Conference paper

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Synthesis and antiproliferative evaluation of novel azido nucleosides and their phosphoramidate derivatives

DOI 10.1515/pac-2016-1218

Abstract: New xylofuranosyl and glucopyranosyl nucleoside phosphoramidates were synthesized as potential mimetics of nucleoside 5′-monophosphates. Their access involved *N*-glycosylation of uracil and 2-acetamido-6-chloropurine with 5′/6′-azido-1,2-di-*O*-acetyl glycosyl donors and subsequent Staudinger-phosphite reaction of the resulting azido nucleosides. The coupling of the purine derivative with the pyranosyl donor furnished N^9- and N^7-linked nucleosides in 1:1 ratio, whereas with the furanosyl donor, the N^9-nucleoside was the major regioisomer formed. When using uracil, only 5′/6′-azido N^1-linked nucleosides were obtained. The purine 5′/6′-azido nucleosides were converted into corresponding phosphoramidates in good yields. The antiproliferative effects of the nucleoside phosphoramidates and those of the azido counterparts on cancer cells were evaluated. While the nucleoside phosphoramidates did not show significant activities, the purine 5′/6′-azido nucleosides displayed potent effects against K562, MCF-7 and BT474 cell lines. The 5′-azidofuranosyl N^9 and N^7-linked purine nucleosides exhibited highest activity towards the chronic myeloid leukemia cell line (K562) with GI_{50} values of 13.6 and 9.7 μM, respectively. Among pyranosyl nucleosides, the N^7-linked nucleoside was the most active compound with efficacy towards all cell lines assayed and a highest effect on K562 cells (GI_{50} = 6.8 μM). Cell cycle analysis of K562 and MCF-7 cells showed that the most active compounds cause G2/M arrest.

Keywords: anticancer activity; azido nucleosides; bioactive molecules; ICS-28; nucleoside/nucleotide analogs; nucleoside phosphoramidates; *N*-glycosylation; Staudinger-phosphite reaction.

Introduction

Nucleoside and nucleotide analogs represent privileged classes of molecules in medicinal chemistry due to their broad spectrum of biological effects, which include anticancer [1, 2], antiviral [2, 3], antibiotic [4–6] or cholinesterase inhibitory properties [7–9].

Article note: A collection of invited papers based on presentations at the XXVIII International Carbohydrate Symposium (ICS-28), New Orleans, July 17–21 2016.

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Their therapeutic potential is well demonstrated and validated by the several compounds of this type in clinical use towards cancer and viral infections. Their bioactivity arises from their ability to mimic natural nucleotides and to be recognized by nucleotide-dependent enzymes. Therefore, these molecules may interfere and inhibit crucial biological pathways, such as nucleic acid synthesis, cell signaling or cell cycle regulation, among other events, the alteration or over-activation of which is a hallmark and drive the progress of some diseases [2].

Various nucleoside or nucleotide analogs can interfere with nucleic acid biosynthesis by incorporation into DNA or RNA and inhibition of DNA or RNA polymerization, which induce inhibition of cellular division or of viral replication. In these cases, the active forms of these molecules are mostly the corresponding nucleoside triphosphate metabolites which are formed intracellularly through mediation of nucleoside/nucleotide kinases.

Other enzymes using nucleotides as substrates include DNA ligases [10, 11], DNA methyltransferases [12, 13], glycosyltransferases [14, 15] or kinases involved in metabolic and signaling pathways [16–26].

Among the nucleoside derivatives that have shown potent biological effects associated with good pharmacokinetic properties for clinical application are the nucleoside phosphoramidates [27]. These nucleotide analogs, in which an amino group replaces a phosphate alkoxy or hydroxyl group, are neutral and moderately lipophilic molecules that show good cell permeability. This aspect makes their use advantageous relatively to that of cytotoxic or antiviral nucleoside and nucleoside monophosphates, which are not able to penetrate into cells due to their hydrophilic nature and often need nucleoside transporters to cross the cell membranes. Moreover, cancer cells and some virus-infected cells frequently decrease their level of nucleoside transporters as a mechanism of resistance [28–30] and therefore modified nucleosides that can enter cells by passive diffusion are desirable. The reported bioactive nucleoside phosphoramidates comprise the phosphoramidate moiety linked to the nucleoside at C-5′ by an oxygen atom and these compounds are converted to nucleoside monophosphates by intracellular enzymes, bypassing the resistance of some cells arising from deficiency of nucleoside kinases and leading ultimately to the active nucleoside triphosphates [27, 31]. Sofosbuvir is probably the best representative compound among antiviral nucleoside phosphoramidates, being the most effective agent towards hepatitis C virus (HCV) [31–33]. Other nucleotide analogs of this type also displayed strong anticancer activities, such as the cytarabine phosphoramidate, which showed higher cytotoxicity than cytarabine towards nucleoside transport-deficient and nucleoside kinase-deficient leukemia cell lines [34].

Owing to their biological potential, the access to new analogous and mimetic structures of nucleosides/nucleotides and the study of their bioactivities remains relevant.

We report herein on the synthesis of new furanosyl and pyranosyl nucleoside phosphoramidates, in which the phosphoramidate system is connected by the nitrogen atom to the sugar moiety at C-5′ or at C-6′, and evaluation of their antiproliferative activities towards cancer cells. Nucleoside phosphoramidates of this type are relatively unexploited with few synthetic reports published in the early nineties [35, 36], whereas concerning their biological interest, the only reported studies were on the incorporation of a guanosine derivative as initiating nucleotide in RNA polymerase-promoted transcriptions [37, 38].

The rational for the access of these molecules is based on their prospective intracellular conversion into nucleoside 5′/6′-phosphoramidic diphosphoric anhydrides, by successive kinase-mediated phosphorylations, and their further incorporation into nucleic acids, eventually stopping chain elongation and inducing cytotoxicity, in analogy with nucleoside triphosphates.

The ability of DNA polymerase I to incorporate nucleoside 5′-phosphoramidic diphosphoric anhydrides (designated as 5′-amino nucleoside-5′-*N*-triphosphates) into DNA by forming a internucleotide phosphoramidate linkage, has been reported [39–42]. These mimetics of nucleotide triphosphates appeared to be sufficiently stable for incorporation in the polynucleotide chain. Recently, studies on their incorporation into RNA, using various DNA-dependent RNA polymerases [43], revealed the propensity of these compounds to inhibit transcription.

Moreover, as potential mimetics of nucleotides, these molecules may act as inhibitors of nucleotide-dependent enzymes.

Protected nucleoside phosphoramidates structurally appropriate for cell penetrability were envisaged. Hence, dialkyl phosphoramidate diesters were accessed taking into account that once inside the cell, the

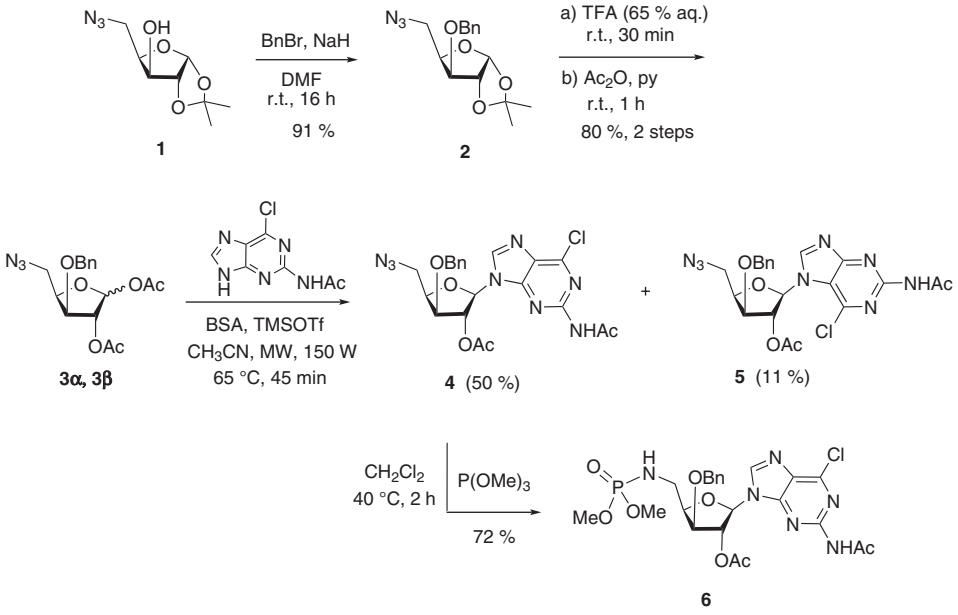
phosphoramidate moiety is susceptible to hydrolysis by phosphodiesterases, a step that normally occurs during intracellular activation of ester-type pronucleotides [27]. However, the resulting deprotected nucleoside phosphoramidates are also prompted to undergo cleavage by cellular phosphoramidases, a process that may take place in competition with kinase-mediated phosphorylation, leading to the corresponding amino nucleoside metabolites. Considering the biological effects reported for 5′-amino nucleosides, which include ability to inhibit thymidine kinases [44] and propensity for incorporation into DNA in HSV-infected cells via phosphorylation to the corresponding mimetics of nucleoside triphosphates [45], resulting in antiviral efficacy [45, 46], it is plausible to predict that such metabolites, if formed, also have potential to exhibit cytotoxicity.

