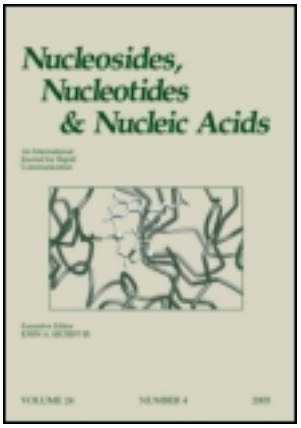


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

# Stereocontrolled Facile Synthesis and Biological Evaluation of (3′S) and (3′R)-3′-Amino (and Azido)-3′-Deoxy Pyranonucleosides

Stella Manta [a], Vanessa Parmenopoulou [a], Christos Kiritsis [a], Athina Dimopoulou [a], Nikolaos Kollatos [a], Ioannis Papasotiriou [b], Jan Balzarini [c] & Dimitri Komiotis [a]

[a] Laboratory of Bio-Organic Chemistry Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

[b] Research Genetic Cancer Center (R.G.C.C. Ltd.), GR, Filotas, Greece

[c] Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

To cite this article: Stella Manta , Vanessa Parmenopoulou , Christos Kiritsis , Athina Dimopoulou , Nikolaos Kollatos , Ioannis Papasotiriou , Jan Balzarini & Dimitri Komiotis (2012): Stereocontrolled Facile Synthesis and Biological Evaluation of (3′S) and (3′R)-3′-Amino (and Azido)-3′-Deoxy Pyranonucleosides, Nucleosides, Nucleotides and Nucleic Acids, 31:7, 522-535

To link to this article: http://dx.doi.org/10.1080/15257770.2012.696759

*Nucleosides, Nucleotides and Nucleic Acids*, 31:522–535, 2012

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2012.696759



# STEREOCONTROLLED FACILE SYNTHESIS AND BIOLOGICAL EVALUATION OF (3′*S*) AND (3′*R*)-3′-AMINO (AND AZIDO)-3′-DEOXY PYRANONUCLEOSIDES

**Stella Manta,[1] Vanessa Parmenopoulou,[1] Christos Kiritsis,[1] Athina Dimopoulou,[1] Nikolaos Kollatos,[1] Ioannis Papasotiriou,[2] Jan Balzarini,[3] and Dimitri Komiotis[1]**

[1]*Laboratory of Bio-Organic Chemistry, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece*
[2]*Research Genetic Cancer Center (R.G.C.C. Ltd.), GR, Filotas, Greece*
[3]*Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

□ *This article describes the synthesis of (3′S) and (3′R)-3′-amino-3′-deoxy pyranonucleosides and their precursors (3′S) and (3′R)-3′-azido-3′-deoxy pyranonucleosides. Azidation of 1,2:5,6-di-O-isopropylidene-3-O-toluenesulfonyl-α-D-allofuranose followed by hydrolysis and subsequent acetylation afforded 3-azido-3-deoxy-1,2,4,6-tetra-O-acetyl-D-glucopyranose, which upon coupling with the proper silylated bases, deacetylation, and catalytic hydrogenation, obtained the target 3′-amino-3′-deoxy-β-D-glucopyranonucleosides. The desired 1-(3′-amino-3′-deoxy-β-D-allopyranosyl)5-fluorouracil was readily prepared from the suitable imidazylate sugar after azidation followed by a protection/deprotection sequence and reduction of the unprotected azido precursor. No antiviral activity was observed for the novel nucleosides. Moderate cytostatic activity was recorded for the 5-fluorouracil derivatives.*

**Keywords** Azido pyranonucleosides; amino pyranonucleosides; cytostatic activity; 5-fluorouracil

## INTRODUCTION

The nucleosides embrace a large family of natural and chemically modified analogs of a great structural diversity and a broad spectrum of biological

Received 11 April 2012; accepted 21 May 2012.

This work was supported in part by the Postgraduate Programs "Biotechnology-Quality Assessment in Nutrition and the Environment," "Application of Molecular Biology–Molecular Genetics–Molecular Markers," Department of Biochemistry and Biotechnology, University of Thessaly and the K.U. Leuven (GOA 10/014). The authors thank Mrs. Lizette van Berckelaer, Mrs. Leentje Persoons, Mrs. Frieda De Meyer, Mrs. Anita Camps, Mrs. Lies Van den Heurck, Mr. Steven Carmans, and Mrs. Leen Ingels for excellent technical assistance.

Address correspondence to Dimitri Komiotis, Laboratory of Bio-organic Chemistry, Department of Biochemistry & Biotechnology, University of Thessaly, 26 Ploutonos Str., 41221 Larissa, Greece. E-mail: dkom@bio.uth.gr

activity.[1–5] The analogs of natural nucleosides and nucleoside antibiotics belong to the most important classes of antiviral drugs, and they are extensively used in the treatment of a variety of cancers. However, the emergence of drug-resistant mutants that render current therapies ineffective, drives interest in the development of novel nucleoside analogs with high potency, low toxicity, and favorable resistance profiles.[6]

The aminosugar nucleosides are well-known bioactive agents,[7–9] of which puromycin, a protein synthesis inhibitor, is one of the most important examples.[10–12] In addition, their azidosugar precursors[13–16] have been attracting growing attention, since 3′-azido-2′,3′-dideoxycytidine (AZC) and guanosine (AZG) nucleosides[14,17,18] exhibit an inhibiting activity on the replication of the human immunodeficiency virus (HIV), while the thymidine derivative (AZT) is a widely utilized anti-HIV drug.[19]

Lately, the pyranosyl nucleosides are among the most recent modifications of the natural nucleosides to receive attention as potential antiviral,[20,21] antibiotic,[22] antioxidant,[23,24] and antitumor[5] agents. Their biological activity is often modulated by the systematic variation of the sugar ring and/or the nucleic base. Modified nucleosides with a six-membered sugar ring containing fluoro, keto,[25–30] exomethylene,[31,32] cyano,[33] and ethynyl[34] functions, proved to have a promising potential in combating rotaviral infections and exhibited cytostatic activity against various cancer cell lines. Experimental data revealed that thymidylate synthase,[35] human poly(A)-specific ribonuclease,[36] and glycogen phosphorylase[37] are among the molecular targets of these compounds.

Based on these findings and as a continuation of our investigations concerning the development of bioactive sugar-modified pyranonucleosides, it was of great interest to design, synthesize, and biologically evaluate novel (3′*S*) and (3′*R*)-3′-amino (and azido)-3′-deoxy pyranonucleosides, i.e., 3′-amino (and azido)-3′-deoxy-$\beta$-D-glucopyranonucleosides and 3′-amino (and azido)-3′-deoxy-$\beta$-D-allopyranonucleosides, bearing 5-fluorouracil, thymine, cytosine, and guanine as heterocyclic bases.



## RESULTS AND DISCUSSION

### Chemistry

Our synthetic plan to the desired 3′-amino-3′-deoxy-$\beta$-D-glucopyranonucleosides **7a,b,e,f** and their azido precursors **6a,b,e,f** was focused on the preparation of the glycosyl donor from a chiral template, carbohydrate precursor, and then the condensation with nucleosidic bases. Synthesis of the key D-glycosyl donor **4**[38] from diacetone-D-glucose (**1**), is shown in Figure 1.

