Synthesis and Enzymatic Evaluation of Nucleosides Derived from 5-Iodo-2'-Halo-2'-Deoxyuridines

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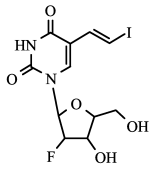
Z. Naturforsch. **58b,** 226 – 230 (2003); received April 24, 2002

The synthesis of new nucleosides by alkenylation of 5-iodo-2'-halo-2'-deoxyuridines with E-(1-tributylstannyl)-propene-1-ol via STILLE-coupling is described. The new compounds are characterized by ^{1}H NMR and elemental analysis. All nucleosides are evaluated by an enzymatic assay to be substrates of herpes simplex virus type 1 thymidine kinase (HSV-1 TK) and compared with uridine, thymidine and (E)-5-(2-iodovinyl)-2'-fluoro-2'-deoxyuridine (IVFRU).

Key words: 5-Iodo-deoxyuridines, STILLE-coupling, HSV-1 Thymidine Kinase

Introduction

(E)-5-(2-iodovinyl)-2'-fluoro-2'-deoxyuridine (IVFRU), a nucleoside analogue that displays potent and selective anti-HSV-1 activity *in vitro* has been shown to be an effective substrate for herpes simplex virus type-1 thymidine kinase (HSV-1 TK) [1,2] (Figure 1). Bearing a fluorine substituent at the 2'-position, IVFRU is also resistant to phosphorylase–mediated deglycosation, a very usual metabolic competitive reaction [3]. In cells where HSV-1 TK is present selective phosphorylation of the nucleoside can result in a trapping. This makes one with radioactive iodine labeled IVFRU-derivative attractive for imaging in gene therapy. The facile non-invasive scintigraphic detection of HSV-1 TK gene expression in tumor cell lines using [^{125}I] and [^{131}I]iodo labeled IVFRU has been reported [4, 5].



IVFRU

Fig. 1. (E)-5-(2-iodovinyl)-2'-fluoro-2'-deoxyuridine.

For utilization of the nucleoside by positron emission tomography (PET) it is urgent to synthesize IVFRU derivatives suitable for labeling with the positron emitter [^{18}F]fluorine. First attempts to introduce this radioisotope at the 2'-position of an uridine by simple ring opening of 2,2'-anhydrouridine with [^{18}F] fluoride resulted in very low yields [6].

Our approach starts with the synthesis of new IVFRU derived nucleosides by introduction of an alkenyl side chain to 5-iodo-2'-halo-2'-deoxyuridines. An hydroxyl group at the end of this side chain could open the way for further derivatisation with view on labelling with [^{18}F]fluoride.

By an enzymatic assay the activity of the new nucleosides to HSV-1 TK should be determined and compared with that of IVFRU, uridine and thymidine.

Results and Discussion

The synthetic route to obtain the new nucleosides is outlined in Figure 2. 2,2'-Anhydrouridine was used as general starting material. Ring opening with hydrogen fluoride in dioxane under anhydrous conditions gave 2'-fluoro-2'-deoxyuridine **1** in 85% yield according to a literature procedure [6]. The acetylation of **1** was carried out by stirring in an acetic anhydride, the 2'-fluoro-2'-deoxy-3',5'-diacetyl-uridine **2** was obtained in 62% yield. By reacting the 2,2'-anhydrouridine with an excess of acetyl chloride in acetonitrile a chlorine atom was introduced in the 2'-position under concurrent acetylation of the 3'- and 5'-position. In this way 2'-chloro-2'-deoxyuridine **3** was synthesized in a high yield of 93%. For introduction of the side chain the 5-position of the uridine had to be activated by a iodine substituent. For iodination of

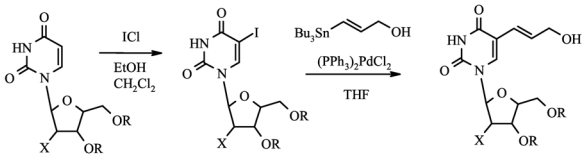


Fig. 2. Synthetic scheme of the new nucleosides.

