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Research Article

Syntheses of 5-(2-radiohaloethyl)- and 5-(2-radiohalovinyl)-2′- deoxyuridines. Novel types of radiotracer for monitoring cancer gene therapy with PET

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Summary

Syntheses of 5-(2-[^{18}F]fluoroethyl)- (**1**), 5-(2-[^{80}Br]bromoethyl)- (**2**), undeprotected (*E*)-5-(2-[^{18}F]fluorovinyl)- (**3**) and (*E*)-5-(2-[^{80}Br]bromovinyl)-2′-deoxyuridines (**4**) as the tracers for monitoring cancer gene therapy with positron emission tomography were described. Decay corrected radiochemical yield and synthesis time including labeling and HPLC purification from end of bombardment for **1** was 9.5% and 2 hours, respectively; yield and time for **2** was 16% and 2 hours, respectively. Chemical (approximate to radiochemical) yield and time for synthesis of **3** was 7.5% and 7 minutes, respectively. Radiochemical yield and synthesis time including labeling and HPLC purification of an analytical sample of **4** was 60% and 30 minutes, respectively. Both **2** and **4** received the side reactions during HPLC purification, i.e. ring closure and cleavage of glycosidic bond, respectively. Application of **2** and **4** needed to be confirmed by *in vitro* or *in vivo* experiments. Radiochemical yield

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of **1** could be optimized by employing a modified protocol for preparation of its precursor. The preparation of fluorovinyl counterparts had demonstrated the potential utility of the stannane, 3-tolyl-3′,5′-di-*O*-acetyl-(*E*)-5-(2-stannyl-vinyl)-2′-deoxyuridine **7**.

Key Words: PET cancer gene therapy; radiofluorination; radiobromination; stannane; tosylate

Introduction

Recently, the application of positron-emitter labelled nucleosides with positron emission tomography (PET) modality to monitor the successful transfaction of gene product *in vivo* during cancer-prodrug therapy has raised considerable attention.[1] In this strategy, the suicide gene was firstly transferred to the cancer cells. Followed by transfaction, the prodrugs such as thymidine analogs were administered to form toxic metabolites, which initiate the subsequent suicidal mechanism. Herpes simplex virus type 1 thymidine kinase (HSV-1 TK) is the most extensively studied suicide gene.[2] Monitoring a successful transfer and expression of HSV-1 TK gene before the introduction of prodrugs is thus of value. Analogs of purine and pyrimidine nucleosides labelled with radioisotopes as substrates for gene probes have been intensively studied by several groups, 1e. [3,4] Typical examples for purine nucleosides are which radiolabelled at the C-8[4] or acyclochain[5] by F-18, e.g. ^{18}F-labelled ganciclovir, 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]-FHBG) and 9-[(3-[^{18}F]-fluoro-1-hydroxy-2-propoxy)methyl]-guanine ([^{18}F]-FHPG). As for the pyrimidine nucleosides, radiolabelling at the side chain of C-5 and at the sugar moieties are mostly encountered, e.g. (*E*)-5-(2-[^{125}I]Iodovinyl)-2′-deoxyuridine,[6] and 2′-fluoro-2′-deoxy-1-*β*-D-arabinofuranosyl-5-[^{131}I]iodouracil (FIAU)[7] and 2'-[^{18}F]fluoro-5-methyldeoxyuracil-*β*-D-arabinofuranoside (FMAU),[8] respectively. Considering the structure and reactivity (SAR), thymidine analogs with the optimal activity against HSV-1 are those base-modified deoxyuridines such as ethyl- or vinyl-groups as substituents at the C-5 of the pyrimidine ring.[9–11] On the other hand, radiolabeling at this side chain with positron emitters have been rarely reported, especially for 5-(2-radiofluoroethyl) deoxyuridine[7] and 5-(2-radiofluoro-vinyl)deoxyuridine, due to either the low radiochemical yield or unavailability of suitable precursors[6] such as organostannan

compounds.[6,12] Additionally, due to the lack of hydroxy group at the C-2′ position of furanose moieties, thymidine derivatives are notoriously labile to phosphorylitic cleavage *in vivo*.[13] Yet, substitution at the C-2 position with fluorine atom in threo or erythro configuration reinforced the hydrolysis and prolonged their half-life *in vivo*.[14] Based on these knowledge, approaching to ideal precursors could be accomplished via two stages: firstly developing syntheses of suitable precursors which can be efficiently radiohalogenated in the subsequent radiochemical synthesis and secondly, if needed, modifying these leading compounds to improve their biostability *in vivo.* Thus, as the first priority, we focused on approaching the former objective. Part of this work had been published recently.[15,16] Here we report the chemical and radiochemical syntheses of these precursors (**1**, **2**, **3**, **4**, **6**, Figures 1, 2).

These positron-emitter labelled nucleosides such as 5-(2-[^{18}F]fluoroethyl)- (FEDU, **1**), 5-(2-[^{80m}Br]bromoethyl)- (BEDU, **2**), (*E*)-5-(2-[^{18}F]fluorovinyl)- (FVDU, **3** after deprotection) and (*E*)-5-(2-[^{80m}Br]bromovinyl)-2′-deoxyuridines (BVDU, **4**) were expected to be the imaging candidates and their stability were also discussed in this paper.

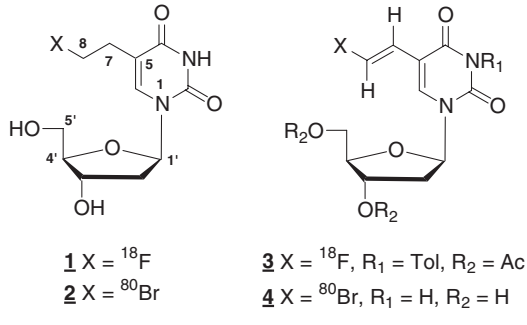


Figure 1. Positron-emitter labelled nucleosides

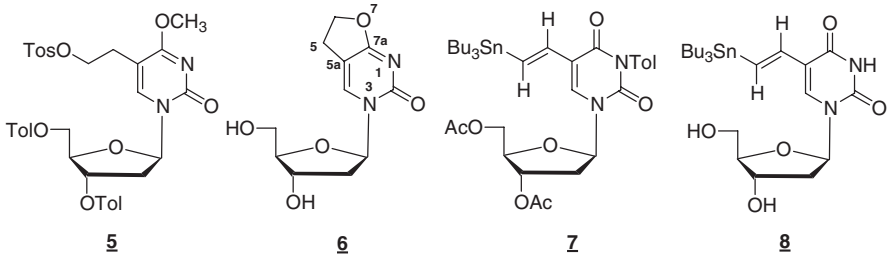


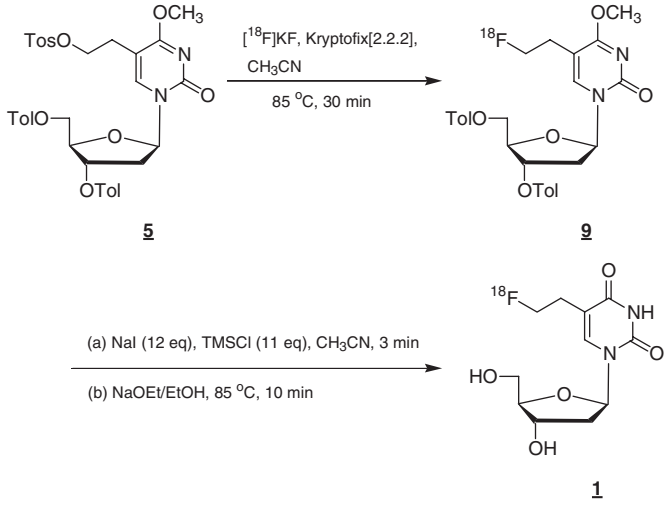
Figure 2. Precursors leading to Positron-emitter labelled nucleosides

Results and discussion

Synthesis of FEDU 1

The preparation of 4-*O*-methyl protected tosylate **5** via an eleven-step synthesis had been described in the previous work.[15] Described therein, the cyclized byproduct **6** were the sole product during the nucleophilic fluorination of the unprotected analog. On behalves of this protection, the undesired ring closure was therefore tackled. The [^{18}F]labelled **9** was prepared by nucleophilic radiofluorination of **5** under the common phase transfer catalyzed conditions (Scheme 1).

