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Design, Synthesis and Biological Evaluation of a Novel Class of Unconventional Aminopyrimidine, Aminopurine and Amino-1,3,5-triazine Methyloxy-nucleosides

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Abstract

A class of unconventional nucleosides (methoxy-nucleosides) combining in their structure unconventional nucleobases such as substituted aminopyrimidines, aminopurines or aminotriazines, with unusual sugars, is described. The allitolyl or altritolyll derivatives were pursued as ribonucleoside mimics, while the tetrahydrofurane analogues as their dideoxynucleoside analogues. The compounds showed poor, if any activity against a broad variety of RNA and DNA viruses, including human immunodeficiency virus (HIV). This inactivity may be due to lack of an efficient metabolic conversion to their corresponding 5'-triphosphates and poor affinity for their target enzymes (DNA/RNA polymerases). Several compounds showed cytostatic activity against proliferating human CD4⁺ T-lymphocyte CEM cells and against several other tumor cell lines including murine leukemia L1210 and human prostate PC3, kidney CAKI-1 and cervix carcinoma HeLa cells. A few compounds were inhibitory to Moloney murine sarcoma virus (MSV) in C3H/3T3 cell cultures, the 2,6-diamino-tri-*O*-benzyl-*D*-allitolyl- and -*D*-altritolyll pyrimidine analogs being the most potent compounds. This series of unconventional nucleosides may represent a novel family of potential antiproliferatives.

Keywords

Nucleosides, Nucleotides, antiproliferation, Medicinal Chemistry, Drug Design.

1. Introduction

Nucleosides, the building blocks of nucleic acids, play crucial roles in many biological processes such as RNA and DNA synthesis, the regulation of gene expression or in immunomodulation, among others. Naturally occurring nucleosides mainly consist of a nucleobase (uracil, thymine, cytosine, adenine or guanine) and a furanose sugar (β -D-ribofuranose or β -D-2-deoxyribofuranose).^[1-3]

Synthetic nucleoside analogues, designed to mimic the naturally-occurring nucleosides, are important chemotherapeutic entities with antiviral, antitumor, antibacterial or antifungal activity.^[4-9] Nucleosides are key leads in drug development and have been used in the clinic, for more than 40 years, for the treatment of patients with cancer or virus-related diseases.^[3,8-10] However, they suffer from several drawbacks and unwanted side-effects such as drug resistance development, toxicity, poor oral bioavailability, etc.^[6-10] which point to the importance of the discovery of new, safer and more selective antitumor and/or antiviral agents.

Several nucleoside analogues show some selectivity against malignancies and viral infections. Cancer cells that continuously undergo mitosis (highly proliferative cells) are usually more sensitive to the cytostatic/toxic activity of nucleoside analogues than resting cells.^[1]

Nucleosides have been extensively modified both, in the nucleobase and/or in the sugar part.^[11,12] The modifications, in general, maintain the relative spatial disposition between the nucleobase and the hydroxymethyl group that is phosphorylated to the corresponding nucleotides (the biologically active form). Moreover, several of the modifications are directed to increase the resistance to enzymatic degradation. At the one hand, some nucleoside analogues incorporate unconventional nucleobases (i.e. ribavirin, bearing a 1,2,4-triazole-carboxamide as adenine or guanine mimetic)^[13] or BVDU that incorporates a 5-bromovinyluracil nucleobase^[14] etc.). On the other hand, some nucleoside analogues incorporate unconventional sugar moieties, i.e. a methylene cyclopropane as in synadenol **1** and synguanol **2** (Fig. 1), that are potent antivirals against human cytomegalovirus HCMV),^[15] or an oxetane moiety as in the antibiotic oxetanocin-A (**3**) or a cyclobutyl as in cyclobut-A (**4**), that are

active against HSV, HCMV, and HIV.^[16,17] Among the different modifications in the sugar part, replacement of the furanose ring by an acyclic aliphatic chain has led to an important group of therapeutic agents, the acyclic nucleosides. One of the most representative compounds of this class of nucleosides is acyclovir (ACV, **5**), a potent and selective anti-herpetic drug^[18] discovered by the 1988 Nobel Prize Gertrude Elion.^[19] ACV and its valyl ester prodrug (valacyclovir) are used in the clinic for the treatment of HSV, VZV and CMV infections.^[18-22]

Figure 1

A special group of acyclic nucleosides are the acyclic nucleoside phosphonates (ANPs)^[23] such as adefovir (PMEA, **6**) and tenofovir (PMPA, **7**), designed as metabolically stable nucleotide analogues bearing a non-

biodegradable phosphonate, via an ether linkage instead of the hydrolysable phosphate ester bond.^[10,23] They are approved for the treatment of HIV and chronic HBV infections.^[10,23] The ANPs mimic a 5'-monophosphate nucleoside and circumvent the first phosphorylation step required for the activation of nucleosides, which is often the bottleneck in the activation sequence to obtain the corresponding triphosphates.

More recently, a novel class of acyclic nucleoside phosphonates has been described, designated 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines, such as PMEO-DAPym (**8**) or PMPO-DAPym (**9**),^[10,24,25] to be active against HIV and HBV.^[23,24] In these nucleoside phosphonates the base is a pyrimidine ring (a 2,4-diaminopyrimidine, DAPym) linked to the aliphatic phosphonate, by an ether bond, at the C-6 position of the pyrimidine base instead of the N-1 or N-9 position of the pyrimidine or purine base.^[25,26] A unique feature of these ANPs compounds is that they mimic a purine ring that retains the most important part of the purine base for recognition by the phosphorylating enzymes (mainly kinases). Therefore, the PMEO compounds have been considered as open-ring mimics of the PME-2,6-diaminopurine (PME-DAP, **10**) and guanine derivatives, being potent anti-HIV compounds.^[25-31] Moreover, PMEO-DAPym-diphosphate (PMEO-DAPym-pp) is specifically recognized by HIV-1 reverse transcriptase (HIV-1 RT) as a purine instead of a pyrimidine derivative and incorporated into the growing DNA chain as a purine nucleotide analog instead of a pyrimidine nucleotide.^[28] Interestingly, and in contrast with the PMEO-DAPyms, the pyrimidine derivatives of PME-analogues are generally devoid of antiviral activity.^[29]

Inspired by the uniqueness of PMEO-DAPyms and with the aim to discover novel families of nucleoside analogues (of unknown structures), we designed a novel class of unconventional nucleosides, of general structure **I** (Fig. 2).

Figure 2. General structure of target nucleosides

These compounds combine in their structure unconventional bases such as diaminopyrimidines as in PMEO-DAPYm or aminotriazines, with unconventional sugars as intact tetrahydrofuran rings (but distinct from ribose and deoxyribose). With such nucleosides we aimed to explore their biological potential.

Herein we describe the synthesis, the conformational studies, and antiviral and cytostatic evaluation in cell culture, of a series of nucleoside analogues of aminopyrimidine-, aminopurine- and amino-1,3,5-triazine-bearing unusual sugar moieties that led to the discovery of a novel family of potential antiproliferative nucleosides.

2. Results and Discussion

2.1. Chemistry

In an effort to discover novel nucleoside analogues as potential antiviral or anticancer hits, we first pursued the tetrahydrofuran nucleosides of 2,6-diaminopyrimidines bearing OH groups at the 3' and 4' positions (**13**), as ribofunanosyl nucleoside mimics. For the synthesis of these key nucleosides the procedure shown in Scheme 1 was devised. Coupling of benzyl key intermediate **11**^[32] with 2,4-diamino-6-chloropyrimidine would give nucleoside **12**, which after debenzylation may provide the desired unprotected nucleoside **13**.

Scheme 1. Synthesis of key nucleosides

First, it was necessary to prepare the key intermediate **11** as shown in Scheme 2. Thus, acid-mediated cyclization of **14**,^[33] prepared in five steps from commercially available D-ribose diethyl dithioacetal as reported,^[32] in the presence of catalytic amount of H_2SO_4 at 50°C gave **15a,b**^[33] in 58 % yield, as an intractable mixture of the 2,3-*cis*- and 2,3-*trans*-tetrahydrofuran isomers.

Scheme 2. Reagents and conditions: (i) H_2SO_4 THF; (ii) NaH 60 %, BnBr, THF; (iii) NaOAc, AcOH, KBr, AcOOH, 10°C , 4h

Although Delf and co-workers^[33] reported that under the above-mentioned conditions the rate of isomers 2,3-*trans* vs 2,3-*cis* was 20:1, in our hands all attempts resulted in a 2:1 ratio. Treatment of **15a,b**^[33] with benzyl bromide in the presence of 60% NaH, followed by Fleming-Tamao oxidation (KBr,

AcOOH)^[33] of the corresponding fully protected silane (**16a,b**), gave **11a,b** (63% overall yield from **15a,b**) as an unseparable 6:1 mixture of allitol (**11a**) and altritol (**11b**) as determined by NMR.