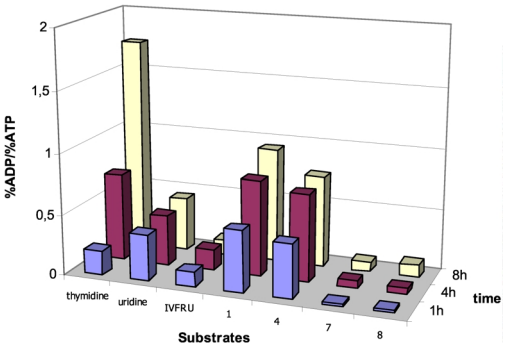


Fig. 3. Results of the enzymatic assay.

1 iodine monochloride in methanol was used and 5-iodo-2'-fluoro-2'-deoxyuridine **4** was obtained in 67% yield. This procedure seems to be more simple than the iodination of **1** with sodium iodide and nitric acid described in literature [7]. In case of the reaction of the acetylated uridines **2** and **3** with iodine monochlorid at the same conditions the complete deprotection of the hydroxyl groups was observed. By using an aprotic solvent like dichloromethane the 5-iodinated acetylated nucleosides **5** and **6** were isolated in high yields of 88% and 93% respectively. 5-Iodo-2'-chloro-2'-deoxyuridine **7** was obtained by deprotection of **6** with potassium carbonate and water in 61% yield.

The palladium catalyzed coupling of aliphatic and aromatic alkenylstannanes with activated heterocycles or nucleosides according to STILLE was already described, uridines have been activated by an iodine or trifluoromethylsulfonyl substituent [8 – 11]. A comprehensive review about C-alkenylation of pyrimidine nucleosides and analogues was published [12]. Suitable solvents for these reactions are acetonitrile, dioxane or tetrahydrofuran, as catalysts Pd(0) and Pd(II) complexes were used. Yields and reaction conditions differ considerably and are strong depending from the reactants. Recently STILLE coupling with a tributylstannyl substituted alkene bearing a free hydroxyl group has been described [18, 19], we utilized this reaction principle first time to uridines.

The coupling of the 5-iodinated uridines with E-(1-tributylstannyl)-propene-1-ol [13] was carried out with $(PPh_3)_2Pd(II)Cl_2$ in dry tetrahydrofuran. In case of non-protected nucleosides **4** and **7** the yields of 5-alkenylated products did not exceed 26%. This is remarkable because in a similar reaction **4** was coupled with 1-E-(tributylstannyl)-2-(trimethylsilyl)-ethene and an yield of 80% was achieved [4]. The reaction may be impeded by the hydroxyl function at the ethene and by the poor solubility of **4** and **7** in tetrahydrofuran. Utilization of acetonitrile instead of THF did not give an improvement of the yields. In the reaction of the acetylated uridine **5** with E-(1-tributylstannyl)-propene-1-ol the yield of the 5-alkenylated nucleoside **9** increased to 60%. Using another catalyst like $(PPh_3)_4Pd$ or the addition of CuI as recommended in literature [8] did not improve the yields for the coupling. Higher or lower reaction temperature than 60 °C resulted in either formation of more by-products or more unreacted material.

In summary, alkenylation on the 5-position of acetylated 2'-fluoro-2'-deoxy-uridine with a stannylated 3-hydroxy-(E)-1-propenyl side chain is a new way to obtain new 3-hydroxy-(E)-1-propenyl substituted uridine derivatives.

In order to evaluate the new nucleosides as substrates of the HSV-1 TK and to compare them with IFVRU we used an enzymatic assay. The nucleosides were incubated in presence of HSV-1 TK with adenosine triphosphate (ATP) and samples were taken and investigated by HPLC in a special time regime. The ratio of ADP and ATP was used to provide an information of the possible substrate affinity to HSV-1 TK.

The result of the enzymatic investigations is shown in Figure 3. Thymidine was the standard substrate and the enzymatic activity was compared with those of uridine, IVFRU and the nucleosides **1**, **4**, **7**, **8**.

As result of the enzymatic investigations it is obvious, that all derivatives were less active to HSV1-TK than the thymidine standard. It is noteworthy that 2'-fluoro-2'-deoxuridine **1** is even more active than uridine. An additional iodine atom at the 5-position (compound **4**) gave no significant change of substrate activity. In contrast, the chlorine atom at the 2'-position in compound **7** lead to a noticeable poor substrate activity. IVFRU showed also a relative low activity compared to that of thymidine and uridine, but there was no great difference between IVFRU and the

new synthesized hydroxypropenyl substituted nucleoside **8**.

