

Synthesis and biological properties of 2-oxabicyclo[4.1.0]heptane nucleosides containing uracil, and thymine

Antonino Corsaro,^{*a} Ugo Chiacchio,^a Venerando Pistarà,^a Luisa Borrello,^a Giovanni Romeo,^b and Renato Dalpozzo^c

^aDipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, Catania 95125, Italy, ^bDipartimento Farmaco-Chimico, Università di Messina, Via SS. Annunziata, Messina 98168, Italy, ^cDipartimento di Chimica, Università della Calabria, Via P. Bucci, Arcavacata di Rende (CS) 87036, Italy

E-mail: acorsaro@unict.it

Dedicated to Professor Giuseppe Bartoli on his 65th birthday

Abstract

α - and β -2-Oxabicyclo[4.1.0]heptane nucleosides **8-13** containing uracil and thymine have been synthesized starting from *L-threo*-hex-4-enopyranosides **5**. The nucleosidation reaction performed with the *O*-acetyl derivative **7** affords only the β -anomer **8** while the nucleosidation of the *O*-methyl derivative **7** leads to the formation of α - and β -anomers. Antiviral, cytotoxicity and apoptotic activity have been investigated: no significant activity has been observed.

Keywords: Anti-HIV drugs, cyclopropanes, carbohydrates, nucleosides

Introduction

There has recently been a renewed interest in the synthesis of nucleosides with a six-membered carbohydrate moiety (Figure 1), due to their potential antiviral¹⁻⁴ and antibiotic⁵ activities and as building blocks in nucleic acid synthesis.⁶ Antiviral properties have been found in 1,5-anhydrohexitols **1** which exhibit a marked and selective activity against HSV-1, HSV-2, VZV, and CMV at relatively low concentrations. This activity is, probably, linked to a conformational flexibility comparable to that of natural nucleosides.²

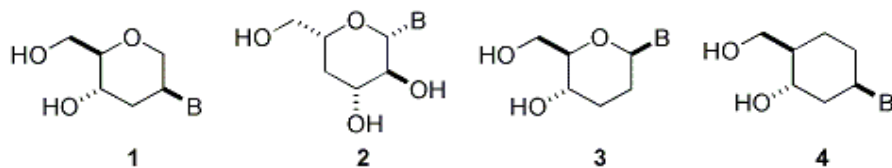


Figure 1. Structures of some anhydrohexitols (B = nucleobase)

The biological activity of nucleoside analogues is often modulated by the nature of the spacer (the furanose ring in natural compounds) which exerts a profound effect on the conformation and puckering of the molecule in natural nucleosides and which is able to control the outcome of the interactions between these compounds and the cellular kinases.

The correlation between the conformational preference required by the enzyme and the particular sugar conformation proves troublesome due to the flexibility of the furanose ring. Such flexibility is responsible for significant differences between the conformation in solid state and in solution.⁷ For this reason, conformationally restricted nucleoside analogues were synthesized with the aim to reduce their flexibility, so improving the interaction between substrates and enzymes. For instance, conformationally locked oxa- or carbocyclic nucleosides, built on a bicyclo[3.1.0]hexanes template, have demonstrated conformational preferences for a number of enzymes.⁸ Furthermore, the biological effects of modified nucleosides depend importantly from the relative distance and arrangement of the hydroxymethyl group and the base moiety.

In this context, we have devised the synthesis of a series of new nucleosides featuring the presence of a pyranose ring fused with a cyclopropane system, in order to construct a spacer unit which could control the conformational mobility while maintaining the base unit and the hydroxymethyl group in a disposition suitable to interact with the enzymes. In fact AM1 calculations performed on the synthesized 2-oxabicyclo[4.1.0]heptane nucleoside **13b** and on the 1,5- β -pyranose nucleoside **2** (Figure 2) chosen as references, indicate that the introduction of the cyclopropane ring at C4 and C5, into **13b** with respect to **2**, induces a puckering of the system which leads to a reduced conformational freedom and a lower distance between the base moiety and the hydroxymethyl group.

