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Research Article

Synthesis and biological evaluation of a lipophilic, fluorine-18-labeled 5-ethynyl-2′-deoxyuridine derivative

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Abstract: The synthesis and preliminary biological evaluation of a lipophilic, fluorine-18-labeled 5-ethynyl-2′-deoxyuridine derivative [^{18}F]-**3** is described. Initially, 5-ethynyl-2′-deoxyuridine **5** was synthesized by coupling trimethylsilyl protected acetylene to 5-iodo-2′-deoxyuridine **4**, followed by deprotection in alkaline conditions. Compound **5** was then reacted with 4-(4′-iodophenyl)phenol to give 5-[4(4′-hydroxyphenyl)phenyl]ethynyl-2′-deoxyuridine **6**. Compound **6** was reacted with $BrCH_2CH_2^{19/18}F$ as alkylating agent to give stable or radiolabeled **3**. The crude products were purified using reversed phase-high performance liquid chromatography to obtain compound **3** and [^{18}F]-**3** in 33 and 7.4% yield (decay corrected), respectively. The synthesis time to obtain pure [^{18}F]-**3** was about 60 min (starting from $BrCH_2CH_2^{18}F$). The specific radioactivity of the tracer was between 74 and 222 GBq/μmol. The log $P_{7.4}$ of [^{18}F]-**3** was found to be 2.4. However, biodistribution study in normal mice showed low uptake of the tracer in the brain. The affinity of compounds **6** and **3** for varicella-zoster virus thymidine kinase enzyme (VZV-TK) was examined *in vitro* and the results revealed that the fluorinated analog **3** has a poor affinity for the enzyme in contrast to the phenol precursor **6**. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: BCNAs; VZV-tk; gene expression imaging; PET

Introduction

Several radiofluorinated acyclo-guanosine (e.g. 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]-FHBG)) and pyrimidine (e.g. 2′-deoxy-2′-[^{18}F]fluoro-5-fluoro-1-β-D-arabinofuranosyluracil ([^{18}F]-FFAU)) nucleoside analogs have been developed and evaluated as potential agents for imaging herpes simplex virus type 1 thymidine kinase (HSV1-tk) reporter gene expression using positron emission tomography (PET).[1,2] However, since none of these tracer agents were found to cross blood–brain barrier (BBB), the widely used HSV1-tk reporter gene system is restricted to applications outside the brain only. This lack of BBB penetration can be primarily attributed to the low log partition coefficient (log *P*) value of these tracers.[3]

Bi-cyclic nucleoside analogs (BCNAs) are novel, lipophilic, potent and selective inhibitors of varicella zoster virus (VZV) with calculated log *P* (*C*log *P*) values ranging between 0.4 and 4.6.[4] They are synthesized in a two-step reaction. The first step consists of a Sonogashira coupling and usually involves coupling of alkyl- or alkylphenyl acetylenes to the 5-position of the 2′-deoxyuridine, under co-catalysis of copper and palladium. The second step is a cyclization that can be achieved *in situ* or after isolation of the intermediate compound, in basic conditions and in the presence of copper.[5,6] Since the discovery of BCNAs in 1999, several studies have been carried out to explore the structure–activity relationship of BCNAs with regard to their anti-viral activity.[4,7] Although the bi-cyclic system compounds (2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one nucleosides) are the lead anti-viral compounds, some of the uncyclized (intermediate) compounds that are precursors for BCNAs were also found to have good anti-viral activity.[8]

With the aim to synthesize PET reporter probes based on BCNAs that can cross BBB for VZV-thymidine kinase (VZV-tk) gene expression imaging in brain, we

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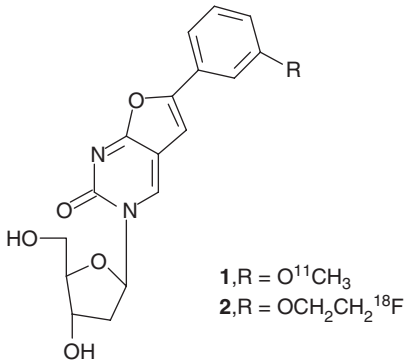

Figure 1 Chemical structures of radiolabeled BCNAs **1** and **2**.

