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Original Scientific Article

Synthesis and Biological Activity of Reversed Pyrimidine Nucleosides

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Abstract. An efficient approach to reversed nucleosides which enables their synthesis in gram quantities is described. *N*-1′-Pyrimidine reversed nucleosides were prepared by treating of the sodium salt of pyrimidine bases with protected 5-tosyl ribose. Additionally, *N*-1′,*N*-3′-disubstituted reversed nucleosides were isolated in the condensation reactions with the 5-halogen pyrimidines. Using the Sonogashira coupling of 5′-iodouracil reversed nucleoside with ethynyltrimethyl silane gave 5′-ethynyl derivative which was further transformed into 5′-acetyl reversed nucleoside. Biological activity of deprotected reversed nucleosides was validated on the panel of six human carcinoma cell lines (HeLa, MIAPaCa2, Hep2, NCI-H358, CaCo-2, and HT-29). 5′-Iodouracil derivative displayed moderate growth inhibition activity against human colon carcinoma (CaCo-2) cells.

Keywords: uracil, 5-halogenuracil, D-ribose, reversed nucleosides, antitumor activity

INTRODUCTION

Modified nucleosides represent a well known class of chemotherapeutic agents for treatment of viral[1–4] and cancer[5,6] diseases. In the quest for new derivatives with a potent biological activity, many structural variations at the base and/or sugar moiety of natural nucleosides have been explored.[7,8] The practical applicability of nucleoside analogues in chemotherapy largely depends on the stability of the drug in organism, because their catabolism usually includes degradation of nucleosidic linkage. Reversed or iso-nucleosides constitute a class of nucleoside analogues in which the nucleobase is linked to the sugar moiety through a carbon atom other than ribofuranose-C1. Hence, this class of compounds appears particularly interesting as drug candidates[9–13] due to the lack of glycosidic linkage which makes them more stable to hydrolytic cleavage. In addition, the reversed nucleosides represent the largest pool of chiral synthons for the synthesis of aliphatic nucleoside analogues.[14–20]

In our previous communication we have reported on the synthesis of several partially and fully deprotected reversed and double headed nucleosides the former incorporating uracil or 5-iodouracil attached by N1′ at the C5 position of ribofuranose.[19,21] In this work we present detailed experimental conditions for the synthesis of such reversed nucleosides and extend the synthesis to the highly interesting reversed nucleoside **13** incorporating 5-fluorouracil, the well-known anticancer drug. We also report on the preparation of the novel type of the nucleoside derivatives **9**, **11** and **15** containing the ribose fragments attached at both, the N1′ and N3′ positions of 5′-iodo and 5′-fluorouracil bases. The example of further synthetic modification of the reversed 5′-iodouracil nucleoside **10** into protected 5′-ethinyl derivative **16** by the Sonogashira coupling reaction is also presented. Upon deprotection it becomes a versatile synthon for the click chemistry. The described synthetic studies enabling preparation of reversed nucleosides in the gram scale quantities are the prerequisite for biological testing and also open new perspectives for their synthetic transformations into novel optically active aliphatic or double headed nucleoside analogues, or sulfonamido and 1,2,3-triazolyl substituted reversed nucleoside derivatives.[22] The prepared reversed nucleosides were tested for the antiproliferative activity on the panel of six human carcinoma cell lines (HeLa, MIAPaCa2, Hep2, NCI-H358, CaCo-2, and HT-29) and 5′-iodouracil derivative **14** showed promising growth

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inhibition activity against human colon carcinoma (Ca-Co-2) cells.

EXPERIMENTAL

General

Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on DC-plastikfolien Kieselgel 60 F_{254} and preparative thick layer (2 mm) chromatography was done on Merck 60 F_{254}. Flash column chromatography was performed on silica gel Merck 0.040−0.063 mm. Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. UV Spectra were taken on a Philips PU8700 UV/VIS spectrophotometer. IR spectra were obtained as KBr pellets on a Perkin-Elmer 297 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on Varian Gemini 300 (300/75 MHz) or Bruker AV 300 and 600 MHz spectrometers using TMS or DMSO-d_6 as the internal standard. The order of C-atoms and protons were confirmed on the basis of 2D NMR HETCOR, COSY, and NOESY. Elemental analyses were done on a Perkin-Elmer 2400 Series II CHNS analyzer.

The Following Compounds were Prepared according to Literature Procedures

Methyl 2,3-O-isopropylidene-β-D-ribofuranoside (2)[23,24]
From D-ribose **1** (5.3 g, 32.84 mmol), compound **2** was obtained in 73 % yield (4.99 g) as oil:
^{1}H NMR ($CDCl_3$) δ/ppm: 4.97 (s, 1H, H-1), 4.82 (d, 1H, J = 6.0 Hz, H-2), 4.59 (d, 1H, J = 6.0 Hz, H-3), 4.40 (t, 1H, J = 3.1 Hz, OH), 3.64 (m, 2H, H-4, H-5$_a$), 3.46 (m, 1H, H-5$_b$), 3.42 (s, 3H, OCH_3), 1.49 (s, 3H, CCH_3), 1.32 (s, 3H, CCH_3); ^{13}C NMR ($CDCl_3$) δ/ppm: 112.03 (s, O−C−O), 110.01 (s, C-1), 84.92 (d, C-4), 85.85 (d, C-3), 82.00 (d, C-2), 64.02 (t, C-5), 55.59 (q, OCH_3), 26.50 (q, CCH_3), 24.81 (q, CCH_3).

Methyl 2,3-O-isopropylidene-5-O-p-toluenesulfonyl-β-D-ribofuranoside (3)[23,24]
From protected methyl ribofuranoside **2** (4.99 g, 24.43 mmol) compound **3** was obtained in 76 % yield (6.7 g) as a white crystals: R_f = 0.3 (CH_2Cl_2/MeOH 20:1); m.p. = 77−82 °C; ^{1}H NMR (DMSO-d_6) δ/ppm: 7.80 (d, 2H, J = 8.3 Hz, Ph), 7.50 (d, 2H, J = 8.0 Hz, Ph), 4.91 (s, 1H, H-1), 4.62 (d, 1H, J = 5.9 Hz, H-2), 4.50 (d, 1H, J = 5.9 Hz, H-3), 4.20 (t, 1H, J = 7.0 Hz, H-4), 4.05 (d, 2H, J = 7.0 Hz, 2 H-5), 3.10 (s, 3H, OCH_3), 2.42 (s, 3H, $\underline{C}H_3$-Ph), 1.35 (s, 3H, CCH_3), 1.21 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 145.67 (s, Ph), 132.52 (s, Ph), 130.71 (d, Ph), 128.16 (d, Ph), 112.20 (s, O−C−O), 109.25 (d, C-1), 84.61 (d, C-4), 83.61(d, C-2), 80.92 (d, C-3), 70.81 (t, C-5), 54.77 (q, OCH_3), 26.61 (q, CCH_3), 25.04 (q, CCH_3), 21.55 (q, Ph-CH_3).

5-Iodopyrimidine-2,4(1H,3H)-dione (6)[25−27]
From uracil **4** (5 g, 0.045 mol) compound **6** was obtained in 86 % yield (9.1 g) as a white crystals: ^{1}H NMR (DMSO-d_6) δ/ppm: 11.43 (brs, 1H, NH-3), 11.14 (brs, 1H, NH-1), 7,87 (d, 1H, J = 5.9 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.38 (s, C-4), 151.16 (s, C-2), 146.92 (d, C-6), 67.41 (s, C-5).