Concerning the sugar moiety, since fully deprotected structures would be rather hydrophilic, hampering their penetration into cells, *O*-acetyl protection and a benzyloxy substituent were kept. While a benzyl group, besides introducing some hydrophobicity, may also allow additional interactions with aromatic amino acid residues of a biological target, the acetate groups are liable to intracellular cleavage by esterases. Ester protection is an approach that was shown to cause a significant improvement on the bioavailability, membrane permeability and sometimes on the bioactivity of nucleosides/nucleotides [47–49].

Motivated by the known anticancer activities of 3′- and 4′-azido nucleosides [50–52], in this work, the anticancer potential of the intermediate 5′- and 6′-azido furanosyl/pyranosyl nucleosides, which belong to a group of azido derivatives whose biological profile is less studied, was also evaluated.

Results and discussion

Azido sugars are useful synthetic intermediates in carbohydrate chemistry [53, 54], particularly for the preparation of various amino-functionalized derivatives, and were the suitable templates used herein for the further access to nucleoside phosphoramidates. Thus, the 5-azido-1,2-di-*O*-acetyl-3-*O*-benzyl xylofuranose derivative **3** was the glycosyl donor precursor for the synthesis of xylofuranosyl-based nucleosides and related phosphoramidates. Its preparation involved the benzylation of the 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (**1**) and subsequent acid-mediated hydrolysis of the acetonide functionality of **2**, which was followed by acetylation (Scheme 1). The 1,2-di-*O*-acetyl derivative **3** was obtained as a anomeric mixture



Scheme 1: Synthesis of 5′-azido xylofuranosyl purine nucleosides and a related nucleoside phosphoramidate.

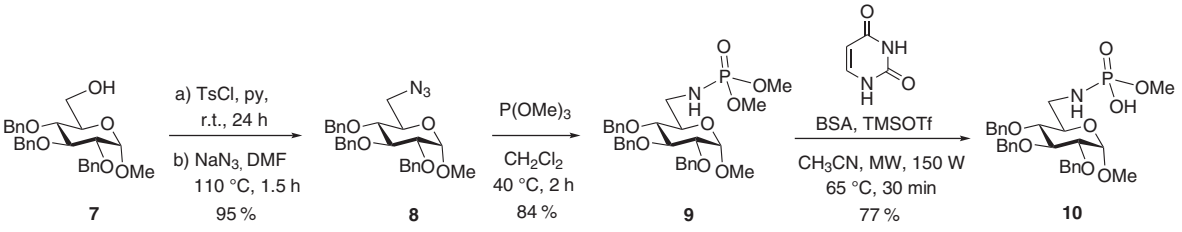
(**3α**/**3β** ratio, 1:1) in 80 % yield and it was coupled with silylated 2-acetamido-6-chloropurine in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) under microwave irradiation (MW), affording the β-N^9-linked azido nucleoside **4** and its N^7 regioisomer **5** in 50 % and 11 % yields, respectively. The assignment of the regiochemistry of the *N*-glycosidic bonds of **4** and **5** was supported on HMBC experiments, which in case of the N^9 nucleoside (**4**), showed a correlation between the anomeric proton (H-1′) and C-4 of the purine system. Moreover, the signals for H-1′, H-8, C-4 and C-8, which are deshielded in the N^7 nucleoside **5**, are also diagnostic $^1H/^{13}C$ NMR features for providing an unambiguous N^9/N^7 regiochemical elucidation.

The conversion of the azido N^9-nucleoside **4** into the corresponding phosphoramidate by the Staudinger-phosphite reaction was subsequently performed. Preliminary trials were carried out with a primary sugar azide, namely the tri-*O*-benzylated 6-azido derivative of methyl glucopyranoside (**8**), which was prepared from the partially protected glycoside **7** through tosylation and further nucleophilic displacement with sodium azide (Scheme 2). The treatment of **8** with trimethyl phosphite in dichloromethane was effective when the reaction was performed under reflux, giving the sugar phosphoramidate **9** in 84 % yield, while virtually no conversion was detected after one hour at room temperature. Hence, using the validated reaction conditions, the nucleoside phosphoramidate **6** was obtained in 72 % yield from **4**.

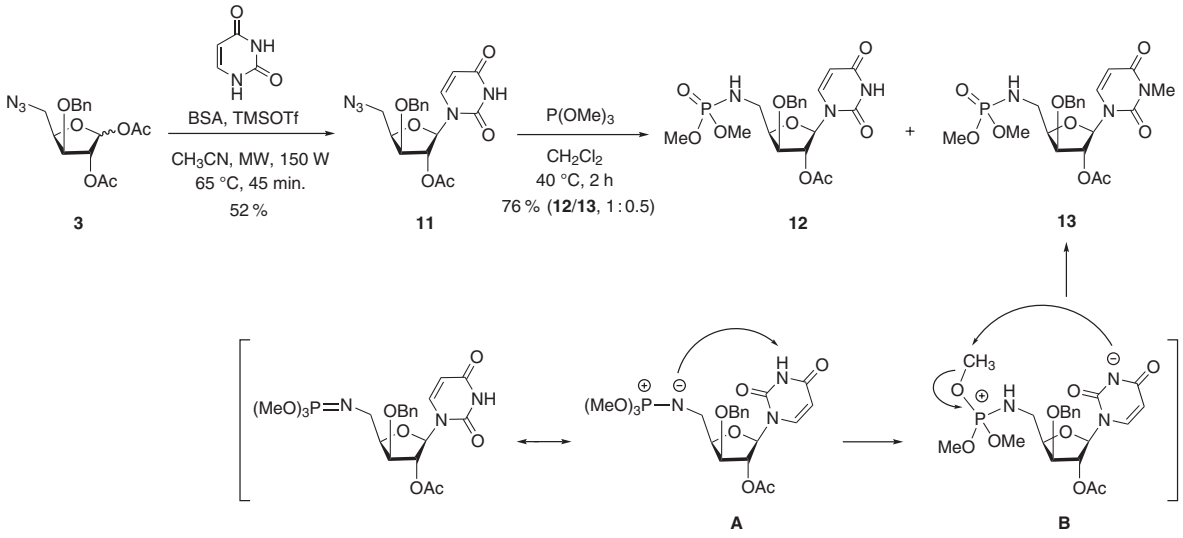
It is noteworthy to mention that in initial experiments in which the glucopyranoside 6-phosphoramidate **9** was subjected to the previously mentioned conditions for *N*-glycosylation, which are also reported to be effective for methyl glycosides, only demethylation at the phosphoramidate moiety leading to **10** occurred (Scheme 2). This transformation most probably follows a similar mechanism than that proposed for the dealkylation of phosphonate esters with trimethylsilyl chloride or bromide [55, 56]. It may involve the nucleophilic attack by a phosphoryl oxygen lone pair of **9** to the TMSOTf silicon center. The displaced triflate anion subsequently captures a methyl group reforming the P–O double bond. The resulting methyl trimethylsilyl phosphoramidate intermediate is then hydrolyzed into **10** during the work-up. This conversion, mediated by the Lewis acid used for *N*-glycosylation, indicated the need of synthesizing firstly the azido nucleosides and performing the Staundinger-phosphite reaction afterwards.

Coupling of the 1,2-di-*O*-acetyl glycosyl donor **3** with uracil under similar reaction conditions to those used for the purine derivative, afforded the xylofuranosyl uracil **11** in moderate yield (Scheme 3). The N1-C1′ linkage in **11** was assigned based on key HMBC correlations between H-1′ and both C-2/C-6. Treatment of **11** with trimethyl phosphite led to the corresponding nucleoside phosphoramidate **12** along with the N^3-methyl derivative **13** in 76 % total yield and a **12**/**13** ratio of 1:05. These products were inseparable by column chromatography and were clearly identified by NMR and HRMS analysis of the mixture. For the minor structure (**13**), a set of methyl protons, aside from those of the dimethylphosphoramidate moiety, were detected at $\delta = 3.30$ ppm exhibiting HMBC correlations with C-2 and C-4, in accordance with a N^3-methyl uracil derivative, whereas the signal for the corresponding carbon atom appeared at $\delta = 27.8$ ppm.

The formation of **13** likely arises from rearrangement of the intermediate phosphorimidate either by an inter- or intramolecular process. The thermal or Lewis acid-catalyzed rearrangement of phosphorimidates in which a alkyl group is transferred to the phosphoramidite nitrogen, leading to the *N*,*N*-disubstituted phosphoramidates, has been reported [57, 58]. In the present case, *N*-alkylation take places at the uracil moiety. As shown for the aza-ylide resonance form (A), an intramolecular rearrangement may occur through



Scheme 2: Synthesis of a 6-phosphoramidate derivative of methyl glucopyranoside and subsequent mono-dealkylation at the phosphoramidate moiety.



Scheme 3: Synthesis of a 5′-azido xylofuranosyl uracil nucleoside and related nucleoside phosphoramidates.