Identification of **9** was performed on the profile during development of radio-TLC. Reference **9** was synthesized from the 5-(2-hydroxyethyl) analog with DAST.[15] Optimal conditions were achieved by using 19 equivalents of Kryptofix[2.2.2] for the nucleophilic radiofluorination of **5**. The time needed for preparation of [^{18}F]KF, nucleophilic radiofluorination of **5**, cleavage of the 4-methoxy group and deprotection in **9** and subsequent C-18 HPLC analysis of **1** was 2 h from end-of-bombardment (EOB). The radioactivity and the radiochemical yield obtained at end-of-synthesis (EOS) were 49 MBq and 4.5% (9.5%, decay corrected radiochemical yield), respectively, calculated from 1.1 GBq $^{18}F^-$ (no carrier added). Since the β- and α-radiolabeled anomers **1β**, **1α** could not be separated by the RP-18 HPLC, an anomerically pure tosylate was required to carry out the labeling experiment. The

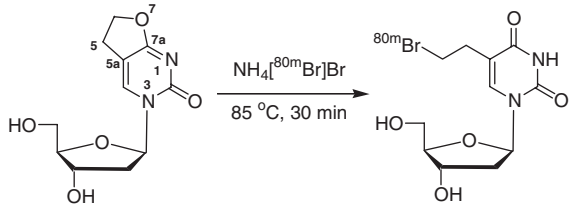


Scheme 1. Nucleophilic radiofluorination of tosylate 5

radiochemical yield of **1β** could be further optimized by improving the synthesis of its precursor.[15] During fluorination in a nonradioactive experiment, not only 5-vinyldeoxyuridine but also 5-(2-chloroethyl)-deoxyuridine formed as the by-products and yielded in 24 and 26%, respectively, compared to 5% of **1**. Appearance of the chloro derivative was due to chlorinated impurities. Preparation of the tosylate **5** was inevitable to be contaminated by chlorinated impurities, originated from the previous step during glycosylation.[15] We had also reported that these chlorinated impurities could be prevented by using an adequate silylating reagent *N,O*-bistrimethylsilyltrifluoroacetimide.[15] Thus the optimized tosylate would enhance the radiochemical yield in radiofluorination.

Synthesis of [^{80m}Br]BEDU 2

Though the direct nucleophilic bromination of the tosylate **5** is facial, the following deprotection will remove this radiohalogen. We proposed the cyclized ring compound **6** might be a candidate for introduction of radiohalogen during ring opening (Scheme 2). In the radiolabeling experiment, 11 MBq [^{80m}Br]BEDU **2** were obtained from 93 MBq [^{80m}Br]NH_4Br (^{80}Br/^{80m}Br ~ 7:1). The half-life of ^{80}Br is 17.66 m, while for ^{80m}Br is 4.42 h and ^{80m}Br decays via IT to ^{80}Br, which contains 2.2% β^+. Both isotopes ^{80}Br and ^{80m}Br share the neutron cross sections during n,γ reaction in the order of 8.5 and 2.6 Barns, respectively, and both contributed to the radioactivity after EOB. Since the total time for the procedure including nucleophilic radiobromo substitution of **6** and subsequent HPLC separation was within 2 h (6.7 times of $T_{1/2}$ of ^{80}Br), the final activity from the starting ^{80}Br should be about 8 MBq. This would be less than that from ^{80m}Br as ascertained by the presence of 11 MBq [^{80m}Br]BEDU **2**. Calculating decay corrected radiochemical yield



6 **2**

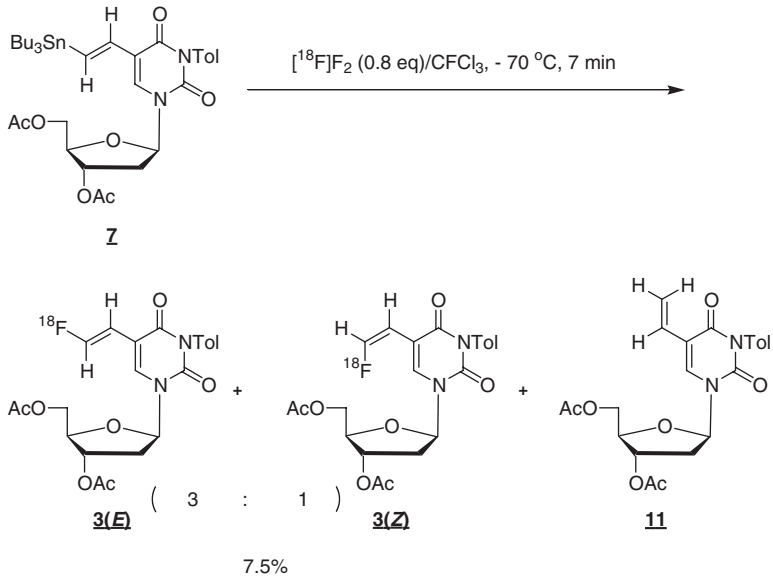
Scheme 2. Ring opening reaction

was thus based on the decay profile of ^{80m}Br. The overall radiochemical yield was 12% (decay corrected yield: 16%). However, one unknown radioactive compound with fast migrating properties, which eluted from RP-18 HPLC during a repeated chromatography of purified **2**, was suspected to be [^{80}Br]HBr. Indeed the reverse reaction, a ring closure reaction, was demonstrated by using reference BEDU **2** as a probe and the intramolecular cyclized product **6** was easily prepared in 82% yield. We utilized this instability to initially prepare **6** from reference **2** by ring closure and then introduced the radioactivity by ring opening. The reference **2** was prepared by the selective nucleophilic bromination of 5-(2-hydroxyethyl)-2′-deoxyuridine according to literature.[9(b)] In a ring opening reaction of **6** with NH_4Cl, we were able to obtain 5-(2-chloroethyl)-2′-deoxyuridine (**10**, CEDU) in high yield (90%) after 24 h. When the story comes to NH_4F, the poor nucleophile F^- did not yield any significant amount of fluorinated product. The chloride ion was thus the most efficient reagent for the ring opening of **6** and preparation of [^{34m}Cl]CEDU **10** might be envisaged. The bromide ion could also act as the ring-opening reagent, while the inherent reactivity of the bromo analog **2** limited its radiochemical yield. The ring opening reaction was also applicable to other isotopes of ^{80m}Br such as ^{82}Br or some other clinically relevant isotopes, e.g. ^{75}Br, ^{76}Br and ^{77}Br.

Synthesis of protected [^{18}F]FVDU 3(E)+3(Z)

Preparation of the organostannane **7** described in the previous paper was a four-step synthesis starting from 5-iododeoxyuridine and the yield was 50%.[16] Using the typical reaction conditions for radiofluorination of arylstannanes,[17] 0.8 eq of [^{18}F]F_2 were bubbled through **7** (25 μmol) in $CFCl_3$ at − 70°C (Scheme 3). Radioactivity of F_2 from EOB was estimated to be 0.25 GBq, while 0.38 GBq for the reaction mixture was measured by ionization chamber.