have carried out the synthesis and preliminary biological evaluation of two radiolabeled BCNAs, **1** and **2** whose chemical structures are provided in Figure 1.[9] These tracers were found not to be taken up in the brain despite fulfilling generally accepted requirements for BBB penetration, with log $P_{7.4}$ values around 1.25.[9] *In vitro* evaluation of these tracers in VZV-TK expressing 293 T (human embryonic kidney) cell line revealed that **1** and **2** can still be useful imaging agents for VZV infection and reporter gene expression outside the brain *in vivo*, and this thus constitutes a new PET reporter gene/reporter probe system.[9]

As part of our ongoing research to develop lipophilic radiolabeled nucleoside analogs, which can cross the BBB, we report here the synthesis of a fluorine-18-labeled 5-ethynyl-2′-deoxyuridine derivative (a BCNA precursor), namely, 5-[4(4′-[^{18}F]fluoroethoxyphenyl)-phenyl]ethynyl-2′-deoxyuridine ([^{18}F]-**3**) and its biodistribution in normal mice. Further, we aimed to synthesize a radiolabeled BCNA precursor [^{18}F]-**3**, which has bi-phenyl ring as side chain in view of the good anti-viral activity and high *C*log *P* value (2.24) of the bi-phenyl BCNA that is reported in the literature.[10]

Results and discussion

The phenol precursor 5-[4(4′-hydroxyphenyl)phenyl]-ethynyl-2′-deoxyuridine **6** was synthesized following a three-step synthesis route (Figure 2). As described above, the general synthetic pathway for BCNAs involves a Pd-catalysed coupling between 5-iodo-2′-deoxyuridine and the appropriate aryl acetylenes.[5] Unfortunately, the aryl acetylene 4-(4′-hydroxyphenyl)-phenylacetylene required for the synthesis of **6** is not commercially available. Therefore, we synthesized 5-ethynyl-2′-deoxyuridine **5** that has an alkynyl moiety on the nucleoside and used it as a synthon for the subsequent coupling with the iodo derivative 4-(4′-iodophenyl)phenol. Firstly, 5-ethynyl-2′-deoxyuridine **5** was synthesized in an overall yield of 44% by coupling trimethylsilyl protected acetylene to 5-iodo-2′-deoxyuridine **4** in triethylamine (TEA) and subsequent deprotection in alkaline conditions, following a reported procedure.[11] Compound **5** was then reacted with 4-(4′-iodophenyl)phenol under co-catalysis of Pd and Cu to give 5-[4(4′-hydroxyphenyl)phenyl]ethynyl-2′-deoxyuridine **6**, in a rather low yield (10%).

Compound **6** was used as the precursor for the synthesis of both non-radioactive and radiolabeled **3**. Fluoroethylation of phenol **6** was achieved by heating with fluoroethyl bromide (FEtBr) in the presence of potassium carbonate (K_2CO_3) as a base in DMF as a solvent (Figure 2). The crude product was purified using semi-preparative reversed phase-high performance liquid chromatography (RP-HPLC) to obtain pure compound **3** in 33% yield. However, for the radiolabeling experiments cesium carbonate (Cs_2CO_3), which has better solubility in organic solvents, was used instead of K_2CO_3. Moreover, it was observed during the radiosynthesis of **1** and **2** that Cs_2CO_3 results in faster alkylation compared to K_2CO_3.[9] Further, the optimal temperature for radiolabeling was found to be 90°C. Therefore, radiolabeling of the phenol precursor **6** was performed by heating with [^{18}F]fluoroethyl bromide ([^{18}F]FEtBr) in the presence of Cs_2CO_3 in DMF. The labeled compound [^{18}F]-**3** was isolated on semi-preparative RP-HPLC and using UV and radiometric monitoring of the eluate. The radiochemical yield (RCY) was 7.4% (decay corrected to starting [^{18}F]FEtBr) and the radiochemical purity was found to be >99%. The specific radioactivity of [^{18}F]-**3** was between 74 and 222 GBq/μmol at the end of synthesis. The identity of the purified tracer was confirmed by co-elution with authentic non-radioactive compound after co-injection on an analytical HPLC system that consisted of a XTerra™ RP C_{18} column (5 μm, 4.6 mm × 250 mm; Waters, Milford, USA), eluted with 35% acetonitrile in 0.05 M sodium acetate buffer (pH 5.5) at a flow rate of 1 mL/min. (Rt = 9 min).