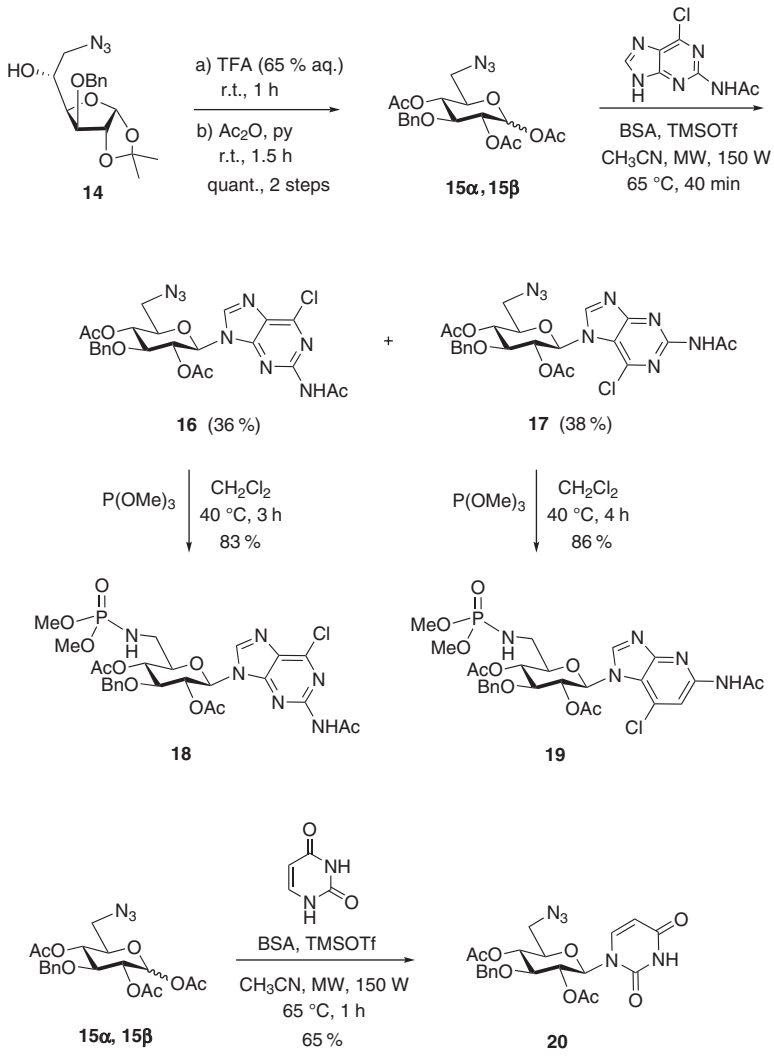
deprotonation of the uracil moiety by the negatively-charged nitrogen atom and further transferring of a methyl group to the uracil N^3 atom with accompanying formation of the double P–O bond (B).

For the synthesis of azido nucleosides and related phosphoramidates embodying a glucopyranosyl unit, a tri-*O*-acetylated 6-azido-3-*O*-benzyl glycosyl donor (**15**) was prepared as a anomeric mixture (α/β ratio 1:0.7) through cleavage of the isopropylidene group of the 6-azido-3-*O*-benzyl glucofuranose precursor **14** [59], and subsequent acetylation (Scheme 4). MW-assisted TMSOTf-mediated *N*-glycosylation of silylated 2-acetamido-6-chloropurine with **15** furnished the purine N^9-linked and N^7-linked 6′-azido nucleosides **16** and **17** in ca. 1:1 ratio and a combined 74 % yield, whose structures were assigned based on their characteristic NMR distinguishing data as for nucleosides **4**/**5**. Their reactions with trimethyl phosphite furnished the corresponding phosphoramidates **18** and **19** in 83 % and 86 % yields, respectively.

Similarly to **3**, the 6-azido glucofuranosyl donor **15** was converted into its uracil N^1-linked nucleoside **20** in good yield using the previously described *N*-glycosylation protocol.

The nucleoside phosphoramidates (**6**, **18**, **19)** as well as the azido nucleosides (**4**, **5**, **11**, **16**, **17**, **20**) were evaluated for their antiproliferative effects in chronic myeloid leukemia cell line K562 and breast cancer cell lines MCF-7 and BT474 (Table 1). None of the phosphoramidate derivatives showed anticancer activity at concentrations below 100 μM. However, moderate activities were displayed by the azido nucleosides possessing a 2-acetamido-6-chloropurine moiety. The xylofuranosyl-based nucleosides (**4**, **5**) displayed higher antiproliferative action in the leukemia cell line, with approximately similar effects from both N^9 and N^7-regioisomers (GI_{50} = 13.6 μM and 9.7 μM, respectively). The activity of the glucopyranosyl nucleosides proved to be influenced by the regiochemistry of the *N*-glycosidic bond. While the purine N^9-linked nucleoside **16** showed weak effects towards the K562 and the BT474 cell lines, its N^7-regioisomer **17** was the most active compound of this series, with significant activities in all cancer cells. K562 cells were the most sensitive to nucleoside **17**, with a GI_{50} value of 6.8 μM. Although the activity of **17** in K562 is 10 fold lower than that of the antileukemic agent imatinib, the result obtained is satisfactory and motivates further structural optimization.

Finally, flow cytometric analysis of cells treated with the most active compounds was performed in order to determine their effect on the cell cycle (Fig. 1). The experiment revealed that 6′-azido nucleoside **17** (6.25 μM concentration) causes a significant G2/M arrest in K562 and MCF-7 cells after 24 h incubation. The 5′-azido furanosyl nucleoside **4** (25 μM concentration) showed the same effect in K562 cells (>20 % G2/M cells than in control cells), while it did not produce any alteration in MCF-7 cells. In addition, increased subdiploid populations were observed in both cell lines treated with **17**, while **4** affected only K562 cells.



Scheme 4: Synthesis of 6′-azido glucopyranosyl nucleosides and related nucleoside phosphoramidates.

Table 1: Antiproliferative activity of the new azido nucleosides and nucleoside phosphoramidates in cancer cell lines.

Compound	GI_{50} (μM)[a]		
	K562	MCF-7	BT474
4	13.6	>100	93.1
5	9.7	>100	>100
6	>100	>100	>100
11	>100	>100	>100
16	48.5	>100	50.9
17	6.8	29.6	13.6
18	>100	>100	>100
19	98.1	>100	>100
20	>100	>100	>100
imatinib	0.7	>10	>10
5-fluorouracil	>50	2.4	NT

[a]All values were obtained from at least two determinations.

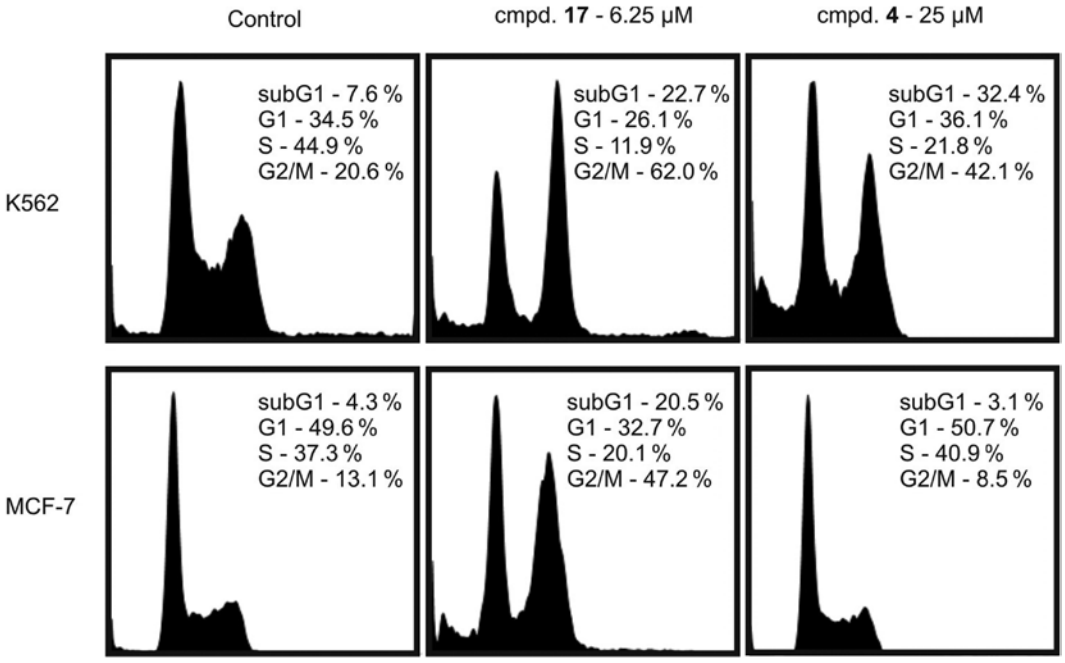


Fig. 1: Effect of **17** (6.25 μM) and **4** (25 μM) on the cell cycle of K562 and MCF-7 cancer cell lines following 24 h treatment. Flow cytometric analysis of the cell cycle (propidium iodide staining) was quantified by ModFit software, version 4.1.7.

Conclusions

Uracil and purine 5′-azido xylofuranosyl and 6′-azido glucopyranosyl-based nucleosides were synthesized from easily accessed azido-1,2-di-*O*-acetyl glycosyl donors and the purine derivatives were converted into the corresponding nucleoside 5′/6′-phosphoramidates. The latter compounds, planned as potential nucleotide mimetics, were devoid of any noticeable antiproliferative effect, which is probably due to the lability of the phosphoramidate function. In contrast, the purine 5′/6′-azido nucleosides showed significant anticancer activities, namely the furanosyl derivatives which displayed selectivity towards K562 cells and the N^7-linked pyranosyl nucleoside with action on all cell lines, especially towards K562 and BT474 cells. The uracil nucleosides were nearly inactive. These findings indicate that the azido functionality as well as the 2-chloro-6-acetamido purine unit are important for the detected bioactivities. These results motivate compounds' structural optimization aiming at improving their bioactivities and studying which structural features are essential for a selective action.