Radio-TLC of an analytical sample of the mixture was developed and the activity was measured (Figure 3). Since our HPLC purification was planned to be carried out after deprotection, the radiochemical yield of **3** was attempted to be estimated by the TLC profile. We assumed the radiolabeling agent [^{18}F]F_2 would not disturb the measurement of radiofluorinated product for its easy volatility. Furthermore the hydrolysed product [^{18}F]F^- with a high polarity was easily identified from the immobilization of the migrating zone (P1). The unknown peak P3 was assumed to be fluoroorganostannanes. According to the



Scheme 3. Radiofluorination of stannane 7

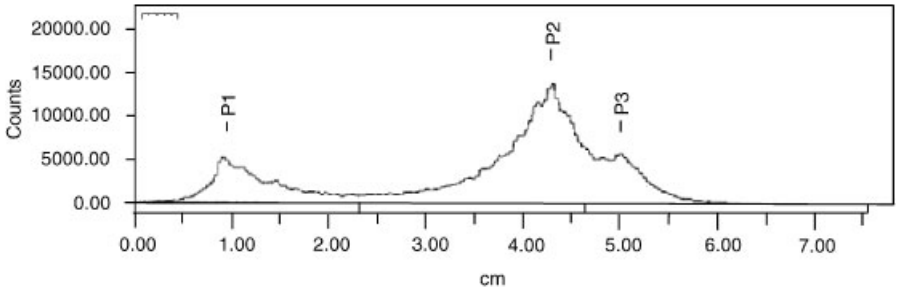


Figure 3. Radio-TLC of the crude product from the reaction of $[^{18}F]F_2$ with 7

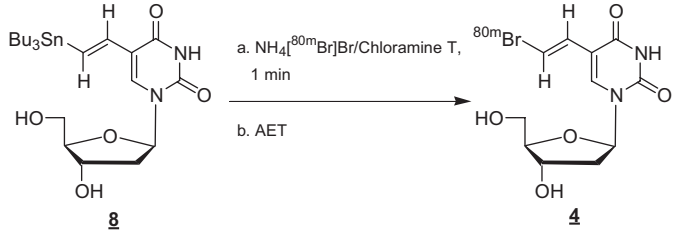
approximate calculation, about 0.2 GBq corresponding to the product peak P2, which was about 50% of the total radioactivity of the mixture. However, due to the inherent availability of one fluorine atom being ^{18}F in F_2, the radiochemical yield of 50% is not possible. Nevertheless the radiochemical yield could be referred to the yield of the nonradioactive **3**. The mixture after 48 h for decay was submitted to purification by chromatography on SiO_2 to afford the fractions corresponding to that of P2 of the above radio-TLC profile. The collected fractions were analyzed by ESI-MS and ^1H- and ^{19}F-NMR. The amount of compound **3** obtained was about 0.9 mg according to the proportion of compounds **3** and **11** on ESI-MS in a behavior of 1:7. Thus chemical yield of

compound **3** to the initial carrier F_2 was 7.5%. This should be relevant to the radiochemical yield. The time for synthesis was 7 min. The estimation of radiochemical yield in Figure. 3 reveled the major activity in P2 was not originated from compound **3**. To have a better calculation of radiochemical yield, these fractions needed to be purified by HPLC. The chemical shifts and coupling constants from ^{19}F NMR analysis demonstrated the presence of two isomers, (*E*)-fluorovinyl analog **3(*E*)** and (*Z*)-fluorovinyl analog **3(*Z*)** in a 2:1 ratio.[16] At our hands, we did not try to separate the (*E*), (*Z*) mixture. We kept the work concerning the deprotection together with the following separation by HPLC as an on-going program.

Synthesis of BVDU (4)

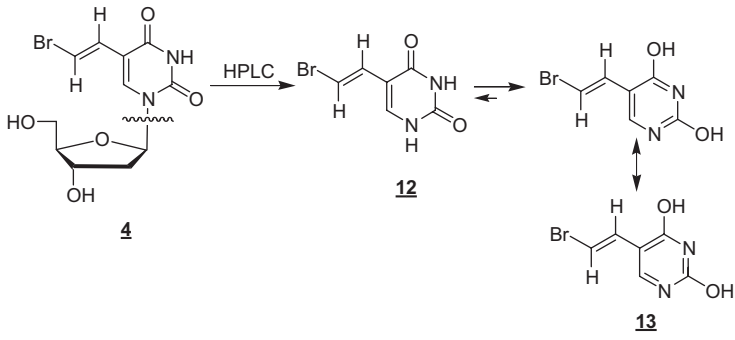
A radioactive experiment of the stannane **8** with [^{80}Br]NH_4Br and CAT was carried out according to the published procedure for the arabinosyl analog of **8**[12](Scheme 4). CAT was used to oxidize the bromide ion Br^- to bromine Br^0. This oxidation was quenched by aminoethanethiohydrochloride (AET). The best yield (99%, determined by radio-TLC) of radiobrominated **4** was obtained 1 min after addition of the oxidant CAT. However, during HPLC analysis, instability of **4** was observed. A deglycosylated product, (*E*)-5-2-[^{80}Br]bromovinyl uracil (BVA) **12**, was obtained after HPLC purification. This phenomenon might be explained in terms of an equilibration between the two tautomeric isomers **12** and **13** (Scheme 5). The bromo substitutent might favor the formation of imide tautomer **13** by acquiring the resonance stabilization.

To overcome this side reaction, modification of the glycosidic bond should be necessary. On the other hand, chlorination as side reaction



8 **4**

Scheme 4. Electrophilic radiobromination



Scheme 5. Proposed resonance stabilization leading to cleavage

had been noted during oxidation by CAT in radiobromination. This side reaction could probably occur, though we did not observe another peak besides **4** from UV chromatogram in HPLC. The time required for radiochemical synthesis and HPLC analysis was 30 min. The decay-corrected radiochemical yield of **4** was 60% from EOB based on the calculation of an analytical sample as described for [^{80m}Br]BEDU **2**.

Conclusions

Syntheses of ^{18}F and ^{80m}Br labelled 5-(2-haloethyl)- and 5-(2-halovinyl)-deoxyuridine analogs **1**, **2**, **3**, **4** were reported. Comparing with the carrier-added reactions for compounds **2**, **3** and **4**, the product **1** was prepared from no-carrier-added source $^{18}F^-$ and could provide a more high specific activity. Radiochemical yield of products prepared in this study was decreasing in the order of **4** > **2** ≥ **1** ≥ **3**. Radioactivity of **1**, **2** and **4** obtained in this study were sufficient to carry out *in vitro* or *in vivo* experiment. On the other hand, compound **3** after deprotection was believed to have enough quantities of activity for the same purpose. Preparation of **2** and **4** could be further optimized by using appropriate columns. Whether the cyclization of compound **2** or the cleavage of compound **4** limited their applications is still needed to be confirmed by *in vitro* or *in vivo* experiments. For serving as medical-interested monitoring tools, the two fluorinated compounds **1** and **3** provided alternatives to 8-[^{18}F]fluoroganciclovir, [^{18}F]-FHPG and [^{18}F]-FMAU. A relative more-step required procedure for preparing the precursor **5** was probably an efficient approach. The preparation of fluorovinyl

counterparts had demonstrated the potential utility of stannane **8**. The only five-step required synthesis of **8** showed another advantage compared to the alkyl tosylate **5**. All the precursors presented here, **5**, **6**, **7** and **8** fulfilled the requirements of least steps for deprotection.

