Synthesis and Biological Activity of Reversed Pyrimidine Nucleosides

Nataša Župančić,a Željka Ban,b Josipa Matić,b Dijana Saftić,b Ljubica Glavaš-Obrovac,c and Biserka Žinićb,*

aTAPI Research and Development, PLIVA Croatia Ltd., Prilaz baruna Filipovića 25, 10 000 Zagreb, Croatia
bDivision of Organic Chemistry and Biochemistry, Rudjer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia
cDepartment of Clinical Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, J. J. Strossmayer University of Osijek, Hultzlerova 4, 31000 Osijek, Croatia

RECEIVED SEPTEMBER 9, 2014; REVISED SEPTEMBER 22, 2014; ACCEPTED SEPTEMBER 23, 2014

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 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 ethynyltrimethylsilane 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 viral1−4 and cancer5,6 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 candidates9−13 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′-ethyl 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
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-plastifikolien Kieselgel 60 F254 and preparative thick layer (2 mm) chromatography was done on Merck 60 F254. 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. 1H and 13C NMR spectra were recorded in dimethyl sulfoxide (DMSO-δ6 or CDCl3 on Varian Gemini 300 (300/75 MHz) or Bruker AV 300 and 600 MHz spectrometers using TMS or DMSO-δ6 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:

\[ ^1H\text{NMR (CDCl}_3\text{)} \delta/\text{ppm: 4.97 (s, 1H, H-1), 4.82 (d, 1H, J = 6.0 Hz, H-2), 4.59 (d, 1H, J = 6.0 Hz, H-3), 4.40 (t, 1H, J = 3.1 Hz, OH), 3.64 (m, 2H, H-4, H-5a), 3.46 (m, 1H, H-5b), 3.42 (s, 3H, OCH}_3\text{), 1.49 (s, 3H, CCH}_3\text{), 1.32 (s, 3H, CCH}_3\text{); } ^{13}\text{C NMR (CDCl}_3\text{)} \delta/\text{ppm: 145.67 (s, Ph), 132.52 (s, Ph), 130.71 (d, Ph), 128.16 (d, Ph), 112.20 (s, O-C-O), 109.25 (d, C-1), 84.61 (d, C-4), 83.61(d, C-2), 80.92 (d, C-3), 70.81 (t, C-5), 54.77 (q, OCH}_3\text{), 26.61 (q, CCH}_3\text{), 25.04 (q, CCH}_3\text{), 21.55 (q, Ph-CH}_3\text{).} \]

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: \[ ^1H\text{NMR (DMSO-δ6)} \delta/\text{ppm: 11.43 (brs, 1H, NH-3), 11.14 (brs, 1H, NH-1), 7.87 (d, 1H, J = 5.9 Hz, H-6); } ^{13}\text{C NMR (DMSO-δ6)} \delta/\text{ppm: 161.38 (s, C-4), 151.16 (s, C-2), 146.92 (d, C-6), 67.41 (s, C-5).} \]

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 hydrate (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 Na2SO4 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 (CH2Cl2:MeOH 60:1), compound 7 (1.42 g) was obtained in a yield of 37 %, as a white solid: \( R_f = 0.26 \) (CH2Cl2:MeOH 20:1); m.p. 187–188 °C; UV (96 % EtOH) \( \lambda_{max}/nm: 207, 228 \) and 263, log \( \varepsilon/dm^3 mol^{-1} cm^{-1}: 3.96, 3.39 \) and 4.03; IR(KBr) \( \tilde{\nu}/cm^{-1}: 3145 (w), 3090 (m), 2925 (m), 1740 (s), 1705 (s), 1645 (s), 1420 (m), 1375 (m), 1245 (m), 1215 (m), 1090 (m), 1060 (m), 1025 (m), 955 (m); \[ ^1H\text{NMR (CDCl}_3\text{)} \delta/\text{ppm: 112.03 (s, O-C-O), 110.01 (s, C-1), 84.92 (d, C-4), 85.85 (d, C-3), 82.00 (d, C-2), 64.02 (t, C-5), 55.59 (q, OCH}_3\text{), 26.50 (q, CCH}_3\text{), 24.81 (q, CCH}_3\text{).} \]