Basing on these results it can be assumed that the substrate activity will not be altered in a big scale when the hydroxyl group will be replaced by fluoride and later by [^{18}F]fluorine what is encouraging for the following work.

Experimental Section

Synthesis

Commercial available reagents and solvents were supplied from FLUKA, MERCK and ALDRICH. IVFRU, 2'-fluoro-2'-deoxyuridine **1** and 2'-chloro-3',5'-diacetyluridine **3** were synthesized according to literature [4,6,14]. Preparative HPLC separations were performed on a RP 18 column (diameter: 16 mm; length: 25 cm) at a flow rate of 5 ml/min, isocratic mixtures of acetonitrile and water as eluents and UV detection at 254 nm. ^{1}H NMR investigations were carried out on a 400 MHz VARIAN UNITY INOVA spectrometer in deuterated solvents. Elemental analyses were performed on a LECO CNHS 932 elemental analyzer.

2'-Fluoro-3',5'-diacetyl-2'-deoxyuridine (**2**)

600 mg of **1** (2.44 mmol) suspended in 12 ml of acetic anhydride was stirred with 40 mg of 4-(dimethylamino)pyridine overnight at room temperature. The solvent was evaporated to dryness and the residue was recrystallized from 10 ml ethanol.

Yield: 500 mg (62%). – M.p. 172 – 173 °C. – ^{1}H NMR (δ, ppm, DMSO-d_6): 2.02 (s, 3H, -$COCH_3$); 2.10 (s, 3H, -$COCH_3$); 4.17/4.35 (m, 1H, 5'-H); 4.26 (m, 1H, 4'-H); 5.22 (m, 1H, 3'-H); 5.47/5.60 (dd, 1H, 2'-H); 5.68 (d, 1H, 5-H), 5.89 (d, 1H, 1'-H), 7.72 (d, 1H, 6-H); 11.46, (s, 1H, -NH). – $C_{13}H_{15}N_2O_7F$ (330.27): calcd. C 47.27, H 4.55, N 8.48; found C 47.30, H 4.54, N 8.46.

5-Iodo-2'-fluoro-2'-deoxyuridine (**4**)

300 mg of **1** (1.22 mmol) dissolved in 18 ml of dry methanol was heated in a closed vial with 238 mg (1.46 mmol) of iodine monochloride at 80 °C for 24 hours. After cooling the solution was evaporated to dryness and purified by column chromatography (silica gel, chloroform/methanol = 6:1).

Yield: 303 mg (67%). – M.p. 210 – 213 °C. – ^{1}H NMR (δ, ppm, DMSO-d_6): 3.59/3.80 (dd, 2H, 5'-H); 3.88 (d, 1H, 4'-H); 4.17 (d, 1H, 3'-H); 4.94/5.07 (dd, 1H, 2'-H); 5.38 (t, 1H, 5'-OH); 5.60 (d, 1H, 3'-OH); 5.85 (d, 1H, 1'-H); 8.49 (s, 1H, 6-H); 11.71 (s, 1H, -NH). – $C_9H_{10}N_2O_5FI$ (372.09): calcd. C 29.05, H 2.71, N 7.53; found C 29.29, H 2.97, N 7.45.

5-Iodo-2'-fluoro-3',5'-diacetyl-2'-deoxyuridine (**5**)

419 mg of **2** (1.27 mmol) was dissolved in 200 ml of dry dichloromethane and 309 mg (1.9 mmol) of iodine monochloride was added. The mixture was refluxed for 6 hours and evaporated to dryness after cooling. The residue was evaporated 3 times with 20 ml of dichloromethane to remove excess iodine. The resulting yellow foam was purified by column chromatography (silica gel, ethyl acetate / hexane = 7:3).

Yield: 507 mg (88%). – M.p. 71 – 73 °C. – ^{1}H NMR (δ, ppm, $CDCl_3$): 2.13 (s, 3H, -$COCH_3$); 2.19 (s, 3H, -$COCH_3$); 4.35 (m, 1H, 4'-H); 4.43 (d, 2H, 5'-H); 5,13 (m, 1H, 3'-H); 5.24/5.37 (dd, 1H, 2'-H); 5.89 (d, 1H, 1'-H); 7.88 (s, 1H, 6-H); 9.93 (s, 1H, -NH). – $C_{13}H_{14}N_2O_7FI$ (456.17): calcd. C 34.23, H 3.09, N 6.14; found C 34.64, H 2.93, N 5.94.

