.21 (1H, t, J = 9.4 Hz, H-4), 5.57 (1H, d, J = 3.8 Hz, H-1) and 5.60 (1H, t, J = 9.7 Hz, H-3); ^{13}C NMR (CDCl_3) (α -anomer) δ 20.6 (OAc), 20.7 (2 \times OAc), 52.9 (CO_2CH_3), 68.1 (C–H), 69.0 (C–H), 69.5 (C–H), 70.7 (C–H), 90.3 (C-1), 168.3 (C=O), 169.7 (C=O), 170.0 (C=O) and 170.1 (C=O); ^1H NMR (500 MHz, CDCl_3) (β -anomer) δ 2.05 (3H, s, OAc), 2.07 (3H, s, OAc), 2.11 (3H, s, OAc), 3.78 (3H, s, CO_2CH_3), 4.15 (1H, d, J = 10.0 Hz, H-5), 4.35 (1H, br d, OH), 4.82 (1H, t, J = 8.1 Hz, H-2), 4.94 (1H, m, H-1), 5.25 (1H, t, J = 9.5 Hz, H-4) and 5.33 (1H, t, J = 9.4 Hz, H-3); ^{13}C NMR (125.8 MHz, CDCl_3) (β -anomer) δ 20.5 (OAc), 20.6 (OAc), 20.6 (OAc), 53.1 (CO_2CH_3), 69.4 (C–H), 71.4 (C–H), 72.7 (C–H), 73.0 (C–H), 95.6 (C-1), 167.5 (C=O), 169.5 (C=O), 170.2 (C=O) and 170.8 (C=O).

Methyl 2,3,4-tri-O-acetyl-1-O-(trichloroacetimidoyl)- α -D-glucopyranuronate 6. Methyl 2,3,4-triacetyl- α , β -glucopyranuronate **5** (386 mg, 1.15 mmol) was dissolved in anhydrous CH_2Cl_2 (6 mL) and CCl_3CN (1.2 mL, 11.5 mmol) and then Cs_2CO_3 (67 mg, 0.207 mmol) were added. The mixture was stirred at room temperature for 2 h and then passed through a pad of silica, which was washed with petrol–ethyl acetate (50 : 50). The solvent was removed *in vacuo*, following which, MPC [petrol–ethyl acetate (80 : 20) \rightarrow (50 : 50)] of the crude residue afforded methyl 2,3,4-tri-O-acetyl-1-O-(trichloroacetimidoyl)- α -D-glucopyranuronate **6** (449 mg, 82%) as a white solid; mp 108.6–110.2 °C, lit.⁷ 108 °C; IR (cm^{-1}) 1737 (C=O), 2958 (C–H), 3349 (N–H); ^1H NMR (CDCl_3) δ 2.01 (3H, s, OAc), 2.04 (3H, s, OAc), 2.05 (3H, s, OAc), 3.74 (3H, s, CO_2CH_3), 4.49 (1H, d, J = 10 Hz, H-5), 5.14 (1H, dd, J = 4.0 Hz and 10.0 Hz, H-2), 5.27 (1H, t, J = 10.0 Hz, H-4), 5.62 (1H, t, J = 10.0 Hz, H-3), 6.63 (1H, d, J = 3.5 Hz, H-1) and 8.72 (1H, s, NH); ^{13}C NMR (CDCl_3) δ 20.4, 20.5, 20.7 (3 \times OAc), 53.1 (CO_2CH_3), 68.9 (C–H), 69.1 (C–H), 69.5 (C–H), 70.5 (C–H), 92.6 (C–H), 160.6 (C=N), 167.2 (C=O), 169.5 (C=O), 169.7 (C=O) and 169.8 (C=O).

(2R,3R,4S,5S,6S)-2-(((2R,3S,5R)-3-Fluoro-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate 7. 3'-Deoxy-3'-fluorothymidine **1** (113 mg, 0.47 mmol) and methyl 2,3,4-triacetyl-1-O-(trichloroacetimidoyl)- α -D-glucopyranuronate **6** (215 mg, 0.47 mmol) were solubilised in anhydrous CH_2Cl_2 (2 mL) with 4 Å molecular sieves (70 mg) and cooled to –40 °C. Trimethylsilyl trifluoromethanesulfonate (26 mg, 21 μL , 0.116 mmol) was introduced dropwise, and stirring continued for 2 h at –40 °C. The solution was allowed to reach ambient temperature, and was stirred at room temperature for 18 h. The reaction mixture was concentrated directly onto silica and preliminary purification [dichloromethane–methanol (100 : 0) \rightarrow (96 : 4)] of the crude residue was carried out, followed by semi-preparative HPLC, to afford title compound **7** (60 mg, 23%) as a white solid; R_f = 0.60 (10% CH_3OH – CH_2Cl_2); IR