Moreover, with the preliminary studies highlighting a G2/M cell cycle arrest induced by the active azido nucleosides, further studies will be carried out to disclose their mechanism of action. That probably involves interference with DNA repair or replication machinery. Most likely, it differs from that of the known anticancer and antiviral azido nucleosides, since these new compounds lack a terminal hydroxyl group for further conversion into nucleoside triphosphates, not enabling their incorporation into DNA.

Experimental section

Chemistry

General methods

The reactions were monitored by TLC on Merck 60 F_{254} silica gel aluminium plates and the spots were detected under UV light (254 nm) and/or by spraying with a solution of 10 % H_2SO_4 in EtOH. For column chromatography,

silica gel 60 G (0.040–0.063 mm, E. Merck) was used. NMR spectra were acquired with a BRUKER Avance 400 spectrometer operating at 400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C or at 161.91 MHz for ^{31}P. Chemical shifts are expressed in parts per million and are reported relative to internal TMS, in the case of $CDCl_3$, or relative to the respective solvent peak as reference. ^{31}P Spectra were referenced according to the IUPAC recommendations for chemical shift referencing [60]. High-resolution mass measurements were performed on a High Resolution QqTOF Impact II mass spectrometer equipped with an ESI ion source (Bruker Daltonics). Spectra were recorded in positive ESI mode with external calibration. Melting points were determined with a Stuart Scientific SMP 3 apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 343 polarimeter.

5-Azido-3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (2)

To a solution of 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (222 mg, 1.03 mmol) in DMF (5 mL) at 0 °C, NaH (60 %, 82 mg, 2.06 mmol) was added. The suspension was stirred at 0 °C for 10 min., whereupon benzyl bromide (0.25 mL, 2.06 mmol) was added. The mixture was stirred at room temperature overnight. After completion of the reaction, as shown by TLC (petroleum ether/EtOAc, 3:2), water was added at 0 °C and the mixture was transferred to a separatory funnel. The aqueous phase was extracted with diethyl ether. The combined organic phases were dried with anhydrous magnesium sulfate, filtered and the solvent was evaporated under vacuum. The crude was subjected to flash column chromatography on silica-gel (petroleum ether/EtOAc, 7:1) to give the title compound as a colorless oil (286 mg, 91 %).

^{1}H RMN ($CDCl_3$, 400 MHz)*: δ 7.39–7.26 (m, 5 H, Ph), 5.92 (d, 1 H, H-1, $J_{1,2}$ = 3.8), 4.67 (d, part A of AB system, 1 H, H-a from CH_2Ph, $J_{a,b}$ = 11.8), 4.62 (d, 1 H, H-2, $J_{1,2}$ = 3.8), 4.50 (d, part B of AB system, 1 H, H-b from CH_2Ph) 4.30 (ddd, 1 H, H-4), 3.94 (d, 1 H, H-3, $J_{3,4}$ = 3), 3.58 (dd, part A of ABX system, 1 H, H-5$_a$, $J_{5a,5b}$ = 12.4, $J_{4,5a}$ = 6.7), 3.47 (dd, part B of ABX system, 1 H, H-5b, $J_{4,5b}$ = 6.4), 1.49 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3). ^{13}C (RMN, 100 MHz)*: δ 137.2 (C$_q$, arom), 128.6, 128.1, 128.8 (*C*H, Ph), 111.9 (C$_q$, *i*-Pr), 105.2 (C-1), 82.1 (C-2), 81.5 (C-3), 78.8 (C-4), 71.9 (*C*H$_2$, Bn), 49.3 (C-5), 26.8, 26.3 (2 × *C*H$_3$).

*NMR data were in agreement with the reported data [61].

1,2-Di-*O*-acetyl-5-azido-3-*O*-benzyl-5-deoxy-α,β-D-xylofuranose (3α, 3β)

A solution of 5-azido-3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (109 mg, 0.36 mmol) in aqueous trifluoroacetic acid (65 %, 3 mL) was stirred at room temp. for 30 min. The solvents were co-evaporated with toluene. The resulting residue was treated with pyridine (2 mL) and acetic anhydride (1.5 mL) and the mixture was stirred at room temp. for 1 h. The solvents were co-evaporated with toluene and the crude was purified by flash column chromatography (n-hexane/EtOAc, 6:1) to give the title compound (100 mg, 80 %, two steps, anomeric mixture, α/β ratio, 1:1) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44–7.29 (m, 10 H, C*H*, Ph, α, β), 6.44 (d, 1 H, H-1 α, $J_{1,2\,(\alpha)}$ = 4.6), 6.17 (br. d, 1 H, H-1 β), 5.35–5.30 (m, 2 H, H-2 α, H-2 β), 4.81, 4.72 (2 d, 2 × part A of AB system, 2 H, H-a, CH_2, Bn, α, β, $J_{a,b\,(\alpha)}$ = 12.0, $J_{a,b\,(\beta)}$ = 12.0), 4.62, 4.59 (2 d, 2 × part B of AB system, 2 H, H-b, CH_2, Bn, α, β), 4.48–4.39 (m, 4 H, H-4 α, H-4 β), 4.29 (t, 1 H, H-3α, $J_{2,3} \sim J_{3,4} \sim 5.4$), 4.07 (br. dd, 1 H, H-3 β), 3.65–3.51 (m, 4 H, CH_2-5, α, β), 2.14, 2.11, 2.10, 2.09 (4 s, 12 H, CH_3, OAc, α, β). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.7, 169.6, 169.4 (*C*O, Ac, α, β), 137.3, 134.2 (2 × Cq, Ph, α, β), 128.7, 128.6, 128.6, 128.3, 128.2, 128.0 (*C*H, Ph, α, β), 99.7 (C-1 β), 94.0 (C-1 α), 81.9 (C-4 β), 80.2 (C-3 β), 79.6, 79.4 (C-2 β, C-3 α), 77.9 (C-4 α), 76.6 (C-2 α), 72.7, 72.1 (CH_2Ph, α, β), 50.7, 50.4 (C-5, α, β), 21.3, 21.1, 21.0, 20.7 (*C*H$_3$, OAc, α, β). HRMS: calcd for $C_{16}H_{19}N_3O_6$ [*M* + Na]$^+$ 372.1166, found 372.1181.

General procedure for *N*-glycosylation of 2-acetamido-6-chloropurine or uracil with 1-*O*-acetyl glycosyl donors

To a suspension of nucleobase (0.255 mmol) in anhydrous acetonitrile (2 mL), *N*,*O*-bis(trimethylsilyl)acetamide (BSA, 0.13 mL, 0.51 mmol) was added and the mixture was stirred under N_2 at room temp. for 20 min. A

solution of 1-*O*-acetyl glycosyl donor (0.17 mmol) in anhydrous acetonitrile (2 mL) was added to the previous solution, which was followed by dropwise addition of trimethylsilyl triflate (TMSOTf, 0.2 mL, 1.1 mmol). The mixtute was stirred under microwave irradion (150 W, P max = 250 Psi) at 65 °C for 40–60 min. It was then diluted with DCM and neutralized with sat. aq. $NaHCO_3$ soln. The aqueous phase was extracted with dichloromethane (3×) and the combined organic phases were dried with anhydrous $MgSO_4$. After filtration and concentration under vacuum, the residue was purified by flash column chromatography on silica gel.

2-Acetamido-9-(2-*O*-acetyl-5-azido-3-*O*-benzyl-5-deoxy-β-D-xylofuranosyl)-6-chloropurine (4) and 2-acetamido-7-(2-*O*-acetyl-5-azido-3-*O*-benzyl-5-deoxy-β-D-xylofuranosyl)-6-chloropurine (5)

Compounds **4** and **5** were obtained according to the general procedure, starting from 1,2-di-*O*-acetyl-5-azido-3-*O*-benzyl-5-deoxy-α,β-D-xylofuranose (**3**, 59 mg, 0.17 mmol) and 2-acetamido-6-chloropurine (54 mg, 0.255 mmol). *N*-Glycosylation of the corresponding silylated purine with **3** in the presence of TMSOTf (0.2 mL, 1.1 mmol) was complete within 45 min. Purification by flash column chromatography on silica gel (EtOAc/hexane, from 1:1 to 3:1) afforded the N^9 nucleoside **4** (42.1 mg, 50 %) and its N^7 regioisomer **5** (11 mg, 13 %) as colorless oils.