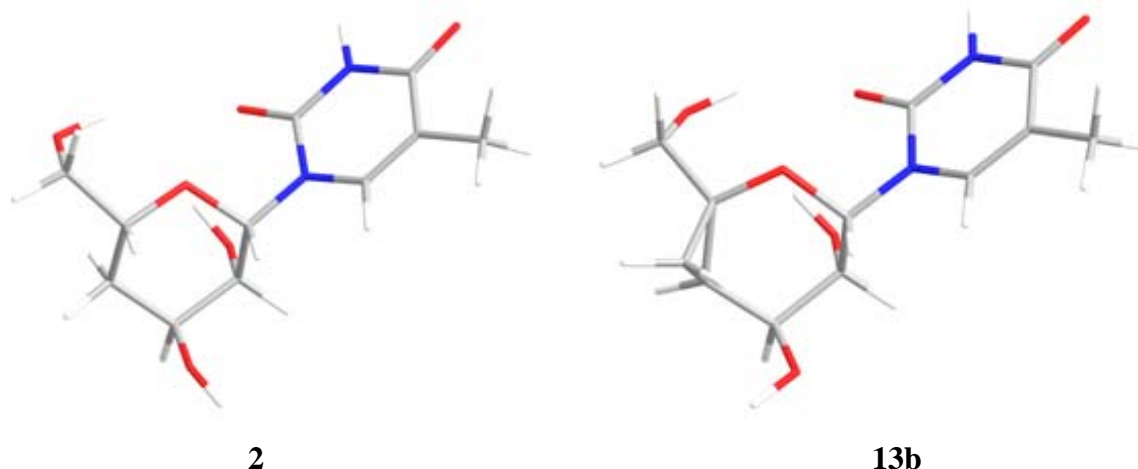
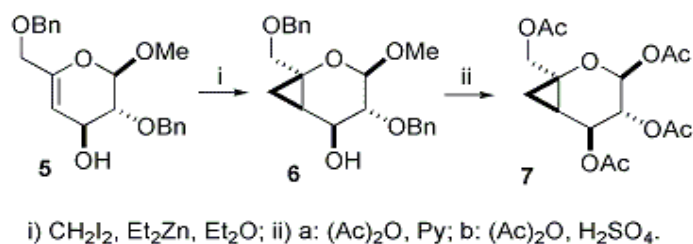


Figure 2. AM1 optimized structures for compounds **2** and **13b**

According to these considerations, and following our interest in the chemical valorization of lactose,⁹ a natural disaccharide easily and cheaply available in large amounts as a waste product of the cheese industry, we report here the synthesis and the biological properties of the 2-oxabicyclo[4.1.0]heptane nucleosides **8-13**, not yet reported in the literature.

Results and Discussion

Recently we have described the cyclopropanation reactions¹⁰ of *L-threo*-hex-4-enopyranosides **5**, according to a Furukawa- modified^{11a} Simmons-Smith procedure,^{11b} as a valuable tool to obtain 2-oxabicyclo[4.1.0]heptane derivatives **6**, with a nearly quantitative yield (Scheme 1). These derivatives have then been utilized as the starting material for the preparation of nucleosides **8-13**.

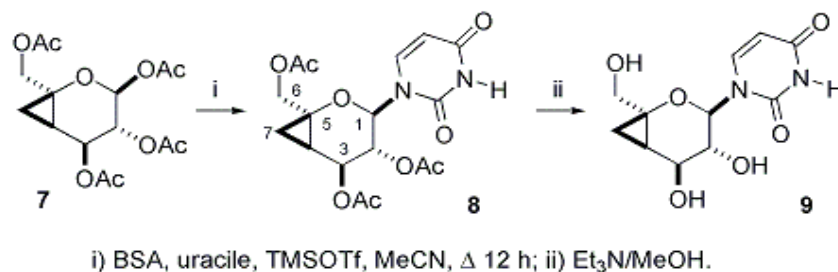


Scheme 1

The cyclopropane derivative **6** was converted in the more reactive peracetylated compound **7** (Scheme 1) and used for the subsequent nucleosidation reaction with silylated uracil, under Vorbrüggen conditions,¹² with TMSOTf in dry acetonitrile (Scheme 2). The reaction afforded

exclusively the nucleoside **8** with good yield (80% isolated) and high stereoselectivity. The stereochemistry of **8** was assessed on the basis of mono- and two-dimensional ^1H -NMR experiments, and NOE effects. In particular, the diagnostic NOE enhancement observed for H-1 (4.4%) when irradiating H-3 proton and vice versa, clearly indicates a *trans* relationship between the nucleobase and the substituent at C-6. Moreover, the H-1 proton resonates as a doublet (5.88 ppm) with a coupling constant $J_{1,2} = 10.0$ Hz, typical of a *trans*_{ax/ax} disposition between H-1 and H-2 protons.

The deprotection of **8** with Et_3N in methanol afforded the modified nucleoside **9** with a good yield (85%) (Scheme 2).



Scheme 2

The exclusive production of **8** is explainable in terms of the reversible formation of the σ -complex **A** which undergoes the attack of the persilylated base on the same side of the cyclopropane ring (Figure 3).

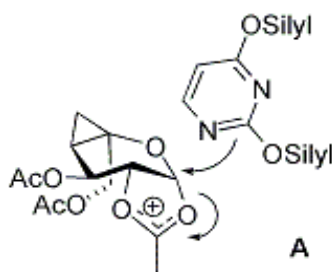
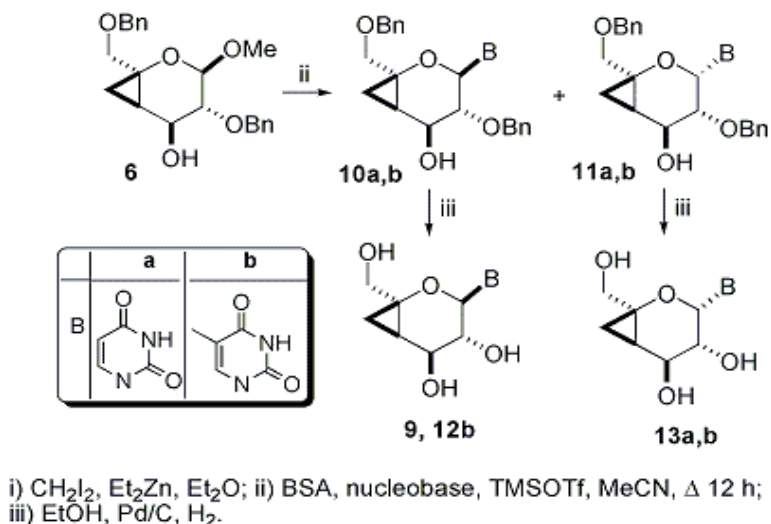