Thus, oxidation of the commercially available 1,2:5,6-di-*O*-isopropylidene-$\alpha$-D-glucofuranose (**1**) followed by stereoselective reduction

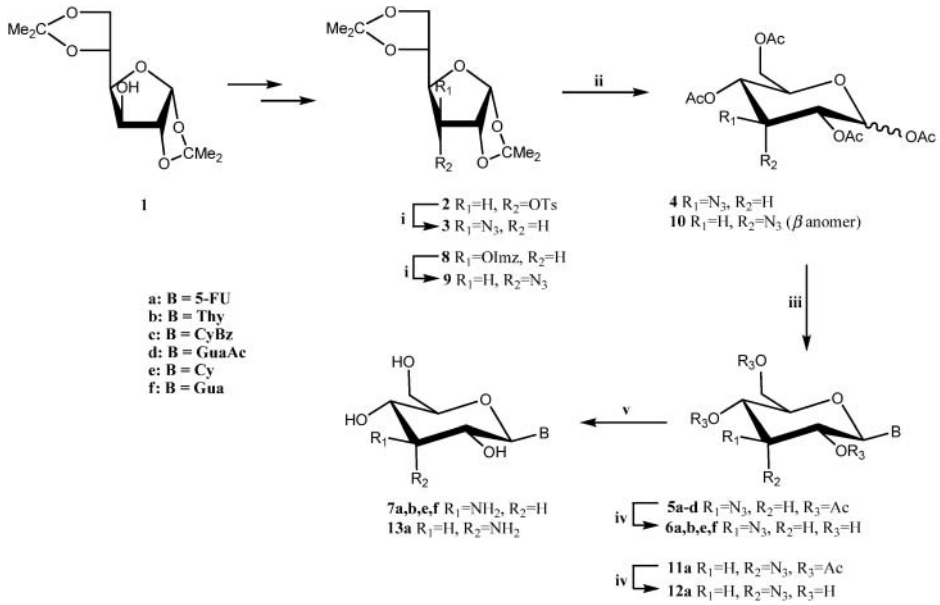


**FIGURE 1** (i) $NaN_3$, DMF; (ii) (a) MeOH, $H_2O$, Amberlite IR 120 ($H^+$); (b) pyridine, $Ac_2O$; (iii) nucleobase, HMDS, $Me_3SiOSO_2CF_3$, saccharine, $CH_3CN$; (iv) ammonia/MeOH; (v) $H_2$, 10% Pd/C, triethylamine, EtOAc, EtOH, 20°C, 24 hours.

and tosylation, gave the corresponding 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-allofuranose (**2**).[39,40] The *endo*-sulfonate group of furanose **2**[39,40] was readily displaced with azide ion in boiling *N*,*N*-dimethylformamide (DMF). Hydrolysis of the resulting azide **3** using the Amberlite IR 120 ($H^+$) resin followed by the subsequent treatment with $Ac_2O$ in pyridine afforded a mixture of the anomeric acetates **4**[38] (ratio of α to β anomer, 0.57:0.43). The anomeric mixture **4**[38] was readily converted into the protected 1-(2′,4′,6′-tri-*O*-acetyl-3′-azido-3′-deoxy-β-D-glucopyranosyl) nucleosides **5a–d**,[41] upon reaction with silyl-protected 5-fluorouracil, thymine, $N^4$-benzoylcytosine, and $N^2$-acetylguanine, respectively, in the presence of trimethylsilyltrifluoromethane-sulfonate ($Me_3SiOSO_2CF_3$) as an activator.[42] $^1$H NMR data obtained for the newly synthesized nucleosides **5** showed a large coupling between protons H-1′, H-2′ and H-2′, H-3′ ($J_{1',2'}$ = 9.2–9.4 Hz, $J_{2',3'}$ = 9.5–9.9 Hz), thus revealing the β-configuration of the sugar moiety and an equatorially oriented azido group. Removal of all the protecting groups of **5a–d**, with saturated methanolic ammonia,[43] furnished the target products **6a,b,e,f**, in good yields (71–81%). The infrared (IR) spectra of the **6a,b,e,f** exhibited characteristic absorption bands at 2107–2115 $cm^{-1}$ due to the presence of the azido group. In the final step, the azido derivatives **6a,b,e,f** were reduced to form the desired amino analogs **7a,b,e,f** by hydrogen in the presence of palladium-*on*-carbon (Table 1).

**TABLE 1** Cytostatic activity of **6a**,**b**,**e**,**f**, **7a**,**b**,**e**,**f**, **12a**, and **13a** against suspension and monolayer tumor cell lines

| Compound | $IC_{50}$[a] ($\mu$M) | | |
|---|---|---|---|
| | L1210 | CEM | HeLa |
| **6a** | $174 \pm 21$ | >250 | >250 |
| **6b** | >250 | >250 | >250 |
| **6e** | >250 | >250 | >250 |
| **6f** | ≥250 | >250 | $171 \pm 6$ |
| **7a** | $92 \pm 17$ | >250 | $176 \pm 16$ |
| **7b** | >250 | >250 | ≥250 |
| **7e** | >250 | >250 | $232 \pm 25$ |
| **7f** | >250 | >250 | $244 \pm 8$ |
| **12a** | >250 | >250 | ≥250 |
| **13a** | $119 \pm 10$ | >250 | $174 \pm 4$ |

[a]50% inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%.



The target amino pyranonucleosides **7a,b,e,f** and their key intermediates **6a,b,e,f** proved to be slightly cytostatic against the murine leukemia L1210, the human lymphocyte CEM, and the human cervix carcinoma HeLa cells, except the 5-fluorouracil analog **7a** that proved to be the most cytotoxic against L1210 cells ($IC_{50}$: 92 $\mu$M).

To further explore the impact of the azido and amino orientation on the biological activity, the most potent 5-fluorouracil nucleoside **7a** was chosen and its (3′*R*)-diastereomer was also synthesized. Thus, 3-imidazylate ester **8**[44] appeared to be a suitable precursor for its synthesis, since the tosylate ester of diacetone-D-glucose proved to be particularly resistant to nucleophilic displacement reactions, presumably due to the *exo*-orientation of the sulfonate group[40,45] (Figure 1). Ester **8** was converted at 50°C in DMF to the azide **9** in 79% yield with inversion of configuration at the $C^3$ center. Azidolysis was followed by the quantitative cleavage of the isopropylidene protecting groups of **9** and the subsequent direct standard acetylation afforded peracetylated allopyranose **10**,[46] existing exclusively as its $\beta$-pyranose form. The desired 5-fluorouracil nucleoside **11a** was obtained by condensing the glycosyl donor 3-azido-3-deoxy-1,2,4,6-tetra-*O*-acetyl-$\beta$-D-allopyranose (**10**)[46] with silylated 5-fluorouracil in the presence of $Me_3SiOSO_2CF_3$ as a catalyst.[42] As expected, its $^1$H NMR spectra showed a large coupling between protons H-1′ and H-2′ ($J_{1',2'} = 9.4$ Hz) and a small coupling between protons H-2′ and H-3′ ($J_{2',3'} = 3.3$ Hz), indicating an equatorially oriented base ring and an axial oriented azido group, respectively. Deacetylation performed with the saturated methanolic ammonia,[43] afforded the unprotected nucleoside **12a**, in good yield (72%). In the final step, catalytic hydrogenation of the azide precursor provided the target amino allopyranonucleoside **13a**, in 78% yield.

## Biological Evaluation

The compounds have been evaluated against a broad panel of DNA and RNA viruses, but were found to be inactive at the highest tested concentration (100–200 $\mu$M). Also, none of the compounds showed pronounced cytostatic or cytotoxic activity in the monolayer cell cultures used for the antiviral assays. Among all the nucleosides tested, the fluorouracil derivatives **6a**, **7a**, and **13a** proved moderately cytostatic against L1210 ($IC_{50}$: 92–174 $\mu$M) and Hela cells ($IC_{50}$: 174–176 $\mu$M), but not CEM cells ($IC_{50} > 250$ $\mu$M). The guanine analogs **6f** and **7f** also proved moderately cytostatic against HeLa cells ($IC_{50}$: 171–244 $\mu$M).