Alkylation of 2,4-diamino-6-chloropyrimidine (**17**) with the *in situ* generated sodium alkoxide of **11a,b** (60% NaH, THF) at 90°C (Scheme 3) gave a 4:1 mixture of **12a** and **12b** in 82% yield, that could not be separated. Next, removal of the benzyl groups of **12a,b** was attempted. Initial attempts of hydrogenolysis (H₂, 10% Pd/C) of the mixture **12a,b** failed to produce the desired unprotected nucleosides rendering unreacted starting compound. However, when the mixture **12a,b** was hydrogenated in the presence of 20% Pd(OH)₂/C and a catalytic amount of HCl, the deprotected nucleoside **13a** was obtained, as the sole product, in 80% yield. Unexpectedly, no traces of the deprotected **13b** isomer were detected.

Scheme 3. Reagents and conditions: (i) NaH 60%, dry THF, 90 °C, 16h; (ii) H₂, Pd(OH)₂/C 20%, MeOH, HCl, 30 °C, 40 psi

Next, in view of the novelty of the structures the synthesis of other aminopyrimidine nucleosides was also pursued. Thus, alkylation of 2-amino-

4,6-dichloropyrimidine (**18**) with **11a,b** (Scheme 4), using the same reaction conditions described above (NaH, THF, 90°C), gave a 5:1 mixture of compounds **19a,b**, that was separated by preparative centrifugal circular thin-layer chromatography, to give the individual isomers **19a** and **19b** in 71% and 15% yield, respectively. The structures of **19a** and **19b** were unequivocally assigned on the basis of their corresponding spectroscopic data, using a combination of mono- and bidimensional ^1H and ^{13}C NMR (NOESY, g-HMBC, g-HSQC). The relative stereochemistry of **19a** and **19b** was determined by Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiments. A correlation between H2' and H5' in compound **19a**, confirmed the 2',3'-*trans* configuration, whereas, no correlation between these protons were observed in the isomer **19b**, thus indicating a 2',3'-*cis* configuration for this compound. Removal of the benzyl groups of **19a** by hydrogenolysis in the presence of 10% Pd/C gave the fully deprotected dehalogenated derivative **20a** in 50% yield. However, all the attempts to remove the benzyl groups of **19b** under different conditions (H_2 , 10% Pd/C, 5% Pd/C or 20% $\text{Pd}(\text{OH})_2$, in the presence or absence of catalytic amounts of HCl) rendered complex mixtures without any formation of the desired derivative **20b**.

Scheme 4. Reagents and conditions: (i) NaH 60%, dry THF, 90 °C, 16 h ; (ii) H_2 , Pd(C) 10%, MeOH, 30 °C, 40 psi

Compounds **13a** and **20b** were evaluated for inhibition of the replication of a wide variety of viruses in cell culture, but were generally found to show poor if any inhibitory activity.

Next we explored the synthesis of the corresponding aminopyrimidine dideoxynucleoside analogues. The synthesis of compounds **26-28** and **31** (Scheme 5) was carried out following a similar procedure to that described for the synthesis of **13** and **20**. Thus, treatment of (2*RS*,5*SR*)-**21**^[34] with 2 equiv. of the appropriate aminopyrimidine [2,4-diamino-6-chloropyrimidine (**17**), 2-amino-4,6-dichloropyrimidine (**18**), 4-amino-6-chloropyrimidine (**22**) or 4-amino-2-chloropyrimidine (**29**)] in the presence of 60% NaH in a pressure tube, afforded the corresponding (2'*RS*,5'*SR*)-benzyl derivatives **23** (55%), **24** (79%), **25** (58%) and **30** (37%) in moderate to good yields. Hydrogenolysis of **23-25** and **30** [Pd(OH)₂/C; HCl at 30 °C, 40 psi] yielded the desired (2*RS*,5*SR*)-dideoxy derivatives **26** (51%), **27** (74%), **28** (20%) and **31** (28%), respectively. All compounds were racemic mixtures (2'*RS*,5'*SR*) that could not be separated.

Scheme 5. Reagents and conditions: (i) NaH 60 %, DMF or THF, pressure tube; (ii) H₂ / Pd(OH)₂, 37% HCl, 30 °C, 40 psi

A similar alkylation of pyrimidines bearing C=O, or OH substituents (**32-35**) under different conditions (large excess of pyrimidines in the presence of 60% NaH in acetonitrile or THF, at 70°C or 100°C in a pressure tube or under microwave irradiation at 100°C) failed to produce the desired nucleosides. Only unreacted starting material was recovered. Interestingly, when 2-amino-4-chloro-6-methoxypyrimidine (**36**) or 4-chloro-6-methoxypyrimidine (**37**) were alkylated with **21** (60% NaH in a pressured tube at 100 °C), the desired nucleosides **39** and **40** were obtained in 54% and 45% yield, respectively. In both reactions the dimer compounds **43** and **44** (9% and 8% yield, respectively) were observed as by-products (Scheme 6). These unexpected derivatives may result from transesterification by the alcoholate anion of **21**. Transesterifications by alcoholate anions have been described in the literature.^[35b,c] A similar transesterification of a methoxy-pyrimidine ring by a benzylate anion to give a benzyl ether has been reported by Delacotte et al.³⁵ upon heating at 95 °C. Also Brown et al.^[36] reported transesterifications in the presence of silver oxide as catalyst.

Scheme 6. Reagents and conditions: (i) NaH 60 %, DMF or THF pressure tube; (ii) H₂ / Pd(OH)₂, 37% HCl, 30 °C, 40psi.

Hydrogenolysis of benzyl derivative **39**, in the presence of catalytic amounts of Pd(OH)₂/C and HCl at 30 °C and 40 psi, resulted in a complex reaction mixture, and no desired deprotected nucleoside **41** was detected. Instead, analogous treatment of derivative **40** gave the unprotected **42** (10%), although in low yield. To avoid the hydrogenolysis step, we replaced the benzyl group by a *tert*-butyldiphenylsilyl protecting (TBDPS) group, removable under acidic conditions, and used **38** as starting compound. Thus, treatment of **38**^[37] with

pyrimidines **36** or **37**, under the standard conditions, afforded the deprotected nucleosides **41** and **42** in 10% and 12% yield, respectively.

Scheme 7. Reagents and conditions: (i) NaH 60 %, dry DMF, 100 °C, microwave; (ii) H₂ / Pd(OH)₂, 37% HCl, 30 °C, 40psi; (iii) NaH 60 %, dry DMF 100°C, pressure tube.

Next, we explored the synthesis of aza-analogues of the so far mentioned nucleosides. Disubstituted triazines are much less reactive and the substitutions of the chloride atom requires stronger conditions.^[38] Thus, alkylation of the 2-chloro-4,6-diamino-1,3,5-triazine (**45**) with **21** (Scheme 7) was carried out by treatment with 60% NaH under microwave irradiation at 100 °C for 3 hours, to give **47** in 62% yield. Hydrogenolysis of **47** (H₂, 20% Pd(OH)₂/C, HCl), to remove the benzyl group gave an unseparable 2:1 mixture of the desired compound **48** and ameline (**49**). To avoid the hydrogenolysis step and thus to prevent the ameline formation, we used TBDPS-protected compound **38**. Treatment of **38** with **45** gave a complex mixture that could not be identified. Finally, the unprotected derivative **46** was used as starting compound. Thus, **46** was reacted with 2-chloro-4,6-diamino-1,3,5-triazine (**45**) to give the deprotected derivative **48** in moderate yield (42%) together with the disubstituted **50** (14%) as minor compound.

The results obtained with the synthesis of amino pyrimidine nucleosides were taken into consideration to expand the synthetic application to purine methoxy-nucleoside analogues.

Scheme 8. Reagents and conditions: (i) NaH 60 %, DMF, pressure tube; (ii) H_2 / $\text{Pd}(\text{OH})_2$, 37% HCl, 30 °C, 40 psi

An efficient method for the synthesis of 6-oxy-substituted purines uses 1,4-diazabicyclo[2.2.2]octane (DABCO) as the leaving group to achieve the displacements at C-6.^[39] 6-DABCO purines show faster rate of displacement as compared to chlorine or quinuclidine.^[38] Thus, treatment of DABCO-purines **51** or **52**^[39] (Scheme 8) with the sodium alkoxide of **21**, generated *in situ* upon treatment with 60% NaH at 100°C overnight, gave the protected derivatives **53** and **54** in moderate yields (30% and 53%, respectively). Subsequently, hydrogenolysis (20% $\text{Pd}(\text{OH})_2/\text{C}$ and HCl) of **53** and **54** afforded the target purine derivatives **55** (41% yield) and **56** (35% yield). All dideoxynucleoside analogues described so far were racemic mixtures (2'*RS*,5'*SR*).