General Procedures for the Preparation of Reversed Nucleosides 7−11

The sodium salt of base was prepared by stirring a suspension of an equimolar amount of the pyrimidine base **4**−**6** (1 mmol) and sodium hydride (50 % in oil suspension, 1 mmol) in DMF (3−4 mL/mmol) at room temperature for 1 h and warming at 60−80 °C for 0.5 h. A solution of the methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside (**3**) (0.8 mmol) in DMF (1.7 mL/mmol of sugar) was added dropwise to this suspension at room temperature. The reaction mixture was stirred and heated at 100 °C for 20 hours. The resulting clear solution was evaporated and the residue was dissolved in hot chloroform. The suspension was filtered through Celite and filtrate was washed with water, dried over Na_2SO_4 and evaporated.

Methyl 5-deoxy-5-(2,4-dioxopyrimidin-1H-1-yl)-2,3-O-isopropylidene-β-D-ribofuranoside (7)
Method A: Following the general procedure from uracil **4** (1.8 g, 16 mmol) and after purification of the crude mixture by flash chromatography (CH_2Cl_2:MeOH 60:1), compound **7** (1.42 g) was obtained in a yield of 37 %, as a white solid: R_f = 0.26 (CH_2Cl_2/MeOH 20:1); m.p. 187−188 °C; UV (96 % EtOH) λ_{max}/nm: 207, 228 and 263, log ε/dm^3 mol^{-1} cm^{-1}: 3.96, 3.39 and 4.03; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3145 (w), 3090 (m), 2995 (m), 2925 (m), 1740 (s), 1705 (s), 1465 (s), 1420 (m), 1375 (m), 1245 (m), 1215 (m), 1090 (m), 1060 (m), 1025 (m), 955 (m); ^{1}H NMR ($CDCl_3$) δ/ppm: 9.38 (brs, 1H, NH-3'), 7.25 (d, 1H, J = 7.9 Hz, H-6'), 5.71 (dd, 1H, J = 7.9 Hz, J = 2.1 Hz, H-5'), 5.00 (s, 1H, H-1), 4.65 (brs, 2H, H-2 and H-3), 4.49 (dd, 1H, J = 5.3, J = 8.2 Hz, H-4), 4.21 (dd, 1H, J = 5.3, J = 13.8 Hz, H-5a), 3.43 (dd, 1H, J = 8.2, J = 13.8 Hz, H-5b), 3.41 (s, 3H, OCH_3), 1.47 (s, 3H, CCH_3), 1.32 (s, 3H, CCH_3); ^{13}C NMR ($CDCl_3$) δ/ppm: 163.71 (s, C-4'), 150.90 (s, C-2'), 145.04 (d, C-6'), 112.92 (s, O−C−O), 110.61 (d, C-1), 102.03 (d, C-5'), 84.93 (d, C-3), 84.37 (d, C-4), 81.83 (d, C-2), 55.93 (q, OCH_3), 51.47 (t, C-5), 26.41 (q, CCH_3), 25.00 (q, CCH_3). Anal. Calcd. mass fractions of elements, w/%, for $C_{13}H_{18}N_2O_6$ (M_r = 298.29) are: C 52.34, H 6.08, N 9.39; found: C 52.14, H 6.21, N 9.5.
Method B: Compound **10** (143 mg, 0.34 mmol) was dissolved in methanol (50 mL) and 0.1 M aqueous NaOH (3.4 mL) was added. The reaction mixture was

cooled to 5 ºC and purged with argon. Palladium on carbon catalyst (79 mg) was added and the reaction mixture was treated with hydrogen gas (42 psi) in a Parr hydrogenation apparatus for 4 h. The mixture was filtered through a Celite pad and washed with boiling methanol (20 mL). The combined methanol filtrates were concentrated under reduced pressure, dissolved in dichloromethane, washed with water, dried over Na_2SO_4 and evaporated. The product was crystallized from methanol to afford 82.6 mg (82 %) of **3**. The spectral properties were identical with a sample synthesized by method A.

Methyl 5-deoxy-5-(2,4-dioxo-5-fluoropyrimidin-1H-1-yl)-2,3-O-isopropylidene-β-D-ribofuranoside (8) and 5-fluoro-1,3-bis[(tetrahydro-4-methoxy-2,2-dimethyl-furo[3,4-d][1,3]dioxol-6-yl)methyl]pyrimidine-2,4(1H,3H)-dione (9)

Following the general procedure from 5-fluorouracil **5** (1.2 g, 9.2 mmol) and after purification of the crude mixture by flash chromatography (CH_2Cl_2/MeOH 60:1), *N*-1′-regioisomer **8** (537 mg) was obtained in a yield of 23 % and *N*-1′,*N*-3′-disubstituted nucleoside **9** (931 mg) was obtained in a yield of 25 %, both in the form of foam:

N-1′-regioisomer **8**: R_f = 0.51 (CH_2Cl_2/MeOH 20:1); UV (MeOH) λ_{max} 237 and 288 nm, log ε/dm^3 mol^{-1} cm^{-1}: 4.02 and 4.11; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3450 (w), 3220 (w), 3090 (w), 3080 (w), 2940 (w), 1741 (s), 1665 (s), 1385 (m), 1240 (m), 1215 (m), 1005 (m), 1090 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.87 (s, 1H, NH-3'), 8.08 (d, 1H, $J_{H\text{-}F}$ = 6.9 Hz, H-6'), 4.95 (s, 1H, H-1), 4.74 (d, 1H, J = 6.0 Hz, H-2), 4.62 (d, 1H, J = 5.9 Hz, H-3), 4.34 (t, 1H, J = 7.2 Hz, H-4), 3.86 (dd, 1H, J = 13.9, J = 7.6 Hz, H-5a), 3.59 (dd, 1H, J = 13.9, J = 6.9 Hz, H-5b), 3.28 (s, 3H, OCH_3), 1.37 (s, 3H, CCH_3), 1.25 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 157.71 (d, $J_{C\text{-}F}$ = 26 Hz, C-4'), 150.11 (s, C-2'), 139.85 (d, $J_{C\text{-}F}$ = 230 Hz, C-5'), 130.38 (d, $J_{C\text{-}F}$ = 34 Hz, C-6'), 111.94 (s, O−C−O), 109.43 (d, C-1), 84.72 (d, C-3), 83.45 (d, C-4), 81.32 (d, C-2), 54.99 (q, OCH_3), 50.37 (t, C-5), 26.31 (q, CCH_3), 24.84 (q, CCH_3). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{13}H_{17}N_2O_6F$ (M_r = 316.28) are: C 49.37, H 5.42, N 8.86; found: C 49.19, H 5.36, N 8.90.