## EXPERIMENTAL

### General Methods

Thin-layer chromatography (TLC) was performed on Merck precoated 60F254 plates. The reactions were monitored by the TLC on a silica gel, with detection by the UV light (254 nm) or by charring with sulfuric acid. Flash column chromatography (FCC) was performed using silica gel (240–400 mesh, Merck). The $^1$H and $^{13}$C NMR spectra were obtained at room temperature with a Bruker 400 spectrometer at 400 and 100 MHz, respectively, using chloroform-*d* ($CDCl_3$), methanol-$d_4$ ($CD_3OD$), and dimethylsulfoxide-$d_6$ (DMSO-$d_6$) with internal tetramethylsilane (TMS). The chemical shifts ($\delta$) were given in ppm and measured downfield from TMS, and spin–spin coupling constants were in Hz. The UV–vis spectra were recorded on a PG T70 UV–VIS spectrometer and the mass spectra were obtained on a Thermo Quest Finnigan AQA Mass Spectrometer (electrospray ionization). The optical rotations were measured using an Autopol I polarimeter. The IR spectra were obtained with a Thermo Scientific Nicolet IR100 FTIR spectrometer. The acetonitrile ($CH_3CN$) was distilled from calcium hydride and stored over 3E molecular sieves. The DMF was stored over 3E molecular sieves.

***3-Azido-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (3)***

A solution of the tosylate **2**[39,40] (5.00 g, 12.1 mmol) in DMF (60.5 mL) containing suspended sodium azide ($NaN_3$) (2.35 g, 36.2 mmol) was heated under reflux for 4 hours. The DMF was removed under reduced pressure, and the residue was dissolved in ethyl acetate (EtOAc). The organic phase was washed with $H_2O$ (2×), dried over an anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by FCC (hexane–EtOAc 9:1) to give compound **3** (2.68 g, 78%, $R_f = 0.29$ in hexane–EtOAc 9:1) as an oil. The $^1$H NMR spectra agreed fully with the recorded data.[40] $[\alpha]_D^{22}$ − 36 (*c* 0.65, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 5.73 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.61 (d, 1H, H-2), 4.24 (m, 1H, H-5), 4.16–4.07 (m, 3H, H-3, H-6a, H-6b),

3.98 (dd, 1H, $J_{3,4}$ = 4.8 Hz, $J_{4,5}$ = 8.6 Hz, H-4), 1.51, 1.43, 1.38, 1.32 (4s, 12H, $4CH_3$); ESI-MS ($m/z$): 286.27 [M+H$^+$]. Anal. calcd. for $C_{12}H_{19}N_3O_5$: C, 50.52; H, 6.71; N, 14.73. Found: C, 50.81; H, 6.43; N, 14.84.

***3-Azido-3-deoxy-1,2,4,6-tetra-O-acetyl-D-glucopyranose (4)*[38]**

To a solution of compound **3** (2.68 g, 9.40 mmol) in methanol (MeOH) (13.5 mL) and $H_2O$ (81 mL) was added Amberlite IR 120 ($H^+$) (4.7 g) resin and the mixture was refluxed overnight. The reaction mixture was filtered and evaporated to dryness. The residue was then dissolved in a mixture of pyridine (33 mL) and acetic anhydride ($Ac_2O$) (17 mL). The reaction was carried out at room temperature for 1 hour, then was quenched with MeOH at 0°C, and was concentrated in vacuum. The residue was diluted with EtOAc and washed with saturated sodium bisulfate, sodium bicarbonate, and water. The organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to give anomeric acetates **4**[38] (2.98 g, 85%, $R_f$ = 0.27 in hexane–EtOAc 8:2) as a yellow foam. $[\alpha]_D^{22}$ − 2 ($c$ 0.20, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 6.32 and 5.69 ($\alpha$: d, $J_{1',2'}$ = 3.6 Hz, 0.57H, H-1; $\beta$: d, $J_{1',2'}$ = 8.2 Hz, 0.43H, H-1), 5.08–4.94 (m, 2H), 4.28–3.67 (m, 4H), 2.19–2.09 (2s, 12H, OAc). ESI-MS ($m/z$): 374.30 [M+H$^+$]. Anal. calcd. for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 45.32; H, 4.79; N, 11.57.

***General Procedure for Preparation of 3′-Azido-3′-deoxy-β-D-glucopyranonucleosides 5a–d***

A mixture of the proper base (6.97 mmol), hexamethyldisilazane (HMDS) (8.64 mmol, 1.24 eq), and saccharine (0.32 mmol, 0.046 eq) in anhydrous $CH_3CN$ (28 mL) was refluxed for 30 minutes under nitrogen. 3-Azido-3-deoxy-1,2,4,6-tetra-*O*-acetyl-D-glucopyranose (**4**)[38] (5.36 mmol) and $Me_3SiOSO_2CF_3$ (7.50 mmol, 1.4 eq) were then added and the reaction mixture was refluxed for 2 hours, cooled, neutralized with aqueous sodium bicarbonate, and extracted with dichloromethane ($CH_2Cl_2$, 1000 mL). The organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by FCC to give compounds **5**, in 60–87% yields as yellow oils.

*1-(2′,4′,6′-Tri-O-acetyl-3′-azido-3′-deoxy-β-D-glucopyranosyl)5-fluorouracil (**5a**).* Yield 87%; $R_f$ = 0.37 (EtOAc–hexane 1:1); $[\alpha]_D^{22}$ + 2 ($c$ 0.40, $CHCl_3$); $\lambda_{max}$ 265 nm ($\varepsilon$ 2219); $^1$H NMR ($CDCl_3$): $\delta$ 7.43 (d, 1H, $J_{6,F5}$ = 5.7 Hz, H-6), 5.82 (dd, 1H, $J_{1',2'}$ = 9.3 Hz, $J_{1',F5}$ = 1.4 Hz, H-1′), 5.05 (t, 1H, $J$ = 10.1 Hz, H-4′), 4.98 (t, 1H, $J$ = 9.6 Hz, H-2′), 4.28−4.11 (m, 2H, H-6a′, H-6b′), 3.93−3.86 (m, 2H, H-3′, H-5′), 2.17, 2.11 (2s, 9H, 3OAc). ESI-MS ($m/z$): 444.37 [M+H$^+$]. Anal. calcd. for $C_{16}H_{18}FN_5O_9$: C, 43.35; H, 4.09; N, 15.80. Found: C, 43.14; H, 4.41; N, 16.14.

*1-(2′,4′,6′-Tri-O-acetyl-3′-azido-3′-deoxy-β-D-glucopyranosyl)thymine (**5b**).* Yield 85%; $R_f$ = 0.40 (EtOAc–hexane 8:2); $[\alpha]_D^{22}$ − 2 ($c$ 0.27, $CHCl_3$); $\lambda_{max}$ 260 nm ($\varepsilon$ 3479); $^1$H NMR ($CDCl_3$): $\delta$ 8.40 (br s, 1H, NH), 7.13 (s, 1H,

H-6), 5.81 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 5.03 (t, 2H, $J = 9.9$ Hz, H-2′, H-4′), 4.26–4.08 (m, 2H, H-6a′, H-6b′), 3.89–3.83 (m, 2H, H-3′, H-5′), 2.16, 2.10, 2.08 (3s, 9H, 3OAc), 1.96 (s, 3H, 5-$CH_3$). ESI-MS ($m/z$): 440.40 [M+$H^+$]. Anal. calcd. for $C_{17}H_{21}N_5O_9$: C, 46.47; H, 4.82; N, 15.94. Found: C, 46.13; H, 4.56; N, 16.23.