Finally, we considered of interest to study the capability of a representative example of this new kind of methoxy-nucleosides to interact with DNA polymerases (i.e. the DNA polymerase activity of HIV reverse transcriptase). Therefore, we prepared compound **59** (the triphosphate derivative of **26**) (Scheme 9). For the synthesis of the target-TP we used the Yoshikawa methodology^[40] which involves the phosphorylation of unprotected nucleoside **26**, with phosphorous oxychloride in the presence of Proton Sponge as base, and trimethyl phosphate as solvent, followed by *in situ* reaction of the phosphorodichlorate intermediate (**57**) with pyrophosphate to yield the cyclic triphosphate (**58**) which is finally hydrolyzed to **59** (8% yield) upon treatment with a 1M solution of triethylammonium bicarbonate (TEAB).

Scheme 9. Reagents and conditions: (i) Proton sponge, trimethyl phosphate, POCl_3 , 5°C , 16 h; (ii) NBu_3 , tributylammonium pyrophosphate, DMF, 0°C , 30 min; (iii) 0.2M triethylammonium trimethyl phosphate solution, r.t.

2.2. Conformational studies

With the aim to determine whether the conformational freedom of the furanose ring was restricted in these nucleosides, we carried out conformational studies in the solution state for compounds **20a** and **27**.

For this type of nucleosides three conformational features must be considered: the puckering of the furanose ring, the conformation of the exocyclic bonds $\text{C4}'\text{-C5}'$ and $\text{C1}'\text{-C7}$ and the conformation around the glycosidic bond. The puckering of the furanose ring in solution can be determined using the concept of pseudorotation,^[41] in which the conformation of the sugar ring is fully described by two parameters: a phase angle of pseudorotation (P) and a puckering amplitude (τ_m). In solution, it is often accepted a two-state $\text{N} \rightleftharpoons \text{S}$ conformational equilibrium as model. Therefore, from the experimental three-bond proton-proton coupling constants values ($^3J_{\text{H,H}}$), and by using the PSEUROT program,^[42] information about the geometry and population of both North and South-type conformers can be obtained. Five parameters are needed

to describe such a two-state conformational equilibrium: P and τ_m of both North and South conformers and the mole fraction (X_n) of one of them.

For compound **27**, eight observables are available and thus the system is fully defined. However, for compound **20a**, only three observables are available and consequently, two variables must be constrained in the calculation. The results obtained for both compounds are shown in Table 1. The conformational parameters for compound **20a**, $P_N = 43.0$ $P_S = 133.5$ $X_n = 0.34$, indicated that the furanose ring adopts an equilibrium between ${}_4E$ and ${}_1E$ shifted to the south conformer. For compound **27** the conformational parameters ($P_N = 8.1$, $P_S = 158.5$) indicated an equilibrium in the usual ranges for dideoxynucleosides ($P_N = 0-36$ and $P_S = 144-180$).^[43]

The conformation around C5'-C6' bond can be studied from the experimental coupling constants ${}^3J_{H5',H6'a}$ and ${}^3J_{H5',H6'b}$, by considering an equilibrium between three staggered rotamers $g+$, t and $g-$. The experimental values ${}^3J_{H5',H6'a} = 4.2$ and ${}^3J_{H5',H6'b} = 5.0$ Hz for compound **20a** and ${}^3J_{H5',H6'a} = 5.2$ and ${}^3J_{H5',H6'b} = 5.2$ Hz for compound **27** suggested no preferred rotamers for this conformational feature in both compounds.

The conformation around the C1'-C2' bond was studied using the same model. For compound **20a**, ${}^3J_{H1'a,H2'} = 3.2$ and ${}^3J_{H1'b,H2'} = 6.7$ Hz indicated that $g+$ (47%) and t (43%) rotamers were preferred. Similar conclusions were obtained for compound **27**. Thus, the coupling constants values ${}^3J_{H1'a,H2'} = 3.7$ and, ${}^3J_{H1'b,H2'} = 6.7$ Hz indicated that $g+$ (43%) and t (50%) rotamers were also preferred (Figure 3).

Figure 3. MM conformers, as determined by PSEUROT program, of compounds **20a** and **27**

Finally, the conformation of the “glycosidic bond” was determined from the cross-peaks observed for the signals corresponding to proton H5 of the base in the NOESY experiments. Thus, while, for compound **27**, H5 showed cross-peaks with some protons on the β -face of the furanose (H6’a, H6’b and H3’a), for compound **20a**, only a tiny correlation was observed with H6’a. These data point to a major *anti* conformation for the glycosidic bond in both compounds.

2.3. Biological evaluation

None of the compounds were significantly inhibitory against a variety of DNA and RNA viruses. Given the structural similarity of several compounds with the antiretroviral 2’,3’-dideoxynucleoside analogues (NRTI), the compounds were also evaluated for their inhibitory activity against HIV-1 and HIV-2 in CD₄⁺ T-lymphocytic CEM cell cultures (Table 2). Unfortunately, they were also devoid of anti-HIV activity at subtoxic concentrations.

Although none of the compounds showed any anti-HIV activity at subtoxic concentrations, it could not be excluded that the antiviral inactivity was due to a

lack of metabolic conversion to their corresponding 5'-triphosphates. Therefore, compound **59**, the triphosphate derivative of compound **26**, was synthesized and evaluated for its inhibitory activity against HIV-1 reverse transcriptase. PMEODAPym-pp was included as a positive control. As shown in Table 5, **59** affects HIV-1 RT only when the poly rU.dA template-primer had been used (as with PMEODAPym-pp). Since HIV-1 RT is inhibited by compound **59** solely in the presence of this template-primer (as also earlier demonstrated to be the case for PMEODAPym-pp), it is tempting to assume that this compound – alike PMEODAPym-pp - acts as an adenine nucleotide mimic. However, it should be recognized that the IC₅₀ value for **59** was quite high in the presence of poly rU.dA and dATP and therefore, more research would be required to further justify this assumption.

It should be mentioned that compounds **12a,b**, **19a**, **39**, **40**, **43**, **44** and **54** proved cytostatic against the CD₄⁺ T-lymphocytic CEM tumor cell cultures (50% cytostatic concentration ranging between 13 and 50 μM). These particular compounds were also cytostatic against several other tumor cell lines, including murine leukemia L1210, human prostate PC3, kidney CAKI-1 and cervix carcinoma HeLa cells. There was a trend to a preferential cytostatic activity against leukemia and lymphoma tumor cell types *versus* the solid carcinoma tumor cell types (Table 3). A few compounds (i.e. **12a,b**, **31**, **40** and **54**) were able to inhibit murine fibroblast C3H/3T3 cell transformation by Moloney murine sarcoma virus (MSV), in particular compound **12a,b** (EC₅₀: 13 μM) (Table 4).

3. Conclusions

In conclusion, we have described for the first time a series of unconventional aminopyrimidine, aminopurine and amino-1,3,5-triazine methyloxy-nucleosides of hitherto unknown structure. These unconventional nucleosides have been prepared by alkylation of the corresponding chloro-substituted nucleobases with conveniently protected and *in situ* generated sodium alkoxide of D-allitol, D-altritol or 2,5-bis-hydroxymethyl tetrahydrofurane. The allitollyl or altritollyl derivatives were pursued as unusual ribonucleoside mimics, while the tetrahydrofurane analogues can be considered as their dideoxynucleoside analogues. The tetrahydrofurane ring in the polyhydroxylated nucleosides

adopts an equilibrium between 4E and 1E shifted to the south conformer. While in the corresponding dideoxyanalogues the equilibrium is in the usual ranges for dideoxynucleosides (between 3E and 2E).

None of the compounds showed specific antiviral (including anti-HIV) activity at subtoxic concentrations in cell culture. This might be due to lack of efficient metabolic conversion to the triphosphate derivatives and/or to a much lower affinity for the target polymerases.

On the other hand, several compounds proved cytostatic in particular against leukemia/lymphoma tumor and human prostate carcinoma cell lines. Compounds **12a,b**, **31**, **40** and **54** inhibited murine fibroblast C3H/3T3 cell transformation by Moloney murine sarcoma virus (MSV), **12a,b** (EC_{50} : 13 μ M) being superior to the others.