Data for **4**: $[\alpha]_D^{20} = +26$ (c = 1, in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.27 (br. s, 2 H, H-8, NH), 7.36–7.16 (m, 5 H, Ph), 6.21 (br.s, 1 H, H-1′), 5.49 (br.s, 1 H, H-2′), 4.70 (d, 1 H, part A of AB system, H-a, Bn, $J_{a,b} = 11.7$), 4.59 (d, 1 H, part B of AB system, H-b, Bn), 4.39 (ddd, 1 H, H-4′), 4.07 (d, 1 H, H-3′, $J_{3',4'} = 3.5$), 3.74 (dd, part A of ABX system, 1 H, H-5′a, $J_{4',5'a} = 6.8$, $J_{5'a,5'b} = 12.7$), 3.63 (dd, part B of ABX system, 1 H, H-5′b, $J_{4',5'b} = 6.1$, $J_{5'a,5'b} = 12.7$), 2.50 (s, 3 H, CH_3, NHAc), 2.18 (s, 3 H, CH_3, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.8 (*C*O, NHAc), 169.6 (*C*O, Ac), 152.2 (C-2 or C-6), 152.0 (C-4), 151.4 (C-2 or C-6), 142.3 (C-8), 136.1 (Cq, Ph), 128.8, 128.6, 128.3 (*C*H, Ph),128.0 (C-5), 88.0 (C-1′), 81.8 (C-4′), 80.0 (C-3′), 79.3 (C-2′), 72.6 (*C*H_2, Bn) 49.3 (C-5′), 25.2 (*C*H_3, NHAc), 20.8 (*C*H_3, OAc). HRMS: calcd for $C_{21}H_{21}ClN_8O_5$ [*M* + H]$^+$ 501.1396, found 501.1374; calcd for [*M* + Na]$^+$ 523.1216, found 523.1194.

Data for **5**: $[\alpha]_D^{20} = +26$ (c = 0.8, in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.51 (br. s, 1 H, H-8), 8.06 (s, 1 H, NH), 7.37–7.11 (m, 5 H, Ph), 6.53 (br.s, 1 H, H-1′), 5.38 (br.s, 1 H, H-2′), 4.62 (d, 1 H, part A of AB system, H-a, Bn, $J_{a,b} = 11.8$), 4.54 (d, 1 H, part B of AB system, H-b, Bn), 4.42 (ddd, 1 H, H-4′), 4.03 (d, 1 H, H-3′, $J_{3',4'} = 3.3$), 3.79 (dd, part A of ABX system, 1 H, H-5′a, $J_{4',5'a} = 7.0$, $J_{5'a,5'b} = 12.7$), 3.66 (dd, part B of ABX system, 1 H, H-5′b, $J_{4',5'b} = 6.0$, $J_{5'a,5'b} = 12.7$), 2.61 (s, 3 H, CH_3, NHAc), 2.19 (s, 3 H, CH_3, OAc).^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.3 (*C*O, NHAc), 169.4 (*C*O, Ac), 163.8 (C-4), 152.7 (C-2 or C-6), 147.9 (C-8), 142.8 (C-2 or C-6), 136.0 (Cq, Ph), 128.8, 128.7, 128.5 (*C*H, Ph), 118.0 (C-5), 90.3 (C-1′), 82.4 (C-4′), 80.0 (C-3′), 79.8 (C-2′), 72.7 (*C*H_2, Bn) 49.1 (C-5′), 25.4 (*C*H_3, NHAc), 20.8 (*C*H_3, OAc). HRMS: calcd for $C_{21}H_{21}ClN_8O_5$ [*M* + H]$^+$ 501.1396, found 501.1380.

1-(2-*O*-Acetyl-5-azido-3-*O*-benzyl-5-deoxy-β-D-xylofuranosyl)uracil (11)

Compound **11** was obtained according to the general procedure, starting from 1,2-di-*O*-acetyl-5-azido-3-*O*-benzyl-5-deoxy-α,β-D-xylofuranose (**3**, 30 mg, 86 μmol) and uracil (15 mg, 129 μmol). *N*-Glycosylation of the corresponding silylated uracil with **3** in the presence of TMSOTf (0.1 mL, 0.56 mmol) was complete within 45 min. Purification by flash column chromatography on silica gel (EtOAc/hexane, 1:1) afforded the title compound (18 mg, 52 %) as a colorless oil.

$[\alpha]_D^{20} = -2$ (c = 0.8, in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.93 (br. s, 1 H, NH), 7.55 (d, 1 H, H-6, $J_{5,6} = 8.1$), 7.43–7.22 (m, 5 H, Ph), 6.14 (br.s, 1 H, H-1′), 5.64 (d, 1 H, H-5), 5.19 (br.s, 1 H, H-2′), 4.75 (d, 1 H, part A of AB system, H-a, Bn, $J_{a,b} = 11.5$), 4.59 (d, 1 H, part B of AB system, H-b, Bn), 4.23 (ddd, 1 H, H-4′), 3.94 (d, 1 H, H-3′, $J_{3',4'} = 2.7$), 3.69 (dd, part A of ABX system, 1 H, H-5′a, $J_{4',5'a} = 6.9$, $J_{5'a,5'b} = 12.7$), 3.59 (dd, part B of ABX system, 1 H, H-5′b, $J_{4',5'b} = 6.1$), 2.16 (s, 3 H, CH_3, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.8 (*C*O, Ac), 163.0 (C-4), 150.2 (C-2), 140.2 (C-6), 136.3 (Cq, Ph), 128.9, 128.8, 128.6 (*C*H, Ph), 102.9 (C-5), 88.8 (C-1′), 80.9 (C-4′), 79.8 (C-3′), 79.6 (C-2′), 72.1 (*C*H_2, Bn), 49.0 (C-5′), 20.9 (*C*H_3, OAc). HRMS: calcd for $C_{18}H_{19}N_5O_6$ [*M*+H]$^+$ 402.1408, found 402.1416; calcd for [*M* + Na]$^+$ 424.1228, found 424.1240.

General procedure for the Staudinger-phosphite reaction of azido derivatives with trimethyl phosphite

To a solution of azido derivative (0.05 mmol) in dichloromethane (5 mL), trimethyl phosphite (0.06 mmol, 7 μL) was added. The solution was stirred under reflux for 2–4 h, until complete conversion, as indicated by TLC. The solution was concentrated under vacuum and the residue was purified by flash column chromatography on silica gel.

2-Acetamido-9-[2-*O*-acetyl-3-*O*-benzyl-5-deoxy-5-(dimethoxyphosphoryl)amino-β-D-xylofuranosyl]-6-chloropurine (6)

Compound **6** was obtained according to the general procedure, treating 2-acetamido-9-(2-*O*-acetyl-5-azido-3-*O*-benzyl-5-deoxy-β-D-xylofuranosyl)-6-chloropurine (**4**, 25 mg, 0.05 mmol) with trimethyl phosphite (7 μL, 0.06 mmol). The reaction was complete within 2 h. Purification by flash column chromatography on silica gel (from EtOAc to EtOAc /MeOH, 4:1) afforded the title compound (21 mg, 72 %) as a colorless oil.

$[\alpha]_D^{20}$ = +6 (c = 1.2, in $CHCl_3$). ^{1}H NMR (MeOD, 400 MHz): δ 8.43 (br. s, 1 H, H-8), 7.28–7.07 (m, 5 H, Ph), 6.32 (br.s, 1 H, H-1′), 5.71 (br.s, 1 H, H-2′), 4.61 (br. s, 2 H, CH_2, Bn), 4.41 (ddd, 1 H, H-4′), 4.22 (d, 1 H, H-3′, $J_{3',4'}$ = 3.5), 3.69 (d, 6 H, 2 × OCH_3, $J_{CH3,P}$ = 11.1), 3.46–3.33 (m, 2 H, CH_2-5′a), 2.29 (s, 3 H, CH_3, NHAc), 2.17 (s, 3 H, CH_3, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1 (*C*O, NHAc, *C*O, Ac), 153.6 (C-2 or C-6), 152.0 (C-4), 151.4 (C-2 or C-6), 145.1 (C-8), 138.5 (Cq, Ph), 129.4, 129.1, 129.0 (*C*H, Ph, C-5), 89.6 (C-1′), 85.0 (C-4′, $J_{4',P}$ = 4.6), 81.7 (C-3′), 80.1 (C-2′), 73.5 (*C*H_2, Bn), 53.9, 53.8 (2 d, O*C*H_3, $J_{CH3,P}$ = 5.3), 41.2 (C-5′), 24.8 (*C*H_3, NHAc), 20.7 (*C*H_3, OAc). ^{31}P NMR (MeOD, 162 MHz) δ: 12.79. HRMS: calcd for $C_{23}H_{28}ClN_6O_8P$ $[M+H]^+$583.1468, found 583.1489; calcd for $[M+Na]^+$605.1287, found 605.1311.

1-[2-*O*-acetyl-3-*O*-benzyl-5-deoxy-5-(dimethoxyphosphoryl)amino-β-D-xylofuranosyl]uracil (12) and 1-[2-*O*-acetyl-3-*O*-benzyl-5-deoxy-5-(dimethoxyphosphoryl)amino-β-D-xylofuranosyl]-3-methyluracil (13)

Compounds **12** and **13** were obtained according to the general procedure, treating 1-(2-*O*-acetyl-5-azido-3-*O*-benzyl-5-deoxy-β-D-xylofuranosyl)uracil (**11**, 14 mg, 35 μmol) with trimethyl phosphite (10 μL, 85 μmol). The reaction was complete within 2 h. Purification by flash column chromatography on silica gel (from EtOAc/hexane, 2:1 to EtOAc /MeOH, 9:1) afforded a mixture of the title compounds (13 mg, ratio **12**/**13**, 1:0.5, 76 %) which could not be separated despite repeated attempts.