*1-(2′,4′,6′-Tri-O-acetyl-3′-azido-3′-deoxy-β-D-glucopyranosyl)$N^4$-benzoylcytosine (**5c**).* Yield 82%; $R_f = 0.27$ (EtOAc–hexane 8:2); $[\alpha]_D^{22} + 2$ ($c$ 0.20, $CHCl_3$); $\lambda_{max}$ 264 nm ($\varepsilon$ 4151); $^1$H NMR ($CDCl_3$): $\delta$ 8.14–7.53 (m, 7H, Bz, H-5 and H-6), 6.03 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1′), 5.11–4.99 (m, 2H, H-2′, H-4′), 4.27–4.09 (m, 2H, H-6a′, H-6b′), 3.97–3.92 (m, 2H, H-3′, H-5′), 2.17, 2.09 (2s, 9H, 3OAc). ESI-MS ($m/z$): 529.49 [M+$H^+$]. Anal. calcd. for $C_{23}H_{24}N_6O_9$: C, 52.27; H, 4.58; N, 15.90. Found: C, 52.66; H, 4.24; N, 16.05.

*9-(2′,4′,6′-Tri-O-acetyl-3′-azido-3′-deoxy-β-D-glucopyranosyl)$N^2$-acetylguanine (**5d**).* Yield 60%; $R_f = 0.38$ (EtOAc); $[\alpha]_D^{22} - 4$ ($c$ 0.20, $CHCl_3$); $\lambda_{max}$ 270 nm ($\varepsilon$ 2018); $^1$H NMR ($CD_3OD$): $\delta$ 8.11 (s, 1H, H-8), 5.80 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1′), 5.71 (t, 1H, $J = 9.5$ Hz, H-2′), 5.16 (t, 1H, $J = 9.9$ Hz, H-4′), 4.28–4.12 (m, 4H, H-6a′, H-6b′, H-3′, H-5′), 2.25, 2.17, 2.04, 1.90 (4s, 12H, 4OAc). ESI-MS ($m/z$): 507.40 [M+$H^+$]. Anal. calcd. for $C_{19}H_{22}N_8O_9$: C, 45.06; H, 4.38; N, 22.13. Found: C, 45.24; H, 4.14; N, 22.32.

### *General Procedure for Preparation of 3′-Azido-3′-deoxy-β-D-glucopyranonucleosides 6a,b,e,f*

The protected nucleosides **5** (1 mmol) were treated with ammonia/MeOH (saturated at 0°C, 41.8 mL) overnight at room temperature. The solvent was evaporated under reduced pressure to afford pure **6**, in 71–81% yields as white foams.

*1-(3′-Azido-3′-deoxy-β-D-glucopyranosyl)5-fluorouracil (**6a**).* Yield 78%; $R_f =$ 0.36 (EtOAc); $[\alpha]_D^{22} - 4$ ($c$ 0.20, MeOH); $\lambda_{max}$ 266 nm ($\varepsilon$ 1467); IR (Nujol, $cm^{-1}$): 2111 (N3); $^1$H NMR (DMSO-$d_6$): $\delta$ 8.15 (d, 1H, $J_{6,F5} = 7.2$ Hz, H-6), 5.83 (d, 1H, $J_{4'\text{-OH},4'} = 5.6$ Hz, 4′-OH), 5.62 (d, 1H, $J_{2'\text{-OH},2'} = 6.5$ Hz, 2′-OH), 5.47 (dd, 1H, $J_{1',2'} = 8.9$ Hz, $J_{1',F5} = 1.7$ Hz, H-1′), 4.59 (t, 1H, $J = 5.7$ Hz, 6′-OH), 3.75–3.66 (m, 1H, H-2′), 3.65–3.58 (m, 1H, H-4′), 3.55–3.42 (m, 4H, H-3′, H-5′, H-6a′, H-6b′); $^{13}$C NMR (DMSO-$d_6$): 157.1, 149.3, 139.5, 126.9, 95.1, 86.4, 70.4, 64.2, 63.7, 55.6; ESI-MS ($m/z$): 318.25 [M+$H^+$]. Anal. calcd. for $C_{10}H_{12}FN_5O_6$: C, 37.86; H, 3.81; N, 22.08. Found: C, 37.59; H, 3.95; N, 21.85.

*1-(3′-Azido-3′-deoxy-β-D-glucopyranosyl)thymine (**6b**).* Yield 81%; $R_f = 0.48$ ($CH_2Cl_2$–MeOH 8:2); $[\alpha]_D^{22} - 2$ ($c$ 0.20, MeOH); $\lambda_{max}$ 264 nm ($\varepsilon$ 1547); IR (Nujol, $cm^{-1}$): 2107 (N3); $^1$H NMR ($CD_3OD$): $\delta$ 7.52 (s, 1H, H-6), 5.56 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1′), 3.82 (dd, 1H, $J_{6a,6b} = 12.1$ Hz, $J_{5,6a} = 1.9$ Hz, H-6a), 3.70 (dd, 1H, $J_{5,6b} = 4.7$ Hz, H-6b), 3.59 (t, 1H, $J = 9.2$ Hz, H-2′), 3.50–3.44 (m, 3H, H-3′, H-4′, H-5′), 1.87 (s, 3H, 5-$CH_3$); $^{13}$C NMR ($CD_3OD$): 162.4, 148.5, 136.9, 112.3, 96.7, 82.1, 68.1, 66.0, 62.6, 54.8, 11.7; ESI-MS ($m/z$):

314.29 [M+H$^+$]. Anal. calcd. for $C_{11}H_{15}N_5O_6$: C, 42.17; H, 4.83; N, 22.36. Found: C, 41.81; H, 4.97; N, 22.48.

*1-(3′-Azido-3′-deoxy-β-D-glucopyranosyl)cytosine (**6e**).* Yield 71%; $R_f = 0.25$ (EtOAc–MeOH 9:1); $[\alpha]_D^{22} - 20$ ($c$ 0.20, MeOH); $\lambda_{max}$ 259 nm ($\varepsilon$ 1894); IR (Nujol, cm$^{-1}$): 2111 (N3); $^1$H NMR ($CD_3OD$): $\delta$ 7.66 (d, 1H, $J_{5,6} = 7.6$ Hz, H-6), 5.91 (d, 1H, H-5), 5.68 (d, 1H, $J_{1',2'} = 8.8$ Hz, H-1′), 3.83 (m, 1H, H-2′), 3.73–3.46 (m, 5H, H-3′, H-4′, H-5′, H-6a′, H-6b′); $^{13}$C NMR ($CD_3OD$): 167.4, 153.8, 141.7, 96.1, 95.3, 82.3, 68.3, 66.3, 60.8, 56.9; ESI-MS ($m/z$): 299.24 [M+H$^+$]. Anal. calcd. for $C_{10}H_{14}N_6O_5$: C, 40.27; H, 4.73; N, 28.18. Found: C, 40.65; H, 4.37; N, 28.36.

*9-(3′-Azido-3′-deoxy-β-D-glucopyranosyl)guanine (**6f**).* Yield 76%; $R_f = 0.4$ (EtOAc–MeOH 7:3); $[\alpha]_D^{22} - 40$ ($c$ 0.20, MeOH); $\lambda_{max}$ 278 nm ($\varepsilon$ 1203); IR (Nujol, cm$^{-1}$): 2115 (N3); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.87 (s, 1H, H-8), 6.48 (br s, 2H, $NH_2$), 5.84 (d, 1H, $J_{4'\text{-OH},4'} = 6.1$ Hz, 4′-OH), 5.84 (d, 1H, $J_{2'\text{-OH},2'} = 5.6$ Hz, 2′-OH), 5.23 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 4.66 (t, 1H, $J = 5.7$ Hz, 6′-OH), 3.95 (m, 1H, H-2′), 3.68–3.63 (m, 1H, H-4′), 3.52–3.39 (m, 4H, H-3′, H-5′, H-6a′, H-6b′); $^{13}$C NMR (DMSO-$d_6$): 156.2, 155.3, 148.8, 136.1, 116.7, 97.8, 81.5, 68.9, 66.5, 62.3, 55.9; ESI-MS ($m/z$): 339.27 [M+H$^+$]. Anal. calcd. for $C_{11}H_{14}N_8O_5$: C, 39.06; H, 4.17; N, 33.12. Found: C, 38.81; H, 3.90; N, 32.93.