N-1′,*N*-3′-disubstituted nucleoside **9**: R_f = 0.72 (CH_2Cl_2/MeOH 20:1); UV (MeOH) λ_{max} 238 and 290 nm, log ε/dm^3 mol^{-1} cm^{-1}: 3.92 and 3.98; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3090 (w), 3080 (w), 2940 (w), 1740(s), 1660 (s), 1465 (m), 1380 (m), 1245 (m), 1210 (m), 1166 (m), 1015 (m), 1090 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.19 (d, 1H, $J_{H\text{-}F}$ = 6.5 Hz, H-6'), 4.95 (s, 1H, H-1), 4.93 (s, 1H, H-1"), 4.76 (d, 1H, J = 5.9 Hz, H-2), 4.69 (d, 1H, J = 5.9 Hz, H-2"), 4.62 (d, 1H, J = 5.9 Hz, H-3), 4.60 (d, 1H, J = 5.9 Hz, H-3"), 4.38 (t, 1H, J = 7.2 Hz, H-4), 4.24 (dd, 1H, J = 9.0 Hz, J = 4.9 Hz, H-4"), 4.80 (dd, 1H, J = 13.2 Hz, J = 9.2 Hz, H-5"a), 4.01 (dd, 1H, J = 14.0 Hz, J = 6.7 Hz, H-5a), 3.90 (dd, 1H, J = 13.2, 5.0 Hz, H-5"b), 3.70 (dd, 1H, J = 14.0 Hz, J = 7.6 Hz, H-5b), 3.30 (s, 3H, OCH_3), 3.28 (s, 3H, OCH_3), 1.37 (s, 3H, CCH_3), 1.34 (s, 3H, CCH_3), 1.25 (s, 3H, CCH_3), 1.22 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 159.97 (s, C-4'), 150.99 (s, C-2'), 149.15 (d, C-6'), 128.26 (d, C-5'), 111.61 (s, O−C−O), 111.46 (s, O−C−O), 109.46 (d, C-1), 108.2 (d, C-1"), 84.60 (d, C-3 or C-3 "), 84.50 (d, C-3 or C-3"), 83.13 (C-4 and C-4"), 81.69 (C-2 or C-2"), 81.11 (C-2" or C-2), 55.06 (q, OCH_3), 54.50 (q, OCH_3), 51.52 (t, C-5), 44.55 (t, C-5"), 26.24 (q, CCH_3), 24.72 (q, CCH_3). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{22}H_{31}N_2O_{10}F$ (M_r = 502.49) are: C 52.59, H 6.22, N 5.57; found: C 52.65, H 6.30, N 5.58.

Methyl 5-deoxy-5-(2,4-dioxo-5-iodopyrimidin-1H-1-yl)-2,3-O-isopropylidene-β-D-ribofuranoside (10) and 5-iodo-1,3-bis[(tetrahydro-4-methoxy-2,2-dimethyl-furo[3,4-d][1,3]dioxol-6-yl)methyl]pyrimidine-2,4(1H,3H)-dione (11)

Following the general procedure from 5-iodouracil **6** (1.55 g, 6.5 mmol) and after purification of the crude mixture by flash chromatography (CH_2Cl_2/MeOH 60:1), *N*-1′-regioisomer **10** (1.27 g) was obtained in a yield of 58 % as a white solid and *N*-1′,*N*-3′-disubstituted nucleoside **11** (47 mg) was obtained in a yield of 1.5 % as a yellow foam.

N-1′-regioisomer **10**: R_f = 0.37 (CH_2Cl_2/MeOH 20:1); m.p. 182−183 °C; UV(MeOH) λ_{max}/nm: 215 and 288, log ε/dm^3 mol^{-1} cm^{-1}: 4.13 and 3.91; IR (KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3190 (m), 3100 (m), 3050 (m), 2995 (m), 1715 (s), 1655 (s), 1655 (s), 1450 (m), 1435 (m), 1400 (m), 1385 (m), 1360 (m), 1345 (m), 1240 (m), 1200 (m) 965 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.70 (brs, 1H, NH-3'), 8.10 (s, 1H, H-6'), 4.95 (s, 1H, H-1), 4.74 (d, 1H, J = 5.8 Hz, H-2), 4.62 (d, 1H, J = 5.8 Hz, H-3), 4.35 (m, 1H, H-4), 3.94 (dd, 1H, J = 6.7 Hz, J = 13.8 Hz, H-5a), 3.62 (dd, 1H, J = 7.6 Hz, J = 13.8 Hz, H-5b), 3.31 (s, 3H, OCH_3), 1.37 (s, 3H, CCH_3), 1.26 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 160.81 (s, C-4'), 150.67 (s, C-2'), 150.15 (d, C-6'), 111.66 (s, O-C-O), 109.37 (d, C-1), 84.51 (d, C-3), 83.24 (d, C-4), 81.05 (d, C-2), 68.08 (s, C-5'), 55.05 (q, OCH_3), 50.41 (t, C-5), 26.21 (q, CCH_3), 24.72 (q, CCH_3). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{13}H_{17}N_2O_6I$ (M_r = 424.19) are: C 36.81, H 4.04, N 6.61; found: C 36.65, H 3.96, N 6.78.

N-1′,*N*-3′-disubstituted nucleoside **11**: R_f = 0.62 (CH_2Cl_2/MeOH 20:1); UV(MeOH) λ_{max}/nm: 217 and 290 (log ε/dm^3 mol^{-1} cm^{-1}: 4.02 and 3.97); IR (KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 2990 (m), 2970 (m), 1710 (s), 1665 (br, s), 1630 (m), 1445 (br, m), 1385 (m), 1375 (m), 1340 (w), 1275 (m), 1240 (m), 1215 (m), 1160 (br, s), 870 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.19 (s, 1H, H-6'), 4.95 (s,

1H, H-1), 4.93 (s, 1H, H-1"), 4.76 (d, 1H, *J* = 5.9 Hz, H-2), 4.69 (d, 1H, *J* = 5,9 Hz, H-2"), 4.62 (d, 1H, *J* = 5.9 Hz, H-3), 4.60 (d, 1H, *J* = 5.9 Hz, H-3"), 4.38 (pt, 1H, *J* = 7.2 Hz, H-4), 4.24 (dd, 1H, *J* = 9.0, 4.9 Hz, H-4"), 4.08 (dd, 1H, *J* = 13.2, 9.2 Hz, H-5"a), 4.01 (dd, 1H, *J* = 14.0, 6.7 Hz, H-5a), 3.90 (dd, 1H, *J* = 13.2, 5.0 Hz, H-5"b), 3.70 (dd, 1H, *J* = 14.0, 7.6 Hz, H-5b), 3.30 (s, 3H, OCH_3), 3.28 (s, 3H, OCH_3), 1.37 (s, 3H, OCH_3), 1.34 (s, 3H, OCH_3), 1.25 (s, 3H, OCH_3), 1.22 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 159.97 (s, C-4'), 150.99 (s, C-2'), 149.08 (d, C-6'), 111.62 (s, O−C−O), 111.46 (s, O−C−O), 109.46 (d, C-1), 108.72 (d, C-1"), 84.60 (d, C-3), 84.50 (d, C-3"), 83.13 (brd, C-4, C-4"), 81.69 (d, C-2), 81.11 (d, C-2"), 67.11 (s, C-5'), 55.07 (q, OCH_3), 54.51 (q, OCH_3), 51.52(t, C-5), 44.55 (t, C-5"), 26.24 (q, CCH_3), 26.20 (q, CCH_3), 24.72 (q, CCH_3). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{22}H_{31}N_2O_{10}I$ (M_r = 610.39) are: C 43.29, H 5.12, N 4.59; found: C 43.15, H 5.07, N 4.70.

General Procedure for the Hydrolysis of Isopropylidene Protecting Group of Reversed Nucleosides 7−10 and 17

To a solution of reversed nucleoside (1 mmol) in methanol (11−15 mL/mmol) Amberlite IR-120 (H^+) ion exchange resin (3.3 g/mmol), that has been washed several times with absolute methanol, was added. The mixture was refluxed for 8 h, cooled and filtered through a Celite pad, and the resin was washed with methanol (≈20 mL). The filtrate and washings were combined and evaporated.