Figure 3. Proposed σ -complex between the persilylated uracil and the cyclopropanated sugar **7**.

With the aim to obtain the modified nucleoside featuring a *cis*-disposition between the base and the hydroxymethyl group at C-6, and in order to compare the biological activities of *cis*- and *trans*-derivatives, we have performed the nucleosidation reaction of the more hindered cyclopropanated dibenzyl-methyl ether **6**¹⁰ with silylated uracil and thymine (Scheme 3). A mixture of the two α - and β -anomers **10a,b** and **11a,b** in a 3:1 ratio (40% total yield) was obtained: the pure compounds were isolated by flash and HPLC chromatography.



Scheme 3

The stereochemistry was confirmed by mono- and two-dimensional ^1H -NMR experiments, and NOE measurements. In the case of the α -nucleosides (**10a,b**), the anomeric proton appears as a doublet (5.66 ppm) with a coupling constant $J_{1,2} = 9.5$ Hz, typical of a $\text{trans}_{\text{ax/ax}}$ disposition between H-1 and H-2 protons; on the contrary in β -nucleosides (**11a,b**), H-1 appears as a doublet (5.93 ppm) with a small coupling constant $J_{\text{ax/eq}}$ (2.5 Hz). Moreover, for **10a,b** diagnostic NOE enhancements were observed for H-1 when irradiating H-3 (2.5%). No NOE effect was observed in β -anomers **11a,b** between H-1 and H-3 protons (Figure 4).

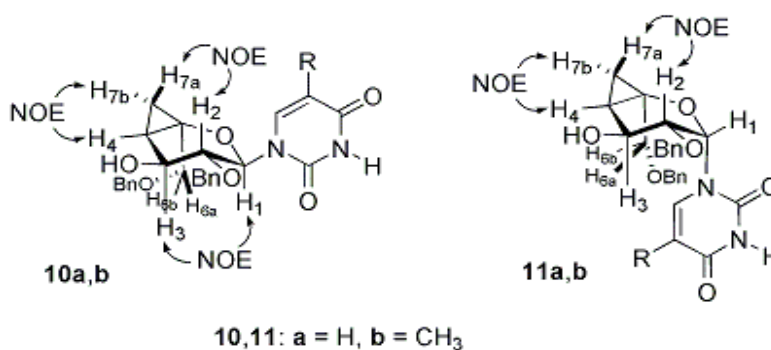


Figure 4. NOE enhancements for **10a,b** and **11a,b**.

The fully deprotected **12a,b** and **13a,b** nucleosides were then obtained in a nearly quantitative yields by catalytic debenzoylation reaction (Scheme 3).

Biological assays

The synthesized compounds were tested for their antiviral activity against HSV-1, HSV-2 in Vero (African Green Monkey) cells, HTLV-1 in human cells (lymphomonocytes of peripheral blood) and for their toxicity in vitro. None of these derivatives reached the inhibitory concentration 50 at the highest concentrations tested (e.g. 320 μM), indicating a lack of significant antiviral activity.

The apoptotic activity was also tested in Molt-3 cells. Only **13b** showed a moderate activity (32%) at the concentration 500 μM after 48 h. No significant toxicity, when evaluated using the classical trypan blue test, was detected until 3 days of treatment in lymphoid Molt-3 cell line assayed.

Conclusions

The synthesis of α - and β -2-oxabicyclo[4.1.0]heptane nucleosides **8-13** containing the uracil and thymine was achieved starting from *L-threo*-hex-4-enopyranosides **5**. The nucleosidation of *O*-acetyl derivative **7** affords only β -anomer **8** while the nucleosidation of *O*-methyl derivative **7** leads to the formation of α - and β -anomers.

Only **13b** exhibits a moderate apoptotic activity (28%) at the concentration 500 μM after 48 h.

Experimental Section

General Procedures. Melting points were determined with a Kofler apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer elemental analyzer. NMR spectra were recorded on a Varian instrument at 200 or 500 MHz (^1H) and at 50 or 125 MHz (^{13}C) using CDCl_3 , CD_3OD or CD_3CN as solvent; chemical shifts are given in ppm from TMS as internal standard. Thin-layer chromatographic separations were performed on Merck silica gel 60-F₂₅₄ precoated aluminium plates. Preparative separations were by column- and flash chromatography using Merck silica gel 0.063-0.200 mm and 0.035-0.070 mm, respectively, with chloroform-methanol mixtures as eluents. HPLC purifications were made with a preparative column (Microsorb Dynamax 100Å, 21.4 x 250 mm).

