Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 5448–5456
Bioorganic & Medicinal Chemistry

# Exomethylene pyranonucleosides: Efficient synthesis and biological evaluation of 1-(2,3,4-trideoxy-2-methylene-β-D-*glycero*-hex-3-enopyranosyl)thymine

George Agelis,[a] Niki Tzioumaki,[a] Tanja Botić,[b] Avrelija Cencič[b,c] and Dimitri Komiotis[a,*]

[a]*Department of Biochemistry and Biotechnology, Laboratory of Organic Chemistry, University of Thessaly, 26 Ploutonos Street, 41221 Larissa, Greece*
[b]*Department of Microbiology, Biochemistry and Biotechnology, Faculty of Agriculture, University of Maribor, Vrbanska c.30, 2000 Maribor, Slovenia*
[c]*Department of Biochemistry, Medical Faculty, University of Maribor, Slovenia*

Received 17 October 2006; revised 22 May 2007; accepted 23 May 2007
Available online 26 May 2007

**Abstract**—A new series of unsaturated pyranonucleosides with an exocyclic methylene group and thymine as heterocyclic base have been designed and synthesized. D-Galactose (**1**) was readily transformed in three steps into the corresponding 1-(β-D-galactopyranosyl)thymine (**2**). Selective protection of the primary hydroxyl group of **2** with a *t*-butyldimethylsilyl (TBDMS) group, followed by specific acetalation, and oxidation gave 1-(6-*O*-*t*-butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-*lyxo*-hexopyranosyl-2-ulose)thymine (**5**). Wittig reaction of the ketonucleoside **5**, deprotection and tritylation of the 6′-hydroxyl group gave 1-(2-deoxy-2-methylene-6-*O*-trityl-β-D-*lyxo*-hexopyranosyl)thymine (**9**). Exomethylene pyranonucleoside **9** was converted to the olefinic derivative **10**, which after detritylation afforded the title compound 1-(2,3,4-trideoxy-2-methylene-β-D-*glycero*-hex-3-enopyranosyl)thymine (**11**). These novel synthesized compounds were evaluated for antiviral activity against rotaviral infection and cytotoxicity in colon cancer. As compared to AZT, compounds 1-(2-deoxy-2-methylene-β-D-*lyxo*-hexopyranosyl)thymine (**7**) and 1-(β-D-*lyxo*-hexopyranosyl-2-ulose)thymine (**8**) showed to be more efficient, in rotavirus infections and in treatment of colon cancer.


## 1. Introduction

Rotaviruses are the major viral cause of infantile gastroenteritis and first contact with host represents the gastrointestinal tract. In many cases they enter via contaminated food or water. Rotaviruses represent 80% of recognized viral etiologies and 140 million cases of diarrhea per year.[1] They strike young children with similar frequency throughout the world, but the mortality rate is high especially in developing countries where 870,000 deaths per year have been reported (WHO, 1997). Therefore, it is important to find an effective anti-rotaviral therapy. As to our knowledge there is no efficient therapy or golden standard among nucleoside analogues against rotavirus infection.

Nucleoside analogues display a wide range of biological activities as anti-tumor, antiviral, and chemotherapeutic agents.[2–5] Consequently, a large number of modifications have been made to both the base and sugar moieties of natural nucleosides, accordingly, with the objective of increasing the therapeutic index of established antiviral agents.[6] Over the past two decades, nucleoside chemistry has evolved to facilitate efficient routes to effective agents for the treatment of cancer and antiviral agents against several types of viruses including rotavirus.[7–11]

Lately, nucleosides with a six-membered carbohydrate moiety have been evaluated for their potential antiviral[12–15] and antibiotic[16] properties and as building blocks in nucleic acid synthesis.[17,18] Among them, the unsaturated ketonucleosides are well established for their antineoplastic activity and immunosuppressive effects.[19–21] It appeared that the presence of a carbon–carbon double bound in α,β disposition to the keto group of the sugar moiety enhances activity and the presence

*Keywords*: Exomethylene nucleosides; Ketonucleosides; Unsaturated nucleosides; Olefination; Antiviral, anti-tumor activity.
* Corresponding author. Tel.: +30 2410 565285; fax: +30 2410 565299; e-mail: dkom@bio.uth.gr

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.05.055

of a primary hydroxyl group seemed not to be a prerequisite for biological activity.[19,22,23] Those nucleosides not only exhibit growth inhibitory activity against a variety of tumor cells[23,24] in vitro and L1210 leukemia[25,26] in vivo, but they also may constitute important synthetic intermediates in the nucleoside field owing to their chemical reactivity in various media.[27,28]

We have recently reported[10,11] the synthesis of a new generation of unsaturated ketopyranosyl nucleoside derivatives and a number of them proved to be more efficient in rotavirus and anti-tumor growth inhibition as compared to AZT and to 5FU, respectively. Our findings indicated that some of the new ketonucleoside analogues interfere with the replication of rotavirus in cell model of Caco-2 cells and by that limit the destruction of cells originated from human colon.

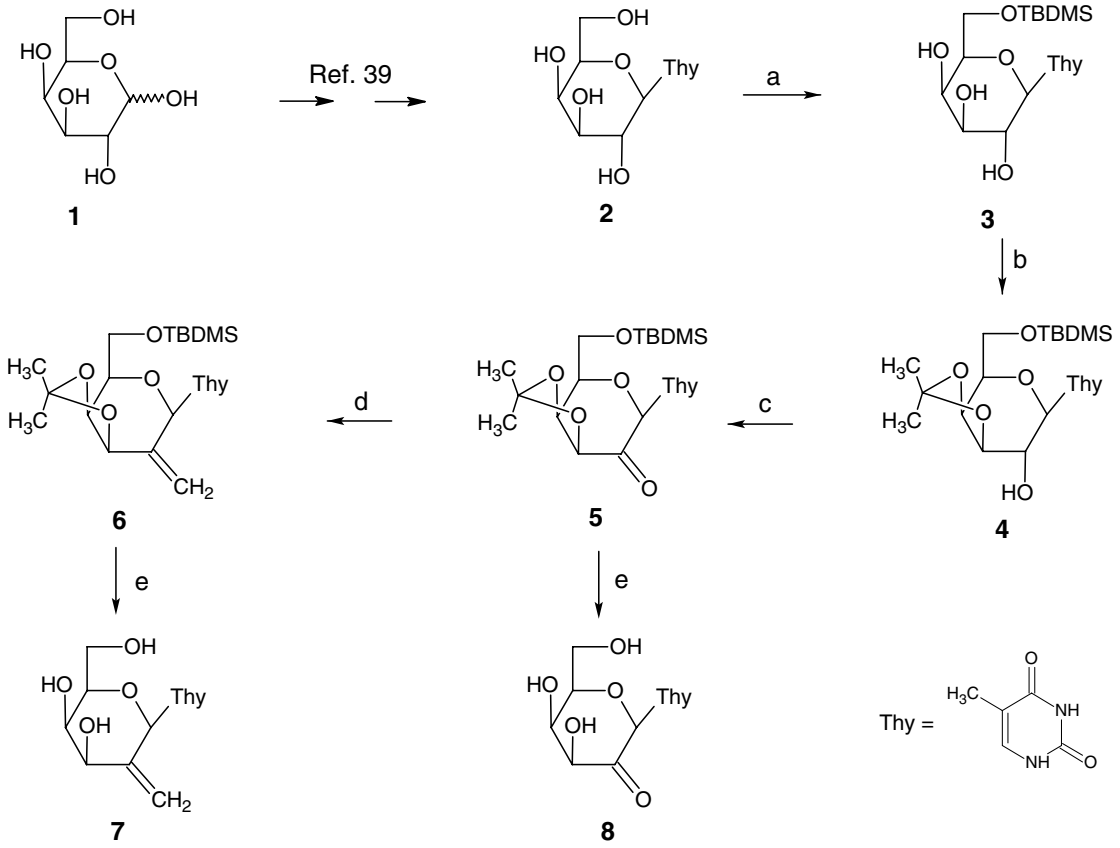
Furthermore, modified nucleosides containing an exocyclic methylene or fluoromethylene group in position 2′, 3′ or 4′ exhibited potent anti-tumor and antiviral activities. Their satisfactory antiviral and anti-cancer properties were attributed, at least in part, to the ability of these nucleosides to irreversibly inactivate ribonucleotide reductase after phosphorylation via specific nucleoside kinases.[29–38] Based on these findings we designed, synthesized, and pharmacologically evaluated the rotavirus and anti-tumor growth inhibition of a new series of unusual nucleosides, i.e., the exomethylene and unsaturated exomethylene pyranonucleosides shown in Schemes 1 and 2.