Methyl 5-deoxy-5-(2,4-dioxopyrimidin-1H-1-yl)-β-D-ribofuranoside (12)

Following the general procedure from reversed nucleoside **7** (80 mg, 0.27 mmol) the resulting mixture was purified by preparative TLC with CH_2Cl_2/MeOH (9:1) as eluents. The major band was eluted, evaporated to afford 62 mg (89 %) of **12** as a foam: R_f = 0.32 (CH_2Cl_2/MeOH 9:1); UV(MeOH) λ_{max}/nm: 208 and 265, log ε/dm^3 mol^{-1} cm^{-1}: 3.72 and 3.80; IR (KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3420 (s), 2940 (m), 1685 (s), 1530 (m), 1385 (m), 1350 (m), 1255 (m) 1130 (m), 1085 (m), 1025 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: (anomers β:α 10:1) β-anomer: 11.40 (brs, 1H, NH-3'), 7.50 (d, 1H, *J* = 7.9 Hz, H-6'), 5.54 (d, 1H, *J* = 7.9 Hz, H-5'), 5.08 (brs, 2H, OH-2 and OH-3), 4.83 (s, 1H, H-1), 4.01−3.62 (m, 5H, H-2, H-3, H-4 and 2 H-5), 3.40 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: (β-anomer) 163.94 (s, C-4'), 151.18 (s, C-2'), 146.62 (d, C-6'), 108.58 (d, C-1), 100.62 (d, C-5') 79.63 (d, C-4), 74.38 (d, C-2), 72.46 (d, C-3), 54.97 (q, OCH_3), 50.74 (t, C-5). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{10}H_{14}N_2O_6$ (M_r = 258.23) are: C 46.51, H 5.46, N 10.85; found: C 46.31, H 5.69, N 10.73.

Methyl 5-deoxy-5-(2,4-dioxo-5-fluoropyrimidin-1H-1-yl)-β-D-ribofuranoside (13)

Following the general procedure from reversed nucleoside **8** (250 mg, 0.79 mmol) the product **13** was obtained in 79 % (72 mg) yield as a foam: R_f = 0.21 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max}/nm: 235 and 288, log ε/dm^3 mol^{-1} cm^{-1}: 3.90 and 4.10; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3445 (m), 3210 (m), 3080 (w), 2945 (w), 1745 (s), 1665 (s), 1475 (w), 1380 (m), 1230 (m), 1210 (m), 1150 (w), 1130 (m), 1045 (m), 1035 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: β-anomer: 7.91 (d, 1H, $J_{H\text{-}F}$ = 6.8 Hz, H-6'), 5.34−5.01 (brs, 1H, OH-2), 4.64 (s, 1H, H-1$_\beta$), 4.12−3.57 (m, 6H, OH-3, H-2, H-3, H-4, 2H-5), 3.25 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: β-anomer: 157.94 (d, $^3J_{C\text{-}F}$ = 23 Hz, C-4'), 150.11 (s, C-2'), 139.31 (d, $J_{C\text{-}F}$ = 227 Hz, C-5'), 130.68 (d, $^3J_{C\text{-}F}$ = 34 Hz, C-6'), 108.45 (d, C-1), 79.36 (d, C-4), 74.26 (d, C-2), 72.28 (d, C-3), 54.79 (q, OCH_3), 50.42 (t, C-5); α-anomer: ^{13}C NMR (DMSO-d_6) δ/ppm: 157.94 (d, $^3J_{C\text{-}F}$ = 23 Hz, C-4'), 150.11 (s, C-2'), 139.31 (d, $J_{C\text{-}F}$ = 227 Hz, C-5'), 130.68 (d, $^3J_{C\text{-}F}$ = 34 Hz, C-6'), 102.83 (d, C-1), 81.00 (d, C-4), 70.99 (d, C-3 or C-2), 70.04 (d, C-3 or C-2), 54.83 (q, OCH_3), 50.42 (t, C-5). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{10}H_{13}N_2O_6F$ (M_r = 276.22) are: C 43.48, H 4.74, N 10.14; found: C 43.51, H 4.70, N 10.19.

Methyl 5-deoxy-5-(2,4-dioxo-5-iodopyrimidin-1H-1-yl)-β-D-ribofuranoside (14)

Following the general procedure from reversed nucleoside **10** (498 mg, 1.15 mmol) the product was crystallized from methanol to afford 381 mg (89 %) of **14** as a white crystals: R_f = 0.48 (CH_2Cl_2/MeOH 9:1); m.p. 99−101 °C; UV(MeOH) λ_{max}/nm: 213 and 287, log ε/dm^3 mol^{-1} cm^{-1}: 4.14 and 3.98; IR (KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3430 (m), 3050 (w), 2970 (w), 1730 (s), 1715 (s), 1665 (s), 1610 (m), 1445 (w), 1420 (w), 1340 (w), 1300 (w), 1255 (m), 1125 (m), 1100 (w), 1080 (w), 1025 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: (anomers β:α 10:1) β-anomer: 11.65 (brs, 1H, NH-3'), 8.02 (s, 1H, H-6'), 5.13 (d, 1H, *J* = 4.1 Hz, OH-2), 4.99 (d, 1H, *J* = 5.3 Hz, OH-3), 4.64 (s, 1H, H-1), 4.06−3.68 (m, 5H, H-2, H-3, H-4 and 2H-5), 3.25 (s, 3 H, CH_3O); ^{13}C NMR (DMSO-d_6) δ/ppm: (β-anomer) 161.06 (s, C-4'), 150.85 (s, C-2'), 150.85 (d, C-6'), 108.64 (d, C-1), 79.69 (d, C-4), 74.44 (d, C-2), 72.18 (d, C-3), 67.50 (s, C-5'), 55.14 (q, CH_3O), 50.17 (t, C-5). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{10}H_{13}N_2O_6I$ (M_r = 384.12) are: C 31.27, H 3.41, N 7.29; found: C 31.41, H 3.68, N 7.37.

5-Fluoro-1,3-bis[(tetrahydro-3,4-dihydroxy-5-methoxyfuran-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (15)

Following the general procedure from reversed nucleoside **9** (385 mg, 0.77 mmol) the product **15** was obtained in 65 % (210 mg) yield as a foam: R_f = 0.35 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max}/nm: 237 and

291, log ε/dm^3 mol^{-1} cm^{-1}: 3.91 and 4.10; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3440 (m), 3215 (m), 3000 (w), 2945 (w), 1740 (s), 1655 (s), 1455 (w), 1370 (m), 1230 (m), 1210 (m), 1135 (m), 1040 (m), 1030 (w), 955 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: (anomers β:α 10:3) β-anomer: 8.07 (d, 1H, J = 6.4 Hz, H-6'α), 8.03 (d, 1H, J = 6.5 Hz, H-6β), 5.08 (brs, 4H, OH), 4.64 (s, 1H, H-1), 4.59 (s, 1H, H-1"), 4.14−3,29 (m, 10H, H-2, H-3, H-4, 2H-5, H-2", H-3", H-4", 2H-5"), 3.26 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: (β-anomer) 156.84 (d, $^3J_{C\text{-}F}$ = 15 Hz, C-4'), 149.66 (s, C-2'), 140.14, 138.64 (d, $J_{C\text{-}F}$ = 226 Hz, C-5'), 129.64 (d, $J_{C\text{-}F}$ = 33 Hz, C-6'), 108.50 (d, C-1), 108.20 (d, C-1"), 79.22 (d, C-4), 78.18 (d, C-4"), 74.50 (d, C-2 or 2"), 74.28 (d, C-2 or 2"), 73.40 (d, C-3 or C-3"), 72.32 (d, C-3 or C-3"), 54.84 (q, OCH_3), 54.39 (q, OCH_3), 51.74 (t, C-5), 44.70 (t, C-5"). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{16}H_{23}N_2O_{10}F$ (M_r = 422.36) are: C 45.50, H 5.49, N 6.63; found: C 45.42, H 5.41, N 6.72.