In summary, this series of unconventional methoxy-nucleosides may be considered as a novel family of potential antiproliferative candidates that may be further investigated for structural optimization.

4. Experimental section

4.1. Chemical synthesis

All experiments that involved water-sensitive compounds were carried out under scrupulously dry conditions. Hygroscopic solids were vacuum pre-dried in the presence P_2O_5 for 24h. THF was distilled from Na/benzophenone and stored over molecular sieves (4 Å). Anhydrous DMF was purchased from Aldrich and stored over molecular sieves (4 Å). Microwave reactions were performed in a Biotage Initiator™ 2.0 single-mode cavity instruments from Biotage (Uppsala, Sweden). Experiments were carried out in sealed microwave process vials using standard absorbance level (400 W maximum power). The temperature was measured with an IR sensor on the outside of the reaction vessel. The reactions were monitored by Analytical TLC on silica gel 60 F_{254} (Merck) pre-coated plates (0.2 mm). Products were previously under UV light (254 nm) and/or by heating after treatment with a 5 % solution of H_2SO_4 in ethanol. Preparative TLC was performed on pre-coated silica gel 20 X 20 cm glass plates (Altech 02013, Silica Gel GF with UV 254, 1000 microns layer

thickness; Sigma-Aldrich). Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron[®] Harrison Research 8924 model (Kieselgel 60 PF₂₅₄ gipshaltig, Merck), with a layer thickness of 1 mm and 2 mm and a flow rate of 4 or 8 mL/min, respectively). Flash chromatography was performed by HPFC using a force flow Isolera One (Biotage) with flash silica gel SNAP cartridges of KP-Sil of 50 g (39 x 81 mm) or 100 g (39 x 157 mm). Reverse phase chromatography was performed: (a) by HPFC in an Isolera One (Biotage) using SNAP 12g KP-C18-HS cartridges, or (b) by solid phase extraction chromatography (SPE) under vacuum in the manifold using DSC-Si 1 g (Supelco) cartridges. Samples were lyophilized using mixtures water/acetonitrile on a Telstar 8-80 instrument.

The purity of the compounds was checked by analytical RP-HPLC on: (a) a Waters 2690 instrument equipped with a Diode Array and C18 Sunfire column (4.6 mm x 50 mm, 3.5 μ m); as mobile phase, A:B mixtures were used, where A = CH₃CN (0.04% TFA) and B = H₂O (0.05% TFA); (b) an Agilent Technologies 1120 Compact LC instrument equipped with a Diode Array and a ACE 5 C18-300 column (4.6 mm x 150 mm, 3.5 μ m), using as mobile phase A:B mixtures where A = CH₃CN and B = H₂O (0.05% TFA). Flow was in all cases 1 mL/min and the samples were analyzed at 217 and 254 nm in a gradient mode. Gradients used : (a) gradient 1: from A:B mixtures 80:20 to 100:0 in 5 minutes; (b) gradient 2: A:B mixtures 40:60 to 70:30 in 8 minutes; (c) gradient 3: A:B mixtures 10:90 to 100:0 in 10 minutes; (d) gradient 4: A:B mixtures from 2:98 to 30:70 in 10 minutes; (e) gradient 5: A:B mixtures 50:50 to 100:0 in 10 minutes. HPLC/MS was performed by a HPLC Waters 2695 instrument connected to a Waters Micromass ZQ 2000 spectrometer, and a photodiode array detector. The column used was a Sunfire C-18 (4.6 mm x 50 mm, 3.5 μ m) and the flow was 1 mL/min. Solvents used were mixtures of A: CH₃CN (0.08% formic acid) and B: H₂O (0.1% formic acid). The gradient used was from 10% A to 100% A in 10 min. Detection was at 214 and 254 nm. The triphosphate **59** was analyzed in a HPLC Waters 600 instrument previous with a UV/Visible detector and an ionic exchange column Waters Spherisorb[®] SAX (4.6 mm x 250 mm, 10.0 μ m). Mobile phase was 0.5 M de KH₂PO₄ solution in isocratic mode for 15 min. Flow was 1.5 mL/min and the detection at 220 y 254 nm.

Optical rotations were measured in a Perkin Elmer 241 polarimeter at $23 \pm 2^\circ$

C. Electrospray mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MSHP1100). HRMS (EI+) was carried out in an Agilent 6520 Accurate-Mass Q-TOF LC/MS spectrometer using methanol water (0.1 % acetic acid). NMR spectra were recorded with a Varian Inova-300, Varian Inova-400 or Varian System-500 spectrometers operating at 300, 400 or at 500 MHz for ^1H NMR and at 75, 100 or at 125 MHz for ^{13}C NMR, and a Mercury-400 operating at 162 MHz for ^{31}P NMR with Phosphoric acid as internal standard. Chemical shift values are reported in parts per million (δ). Coupling constants (J values) are reported in Hertz (Hz), and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad signal). Complex ^1H - and ^{13}C -NMR spectra were assigned by homonuclear ^1H - ^1H COSY and NOESY experiments and by heteronuclear ^1H - ^{13}C HSQC and HMBC experiments.

2,5-Anhydro-3,4-di-O-benzyl-1-deoxy-1-dimethylphenylsilyl-D-allitol and 2,5-anhidro-3,4-di-O-benzyl-1-deoxy-1-dimethylphenylsilyl-D-altritol (15a and 15b):^[33] To a solution of mixture 3,4-Di-O-benzyl-1-deoxy-1-dimethylphenylsilyl-D-allitol and 3,4-di-O-benzyl-1-deoxy-1-dimethylphenylsilyl-D-altritol^[33] (960 mg, 1.99 mmol) in dry THF (7 mL), concentrated H_2SO_4 (49.5 μL) was added and stirred at 50 $^\circ\text{C}$ for 48 h, diluted with ethyl acetate (15 mL) and washed with saturated NaHCO_3 (3 x 10 mL). The layers were separated and the organic phase dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by HPFC in the Biotage (hexane/ethyl acetate, 7:3). The fastest moving fractions gave **15a** and **15b** (532 mg, 58%) as an intractable mixture (ratio 2,3-*trans* (**1a**) y 2,3-*cis* (**1b**), 2:1) as determined by ^1H -NMR, after purification. Analytical and spectroscopic data (NMR) are similar to those described in the literature [33]. ^1H -NMR (400 MHz, CDCl_3) δ : 0.30, 0.33 (s, 6H, CH_3 , $\text{SiCH}_{3\text{trans}}$), 0.31, 0.33 (s, 6H, CH_3 , $\text{SiCH}_{3\text{cis}}$), 1.00 (dd, 1H, $J_{1a,2} = 9.7$ Hz, $J_{1a,1b} = 14.4$ Hz, H-1a_{trans}), 1.12 (dd, 1H, $J_{1b,2} = 4.9$ Hz, $J_{1a,1b} = 14.4$ Hz, H-1b_{trans}), 1.16 (dd, 1H, $J_{1b,2} = 5.8$ Hz, $J_{1a,1b} = 14.5$ Hz, H-1b_{cis}), 1.44 (dd, 1H, $J_{1a,2} = 9.2$ Hz, $J_{1a,1b} = 14.5$ Hz, H-1a_{cis}), 3.42 (dd, 1H, $J_{5,6a} = 2.9$ Hz, $J_{6a,6b} = 11.9$ Hz, H-6a_{trans}), 3.45 (m, 1H, H-3_{trans}), 3.47 (m, 1H, H-3_{cis}), 3.63 (dd, 1H, $J_{5,6b} = 3.1$ Hz, $J_{6a,6b} = 11.9$ Hz, H-6b_{trans}), 3.72 (dd, 1H, $J_{5,6b} = 2.9$ Hz, $J_{6a,6b} = 11.9$ Hz,

H-6a_{cis}), 3.75 (t, 1H, $J_{3,4} = 3.9$ Hz, H-4_{cis}), 3.85 (t, 1H, $J_{3,4} = 5.5$ Hz, H-4_{trans}), 3.95-4.09 (m, 4H, H-2_{trans,cis}, H-5_{cis}, H-6b_{cis}), 4.18 (m, 1H, H-5_{trans}), 4.41-4.80 (d, 8H, $J = 11.8$ Hz, CH₂Ph_{trans,cis}), 7.25-7.53 (m, 30H, Ar_{trans,cis}). HPLC (Agilent 1120, gradient 3): $t_R = 10.84$ min (95%). HRMS (ES+) m/z : Calcd for C₂₈ H₃₄ O₄ Si 462.2224. Found 462.2226.