Starting materials. Thymine, uracil and all the reagents were purchased from Aldrich Co. All solvents were dried according to literature methods.

(1R,3R,4R,5S,6R)-1-(Acetoxymethyl)-2-oxa-bicyclo[4.1.0]heptane-3,4,5-triyl triacetate (7). To a solution of compound **6**¹⁰ (750 mg, 2 mmol) in pyridine (9.5 ml), was added acetic anhydride (1.9 ml, 19.8 mmol) under stirring at room temperature. After the disappearance of the starting product (3 h), the reaction mixture was evaporated under reduced pressure and co-evaporated with toluene; then was dissolved in acetic acid (9.5 ml), acetic anhydride (1.9 ml,

20.8 mmol) was added and, dropwise at 0 °C, sulfuric acid (0,039 ml). The reaction mixture was stirred at room temperature for 16 h, then diluted with ice and extracted (2 x 10 ml) with CH₂Cl₂. The organic phases were washed with a solution of NaHCO₃ and dried over Na₂SO₄, filtered and evaporated under vacuum. The residue, purified by flash chromatography, affords the exclusive α -anomer **7** (75% yield) as a yellow syrup: ¹H NMR (CDCl₃, 500 MHz) δ 0.98 (dd, 1H, J = 6.8, 10.0 Hz, H-7a), 1.06 (dd, 1H, J = 6.8, 7.0 Hz, H-7b), 1.93 (ddd, 1H, J = 6.8, 7.0, 10.0 Hz, H-4), 2.04, 2.09, 2.16, and 2.17 (s, each 3H, 4 x CH₃), 4.05-4.13 (AB system, 2H, J_{AB} = 12.3 Hz, H-6a, H-6b), 4.84 (dd, 1H, J = 2.8, 9.0 Hz, H-2), 5.48 (dd, 1H, J = 7.0, 9.0 Hz, H-3), 6.03 (d, 1H, J = 2.8 Hz, H-1); ¹³C NMR (CDCl₃, 50 MHz) δ 15.68 (C-7), 20.32 (C-4), 20.64, 20.86, 21.06, and 21.12 (4 x CH₃), 60.22 (C-5), 66.83 (C-3), 68.04 (C-6), 68.92 (C-2), 89.67 (C-1), 169.35, 170.03, 170.68, 170.87 (C=O); Anal. Calc. for C₁₅H₂₀O₉: C, 52.32; H, 5.85%. Found: C, 52.37; H, 5.82.

General procedure for the reaction between silylated bases (uracil and thymine) with cyclopropanated sugar **6 and **7**.** A suspension of base (0.62 mmol) in dry acetonitrile (3 mL) was treated with 2.48 mmol of bis(trimethylsilyl)acetamide (BSA) and refluxed for 15 min under stirring. To the clear solution obtained was added at room temperature a solution of the cyclopropanated sugar (0.52 mmol) in dry acetonitrile (3 mL), 0.78 mmol of trimethylsilyl triflate (TMSOTf) dropwise, and the reaction mixture was refluxed for 12 h. After being cooled at 0 °C, the solution was neutralized by careful addition of aqueous 5% sodium bicarbonate and then concentrated under vacuum. After addition of dichloromethane (8 mL) the organic phase was separated, washed with water (2x10 mL), dried over sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash chromatography to give the cyclopropanated nucleoside (**9**), and for the anomeric mixture of compounds **10a,b** and **11a,b**, by HPLC (2-propanol/*n*-hexane).

(1R,3R,4R,5S,6R)-1-(Acetoxymethyl)-3-[2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl]-2-oxa-bicyclo[4.1.0]heptane-4,5-diyl diacetate (8**).** Amorphous solid, yield 80%, $[\alpha]_D^{25} +52.63^\circ$ (c 0.1, CHCl₃). ¹H NMR (CDCl₃, 500 MHz) δ 1.03 (dd, 1H, J = 7.0, 10.5 Hz, H-7a), 1.16 (dd, 1H, J = 7.0, 7.5 Hz, H-7b), 1.93 (ddd, 1H, J = 7.5, 8.0, 10.5 Hz, H-4), 2.04, 2.09, and 2.15 (s, each 3H, 3 x CH₃), 3.78-4.51 (AB system, 2H, J_{AB} = 12.3 Hz, H-6a, H-6b), 4.95 (dd, 1H, J = 8.0, 10.0 Hz, H-2), 5.56 (dd, 1H, J = 7.5, 8.0 Hz, H-3), 5.72 (dd, 1H, J = 8.3, 2.1 Hz, H-5') 5.89 (d, 1H, J = 10.0 Hz, H-1), 7.22 (d, 1H, J = 8.3 Hz, H-6'), 8.54 (bs, 1H, NH); ¹³C NMR (CDCl₃, 50 MHz) δ 14.18 (C-7), 16.09 (C-4), 20.77, 20.89, and 21.16 (3 x CH₃), 60.39 (C-5), 67.33 (C-6), 69.38 (C-3), 71.47 (C-2), 79.46 (C-1), 103.07 (C-5'), 139.31 (C-6'), 150.10 (C-2'), 162.24 (C-4'), 169.78, 170.86, and 171.05 (C=O); Anal. Calc. for C₁₇H₂₀N₂O₉: C, 51.52; H, 5.09%. Found: C, 51.59; H, 5.11.