### *General Procedure for Preparation of 3′-Amino-3′-deoxy-β-D-glucopyranonucleosides 7a,b,e,f*

After two vacuum/$H_2$ cycles to remove air from the reaction tube, the stirred mixture of nucleosides **6** (1 mmol), Pd/C (139 mg), and triethylamine (272 $\mu$L, 1.96 mmol) in EtOAc (31 mL) and ethanol (EtOH) (31 mL) was hydrogenated at ambient pressure (balloon) and temperature (ca. 20°C) for 24 hours. The reaction mixture was filtered and the filtrate was concentrated in vacuum to give pure **7**, in 60–79% yields as white foams.

*1-(3′-Amino-3′-deoxy-β-D-glucopyranosyl)5-fluorouracil (**7a**).* Yield 76%; $R_f = 0.41$ ($CH_2Cl_2$/MeOH 1:1); $[\alpha]_D^{22} + 2$ ($c$ 0.20, DMSO); $\lambda_{max}$ 260 nm ($\varepsilon$ 1147); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.88 (d, 1H, $J_{6,F5} = 6.9$ Hz, H-6), 5.36 (d, 1H, $J_{1',2'} = 9.0$ Hz, H-1′), 3.49–3.12 (m, 8H, H-2′, H-4′, H-5′, H-6a′, H-6b′, 3OH), 2.68 (t, 1H, $J = 9.3$ Hz, H-3′), 1.74 (s, 2H, $NH_2$); $^{13}$C NMR (DMSO-$d_6$): 155.1, 149.8, 138.6, 127.2, 95.3, 81.3, 72.7, 66.8, 64.6, 55.8; ESI-MS ($m/z$): 292.25 [M+H$^+$]. Anal. calcd. for $C_{10}H_{14}FN_3O_6$: C, 41.24; H, 4.85; N, 14.43. Found: C, 40.99; H, 4.56; N, 14.80.

*1-(3′-Amino-3′-deoxy-β-D-glucopyranosyl)thymine (**7b**).* Yield 72%; $R_f = 0.2$ (EtOAc–MeOH 4:6); $[\alpha]_D^{22} + 4$ ($c$ 0.20, DMSO); $\lambda_{max}$ 262 nm ($\varepsilon$ 1554); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.50 (s, 1H, H-6), 5.32 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1′), 4.54 (br s, 1H, OH), 3.66–3.42 (m, 6H, H-4′, H-5′, H-6a′, H-6b′, 2OH), 3.06 (t, 1H, $J = 9.5$ Hz, H-2′), 2.67 (t, 1H, $J = 9.3$ Hz, H-3′), 1.77 (s, 2H, $NH_2$), 1.73 (s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): 160.9, 151.7, 136.4, 111.2, 98.1, 84.8,

70.1, 68.0, 60.1, 52.2, 13.0; ESI-MS ($m/z$): 288.32 [M+H$^+$]. Anal. calcd. for $C_{11}H_{17}N_3O_6$: C, 45.99; H, 5.96; N, 14.63. Found: C, 45.74; H, 6.11; N, 14.27.

*1-(3′-Amino-3′-deoxy-β-D-glucopyranosyl)cytosine (**7e**).* Yield 79%; $R_f = 0.28$ (MeOH); $[\alpha]_D^{22}$ + 1 ($c$ 0.20, DMSO); $\lambda_{max}$ 270 nm ($\varepsilon$ 3031); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.49 (d, 1H, $J_{5,6} = 7.5$ Hz, H-6), 5.70 (d, 1H, H-5), 5.47 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 3.66–3.40 (m, 7H, H-4′, H-5′, H-6a′, H-6b′, 3OH), 3.01 (t, 1H, $J = 9.4$ Hz, H-2′), 2.68 (t, 1H, $J = 9.3$ Hz, H-3′), 1.87 (s, 2H, $NH_2$); $^{13}$C NMR (DMSO-$d_6$): 163.9, 153.7, 140.3, 97.7, 91.7, 85.4, 70.1, 66.8, 61.3, 54.8; ESI-MS ($m/z$): 273.25 [M+H$^+$]. Anal. calcd. for $C_{10}H_{16}N_4O_5$: C, 44.12; H, 5.92; N, 20.58. Found: C, 44.41; H, 5.74; N, 20.31.

*1-(3′-Amino-3′-deoxy-β-D-glucopyranosyl)guanine (**7f**).* Yield 60%; $R_f = 0.18$ (MeOH); $[\alpha]_D^{22}$ + 2 ($c$ 0.20, DMSO); $\lambda_{max}$ 273 nm ($\varepsilon$ 2764); $^1$H NMR (DMSO-$d_6$): $\delta$ 8.16 (br s, 1H, NH), 7.86 (s, 1H, H-8), 6.49 (s, 2H, $NH_2$), 6.14 (s, 1H, OH), 5.56 (s, 1H, OH), 5.22 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 4.67–4.58 (m, 2H, H-2′, H-4′), 3.67–3.49 (m, 5H, H-3′, H-5′, H-6a′, H-6b′, OH), 1.86 (s, 2H, 3′-$NH_2$); $^{13}$C NMR (DMSO-$d_6$): 156.6, 151.7, 151.3, 136.2, 116.5, 96.5, 81.4, 70.8, 67.9, 62.0, 55.9; ESI-MS ($m/z$): 313.29 [M+H$^+$]. Anal. calcd. for $C_{11}H_{16}N_6O_5$: C, 42.31; H, 5.16; N, 26.91. Found: C, 42.59; H, 5.56; N, 26.57.

***3-Azido-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (9)***

A solution of the imidazylate sugar **8**[44] (1.00 g, 2.56 mmol) in DMF (12.8 mL) was added slowly to a solution of $NaN_3$ (499 mg, 7.68 mmol) at 50°C. The reaction was complete after 12 hours stirring at 50°C. DMF was removed under reduced pressure, and the residue was dissolved in EtOAc. The organic phase was washed with $H_2O$ (2×), dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by FCC (hexane–EtOAc 3:1) to give compound **9** (576 mg, 79%, $R_f = 0.22$ in hexane–EtOAc 3:1) as a colorless liquid. The $^1$H NMR spectra agreed fully with the recorded data.[47] $^1$H NMR ($CDCl_3$): $\delta$ 5.80 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.74 (t, $J = 4.6$ Hz, H-2), 4.24–3.98 (m, 4H, H-4, H-5, H-6a, H-6b), 3.54 (dd, $J_{2,3} = 4.7$ Hz, $J_{3,4} = 9.0$ Hz, H-3), 1.60, 1.50, 1.40, 1.39 (4s, 12H, 4$CH_3$). ESI-MS ($m/z$): 286.32 [M+H$^+$]. Anal. calcd. for $C_{12}H_{19}N_3O_5$: C, 50.52; H, 6.71; N, 14.73. Found: C, 50.78; H, 7.08; N, 14.57.