NMR data for **12**: ^{1}H NMR ($CDCl_3$, 400 MHz)*: δ 9.01 (s, NH), 7.58 (d, 1 H, H-6, $J_{5,6}$ = 8.0), 7.46–7.22 (m, 5 H, Ph), 6.08 (br.s, 1 H, H-1′), 5.62 (d, 1 H, H-5), 5.15 (br.s, 1 H, H-2′), 4.72 (d, 1 H, part A of AB system, H-a, Bn, $J_{a,b}$ = 11.5), 4.56 (d, 1 H, part B of AB system, H-b, Bn), 4.17 (ddd, 1 H, H-4′), 3.90 (d, 1 H, H-3′, $J_{3',4'}$ = 2.8), 3.71, 3.68 (2 d, 6 H, 2 × OCH_3, $J_{CH3,P}$ = 11.0), 3.41–3.21 (m, 2 H, CH_2-5′), 2.99 (t, 1 H, N*H*), 2.14 (s, 3 H, CH_3, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz)*: δ 169.8 (*C*O, Ac), 163.0 (C-4), 150.2 (C-2), 140.3 (C-6), 136.4 (Cq, Ph), 128.9, 128.8, 128.5 (*C*H, Ph), 102.7 (C-5), 88.7 (C-1′), 82.4 (C-4′), 79.9, 79.8 (C-3′ C-2′), 72.9 (*C*H_2, Bn), 53.5, 53.4 (2 d, O*C*H_3, $J_{CH3,P}$ = 5.6), 39.9 (C-5′), 21.0 (*C*H_3, OAc).

NMR data for **13**: ^{1}H NMR ($CDCl_3$, 400 MHz)*: δ 7.55 (d, 1 H, H-6, $J_{5,6}$ = 8.0), 7.46–7.22 (m, 5 H, Ph), 6.08 (br.s, 1 H, H-1′), 5.70 (d, 1 H, H-5), 5.15 (br.s, 1 H, H-2′), 4.70 (d, 1 H, part A of AB system, H-a, Bn, $J_{a,b}$ = 11.5), 4.56 (d, 1 H, part B of AB system, H-b, Bn), 4.17 (ddd, 1 H, H-4′), 3.90 (d, 1 H, H-3′, $J_{3',4'}$ = 2.8), 3.71, 3.68 (2 d, 6 H, 2 × OCH_3, $J_{CH3,P}$ = 11.0), 3.41–3.21 (m, 4 H, CH_2-5′, N-CH_3), 2.94 (t, 1 H, N*H*), 2.15 (s, 3 H, CH_3, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz)*: δ 169.8 (*C*O, Ac), 162.8 (C-4), 151.1 (C-2), 137.9 (C-6), 136.5 (Cq, Ph), 128.9, 128.7, 128.5 (*C*H, Ph), 101.9 (C-5), 89.7 (C-1′), 82.5 (C-4′), 79.9, 79.8 (C-3′, C-2′), 72.9 (*C*H_2, Bn), 53.5, 53.4 (2 d, O*C*H_3, $J_{CH3,P}$ = 5.6), 39.9 (C-5′), 27.8 (N*C*H_3), 21.0 (*C*H_3, OAc).

*Data extracted from the spectrum of the mixture containing **12**/**13**.

^{31}P NMR (MeOD, 162 MHz) δ: 11.22, 11.23. HRMS: calcd for $C_{20}H_{26}N_3O_9P$ $[M+H]^+$484.1479, found 484.1488; calcd for $[M+Na]^+$506.1299, found 506.1298. HRMS: calcd for $C_{21}H_{28}N_3O_9P$ $[M+H]^+$ 498.1636, found 498.1641; calcd for $[M+Na]^+$ 520.1455, found 520.1454.

Methyl 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranoside (8)

To a solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**7**, 52 mg, 0.11 mmol) in dichloromethane and pyridine (3 mL, 1:1) under nitrogen, tosyl chloride (40 mg, 0.21 mmol) was added and the solution was stirred at room temp. for 24 h. Then, water was added, and the mixture was extracted with ethyl acetate. The combined organic layers were washed with water and dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent under vacuum, the residue was dissolved in *N*,*N*-dimethylformamide (DMF, 3 mL) and sodium azide (40 mg, 0.62 mmol) was added. The mixture was stirred at 110 °C for 1.5 h. Then water was added, and the mixture was extracted with ethyl acetate. The combined organic layers were washed with water and dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the residue was subjected to column chromatography (EtOAc/hexane, 1:4) to afford the title compound (52 mg, 95 %) as a colorless oil.

NMR data: ^{1}H NMR (400 MHz, $CDCl_3$)*: δ 7.47–7.20 (m, 15 H, C*H*, Ph), 5.00 (d, part A of AB system, H-a, CH_2Ph, $J_{a,b}$ = 10.8), 4.90 (d, part A of AB system, H-a, CH_2Ph, $J_{a,b}$ = 10.9), 4.85–4.77 (m, 2 H, H-a, H-b, CH_2Ph), 4.67 (d, part B of AB system, H-b, CH_2Ph), 4.61 (d, 1 H, H-1, $J_{1,2}$ = 3.5), 4.57 (d, part B of AB system, H-b, CH_2Ph), 3.98 (t, 1 H, H-3, $J_{2,3}$ = $J_{3,4}$ = 9.3), 3.78 (ddd, 1 H, H-5), 3.54 (dd, 1 H, H-2), 3.48–3.38 (m, 5 H, H-4, H-6a, OCH_3), 3.33 (dd, part B of ABX system, 1 H, H-6 b, $J_{5,6b}$ = 5.7, $J_{6a,6b}$ = 13.1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 138.1, 138.0 (3 × Cq, Ph), 128.6, 128.6, 128.2, 128.1, 128.1, 128.1, 128.1, 127.8 (*C*H, Ph), 98.2 (C-1), 82.0 (C-3), 80.1 (C-2), 78.4 (C-4), 75.9, 75.3, 73.6 (CH_2, Bn), 70.0 (C-5), 55.5 (CH_3, OMe), 51.5 (C-6).

*NMR data were in agreement with the reported data [62].

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-(dimethoxyphosphoryl)amino-α-D-glucopyranoside (9)

Compound **9** was obtained according to the general procedure, treating methyl 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranoside (**8**, 50 mg, 0.1 mmol) with trimethyl phosphite (25 µL, 0.21 mmol). The reaction was complete within 2 h. Purification by flash column chromatography on silica gel (from EtOAc to EtOAc/MeOH, 9:1) afforded the title compound (49 mg, 84 %) as a colorless oil.

$[\alpha]_D^{20}$ = +10 (c = 0.9, in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.24 (m, 15 H, C*H*, Ph), 4.99 (d, part A of AB system, H-a, CH_2Ph, $J_{a,b}$ = 10.8), 4.89 (d, part A of AB system, H-a, CH_2Ph, $J_{a,b}$ = 10.8), 4.84–4.76 (m, 2 H, H-a, H-b, CH_2Ph), 4.65 (d, part B of AB system, H-b, CH_2Ph), 4.62 (d, part B of AB system, H-b, CH_2Ph), 4.51 (d, 1 H, H-1, $J_{1,2}$ = 3.5), 3.99 (t, 1 H, H-3), 3.72–3.60 (m, 7 H, 2 × OCH_3, $J_{CH3,P}$ = 10.9, H-5), 3.47 (dd, 1 H, H-2, $J_{2,3}$ = 9.6), 3.36 (s, 3 H, OCH_3), 3.34 (t, 1 H, H-4, $J_{3,4}$ = $J_{4,5}$ = 9.5), 3.17 (m, 1 H, H-6a, $J_{5,6a}$ = 3.0), 2.97 (m, 1 H, H-6b), 3.03–2.85 (m, 2H, H-6b, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 138.1, 138.1 (3 × Cq, Ph), 128.6, 128.6, 128.5, 128.2, 128.1, 128.0, 127.8 (*C*H, Ph), 98.1 (C-1), 81.9 (C-3), 80.0 (C-2), 78.6 (C-4), 75.9, 75.1, 73.5 (CH_2, Bn), 70.2 (C-5, $J_{5,P}$ = 6.8), 55.4 (CH_3, OMe), 53.2, 53.2 (2 d, OCH_3, $J_{CH3,P}$ = 5.6), 42.2 (C-6). ^{31}P NMR (MeOD, 162 MHz): δ 11.52. HRMS: calcd for $C_{30}H_{38}NO_8P$ $[M+H]^+$572. 2408, found 572. 2414; calcd for $[M+Na]^+$594.2227, found 594.2239.

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-(methoxyphosphoryl)amino-α-D-glucopyranoside (10)

Compound **10** was obtained according to the general procedure, starting from methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-(dimethoxyphosphoryl)amino-α-D-glucopyranoside (**9**, 42 mg, 0.074 mmol) and uracil (12.4 mg, 0.11 mmol). The reaction in the presence of TMSOTf (0.09 mL, 0.48 mmol) was complete within 30 min. Puri-

fication by flash column chromatography on silica gel (EtOAc/methanol, from 4:1 to 2:1) afforded the title compound (31.4 mg, 77 %) as a colorless oil.

The conversion also occurred without silylated nucleobase, being the title compound obtained in similar yield (81 %).