1-{(1R,3R,4R,5S,6R)-4-(Benzyloxy)-1-(benzyloxymethyl)-5-hydroxy-2-oxa-bicyclo[4.1.0]-heptan-3-yl}pyrimidine-2,4(1H,3H)-dione (10a**).** Amorphous solid, yield 33%, $[\alpha]_D^{25} +123.90^\circ$ (c 0.1, CHCl₃). ¹H NMR (CDCl₃, 500 MHz) δ 0.95 (dd, 1H, J = 6.0, 10.5 Hz, H-7a), 1.03 (dd, 1H, J = 6.0, 7.0 Hz, H-7b), 1.59 (ddd, 1H, J = 6.0, 7.0, 10.5 Hz, H-4), 3.36 (dd, 1H, J = 7.0, 9.5

Hz, H-2), 3.42-3.69 (AB system, 2H, $J_{AB} = 10.5$ Hz, H-6a, H-6b), 4.38 (dd, 1H, $J = 6.5, 7.0$ Hz, H-3), 4.52-4.81 (2 x AB system, each 2H, $J_{AB} = 12.0$ Hz, OCH_2Ph), 5.43 (d, 1H, $J = 8.0$ Hz, H-5'), 5.72 (d, 1H, $J = 9.5$ Hz, H-1), 6.89 (d, 1H, $J = 8.0$ Hz, H-6'), 7.13-7.38 (m, 10H, aromatic H), 8.19 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.25 (C-7), 19.97 (C-4), 63.62 (C-5), 70.33 (C-6), 73.10 and 73.29 (CH_2), 77.73 (C-3), 78.23 (C-2), 80.79 (C-1), 102.34 (C-5'), 127.67, 127.88, 128.03, 128.20, 128.39 and 128.51 (aromatic CH), 137.18 and 137.73 (aromatic C), 139.20 (C-6'), 150.12 and 162.48 (C=O); Anal. Calc. for $C_{25}H_{26}N_2O_6$: C, 66.65; H, 5.82%. Found: C, 66.71; H, 5.85.

1-((1R,3S,4R,5S,6R)-4-(Benzyloxy)-1-(benzyloxymethyl)-5-hydroxy-2-oxa-bicyclo[4.1.0]-heptan-3-yl)pyrimidine-2,4(1H,3H)-dione (11a). Amorphous solid, yield 15%, $[\alpha]_D^{25} -83.55^\circ$ (c 0.5, $CHCl_3$). 1H NMR ($CDCl_3$, 500 MHz) δ 0.89 (dd, 1H, $J = 6.5, 10.5$ Hz, H-7a), 1.05 (dd, 1H, $J = 6.5, 7.0$ Hz, H-7b), 1.61 (ddd, 1H, $J = 6.5, 7.0, 10.5$ Hz, H-4), 3.31 (dd, 1H, $J = 2.5, 7.0$ Hz, H-2), 3.38-3.55 (AB system, 2H, $J_{AB} = 11.5$ Hz, H-6a, H-6b), 4.49 (dd, 1H, $J = 7.0, 7.5$ Hz, H-3), 4.52-4.81 (2 x AB system, each 2H, $J_{AB} = 12.0$ Hz, OCH_2Ph), 5.66 (d, 1H, $J = 8.0$ Hz, H-5'), 5.85 (d, 1H, $J = 2.5$ Hz, H-1), 7.09 (d, 1H, $J = 8.0$ Hz, H-6'), 7.21-7.38 (m, 10H, aromatic H), 8.25 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 16.25 (C-7), 21.27 (C-4), 62.62 (C-5), 67.73 (C-3), 70.29 (C-6), 73.25 and 73.43 (CH_2), 79.32 (C-2), 83.56 (C-1), 103.34 (C-5'), 126.88, 127.08, 127.93, 128.02, 128.30 and 128.41 (aromatic CH), 137.68 and 137.93 (aromatic C), 138.27 (C-6'), 160.42 and 161.39 (C=O); Anal. Calc. for $C_{25}H_{26}N_2O_6$: C, 66.65; H, 5.82%. Found: C, 66.71; H, 5.85.