Experimental

General

Chemicals were purchased from Aldrich. Acetonitrile was DNA-synthesis grade from Merck-Schuchardt. Fluorine gas was purchased from Messer-Griessheim (mixture of 3% F_2 in Ne). Melting points were determined with a Büchi 535 apparatus and not corrected. Elemental analysis was performed at the Max Planck Institut für Medizinische Forschung in Heidelberg. Electrospray ionization mass analysis (ESI-MS) were recorded with a Finnigan TSQ 7000 triple-quadrupole system. ^{1}H NMR and ^{13}C NMR (DEPT-135) were recorded with a Bruker AC-250 spectrometer in the Central Department of Spectroscopy of DKFZ. Coupling constants (*J*) were expressed in Hz. Cation exchange resin (H^+ form, AG 50W-X8, 200-400 mesh) was purchased from Bio-Rad. Radio-TLC was analysed by digital autoradiography employing the multiwire proportional chamber LB 287 DAR of EG & G, Berthold. Radioactive ^{18}F and ^{80}Br were obtained from the Scanditronix MC 32 NI cyclotron and the TRIGA Heidelberg II nuclear reactor, both at the German Cancer Research Center (DKFZ). The neutron flux was 2×10^{12} n/cm^2 S.

Radiochemical synthesis

1-(2-*deoxy*-β-D-*erythro*-*pentafuranosyl*)-5-(2-[^{18}*F*]*fluoroethyl*)*pyrimidin*-2,4(3 *H*)*dione* **1**

(1) K [^{18}F]F Production

[^{18}F]F^- was produced by irradiation of 1.5 ml of enriched water [^{18}O]H_2O (98%) with 18 MeV protons at a beam current of 5-6 μA for 1 h. The yield was 22 GBq. We used an amount of 1.1 GBq of [^{18}F]F^- for carrying out the following fluorination reaction. A mixture of [^{18}F]F^- (1.1 GBq, measured by an ion chamber), Kryptofix[2.2.2] (26 mg, 0.069 mmol) in CH_3CN (800 μl), K_2CO_3 (2.6 mg, 0.026 mmol) in distilled water (100 μl), and CH_3CN (5 ml) were heated in the reaction

vessel at 105°C. It was heated until the solvents had completely evaporated. After drying, CH_3CN (1 ml) was added and heated to 105°C. Repeating this process and finally the solution was cooled to rt. The entire procedure required 30 min.

(2) HPLC methods

(i) Analytical column: Knauer Eurosphere 100-C18 column, 250 × 4.6 mm (5 μm). Merck Hitachi L6200 solvent pump with a U6 K Waters injection valve with a 5 ml sample loop.

(ii) UV and radioactivity detection was carried out using Waters 996 PAD programmable multi wavelength detector at 260 nm and Canberra FLOW-ONE A200 detection system (cell size 500 μl).

(iii) HPLC buffers A = 50 mM Na_3PO_4/H_3PO_4 at pH = 5.7 and B = 95% ethanol was applied at a flow rate of 1 ml min^{-1}, gradient: 0–30% B 0–10 min, isocratic: 30% B 10–25 min.

(3) Synthesis

The experiment was carried out in an automatic synthesis module designed for radiolabelling experiments. The K[^{18}F]F, tosylate **5** (5 mg, 7.4 μmol), a solution of Kryptofix[2.2.2] (26 mg, 0.069 mmol) in CH_3CN (800 μl), and CH_3CN (5 ml) were introduced into the reaction vessel. The mixture was heated to 85°C for 30 min, transferred to a cartridge packed with Al_2O_3 (neutral) and filtered. After collecting the filtrate, the cartridge was further washed with CH_3CN (3 ml). The combined filtrates collected were then transferred into a 10 ml round-bottomed flask. To this stirred solution were sequentially added 14 mg (0.092 mmol, 12 eq) NaI and 10.4 μl (0.082 mmol, 11 eq) $(CH_3)_3SiCl$. Stirring was continued at rt for 3 min. The solvent was then evaporated at 50°C under reduced pressure (20 mbar). To the residue was added 1 ml of dry ethanol and 40 μl (40 μmol, 5.4 eq) of 1N NaOEt/EtOH and the solution was stirred at 85°C for 10 min. It was then neutralized with cation exchange resin (H^+), filtered through a short cartridge (0.45 μm filter, milipore) and washed with ethanol (0.5 ml). The combined filtrates (1.3 ml) were concentrated and chromatographed by RP-18 HPLC with the analytical column. The radiolabeled product **1** was obtained in an amount of 49 MBq (measured by an ionization chamber). The t_R of **1** was 14.5 min.

1-(2-deoxy-β-D-erythro-pentafuranosyl)-5-(2-[^{80}Br]bromoethyl) pyrimidin-2,4(3 H)dione **2**

(1) [^{80}Br]bromide production

Solid target ($NH_4^{79}Br$) was contained in a capsule with a volume 1 ml. Enriched $NH_4^{79}Br$ (90%, 1 mg, 0.01 mmol) was irradiated with slow

thermal neutrons (0.1 eV) according to $^{79}Br(n,\gamma)^{80m}Br$. The irradiation was continued for 4 h. An amount of 0.74 GBq (EOB, measured by an ionization chamber) was obtained. Multichannel analyzer (MCA, 2080 channel) coupled with a Ge(Li) detector with 4000 Volt was used to analyze the radioactivity at various radiation (γ-) energy. The range from 504 to 521 keV and from 609 to 631 keV was chosen for counting of radioactivity. The detection efficiency was 30%.

(2) HPLC methods

Semi-preparative column: Hibar RP-18, 250 × 10 mm (10 μm material). Flow rate was 3 ml min^{-1}. Elution buffer, gradient elution mode, pump, UV and radioactivity detection was carried out as above described for synthesis of [^{18}F]FEDU **1**.

(3) Synthesis

Starting material **6** (5 mg, 0.02 mmol) was dissolved in DMF (1 ml) with stirring at rt. $NH_4[^{80+80m}Br]Br$ (0.74 GBq) was added, and the mixture was stirred at 85°C for 30 min. The stirring was continued for 30 min. One product ($R_f = 0.59$) corresponding to reference **2** was observed by radio-TLC ($MeOH/CHCl_3$ 1:4). The mixture was purified with the semi-preparative HPLC (RP18). The retention time (t_R) of **2** was 18.0 min. An amount of 300 μl of the reaction solution (1 ml) was chromatographed and separated fraction was collected. This process had been repeated twice. The radioactivity of the three combined fractions was measured by a Ge(Li) detector. The total time for the procedure including nucleophilic radiobromination (30 min) and subsequent C-18 HPLC separation (90 min) was 120 min. After calibrating the detector efficiency, an amount of 11 MBq was obtained. The radiochemical yield of **2** was 12% based on 72 MBq of ^{80m}Br; decay corrected radiochemical yield was 16%.

1-(3,5-di-O-acetyl-2-deoxy-β-D-erythro-pentafuranosyl)-3-tolyl-(E)-,(Z)-5-(2-[^{18}F]fluorovinyl)pyrimidin-2,4-dione ***3(E)***, ***3(Z)***

(1) [^{18}F]F_2 Production

A stainless steel target with volume 145 ml was filled with ^{20}Ne containing 3% F_2. The amount of fluorine in the vessel was 0.6 mmol. The fluorine was further diluted to 0.7% by pressurizing with the target gas ^{20}Ne to 15 bar. The target was irradiated with ~14 MeV deuterons at a beam current 15 μA for 2 h to generate fluorine-18 in 0.25 GBq/μAh yield. An amount of 19 μmol (70 ml of the total volume at 1 atm) carrier-added [^{18}F]F_2, corresponding to 0.25 GBq by estimation, was used for the radiofluorination of **7**.