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: \( R_f = 0.3 (\text{CH}_2\text{Cl}_2/\text{MeOH} 20:1); \) m.p. 77–82 °C; \[ ^1H\text{NMR (DMSO-δ6)} \delta/\text{ppm: 7.80 (d, 2H, J = 8.3 Hz, Ph), 7.50 (d, 2H, J = 8.0 Hz, Ph), 4.91 (s, 1H, H-1), 4.62 (d, 1H, J = 5.9 Hz, H-2), 4.50 (d, 1H, J = 5.9 Hz, H-3), 4.20 (t, 1H, J = 7.0 Hz, H-4), 4.05 (d, 2H, J = 7.0 Hz, 2 H-5), 3.10 (s, 3H, OCH}_3\text{), 2.42 (s, 3H, CH}_3-\text{Ph), 1.35 (s, 3H, CCH}_3\text{), 1.21 (s, 3H, CCH}_3\text{); } ^{13}\text{C NMR (DMSO-δ6)} \delta/\text{ppm: 145.67 (s, Ph), 132.52 (s, Ph), 130.71 (d, Ph), 128.16 (d, Ph), 112.20 (s, O-C-O), 109.25 (d, C-1), 84.61 (d, C-4), 83.61(d, C-2), 80.92 (d, C-3), 70.81 (t, C-5), 54.77 (q, OCH}_3\text{), 26.61 (q, CCH}_3\text{), 25.04 (q, CCH}_3\text{), 21.55 (q, Ph-CH}_3\text{).} \]

Croat. Chem. Acta 88 (2015) 43.
cooled to 5 °C 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 Na2SO4 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-dimethylfuro[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 (CH2Cl2/MeOH 60:1), N′-1′-regiosomer 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′-regiosomer 8:  
Rt = 0.51 (CH2Cl2/MeOH 20:1); UV (MeOH) λmax 237 and 288 nm, log ε/dm3 mol−1 cm−1: 4.02 and 4.11; IR(KBr) δmax/cm−1: 3450 (w), 3220 (w), 3090 (w), 2940 (w), 1741 (s), 1665 (s), 1385 (m), 1240 (m), 1215 (m), 1005 (m), 1090 (m); 1H NMR (DMSO-d6) δ/ppm: 11.70 (brs, 1H, H-1′), 3.62 (dd, 1H, J = 6.9 Hz, H-5a), 3.59 (dd, 1H, J = 13.9, H-3), 3.28 (s, 3H, OCH3), 1.37 (s, 3H, CH3), 1.25 (s, 3H, CH3); 13C NMR (DMSO-d6) δ/ppm: 157.71 (d, Jc-c = 26 Hz, C′-4), 150.11 (s, C′-2), 139.85 (d, Jc-c = 230 Hz, C′-5), 130.38 (d, Jc-c = 34 Hz, C′-6), 111.94 (s, O-C=O), 108.43 (d, C-1), 84.72 (d, C-3), 83.45 (d, C-4), 81.32 (d, C-2), 54.99 (q, OCH3), 50.37 (t, C-5), 26.31 (q, CH2), 24.84 (q, CH2). Anal. Caled. mass fractions of elements, w%, for C13H10N2O3F (Mr = 316.28) are: C 49.37, H 5.42, N 8.66; found: C 49.19, H 5.36, N 8.90.