1-((1R,3R,4R,5S,6R)-4-(Benzyloxy)-1-(benzyloxymethyl)-5-hydroxy-2-oxa-bicyclo[4.1.0]-heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (10b). Amorphous solid, yield 30%, $[\alpha]_D^{25} +26.82^\circ$ (c 0.7, $CHCl_3$). 1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (dd, 1H, $J = 6.5, 10.0$ Hz, H-7a), 1.01 (dd, 1H, $J = 6.5, 7.0$ Hz, H-7b), 1.60 (ddd, 1H, $J = 6.5, 7.0, 10.0$ Hz, H-4), 1.77 (d, $J = 1.0$ Hz, CH_3), 3.28 (dd, 1H, $J = 7.5, 9.5$ Hz, H-2), 3.34-3.65 (AB system, 2H, $J_{AB} = 10.5$ Hz, H-6a, H-6b), 4.55 (dd, 1H, $J = 7.5, 8.0$ Hz, H-3), 4.52-4.58 (AB system, 2H, $J_{AB} = 12.0$ Hz, C6- OCH_2Ph), 4.56-4.71 (AB system, 2H, $J_{AB} = 12.0$ Hz, C2- OCH_2Ph), 5.66 (d, 1H, $J = 9.5$ Hz, H-1), 6.75 (d, 1H, $J = 1.0$ Hz, H-6'), 7.17-7.33 (m, 10H, aromatic H), 8.64 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.50 (CH_3), 15.15 (C-7), 23.14 (C-4), 62.80 (C-5), 71.67 (C-3), 73.13, 73.18 and 73.55 (CH_2), 79.08 (C-2), 80.95 (C-1), 110.77 (C-5'), 127.60, 127.67, 128.10, 128.35 and 128.45 (aromatic CH), 135.27 (C-6') 137.25, 137.82 (aromatic C), 150.41 (C-2'), 163.35 (C-4'); Anal. Calc. for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08%. Found: C, 67.33; H, 6.12.

1-((1R,3S,4R,5S,6R)-4-(Benzyloxy)-1-(benzyloxymethyl)-5-hydroxy-2-oxa-bicyclo[4.1.0]-heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (11b). Amorphous solid, yield 10%, $[\alpha]_D^{25} -59.09^\circ$ (c 0.2, $CHCl_3$). 1H NMR ($CDCl_3$, 500 MHz) δ 0.77 (dd, 1H, $J = 6.5, 10.0$ Hz, H-7a), 1.23 (dd, 1H, $J = 6.5, 7.0$ Hz, H-7b), 1.53 (ddd, 1H, $J = 6.5, 7.0, 10.0$ Hz, H-4), 1.73 (d, $J = 1.5$ Hz, CH_3), 2.45 (bs, 1H, OH), 3.41 (dd, 1H, $J = 2.5, 4.5$ Hz, H-2), 3.50-3.81 (AB system, 2H, $J_{AB} = 11.0$ Hz, H-6a, H-6b), 4.46-4.60 (AB system, 2H, $J_{AB} = 11.5$ Hz, C3- OCH_2Ph), 4.51 (dd, 1H, $J = 4.5, 7.0$ Hz, H-3), 4.56-4.63 (AB system, 2H, $J_{AB} = 12.0$ Hz, C6- OCH_2Ph), 5.93 (d, 1H, $J = 2.5$ Hz, H-1), 7.19-7.63 (m, 10H, aromatic H), 7.63 (d, 1H, $J = 1.5$ Hz, H-6'), 8.46 (bs, 1H, NH); ^{13}C

NMR (CDCl₃, 50 MHz) δ 9.00 (C-7), 12.25 (CH₃), 17.59 (C-4), 62.84 (C-5), 63.04 (C-3), 72.95 and 73.01 (CH₂), 73.74 (C-2), 74.23 (CH₂), 76.78 (C-1), 109.25 (C-5'), 127.60, 127.77, 128.20, 128.35 and 128.45 (aromatic CH), 136.80 and 137.90 (aromatic C), 138.26 (C-6'), 150.12 (C-2'), 163.52 (C-4'); Anal. Calc. for C₂₆H₂₈N₂O₆: C, 67.23; H, 6.08%. Found: C, 67.36; H, 6.04.

1-[(1R,3R,4R,5S,6R)-4,5-Dihydroxy-1-(hydroxymethyl)-2-oxa-bicyclo[4.1.0]heptan-3-yl]pyrimidine-2,4(1H,3H)-dione (9). A solution of **8** (210 mg, 0.53 mmol) in methanolic ammonia (12 ml) was stirred at room overnight, then concentrated under reduce pressure and purified by flash chromatography to give **5** as a yellow syrup (78% yield): ¹H NMR (CD₃CN, 500 MHz) δ 0.77 (dd, 1H, *J* = 6.0, 10.5 Hz, H-7a), 0.91 (dd, 1H, *J* = 6.5, 7.0 Hz, H-7b), 1.52 (ddd, 1H, *J* = 6.5, 7.0, 10.5 Hz, H-4), 3.28-3.70 (AB system, 2H, *J*_{AB} = 12.0 Hz, H-6a, H-6b), 3.38 (dd, 1H, *J* = 8.0, 10.0 Hz, H-2), 4.17 (dd, 1H, *J* = 7.0, 8.0 Hz, H-3), 5.44 (d, 1H, *J* = 10.0 Hz, H-1), 5.63 (d, 1H, *J* = 8.2 Hz, H-5'), 7.41 (d, 1H, *J* = 9.2 Hz, H-6'); ¹³C NMR (CD₃CN, 50 MHz) δ 15.26 (C-4), 24.00 (C-7), 66.90 (C-5), 72.18 (C-6), 72.24 (C-3), 72.73 (C-2), 83.02 (C-1), 103.06 (C-5'), 141.72 (C-6'), 158.43 (C-2'), 161.22 (C-4'); Anal. Calc. for C₁₁H₁₄N₂O₆: C, 58.89; H, 5.22%. Found: C, 58.83; H, 5.19.