(2) Synthesis

The stannylated precursor **7** (19 mg, 25 μmol) was dissolved in $CFCl_3$ (20 ml) and cooled to –70°C. The 19 μmol $[^{18}F]F_2$ were bubbled through the cooled solution with a flow rate 10 ml min^{-1} for 7 min. A white suspension was formed. The radioactivity of the crude mixture was 0.38 GBq as measured by an ionization chamber.

*1-(2-deoxy-β-D-erythro-pentafuranosyl)-(E)-5-(2-[^{80}Br]bromovinyl)pyrimidin-2,4(3H)dione (**4**, BVDU)*

(1) [^{80}Br]Bromide production

Enriched $NH_4^{79}Br$ (91%, 1 mg, 0.01 mmol) was introduced into a capsule with volume 1 ml, and EtOH (400 μl) and H_2O (50 μl) were added. A supersonic vibrator then mixed the mixture. A clear solution was obtained. This solution was used as a liquid target, and was irradiated with thermal neutrons for 4 h. An amount of 0.37 GBq (EOB) of $NH_4[^{80}Br]Br$ was obtained. A portion of the stock solution was used as reference for counting of the decay-corrected yield.

(2) HPLC methods

(i) Semi-preparative column: Hibar Lichrospher Si100 column, 250 × 10 mm (10 μm material).

(ii) Waters 510 solvent pump with a motor valve with a 5 ml sample loop. UV and radioactivity detection was carried out using a Knauer variable wavelength detector at 260 nm and radioisotope calibrator CRC-100R.

(iii) EtOH/CH_2Cl_2 1:9 was applied at a flow rate of 4 ml min^{-1}

(3) Synthesis

Compound **8** (6 mg, 0.01 mmol) was dissolved in ethanol (100 μl) with stirring at rt. To this stirred solution was added the solution of $NH_4[^{80}Br]Br$ (0.37 GBq) and then CAT (9 mg, 0.04 mmol, 4 eq). The stirring was continued at rt for 1 min. Quenching reagent AET (27 mg, 0.24 mmol, 24 eq) was then added. Only one spot ($R_f = 0.59$) corresponding to the reference **4** was observed by radio-TLC (solvent: MeOH/$CHCl_3$ 1:4). Yield of this spot was 99%. Methanol (1 ml) was added to the mixture and one tenth of this volume (150 μl) was injected into HPLC. The retention time of **4** was $t_R = 12.4$ min in the radioactive-chromatogram. An amount of 250 μl from the separated fraction (16 ml) was further chromatographed by HPLC purification. An increased peak of [^{80}Br]BVU (t_R=5.1 min) **11** and a decreased peak of **4** were observed. The radiolabelled **4** was obtained in 60% radiochemical yield (decay corrected) based on the calculation of the analytical volume.

5.3. Precursor syntheses

1-(2-deoxy-β-D-erythro-pentafuranosyl)-5-(2-fluoroethyl)pyrimidin-2,4(3 H)-dione **1**

Preparation of this compound had been reported by Griengl *et al.*[9(a)] 1*β*: pale yellow foam [lit.[9(a)], 152–153°C], anal. $C_{11}H_{15}FN_2O_5$, MW: 274.25, ESI + Q1MS, M = 274 (*m/z*); $[M+H]^+$ = 274.8, $[M+Na]^+$ = 296.9, $[2M+Na]^+$ = 571.1, $[3M+Na]^+$ = 845.1; ESI - Q1MS, M = 274 (*m/z*); $[M\text{-}H]^-$ = 272.7, $[M+{}^{35}Cl]^-$ = 308.7, $[2M\text{-}H]^-$ = 547.0, $[2M+{}^{35}Cl]^-$ = 582.9, $[3M\text{-}H]^-$ = 821.1; ^{1}H-NMR (500 MHz, CD_3OD) δ 2.21 (ddd, $J_{2'(a),2'(b)}$ = 13.6, $J_{2'(a),1'}$ = 7.2, $J_{2'(a),3'}$ = 6.3 Hz, 1 H, H-2′(a)), 2.28 (ddd, $J_{2'(b),2'(a)}$ = 13.6, $J_{2'(b),1'}$ = 6.3, $J_{2'(b),3'}$ = 3.7 Hz, 1 H, H-2′(b)), 2.69 (ddddd, $J_{7,F}$ = 23.1, $J_{7,8(a)}$ = 6.2, $J_{7,8(b)}$ = 6.2, $J_{7(a),7(b)}$ = 2.2 Hz, $^4J_{7,6}$ = 0.9 Hz, 2 H, H-7), 3.72 (dd, $J_{5'(a),5'(b)}$ = 12.0, $J_{5'(a),4'}$ = 3.7 Hz, 1 H, H-5′(a)), 3.78 (dd, $J_{5'(b),5'(a)}$ = 12.0, $J_{5'(b),4'}$ = 3.3 Hz, 1 H, H-5′(b)), 3.91 (ddd, $J_{4',5'(a)}$ = 3.7, $J_{4',3'}$ = 3.7, $J_{4',5'(b)}$ = 3.3 Hz, 1 H, H-4′), 4.39 (dddd, $J_{3',2'(a)}$ = 6.3, $J_{3',2'(b)}$ = 3.7, $J_{3',4'}$ = 3.7, $J_{3',1'}$ = 0.4 Hz, 1 H, H-3′), 4.51 (dddd, $J_{8,F}$ = 47.2, $J_{8,7(a)}$ = 6.2 Hz, $J_{8,7(b)}$ = 6.2, $J_{8(a),8(b)}$ = 2.6 Hz, 2 H, H-8), 6.26 (ddd, $J_{1',2'(a)}$ = 7.2, $J_{1',2'(b)}$ = 6.3, $J_{1',3'}$ = 0.4 Hz, 1 H, H-1′), 7.89 (t, $^4J_{6,7}$ = 0.9 Hz, 1 H, H-6). ^{13}C-NMR (125.76 MHz, CD_3OD): *δ* 29.10 (d, $J_{7,F}$ = 21.6 Hz, C-7), 41.30(C-2′), 62.77 (C-5′), 72.13 (C-3′), 82.71 (d, $J_{8,F}$ = 167.0 Hz, C-8), 88.39 (C-1′), 91.15 (C-4′), 110.02 (d, $J_{5,F}$ = 6.0 Hz, C-5), 140.68 (C-6), 152.31 (C-2), 165.96 (C-4). ^{19}F-NMR (235.34 MHz, CD_3OD): *δ* -143.11.