Methyl 5-deoxy-5-{2,4-dioxo-5-[(2-trimethylsilyl)ethynyl]pyrimidin-1H-1-yl}-2,3-O-isopropylidene-β-D-ribofuranoside (16)

A suspension of **10** (200 mg, 0.47 mmol), bis(triphenylphosphine)palladium(II) chloride (33 mg, 0.047 mmol) and cooper(I) iodide (0.9 mg, 0.0047 mmol) in a degassed triethylamine (19 mL) was stirred vigorously and purged with argon. Excess ethynyltrimethylsilane (55 mg, 0.56 mmol) was added and the reaction mixture was stirred under argon in an oil bath at 50 °C for 8 h. The reaction mixture was cooled and evaporated. The colored residue was dissolved in chloroform (50 mL), washed with 5 % disodium EDTA/H_2O (2x20 mL) and water (20 mL), dried over Na_2SO_4 and evaporated. The resulting mixture was purified by flash column chromatography on silica gel. The product was eluted with CH_2Cl_2/MeOH (20:1) and (9:1), evaporated and crystallized from chloroform to afford 132 mg (71 %) of **16** as a white crystals: R_f = 0.39 (CH_2Cl_2/MeOH 20:1); m.p. 207−208 °C; UV (MeOH) λ_{max}/nm: 233 and 296, log ε/dm^3 mol^{-1} cm^{-1}: 4.12 and 4.19; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3195 (m), 3095 (m), 2990 (w), 2950 (m), 2160 (w), 1745 (m), 1715 (s), 1695 (s), 1625 (m), 1450 (m), 1385 (m), 1375 (m), 1355 (m), 1245 (m), 1230 (s), 1210 (m), 1160 (m), 1105 (m), 1080 (s), 1060 (m), 1040 (m); ^{1}H NMR ($CDCl_3$) δ/ppm: 9.85 (brs, 1H, NH-3'), 7.54 (s, 1H, H-6'), 5.02 (s, 1H, H-1), 4.68 (brs, 2H, H-2 and H-3), 4.51 (dd, 1H, $J_{4,5a}$ = 5.6, $J_{4,5b}$ = 8.5 Hz, H-4), 4.21 (dd, 1H, $J_{5a,4}$ = 5.6, $J_{a,b}$ = 14.1 Hz, H-5a), 3.49 (dd, 1H, $J_{5b,4}$ = 8.5, $J_{b,a}$ = 14.1 Hz, H-5b), 3.44 (s, 3H, CH_3O), 1.47 (s, 3H, CH_3C), 1.33 (s, 3H, CH_3C), 0.21 (s, 9H, $(CH_3)_3Si$); ^{13}C NMR ($CDCl_3$) δ/ppm: 161.57 (s, C-4'), 149.67 (s, C-2'), 148.47 (d, C-6'), 112.76 (s, O−C−O), 110.61 (d, C-1), 99.55 (s, C≡C), 99.15 (s, C≡C), 94.98 (s, C-5'), 84.82 (d, C-3), 83.69 (d, C-4), 81.49 (d, C-2), 55.93 (q, CH_3O), 51.47 (t, C-5), 26.19 (q, CH_3C), 24.77 (q, CH_3C), 0.00 ($(CH_3)_3Si$). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{18}H_{26}N_2O_6Si$ (M_r = 394.50) are: C 54.80, H 6.64, N 7.10; found: C 54.58, H 6.61, N 7.07.

Methyl 5-deoxy-5-(2,4-dioxo-5-acetylpyrimidin-1H-1-yl)-β-D-ribofuranoside (17)

Method A: Following the general procedure for the hydrolysis of isopropylidene protecting group: from reversed nucleoside **16** (369 mg, 0.94 mmol) the product was crystallized from methanol to afford 217 mg (78 %) of **17** as white crystals: R_f = 0.42 (CH_2Cl_2/MeOH 9:1); m.p. 189−190 °C; UV (MeOH) λ_{max}/nm: 228 and 290, log ε/dm^3 mol^{-1} cm^{-1}: 3.99 and 4.06; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3440 (w), 3000 (w), 1730 (s), 1710 (s), 1685 (s), 1675 (s), 1605 (m), 1470 (m), 1430 (w), 1385 (m), 1365 (m), 1330 (m), 1250 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: β-anomer: 11.65 (brs, 1H, NH-3'), 8.31 (s, 1H, H-6'), 5.16 (d, 1H, J = 4.3 Hz, OH), 5.05 (d, 1H, J = 6.5 Hz, OH), 4.63 (s, 1H, H-1), 4.16 (dd, 1H, J = 13.7 Hz, J = 3.4 Hz, H-5a), 4.04−3.91 (m, 1H, H-5b), 3.91−3.77 (m, 2H, H-4, H-3), 3.74 (t, 1H, J = 4.1 Hz, H-2), 3.22 (s, 3H, OCH_3), 2.46 (s, 3H, $COCH_3$); ^{13}C NMR (DMSO-d_6) δ/ppm: β-anomer: 194.40 (s, $COCH_3$), 162.14 (s, C-4'), 153.20 (d, C-6'), 150.23 (s, C-2'), 111.45 (s, C-5'), 109.09 (d, C-1), 79.80 (d, C-4), 74.22 (d, C-2), 72.35 (d, C-3), 55.53 (q, CH_3O), 51.30 (t, C-5), 30.54 (q, CH_3CO). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{12}H_{16}N_2O_7$ (M_r = 300.27) are: C 48.00, H 5.37, N 9.33; found: C 47.93, H 5.40, N 9.40.

Method B: Reversed nucleoside **16** (400 mg, 1.01 mmol) was stirred at room temperature for 4 h in a 2:1 mixture of TFA/H_2O (20 mL). After evaporation of volatiles, the crude residue was purified by flash chromatography (CH_2Cl_2/MeOH 9:1). The product was crystallized from methanol to afford 237 mg (78 %) of **17** as white crystals. The spectral properties were identical with a sample synthesized by method A.

Method C: Reversed nucleoside **18** (350 mg, 1.09 mmol) was stirred at room temperature for 3 h in a 2:1 mixture of TFA/H_2O (20 mL). After evaporation of volatiles, the crude residue was purified by flash chromatography (CH_2Cl_2/MeOH 9:1). The product was crystallized from methanol to afford 317 mg (97 %) of **17** as white crystals. The spectral properties were identical with a sample synthesized by method A.