## 2. Results and discussion

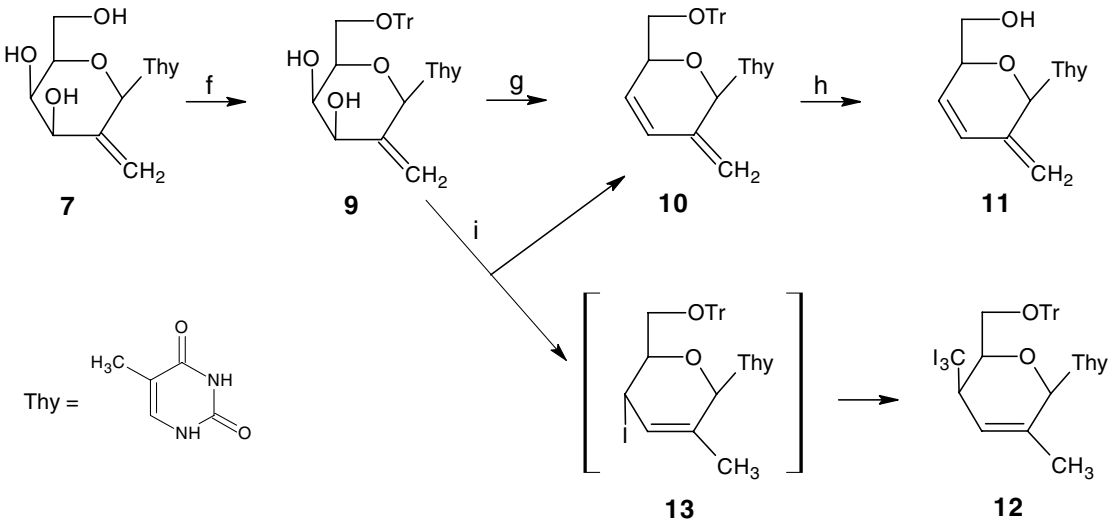
### 2.1. Synthesis

Commercially available D-galactose (**1**) was readily transformed in three steps into the corresponding 1-(β-D-galactopyranosyl)thymine[39] (**2**) in which the primary hydroxyl group was selectively protected with TBDMS group to yield **3**. Specific acetalation of **3** using 2,2-dimethoxypropane[40] in acetone led to the 3′,4′-*O*-isopropylidene derivative **4**. Oxidation of the free hydroxyl with the pyridinium dichromate (PDC)/acetic anhydride ($Ac_2O$) method[41] gave the desired 2′-ketonucleoside **5** isolated as a white solid. The $^1$H NMR spectrum of **5** showed the H-1′ signal as sharp singlet at 6.18 ppm and $J_{3',4'} = 5.4$ as a doublet, confirming the absence of a proton at C-2′.

Wittig reaction of the keto intermediate **5** with sodium hydride (NaH) and methyl triphenylphosphonium bromide ($Ph_3PCH_3Br$) at 0 °C, in the presence of *t*-amyl alcohol in tetrahydrofuran (THF), resulted in **6** in excellent yield (92%).[36] The $^1$H NMR spectrum of **6** showed the presence of two vinylic protons at 5.62 and 5.49 ppm each as a broad singlet, corresponding to the 2′-exom-




**Scheme 1.** Reagents and conditions: (a) TBDMSCl, pyridine, 0–25 °C, 5 h; (b) acetone, 2,2-dimethoxypropane, *p*-toluenesulfonic acid, 25 °C, 3 h; (c) PDC, $Ac_2O$, dry $CH_2Cl_2$, 25 °C, under nitrogen, 4 h; (d) $Ph_3PCH_3Br$, NaH, *t*-amyl alcohol in THF, 0 °C, 2 h, 25 °C, 30 min, under nitrogen; (e) TFA 90% in MeOH, 25 °C, 10 min.




**Scheme 2.** Reagents and conditions: (f) TrCl, pyridine, 25 °C, 4 h; (g) dry Tol/DMF (4:1), iodine–imidazole–$Ph_3P$, 100 °C, 1 h; (h) formic acid/diethyl ether, 1:2, 25 °C, 7 min; (i) dry Tol/DMF (4:1), $CHI_3$–imidazole–$Ph_3P$, 100 °C, 1 h.

ethylene group. Compounds **5** and **6** were converted to the fully unprotected derivatives **8** and **7**, respectively, by treatment with trifluoroacetic acid (TFA) 90% in methanol (MeOH), for 10 min (Scheme 1).