1α

coloruless foam [lit.[9(a)], 128°C]. anal. $C_{11}H_{15}FN_2O_5$, MW: 274.25, ESI + Q1MS, M = 274 (*m/z*); $[M+H]^+$ = 274.8, $[M+Na]^+$ = 296.8, $[2M+H]^+$ = 549.0, $[2M+Na]^+$ = 571.0, $[3M+Na]^+$ = 845.1; ESI - Q1MS, M = 274 (*m/z*); $[M\text{-}H]^-$ = 272.7, $[M+{}^{35}Cl]^-$ = 308.7, $[2M\text{-}H]^-$ = 547.0, $[2M+{}^{35}Cl]^-$ = 583.0; ^{1}H-NMR (500 MHz, CD_3OD) *δ* 2.05 (dddd, $J_{2'(a),2'(b)}$ = 14.6, $J_{2'(a),1'}$ = 2.6, $J_{2'(a),3'}$ = 2.1 $J_{2'(a),4'}$ = 0.7 Hz, 1 H, H-2′(a)), 2.66 (ddd, $J_{2'(b),2'(a)}$ = 14.6, $J_{2'(b),1'}$ = 7.4, $J_{2'(b),3'}$ = 6.2 Hz, 1 H, H-2′(b)), 2.69 (ddd, $J_{7,F}$ = 23.5, $J_{7,8}$ = 6.2, $^4J_{7,6}$ = 0.9 Hz, 2 H, H-7), 3.56 (dd, $J_{5'(a),5'(b)}$ = 12.0, $J_{5'(a),4'}$ = 4.6 Hz, 1 H, H-5′(a)), 3.59 (dd, $J_{5'(b),5'(a)}$ = 12.0, $J_{5'(b),4'}$ = 4.4 Hz, 1 H, H-5′(b)), 4.28 (dddd, $J_{4',5'(a)}$ = 4.6, $J_{4',5'(b)}$ = 4.4, $J_{4',3'}$ = 2.3, $J_{4',2'(a)}$ = 0.7 Hz, 1 H, H-4′), 4.35 (ddd, $J_{3',2'(b)}$ = 6.2, $J_{3',4'}$ = 2.3, $J_{3'2'(a)}$ = 2.1, Hz, 1 H, H-3′), 4.50 (dt, $J_{8,F}$ = 47.3, $J_{8,7}$ = 6.2 Hz, 2 H, H-8), 6.19 (dd, $J_{1',2'(b)}$ = 7.4, $J_{1',2'(a)}$ = 2.6 Hz, 1 H, H-1′), 7.85 (t, $^4J_{6,7}$ = 0.9 Hz, 1 H, H-6). ^{13}C-NMR (125.76 MHz, CD_3OD): *δ* 29.20 (d, $J_{7,F}$ = 21.6 Hz, C-7), 41.73 (C-2′),

63.48 (C-5′), 72.47 (C-3′), 82.61 (d, $J_{8,F} = 166.9$ Hz, C-8), 86.51 (C-1′), 88.87 (C-4′), 110.84 (d, $J_{5,F} = 6.0$ Hz, C-5), 139.94 (C-6), 152.18 (C-2), 165.69 (C-4).

*1-(2-deoxy-β-*D*-erythro-pentafuranosyl)-5-(2-bromoethyl)pyrimidin-2,4(3 H)-dione* **2**

Compound **2** was prepared according to the literature[9(b)] with slight modification. Mixture of α,β-anomers of 5-(2-hydroxyethyl)-2′-deoxyuridine (500 mg, 1.84 mmol)[9(b)] and triphenyl phosphine (1.5 g, 5.71 mmol, 3 eq) in DMF (8 ml) was stirred at rt for 20 min (lit.: 10 min). NBS (655 mg, 3.68 mmol, 2 eq) was added and it was stirred at rt for 30 min. After adding quenching reagent *n*-butanol (2 ml), the solution was concentrated under vacuum (4 mbar) at 45°C. The residue was purified by silica gel column chromatography with $MeOH/CHCl_3$ 1:9 to give product **2** (*β*/α 5:1) in 40% yield (247 mg). The left solvent DMF might be not completely removed after chromatography. A repeated chromatography with $MeOH/CHCl_3$ 1:9 could solve this problem, pink crystals, 157-159°C [lit.[9(b)], 161-163 C] anal. $C_{11}H_{15}BrN_2O_5$, calcd: C 39.42, H 4.51, N 8.36, Br 23.84; found: C 39.51, H 4.80, N 8.37, Br 24.15. anal. $C_{11}H_{15}BrN_2O_5$, MW: 335.16, ESI + Q1MS, M = 335 (*m/z*); $[M + Na]^+ = 356.9$, 358.9, $[2M + Na]^+ = 693.0$, $[2M\text{-}H + 2Na]^+ = 715.0$; ESI - Q1MS, M = 335 (*m/z*), $[M + {}^{35}Cl]^- = 370.9$; ^{1}H-NMR (250 MHz, CD_3OD) δ 2.19 (ddd, $J_{2'(a),2'(b)} = 13.6$, $J_{2'(a),3'} = 7.0$, $J_{2'(a),1'} = 6.6$ Hz, 1 H, H-2′(a)), 2.27 (ddd, $J_{2'(b),2'(a)} = 13.6$, $J_{2'(b),1'} = 6.3$, $J_{2'(b),3'} = 4.0$ Hz, 1 H, H-2′(b)), 2.80 (dddd, $J_{7(a),7(b)} = 21.8$, $J_{7(a),8(a)} = 7.3$, $J_{7(a),8(b)} = 7.3$, $^4J_{7(a),6} = 0.8$ Hz, 1 H, H-7(a)), 2.86 (dddd, $J_{7(b),7(a)} = 21.8$, $J_{7(b),8(a)} = 6.8$, $J_{7(b),8(b)} = 6.8$, $^4J_{7(b),6} = 0.8$ Hz, 1 H, H-7(b)), 3.55 (ddd, $J_{8,7(a)} = 7.3$ Hz, $J_{8,7(b)} = 6.8$, $J_{8(a),8(b)} = 1.5$ Hz, 2 H, H-8), 3.71 (dd, $J_{5'(a),5'(b)} = 12.1$, $J_{5'(a),4'} = 3.6$ Hz, 1 H, H-5′(a)), 3.79 (dd, $J_{5'(b),5'(a)} = 12.1$, $J_{5'(b),4'} = 3.1$ Hz, 1 H, H-5′(b)), 3.91 (ddd, $J_{4',3'} = 3.7$, $J_{4',5'(a)} = 3.6$, $J_{4',5'(b)} = 3.1$ Hz, 1 H, H-4′), 4.39 (ddd, $J_{3',2'(a)} = 7.0$, $J_{3',2'(b)} = 4.0$, $J_{3',4'} = 3.7$ Hz, 1 H, H-3′), 6.25 (dd, $J_{1',2'(a)} = 6.6$, $J_{1',2'(b)} = 6.3$ Hz, 1 H, H-1′), 7.92 (t, $^4J_{6,7(a)} = 0.8$, $^4J_{6,7(b)} = 0.8$ Hz, 1 H, H-6). ^{13}C-NMR (62.90 MHz, CD_3OD): δ 31.70 (d, $J_{7,F} = 21.6$ Hz, C-7), 31.92 (C-8), 41.43(C-2′), 62.78 (C-5′), 72.11 (C-3′), 86.46 (C-1′), 88.92 (C-4′), 112.45 (C-5), 140.10 (C-6), 152.12 (C-2), 165.49 (C-4).