The slowest moving fractions gave **3,4-di-O-benzyl-1,2-dideoxy-D-ribo-hex-1-enitol**^[33] (260 mg, 40%). MS (ES+) m/z : 351 (M)⁺. ¹H-NMR (300 MHz, CDCl₃) δ : 3.10 (bs, 2H, OH), 3.61 (t, 1H, $J_{3,4} = J_{4,5} = 6.1$ Hz, H-4), 3.71-3.78 (m, 3H, H-5, H-6), 4.08 (dd, 1H, $J_{2,3} = 7.7$ Hz, $J_{3,4} = 6.1$ Hz, H-3), 4.52 (d, 2H, $J = 11.8$ Hz, CH₂Ph), 4.62 (d, 2H, $J = 11.8$ Hz, CH₂Ph), 5.36-5.46 (m, 2H, H-1), 5.91 (ddd, 1H, $J_{1a,2} = 16.7$ Hz, $J_{1b,2} = 11.0$ Hz, $J_{2,3} = 7.7$ Hz, H-2), 7.25-7.40 (m, 10H, Ar).

2,5-Anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-dimethylphenylsilyl-D-allitol and 2,5-anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-dimethylphenylsilyl-D-altritol

(**16a** and **16b**): To a mixture of **15a,b** (1.05 g, 2.26 mmol) and BnBr (0.54 mL, 4.52 mmol) in dry THF (15 mL), stirred at 0 °C under argon, 60% NaH (181 mg, 4.52 mmol) was added. The reaction was stirred at room temperature overnight, diluted with ethyl ether (15 mL), and then a saturated solution of NH₄Cl (5 mL) was slowly added. The layers were separated and the organic phase washed with saturated NaCl (2 x 10 mL), dried (Na₂SO₄), filtered and evaporated to dryness. The residue was purified by HPFC in the Biotage (hexane/ethyl acetate, 9:1) to give a mixture of **16a** and **16b** yield 1.02 g (82%) as an oil. Ratio 2,3-*trans*:2,3-*cis*, 4:1 (by ¹H-NMR and HPLC). ¹H-RMN (500 MHz, CDCl₃) δ : 0.31, 0.32 (s, 6H, CH₃, SiCH₃_{trans}), 0.32, 0.33 (s, 6H, CH₃, SiCH₃_{cis}), 1.04 (dd, 1H, $J_{1a,2} = 9.0$ Hz, $J_{1a,1b} = 14.5$ Hz, H-1a_{trans}), 1.14-1.30 (m, 2H, H-1b_{trans,cis}), 1.46 (dd, 1H, $J_{1a,2} = 8.0$ Hz, $J_{1a,1b} = 14.5$ Hz, H-1a_{cis}), 3.40-3.51 (m, 4H, H-3_{trans}, H-6a,b_{trans}, H-6a_{cis}), 3.56 (m, 1H, H-6b_{cis}), 3.76 (t, 1H, $J_{2,3} = J_{3,4} = 4.1$ Hz, H-4_{cis}), 3.88 (dd, 1H, $J_{2,3} = 5.2$ Hz, $J_{3,4} = 4.5$ Hz, H-4_{trans}), 4.04-4.20 (m, 4H, H-2_{trans,cis}, H-5_{trans,cis}), 4.30-4.80 (m, 12H, CH₂Ph_{trans,cis}), 7.24-7.58 (m, 40H, Ar_{trans,cis}). ¹³C-NMR (125 MHz, CDCl₃) δ : -2.3, -1.5 (CH₃, SiCH₃_{trans}), -2.2, -1.8 (CH₃, SiCH₃_{cis}), 17.1 (C-1_{cis}), 21.6 (C-1_{trans}), 70.5 (C-6_{cis}), 70.9 (C-6_{trans}), 71.9, 72.1, 73.5 (CH₂Ph_{trans}), 72.6, 73.0, 73.5 (CH₂Ph_{cis}), 77.6 (C-4_{trans}), 78.8 (C-4_{cis}, C-3_{cis}), 79.0 (C-2_{trans}), 79.4 (C-2_{cis}), 80.5 (C-5_{cis}), 81.3 (C-5_{trans}), 83.6 (C-3_{trans}), 127.5-133.8 (CH Ar_{trans,cis}), 138.1, 138.3, 138.4, 139.6 (C Ar_{trans}), 138.2, 138.5,

138.8, 139.5 (C Ar_{cis}). HPLC (Waters 2690, gradient 1): t_R = 4.58 min (21%), 7.74 (78%). HRMS (ES+) m/z : Calcd for C₃₅H₄₀O₄Si 552.2688. Found 552.2696.

2,5-Anhydro-3,4,6-tri-O-benzyl-D-allitol and 2,5-anhydro-3,4,6-tri-O-benzyl-D-altritol (11a and 11b): To a solution of NaOAc (1.97 g, 24.05 mmol) in AcOH (9 mL) the phenylsilane mixture **16a,b** (1.02 g, 1.85 mmol) was added. KBr (0.26 g, 2.22 mmol) was added and the reaction cooled to 10 °C, and AcOOH (18 mL, 32 % in AcOH) (18 mL) was slowly added, under exclusion of light. During the addition oxygen was liberated. The reaction was stirred at 10 °C for 4 h., then diluted with ethyl acetate (20 mL) and poured into a cooled (0 °C) solution of saturated Na₂S₂O₃ (20 mL). The organic phase was separated and neutralized with NaHCO₃, until the complete stop of gas liberation. The organic phase was washed with H₂O (2 x 50 mL), dried (Na₂SO₄) anhidro, filtered and concentrated. The residue was purified by HPFC in the Biotage (hexane/ethyl acetate, 3:2) to give the mixture **11a** and **11b** (632 mg, 77%) as an oil. Ratio 2,3-*trans* (**11a**) and 2,3-*cis* (**11b**) (6:1). ¹H-NMR (400 MHz, CDCl₃) δ : 2.70 (bs, 1H, OH), 3.50 (m, 2H, H-1a, H-6a), 3.70 (dd, 1H, $J_{5,6b}$ = 3.2 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6b), 3.80 (dd, 1H, $J_{1b,2}$ = 2.7 Hz, $J_{1a,1b}$ = 12.1 Hz, H-1b), 4.03 (dd, 1H, $J_{2,3}$ = 4.0 Hz, $J_{3,4}$ = 5.2 Hz, H-3), 4.11 (dd, 1H, $J_{3,4}$ = 5.2 Hz, $J_{4,5}$ = 6.1 Hz, H-4), 4.16-4.23 (m, 2H, H-2, H-5), 4.43 (d, 1H, J = 11.9 Hz, CH₂Ph), 4.47 (d, 1H, J = 11.8 Hz, CH₂Ph), 4.53 (d, 1H, J = 11.9 Hz, CH₂Ph), (d, 1H, J = 11.8 Hz, CH₂Ph), 4.61 (s, 2H, CH₂Ph), 7.22-7.37 (m, 15H, Ar). ¹³C-NMR (100 MHz, CDCl₃) δ : 62.2 (C-1_{cis}), 63.4 (C-1_{trans}), 69.7 (C-6_{trans}), 70.3 (C-6_{cis}), 72.7, 73.2, 73.6 (CH₂Ph_{cis}), 72.2, 72.4, 73.6 (CH₂Ph_{trans}), 78.0, 78.1 (C-3_{trans}, C-4_{trans}), 78.2, 78.7 (C-3_{cis}, C-4_{cis}), 81.0 (C-2_{trans}), 79.7 (C-2_{cis}), 80.9 (C-5_{cis}), 83.0 (C-5_{trans}), 127.8-128.7 (CH Ar_{trans,cis}), 137.6, 137.9, 138.0 (C Ar_{trans}), 137.7, 137.9, 138.1 (C Ar_{cis}). HPLC (Agilent 1120, gradient 3): t_R = 9.52min (95%). HRMS (ES+) m/z : Calcd. For C₂₇H₃₀O₅ 434.2108. Found 434.2093.

General procedure for the synthesis of D-allitoyl-, D-altritoyl-, and tetrahydrofuran-2'-yl pyrimidine/purine derivatives: To a cooled (0 °C) solution of **11a,b**, **21**, **38** or **46** (0.46 mmol) in dry THF (10 mL), under argon, 60% NaH (36.8 mg, 0.92 mmol) was added. The mixture was stirred at room

temperature for 1h, then, the corresponding chloropyrimidine/purine (0.92 mmol) was added, and the reaction was stirred at 90-100 °C, either in a pressure tube or in the microwave, for 3 h-overnight. After cooling to room temperature, MeOH was added (to remove the excess of NaH) and evaporated to dryness. The residue was dissolved in EtOAc (10 mL), washed with 1N HCl (3 x 5 mL) and the organic phase dried (Na₂SO₄), filtered and evaporated to dryness and purified. The purification method, eluents and yields are indicated in each case.