***3-Azido-3-deoxy-1,2,4,6-tetra-O-acetyl-β-D-allopyranose (10)[46]***

Compound **10**[46] was prepared from compound **9** according to the general method described for compound **4**. Yield 69%; $R_f = 0.22$ (EtOAc–hexane 1:3); $[\alpha]_D^{22}$ − 16 ($c$ 0.20, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 5.97 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.99–4.94 (m, 2H, H-2, H-4), 4.48 (t, 1H, $J_{2,3} = J_{3,4} = 3.4$ Hz, H-3), 4.29 (dd, 1H, $J_{5,6} = 4.4$ Hz, $J_{6,6'} = 12.4$ Hz, H-6a), 4.21–4.13 (m, 2H, H-5, H-6b), 2.14, 2.13, 2.09 (3s, 12H, 4OAc). ESI-MS ($m/z$): 374.33 [M+H$^+$]. Anal. calcd. for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 45.35; H, 4.87; N, 11.36.

***1-(2′,4′,6′-Tri-O-acetyl-3′-azido-3′-deoxy-β-D-allopyranosyl)5-fluorouracil (11a)***

Compound **11a** was prepared according to the general method described for compounds **5a–d**. Yield 60%; $R_f = 0.34$ (EtOAc–hexane 1:1); $[\alpha]_D^{22} + 8$ (*c* 0.20, $CHCl_3$); $\lambda_{max}$ 265 nm ($\varepsilon$ 3169); $^1$H NMR ($CDCl_3$): $\delta$ 8.67 (br s, 1H, NH), 7.36 (d, 1H, $J_{6,F5} = 5.8$ Hz, H-6), 6.10 (dd, 1H, $J_{1',2'} = 9.4$ Hz, $J_{1',F5} = 1.2$ Hz, H-1′), 5.07 (dd, 1H, $J_{2',3'} = 3.3$ Hz, H-2′), 5.00 (dd, 1H, $J_{3',4'} = 3.1$ Hz, $J_{4',5'} = 9.7$ Hz, H-4′), 4.51 (t, 1H, H-3′), 4.31–4.17 (m, 3H, H-5′, H-6a′, H-6b′), 2.17, 2.12, 2.11 (3s, 9H, 3OAc). ESI-MS (*m/z*): 444.35 [M+H$^+$]. Anal. calcd. for $C_{16}H_{18}FN_5O_9$: C, 43.35; H, 4.09; N, 15.80. Found: C, 43.55; H, 4.24; N, 16.06.

***1-(3′-Azido-3′-deoxy-β-D-allopyranosyl)5-fluorouracil (12a)***

Compound **12a** was prepared according to the general method described for compounds **6a,b,e,f**. Yield 72%; $R_f = 0.22$ ($CH_2Cl_2$–MeOH 8.5:1.5); $[\alpha]_D^{22} + 6$ (*c* 0.20, MeOH); $\lambda_{max}$ 266 nm ($\varepsilon$ 1671); IR (Nujol, cm$^{-1}$): 2115 (N3); $^1$H NMR ($CD_3OD$): $\delta$ 7.90 (d, 1H, $J_{6,F5} = 6.7$ Hz, H-6), 5.78 (dd, 1H, $J_{1',2'} = 9.3$ Hz, $J_{1',F5} = 1.6$ Hz, H-1′), 4.21 (t, 1H, $J = 3.2$ Hz, H-3′), 3.92 (dd, 1H, H-2′), 3.86–3.68 (m, 4H, H-4′, H-5′, H-6a′, H-6b′). $^{13}$C NMR ($CD_3OD$): 157.8, 149.6, 141.2, 126.4, 95.3, 81.8, 71.3, 65.9, 60.2, 56.7; ESI-MS (*m/z*): 318.26 [M+H$^+$]. Anal. calcd. for $C_{10}H_{12}FN_5O_6$: C, 37.86; H, 3.81; N, 22.08. Found: C, 38.05; H, 4.02; N, 22.45.

***1-(3′-Amino-3′-deoxy-β-D-allopyranosyl)5-fluorouracil (13a)***

Compound **13a** was prepared according to the general method described for compounds **7a,b,e,f**. Yield 78%; $R_f = 0.18$ (EtOAc–MeOH 8:2); $[\alpha]_D^{22} + 2$ (*c* 0.20, DMSO); $\lambda_{max}$ 264 nm ($\varepsilon$ 1012); $^1$H NMR (DMSO-$d_6$): $\delta$ 8.04 (d, 1H, $J_{6,F5} = 7.2$ Hz, H-6), 5.85 (dd, 1H, $J_{1',2'} = 8.7$ Hz, $J_{1',F5} = 1.4$ Hz, H-1′), 5.63 (t, 1H, $J = 4.6$ Hz, H-3′), 5.31 (dd, 1H, H-2′), 5.15 (dd, 1H, $J_{4',5'} = 8.3$ Hz, H-4′), 3.82–3.55 (m, 6H, H-5′, H-6a′, H-6b′, 3OH), 1.22 (s, 2H, $NH_2$); $^{13}$C NMR (DMSO-$d_6$): 157.8, 149.5, 142.4, 125.6, 96.5, 84.7, 71.8, 70.2, 60.0, 53.5; ESI-MS (*m/z*): 292.25 [M+H$^+$]. Anal. calcd. for $C_{10}H_{14}FN_3O_6$: C, 41.24; H, 4.85; N, 14.43. Found: C, 40.87; H, 5.06; N, 14.72.

## Antiviral Activity Assays

The antiviral assays, other than the anti-HIV assays, were based on the inhibition of virus-induced cytopathicity or plaque formation in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, human cytomegalovirus (HCMV), and varicella-zoster virus (VZV)], Vero (parainfluenza-3, reovirus-1, Sindbis virus, and Coxsackie B4), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) or MDCK [influenza A (H1N1; H3N2) and influenza B] cell cultures. The confluent cell cultures (or nearly confluent for MDCK cells)

in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque-forming units (PFU) (for VZV) in the presence of varying concentrations (100, 20, ... $\mu$M) of the test compounds. The viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The antiviral activity was expressed as the $EC_{50}$ or the compound concentration required to reduce virus-induced cytopathogenicity or viral plaque (VZV) plaque formation by 50%. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. Alternatively, the cytostatic activity of the test compounds was measured based on the inhibition of cell growth. HEL cells were seeded at a rate of $5 \times 10^3$ cells/well into 96-well microtiter plates and allowed to proliferate for 24 hours. Then, the medium containing different concentrations of the test compounds was added. After three days of incubation at 37°C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the $CC_{50}$, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/$cm^3$) cells were infected with 100 $CCID_{50}$ of HIV($III_B$) or HIV-2(ROD)/mL and seeded in 200 $\mu$L wells of a microtiter plate containing appropriate dilutions of the test compounds. After four days of incubation at 37°C, the HIV-induced CEM giant cell formation was examined microscopically.



### Antiproliferative Assays

The cytostatic effects of the test compounds on murine leukemia cells (L1210), human T-lymphocyte cells (CEM), and human cervix carcinoma cells (HeLa) were evaluated as follows: an appropriate number of cells suspended in growth medium were allowed to proliferate in 200-$\mu$L-wells of 96-well-microtiter plates in the presence of variable amounts of test compounds at 37°C in a humidified $CO_2$-controlled atmosphere. After 48 hours (L1210), 72 hours (CEM), or 96 hours (HeLa), the number of cells was counted in a Coulter counter. The $IC_{50}$ value was defined as the compound concentration required to inhibit cell proliferation by 50%.

## REFERENCES

1. Galmarini, C.M.; Mackey, J.R.; Dumontet, C. Nucleoside analogues and nucleobases in cancer treatment. *Lancet Oncol.* **2002**, 3, 415–424.
2. De Clercq, E. Highlights in the discovery of antiviral drugs: A personal retrospective. *J. Med. Chem.* **2010**, 53, 1438–1450.
3. De Clercq, E. In search of a selective therapy of viral infections. *Antiviral Res.* **2010**, 85, 19–24.