Methyl 5-deoxy-5-(2,4-dioxo-5-ethynylpyrimidin-1H-1-yl)-2,3-O-isopropylidene-β-D-ribofuranoside (18)

A solution of (trimethylsilyl)ethynyl derivative **16** (107 mg, 0.27 mmol) in 0.2 M solution of sodium methoxide in methanol (5.5 ml) was stirred 30 min at room temperature. The solution was carefully neutralized by addition of Amberlite IR-120 (H^+) ion ex-

change resin until moistened pH paper indicated pH ≈ 6. The mixture was filtered and the resin was washed with methanol. The combined filtrate was evaporated and the product was crystallized from CH_2Cl_2/hexane to afford 72 mg (88 %) of **18** as white crystals: R_f = 0.43 (diethyl ether); m.p. 211−212 °C; UV (MeOH) λ_{max}/nm: 227 and 290, log ε/dm^{3}mol^{-1}cm^{-1}: 3.80 and 3.87; IR(KBr) $\tilde{\upsilon}_{max}$/cm^{-1}: 3270 (m), 3200 (w), 2100 (vw), 1710 (s), 1680 (s), 1630 (s), 1460 (s), 1435 (m), 1405 (w), 1385 (m), 1375 (m), 1370 (m), 1240 (m), 1205 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ/ppm: 8.75 (brs, 1H, NH-3'), 7.57 (s, 1H, H-6'), 5.01 (s, 1H, H-1), 4.66 (brs, 2H, H-2 and H-3), 4.50 (dd, 1H, $J_{4,5a}$ = 5.1 Hz, $J_{4,5b}$ = 8.7 Hz, H-4), 4.22 (dd, 1H, $J_{5a,4}$ = 5.1 Hz, $J_{a,b}$ = 14.1 Hz, H-5a), 3.49 (dd, 1H, $J_{5b,4}$ = 8.7 Hz, $J_{b,a}$ = 14.1 Hz, H-5b), 3.44 (s, 3H, CH_3O), 3.17 (s, 1H, C≡CH), 1.47 (s, 3H, CH_3C), 1.33 (s, 3H, CH_3C); ^{13}C NMR ($CDCl_3$) δ/ppm: 161.31 (s, C-4'), 149.48 (s, C-2'), 148.85 (d, C-6'), 113.03 (s, O−C−O), 110.93 (d, C-1), 98.61 (s, C-5'), 84.98 (d, C-3), 83.95 (d, C-4), 81.92 (s, C≡CH), 81.70 (d, C-2), 74.25 (d, C≡CH), 56.20 (q, CH_3O), 51.85 (t, C-5), 26.38 (q, CH_3C), 24.87 (q, CH_3C). Anal. Calcd. mass fractions of elements, *w*/%, for $C_{15}H_{18}N_2O_6$ (M_r = 322.31) are: C 55.89, H 5.63, N 8.69; found: C 55.68, H 5.73, N 8.62.

Cell culturing and MTT test[28,29]

Reversed nucleoside derivatives **12**−**15** and **17**, in a parallel with 5-fluorouracil **5** as a standard antitumor drug, were selected for preliminary *in vitro* testing on cytotoxicity using 6 different human tumor cell lines: cervix adenocarcinoma (HeLa), pancreatic carcinoma (MIAPaCa2), laryngeal carcinoma (Hep-2), human caucasian bronchioalveolar carcinoma (NCI-H358), and colon carcinoma (HT-29, CaCo2). The cells were grown in monolayer at 37 °C in a humidified atmosphere with 5 % CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % (*v*/*v*) fetal bovine serum, 2 mM glutamine, 100 U penicillin and 100 mg/mL streptomycin. Cell lines were incubated with four 10-fold dilutions (10^{-4} to 10^{-7} M). After 72 hours of incubation the cell growth rate was evaluated using the MTT assay.

For the MTT test, cells were seeded on 96 micro well flat bottom plates (Greiner, Austria) at 2×10^4 cells/mL. After 72 hours of incubation with the tested compounds MTT (Merck, Germany) was added. DMSO (Merck, Germany) was used to dissolve the formed MTT-formazane crystals. Absorbency was measured at 570 nm on Stat fax 2100 plate reader (Awareness Technology Inc. USA). All experiments were performed three times in triplicates. The percentage of treated tumor cells growth inhibition was calculated relative to the growth of untreated (control) cells.

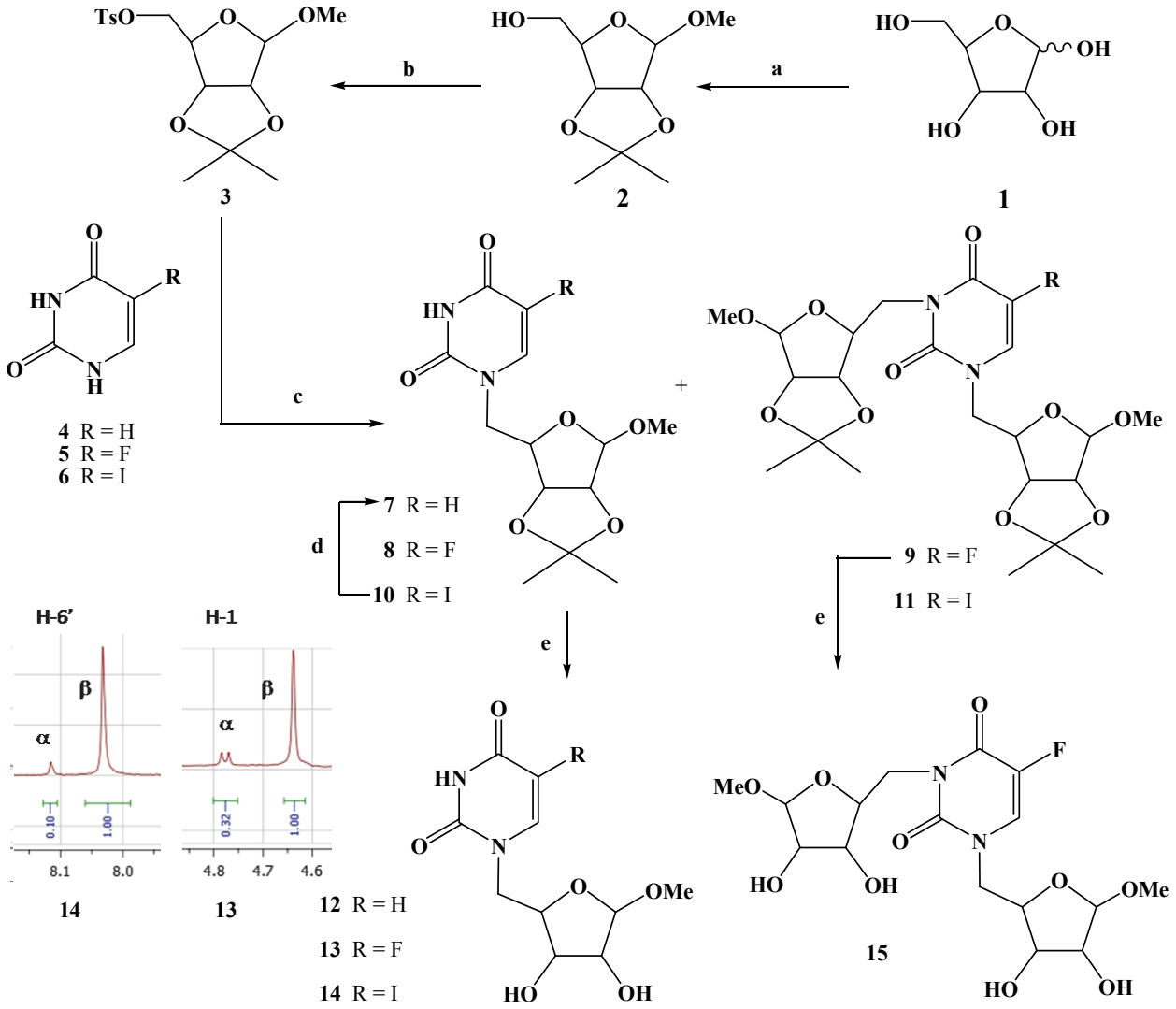
RESULTS AND DISCUSSION

The synthetic approach to reversed nucleoside analogues is based on the preparation of the already known, suitably protected methyl ribofuranoside **2** (73 %) and its transformation into 5-tosyl derivative **3** (76 %) by adopting the methods described in the literature (Scheme 1).[23,24] Following our previously described approach to reversed nucleosides, the sodium salts of the uracil derivatives **4**−**6** were reacted with ribofuranoside **3** giving the corresponding reversed nucleosides **7**, **8** and **10** (Scheme 1).[19,21]