$[\alpha]_D^{20}$ = +14 (c = 1, in $CHCl_3$). ^{1}H NMR (400 MHz, MeOD): δ 7.44–7.18 (m, 15 H, C*H*, Ph), 4.90 (d, part A of AB system, H-a, CH_2Ph), 4.81 (d, part A of AB system, H-a, CH_2Ph, $J_{a,b}$ = 10.9), 4.48–4.63 (m, 5 H, H-1, 2 × H-b, H-a, H-b, CH_2Ph), 3.87 (t, 1 H, H-3, $J_{2,3} = J_{3,4}$ = 9.4), 3.64 (ddd, 1 H, H-5), 3.56–3.46 (m, 4 H, H-2, 2 × OCH_3, $J_{CH3,P}$ = 11.0), 3.44–3.36 (m, 4 H, H-4, OCH_3), 3.28 (ddd, 1 H, H-6a, $J_{6a,6b}$ = 13.1, $J_{5,6a}$ = 2.6, $J_{6a,P}$ = 6.3), 2.99 (dt, 1 H, H-6b, $J_{5,6b} = J_{6b,P}$ = 7). ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.1, 139.9, 139.6 (3 × Cq, Ph), 129.5, 129.3, 129.3, 129.2, 128.9, 128.6, 127.6 (*C*H, Ph), 99.0 (C-1), 83.0 (C-3), 81.6 (C-2), 80.4 (C-4), 76.5, 75.9, 74.0 (CH_2, Bn), 68.6 (C-5), 55.6 (CH_3, OMe), 52.3 (OCH_3, $J_{CH3,P}$ = 5.7), 43.7 (C-6). ^{31}P NMR (MeOD, 162 MHz): δ 7.86. HRMS: calcd for $C_{29}H_{36}NO_8P$ $[M+H]^+$ 558.2251, found 558.2250; calcd for $[M+Na]^+$580.2071, found 580.2074.

1,2,4-Tri-*O*-acetyl-6-azido-3-*O*-benzyl-6-deoxy-α,β-D-glucopyranose (15α, 15β)

A solution of 6-azido-3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**14**, 306 mg, 0.91 mmol) in aqueous trifluoroacetic acid (60 %, 5 mL) was stirred at room temp. for 1 h. The solvents were co-evaporated with toluene. The resulting residue was treated with pyridine (4 mL) and acetic anhydride (3 mL) and the mixture was stirred at room temp. for 90 min. The solvents were co-evaporated with toluene and the crude was purified by flash column chromatography (n-hexane/EtOAc, 1:1) to give the title compound (377 mg, 98 %, two steps, anomeric mixture, α/β ratio, 1:0.7) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.41–7.17 (m, 8.5 H, Ph), 6.33 (d, 1 H, H-1α, $J_{1,2\,(\alpha)}$ = 3.5), 5.65 (d, 0,7 H, H-1β, $J_{1,2\,(\beta)}$ = 8.4), 5.15 (t, 0.7 H, H-2 β), 5.11–5.01 (m, 2.7 H, H-2α, H-4α, H-4β), 4.71 (d, 1 H, H-a, Bn, α, $J_{a,b}$ = 11.9), 4.65–4.58 (m, 2.4 H, H-b, Bn, α, CH_2Ph β), 4.02–3.90 (m, 2 H, H-5α, H-3α), 3.75 (t, 0.7 H, H-3 β, $J_{2,3} \sim J_{3,4} \sim$ 9.3), 3.69 (ddd, 0.7 H, H-5 β, $J_{5,6a}$ = 6.6, $J_{5,6b}$ = 2.8, $J_{4,5}$ = 9.9), 3.39–3.22 (m, CH_2-6 α, CH_2-6 β, $J_{6a,6b(\alpha)}$ = 13.2), 2.18, 2.11, 1.99, 1.99, 1.98 (5 × s, 15.3 H, CH_3, OAc, α, β). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.7, 169.5, 169.5, 169.3, 169.2, 168.8 (6 × *C*O, OAc,α, β), 138.0, (Cq, Bn, α), 137.6 (Cq, Bn, β), 128.6, 128.6, 128.1, 127.9, 127.9, 127.6 (CH, Ph, α, β), 91.9 (C1-β), 89.3 (C1-α), 79.9 (C-3 β), 76.9 (C-5 α), 74.9 (*C*H_2, Bn, α) 74.3 (C-5 β, *C*H_2, Bn, β), 71.5, 71.5, 71.4 (C-3α, C-2 α, C-2 β), 70.5, 70.4 (C-4 α, C-4 β), 51.1 (C-6 α), 51.0 (C-6 β), 21.0, 20.9, 20.9, 20.8, 20.8, 20.7 (6 × *C*H_3, OAc, α, β). HRMS: calcd for$C_{19}H_{23}N_3O_8$ $[M+Na]^+$ 444.1377, found 444.1380.

2-Acetamido-9-(2,4-di-*O*-acetyl-6-azido-3-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-6-chloropurine (16) and 2-acetamido-7-(2,4-di-*O*-acetyl-6-azido-3-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-6-chloropurine (17)

Compound **16** and **17** were obtained according to the general procedure, starting from 1,2,4-tri-*O*-acetyl-6-azido-3-*O*-benzyl-6-deoxy-α,β-D-glucopyranose (**15**, 200 mg, 0.48 mmol) and 2-acetamido-6-chloropurine (172 mg, 0.81 mmol). *N*-Glycosylation of the corresponding silylated purine with **15** in the presence of TMSOTf (0.64 mL, 3.74 mmol) was complete within 40 min. Purification by flash column chromatography on silica gel (EtOAc/hexane, from 1:4 to 3:1) afforded the N^9 nucleoside **16** (98.5 mg, 36 %) as a colorless oil and its N^7 regioisomer **17** (104.3 mg, 38 %) as a white solid.

Data for **16**: $[\alpha]_D^{20}$ = +19 (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.73 (br.s, 1 H, N*H*), 8.25 (s, H-8), 7.38–7.20 (m, 5 H, Ph), 5.81 (d, 1 H, H-1′, $J_{1',2'}$ = 9.3), 5.60 (t, 1 H, H-2′, $J_{1',2'} = J_{2',3'}$ = 9.3), 5.26 (t, 1 H, H-4′, $J_{3',4'} = J_{4',5'}$ = 9.5), 4.68 (br.s, 2 H, CH_2, Bn), 4.00 (t, 1 H, H-3′), 3.90 (ddd, 1 H, H.5′), 3.42 (dd, part A of ABX system, 1 H, H-6′a, $J_{5',6'a}$ = 2.2, $J_{6'a,6'b}$ = 13.5), 3.33 (dd, part B of ABX system, 1 H, H-6′b, $J_{5',6'b}$ = 5.9), 2.54 (s, 3 H, CH_3, NHAc), 2.02 (s, 3 H, CH_3, OAc-4), 1.73 (s, 3 H, CH_3, OAc-2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (*C*O, NHAc), 169.5 (*C*O, Ac-4), 169.1 (*C*O, Ac-2), 152.7 (C-4), 152.4, 151.6 (C-2, C-6), 142.5 (C-8), 137.4 (Cq, Ph), 128.6, 128.1, 128.0 (*C*H, Ph), 128.0 (C-5), 80.7 (C-1′), 80.2 (C-3′), 76.3 (C-5′), 75.0 (*C*H_2, Bn), 71.8 (C-2′), 70.2 (C-4′), 50.7 (C-6′), 25.3 (*C*H_3, NHAc), 20.8 (*C*H_3, OAc-4), 20.3 (CH_3, OAc-2). HRMS: calcd for $C_{24}H_{25}ClN_8O_7$ $[M+H]^+$ 573.1608, found 573.1613.

Data for **17**: m. p. = 108.3–110 °C. $[\alpha]_D^{20}$ = +13 (c = 1, in CH_2Cl_2/MeOH, 2:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.70 (br. s, 1 H, N*H*), 8.54 (s, 1 H, H-8), 7.40–7.21 (m, 5 H, Ph), 6.06 (br.s, 1 H, H-1′), 5.69 (br.t, 1 H, H-2′), 5.26 (t, 1 H, H-4′, $J_{3',4'} = J_{4',5'} = 9.6$), 4.69 (br.s, 2 H, C$H_2$, Bn), 4.00 (t, 1 H, H-3′, $J_{2',3'} \sim J_{3',4'}$), 3.94 (ddd, 1 H, H.5′), 3.45–3.33 (m, CH_2-6′), 2.56 (s, 3 H, CH_3, NHAc), 2.03 (s, 3 H, CH_3, OAc-4), 1.81 (s, 3 H, CH_3, OAc-2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.2 (*C*O, NHAc), 169.4 (*C*O, Ac-4), 169.1 (*C*O, Ac-2), 163.3 (C-4), 152.9 (C-2 or C-6), 147.7 (C-8), 143.2 (C-2 or C-6), 137.2 (Cq, Ph), 128.6, 128.2, 127.8 (*C*H, Ph), 118.5 (C-5), 82.5 (C-1′), 80.5 (C-3′), 76.4 (C-5′), 75.0 (*C*H$_2$, Bn), 71.4 (C-2′), 70.2 (C-4′), 50.8 (C-6′), 25.2 (*C*H$_3$, NHAc), 20.8 (*C*H$_3$, OAc-4), 20.4 (C*H*$_3$, OAc-2). HRMS: calcd for $C_{24}H_{25}ClN_8O_7$ $[M+H]^+$ 573.1608, found 573.1629.