General procedure for the debenylation of cyclopropanated nucleosides 10a,b and 11a,b.

To a solution of cyclopropanated nucleosides **10a,b** and **11a,b** (0.90 mmol) in ethanol (3 mL) was added a spatula tip of Pd/C and the suspension was stirred under H₂ atmosphere for 12 h. After this time the suspension was filtered and the filtrate was evaporated at reduced pressure to give, with nearly quantitative yields, the corresponding deprotected derivatives **9**, **12b** and **13a,b**.

1-[(1R,3R,4R,5S,6R)-4,5-Dihydroxy-1-(hydroxymethyl)-2-oxa-bicyclo[4.1.0]heptan-3-yl]-5-methylpyrimidine-2,4(1H,3H)-dione (12b). Amorphous solid, yield 90%, [α]_D²⁵ +69.22 (c 0.3, MeOH). ¹H NMR (CD₃OD, 500 MHz) δ 0.84 (dd, 1H, *J* = 6.0, 10.0 Hz, H-7a), 1.02 (t, 1H, *J* = 7.0 Hz, H-7b), 1.58 (ddd, 1H, *J* = 6.5, 7.0, 10.0 Hz, H-4), 1.87 (d, *J* = 1.0 Hz, CH₃), 3.39-3.75 (AB system, 2H, *J*_{AB} = 12.5 Hz, H-6a, H-6b), 3.47 (dd, 1H, *J* = 8.0, 9.5 Hz, H-2), 4.21 (dd, 1H, *J* = 7.0, 8.0 Hz, H-3), 5.52 (d, 1H, *J* = 9.5 Hz, H-1), 7.41 (d, 1H, *J* = 1.0 Hz, H-6'); ¹³C NMR (CD₃OD, 50 MHz) δ 12.37 (CH₃), 15.47 (C-7), 23.60 (C-4), 66.74 (C-6), 53.15 (C-5), 71.72 (C-3), 76.78 (C-2), 83.08 (C-1), 112.85 (C-5'), 135.11 (C-6'), 157.02 (C-2'), 162.21 (C-4'); Anal. Calc. for C₁₂H₁₆N₂O₆: C, 50.70; H, 5.67%. Found: C, 50.77; H, 5.62.

1-[(1R,3S,4R,5S,6R)-4,5-Dihydroxy-1-(hydroxymethyl)-2-oxa-bicyclo[4.1.0]heptan-3-yl]pyrimidine-2,4(1H,3H)-dione (13a). Amorphous solid, yield 89%, [α]_D²⁵ -38.75 (c 0.08, MeOH). ¹H NMR (CD₃OD, 500 MHz) δ 0.80 (dd, 1H, *J* = 6.0, 10.0 Hz, H-7a), 1.02 (dd, 1H, *J* = 6.0, 7.0 Hz, H-7b), 1.48 (ddd, 1H, *J* = 6.5, 7.0, 10.0 Hz, H-4), 3.48-3.80 (AB system, 2H, *J*_{AB} = 11.5 Hz, H-6a, H-6b), 3.35 (dd, 1H, *J* = 2.0, 4.5 Hz, H-2), 4.13 (dd, 1H, *J* = 4.5, 7.5 Hz, H-3), 5.48 (d, 1H, *J* = 2.0 Hz, H-1), 5.59 (d, 1H, *J* = 8.2 Hz, H-5'), 7.89 (d, 1H, *J* = 1.5 Hz, H-6'); ¹³C NMR (CD₃OD, 50 MHz) δ 15.31 (C-4), 23.75 (C-7), 53.27 (C-5), 66.84 (C-6), 71.62 (C-3), 78.28 (C-2), 82.58 (C-1), 111.85 (C-5'), 134.61 (C-6'), 155.12 (C-2'), 161.11 (C-4'); Anal. Calc. for C₁₁H₁₄N₂O₆: C, 48.89; H, 5.22%. Found: C, 48.95; H, 5.26.

1-((1R,3S,4R,5S,6R)-4,5-Dihydroxy-1-(hydroxymethyl)-2-oxa-bicyclo[4.1.0]heptan-3-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (13b). Amorphous solid, yield 90%, $[\alpha]_D^{25}$ -131.54 (c 0.2, MeOH). ^1H NMR (CD_3OD , 500 MHz) δ 0.76 (dd, 1H, $J = 6.5, 10.0$ Hz, H-7a), 0.97 (t, 1H, $J = 6.5$ Hz, H-7b), 1.38 (ddd, 1H, $J = 6.5, 7.0, 10.0$ Hz, H-4), 1.88 (d, $J = 1.5$ Hz, CH_3), 3.51-3.85 (AB system, 2H, $J_{\text{AB}} = 12.0$ Hz, H-6a, H-6b), 3.47 (dd, 1H, $J = 1.5, 7.5$ Hz, H-2), 4.33 (dd, 1H, $J = 7.0, 7.5$ Hz, H-3), 5.78 (d, 1H, $J = 1.5$ Hz, H-1), 7.89 (d, 1H, $J = 1.5$ Hz, H-6'); ^{13}C NMR (CD_3OD , 50 MHz) δ 12.37 (CH_3), 15.47 (C-7), 23.60 (C-4), 66.74 (C-6), 53.15 (C-5), 71.72 (C-3), 76.78 (C-2), 83.08 (C-1), 112.85 (C-5'), 135.11 (C-6'), 157.02 (C-2'), 162.21 (C-4'); Anal. Calc. for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_6$: C, 50.70; H, 5.67%. Found: C, 50.77; H, 5.62.