N′-1′,N′-3′-disubstituted nucleoside 9:  
Rt = 0.72 (CH2Cl2/MeOH 20:1); UV (MeOH) λmax 238 and 290 nm, log ε/dm3 mol−1 cm−1: 3.92 and 3.98; IR(KBr) δmax/cm−1: 160.81 (s) 3090 (w), 2940 (w), 1740 (s), 1660 (s), 1465 (m), 1380 (m), 1245 (m), 1215 (m), 1160 (br, s), 870 (s); 1H NMR (DMSO-d6) δ/ppm: 11.70 (brs, 1H, H-1′), 3.62 (dd, 1H, J = 6.9 Hz, H-5a), 3.59 (dd, 1H, J = 13.9, H-3), 3.28 (s, 3H, OCH3), 1.37 (s, 3H, CH3), 1.25 (s, 3H, CH3); 13C NMR (DMSO-d6) δ/ppm: 157.71 (d, Jc-c = 26 Hz, C′-4), 150.11 (s, C′-2), 139.85 (d, Jc-c = 230 Hz, C′-5), 130.38 (d, Jc-c = 34 Hz, C′-6), 111.94 (s, O-C=O), 108.43 (d, C-1), 84.72 (d, C-3), 83.45 (d, C-4), 81.32 (d, C-2), 54.99 (q, OCH3), 50.37 (t, C-5), 26.31 (q, CH2), 24.84 (q, CH2). Anal. Caled. mass fractions of elements, w%, for C13H10N2O3F (Mr = 316.28) are: C 49.37, H 5.42, N 8.66; found: C 49.19, H 5.36, N 8.90.

N′-1′,N′-3′-disubstituted nucleoside 10:  
Rt = 0.37 (CH2Cl2/MeOH 20:1); m.p. 182–183 °C; UV(MeOH) λmax/nm: 215 and 288, log ε/dm3 mol−1 cm−1: 4.13 and 3.91; IR (KBr) δmax/cm−1: 3190 (m), 3100 (m), 3050 (m), 2995 (m), 1715 (s), 1655 (s), 1450 (m), 1435 (m), 1400 (m), 1385 (m), 1360 (m), 1345 (m), 1240 (m), 1200 (m) 965 (m); 1H NMR (DMSO-d6) δ/ppm: 11.70 (brs, 1H, H-1′), 3.61 (s, 1H, H-3), 3.60 (s, 2H, OCH2), 2.62 (dd, 1H, J = 6.7 Hz, H-5a), 2.31 (3H, 1H, H-5a), 2.31 (s, 3H, OCH3), 1.37 (s, 3H, CH3), 1.26 (s, 3H, CH3); 13C NMR (DMSO-d6) δ/ppm: 160.81 (s, C′-4), 150.67 (s, C′-2), 150.11 (d, C′-5), 146.99 (d, C-2), 84.50 (d, C-3), 83.45 (d, C-4), 81.50 (d, C-2), 54.99 (q, OCH3), 50.37 (t, C-5), 26.31 (q, CH2), 24.84 (q, CH2). Anal. Caled. mass fractions of elements, w%, for C13H10N2O3F (Mr = 316.28) are: C 49.37, H 5.42, N 8.66; found: C 49.19, H 5.36, N 8.90.

N′-1′,N′-3′-disubstituted nucleoside 11:  
Rt = 0.62 (CH2Cl2/MeOH 20:1); UV(MeOH) λmax/nm: 217 and 290 (log ε/dm3 mol−1 cm−1: 4.02 and 3.97); IR (KBr) δmax/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); 1H NMR (DMSO-d6) 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, 2.4 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, OCH3), 3.28 (s, 3H, OCH3), 1.37 (s, 3H, OCH3), 1.34 (s, 3H, OCH3), 1.25 (s, 3H, OCH3), 1.22 (s, 3H, OCH3); 13C NMR (DMSO-d6) δ/ppm: 159.97 (s, C-4’), 150.99 (s, C-2’), 149.08 (d, C-6’), 111.62 (s, C-O-C’), 111.46 (s, C-O-C”), 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, OCH3), 54.51 (q, OCH3), 51.92(t, C-5”), 45.55 (t, C-5”), 26.24 (q, CCH3), 26.20 (q, CCH3), 24.72 (q, CCH3). Anal. Calcd. mass fractions of elements, w%, for C22H31N2O10I (Mf = 610.39) are: C 43.29, H 5.12, N 10.19; found: C 43.15, H 5.07, N 4.70.