4. Komiotis, D.; Manta, S.; Tsoukala, E.; Tzioumaki, N. Antiviral unsaturated nucleosides. *Curr. Med. Chem.: Anti-Infect. Agents* **2008**, 7, 219–244.
5. Tsoukala, E.; Manta, S.; Kiritsis, C.; Komiotis, D. Keto and exomethylene pyranonucleosides as antitumor agents. *Mini Rev. Med. Chem.* **2012**, 12, 255–275.
6. Meng, W.D.; Qing, F.L. Fluorinated nucleosides as antiviral and anti-tumor agents. *Curr. Top. Med. Chem.* **2006**, 6, 1499–1528.
7. Watanabe, K.A.; Beranek, J.; Friedman, H.A.; Fox, J.J. Nucleosides. XXVII. 3′-Amino-3′-deoxyhexopyranosyl nucleosides. II. Synthesis of 1-(3′-amino-3′-deoxy-beta-D-glucopyranosyl) pyrimidines and related compounds. *J. Org. Chem.* **1965**, 30, 2735–2739.
8. Friedman, H.A.; Watanabe, K.A.; Fox, J.J. Nucleosides. 43. 3′-Amino-3′-deoxyhexopyranosyl nucleosides. V. Studies on the preparation of aminoacyl derivatives of amino sugar nucleosides. *J. Org. Chem.* **1967**, 32, 3775–3780.
9. Beranek, J.; Friedman, H.A.; Watanabe, K.A.; Fox, J.J. Nucleosides XXVIII. 3′-Amino-3′-deoxyhexopyranosyl nucleosides. Part III. Synthesis of 3′-amino-3′-deoxyhexopyranosyl adenines. *J. Heterocyclic Chem.* **1965**, 2, 188–191.
10. Suhadolnik, R.J. *Nucleoside Antibiotics*, Wiley-Interscience: New York, 1970; pp. 3–50.
11. Nakanishi, T.; Tomita, F.; Suzuki, T. Production of a new aminonucleoside, 9-(2′-Amino-2′-deoxypentofuranosyl) guanine, by aerobacter sp. *Agric. Biol. Chem.* **1974**, 38, 2465–2469.
12. Botta, O.; Moyroud, E.; Lobato, C.; Strazewski, P. Synthesis of 3′-azido- and 3′-amino-3′-deoxyadenosine in both enantiomeric forms. *Tetrahedron* **1998**, 54, 13529–13546.
13. Lin, T.S.; Chen, M.S.; McLaren, C.; Gao, Y.S.; Ghazzouli, I.; Prusoff, W.H. Synthesis and antiviral activity of various 3′-azido, 3′-amino, 2′,3′-unsaturated, and 2′,3′-dideoxy analogues of pyrimidine deoxyribonucleosides against retroviruses. *J. Med. Chem.* **1987**, 30, 440–444.
14. Pathak, T. Azidonucleosides: Synthesis, reactions, and biological properties. *Chem. Rev.* **2002**, 102, 1623–1668.
15. Timoshchuk, V.A.; Hogrefe, R.I.; Vaghefi, M.M. Improved and reliable synthesis of 3′-azido-2′,3′-dideoxyguanosine derivatives. *Nucleosides Nucleotides Nucleic Acids* **2004**, 23, 171–181.
16. Gadthula, S.; Chu, C.K.; Schinazi, R.F. Synthesis and anti-HIV activity of beta-D-3′-azido-2′,3′-unsaturated nucleosides and beta-D-3′-azido-3′-deoxyribofuranosylnucleosides. *Nucleosides Nucleotides Nucleic Acids* **2005**, 24, 1707–1727.
17. Gurjar, M.K.; Pawar, S.M.; Rama Rao, A.V. A synthesis of 1,5-di-*O*-acetyl- 3-azido-2,3-dideoxy-D-ribofuranose. *J. Carbohydr. Chem.* **1988**, 7, 271–275.
18. Zhang, H.W; Detorio, M.; Herman, B.D; Solomon, S.; Bassit, L.; Nettles, J.H.; Obikhod, A.; Tao, S.J.; Mellors, J.W.; Sluis-Cremer, N.; Coats, S.J.; Schinazi, R.F. Synthesis, antiviral activity, cytotoxicity and cellular pharmacology of l-3′-azido-2′,3′-dideoxypurine nucleosides. *Eur. J. Med. Chem.* **2011**, 46, 3832–3844.
19. Zhang, H.W.; Coats, S.J.; Bondada, L.; Amblard, F.; Detorio, M.; Asif, G.; Fromentin, E.; Solomon, S.; Obikhod, A.; Whitaker, T.; Sluis-Cremer, N.; Mellors, J.W.; Schinazi, R.F. Synthesis and evaluation of 3′-azido-2′,3′-dideoxypurine nucleosides as inhibitors of human immunodeficiency virus. *Bioorg. Med. Chem. Lett.* **2010**, 20, 60–64.
20. Verheggen, I.; Van Aerschot, A.; Toppet, S.; Snoeck, R.; Janssen, G.; Balzarini, J.; DeClercq, E.; Herdewijn, P. Synthesis and antiherpes virus activity of 1,5-anhydrohexitol nucleosides. *J. Med. Chem.* **1993**, 36, 2033–2040.
21. 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. Synthesis, biological evaluation, and structure analysis of a series of new 1,5-anhydrohexitol nucleosides. *J. Med. Chem.* **1995**, 38, 826–835.
22. Haouz, A.; Vanheusden, V.; Munier-Lehmann, H.; Froeyen, M.; Herdewijn, P.; Van Calenbergh, S.; Delarue, M. Enzymatic and structural analysis of inhibitors designed against mycobacterium tuberculosis thymidylate kinase, new insights into the phosphoryl transfer mechanism. *J. Biol. Chem.* **2003**, 278, 4963–4971.
23. Spanou, C.; Manta, S.; Komiotis, D.; Dervishi, A.; Kouretas, D. Antioxidant activity of a series of fluorinated pyrano-nucleoside analogues of $N^4$-benzoyl cytosine and $N^6$-benzoyl adenine. *Int. J. Mol. Sci.* **2007**, 8, 695–704.
24. Spanou, C.; Tzioumaki, N.; Manta, S.; Margaris, P.; Kouretas, D.; Komiotis, D.; Liadaki, K. Unsaturated keto and exomethylene pyranonucleoside analogues of thymine and uracil exhibit potent antioxidant properties. *Pharmacol. Pharm.* **2011**, 2, 122–126.