The olefination of the 3,4-vicinal diol proved to be a crucial step for our synthetic procedure and several possible synthetic approaches to the olefinic nucleoside **10** were investigated. Selective protection of the 6′-hydroxyl group of 1-(2-deoxy-2-methylene-β-D-galactopyranosyl)thymine (**7**) by a trityl group (Tr) afforded **9** in high yield (85%). The Corey–Winter[42,43] reaction by treating the diol **9** with thiocarbonyldiimidazole followed by reductive olefination with trimethylphosphite, the Barton[44] deoxygenation via xanthate ester upon treatment with tributyltin hydride, as the Hanessian[45] method with *N*,*N*-dimethylformamide dimethylacetal and methyl iodide, afforded an untreatable mixture of products. In contrast, the Garegg–Samuelsson method,[46–49] with iodine-triphenylphosphine ($Ph_3P$)-imidazole,[49] led to the direct conversion of diol **9** to the desired olefinic compound **10** in 56% yield. Noteworthy, when the same reaction was attempted with iodoform ($CHI_3$)–$Ph_3P$–imidazole[50–52] in toluene/dimethylformamide, 4:1, at 100 °C, **10** was obtained in only 26% yield along with the tentatively proposed byproduct **12**, as it has been characterized by NMR, FAB-MS, and chemical analysis. Compound **12** was possibly formed[53] via the intermediate **13** and its $^1H$ NMR spectrum showed the disappearance of the two exomethylene proton signals, the presence of one vinylic proton signal due to the H-3′ at 7.10 ppm as a doublet ($J_{3',4'} = 1.3$ Hz), and a three protons singlet due to the methyl group in 2′-position at 1.90 ppm. Finally, detritylation of **10** was performed with formic acid/diethyl ether,[54] 1:2, for 7 min, to afford the corresponding title nucleoside derivative 1-(2,3,4-trideoxy-2-methylene-β-D-*glycero*-hex-3-enopyranosyl)thymine (**11**) (Scheme 2).

Compounds **7**, **8**, **10**, and **11** were evaluated for antiviral and cytotoxic activity.

## 2.2. Biological activity

The biological activity of compounds was evaluated in intestinal epithelial cell cultures of the human colon adenocarcinoma cell line Caco-2, non-tumorigenic human fetal cell line H4, and embryonic monkey kidney cells MA 104. Antiviral activity of synthesized compounds was evaluated against rotavirus infection of Caco-2 and MA 104 cells and potential anti-tumor activity was evaluated by cytotoxicity and anti-proliferation test, using tumorous Caco-2 and H4, non-tumorigenic cell counterpart, respectively.

**2.2.1. Antiviral activity.** To examine the potential antiviral properties of the compounds, two different approaches were followed to test the possible inhibitory activity of the compounds on rotavirus infection of Caco-2 and MA 104 cells, as described in experimental part. Only compounds **7** and **8** showed a significant protection against rotavirus on Caco-2 cells by neutralization of virus before its attachment on cell surface at very low dose of 2.46 and 2.44 μM, respectively (Table 1) in comparison to golden standard used (AZT, 74.62 μM). The same antiviral activity was observed also on MA 104 cells by compounds **7** and **8**. None of the tested compounds was able to elicit antiviral activity by inhibition of virus infectivity in Caco-2 cells (Table 1). On the contrary, the inhibition of virus replication following virus attachment was different on MA 104 cells as the one observed for Caco-2 cells (Table 2 vs Table 1). Compounds **7**, **8**, and **10** achieve antiviral activity against rotavirus at the concentrations of 7.03, 6.98, and 4.06 μM. In comparison to standard (AZT), concentration used for compounds was three to four times lower, except for the compound **11**, where the $IC_{50}$ was achieved at the concentration of 79.92 μM (Table 2). From the results obtained in this study and results of tissue selectivity index (TSI values) it can be concluded that the antiviral effect of the compounds depends on the mechanisms of establishment of antiviral state in infected cells and is cell line specific.

**Table 1.** Antiviral activity of compounds **7**, **8**, **10**, **11** and **AZT** against rotavirus RF strain on Caco-2 cells ($IC_{50}$)

| Compound | Treatment A[a] | | | Treatment B[b] | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ | | $CC_{50}/IC_{50}$[c] | $IC_{50}$ | | $CC_{50}/IC_{50}$ |
| | mg/mL | μM | | mg/mL | μM | |
| **7** | 0.0007 | 2.46 | 28.61 | n.e. | n.e. | n.e. |
| **8** | 0.0007 | 2.44 | 28.65 | n.e. | n.e. | n.e. |
| **10** | n.e. | n.e. | n.e. | n.e. | n.e. | n.e. |
| **11** | n.e. | n.e. | n.e. | n.e. | n.e. | n.e. |
| **AZT** | 0.0200 | 74.62 | 0.75[d] | 0.006 | 22.39 | 2.5 |

n.e., no effect observed.
[a] Neutralization of the virus in the solution before its attachment.
[b] Inhibition of infectivity following virus attachment.
[c] $CC_{50}/IC_{50}$ values were calculated using $CC_{50}$ values in Table 3.
[d] $CC_{50}$ for AZT on Caco-2 cells = 56.1 μM.

**Table 2.** Antiviral activity of compounds **7**, **8**, **10**, **11** and **AZT** against rotavirus RF strain on MA 104 cells ($IC_{50}$)

| Compound | Treatment A[a] | | | Treatment B[b] | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ | | $CC_{50}/IC_{50}$[c] | $IC_{50}$ | | $CC_{50}/IC_{50}$ |
| | mg/mL | μM | | mg/mL | μM | |
| **7** | 0.0007 | 2.46 | 85.80 | 0.002 | 7.03 | 30.03 |
| **8** | 0.0007 | 2.44 | 85.90 | 0.002 | 6.98 | 30.03 |
| **10** | n.e. | n.e. | n.e. | 0.002 | 4.06 | 60.00 |
| **11** | n.e. | n.e. | n.e. | 0.020 | 79.92 | 5.99 |
| **AZT** | 0.020 | 74.62 | 3.01[d] | 0.006 | 22.39 | 10.03 |

n.e., no effect observed.
[a] Neutralization of the virus in the solution before its attachment.
[b] Inhibition of infectivity following virus attachment.
[c] $CC_{50}/IC_{50}$ values were calculated using $CC_{50}$ values in Table 3.
[d] $CC_{50}$ for AZT on MA 104 cells = 224.5 μM.

**2.2.2. Cytotoxic and growth inhibition.** All tested compounds have potential anti-tumor activity but at different doses. Cytotoxic effect ($IC_{50}$) measured in H4 and Caco-2 cells for compounds **7** and **8** showed that tested compounds are four times more cytotoxic and 2.5 times more selective (see TSI values) against colon carcinoma cells than standard 5FU compound (Table 3). $CC_{50}$ for both compound were 69.9 and 70.4 μM, respectively, in comparison to 5FU where concentration of 384.4 μM was needed to obtain same anti-tumor activity against Caco-2 cells. Higher concentration of compounds **10** and **11** resulted in intensive destruction of H4 cells, whereas in the case of Caco-2 cells, cytotoxic activity was observed not before concentration of 1015.1 and 1998.0 μM (Table 3).

Additionally, cell growth inhibition assay was performed in Caco-2 cells. Compounds **8** and **10** diminished the growth of Caco-2 cells as determined by cell colony numbers after 10 days of incubation at very low concentration of 0.70 and 0.40 μM, respectively, in comparison to 5FU, whereas $IC_{50}$ for compound **7** was achieved at higher concentration of 21.1 μM. From the results obtained it can be stated that compound **8** was the most efficient in achieving anti-tumor activity against Caco-2 cells if results of cytotoxic activity on H4 are as well taken into the account.