2,6-Diamino-4-(2',5'-anhydro-3',4',6'-tri-O-benzyl-D-allitoly)pyrimidine and 2,6-diamino-4-(2',5'-anhydro-3',4',6'-tri-O-benzyl-D-altritoly)pyrimidine

(**12a** and **12b**): Following the general procedure **11a,b** (50 mg, 0.12 mmol) reacted with 2,4-diamino-6-chloropyrimidine (**17**) (34 mg, 0.24 mmol), overnight. To give, after purification by CCTLC on the chromatotron (dichloromethane/methanol, 9:1) a (4:1) mixture of **12a** and **12b** (54 mg, 82%) as an oil. ¹H-NMR (500 MHz, CDCl₃) δ: 3.52 (dd, 1H, *J*_{5',6'a} = 4.4 Hz, *J*_{6'a,6'b} = 10.6 Hz, H-6'a_{trans}), 3.58 (dd, 1H, *J*_{5',6'b} = 4.0 Hz, *J*_{6'a,6'b} = 10.5 Hz, H-6'b_{trans}), 3.93 (m, 2H, H-3'_{trans}, H-4'_{trans}), 4.06 (m, 1H, H-4'_{cis}), 4.16 (m, 1H, H-3'_{cis}), 4.26 (m, 2H, H-1'a_{trans}, H-5'_{trans}), 4.33 (m, 2H, H-1'b_{trans}, H-2'_{trans}), 4.48-4.65 (m, 6H, CH₂Ph_{trans,cis}), 5.12 (s, 1H, H-5_{trans}), 5.25 (s, 1H, H-5_{cis}), 7.27-7.37 (m, 15H, Ar_{trans,cis}). ¹³C-NMR (125 MHz, CDCl₃) δ: 64.1 (C-1'_{cis}), 65.6 (C-1'_{trans}), 70.0 (C-6'_{cis}), 70.3 (C-6'_{trans}), 71.9, 72.0, 73.5 (CH₂Ph_{trans}), 72.6, 73.4 (CH₂Ph_{cis}), 77.5 (C-4'_{trans}), 77.7 (C-3'_{trans}), 78.2 (C-4'_{cis}), 78.5 (C-3'_{cis}), 78.8 (C-5_{trans}), 79.2 (C-2'_{cis}), 79.8 (C-2'_{trans}), 80.1 (C-5'_{cis}), 81.4 (C-5'_{trans}), 127.5-128.5 (CH Ar_{trans,cis}), 137.8-138.4 (C Ar_{trans,cis}), 162.6 (C-2_{trans}), 162.7 (C-2_{cis}), 165.3 (C-6_{trans}), 165.4 (C-6_{cis}), 171.0 (C-4_{trans}), 171.1 (C-4_{cis}). HPLC (Waters 2690, gradient 2): *t*_R = 3.51 min (79%) and 3.93 min (19%). HRMS (ES+) *m/z*: Calcd for C₃₁H₃₄N₄O₅ 542.2527. Found 542.2529.

2-Amino-4-(2',5'-anhydro-3',4',6'-tri-O-benzyl-D-allitoly)-6-chloropyrimidine and 2-Amino-4-(2',5'-anhydro-3',4',6'-tri-O-benzyl-D-altritoly)-6-chloropyrimidine (19a** and **19b**):** Following the general procedure **11a,b** (200 mg, 0.46 mmol) reacted with 2-amino-4,6-dichloropyrimidine (**18**) (133 mg, 0.92 mmol) in a pressure tube for 6 h. After the work-up and

purification by CCTLC in the chromatotron (hexane/ethyl ether, 1:1), gave from the fastest moving band compound **19b** (40 mg, 15%) as an oil. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 3.51 (dd, 1H, $J_{5',6'a} = 3.7$ Hz, $J_{6'a,6'b} = 10.6$ Hz, H-6'a), 3.60 (dd, 1H, $J_{5',6'b} = 3.6$ Hz, $J_{6'a,6'b} = 10.6$ Hz, H-6'b), 4.07 (dd, 1H, $J_{2',3'} = 5.6$ Hz, $J_{3',4'} = 4.7$ Hz, H-4'), 4.17 (dd, 1H, $J_{3',4'} = 4.7$ Hz, $J_{4',5'} = 5.6$ Hz, H-3'), 4.26 (dt, 1H, $J_{4',5'} = 5.6$ Hz, $J_{5',6'a} = J_{5',6'b} = 3.6$ Hz, H-5'), 4.40 (dt, 1H, $J_{2',3'} = 5.2$ Hz, $J_{1',2'a} = J_{1',2'b} = 6.7$ Hz, H-2'), 4.55 (m, 2H, H-1'), 4.48 (d, 1H, $J = 11.9$ Hz, CH_2Ph), 4.50-4.57 (m, 4H, H-1', CH_2Ph), 4.57 (d, 1H, $J = 11.7$ Hz, CH_2Ph), 4.63 (d, 1H, $J = 11.9$ Hz, CH_2Ph), 4.74 (d, 1H, $J = 11.8$ Hz, CH_2Ph), 5.13 (bs, 2H, NH_2), 6.11 (s, 1H, H-5), 7.27-7.37 (m, 15H, Ar). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 65.9 (C-1'), 70.1 (C-6'), 72.7, 73.4, 73.6 (CH_2Ph), 77.6 (C-3'), 77.7 (C-2'), 79.1 (C-4'), 80.5 (C-5'), 97.4 (C-5), 127.5-128.5 (CH Ar), 137.9, 138.1, 138.2 (C Ar), 160.8 (C-2), 162.2 (C-6), 171.1 (C-4). HPLC (Agilent 1120, gradient 3): $t_R = 10.63$ min (98%). HRMS (ES+) m/z : Calcd for $\text{C}_{31}\text{H}_{32}\text{ClN}_3\text{O}_5$ 561.2051. Found 561.2030.

From the slowest moving band compound **19a** (184 mg, 71%) was isolated as an oil. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 3.49 (dd, 1H, $J_{5',6'a} = 4.2$ Hz, $J_{6'a,6'b} = 10.5$ Hz, H-6'a), 3.54 (dd, 1H, $J_{5',6'b} = 4.1$ Hz, $J_{6'a,6'b} = 10.5$ Hz, H-6'b), 3.88 (m, 1H, H-3'), 3.95 (m, 1H, H-4'), 4.25 (m, 1H, H-5'), 4.27 (m, 1H, H-1'a), 4.33 (m, 1H, H-2'), 4.40 (dd, 1H, $J_{1'b,2'} = 3.3$ Hz, $J_{1'a,1'b} = 11.2$ Hz, H-1'b), 4.48 (d, 1H, $J = 11.9$ Hz, CH_2Ph), 4.50 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.54 (d, 1H, $J = 12.3$ Hz, CH_2Ph), 4.55 (d, 1H, $J = 12.3$ Hz, CH_2Ph), 4.58 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.60 (d, 1H, $J = 11.9$ Hz, CH_2Ph), 5.05 (Bs, 2H, NH_2), 5.95 (s, 1H, H-5), 7.27-7.37 (m, 15H, Ar). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 66.3 (C-1'), 70.3 (C-6'), 72.0, 72.2, 73.6 (CH_2Ph), 77.2 (C-4'), 77.5 (C-3'), 79.2 (C-2'), 81.7 (C-5'), 97.4 (C-5), 127.6-128.6 (CH Ar), 137.7, 137.8, 138.2 (C Ar), 160.8 (C-2), 162.1 (C-6), 170.9 (C-4). HPLC (Agilent 1120, gradient 3): $t_R = 10.47$ min (98%). HRMS (ES+) m/z : Calcd for $\text{C}_{31}\text{H}_{32}\text{ClN}_3\text{O}_5$ 561.2051. Found 561.2030.