General Procedure for the Hydrolysis of Isopropyldene 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/mlmol), that was 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-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: Rs = 0.21 (CH2Cl2/Methanol 9:1); UV (MeOH) λmax/nm: 235 and 288, log ε/dm3 mol−1 cm−1: 3.90 and 4.10; IR(KBr) δmax/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), 1H NMR (DMSO-d6) δ/ppm: β-anomer: 7.91 (d, 1H, JF = 6.8 Hz, H-6”), 5.34–5.01 (brs, 1H, OH-2), 4.64 (s, 1H, H-1’), 4.12–3.57 (m, 6H, OH-3, H-2, H-3, H-4, 2H-5), 3.25 (s, 3H, OCH3); 13C NMR (DMSO-d6) δ/ppm: β-anomer: 157.94 (d, 3JCF = 23 Hz, C-4”), 150.11 (s, C-2”), 139.31 (d, JCF = 227 Hz, C-5”), 130.68 (d, 3JCF = 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, OCH3), 50.42 (t, C-5); α-anomer: 13C NMR (DMSO-d6) δ/ppm: 159.74 (d, 3JCF = 23 Hz, C-4’), 150.11 (s, C-2’), 139.31 (d, JCF = 227 Hz, C-5’), 130.68 (d, 3JCF = 34 Hz, C-6’), 108.23 (d, C-1’), 81.00 (d, C-4’), 70.99 (d, C-4’ and C-2”), 70.04 (d, C-3 or C-2’), 54.83 (q, OCH3), 50.42 (t, C-5’). Anal. Calcd. mass fractions of elements, w%, for C19H19N2O5F (Mf = 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: Rs = 0.48 (CH2Cl2/Methanol 9:1); m.p. 99–101 °C; UV(MeOH) λmax/nm: 213 and 287, log ε/dm3 mol−1 cm−1: 4.14 and 3.98; IR (KBr) δmax/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); 1H NMR (DMSO-d6) 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, 3H, CH3O); 13C NMR (DMSO-d6) 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, CH3O), 50.17 (t, C-5’). Anal. Calcd. mass fractions of elements, w%, for C19H19N2O5I (Mf = 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[4-hydroxy-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: Rs = 0.35 (CH3Cl2/Methanol 9:1); UV (MeOH) λmax/nm: 237 and
Methyl 5-deoxy-5-(2,4-diaza-5-ethynylpyrimidin-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: Rf = 0.42 (CH2Cl2/MeOH 9:1); m.p. 189−190 °C; UV (MeOH) λmax/nm: 228 and 290, log ε/cm3 mol−1 cm−1: 3.99 and 4.06; IR(KBr) δmax/cm−1: 3440 (w), 3000 (w), 1730 (s), 1695 (s), 1625 (s), 1575 (s), 1500 (s), 1470 (s), 1385 (s), 1365 (s), 1330 (s), 1250 (w); 1H NMR (DMSO-d6) 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, OCH3), 2.46 (s, 3H, COCH3); 13C NMR (DMSO-d6) δ/ppm: β-anomer: 194.40 (s, COCH3), 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 or C-2'), 73.40 (d, C-3 or C-3'), 72.32 (d, C-3 or C-3'), 54.84 (q, OCH3), 54.39 (q, OCH3), 51.74 (t, C-5), 44.70 (t, C-5'). Anal. Calcd. mass fractions of elements, w/%, for C16H23N2O10F (Mr = 394.50) are: C 48.00, H 5.37, 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/H2O (20 mL). After evaporation of volatiles, the crude residue was purified by flash chromatography (CH2Cl2/MeOH 9:1). The spectral properties were identical with a sample synthesized by method A.