The lipophilicity of RP-HPLC purified [^{18}F]-**3** was determined by partitioning between 1-octanol and 0.025 M phosphate buffer pH 7.4. The log $P_{7.4}$ value was found to be 2.4, which is higher than the log $P_{7.4}$ of the previously reported BCNA tracers **1** and **2** (Table 1) and is within the optimal range for passive diffusion of a compound over the BBB.[12]

Biodistribution of the tracer was determined in male NMRI mice (body mass 30–40 g). A volume of 0.1 mL of diluted tracer solution (containing approximately 370 kBq [^{18}F]-**3**) was injected into the mice via a tail vein, under anesthesia. The mice were killed by decapitation at 2 or 60 min after injection ($n = 4$ at each time point). All organs and other body parts were

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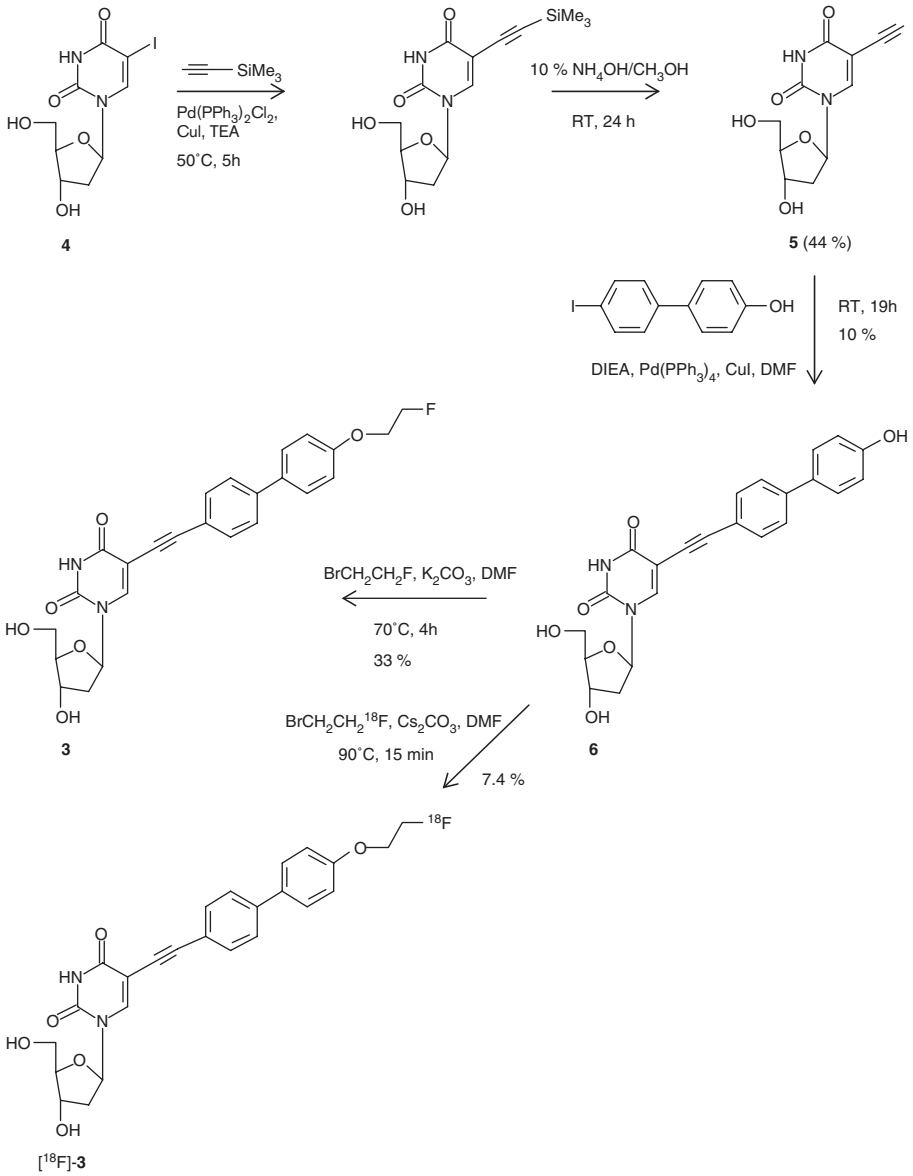

Figure 2 Synthesis scheme of 5-[4(4′-fluoroethoxyphenyl)phenyl]ethynyl-2′-deoxyuridine **3** and [^{18}F]-3 (intermediate compound between 4 and 5 was not isolated).