(cm^{-1}) 1209, 1682, 1748 (C=O), 2956 (C-H); λ_{max} (EtOH)/nm 265; mp 112.4–113.8 °C; ^1H NMR (CDCl_3) δ 1.95 (3H, d, J = 1.2 Hz, Ar-CH₃), 1.98 (3H, s, OAc), 2.00 (3H, s, OAc), 2.03 (3H, s, OAc), 2.10–2.13 (1H, m, H-2a), 2.48–2.57 (1H, m, H-2b), 3.70 (1H, dd, J = 10.5 and 2.0 Hz, H-5a), 3.74 (3H, s, CO₂CH₃), 4.07 (1H, d, J = 9.5 Hz, H-5'), 4.19 (1H, dt, J = 10.6 and 1.9 Hz, H-5b), 4.32 (1H, dt, J = 27.1 and 2.5 Hz, H-4), 4.51 (1H, d, J = 7.9 Hz, H-1'), 4.92 (1H, dd, J = 9.7 and 7.9 Hz, H-2'), 5.02 (1H, dd, J = 53.7 and 4.7 Hz, H-3), 5.12 (1H, dd, J = 9.9 and 9.9 Hz, H-4'), 5.24 (1H, dd, J = 9.6 and 9.6 Hz, H-3'), 6.42 (1H, dd, J = 9.0 and 5.5 Hz, H-1), 7.47 (1H, d, J = 1.0 Hz, Ar-H), 8.49 (1H, s, NH); ^{13}C NMR (CDCl_3) δ 12.4 (Ar-CH₃), 20.5 (OAc), 20.6 (2 \times OAc), 37.9 (d, J = 21.0 Hz, C-2), 53.0 (CO₂CH₃), 69.4 (C-4'), 69.5 (C-5), 70.9 (C-2'), 71.4 (C-3'), 72.9 (C-5'), 83.0 (d, J = 26 Hz, C-4), 84.9 (C-1), 94.3 (d, J = 177 Hz, C-3), 100.6 (C-1'), 111.6, 135.4 (ArC-H), 150.3, 163.6, 166.6, 169.3, 170.0; ^{19}F NMR (470.7 MHz, CDCl_3) δ –173.5; LRMS (ESI-) m/z 559.3 [$\text{M} - \text{H}$][–]; HRMS calcd for C₂₃H₂₈FN₂O₁₃ [$\text{M} - \text{H}$][–] 559.1575, found 559.1578; HPLC 96.7% purity (elution with 0.1% ammonia/MeCN) and 97.7% purity (elution with 0.1% formic acid/MeCN).

(2S,3S,4S,5R,6R)-6-(((2R,3S,5R)-3-Fluoro-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid 2. To a solution of glucuronidated FLT 7 (30 mg, 0.054 mmol) in THF–H₂O (4 : 1, 2.5 mL) at 0 °C, was added a 2 M aqueous solution of KOH (135 μL , 0.27 mmol). The reaction mixture was allowed to warm to ambient temperature, and stirring continued for 2 h, before addition of AcOH to bring the pH to 8, followed by removal of the solvent under reduced pressure. Purification of the crude residue by semi-preparative HPLC afforded title compound 2 (22 mg, 97%) as a white solid; R_f = 0.60 (10% CH₃OH–CH₂Cl₂); IR (cm^{-1}) 1120, 1664, 2071, 2480, 3374 (O–H); λ_{max} (EtOH)/nm 262; mp 150.6–152.2 °C; ^1H NMR (MeOD) δ 1.94 (3H, s, CH₃), 2.27–2.44 (1H, m, H-2a), 2.48–2.58 (1H, m, H-2b), 3.25 (1H, app. t, J = 8.7 Hz, H-2'), 3.25 (1H, app. t, J = 9.0 Hz, H-3'), 3.43 (1H, app. t, J = 9.6 Hz, H-4'), 3.81–3.85 (2H, m, H-5' and H-5a), 4.22 (1H, d, J = 10.5 Hz, H-5b), 4.43–4.47 (2H, m, H-1' and H-4), 5.43 (1H, dd, J = 4.0 and 53.5, H-3), 6.36 (1H, dd, J = 5.5 and 9.0 Hz, H-1), 7.84 (1H, s, Ar-H); ^{13}C NMR (MeOD) δ 12.7 (CH₃), 39.5 (d, J = 21.0 Hz, C-2), 68.9 (C-5), 70.3 (C-4'), 73.1 (C-2'), 75.0 (C-5'), 77.7 (C-3'), 85.5 (d, J = 26.0 Hz, C-4), 86.7 (C-1), 95.8 (d, J = 175.0 Hz, C-3), 104.5 (C-1'), 112.0 (C-Ar), 137.7 (Ar-C-H); ^{19}F NMR (MeOD) δ –175.5; LRMS (ESI-) m/z 419.3 [$\text{M} - \text{H}$][–]; HRMS calcd for C₁₆H₂₀FN₂O₁₀ [$\text{M} - \text{H}$][–] 419.1102, found 419.1102; HPLC 99.6% purity (elution with 0.1% ammonia/MeCN) and 99.7% purity (elution with 0.1% formic acid/MeCN).