2-Acetamido-9-[2,4-di-*O*-acetyl-3-*O*-benzyl-6-deoxy-6-(dimethoxyphosphoryl)amino-β-D-glucopyranosyl]-6-chloropurine (18)

Compound **18** was obtained according to the general procedure, treating 2-acetamido-9-(2,4-di-*O*-acetyl-6-azido-3-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-6-chloropurine (**16**, 70 mg, 0.122 mmol) with trimethyl phosphite (350 µL, 2.97 mmol). The reaction was complete within 3 h. Purification by flash column chromatography on silica gel (from EtOAc to EtOAc /MeOH, 5:1) afforded the title compound (66 mg, 83 %) as a colorless oil.

$[\alpha]_D^{20}$ = +4 (c = 0.4, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.21 (s, H-8), 7.39–7.18 (m, 5 H, Ph), 5.79 (d, 1 H, H-1′, $J_{1,2} = 9.4$), 5.56 (t, 1 H, H-2′, $J_{1',2'} = {}_{2',3'} = 9.4$), 5.11 (t, 1 H, H-4′, $J_{3',4'} = J_{4,5} = 9.6$), 4.69–4.61 (m, 2 H, C$H_2$, Bn), 3.95 (t, 1 H, H-3′), 3.79 (ddd, 1 H, H-5′), 3.74 (d, 3 H, OCH_3, $J_{CH3,P} = 11.1$), 3.67 (d, 3 H, OCH_3, $J_{CH3,P} = 11.1$), 3.18–2.94 (m, 2 H, CH_2-6′), 2.40 (s, 3 H, CH_3, NHAc), 2.04 (s, 3 H, CH_3, OAc-4), 1.69 (s, 3 H, CH_3, OAc-2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.9 (*C*O, Ac-4), 169.1 (*C*O, Ac-2), 152.6 (C-4), 152.5, 151.6 (C-2, C-6), 142.5 (C-8), 137.5 (Cq, Ph), 128.7, 128.2, 127.8 (*C*H, Ph), 127.4 (C-5), 80.5 (C-1′), 80.4 (C-3′), 77.1 (C-5′), 75.0 (*C*H$_2$, Bn), 71.5 (C-2′), 70.7 (C-4′), 53.5, 53.3 (2 d, OCH$_3$, $J_{CH3,P} = 5.5$), 42.0 (C-6′), 25.1 (*C*H$_3$, NHAc), 20.9 (*C*H$_3$, OAc-4), 20.4 (C*H*$_3$, OAc-2). ^{31}P NMR ($CDCl_3$, 162 MHz) δ: 11.49. HRMS: calcd for $C_{26}H_{32}ClN_6O_{10}P$ $[M+H]^+$ 655.1679, found 655.1683.

2-Acetamido-7-[2,4-di-*O*-acetyl-3-*O*-benzyl-6-deoxy-6-(dimethoxyphosphoryl)amino-β-D-glucopyranosyl]-6-chloropurine (19)

Compound **19** was obtained according to the general procedure, treating 2-acetamido-7-(2,4-di-*O*-acetyl-6-azido-3-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-6-chloropurine (**17**, 72 mg, 0.126 mmol) with trimethyl phosphite (360 µL, 3.05 mmol). The reaction was complete within 4 h. Purification by flash column chromatography on silica gel (from EtOAc to EtOAc /MeOH, 3:1) afforded the title compound (71 mg, 86 %) as a colorless oil.

$[\alpha]_D^{20}$ = +17 (c = 1, in CH_2Cl_2/MeOH, 2:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.29 (br. s, 1 H, N*H*), 8.41 (s, 1 H, H-8), 7.39–7.20 (m, 5 H, Ph), 5.99 (br.d, 1 H, H-1′, $J_{1',2'} = 8.9$), 5.62 (t, 1 H, H-2′, $J_{1',2'} = J_{2',3'} = 8.9$), 5.15 (t, 1 H, H-4′, $J_{3',4'} = J_{4',5'} = 9.6$), 4.69, 4.65 (2 d, AB system, 2 H, CH_2, Bn, $J_{a,b} = 11.9$), 3.96 (t, 1 H, H-3′), 3.83 (ddd, 1 H, H.5′), 3.70 (d, 3 H, OCH_3, $J_{CH3,P} = 11.2$), 3.67 (d, 3 H, OCH_3, $J_{CH3,P} = 11.2$), 3.25–3.03 (m, CH_2-6′), 2.58 (s, 3 H, CH_3, NHAc), 2.05 (s, 3 H, CH_3, OAc-4), 1.78 (s, 3 H, CH_3, OAc-2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (*C*O, NHAc), 169.8 (*C*O, Ac-4), 169.2 (*C*O, Ac-2), 162.7 (C-4), 152.7 (C-2 or C-6), 147.4 (C-8), 142.9 (C-2 or C-6), 137.4 (Cq, Ph), 128.7, 128.2, 127.8 (*C*H, Ph), 118.2 (C-5), 82.4 (C-1′), 80.8 (C-3′), 77.4 (C-5′), 75.0 (*C*H$_2$, Bn), 71.4 (C-2′), 70.6 (C-4′), 53.5, 53.4 (2 d, OCH$_3$, $J_{CH3,P} = 6.0$), 42.1 (C-6′), 25.1 (*C*H$_3$, NHAc), 20.9 (*C*H$_3$, OAc-4), 20.5 (C*H*$_3$, OAc-2). ^{31}P NMR ($CDCl_3$, 162 MHz): δ 11.61. HRMS: calcd for $C_{26}H_{32}ClN_6O_{10}P$ $[M+H]^+$ 655.1679, found 655.1685.

1-(2,4-di-*O*-Acetyl-6-azido-3-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)uracil (20)

Compound **20** was obtained according to the general procedure, starting from 1,2,4-tri-O-acetyl-6-azido-3-O-benzyl-6-deoxy-α,β-D-glucopyranose (**15**, 50 mg, 0.12 mmol) and uracil (20 mg, 0.18 mmol). *N*-Glycosylation of the corresponding silylated uracil with **15** in the presence of TMSOTf (0.16 mL, 0.88 mmol) was complete

within 1 h. Purification by flash column chromatography on silica gel (EtOAc/hexane, from 1:2 to 1:1) afforded the title compound (36 mg, 65 %) as a white solid. m.p. = 128.7–130.4 °C.

$[\alpha]_D^{20} = +37$ (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.35 (br. s, 1 H, N*H*), 7.42–7.16 (m, 6 H, H-6, Ph, $J_{5,6} = 8.4$), 5.84–5.78 (m, 2 H, H-1, H-5), 5.19–5.10 (m, 2 H, H-2′, H-4′, $J_{1',2'} = J_{2',3'} = 9.4$, $J_{3',4'} = J_{4',5'} = 9.6$), 3.88 (t, 1 H, H-3′), 3.78 (ddd, 1 H, H.5′), 3.41 (dd, part A of ABX system, 1 H, H-6′a, $J_{5',6'a} = 2.5$, $J_{6'a,6'b} = 13.7$), 3.26 (dd, part B of ABX system, 1 H, H-6′b, $J_{5',6'b} = 5.6$), 2.05 (s, 3 H, CH_3, OAc-4), 1.78 (s, 3 H, CH_3, OAc-2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.6, 169.5 (2× *C*O, Ac), 162.7 (C-4), 150.7 (C-2), 139.4 (C-6), 136.5 (Cq, Ph), 128.7, 128.2, 127.9 (*C*H, Ph), 103.9 (C-5), 80.4 (C-1′), 80.1 (C-3′), 76.2 (C-5′), 75.1 (*C*H$_2$, Bn), 71.3 (C-2′), 70.1 (C-4′), 50.8 (C-6′), 20.8, 20.6 (2× *C*H$_3$, 2× OAc). HRMS: calcd for $C_{21}H_{23}N_5O_8$ $[M+H]^+$ 474.1619, found 474.1616.

Biological assays

Proliferation assay

Cancer cell lines of different histological origin were cultivated according to the recommendations of the supplier. The cells were assayed with compounds using three-fold dilutions in triplicate. Treatment lasted for 72 h, followed by addition of Calcein AM solution, and measurement of the fluorescence of live cells at 485 nm/538 nm (ex/em) with a Fluoroskan Ascent microplate reader (Labsystems). The GI_{50} value, the drug concentration lethal to 50 % of the cancer cells, was calculated from the obtained dose response curves.

Cell cycle analysis

Asynchronous cells were seeded into 96 well plate and then, after preincubation period, treated with tested compound for 24 h. Adherent MCF-7 cells were first washed with PBS, then trypsinized and finally the solution of trypsin inhibitor was added. After incubation, 5× staining solution (17 mM trisodium citrate dihydrate, 0.5 % IGEPAL® CA-630, 7.5 mM spermine tetrahydrochloride, 2.5 mM Tris; pH 7.6 containing 50 µg/mL propidium iodide) was added. K562 cells were stained by 5× staining solution directly (i.e. without a trypsinization). Cell cycle was analyzed by flow cytometry using a 488 nm laser (BD FACS Verse with software BD FACSuite™, version 1.0.6.).

Acknowledgments: 'Fundação para a Ciência e Tecnologia' (FCT) is acknowledged for funding through the FCT Investigator Program (IF/01488/2013), the exploratory project IF/01488/2013/CP1159/CT0006 and the strategic project UID/MULTI/00612/2013. RJ, EŘ and VK gratefully acknowledge support from the Ministry of Education, Youth and Sports of the Czech Republic (the National Program of Sustainability I – Grant LO1204).

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