*3-(2-deoxy-β-*D*-erythro-pentafuranosyl)-5,6-dihydrofuropyrimidin-2-one* **6**

Methanol (60 ml) was saturated with ammonia at 0°C. To the solution was added **2** (320 mg, 0.95 mmol) at rt and it was stirred for 50 min. After concentration of the solution under reduced pressure at

40°C, the crystallized product **6** was obtained in 82% yield (210 mg), colourless crystals, 184–187°C (the sample may contain water), lit.[9(b)] 206–208°C, anal. $C_{11}H_{14}N_2O_5$, calcd: C 51.97, H 5.55, N 11.02; found: C 48.37, H 5.97, N 10.70; MW: 254.24, ESI + Q1MS, M = 254 (*m/z*), $[M+H]^+$: 255.0, $[M+NH_4]^+$: 272.0, $[M+Na]^+$: 277.0, $[2M+H]^+$: 509.2, $[2M+NH_4]^+$: 526.2, $[2M+Na]^+$: 531.2; ESI – Q1MS, M = 254 (*m/z*), $[M\text{-}H]^-$: 252.9, $[M+{}^{35}Cl]^-$: 289.0, $[2M\text{-}H]^-$: 507.2, $[M+Br]^-$: 332.9/334.9, $[2M+Br]^-$: 587.1/589.1; UV (MeOH): λ_{max} = 287.8 nm; λ_{min} = 247.4 nm; TLC ($MeOH/CHCl_3$ 1:4) R_f = 0.33; ^{1}H-NMR (250 MHz, DMSO-d6): δ 1.95 (ddd, $J_{2'(a),2'(b)}$ = 13.2, $J_{2'(a),3'}$ = 6.9, $J_{2'(a),1'}$ = 6.2 Hz, 1 H, H-2′a), 2.20 (ddd, $J_{2'(b),2'(a)}$ = 13.2, $J_{2'(b),1'}$ = 6.0, $J_{2'(b),3'}$ = 3.5 Hz, 1 H, H-2′b), 3.03 (td, $J_{5,6}$ = 8.2, $J_{5,4}$ = 1.6 Hz, 2 H, H-5), 3.57 (ddd, $J_{5'(a),5'(b)}$ = 11.9[¶], $J_{5',5'\ (OH)}$ = 5.3, $J_{5',4'}$ = 3.6 Hz, 2 H, H-5′a + H-5′b), 3.81 (q, $J_{4',5'(a)}$ = 3.6, $J_{4',5'(b)}$ = 3.6, $J_{4',3'}$ = 3.6 Hz, 1 H, H-4′), 4.21 (dddd, $J_{3',2'(a)}$ = 6.9, $J_{3',3'\ (OH)}$ = 4.3, $J_{3',4'}$ = 3.6, $J_{3',2'(b)}$ = 3.5 Hz, 1 H, H-3′), 4.63 (t, $J_{6,5}$ = 8.2 Hz, 2 H, H-6), 4.97 (t, $J_{5'\ (OH),5'}$ = 5.3 Hz, 1 H, 5′-OH), 5.17 (d, $J_{3'(OH),3'}$ = 4.3 Hz, 1 H, 3′-OH), 6.13 (dd, $J_{1',2'(a)}$ = 6.2, $J_{1',2'(b)}$ = 6.0 Hz, 1 H, H-1′), 8.09 (t, $J_{4,5}$ = 1.6 Hz, 1 H, H-4); ^{13}C-NMR (62.90 MHz, DMSO-d6): δ 23.91 (C-5), 40.66 (C-2′), 61.06 (C-5′), 70.07 (C-3′), 71.71 (C-6), 85.85 (C-1′), 87.52 (C-4′), 103.97 (C-5a), 137.29 (C-4), 155.51 (C-2), 177.38 (C-7a)

Typical procedure for the ring-opening reaction of **6** using non-radioactive ammonium halides. NH_4Cl as an example

6 (10 mg, 0.039 mmol) was dissolved in DMF (1 ml) with stirring at rt. To the stirred solution was added NH_4Cl (3 mg, 0.056 mmol, 1.4 eq). The stirring was continued at 85°C for 24 h. After concentration of the solution under vacuum (4 mbar) at 45°C, the residue was chromatographed with $MeOH/CHCl_3$ 1:9 to give product 5-(2-chloroethyl)deoxyuridine **10** in 90% yield (10 mg), colorless needles, 161–164°C [lit.[9(b)], 166-167°C], anal. $C_{11}H_{15}ClN_2O_5$,[‖] calcd: C 45.45, H 5.20, N 9.64; found: C 45.01, H 5.30, N 9.22 anal. anal. $C_{11}H_{15}ClN_2O_5$, MW: 290.70, ESI + Q1MS, M = 290 (*m/z*); $[M+Na]^+$ = 312.8; ESI - Q1MS, M = 290 (*m/z*), $[M\text{-}H]^-$ = 288.7, $[M+{}^{35}Cl]^-$ = 324.6; ^{1}H-NMR (250 MHz, CD_3OD) δ 2.19 (ddd, $J_{2'(a),2'(b)}$ = 13.5, $J_{2'(a),1'}$ = 7.0, $J_{2'(a),3'}$ = 6.0 Hz, 1 H, H-2′(a)), 2.27 (ddd, $J_{2'(b),2'(a)}$ = 13.5, $J_{2''(b),1'}$ = 6.3, $J_{2'(b),3'}$ = 3.9 Hz, 1 H, H-2′(b)), 2.72 (dddd, $J_{7(a),7(b)}$ = 21.6, $J_{7(a),8(a)}$ = 7.4, $J_{7(a),8(b)}$ = 7.4, $^4J_{7(a),6}$ = 0.8 Hz, 1 H, H-7(a)), 2.75 (dddd, $J_{7(b),7(a)}$ = 21.6, $J_{7(b),8(a)}$ = 6.7,

[¶] *J* was measured; but almost no difference in chemical shift values was observed.
[‖] Due to the limited amount of samples obtained, we did not analyse the Cl content.

$J_{7(b),8(b)} = 6.7$, $^4J_{7(b),6} = 0.8$ Hz, 1 H, H-7(b)), 3.63 (ddd, $J_{8,7(a)} = 7.4$ Hz, $J_{8,7(b)} = 6.7$, $J_{8(a),8(b)} = 1.1$ Hz, 2 H, H-8), 3.72 (dd, $J_{5'(a),5'(b)} = 12.1$, $J_{5'(a),4'} = 3.9$ Hz, 1 H, H-5′(a)), 3.78 (dd, $J_{5'(b),5'(a)} = 12.1$, $J_{5'(b),4'} = 3.3$ Hz, 1 H, H-5′(b)), 3.90 (ddd, $J_{4',5'(a)} = 3.9$, $J_{4',3'} = 3.5$, $J_{4',5'(b)} = 3.3$ Hz, 1 H, H-4′), 4.38 (ddd, $J_{3',2'(a)} = 6.0$, $J_{3',2'(b)} = 3.9$, $J_{3',4'} = 3.5$ Hz, 1 H, H-3′), 6.25 (dd, $J_{1',2'(a)} = 7.0$, $J_{1',2'(b)} = 6.3$ Hz, 1 H, H-1′), 7.92 (dd, $^4J_{6,7(a)} = 0.8$, $^4J_{6,7(b)} = 0.8$ Hz, 1 H, H-6). ^{13}C-NMR (62.90 MHz, CD_3OD): δ 31.70 (C-7), 41.43 (C-2′), 43.55 (C-8), 62.78 (C-5′), 72.14 (C-3′), 86.49 (C-1′), 88.94 (C-4′), 111.66 (C-5), 140.23 (C-6), 152.17 (C-2), 165.60 (C-4).