2,6-Diamino-4-[[[(2'RS,5'SR)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy]pyrimidine (**23**):

Following the general procedure **21** [34] (850 mg, 3.82 mmol) reacted with 2,4-diamino-6-chloropyrimidine (**17**) (828 mg, 5.73 mmol) in a pressure tube overnight. After the work-up the residue was purified by reverse phase chromatography using HPFC in the Biotage (water/acetonitrile, 1:1) to give **23** (693 mg, 55%) as an oil. $^1\text{H-NMR}$ (300 MHz,

CDCl₃) δ : 1.75-1.99 (m, 4H, H-3', H-4'), 3.50 (m, 2H, CH_{2a}Oar, CH_{2a}Obn), 4.20 (m, 4H, H-2', H-5', CH_{2b}Obn, CH_{2b}Oar), 4.56 (m, 2H, CH₂Ph), 4.68 (bs, 2H, NH₂), 4.86 (bs, 2H, NH₂), 5.22 (s, 1H, H-5), 7.27-7.37 (m, 5H, Ar). ¹³C-NMR (75 MHz, CDCl₃) δ : 27.9 (C-3', C-4'), 68.2 (CH₂Obn), 73.0 (CH₂Oar), 73.3 (CH₂Ph), 77.7 (C-5), 78.4 (C-2'), 78.8 (C-5'), 127.5, 127.7, 128.4 (CH Ar), 138.5 (C Ar), 162.2 (C-6), 164.9 (C-2), 171.3 (C-4). HPLC (Waters 2690, gradient 2): t_R = 3.51 min (79%). HRMS (ES+) m/z : Calcd for C₁₇H₂₂N₄O₃ 330.1698. Found 330.1692.

2-Amino-6-chloro-4-[(2'*RS*,5'*SR*)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (24): According to the general procedure **21**^[34] (259 mg, 1.16 mmol) reacted with 2-amino-4,6-dichloropyrimidine (**18**) (228 mg, 1.39 mmol) in dry DMF (5 mL) in a pressure tube overnight. Work-up and purification by reverse phase chromatography using HPFC in the Biotage (water/acetonitrile, 1:1) gave **24** (321 mg, 79%) as an oil. ¹H-NMR (400 MHz, DMSO-d₆) δ : 1.64-1.94 (m, 4H, H-3', H-4'), 3.39 (dd, 1H, $J_{5',CH2a}$ = 5.8 Hz, $J_{CH2a,CH2b}$ = 10.2 Hz, CH_{2a}Obn), 3.43 (dd, 1H, $J_{5',CH2b}$ = 4.6 Hz, $J_{CH2a,CH2b}$ = 10.2 Hz, CH_{2b}Obn), 4.04 (m, 1H, H-5'), 4.15 (m, 2H, H-2', CH_{2a}Oar), 4.25 (dd, 1H, $J_{2',CH2b}$ = 6.5 Hz, $J_{CH2a,CH2b}$ = 13.4 Hz, CH_{2b}Oar), 4.48 (m, 2H, CH₂Ph), 6.07 (s, 1H, H-5), 7.08 (bs, 2H, NH₂), 7.27-7.37 (m, 5H, Ar). ¹³C-NMR (100 MHz, DMSO-d₆) δ : 27.3, 27.5 (C-3', C-4'), 68.5 (CH₂Oar), 72.2 (CH₂Ph), 72.5 (CH₂Obn), 76.5 (C-2'), 78.3 (C-5'), 94.3 (C-5), 127.3, 127.4, 128.3 (CH Ar), 138.5 (C Ar), 160.0 (C-2), 162.8 (C-6), 170.5 (C-4). HPLC (Agilent 1120, gradient 3): t_R = 8.43 min (98%). HRMS (ES+) m/z : Calcd for C₁₇H₂ClN₃O₃ 349.1211. Found 349.1193.

6-Amino-4-[(2'*RS*,5'*SR*)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (25): The general procedure was followed with **21**^[34] (85 mg, 0.38 mmol) and 4-amino-6-chloropyrimidine (**22**) (59 mg, 0.46 mmol). The crude was purified by CCTLC in the chromatotron (ethyl acetate/methanol, 9:1) to give **25** (69 mg, 58%) as an oil. ¹H-NMR (400 MHz, CDCl₃) δ : 1.78-2.00 (m, 4H, H-3', H-4'), 3.49 (dd, 2H, $J_{5',CH2a}$ = 5.3 Hz, $J_{CH2a,CH2b}$ = 10.1 Hz, CH_{2a}Obn), 3.52 (dd, 2H, $J_{5',CH2b}$ = 4.9 Hz, $J_{CH2a,CH2b}$ = 10.1 Hz, CH_{2b}Obn), 4.14-4.31 (m, 3H, H-2', H-5', CH_{2b}Oar), 4.38 (dd, 1H, $J_{2',CH2a}$ = 3.2 Hz, $J_{CH2a,CH2b}$ =

10.2 Hz, CH_{2a}Oar), 4.54 (d, 1H, *J* = 12.2 Hz, CH₂Ph), 4.58 (d, 1H, *J* = 12.2 Hz, CH₂Ph), 4.83 (bs, 2H, NH₂), 5.77 (s, 1H, H-5), 7.27-7.37 (m, 5H, Ar), 8.23 (s, 1H, H-2). ¹³C-NMR (100 MHz, CDCl₃) δ: 27.9, 28.0 (C-3', C-4'), 68.7 (CH₂Oar), 72.9 (CH₂Obn), 73.5 (CH₂Ph), 77.7 (C-2'), 79.1 (C-5'), 87.7 (C-5), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 158.0 (C-2), 164.3 (C-6), 170.0 (C-4). HPLC (Waters 2690, gradient 3): *t_R* = 3.17 min (99%). HRMS (ES+) *m/z*: Calcd for C₁₇H₂₁N₃O₃ 315.1571. Found 315.1583.

4-Amino-2-[(2'*RS*,5'*SR*)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (30):

Following the general procedure **21**^[34] (111 mg, 0.50 mmol) reacted with 4-amino-2-chloropyrimidine (**29**) (78 mg, 0.60 mmol). The crude was purified by reverse phase chromatography using HPFC in the Biotage (water/acetonitrile, 1:1) to give **30** (59 mg, 37%) as an oil. ¹H-NMR (400 MHz, CDCl₃) δ: 1.76-2.02 (m, 4H, H-3', H-4'), 3.49 (dd, 1H, *J*_{5',CH2a} = 5.8 Hz, ¹H-RMN (400 MHz, CDCl₃) δ: 1.76-2.02 (m, 4H, H-3', H-4'), 3.49 (dd, 1H, *J*_{5',CH2a} = 5.8 Hz, *J*_{CH2a,CH2b} = 10.0 Hz, CH_{2a}Obn), 3.52 (dd, 1H, *J*_{5',CH2b} = 5.9 Hz, *J*_{CH2a,CH2b} = 10.0 Hz, CH_{2b}Obn), 4.16 (m, 1H, H-5'), 4.28 (m, 3H, H-2', CH₂Oar), 4.53 (d, 1H, *J* = 12.1 Hz, CH₂Ph), 4.58 (d, 1H, *J* = 12.1 Hz, CH₂Ph), 5.04 (bs, 2H, NH₂), 6.06 (d, 1H, *J*_{5,6} = 5.7 Hz, H-5), 7.27-7.37 (m, 5H, Ar), 7.99 (d, 1H, *J*_{5,6} = 5.7 Hz, H-6). ¹³C-NMR (100 MHz, CDCl₃) δ: 28.1, 28.3 (C-3', C-4'), 69.1 (CH₂Oar), 73.1 (CH₂Obn), 73.5 (CH₂Ph), 77.5 (C-2'), 78.9 (C-5'), 99.6 (C-5), 127.7, 127.8, 128.5 (CH Ar), 138.5 (C Ar), 157.3 (C-6), 164.8 (C-4), 165.1 (C-2). HPLC (Agilent 1120, gradient 3): *t_R* = 5.74 min (99%). HRMS (ES+) *m/z*: Calcd for C₁₇H₂₁N₃O₃ 315.1571. Found 315.1583.

2-Amino-6-methoxy-4-[(2'*RS*,5'*SR*)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (39):

The general procedure was followed with **21**^[34] (200 mg, 0.90 mmol) and 2-amino-4-chloro-6-methoxypyrimidine (**36**) (176 mg, 1.10 mmol) in dry DMF (10 mL). The reaction was stirred at a 100 °C in a pressure tube overnight. After the work-up the crude was purified by reverse phase chromatography using HPFC in the Biotage (water/acetonitrile, 3:2). From the fastest moving bands **39** (168 mg, 54%) was isolated as an oil. ¹H-NMR (400 MHz, CDCl₃) δ: 1.80-2.02 (m, 4H, H-3', H-4'), 3.50 (dd, 1H, *J*_{5',CH2a} = 5.4 Hz, *J*_{CH2a,CH2b} = 10.1 Hz, CH_{2a}Obn), 3.53 (dd, 1H, *J*_{5',CH2bObn} = 4.3 Hz,

$J_{\text{CH2a,CH2b}} = 10.1$ Hz, CH2bObn), 3.91 (s, 3H, CH_3), 4.15-4.35 (m, 4H, CH_2Oar , H-2', H-5'), 4.53 (d, 1H, $J = 12.1$ Hz, CH_2Ph), 4.56 (d, 1H, $J = 12.1$ Hz, CH_2Ph), 5.50 (s, 1H, H-5), 7.27-7.37 (m, 5H, Ar). ^{13}C -NMR (100 MHz, CDCl_3) δ : 27.8, 27.9 (C-3', C-4'), 56.2 (CH_3), 70.9 (CH_2Oar), 72.7 (CH_2Obn), 73.4 (CH_2Ph), 77.0 (C-2'), 78.3 (C-5), 79.3 (C-5'), 127.3, 127.9, 128.4 (CH Ar), 138.3 (C Ar), 157.8 (C-2), 169.2 (C-6), 171.1 (C-4). HPLC (Agilent 1120, gradient 3): $t_R = 6.92$ min (97%). HRMS (ES+) m/z : Calcd for $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_4$ 345.1704. Found 345.1689.