5-Iodo-2'-chloro-3',5'-diacetyl-2'-deoxyuridine (**6**)

693 mg of **3** (2 mmol) dissolved in 20 ml of dry dichloromethane was refluxed with 488 mg (3 mmol) iodine monochloride for 8 hours. After evaporation to dryness, the residue was evaporated 3 times with 20 ml of dichloromethane to remove excess iodine. The product was purified by column chromatography (silica gel, hexane/ethylacetate = 1:1) to yield a white foam.

Yield: 890 mg (93%). – M.p. 62 – 66 °C. – ^{1}H NMR (δ, ppm, $CDCl_3$): 2.18 (s, 3H, -$COCH_3$); 2.24 (s, 3H, -$COCH_3$); 4.39 (s, 2H, 5'-H); 4.46 (m, 1H, 4'-H); 4.57 (t, 1H, 3'-H); 5.19 (t, 1H, 2'-H); 6.10 (d, 1H, 1'-H); 7.92 (s, 1H, 6-H); 9.42 (s, 1H, -NH). – $C_{13}H_{14}N_2O_7ClI$ (472.62): calcd. C 33.04, H 2.99, N 5.93; found C 33.70, H 3.14, N 5.74.

5-Iodo-2'-chloro-2'-deoxyuridine (**7**)

472 mg of **6** (1 mmol) was stirred in 60 ml of 1 M potassium carbonate solution for 3 hours at room temperature. After acidification with hydrochloric acid, the solution was evaporated to dryness and the residue extracted with 40 ml of acetonitrile by reflux. The filtrate was evaporated again and the raw product crystallized from chloroform/methanol 4:1.

Yield: 238 mg (61%). – M.p. 230 °C (decomp.). – ^{1}H NMR (δ, ppm, DMSO-d_6): 3.60/3.75 (dd, 2H, 5'-H); 3.94 (m, 1H, 4'-H); 4.23 (m, 1H, 3'-H); 4.58 (t, 1H, 2'-H); 5.44 (t, 1H, 5'-OH); 5.89 (d, 1H, 1'-H); 5.92 (d, 1H, 3'-OH); 8.52 (s, 1H, 6-H); 11.75 (s, 1H, -NH). – $C_9H_{10}N_2O_5ClI$ (388.55): calcd. C 27.82, H 2.59, N 7.21, Cl 9,12; found C 28.21, H 2.63, N 7.46, Cl 8.86.

5-(3-Hydroxy-1(E)-propenyl)-2'-fluoro-2'-deoxyuridine (**8**)

93 mg of **4** (0.23 mmol) was suspended in 5 ml of dry tetrahydrofuran and degassed with argon. 19 mg $(PPh_3)_2PdCl_2$ (0.025 mmol) and 173 mg E-(1-tributylstannyl-propene-1-ol) [13] (0.5 mmol) dissolved in 1 ml of

tetrahydrofuran was added and the mixture was heated at 60 °C in a closed vial for 24 hours. After cooling the solution was filtered and evaporated in a stream of argon to 1 ml. The residue was purified by preparative HPLC (RP 18, acetonitrile/water = 1:9) to yield a white product.

Yield: 20 mg (26%). – M.p. 180 – 183 °C. – ^{1}H NMR (δ, ppm, DMSO-d_6): 3.60/3.77 (dd, 2H, 5'-H); 3.83 (d, 1H, 4'-H); 3,97 (d, 2H, propenyl-CH_2); 4.20 (m, 1H, 3'-H); 4.70 (t, 1H, propenyl-OH); 4.87/5.01 (dd, 1H, 2'-H); 5.37 (t, 1H, 5'-OH); 5.62 (d, 1H, 3'-OH); 5.91 (d, 1H, 1'-H); 6.19 (d, 1H, =CH—); 6.47 (dt, 1H, =CH—); 7.88 (s, 1H, 6-H); 11.46 (s, 1H, -NH). – $C_{12}H_{15}N_2O_6F$ (302.26): calcd. C 47.68, H 5.00, N 9.27; found C 47.27, H 5.15, N 8.95.