## 3. Conclusions

In conclusion, compounds **7** and **8** are potential anti-tumor and anti-rotavirus agents, as first, they exhibit high

**Table 3.** Cytotoxic effect ($CC_{50}$, μM) of compounds **7**, **8**, **10**, **11** and **5FU** on H4, Caco-2, and MA 104 cells and growth inhibition ($IC_{50}$, μM) on Caco-2 cells

| Compound | Cytotoxic effect ($CC_{50}$, μM) | | | TSI[a] | | Growth inhibition ($IC_{50}$, μM) Caco-2 |
|---|---|---|---|---|---|---|
| | H4 | Caco-2 | MA 104 | Caco-2 | MA 104 | |
| **7** | 1758.9 | 70.4 | 211.1 | 24.9 | 8.3 | 21.1 |
| **8** | 1746.8 | 69.9 | 209.6 | 25 | 8.3 | 0.70 |
| **10** | 40.6 | 1015.1 | 243.6 | 0.04 | 0.160 | 0.40 |
| **11** | 2.8 | 1998.0 | 479.5 | 0.0014 | 0.0058 | 79.92 |
| **5FU** | 3843.8 | 384.4 | 230.6 | 10 | 16.67 | 1.50 |

[a] Tumor selectivity index ($CC_{50}$ on H4 cells/$CC_{50}$ on the specific host cells).

anti-tumor activity that is selective to tumor cells where much lower concentration is needed to exhibit the cytotoxic and anti-proliferative effect as compared to normal non-cancerogenic cells, and second, as they have anti-rotavirus activity at much lower concentrations than the observed cytotoxicity effect. The compound **7** is even more interesting, as it is chemically more stable than corresponding keto compound **8**. In contrast to the ketonucleosides,[55,56] the introduction of an additional unsaturation in the exomethylene nucleosides, compounds **10** and **11**, did not enhance growth inhibition of Caco-2 cells, suggesting that the keto and the exomethylene derivatives are acting via different mechanisms. Compounds **7** and **8** showed to be more efficient than currently used nucleoside analogue AZT, in rotavirus infections and furthermore in treatment of colon cancer as used 5FU.

## 4. Experimental

### 4.1. General procedure

Thin-layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash chromatography was performed using Silica gel (240–400 mesh, Merck).

$^1$H NMR spectra were recorded at room temperature with a Brucker 400 MHz spectrometer using chloroform-*d* ($CDCl_3$) and methanol-$d_4$ ($CD_3OD$). Chemical shifts are reported in parts per million ($\delta$) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were obtained with a Micromass Platform LC (ESI-MS). Optical rotations were measured using a Schmidt and Haensch polarimeter. All reactions were carried out in dry solvents. Dichloromethane ($CH_2Cl_2$) was distilled from phosphorus pentoxide and stored over 4E molecular sieves. Acetonitrile, toluene (Tol), and dimethylformamide (DMF) were distilled from calcium hydride and stored over 3E molecular sieves. THF was freshly distilled under nitrogen from sodium/benzophenone before use and pyridine stored over pellets of potassium hydroxide.

**4.1.1. 1-(6-*O*-*t*-Butyldimethylsilyl-β-D-galactopyranosyl)thymine (3).** To a stirred solution of **2** (4.97 g, 17.24 mmol) in pyridine (86 mL) were added successively TBDMSCl, (3.40 g, 22.56 mmol) and a catalytic amount of 4-dimethylaminopyridine. The reaction mixture was stirred for 30 min at 0 °C under nitrogen and then at room temperature for 5 h. After, the reaction mixture was quenched with MeOH and was evaporated under reduced pressure. The resulting residue was purified by flash chromatography ($CH_2Cl_2$/MeOH, 9:1) to give **3** (6.38 g, 92%, $R_f = 0.25$) as a white solid. Mp 140–142 °C; $[\alpha]_D^{22} + 25.5$ (*c* 0.1, MeOH); $\lambda_{max}$ 260 nm; ($\varepsilon$ 3911).

$^1$H NMR ($CD_3OD$): $\delta$ 8.53 (br s, 1H, NH), 7.65 (s, 1H, H-6), 5.50 (d, 1H, H-1′, $J_{1',2'} = 9.2$ Hz), 3.95 (dd, 1H, H-4′, $J_{3',4'} = 3.2$ Hz, $J_{4',5'} = 0.9$ Hz), 3.88-3.78 (m, 3H, H-2′ and H-6a′,6b′) 3.71 (dt, 1H, H-5′, $J_{5',6a'} = J_{5',6b'} = 6.2$ Hz), 3.63 (dd, 1H, H-3′, $J_{2',3'} = 9.4$ Hz), 1.94 (s, 3H, 5-$CH_3$).

Found: C, 50.42; H, 7.74; N, 6.83. Calcd for $C_{17}H_{30}N_2O_7Si$: C, 50.73; H, 7.51; N, 6.96.

ESI-MS (*m*/*z*): Found 403.32 ($M+H^+$).

**4.1.2. 1-(6-*O*-*t*-Butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)thymine (4).** To a stirred suspension of **3** (6.38 g, 15.85 mmol) in anhydrous acetone (280 mL) and 2,2-dimethoxypropane (15.74 mL) was added *p*-toluenesulfonic acid monohydrate (0.58 g, 3.05 mmol). After 3 h the resulting solution was neutralized with triethylamine so pH did not exceed 7. The solution was concentrated and the residue was purified by flash chromatography (ethyl acetate/hexane, 6:4), to give **4** (5.26 g, 75%, $R_f = 0.35$) as a white foam. $[\alpha]_D^{22} + 32.2$ (*c* 0.1, $CHCl_3$); $\lambda_{max}$ 260 nm; ($\varepsilon$ 4332).

$^1$H NMR ($CD_3OD$): $\delta$ 7.43 (s, 1H, H-6), 5.48 (d, 1H, H-1′, $J_{1',2'} = 9.4$ Hz), 4.33 (dd, 1H, H-4′, $J_{3',4'} = 5.5$ Hz, $J_{4',5'} = 2.2$ Hz), 4.20 (dd, 1H, H-3′, $J_{2',3'} = 7.1$ Hz), 4.10 (dt, 1H, H-5′, $J_{5',6a'} = J_{5',6b'} = 6.7$ Hz), 3.91–3.82 (m, 2H, H-6a′,6b′), 3.78 (dd, 1H, H-2′), 1.90 (s, 3H, 5-$CH_3$), 1.56 and 1.36 (2s, 6H, 2× $CH_3$).

Found: C, 54.46; H, 7.48; N, 6.51. Calcd for $C_{20}H_{34}N_2O_7Si$: C, 54.28; H, 7.74; N, 6.33.

ESI-MS (*m*/*z*): Found 443.42 ($M+H^+$).