Table 1 Affinity for VZV-TK or log $P_{7.4}$ value for the synthesized compounds

Compound	IC_{50} (μM)	Log P[b]
1[a]	4.8	1.27 ± 0.04
2[a]	53	1.24 ± 0.03
6	44	—
3	>500	—
[^{18}F]-**3**	—	2.40 ± 0.2

IC_{50} is the 50% inhibitory concentration or compound concentration in μM required to inhibit VZV-TK-catalyzed phosphorylation of 1 μM [CH_3-^{3}H]dThd by 50%.
[a] Data from Ref. 9; IC_{50} values were determined using non-radioactive compounds.
[b] Data are expressed as mean ± SD; $n = 6$.

dissected, weighed, and their radioactivity was counted in a gamma counter. Results are expressed as percentage of injected dose (% of ID), or, where possible, as percentage of injected dose per gram tissue (% of ID/g). For calculation of total radioactivity in blood, blood mass was assumed to be 7% of the body mass.[13]

Table 2 summarizes the biodistribution data of this tracer. The favorable log $P_{7.4}$ value of [^{18}F]-**3** together with its low molecular weight (465 Da) and its neutral character at physiological pH are in favor of uptake of this tracer in the brain.[12,14] However, similar to compounds **1** and **2**,[9] this tracer too did not show significant brain uptake. The activity concentration that was seen in

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Table 2 Biodistribution of **[^{18}F]-3** in normal mice at 2 and 60 min p.i.

Organ	% of ID ($n = 4$)	
	2 min	60 min
Urine	0.1 ± 0.1	1.7 ± 1.9
Kidneys	4.4 ± 0.5	2.8 ± 1.0
Liver	18.3 ± 3.5	14.4 ± 2.7
Spleen + pancreas	3.7 ± 0.1	0.7 ± 0.2
Lungs	1.4 ± 0.3	0.7 ± 0.2
Heart	0.5 ± 0.1	0.3 ± 0.0
Intestines	3.7 ± 0.3	26.9 ± 1.7
Brain	0.2 ± 0.0	0.1 ± 0.0
Blood	54.3 ± 4.6	20.8 ± 2.3
Carcass	16.1 ± 0.6	30.0 ± 1.4
	% of ID/g ($n = 4$)	
Kidneys	5.5 ± 0.7	5.0 ± 1.5
Brain	1.1 ± 0.1	0.5 ± 0.5
Blood	24.8 ± 3.3	8.9 ± 0.8
Carcass	0.5 ± 0.1	1.0 ± 0.1

the brain (1.1% ID/g at 2 min post-injection (p.i.)) can be attributed to the high radioactivity present in blood. Indeed at 2 min p.i. 54% of the injected dose was in blood and this decreased to 21% after 60 min p.i. This indicates rather slow clearance of the tracer from blood. Moreover, there was an increased uptake of radioactivity in the carcass by 60 min p.i. (30% ID), suggesting the progressive accumulation of this tracer either in the muscle or bones. As could be anticipated from its high log $P_{7.4}$ value, the tracer was excreted almost exclusively via the hepatobiliary system.

The affinity of precursor **6** as well as the fluoroethyl derivative **3** for purified, recombinant VZV-TK enzyme was determined *in vitro* in competition with [CH_3-^{3}H]dThd as the natural substrate for VZV-TK, and the results are presented as 50% inhibitory concentration (IC_{50}) values in Table 1. For comparison, the affinity values of previously reported **1** and **2** are also given in Table 1. The phenolic precursor **6** has moderate affinity for the enzyme with IC_{50} value of 44 μM, whereas the fluoroethyl derivative has no measurable affinity at the highest concentration tested (500 μM). This indicates that the affinity of compound **3** for VZV-TK decreases due to the alkylation of the phenol, and/or that the presence of a fluorine atom compromises the affinity.