1-(2-*deoxy*-β-D-*erythro*-*pentafuranosyl*)-(*E*)-5-(2-*bromovinyl*) *pyrimidin*-2,4(3 *H*)-*dione* (*BVDU*) **4**

4 was prepared according to a published method for synthesis of 1-(β-D-arabino-pentafuranosyl)-(*E*)-5-(2-bromovinyl)uracil, an analog of **4**[18]. The yield of **4** was 28% from 5-iodo-2′-deoxyuridine, white crystals, 164-166°C[19], 158°C], anal. $C_{11}H_{13}BrN_2O_5$, calcd: C 39.66, H 3.93, N 8.41; found: C 39.07, H 4.17, N 8.02.**

ESI + Q1MS, M = 333 (*m*/*z*); $[M+H]^+ = 332.9$, 334.9, $[M+Na]^+ = 354.9$, 356.9, $[2M+H]^+ = 667.0$, $[2M+Na]^+ = 689.0$; ESI - Q1MS, M = 333 (*m*/*z*), $[M\text{-}H]^- = 330.9$, 332.9, $[M+{}^{35}Cl]^- = 368.8$, $[2M\text{-}H]^- = 664.6$, $[2M+{}^{35}Cl]^- = 700.9$; ^{1}H-NMR (250 MHz, DMSO-d6) δ (2.11–2.15 m, 2 H, H-2′), 3.53-3.63 (m, 2 H, H-5′), 3.91 (ddd, $J_{4',3'} = 3.7$, $J_{4',5'(a)} = 3.6$, $J_{4',5'(b)} = 3.1$ Hz, 1 H, H-4′), 4.22-4.28 (m, 1 H, H-3′), 5.03 (t, $J_{5'\ (OH),5'} = 5.2$ Hz, 1 H, H-5′), 5.19 (d, $J_{3''(OH),3'} = 4.4$ Hz, 1 H, H-3′), 6.12 (dd, $J_{1',2'(a)} = 6.6$, $J_{1',2'(b)} = 6.6$ Hz, 1 H, H-1′), 6.83 (dd, $J_{8,7} = 13.6$ Hz, 1 H, H-8), 7.22 (d, $J_{7,8} = 13.6$ Hz, 1 H, H-7), 8.06 (s, 1 H, H-6), 11.50 (bs, 1 H, -NH). ^{13}C-NMR (62.90 MHz, DMSO-d6): δ 39.78 (C-2′), 60.93 (C-5′), 69.86 (C-3′), 84.48 (C-1′), 87.47 (C-4′), 106.38 (C-8), 109.59 (C-5), 129.75 (C-7), 139.28 (C-6), 149.16 (C-2), 161.54 (C-4).

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** The impurities were assumed to be EtOH or water. anal. $C_{11}H_{13}BrN_2O_5$, MW: 333.14.

References

1. (a) deVries EFJ, Buursma AR, Hospers GAP, Mulder NH, Vaalburg W. *Curr Pharm Design* 2002; **8** (16): 1435-1450; (b) Nichol C, Kim EE. 2001; *J Nucl Med* 2001; **42**: 1368-1374; (c) Gambhir SS, Herschman HR, Cherry SR, *et al*. *Neoplasia* 2000; **2**: 118-138; (d) Gibson RE, Burns HD, Hamill TG., Eng WS, Francis BE, Ryan C. *Curr Pharm Design* 2000; **6**: 973-989; (e) Gambhir SS, Bario JR, Herschman HR, Phelps ME. *Nucl Med Biol* 1999; **26**: 481-490; (f) Haberkorn U, Altmann A, Morr I, *et al*. *J Nucl Med* 1997; 38 (2): 287–294.
2. Balzarini J, Morin KW, Knaus EE, Wiebe L , deClercq ED. *Gene Therapy* 1995; **2**: 317-322.
3. (a) Gambhir SS, Barrio JR, Wu L. *et al*. *J Nucl Med* 1998; **39**: 2003-2011; (b) Barrio JR, Namavari M, Srinivasan A, *et al*. *J Labelled Compd Radiopharm* 1997; **40**: 348.
4. (a) Namavari M, Barrio JR, Toyokuni T, *et al*. *Nucl Med Biol* 2000; **27** (2): 157-162; (b) Gambhir SS, Bauer E, Black ME, *et al*. *P Natl Acad Sci USA* 2000; **97**: 2785-2790; (c) Gambhir SS, Barrio JR, Phelps ME, *et al*. *P Natl Acad Sci USA* 1999; **96**: 2333–2338.
5. (a) Alauddin MM, Conti PS. *Nucl Med Biol* 1998; **25**: 175–180; (b) Monclus M, Luxen A, Cool V, Damhaut P, Velu T, Goldman S. *Bioorg Med Chem Lett* 1997; **7**: 1879–1882; (c) Alauddin MM, Conti PS, Mazza SM, Hamzeh FM, Lever JR. *Nucl Med Biol* 1996; **23**: 787–792.
6. (a) Morin KW, Atrazheva D, Knaus EE, Wiebe LI. *J Med Chem* 1997; **40**: 2184–2190; (b) Morin KW, Knaus EE, Wiebe LI. *Nucl Med Commun* 1997; **18**: 599–605.
7. (a) Tjuvajev JG, Avril N, *et al*. *Cancer Res* 1998; **58**: 4333–4341; (b) Tjuvajev JG, Finn R, Watanabe K, *et al*. *Cancer Res* 1996; **56**: 4087–4095; (c) Blasberg RG, Tjuvajev J. Int Appl WO 96 1995; 28: 190; *Chem Abstr* 1996; **125**: 321864; (d) Tjuvajev JG, Stockhammer G, Desai R. *et al*. *Cancer Res* 1995; **55**: 6126–6132.
8. Alauddin MM, Conti PS, Fissekis JD. *J Labelled Compd Radiopharm* 2002; **45** (7): 583–590.
9. (a) Griengl H, Wanek E, Schwarz W, Streicher W, Rosenwirth B, deClercq E. *J Med Chem* 1987; **30**: 1199–1204; (b) Griengl H, Bodenteich M, Hayden W. et al. *J Med Chem* 1985; **28**: 1679–1684.
10. (a) Morin KW, Wiebe LI, Knaus EE. *Carbohydrate Res* 1993; **249**: 109–116. (b) Kumar R, Knaues EE, Wiebe LI. *Nucleosides Nucleotides* 1993; **12**: 895–904; (c) Kumar R, Ji G, Wiebe LI, Knaus EE. *J Heterocyclic Chem* 1991; **28**: 711–715; (d) Kumar R, Xu L, Knaues EE, Wiebe LI, Tovell DR, Tyrrell DL, Allen TM. *J Med Chem* 1990; **33**: 717–723. (e) Tandon M, Iwashina T, Xu L, *et al*. *Biochem Archives* 1988; **4**: 131–140.

11. Dyson MR, Coe PL, Walker RT. *J Med Chem* 1991; **34**: 2782–2190.
12. Dougan H, Rennie BA, Lyster DM, Sacks SL. *Appl Radiat Isot* 1994; **45**: 795–801.
13. (a) Mark BL, Parrish JC, Wang Z-X, Wiebe LI, Knaus EE, James MNG. *Acta Crystalographica C* 2001; **57**: 758–760. (b) Naimi E, Wiebe LI, Balzarini J, deClercq E, Knaus EE. *Drug Develop Res* 2001; **52**: 492–499. (c) Balzarini J, Bohman C, Walker RT, deClercq ED. *Mol Pharmacol* 1994; **45**: 1253–1258.
14. Iwashina T, Tovell D R, Xu L, Tyrrell DL, Knaus EE, Wiebe LI. *Drug Design Del* 1988; **3**: 309–321.
15. Yu CS, Oberdorfer F. *Synthesis* 1999; 2057-2064.
16. Yu CS, Oberdorfer F. *Synlett* 2000; 86-88.
17. Adam MJ, Ruth TJ, Jivan S, Pate BD. *J Fluorine Chem* 1984; **25**: 329–337.
18. Bamford M J, Coe PL, Walker RT. *J Med Chem* 1990; **33**: 2494–2501.
19. Eger K, Jalalian M, Schmidt M. *J Heterocycl Chem* 1995; **32**: 211–218.