**4.1.3. 1-(6-*O*-*t*-Butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-*lyxo*-hexopyranosyl-2-ulose)thymine (5).** A mixture of **4** (5.26 g, 11.88 mmol; dried by co-evaporation with Tol), PDC (5.36 g, 14.25 mmol), and $Ac_2O$ (3.36 mL, 35.64 mmol) was stirred in dry $CH_2Cl_2$ (42 mL) for 4 h, under nitrogen at room temperature. Ethyl acetate (15 mL) was added and the resulting slurry was transferred on top of a silica gel column packed in ethyl acetate. The solution was filtered through the column and washed with ethyl acetate (200 mL) until the product was eluted completely. The solvent was evaporated and the residue was rendered free of $Ac_2O$ and pyridine by co-evaporation with Tol (3×). Purification by flash chromatography (ethyl acetate/hexane, 5:5) yielded pure **5** (4.39 g, 84%, $R_f = 0.4$) as a white solid, mp 144–146 °C; $[\alpha]_D^{22} - 26.3$ (*c* 0.1, $CHCl_3$); $\lambda_{max}$ 260 nm; ($\varepsilon$ 8304).

$^1$H NMR ($CDCl_3$): $\delta$ 8.46 (br s, 1H, NH), 7.26 (s, 1H, H-6), 6.18 (s, 1H, H-1′), 4.71 (dd, 1H, H-4′, $J_{3',4'} = 5.4$ Hz, $J_{4',5'} = 1.4$ Hz), 4.65 (d, 1H, H-3′), 4.32-4.29 (m, 1H, H-5′) 3.94-3.86 (m, 2H, H-6a′,6b′), 1.92 (s, 3H, 5-$CH_3$), 1.48 and 1.41 (2s, 6H, 2× $CH_3$).

Found: C, 54.68; H, 7.21; N, 6.52. Calcd for $C_{20}H_{32}N_2O_7Si$: C, 54.52; H, 7.32; N, 6.36.

ESI-MS (*m*/*z*): Found 441.43 ($M+H^+$).

**4.1.4. 1-(6-*O*-*t*-Butyldimethylsilyl-2-deoxy-3,4-*O*-isopropylidene-2-methylene-β-D-*lyxo*-hexopyranosyl)thymine (6).** To a stirred suspension of methyl triphenyl-phosphonium bromide (8.89 g, 24.90 mmol) and *t*-amyl alcohol (2.96 mL, 27.13 mmol) in dry THF (73 mL) was added NaH (0.65 g, 60% in oil, 27.13 mmol) at 0 °C under nitrogen and the reaction mixture was stirred for 2 h at ambient temperature under nitrogen. To this yellow phosphorus ylide was added a solution of **5** (4.39 g, 9.96 mmol) in dry THF (16 mL) dropwise, at 0 °C under nitrogen. After the mixture was stirred for 30 min at ambient temperature under nitrogen, the reaction mixture was quenched with saturated sodium bicarbonate ($NaHCO_3$) solution and extracted with ethyl acetate. The organic layer was washed with water, dried with anhydrous magnesium sulfate ($MgSO_4$), and evaporated. The residue was purified by flash chromatography (ethyl acetate/hexane, 3:7) to give **6** (4.02 g, 92%, $R_f = 0.25$) as a viscous oil. $[\alpha]_D^{22} - 6.32$ (*c* 0.1, $CHCl_3$); $\lambda_{max}$ 260 nm; ($\varepsilon$ 6016).

$^1$H NMR ($CDCl_3$): $\delta$ 8.91 (br s, 1H, NH), 7.28 (s, 1H, H-6), 6.53 (s, 1H, H-1′), 5.62 and 5.49 (br s, 2H, methylene), 4.85 (d, 1H, H-3′, $J_{3',4'} = 7.4$ Hz), 4.38 (dd, 1H, H-4′, $J_{4',5'} = 1.8$ Hz), 3.85-3.74 (m, 2H, H-6a′,6b′), 3.57-3.53 (m, 1H, H-5′), 1.90 (s, 3H, 5-$CH_3$), 1.56 and 1.41 (2s, 6H, 2× $CH_3$).

Found: C, 57.38; H, 7.65; N, 6.24. Calcd for $C_{21}H_{34}N_2O_6Si$: C, 57.51; H, 7.81; N, 6.39.

ESI-MS (*m*/*z*): Found 439.31 ($M+H^+$).

**4.1.5. 1-(2-Deoxy-2-methylene-β-D-*lyxo*-hexopyranosyl)thymine (7).** Compound **6** (3.97 g, 9.05 mmol) was dissolved in 40 mL of 90% TFA in MeOH. The solution was stirred for 10 min at room temperature and then was concentrated at 40 °C under reduced pressure, in order to remove traces of TFA. The residue was purified by flash chromatography (ethyl acetate/MeOH, 9:1) to give **7** (2.19 g, 85%, $R_f = 0.3$) as a white foam. $[\alpha]_D^{22} - 12.4$ (*c* 0.1, MeOH); $\lambda_{max}$ 260 nm; ($\varepsilon$ 5893).

$^1$H NMR ($CD_3OD$): $\delta$ 7.68 (s, 1H, H-6), 6.22 (s, 1H, H-1′), 5.46 and 4.83 (br s, 2H, methylene), 4.37 (br s, 1H, H-3′), 3.93 (m, 1H, H-4′), 3.88–3.84 (m, 1H, H-5′), 3.79–3.70 (m, 2H, H-6a′,6b′), 1.89 (s, 3H, 5-$CH_3$).

Found: C, 50.57; H, 5.45; N, 9.71. Calcd for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.67; N, 9.85.

ESI-MS (*m*/*z*): Found 285.28 ($M+H^+$).

**4.1.6. 1-(β-D-*Lyxo*-hexopyranosyl-2-ulose)thymine (8).** Compound **8** was prepared from **5** (50 mg, 0.11 mmol) as for **7**. Purification by flash chromatography (ethyl acetate/MeOH, 9:1) yielded **8** (25 mg, 79%, $R_f = 0.2$) as a white foam. $[\alpha]_D^{22} = +26.0$ (*c* 0.1, MeOH); $\lambda_{max}$ 260 nm; ($\varepsilon$ 6041).

$^1$H NMR ($CD_3OD$): $\delta$ 7.33 (s, 1H, H-6), 6.31 (s, 1H, H-1′), 4.69 (d, 1H, H-4′, $J_{3',4'} = 3.5$ Hz), 4.37 (d, 1H, H-3′), 4.27 (m, 1H, H-5′) 3.83–3.78 (m, 2H, H-6a′,6b′), 1.87 (s, 3H, 5-$CH_3$).

Found: C, 46.27; H, 4.70; N, 9.63. Calcd for $C_{11}H_{14}N_2O_7$: C, 46.16; H, 4.93; N, 9.79.

ESI-MS (*m*/*z*): Found 287.21 ($M+H^+$).

**4.1.7. 1-(2-Deoxy-2-methylene-6-*O*-trityl-β-D-*lyxo*-hexopyranosyl)thymine (9).** To a solution of **7** (2.19 g, 7.70 mmol) in pyridine (80 mL) were added TrCl (2.58 g, 9.24 mmol) and a catalytic amount of 4-dimethylaminopyridine. The reaction mixture was stirred for 4 h at room temperature. After being quenched with MeOH and concentrated, the resulting residue was purified by flash chromatography (ethyl acetate/hexane, 7:3) to give **9** (3.45 g, 85%, $R_f = 0.6$ in ethyl acetate). $[\alpha]_D^{22} - 15.7$ (*c* 0.1, $CHCl_3$); $\lambda_{max}$ 260 nm; ($\varepsilon$ 7712).