Experimental

Reagents and general conditions

4-(4′-Iodophenyl)phenol was obtained from Apin Chemicals Limited (Abingdon, UK). 1-Bromo-2-fluoroethane (FEtBr) was purchased from ABCR RG (Im Schlehert, Germany). All other reagents and solvents were obtained commercially from Acros Organics (Geel, Belgium) or Aldrich, Fluka, Sigma (Sigma-Aldrich, Bornem, Belgium) or Fischer Bioblock Scientific (Tournai, Belgium). For ascending thin-layer chromatography, pre-coated aluminum backed plates (Silica gel 60 with fluorescent indicator, 0.2 mm thickness; supplied by Macherey-Nagel (Düren, Germany)) were used and developed using 10% CH_3OH in CH_2Cl_2 as mobile phase. ^{1}H-NMR spectra were recorded on a Gemini 200 MHz spectrometer (Varian, Palo Alto, CA, USA) using DMSO-d_6 as solvent. Exact mass measurement was performed on a time-of-flight mass spectrometer (LCT, Micromass, Manchester, UK) equipped with an orthogonal electrospray ionization (ESI) interface, operated in positive mode (ES+) or negative mode (ES−). Samples were infused in acetonitrile/water using a Harvard 22 syringe pump (Harvard instruments, MA, USA). Acquisition and processing of data was done using Masslynx™ software (version 3.5, Waters).

HPLC purification was performed using a Merck Hitachi L6200 intelligent pump (Hitachi, Tokyo, Japan) or on a Waters 600 pump (Waters Corporation, Milford, USA) connected to a UV spectrometer (Waters 2487 Dual λ absorbance detector) set at 254 nm. For the purification and analysis of radiolabeled compounds, the HPLC eluate was passed through a UV detector and a radiometric detector that was connected to a single channel analyzer (Medi-Lab Select, Mechlen, Belgium). The radioactivity measurements during log *P* determination and biodistribution studies were done using an automatic gamma counter (3-inch NaI(Tl) well crystal) coupled to a multichannel analyzer (Wallac 1480 Wizard™ 3″, Wallac, Turku, Finland). The values were corrected for background radiation and physical decay during counting.

Synthesis of 5-ethynyl-2′-deoxyuridine 5

To a suspension of 5-iodo-2′-deoxyuridine **4** (5 g; 14.12 mmol) in 500 mL of TEA, under N_2, $Pd(PPh_3)_2Cl_2$ (198 mg; 0.28 mmol) and CuI (207 mg; 1.09 mmol) were added. A solution of trimethylsilylacetylene (2.77 g; 28.24 mmol) in 2 ml of TEA was added dropwise to the reaction mixture. The reaction mixture was then heated at 50°C for 5 h. The solvent was evaporated, the crude product was dissolved in 200 mL of EtOAc and extracted with 2 × 100 mL of 10% EDTA solution. The organic layers were combined, dried over $MgSO_4$, and evaporated to give 5.05 g of residue. This intermediate trimethylsilyl protected compound was identified using mass spectrometry (Micromass LCT mass spectrometer), ESI+: Theor. mass $[C_{14}H_{19}N_2O_5Si+Na]^+$:

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347; found 347. The residue was then dissolved in 300 mL of 10% NH_4OH/CH_3OH (40:60) and the resulting mixture was stirred at room temperature (RT) for 24 h, followed by purification on a silica gel column that was eluted with gradient mixtures of CH_3OH in CH_2Cl_2 (up to 12%), to give 1.57 g of pure compound in 44% yield. ^{1}H-NMR (DMSO-d_6) δ: 11.62 (brs, 1H, NH), 8.30 (s, 1H, 6-H), 6.11 (s, 1H, 1′-H), 5.26 (d, 1H, 3′-H), 5.14 (t, 1H, 5′-OH), 4.24 (m, 1H, 3′-H), 4.10 (m, 1H, 3′-H), 3.80 (m, 1H, 4′-H), 3.58 (m, 2H, 5′-CH_2), 2.14 (m, 2H, 2′-H). ESI+: theor. mass $[C_{11}H_{12}N_2O_5+Na]^+$: 275.0644; found 275.0637.