It was reported that the condensation of thymine sodium salt with the tosyl monosaccharide **3** gave two regioisomers containing the ribofuranoside attached at N1′ or N3′ position of the thymine ring.[30] However, we were not able to identify formation of the *N*-3′-regioisomer in the reaction of uracil derivatives **4**−**6** with **3** and, exclusively the corresponding *N*-1′-regioisomers **7**, **8** and **10** were isolated. The structures of the reversed nucleosides **7**, **8** and **10** were confirmed by NMR, FTIR and elemental analyses. The formation of the *N*-1′- and not the *N*-3′-regioisomers of **7**, **8** and **10** is apparent from their ^{1}H NMR spectra. In the spectrum of **7**, the signal of C5′ proton appears as a doublet of doublets due to the vicinal H5′-H6′ coupling ($J_{5',6'}$ = 7.9 Hz) and the additional long-range H5'-NH3' coupling ($J_{5',NH\text{-}3'}$ = 2.1 Hz), the latter excluding the *N*-3′-regional isomer structure. In addition, in the spectra of **7**, **8** and **10** the NH proton signals appear at δ 9.38; 11.7 and 11.87 ppm, respectively, being the characteristic chemical shifts of the uracil NH-3′ protons.

5-Fluorouracil **5** is well known to exhibit a strong antitumor activity but its toxicity largely limits the use of **5** as a practical antitumor agent for humans.[31] We examined the possibility to prepare the reversed nucleoside **8** incorporating 5-fluorouracil fragment. The sodium salt of 5-fluorouracil **5** was condensed with tosyl ribofuranoside **3** giving the *N*-1′-regioisomer of reversed nucleoside **8** in 23 % yield and also the novel *N*-1′,*N*-3′-disubstituted nucleoside **9** in 25 % yield. In the ^{1}H NMR spectra of both, **8** and **9** the signal of H-6′ vinyl proton is split into a doublet (**8**: δ = 8.08 ppm, $^3J_{H6'\text{-}F}$ = 6.9 Hz; **9**: δ = 8.19 ppm, $^3J_{H6'\text{-}F}$ = 6.5 Hz) due to the H-F coupling.

Since the yields of the reversed nucleosides **7** and **8** prepared from uracil **4** and 5-fluorouracil **5** sodium salts were relatively low, we examined the condensation of tosyl ribofuranoside **3** with the 5-iodouracil **6** which upon N1′-deprotonation should be better nucleophile compared to the corresponding anions of **4** and **5**, due to electron donating effect of iodine. The *N*-1′-regioisomer **10** was obtained in 58 % yield together with the very small amount of the novel *N*-1',*N*-3'-disubstituted nucleoside derivative **11** (1.5 %). The hydrogenation of **10**



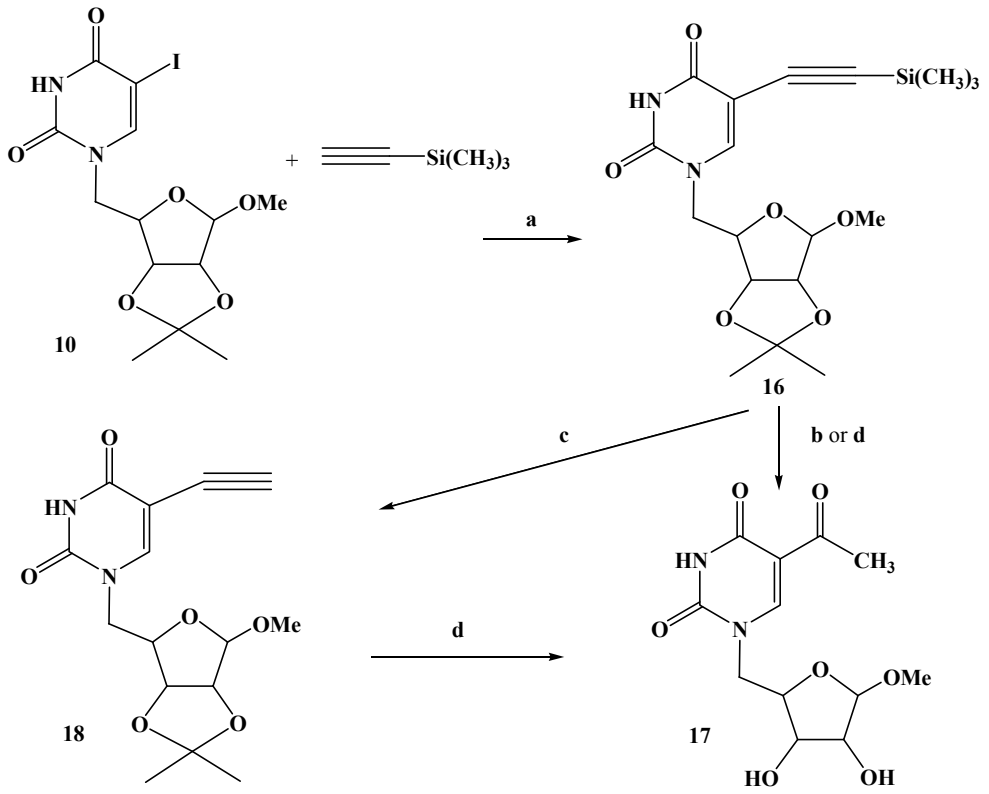
Scheme 1. (a) 1. HCl/MeOH, 2. 2,2-dimethoxypropane, acetone; (b) TsCl/Py; (c) NaH/DMF; (d) H_2, Pd/C, 0.1 M NaOH, MeOH; (e) Amberlite IR-120 (H^+), MeOH, reflux. The ratio of anomers **13** (α/β 3:10) and **14** (α/β 1:10) as determined by ^{1}H NMR spectra (inset).

using Pd/C catalyst afforded the reversed nucleoside **7** in 82 % yield (Scheme 1). By the latter two step preparation, **7** could be prepared in higher yield than in the direct condensation of the sodium salt of uracil **4** with **3**.

The ^{1}H NMR spectra of the isopropylidene protected reversed nucleosides **7**, **8** and **10** as well as those of the equally protected *N*-1′,*N*-3′-disubstituted nucleosides **9** and **11** conclusively show that all posses the β-configuration. In each spectrum, the anomeric C1 proton appears as the singlet due to small coupling constant with the proton at C2 ribose.

The isopropylidene protecting groups of **7**−**10** were removed by using of Amberlite IR-120 (H^+) ion exchange resin in refluxing methanol to yield the corresponding methyl ribofuranoside reversed nucleosides (**12**−**15**) in 65−89 % yields (Scheme 1). The ^{1}H NMR spectrum of **14** reveal the presence of duplicate peaks for H-6' proton due to the presence of an anomeric mixture in the ratio α/β = 1:10 and in the spectrum of **13** signals of protons at C1 position (α/β = 3:10) are well separated as shown in the inset of Scheme 1.