$^1$H NMR ($CDCl_3$): $\delta$ 7.43–7.20 (m, 16H, $3C_6H_5$ and H-6), 6.18 (s, 1H, H-1′), 5.52 and 4.89 (br s, 2H, methylene), 4.78 (br s, 1H, H-3′), 4.11 (m, 1H, H-4′), 3.85–3.83 (m, 1H, H-5′), 3.46–3.39 (m, 2H, H-6a′,6b′), 1.93 (s, 3H, 5-$CH_3$).

Found: C, 70.58; H, 5.43; N, 5.47. Calcd for $C_{31}H_{30}N_2O_6$: C, 70.71; H, 5.74; N, 5.32.

ESI-MS (*m*/*z*): Found 527.31 ($M+H^+$).

**4.1.8. 1-(2,3,4-Trideoxy-2-methylene-6-*O*-trityl-β-D-*glycero*-hex-3-enopyranosyl)thymine (10)**

**4.1.8.1. Method A.** Imidazole (0.89 g, 13.10 mmol), $Ph_3P$ (6.87 g, 26.20 mmol), and iodine (3.33 g, 13.10 mmol) were added to the suspension of **9** (3.45 g, 6.55 mmol) in 50 mL of dry Tol/DMF (4:1). The resulting reaction mixture was heated (100 °C, oil bath) under nitrogen for 1 h where upon the color changed from a yellow–white to a red–brown. The reaction mixture was concentrated in vacuum, the residue diluted with ethyl acetate, washed with aqueous $NaHCO_3$, sodium thiosulfate, and water. The organic phase was dried with $MgSO_4$, the solvent was removed in vacuum, and the residue was purified by flash chromatography (ethyl acetate/hexane, 4:6). The olefinic nucleoside derivative **10** (1.81 g, 56%, $R_f = 0.75$ in ethyl acetate/hexane, 7:3) was obtained as colorless syrup. $[\alpha]_D^{22} - 21.1$ (*c* 0.1, $CHCl_3$); $\lambda_{max}$ 260 nm; ($\varepsilon$ 5282).

$^1$H NMR ($CDCl_3$): $\delta$ 8.31 (br s, 1H, NH), 7.41–7.18 (m, 16H, $3C_6H_5$ and H-6), 6.41 (s, 1H, H-1′), 6.31 (dd, 1H, H-4′, $J_{3',4'} = 10.0$ Hz, $J_{4',5'} = 1.8$ Hz), 5.91 (d, 1H, H-3′), 5.04 and 4.68 (br s, 2H, methylene), 4.63 (m, 1H, H-5′), 3.28–3.03 (m, 2H, H-6a′,6b′), 1.85 (s, 3H, 5-$CH_3$).

Found: C, 75.39; H, 5.58; N, 5.82. Calcd for $C_{31}H_{28}N_2O_4$: C, 75.59; H, 5.73; N, 5.69.

ESI-MS (*m*/*z*): Found 493.23 ($M+H^+$).

**4.1.8.2. Method B.** Imidazole (26 mg, 0.38 mmol), $Ph_3P$ (200 mg, 0.76 mmol), and $CHI_3$ (150 mg, 0.38 mmol) were added to the suspension of **9** (100 mg,

0.19 mmol) in 2 mL of dry Tol/DMF (4:1). The resulting reaction mixture was heated (100 °C, oil bath) under nitrogen for 1 h, was concentrated in vacuum, and the residue diluted with ethyl acetate, washed with aqueous $NaHCO_3$, sodium thiosulfate, and water. The organic phase was dried with $MgSO_4$, the solvent was removed in vacuum, and after purification by flash chromatography (ethyl acetate/hexane, 4:6) were isolated the desired product **10** (24 mg, 26%) and byproduct **12** (35 mg, 21%, $R_f = 0.55$ in ethyl acetate/hexane, 7:3).

Data for compound **12**: $^1$H NMR ($CDCl_3$): $\delta$ 8.71 (br s, 1H, NH), 7.69–7.21 (m, 16H, $3C_6H_5$ and H-6), 7.10 (d, 1H, H-3′, $J_{3',4'} = 1.3$ Hz), 6.69 (s, 1H, H-1′), 4.40-4.38 (m, 1H, H-5′), 3.62–3.53 (m, 2H, H-6a′,6b′), 2.85 (t, 1H, H-4′), 1.90 (s, 3H, 2′-$CH_3$), 1.84 (s, 3H, 5-$CH_3$).

Found: C, 43.47; H, 3.18; N, 3.28. Calcd for $C_{32}H_{29}I_3N_2O_4$: C, 43.36; H, 3.30; N, 3.16.

ESI-MS (*m*/*z*): Found 887.10 ($M+H^+$).

**4.1.9. 1-(2,3,4-Trideoxy-2-methylene-β-D-*glycero*-hex-3-enopyranosyl)thymine (11).** Compound **10** (1.81 g, 3.67 mmol) was dissolved in a mixture of formic acid/diethyl ether (93 mL, 1:2). The mixture was stirred for 7 min at room temperature, diluted with toluene, and co-distilled several times with the same solvent to avoid ester formation.[57] The concentrated residue was purified by flash chromatography (ethyl acetate/hexane, 4:6) to afford pure **11** (0.75 g, 82%, $R_f = 0.25$). $[\alpha]_D^{22} - 18.4$ (*c* 0.1, $CHCl_3$); $\lambda_{max}$ 260 nm; ($\varepsilon$ 4421).

$^1$H NMR ($CDCl_3$): $\delta$ 8.63 (br s, 1H, NH), 7.27 (s, 1H, H-6), 6.55 (s, 1H, H-1′), 6.49 (dd, 1H, H-4′, $J_{3',4'} = 10.1$ Hz, $J_{4',5'} = 2.1$ Hz), 5.80 (d, 1H, H-3), 5.20 and 4.82 (br s, 2H, methylene), 4.52 (m, 1H, H-5′), 3.40-3.30 (m, 2H, H-6a′,6b′), 1.91 (s, 3H, 5-$CH_3$).

Found: C, 57.74; H, 5.46; N, 11.28. Calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19.

ESI-MS (*m*/*z*): Found 251.22 ($M+H^+$).

### 4.2. Methods for measurement of biological activity

**4.2.1. Cells and culture conditions.** The human colon adenocarcinoma cell line Caco-2 was a generous gift of Dr. Rene L'Harridon, INRA, VIM, Jouy-en-Josas, France. Rhesus monkey kidney cell line MA104 and human fetal small intestine cell line H4 were used. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma–Aldrich, Grand Island, USA), supplemented with 5% fetal calf serum (Cambrex, Verviers, Belgium), L-glutamine (2 mmol/L, Sigma, St. Louis, USA), penicillin (100 U/mL, Sigma, St. Louis, USA), and streptomycin (1 mg/mL, Fluka, Buchs, Switzerland) at 37 °C in 5% $CO_2$ atmosphere in tissue culture flasks until confluent. Cell culture medium was regularly changed.