(3aR,5S,6S,7S,7aR)-2-(((2R,3S,5R)-3-Fluoro-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methoxy)-5-(methoxycarbonyl)-2-methyltetrahydro-3aH-[1,3]-dioxolo[4,5-*b*]pyran-6,7-diyl diacetate 4. 3'-Deoxy-3'-fluorothymidine 1 (150 mg, 0.60 mmol), 2,6-lutidine (78 μL , 0.66 mmol) and silver trifluoromethanesulfonate (168 mg, 0.66 mmol) were stirred in anhydrous THF (3 mL) at –36 °C with 4 Å molecular sieves (200 mg) for 45 min. Acetobromo- α -D-glucuronic acid methyl ester 3 (261 mg, 0.66 mmol) in anhydrous THF (2.25 mL) was cooled to –36 °C and then added to the main reaction vessel *via* syringe. The reaction mixture was allowed to

reach ambient temperature over 20 h and then diluted with CH₂Cl₂ (3 mL) and passed through Celite® to collect the precipitate. The filtrate was washed with saturated aqueous NaHCO₃ (3 mL), passed through a phase separator and the solvent removed *in vacuo*. MPC [dichloromethane–methanol (100 : 0) \rightarrow (96 : 4)] of the crude residue afforded the product, which was further triturated with petrol to afford title compound 4 (167 mg, 50%) as a white solid; R_f = 0.37 (10% CH₃OH–CH₂Cl₂); IR (cm^{-1}) 1212, 1369, 1684, 1746 (C=O), 2956 (C–H); λ_{max} (EtOH)/nm 265; mp 120.9–123.0 °C; ^1H NMR (CDCl_3) δ 1.70 (3H, s, CH₃), 1.87 (3H, s, Ar-CH₃), 2.02 and 2.04 (7H, 2 \times s and m, 2 \times OAc, H-2a), 2.54 (1H, m, H-2b), 3.7 (5H, s and m, CO₂CH₃, H-5a, H-5b*), 4.18 (1H, m, H-2'), 4.3–4.4 (2H, 2 \times m, H-4, H-5'*), 5.0–5.3 (3H, 3 \times m, H-3, H-3', H-4'*), 5.95 (1H, d, J = 3.5 Hz, H-1'), 6.34 (1H, m, H-1), 7.47 (1H, s, NCH), 9.22 (1H, s, NH); ^{13}C NMR (CDCl_3) δ 12.4, 20.6, 20.7, 23.5, 38.7, 52.7, 62.5, 67.2, 67.5, 70.7, 74.3, 83.4, 85.2, 95.1, 95.5, 111.3, 123.0, 135.3, 150.4, 163.9, 168.7, 168.7, 169.3; ^{19}F NMR (CDCl_3) δ –174.03; LRMS (ESI-) m/z 559.4 [$\text{M} - \text{H}$][–]; HRMS calcd for C₂₃H₃₃FN₃O₁₃ [$\text{M} + \text{NH}_4$]⁺ 578.1992, found 578.1988; calcd for C₂₃H₃₀FN₂O₁₃ [$\text{M} + \text{H}$]⁺ 561.1726, found 561.1723. * Tentative assignments.

Analysis of [¹⁸F]FLT and [¹⁸F]FLT-glucuronide samples

The clinical study on which patient samples were collected was conducted by the Newcastle upon Tyne Hospitals NHS Foundation Trust (NUTH). The study was approved by the local ethics committee and all patients gave fully informed consent according to the Declaration of Helsinki guidelines. The administration of radioactivity for the PET scans was approved by the Administration of Radioactive Substances Advisory Committee, United Kingdom. Patients received an [¹⁸F]FLT dose and blood samples (10 mL) were collected at 5 time points up to 60 min post-injection. Plasma was separated from the blood by centrifuging at 2000 rpm for 15 min (MSE Falcon 6/300). Protein was precipitated from the plasma sample (3 mL) by the addition of acetonitrile (6 mL). The mixture was centrifuged (5 min at 12 000 rpm, Eppendorf centrifuge 5418). The supernatant was evaporated at 50 °C under nitrogen gas in a TurboVap® LV evaporator (Zymark), and resuspended in HPLC mobile phase (150 μL). An aliquot (80 μL) of the resuspended sample containing [¹⁸F]FLT and [¹⁸F]FLT-glucuronide was separated on a Phenomenex Kinetex™ C18 column (100 mm \times 4.6 mm, 2.6 μM) using an isocratic HPLC method [0.01 M potassium dihydrogen phosphate–acetonitrile (88 : 12 w/w), 1 mL min^{–1}], and detected with a Posi-RAM Model 4 Radio-HPLC Detector (Lablogic). This procedure was performed on sample from six patients on 10 occasions, four patients being studied after two separate [¹⁸F]FLT PET scans.

Analysis of [¹⁹F]FLT and [¹⁹F]FLT-glucuronide samples

An aliquot (10 μL) of mobile phase containing [¹⁹F]FLT (208 μM) and [¹⁹F]FLT-glucuronide (119 μM) was separated using the above HPLC method for [¹⁸F]FLT and [¹⁸F]FLT-glucuronide, with detection by a Waters 2487 Dual λ Absorbance Detector.

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