The 5'-iodo reversed nucleoside **10** is suitable for further functionalization at the uracil ring. It is well known that the coupling of terminal alkynes with 5-iodouracil nucleosides proceeds in high yields in the presence of palladium catalyst.[32] Treatment of **10** with ethynyltrimethylsilane and $(PPh_3)PdCl_2$ in the presence of CuI and triethylamine afforded **16** in 71 % yield (Scheme 2). The structure of **16** was confirmed by the presence of acetylenic band in the IR spectrum (ν 2160 cm^{-1}) and further supported by the



Scheme 2. (a) $PdCl_2(PPh_3)_2$, CuI, Et_3N; (b) Amberlite IR-120 (H+), MeOH, reflux; (c) NaOMe/MeOH, rt; (d) 50 % TFA/H_2O.

^{1}H NMR and elemental analysis (see Experimental part).

Using acidic ion exchange resin in methanol or 50 % aqueous TFA for isopropylidene and trimethylsilyl deprotection of **16** gave 5'-acetyl reversed nucleoside **17** in 78 % yield. As it was described in the literature 5-ethynyl-2'-deoxyuridine could be hydrated by dilute sulphuric acid to give 5-acetyl derivative in high yield.[33,34] Hence, during deprotection of **16**, under acidic conditions besides removal of isopropylidene and trimethylsilyl groups the addition of water on the acetylenic bond occurred giving the 5'-acetyl derivative **17**. Treatment of **16** with 0.2 M sodium methoxide in dry methanol effected removal of the trimethylsilyl group giving 5'-ethynyl reversed nucleoside **18** in 88 % yield. The removal of isopropylidene group of **18** by 50 % aqueous TFA gave the 5'-acetyl **17** in almost quantitative yield (Scheme 2).

The reversed nucleosides **12**−**15** and **17** were evaluated for their antitumor activity *in vitro* against HeLa, MIAPaCa2, Hep2, NCI-H358, CaCo-2, and HT-29 cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay method.[28,29] The reference drug used was 5-fluorouracil. The activity of the samples and the reference drug was assayed under identical conditions at concentrations of 10^{-4} M to 10^{-7} M.

Among the tested compounds only 5'-iodo reversed nucleoside **14** (Figure 1) showed a moderate cytostatic activity against CaCo-2 cell line (50 % growth inhibition c =10^{-4} M and 30 % growth inhibition c = 10^{-6}–10^{-7} M), which indicates that further synthetic variations of **14** may result in the preparation of derivatives with improved cytostatic potential.

CONCLUSIONS

In this work we describe the synthetic approach to reversed nucleosides which enables their preparation in gram quantities. The reaction of the sodium salt of various pyrimidine nucleobases **4**−**6** with a suitably protected ribofuranoside **3**, enable the efficient preparation of the reversed pyrimidine nucleosides (**7, 8, 10**). In some cases also *N*-1,*N*-3-diribofuranosyl substituted nucleosides **9** and **11** were isolated. The 5'-iodo reversed nucleoside **10** was suitable for further functionalization at the uracil and by using the Sonogashira coupling 5'-ethynyl reversed nucleoside **16** was synthesized and transformed to 5'-acetyl derivative **17** under acidic conditions. The reversed nucleosides **12**−**15** and **17** were tested for the antiproliferative activity on the panel of six cell lines (HeLa, MIAPaCa2, Hep2, NCI-H358, CaCo-2, and HT-29). Modest growth inhibition was

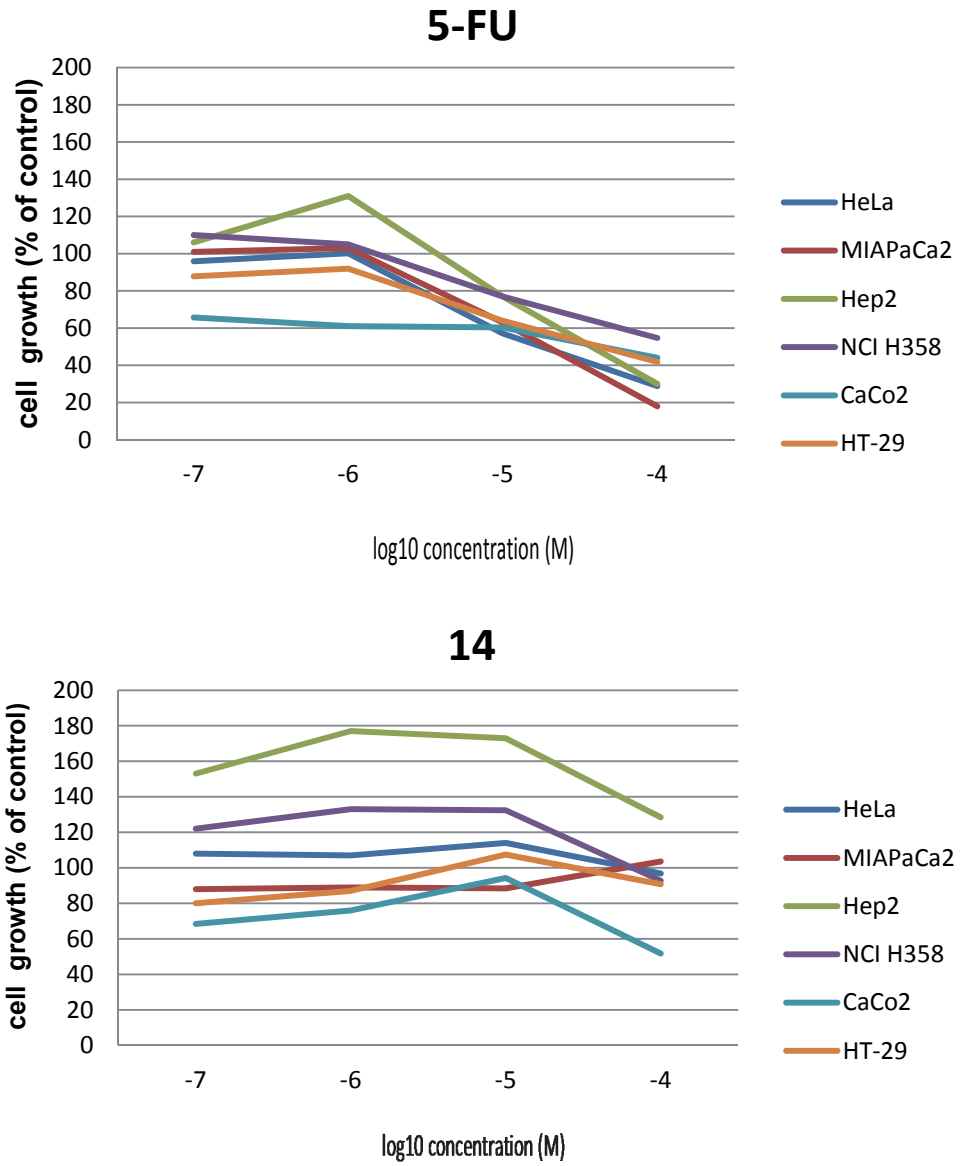


Figure 1. Cytotoxic effects of 5-fluorouracil **5** (5-FU) and 5'-iodo reversed nucleoside **14** on the growth of tumor cell lines after 72 h of incubation in the final concentration range ($10^{-4} - 10^{-7}$ M). Cytotoxicity was analyzed using the MTT survival assay.

obtained only for compound **14** and the CaCo-2 cell line at the highest concentration regime (50 % growth inhibition $c = 10^{-4}$ M).

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