**4.2.2. Pyranonucleosides.** Compounds **7**, **8**, **10**, and **11** were freshly prepared in sterile dimethylsulfoxide (DMSO) at the concentration of 0.5 mg/mL. The final concentration of DMSO was below 0.1% of cell culture medium. All solutions were protected against light.

AZT (Retrovir®) GlaxoSmithKline, USA, a drug used for anti-retroviral therapy (ART) was used as a standard compound in antiviral experiments and 5FU as a standard compound in anti-tumor experiments. Solutions were prepared in the same way as those of unsaturated exomethylene pyranonucleosides.

**4.2.3. Virus propagation.** *The rotavirus RF strain* was propagated in Caco-2 cells in the presence of trypsin (1 μg per mL of DMEM) as described previously.[58] Supernatant containing the virus was collected from the flasks when cytopathic effect (CPE) was observed (24–48 h at 37 °C) by microscopy and clarified by centrifugation. Virus was stored at −70 °C until used.

**4.2.4. Antiviral assay.** The potential antiviral activity of the newly synthesized compounds was tested against rotavirus by investigating:

**4.2.4.1. The inhibition of infectivity following virus attachment.** Washed monolayer of Caco-2 and MA 104 cells was first incubated with rotavirus for 1 h at 37 °C in the atmosphere of 5% $CO_2$ (time for virus to attach to cell membrane receptors). After incubation the remaining virus was washed off with DMEM without supplements and monolayer was treated immediately with the tested compounds or AZT added in 3-fold serial dilutions (initial concentration of 0.5 mg/mL). After 72 h of incubation for rotavirus, the plates were stained with Crystal Violet in ethanol, rinsed with water, and destained with 10% (v/v) acetic acid. The $A_{590}$ was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) to those in control (only virus-infected) wells ($n = 6$). The minimal inhibitory concentration ($IC_{50}$) of the tested compounds was obtained from the concentration–effect curve.

**4.2.4.2. The neutralization of the virus in solution before attachment.** Threefold dilutions of each of the tested compounds or AZT (initial concentration of 0.5 mg/mL) were first co-incubated with rotavirus in DMEM supplemented with trypsin for 12 h prior to the infection of cell monolayer at 37 °C and 5% $CO_2$. Residual viral infectivity was measured after 72 h post-infection. Rotavirus alone was treated in the same way as the control. After 72 h of incubation, the plates were stained with Crystal Violet in ethanol, rinsed with water, and destained with 10% (v/v) acetic acid. The $A_{590}$ was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) to those in control (only virus-infected) wells ($n = 6$). The minimal inhibitory concentration ($IC_{50}$) of the tested compounds was obtained from the concentration–effect curve.

**4.2.5. Growth inhibition assay.** It was performed on Caco-2 cell line by modified method described previously.[59] Briefly, in 96-well plates, six wells of 3-fold

dilutions of each compound or 5FU (initial concentration of 0.5 mg/mL) were applied to monolayers of 10 cells/well in Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum. Incubation was performed at 37 °C in the humidified incubator for 10 days. The colonies were counted in each well and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of colony number in treated wells ($n = 2$) to those in control wells ($n = 24$). The minimal inhibitory concentration ($IC_{50}$) of the tested compounds was obtained from the concentration–effect curve.

**4.2.6. Cytotoxicity assay.** H4 and Caco-2 cells ($6 \times 10^6$ cells per plate) were seeded in P 96-plate and treated with the compounds or 5FU. In assay 3-fold serial dilutions of each compound (initial concentration of 0.5 mg/mL) were tested. Then, the cells were incubated at 37 °C in the humidified incubator for 72 h. The plates were stained with Crystal Violet in ethanol, rinsed with water, and destained with 10% (v/v) acetic acid. The $A_{590}$ was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in treated wells ($n = 2$) to those in control wells ($n = 24$). The minimal inhibitory concentration ($IC_{50}$) of the tested compounds was obtained from the concentration–effect curve.

## Acknowledgments

This work was supported in part by the Greek General Secretariat for Research and Technology Program No. 3245C and Bilateral Scientific-Technology Project between Greece and Slovenia. The authors are grateful to Dr. K. Antonakis (Ec. Nat. Sup. de Chimie de Paris; CNRS-FRE 2463) for encouraging this work and to Dr. T. Halmos (Ec. Nat. Sup. de Chimie de Paris) for the unstinted and fruitful aid, as well as ELPEN pharmaceuticals for financial support.