5-(3-Hydroxy-1(E)-propenyl)-2'-fluoro-3',5'-diacetyl-2'-deoxyuridine (**9**)

310 mg of **5** (0.69 mmol) was dissolved in 5 ml of dry tetrahydrofuran and degassed with argon. 50 mg $(PPh_3)_2PdCl_2$ (0.07 mmol) and 619 mg E-(1-tributylstannyl-propene-1-ol (1.8 mmol) in 1.0 ml tetrahydrofuran was added. After heating at 60 °C in a closed vial for 24 hours the mixture was cooled, evaporated to 1 ml volume and purified by preparative HPLC (RP 18, acetonitrile/water = 3:7).

Yield: 160 mg (60%). – M.p. 62 – 64 °C. – ^{1}H NMR (δ, ppm, DMSO-d_6): 2.02 (s, 3H, -$COCH_3$); 2.09 (s, 3H, -$COCH_3$); 4.02 (t, 2H, propenyl-CH_2), 4.14/4.35 (dd, 2H, 5'-H); 4.27 (t, 1H, 4'-H); 4.81 (t, 1H; propenyl-OH); 5.28 (m, 1H, 3'-H); 5.49/5.62 (dd, 1H, 2'-H); 5.91 (dd, 1H, 1'-H); 6.23 (d, 1H, =CH—); 6.59 (dt, 1H, =CH—); 7.80 (s, 1H, 6-H); 11.56 (s, 1H, -NH). – $C_{16}H_{19}N_2O_8F$ (386.33): calcd. C 49.73, H 4.96, N 7.25; found C 49.17, H 5.15, N 6.92.

5-(3-Hydroxy-1(E)-propenyl)-2'-chloro-2'-deoxyuridine (**10**)

90 mg of **7** (0.23 mmol) was suspended in 5 ml of dry tetrahydrofuran, 36 mg $(PPh_3)_2PdCl_2$ (0.05 mmol) and 158 mg E-(1-tributylstannyl-propene-1-ol (0.46 mmol) were added under argon atmosphere and the mixture was heated in a closed vial for 24 hours at 60 °C. After filtration the volume of the solution was reduced to 1.0 ml and the product was separated by preparative HPLC (RP 18, acetonitrile / water = 5:95).

Yield: 6 mg (8%). – oil. – ^{1}H NMR (δ, ppm, DMSO-d_6): 3.24/3.29 (m, 2H, 5'-H); 4.02 (t, 2H, propenyl-CH_2); 4.07 (t, 1H, 4'-H); 4.38 (m, 1H, 3'-H); 5.19 (m, 1H, 2'-H); 5.90 (s, 1H, -OH); 6.29 (d, 1H, 1'-H); 6.31 (d, 1H, =CH—); 6.68 (dt, 1H, =CH—); 8.01 (s, 1H, 6-H); 11.42 (s, 1H, -NH).

Enzymatic Investigation

For determination of substrate activity a previously published method was used with slight modification [15]. The HSV-1 TK was a gift from L. Scapozza, ETH Zürich. HSV-1 TK activity was assayed as described [16, 17].

HPLC assay: HPLC was performed using an Eurospher RP-18 column (25 × 4 mm) from Knauer with a flow rate of 1 ml/min. The ratios of ADP and ATP were monitored by UV-detection at 254 nm using ion-pair chromatography. A mixture of 0.2 M aqueous NaH_2PO_4, 0.025 M $N(Bu)_4HSO_4$ and methanol (5–10%, depending on the lipophilicity of the compound) was used as eluent. The ratio ADP/ATP gave an preliminary information about substrate affinity and was determined by integration of the UV-active signal. A typically assay was carried out in a final volume of 80 μl containing 50 mM tris-buffer (pH 7.5), 5.0 mM $MgCl_2$, 5.0 mM ATP, 1 – 5 μg enzyme and 1 mM substrate and incubated at 37 °C. Samples of 15 μl for HPLC analysis were taken after 1 h, 4 h and 8 h and diluted with 150 μl of water to terminate the reaction. Every assay was repeated for at least three times. For determination of the occurring minimal independent ATP hydrolysis two blank reactions (no enzyme or no substrate) were performed concurrently.

Acknowledgement

We thank the Free State Saxony (Germany) for financial support of this work. We thank Prof. Dr. Leonardo Scapozza, Institute of Pharmaceutical Sciences, ETH Zürich for supplying the HSV-1 TK.

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