5-[4(4′-Hydroxyphenyl)phenyl]ethynyl-2′-deoxyuridine 6

To a stirred solution of 5-ethynyl-2′- deoxyuridine **5** (1.56 g, 6.2 mmol) in DMF (25 mL) under N_2 at RT was added *N*,*N*-diisopropylethylamine (2.3 mL, 13.0 mmol), tetrakis(triphenylphosphine)palladium(0) (720 mg, 0.6 mmol) and copper(I) iodide (237 mg, 1.25 mmol), followed by the slow addition of a solution of 4-(4′-iodophenyl)phenol (5.5 g, 18.7 mmol) in 5 mL of DMF. After stirring at RT for 19 h, the solvents were removed under reduced pressure and the resulting residue was further stirred in CH_3OH/CH_2Cl_2 (1:1) (15 mL) with an excess of Amberlite IRA-400 (HCO_3^-) for 1 h. The resin was filtered, washed with methanol and the combined filtrates were evaporated to dryness. The crude product was chromatographed on silica gel using gradient mixtures of CH_3OH in CH_2Cl_2 (up to 6%). Appropriate fractions were combined, and evaporated to yield 251 mg of pure product in about 10% yield. HRMS ESI-: theor. mass $[C_{23}H_{19}N_2O_6-H]^-$: 419.1243; found 419.1272.

5-[4(4′-Fluoroethoxyphenyl)phenyl]ethynyl-2′-deoxyuridine 3

To a solution of **6** (10 mg, 0.024 mmol) in 2 mL DMF was added K_2CO_3 (6.6 mg, 0.048 mmol) and dropwise a solution of FEtBr (3.8 μL, 0.029 mmol in 0.2 mL DMF). The reaction mixture was then stirred at 70°C for 4 h and the product was purified using RP-HPLC (Econosphere, 250 mm × 10 mm; Alltech) employing 30% acetonitrile in water as the mobile phase at a flow rate of 3 mL/min to have pure compound in 33% yield. HRMS ESI-: theor. mass $[C_{25}H_{22}N_2O_6F-H]^-$: 465.1462; found 465.1435.

Production of [^{18}F]fluoride, [^{18}F]FEtBr and radiosynthesis of [^{18}F]-3

[^{18}F]F^- was produced [^{18}O(p,n)^{18}F reaction] by irradiation of 0.5 mL of 97% enriched $H_2^{18}O$ (Rotem HYOX^{18}, Rotem Industries, Beer Sheva, Israel) in a niobium target using 18-MeV protons from a Cyclone 18/9 cyclotron (Ion Beam Applications, Louvain-la-Neuve, Belgium). Radiosynthesis was performed in in-house remote-controlled synthesis modules housed in 6-cm lead-shielded cabinets. After the production, [^{18}F]F^- was separated from [^{18}O]H_2O and trapped on a SepPak^{TM} Light Accell plus QMA anion exchange cartridge (Waters), which was pre-conditioned by successive treatments with 0.5 M K_2CO_3 solution (10 mL) and water (2 × 10 mL). The [^{18}F]F^- was then eluted from the cartridge with a solution containing 2.47 mg K_2CO_3 and 27.92 mg Kryptofix 222 dissolved in 0.75 mL of H_2O/CH_3CN (5:95) into a reaction vial.

After evaporation of the solvent from the reaction vial, [^{18}F]F^- was further dried by azeotropic distillation of traces of water using acetonitrile. 2-Bromoethyl triflate ($BrCH_2CH_2OTf$) (5 μL) in *o*-dichlorobenzene (0.7 mL) was added into the vial containing [^{18}F]F^-. The resulting [^{18}F]FEtBr was then distilled at 110°C under a helium flow (3–4 mL/min) and bubbled into another reaction vial containing a solution of 0.2 mg of **3** and 0.5–0.8 mg of Cs_2CO_3 in 0.2 mL anhydrous DMF. The reaction mixture was then heated at 90°C for 15 min. The crude mixture was purified by RP-HPLC (HS Hyper Prep 100 Å silica 8 μm, 10 × 250 mm, Alltech) using 40% ethanol in 0.05 M sodium acetate buffer (pH 5.5) at a flow rate of 3 mL/min. The radiolabeled product was collected at 16.2 min with a decay corrected RCY of 7.4% (to starting [^{18}F]FEtBr). Quality control of the purified tracer was performed using XTerra^{TM} RP C_{18} column (5 μm, 4.6 mm × 250 mm; Waters), eluted with 35% acetonitrile in 0.05 M sodium acetate buffer (pH 5.5) at a flow rate of 1 mL/min (Rt = 9 min). Starting from [^{18}F]FEtBr, the synthesis time to obtain the pure product was about 60 min.