## References and notes

1. Albert, M. J.; Faruque, A. S. G.; Faruque, S. M.; Sack, R. B.; Mahalanabis, D. *J. Clin. Microbiol.* **1999**, *37*, 3458.
2. Zhou, W.; Gumina, G.; Chong, Y.; Wang, J.; Shinazi, R. F.; Chu, C. K. *J. Med. Chem.* **2004**, *47*, 3399.
3. Perigaud, C.; Gosselin, G.; Imbach, J. L. *Nucleosides Nucleotides* **1992**, *11*, 903.
4. Robins, R. K.; Kini, G. D. Purines and Purine Nucleoside Analogues as Antitumor Agents. In *The Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed.; Chapman and Hall: New York, 1990; pp 299–321.
5. MacCoss, M.; Robins, M. J. Anticancer Pyrimidines, Pyrimidine Nucleosides and Prodrugs. In Wilman, D. E. V., Ed.; The Chemistry of Antitumor Agents; Chapman and Hall: New York, 1990; pp 261–298.
6. Len, C.; Violeau, B. *Tetrahedron Lett.* **2005**, *46*, 4835.
7. Verheggen, I.; van Aerschot, A.; Toppet, S.; Snoeck, R.; Janssen, G.; Balzarini, J.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1993**, *36*, 2033.
8. De Clercq, E. *Biochim. Biophys. Acta* **2002**, *1587*, 258.
9. Brady, R. C.; Bernstein, D. I. *Antiviral Res.* **2004**, *61*, 73.
10. Manta, S.; Agelis, G.; Botić, T.; Cencič, A.; Komiotis, D. *Bioorg. Med. Chem.* **2007**, *15*, 980.
11. Manta, S.; Agelis, G.; Botić, T.; Cencič, A.; Komiotis, D. *Eur. J. Med. Chem.* **2007** (in press).
12. Papatheodoridis, G. V.; Dimou, E.; Papadimitropoulos, V. *Am. J. Gastroenterol.* **2002**, *97*, 1618.
13. 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.
14. 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.
15. Maurinsh, Y.; Schraml, J.; de Winter, H.; Blaton, N.; Peeters, O.; Lescrinier, E.; Rozenski, J.; van Aerschot, A.; de Clercq, E.; Busson, R.; Herdewijn, P. *J. Org. Chem.* **1997**, *62*, 2861.
16. Haouz, A.; Vanheusden, V.; Munier-Lechman, H.; Froeyen, M.; Herdewijn, P.; van Galenbergh, S.; Delarue, M. *J. Biol. Chem.* **2003**, *278*, 4963.
17. Vastmans, K.; Pochet, S.; Peys, A.; Kerremans, L.; van Aerschot, A.; Hendrix, C.; Marliere, P.; Herdewijn, P. *Biochemistry* **2000**, *39*, 12757.
18. Vastmans, K.; Froeyen, M.; Kerremans, L.; Pochet, S.; Herdewijn, P. *Nucleic Acids Res.* **2001**, *29*, 3154.
19. Paterson, J.; Uriel, C.; Egron, M. J.; Herscovici, J.; Antonakis, K.; Alaoui, M. *Antimicrob. Agents Chemother.* **1998**, *42*, 779.
20. Alaoui, M.; Lasnes, C.; Antonakis, K.; Chouroulinkov, I. *Mutagenesis* **1986**, *1*, 411.
21. Alaoui, M.; Egron, M. J.; Bessodes, M.; Antonakis, K.; Chouroulinkov, I. *Eur. J. Med. Chem.* **1987**, *22*, 305.
22. Egron, M. J.; Leclercq, F.; Antonakis, K.; Bennani-Baiti, M. I.; Frayssinet, C. *Carbohydr. Res.* **1993**, *248*, 143.
23. Halmos, T.; Cardon, A.; Antonakis, K. *Chem. Biol. Interact.* **1983**, *46*, 11.
24. Antonakis, K.; Chouroulinkov, I. *Biochem. Pharmacol.* **1974**, *23*, 2095.
25. Antonakis, K.; Halmos, T.; Bach, J.; Chouroulinkov, I. *Eur. J. Med. Chem.* **1980**, *15*, 237.
26. Chouroulinkov, I.; Antonakis, K. *C.R. Acad. Sci. Hebd. Seances Acad. Sci. D* **1977**, *285*, 1021.
27. Antonakis, K. *Chimia* **1975**, *29*, 59.
28. Antonakis, K.; Egron, M. J. *Carbohydr. Res.* **1973**, *27*, 468.
29. Baker, C. H.; Banzon, J.; Bollinger, J. M.; Stuble, J.; Samano, V.; Robins, M. J.; Lippert, B.; Jarvi, E.; Resvick, R. *J. Med. Chem.* **1991**, *34*, 1879.
30. Robins, M. J.; Samano, V.; Zhang, W.; Balzarini, J.; de Clercq, E.; Borchardt, R. T.; Lee, Y.; Yuan, C. S. *J. Med. Chem.* **1992**, *35*, 2283.
31. Cory, A. H.; Samano, V.; Robins, M. J.; Cory, J. G. *Biochem. Pharmacol.* **1994**, *47*, 365.
32. Bridges, C. G.; Ahmed, S. P.; Sunkara, P. S.; McCarthy, J. R.; Tyms, A. S. *Antiviral Res.* **1995**, *27*, 325.
33. Takahashi, T.; Nakashima, A.; Kanazawa, J.; Yamaguchi, K.; Akinaga, S.; Tamaoki, T.; Okabe, M. *Cancer Chemother. Pharmacol.* **1998**, *41*, 268.
34. Bisacchi, G. S.; Chao, S. T.; Bachard, C.; Daris, J. P.; Innaimo, S.; Jacobs, G. A.; Kocy, O.; Lapointe, P.; Martel, A.; Merchant, Z.; Slusarchyk, W. A.; Sundeen, J. E.; Young, M. G.; Colonno, R.; Zahler, R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 127.
35. Jeong, L. S.; Yoo, S. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 847.
36. Yoo, S. J.; Kim, H. O.; Lim, Y.; Kim, J.; Jeong, L. S. *Bioorg. Med. Chem.* **2002**, *10*, 215.
37. Gunaga, P.; Baba, M.; Jeong, L. S. *J. Org. Chem.* **2004**, *69*, 3208.
38. Hassan, A. E. A.; Shuto, S.; Matsuda, A. *Tetrahedron* **1994**, *50*, 689.

39. Brevnov, G. M.; Gritsenko, M. O.; Mikhailov, N. S.; Efimtseva, V. E.; Ermolinsky, S. B.; van Aerschot, A.; Herdewijn, P.; Repyk, V. A.; Gromova, S. E. *Nucleic Acids Res.* **1997**, *25*, 3302.
40. Vanheusden, V.; Busson, R.; Herdewijn, P.; van Calenbergh, S. *J. Org. Chem.* **2004**, *69*, 4446.
41. Herscovici, J.; Antonakis, K. *J. Chem. Soc. Chem. Commun.* **1979**, *12*, 561.
42. Corey, E. J.; Winter, R. A. E. *J. Am. Chem. Soc.* **1963**, *85*, 2677.
43. Corey, E. J.; Hopkins, P. B. *Tetrahedron Lett.* **1982**, *23*, 1979.
44. Barret, A. G. M.; Barton, D. H. R.; Bielski, R. *J. Chem. Soc., Perkins Trans 1* **1979**, 2378.
45. Hanessian, S.; Bargiotti, A.; LaRue, M. *Tetrahedron Lett.* **1978**, *19*, 737.
46. Garegg, P. J.; Samuelsson, B. *Synthesis* **1979**, 469.
47. Samuelsson, B.; Liu, Z.; Classon, B. *J. Org. Chem.* **1990**, *55*, 4273.
48. Garegg, P. J.; Samuelsson, B. *Synthesis* **1979**, 813.
49. Luzzio, A. F.; Menes, E. M. *J. Org. Chem.* **1994**, *59*, 7267.
50. Zhang, J.; Clive, D. L. J. *J. Org. Chem.* **1999**, *64*, 1754.
51. Bessodes, M.; Abushanab, E.; Panzica, R. P. *J. Chem. Soc. Chem. Commun.* **1981**, 26.
52. Chun, B. K.; Song, G. Y.; Chu, C. K. *J. Org. Chem.* **2001**, *66*, 4852.
53. Alvarez-Manzaneda, E. J.; Chahboun, R.; Cabrera Torres, E.; Alvarez, E.; Alvarez-Manzaneda, E.; Haidour, A.; Ramos LoJpez, J. M. *Tetrahedron Lett.* **2005**, *46*, 3755.
54. Bessodes, M.; Komiotis, D.; Antonakis, K. *Tetrahedron Lett.* **1986**, *27*, 579.
55. Leclercq, F.; Antonakis, K. *Carbohydr. Res.* **1994**, *263*, 309.
56. Halmos, T.; Komiotis, D.; Antonakis, K. *Carbohydr. Res.* **1985**, *145*, 163.
57. Komiotis, D.; Agelis, G.; Manta, S.; Tzioumaki, N.; Tsoukala, E. *J. Carbohydr. Chem.* **2006**, *25*, 441.
58. Kitaoka, S.; Suzuki, H.; Numazaki, Y.; Konno, T.; Ishida, N. *Tohoku J. Exp. Med.* **1986**, *149*, 437.
59. Cencič, A.; Henry, C.; Lefèvre, F.; Huet, J. H.; Koren, S.; La Bonnardière, C. *Eur. J. Biochem.* **2002**, *269*, 2772.