Antiviral and cytotoxicity assay for HSV

The newly synthesized nucleosides were evaluated for their activity against HSV-1 and HSV-2 by plaque reduction assay in VERO cells using a methodology reported in the literature.¹³ Cytotoxicity assays were conducted in rapidly dividing Vero cells, as reported.¹³

Evaluation of toxicity and apoptosis

Toxicity was evaluated by a standard viability assay, using the trypan blue exclusion test. Normally, apoptosis was evaluated by morphological analysis of the cells, performed following staining with acridine orange as previously described.¹⁴ Briefly, over 600 cells, including those showing typical apoptotic characteristics, were counted using a fluorescence microscope. The identification of apoptotic cells was based on the presence of uniformly stained nuclei showing chromatin condensation and nuclear fragmentation. In some experiments, apoptosis was detected by flow cytometric analysis of isolated nuclei, following staining with propidium iodide, on a Becton Dickinson FAC Scan Analytic Flow Cytometer, as previously described.¹⁵

Acknowledgments

We thank the M.I.U.R. and C.N.R. for their partial financial support.

References

1. Verheggen, I.; Van Aerschot, A.; Toppet, S.; Snoeck, R.; Janssen, G.; Balzarini, J.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1993**, *36*, 2033.
2. Verheggen, I.; Van Aerschot, A.; Van Meervelt, L.; Rozenski, J.; Wiebe, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Claes, P.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1995**, *38*, 826.

3. Ostrowski, T.; Wroblowski, B.; Busson, R.; Rozenski, J.; De Clercq, E.; Bennet, M. S.; Champness, J. N.; Summers, W. C.; Sanderson, M. R.; Herdewijn, P. *J. Med. Chem.* **1998**, *41*, 4343.
4. Haouz, A.; Vanheusden, V.; Munier-Lehmann, H.; Froeyen, M.; Herdewijn, P.; Van Calenbergh, S.; Delarue, M. *J. Biol. Chem.* **2003**, *278*, 4963.
5. Haouz, A.; Vanheusden, V.; Munier-Lehmann, H.; Froyen, M.; Herdewijn, P.; Van Calenbergh, S.; Delarue, M. *J. Biol. Chem.* **2003**, *278*, 4963.
6. Vastmans, K.; Pocket, S.; Peyes, A.; Kerremans, L.; Van Aerschot, A.; Hendrix, C.; Merliere, P.; Herdewijn, P. *Biochemistry* **2000**, *39*, 12757.
7. Jaggannadh, B.; Reddy, D. V.; Kunwar, A. C. *J. BioChem. Biophys. Res. Commun.* **1991**, *179*, 386.
8. Marquez, V. E.; Ezzitouni, A.; Russ, P.; Siddiqui, M. A.; Ford, H. J.; Feldman, R. J.; Mitsuya, H.; George, C.; Barchi, J. J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 2780.
9. Catelani, G.; Corsaro, A.; D'Andrea, F.; Fisichella, S.; Mariani, M.; Pistarà, V. *Environ. Sci. & Pollut. Res.* **2003**, *10*, 325.
10. Corsaro, A.; Chiacchio, U.; Adamo, R.; Pistarà, V.; Rescifina, A.; Romeo, R.; Catelani, G.; D'Andrea, F.; Mariani, M.; Attolino, E., *Tetrahedron*, **2004**, *60*, 3787.
11. (a) Hoberg, J. O.; Bozell, J. J. *Tetrahedron Lett.* **1995**, *36*, 6831. (b) Simmons, H. E.; Cairns, T. L.; Vladuchik, S. A.; Hoiness, C. M. *Org. Reactions* **1973**, *20*, 1.
12. Vorbrüggen, H.; Krolkiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234
13. Schinazi, R. F.; Peters, J.; Williams, C. C.; Chance, D.; Nahmias, A. J. *Antimicrob. Agents Chemother.* **1982**, *22*, 499.
14. Mastino, A.; Sciortino, M. T.; Medici, M. A.; Perri, D.; Ammendolia, M. G.; Grelli, S.; Amici, C.; Pernice, A. *Cell. Death. Differ.* **1997**, *4*, 649.
15. Matteucci, C.; Grelli, S.; De Smaele, E.; Fontana, C.; Mastino, A. *Cytometry*, **1999**, *35*, 145.