Methyl 5-deoxy-5-(2,4-diaza-5-ethynylpyrimidin-1H-1-yl)-β-D-ribofuranoside (18)

A solution of (trimethylsilyl)ethyl 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+).
change until moistened pH paper indicated pH = 6. The mixture was filtered and the resin was washed with methanol. The combined filtrate was evaporated to afford 72 mg (88 %) of 18 as white crystals: \( R_t = 0.43 \) (diethyl ether); m.p. 211–212 °C; UV (MeOH) \( \lambda_{\text{max}} / \text{nm}: 229 \) and 290, log ε/ dm³ mol⁻¹ cm⁻¹: 3.80 and 3.87; IR(KBr) \( \bar{\nu}_{\text{max}} / \text{cm}^{-1}: 3270 \) (m), 3200 (w), 2100 (vw), 1710 (s), 1680 (s), 1630 (s), 1460 (s), 1435 (m), 1405 (w), 1385 (m), 1375 (m), 1240 (m), 1205 (m) cm⁻¹; ¹H NMR (CDCl₃) \( \delta / \text{ppm}: 8.75 \) (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, H-5b), 4.22 (dd, 1H, H-5a), 3.44 (dd, 1H, J₅a,₄ = 5.1 Hz, J₅b,₄ = 14.1 Hz, H-5a), 3.49 (dd, 1H, J₅b,a = 14.1 Hz, H-5b), 3.44 (s, 3H, CH₃O), 3.17 (s, 3H, CH₃C); ¹³C NMR (CDCl₃) \( \delta / \text{ppm}: 148.85 \) (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=CH₂), 56.20 (q, CH₃O), 51.85 (t, C-5), 26.38 (q, CH₃C), 24.87 (q, CH₃C). Anal. calcd. mass fractions of elements, w/%, for C₅H₁₄N₂O₅ (Mₑ = 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**

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 % CO₂ 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 10⁻¹ 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 2×10⁴ 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).²⁵

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 N3′-regioisomer in the reaction of uracil derivatives 4–6 with 3 and, exclusively the corresponding N1′-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 ¹H 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 (J₅b,F = 6.9 Hz) and the additional long-range H5'-NH3' coupling (J₅b,F,N₃′ = 2.1 Hz), the latter excluding the ¹H NMR spectra of the NH proton signals appear at δ 3.98; 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 ¹H NMR spectra of both, 8 and 9 the signal of H-6′ vinyl proton is split into a doublet (8: δ = 8.08 ppm, J₆,F = 6.9 Hz; 9: δ = 8.19 ppm, J₆,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-idouracil 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
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 1H 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 1H 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. Treatment of 10 with ethynyltrimethylsilane and (PPh₃)PdCl₂ in the presence of Cul 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 1. (a) 1. HCl/MeOH, 2. 2,2-dimethoxypropane, acetone; (b) TsCl/Py; (c) NaH/DMF; (d) H₂, 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 1H NMR spectra (inset).
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. 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).

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

Acknowledgements. This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia through Grant No. 098-0982914-2935.