25. Manta, S.; Agelis, G.; Botić, T.; Cencič, A.; Komiotis, D. Fluoro-ketopyranosyl nucleosides: Synthesis and biological evaluation of 3-fluoro-2-keto-$\beta$-D-glucopyranosyl derivatives of $N^4$-benzoyl cytosine. *Bioorg. Med. Chem.* **2007**, 15, 980–987.
26. Manta, S.; Agelis, G.; Botić, T.; Cencič, A.; Komiotis, D. Unsaturated fluoro-ketopyranosyl nucleosides: Synthesis and biological evaluation of 3-fluoro-4-keto-$\beta$-D-glucopyranosyl derivatives of $N^4$-benzoyl cytosine and $N^6$-benzoyl adenine. *Eur. J. Med. Chem.* **2008**, 43, 420–428.
27. Manta, S.; Tzioumaki, N.; Tsoukala, E.; Panagiotopoulou, A.; Pelecanou, M.; Balzarini, J.; Komiotis, D. Unsaturated dideoxy fluoro-ketopyranosyl nucleosides as potent antitumor agents: A convenient synthesis of 2,6-dideoxy-3-fluoro-4-keto-$\beta$-D-glucopyranosyl analogues of uracil, 5-fluorouracil, thymine, $N^4$-benzoyl cytosine and $N^6$-benzoyl adenine. *Eur. J. Med. Chem.* **2009**, 44, 4764–4771.
28. Manta, S.; Tsoukala, E.; Tzioumaki, N.; Goropevšek, A.; Pamulapati, R.T.; Cencič, A.; Balzarini J.; Komiotis, D. Dideoxy fluoro-ketopyranosyl nucleosides as potent antiviral agents: Synthesis and biological evaluation of 2,3- and 3,4-dideoxy-3-fluoro 4- and -2-keto-$\beta$-D-glucopyranosyl derivatives of $N^4$-benzoyl cytosine. *Eur. J. Med. Chem.* **2009**, 44, 2696–2704.
29. Manta, S.; Tsoukala, E.; Tzioumaki, N.; Kiritsis, C.; Balzarini, J.; Komiotis, D. Synthesis of 4,6-dideoxy-3-fluoro-2-keto-$\beta$-D-glucopyranosyl analogues of 5-fluorouracil, $N^6$-benzoyl adenine, uracil, thymine and $N^4$-benzoyl cytosine and evaluation of their antitumor activities. *Bioorg. Chem.* **2010**, 38, 48–55.
30. Tsoukala, E.; Manta, S.; Tzioumaki, N.; Kiritsis, C.; Komiotis, D. Keto-fluorothiopyranosyl nucleosides: A convenient synthesis of 2- and 4-keto-3-fluoro-5-thioxylopyranosyl thymine analogs. *Carbohydr. Res.* **2011**, 346, 2011–2015.
31. Agelis, G.; Tzioumaki, N.; Botić, T.; Cencič, A.; Komiotis, D. Exomethylene pyranonucleosides: Efficient synthesis and biological evaluation of 1-(2,3,4-trideoxy-2-methylene-$\beta$-D-glycero-hex-3-enopyranosyl)thymine. *Bioorg. Med. Chem.* **2007**, 15, 5448–5456.
32. Agelis, G.; Tzioumaki, N.; Tselios, T.; Botić, T.; Cencič, A.; Komiotis, D. Exomethylene pyranonucleosides: Synthesis, conformational analysis and biological evaluation of 1-(2,3,4-trideoxy-4-methylene-$\alpha$-D-glycero-hex-2-enopyranosyl)uracile. *Eur. J. Med. Chem.* **2008**, 43, 1366–1375.
33. Kiritsis, C.; Manta, S.; Parmenopoulou, V.; Balzarini, J.; Komiotis, D. Branched-chain *C*-cyano pyranonucleosides: Synthesis of 3′-*C*-cyano & 3′-*C*-cyano-3′-deoxy pyrimidine pyranonucleosides as novel cytotoxic agents. *Eur. J. Med. Chem.* **2011**, 46, 5668–5674.
34. Kiritsis, C.; Manta, S.; Papasotiriou, I.; Coutouli-Argyropoulou, E.; Trakossas, S.; Balzarini, J.; Komiotis, D. Synthesis and biological evaluation of 3′-*C*-ethynyl and 3′-*C*-(1,4-disubstituted-1,2,3-triazolo) double-headed pyranonucleosides. *Med. Chem.*, **2012**, 8, 320–329.
35. Tzioumaki, N.; Manta, S.; Tsoukala, E.; Vande Voorde, J.; Liekens, S.; Komiotis, D.; Balzarini, J. Synthesis and biological evaluation of unsaturated keto and exomethylene D-arabinopyranonucleoside analogs: Novel 5-fluorouracil analogs that target thymidylate synthase. *Eur. J. Med. Chem.* **2011**, 46, 993–1005.
36. Balatsos, N.A.; Vlachakis, D.; Maragozidis, P.; Manta, S.; Anastasakis, D.; Kyritsis, A.; Vlassi, M.; Komiotis, D.; Stathopoulos, C. Competitive inhibition of human poly(A)-specific ribonuclease (PARN) by synthetic fluoro-pyranosyl nucleosides. *Biochemistry* **2009**, 48, 6044–6051.
37. Tsirkone, V.G.; Tsoukala, E.; Lamprakis, C.; Manta, S.; Hayes, J.M.; Skamnaki, V.T.; Drakou, C.; Zographos, S.E.; Komiotis, D.; Leonidas, D.D. 1-(3-Deoxy-3-fluoro-beta-D-glucopyranosyl) pyrimidine derivatives as inhibitors of glycogen phosphorylase b: Kinetic, crystallographic and modelling studies. *Bioorg. Med. Chem.* **2010**, 18, 3413–3425.
38. Jain, R.; Kamau, M.; Wang, C.; Ippolito, R.; Wang, H.; Dulina, R.; Anderson, J.; Gange, D.; Sofia, M.J. 3-azido-3-deoxy-glycopyranoside derivatives as scaffolds for the synthesis of carbohydrate-based universal pharmacophore mapping libraries. *Bioorg. Med. Chem. Lett.* **2003**, 13, 2185–2189.
39. Tsoukala, E.; Agelis, G.; Dolinsek, J.; Botić, T.; Cencič, A.; Komiotis, D. An efficient synthesis of 3-fluoro-5-thio-xylofuranosyl nucleosides of thymine, uracil, and 5-fluorouracil as potential antitumor or/and antiviral agents. *Bioorg. Med. Chem.* **2007**, 15, 3241–3247.
40. Brimacombe, J.S.; Bryan, J.G.H.; Husain, A.; Stacey, M.; Tolley, M.S. The oxidation of some carbohydrate derivatives, using acid anhydride-methyl sulphoxide mixtures and the pfitzner-moffatt reagent. Facile synthesis of 3-acetamido-3-deoxy-D-glucose and 3-amino-3-deoxy-D-xylose. *Carbohydr. Res.* **1967**, 3, 318–324.
41. Vuilhorgne, M.; Ennifar, S.; Das, C.B. Synthesis and conformational study by $^{13}$C NMR of $\beta$-pyrimidine nucleosides containing one or two hexopyranosyl subunits: Application to the Anthelmycin conformation. *Carbohydr. Res.* **1981**, 97, 19–30.

42. Vorbruggen, H.; Hoefle, G. Nucleoside syntheses. XXIII. On the mechanism of nucleoside synthesis. *Chem. Ber.* **1981**, 114, 1256–1268.
43. Vanheusden, V.; Busson, R.; Herdewijn, P.; Van Calenbergh, S. Synthesis and conformational analysis of 1-[2,4-dideoxy-4-*C*-hydroxymethyl-alpha-l-lyxopyranosyl]thymine. *J. Org. Chem.* **2004**, 69, 4446–4453.
44. Hanessian, S.; Vatèle, J.M. Design and reactivity of organic functional groups: Imidazolylsulfonate (imidazylate)—An efficient and versatile leaving group. *Tetrahedron Lett.* **1981**, 22, 3579–3582.
45. Wolfrom, M.L.; Bernsmann, J.; Horton, D. Synthesis of amino sugars by reduction of hydrazine derivatives. 5-amino-3,6-anhydro-5-deoxy-L-idose derivatives. *J. Org. Chem.* **1962**, 27, 4505–4509.
46. Worch, M.; Wittmann, V. Unexpected formation of complex bridged tetrazoles via intramolecular 1,3-dipolar cycloaddition of 1,2-*O*-cyanoalkylidene derivatives of 3-azido-3-deoxy-D-allose. *Carbohydr. Res.* **2008**, 343, 2118–2129.
47. Baer, H.H; Gan, Y. Synthesis of the methyl 3-amino-3-deoxy-$\alpha$- and $\beta$-D-allopyranosides and -allofuranosides. *Carbohydr. Res.* **1991**, 210, 233–245.