Partition coefficient determination

The RP-HPLC purified labeled product [^{18}F]-**3** was diluted with water for injection to a concentration of about 7.4 MBq/mL. Twenty-five microliters of this diluted solution containing approximately 185 kBq of [^{18}F]-**3** was added to a tube containing a mixture of 1-octanol and 0.025 M phosphate buffer pH 7.4 (2 mL each). The test tube was vortexed at RT for 2 min followed by centrifugation at 3000 rpm (1837*g*) for 5 min (Eppendorf centrifuge 5810, Eppendorf, Westbury, USA). Aliquots of about 60 and 500 μL were drawn from 1-octanol and aqueous phases, respectively, weighed and the radioactivity in the aliquots was measured using an automatic gamma counter. After correcting for density and the mass difference between the two phases, the partition coefficient (*P*) was

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calculated as (radioactivity (cpm/ml) in 1-octanol)/ (radioactivity (cpm/ml) in phosphate buffer pH 7.4).

Biodistribution in normal mice

The biodistribution of [^{18}F]-**3** was determined in male NMRI mice (~6 weeks old). The animal studies were performed according to the Belgian code of practice for the care and use of animals, after approval from the university ethics committee for animals. The RP-HPLC purified tracer [^{18}F]-**3** was diluted with water for injection at least ten times to have EtOH <5%, and further to have a concentration of 3.7 MBq [^{18}F]-**3** per mL. A volume of 0.1 mL of this diluted tracer solution was injected into each mouse via a tail vein, under anesthesia (intraperitoneal injection of 0.1 mL of a solution containing 3 mg ketamine and 0.225 mg xylazine). The mice were killed by decapitation at 2 or 60 min after injection ($n = 4$ at each time point). Blood was collected in a tared tube and weighed. All organs and other body parts were dissected and weighed, and their radioactivity was measured in a gamma counter. Results are expressed as % ID (cpm in organ/ total cpm recovered), or, where possible, as % ID per gram tissue (% ID/g). For calculation of total radioactivity in blood, blood mass was assumed to be 7% of the body mass.

Affinity of test compounds for nucleoside kinase enzyme VZV-TK *in vitro*

The IC_{50} of compounds **6** and **3** against phosphorylation of [CH_3-^{3}H]dThd as the natural substrate for VZV-TK was determined. Briefly, the activity of purified recombinant VZV-TK enzyme was assayed in a 50-μL reaction mixture containing 50 mM Tris–HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 2.5 mM ATP, 1.0 mg/mL bovine serum albumin, 10 mM NaF, [CH_3-^{3}H]dThd (3.7 kBq in 5 μL; 1 μM final concentration) and 5 μL of recombinant enzyme (containing 7.75 ng of VZV-TK protein). The samples were incubated at 37°C for 30 min in the presence or absence of different concentrations of the test compounds. During this time period, the enzyme reaction proceeded linearly. Aliquots of 45 μL of the reaction mixtures were spotted on Whatman DE-81 filter paper disks (Whatman, Maidstone, UK). The filters were washed three times for 5 min in 1 mM ammonium formate and once for 5 min in ethanol. The radioactivity on the filters was determined by liquid scintillation counting.

Conclusion

A lipophilic, fluorine-18-labeled nucleoside analog has been synthesized and evaluated. Despite favorable physical properties for BBB passage, the tracer was not taken up sufficiently in the brain. Due to its progressive uptake in the carcass and due to the lack of affinity for VZV-TK, [^{18}F]-**3** is not a suitable tracer for VZV-tk gene expression imaging *in vivo*. Based on the results from our previous study[9] (using tracers **1** and **2**) and this study, we hypothesize that the presence of the polar sugar moiety might be detrimental for BBB penetration of these tracers, irrespective of their overall lipophilicity. Synthesis and evaluation of the corresponding radiolabeled acyclo derivatives where the sugar is replaced with an acyclic hydroxyethoxymethyl chain is warranted to further explore this hypothesis.

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