REFERENCES

1. E. De Clercq, Annu. Rev. Pharmacol. Toxicol. 51 (2011) 1–24.
2. C. D. Meadows and J. Gervay-Hague, Chem. Med. Chem. 1 (2006) 16–29.
3. C. Mathé and G. L Gosselin, Antiviral Res. 71 (2006) 276–281.
4. D. Komiotis, S. Manta, E. Tsoukala, and N. Tzioumaki, Curr. Med. Chem.: Anti-Infect. Agents 7 (2008) 219–244.
5. C. M. Galmarini, J. R. Mackey, and C. Dumontet, Lancet Oncol. 3 (2002) 415–424.
6. D. Sampath, V. A. Rao, and W. Plunkett, Oncogene 22 (2003) 9063–9074.
7. P. Heredewijn (Ed.), Modified Nucleosides: in Biochemistry, Biotechnology and Medicine, John Wiley & Sons, 2008.
8. P. Merino (Ed.), Chemical Synthesis of Nucleoside Analogues, John Wiley & Sons, 2013.
9. J. W. Beach, L. S. Jeong, A. J. Alves, D. Pohl, H. O. Kim, C.-N. Chang, S.-L. Doong, R. F. Schinazi, Y.-C. Cheng, and C. K. Chu, J. Org. Chem. 57 (1992) 2217–2219.
10. V. Nair, M. St. Clair, J. E. Reardon, H. C. Krasny, R. J. Hazen, M. T. Paff, L. R. Boone, M. Tisdale, I. Najera, R. E. Dornsife, D. R. Everett, K. Borroto-Esoda, J. L. Yale, T. P. Zimmerman, and J. L. Rideout, Antimicrob. Agents Chemother. 39 (1995) 1993–1999.
11. I. Verheggen, A. Van Aerschot, L. Van Meervelt, J. Rozen- 
ski, L. Wiebe, R. Snoeck, G. Andrei, J. Balzarini, P. Claes, 
E. De Clercq, and P. Herdewijn, J. Med. Chem. 38 (1995) 
826–835.
12. J.-F. Wang, X.-D. Yang, L.-R. Zhang, Z.-J. Yang, and L.-H. 
Zhang, Tetrahedron 60 (2004) 8535–8546.
13. T. Bouisset, G. Gosselin, L. Griffe, J.-C. Meillon, and R. Storer, 
Tetrahedron 64 (2008) 6657–6661.
14. M. Kawazu, T. Kanno, S. Yamamura, T. Mizaguchi, and S. Sai-
to, J. Org. Chem. 38 (1973) 2887–2890.
15. A. Holý, Collect. Czech. Chem. Commun. 40 (1975) 187–214.
16. S. N. Mikhailov, L. I. Kolobushkina, A. M. Kritzyn, and V. L. 
Florentiev, Tetrahedron 32 (1976) 2409–2415.
17. A. Holý, Collect. Czech. Chem. Commun. 49 (1984) 2148–2166.
18. V. Škarić and B. Kašnar, Croat. Chem. Acta 58 (1985) 583–592.
19. B. Kašnar, V. Škarić, B. Klaić, and M. Žinić, Tetrahedron Lett. 
34 (1993) 4997–5000.
20. N. F. Zakirova, A. V. Shipitsyn, E. F. Belanov, and M. V. Jasko, 
Bioorg. Med. Chem. Lett. 14 (2004) 3357–3360.
21. B. Kašnar, Nucleosides & Nucleotides 14 (1995) 341–344.
22. Unpublished results in: N. Župančić, PhD Thesis, 2014.
23. N. J. Leonard and K. L. Carraway, J. Heterocyclic Chem. 3 
(1996) 485–489.
24. Adel A.-H. Abdel-Rahman, Ahmed E.-S. Abdel-Megied, Adel 
E.-S. Goda, Ibrahim F. Zeid, and El Sayed H. El Ashry, Nucleo-
sides Nucleotides & Nucleic Acids 22 (2003) 2027–2038.
25. T. B. Johnson and C. O. Johns, J. Biol. Chem. 1 (1906) 305–318.
26. J.-I. Asakura and M. J. Robins, J. Org. Chem. 55 (1990) 
4928–4933.
27. Z. Janeba, J. Balzarini, G. Andrei, R. Snoeck, E. De Clercq, and 
M. J. Robins, Can. J. Chem. 84 (2006) 580–586.
28. N. Horiuchi, K. Nagawa, Y. Sasaky, K. Minato, Y. Fujiwara, K. 
Nezu, Y. Ohe, and N. Sajo, Cancer Chemother. Pharmacol. 22 
(1988) 246–250.
29. G. Mickisch, S. Fajta, H. Bier, R. Tschada, and P. Alken, Urol. 
Res. 19 (1991) 99–103.
30. A. Holý, Collect. Czech. Chem. Commun. 40 (1975) 187–214.
31. J. L. Yamashita, I. Yamawaki, S. Ueda, M. Yasumoto, N. Un-
emi, and S. Hashimoto, Chem. Pharm. Bull. 30 (1982) 
4258–4267.
32. K. Sonogashira, Y. Tohda, and N. Hagihara, Tetrahedron Lett. 
16 (1975) 4467–4470.
33. P. J. Barr, P. Chananont, T. A. Hamor, A. S. Jones, M. K. 
O’leary, and R. T. Walker, Tetrahedron 36 (1980) 1269–1273.
34. F. Amblard, V. Aucagne, P. Guenot, R. F. Schinazi, and L. A. 
Agrofoglio, Bioorg. Med. Chem. 13 (2005) 1239–1248.