The slowest moving bands gave **2-amino-4,6-bis{[(2'RS,5'SR)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (43)** (42 mg, 9%) as an oil. $[\alpha]_D - 0.9^\circ$ (c 0.25, CHCl_3). ^1H -NMR (400 MHz, CDCl_3) δ : 1.80-2.06 (m, 8H, H-3', H-4') 3.48 (dd, 2H, $J_{5',\text{CH2a}} = 4.8$ Hz, $J_{\text{CH2a,CH2b}} = 10.0$ Hz, CH2aObn), 3.51 (dd, 2H, $J_{5',\text{CH2b}} = 5.4$ Hz, $J_{\text{CH2a,CH2b}} = 10.0$ Hz, CH2bObn), 4.14-4.36 (m, 8H, CH_2Oar , H-2', H-5'), 4.55 (m, 4H, CH_2Ph), 5.52 (s, 1H, H-5), 7.27-7.37 (m, 10H, Ar). ^{13}C -NMR (100 MHz, CDCl_3) δ : 27.9, 28.1 (C-3', C-4'), 71.3 (CH_2Oar), 72.8 (CH_2Obn), 73.5 (CH_2Ph), 77.2 (C-2'), 78.9 (C-5), 79.5 (C-5'), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 157.5 (C-2'), 169.7 (C-4', C-6'). HPLC (Agilent 1120, gradient 3): $t_R = 9.06$ min (99%). HRMS (ES+) m/z : Calcd for $\text{C}_{30}\text{H}_{37}\text{N}_3\text{O}_6$ 535.2683. Found 535.2682.

6-Methoxy-4-[(2'RS,5'SR)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (40):

The general procedure was followed with **21**^[34] (200 mg, 0.90 mmol) and 4-chloro-6-methoxypyrimidine (**37**) (159 mg, 1.10 mmol). The reaction was stirred in dry DMF (10 mL) at a 100 °C in a pressure tube overnight. After the work-up the crude was purified by reverse phase chromatography using HPFC in the Biotage (water/acetonitrile, 1:2). From the fastest moving fractions **40** (133 mg, 45%) was isolated as an oil. $[\alpha]_D - 0.1^\circ$ (c 0.5, CHCl_3); ^1H -NMR (400 MHz, CDCl_3) δ : 1.78-2.03 (m, 4H, H-3', H-4'), 3.48 (dd, 1H, $J_{5',\text{CH2a}} = 4.9$ Hz, $J_{\text{CH2a,CH2b}} = 10.0$ Hz, CH2aObn), 3.52 (dd, 1H, $J_{5',\text{CH2b}} = 5.3$ Hz, $J_{\text{CH2a,CH2b}} = 10.0$ Hz, CH2bObn), 3.93 (s, 3H, CH_3), 4.18 (m, 1H, H-5'), 4.25-4.44 (m, 3H, CH_2Oar , H-2'), 6.07 (s, 1H, H-5), 7.27-7.37 (m, 5H, Ar), 8.41 (s, 1H, H-2). ^{13}C -NMR (100 MHz, CDCl_3) δ : 27.9, 28.1 (C-3', C-4'), 54.2 (CH_3), 69.1 (CH_2Oar), 72.8 (CH_2Obn), 73.5 (CH_2Ph), 77.6 (C-2'), 79.1 (C-5'), 90.8 (C-5), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 157.5 (C-2), 170.9 (C-4), 171.3

(C-6). HPLC (Agilent 1120, gradient 3): t_R = 8.56 min (97%). HRMS (ES+) m/z : Calcd for $C_{18}H_{22}N_2O_4$ 330.1592. Found 330.1580.

The slowest moving fractions gave **4,6-bis{[(2'*RS*,5'*SR*)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (44)** (38 mg, 8%) as an oil. $[\alpha]_D - 0.8^\circ$ (c 0.25, $CHCl_3$). 1H -NMR (400 MHz, $CDCl_3$) δ : 1.77-2.03 (m, 8H, H-3', H-4'), 3.49 (dd, 2H, $J_{5',CH2a} = 4.9$ Hz, $J_{CH2a,CH2b} = 10.0$ Hz, $CH_{2a}Obn$), 3.51 (dd, 2H, $J_{5',CH2b} = 5.3$ Hz, $J_{CH2a,CH2b} = 10.0$ Hz, $CH_{2b}Obn$), 4.18 (m, 2H, 2H-5'), 4.29 (m, 4H, $CH_{2a}Oar$, H-2'), 4.38 (m, 2H, $CH_{2b}Oar$), 4.53 (d, 1H, $J = 12.2$ Hz, CH_2Ph), 4.56 (d, 1H, $J = 12.2$ Hz, CH_2Ph), 6.10 (s, 1H, H-5), 7.27-7.37 (m, 10H, Ar), 8.37 (s, 1H, H-2). ^{13}C -NMR (100 MHz, $CDCl_3$) δ : 27.9, 28.1 (C-3', C-4'), 69.1 (CH_2Oar), 72.8 (CH_2Obn), 73.5 (CH_2Ph), 77.6 (C-2'), 79.1 (C-5'), 91.3 (C-5), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 157.3 (C-2), 170.9 (C-4, C-6). HPLC (Agilent 1120, gradient 3): t_R = 10.39 min (97%). HRMS (ES+) m/z : Calcd for $C_{30}H_{36}N_2O_6$ 520.2597. Found 520.2573.

2-Amino-6-methoxy-4-{[(2'*RS*,5'*SR*)-5'-(hydroxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (41): The general procedure was followed with **38**^[37] (120 mg, 0.32 mmol) and 2-amino-4-chloro-6-methoxypyrimidine (**36**) (61 mg, 0.38 mmol) in dry DMF (5 mL). The reaction was heated at 100 °C in a pressure tube overnight. The crude was purified by CCTLC in the chromatotron (ethyl acetate/methanol, 10:1) to give **41** (16 mg, 10%) as an oil. 1H -NMR (400 MHz, $DMSO-d_6$) δ : 1.62-1.94 (m, 4H, H-3', H-4'), 3.35 (m, 2H, CH_2OH), 3.75 (s, 3H, CH_3), 3.86 (m, 1H, H-2'), 4.09 (m, 2H, $CH_{2a}Oar$, H-5'), 4.16 (m, 1H, $CH_{2b}Oar$), 4.65 (bs, 1H, OH), 5.33 (s, 1H, H-5), 6.56 (bs, 2H, NH_2). ^{13}C -NMR (100 MHz, $DMSO-d_6$) δ : 27.2, 27.5 (C-3', C-4'), 53.2 (OCH_3), 63.9 (CH_2OH), 68.1 (CH_2Oar), 76.7 (C-5'), 78.1 (C-5), 80.3 (C-2'), 162.7 (C-2), 171.2 (C-6), 171.7 (C-4). HPLC (Agilent 1120, gradient 3): t_R = 3.77 min (99%). HRMS (ES+) m/z : Calcd for $C_{11}H_{17}N_3O_4$ 256.1224. Found 255.1219.

6-Methoxy-4-{[(2'*RS*,5'*SR*)-5'-(hydroxymethyl)tetrahydrofuran-2'-yl]methoxy}pyrimidine (42): The general procedure was followed with **38**^[37] (120 mg, 0.32 mmol) and 4-chloro-6-methoxypyrimidine (**37**) (56 mg, 0.38 mmol). The reaction was heated at 100 °C in dry DMF (5 mL) in a pressure tube overnight. The crude was purified by CCTLC in the chromatotron (ethyl

acetate/methanol, 10:1) **42** (18 mg, 12%) as an oil. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.79-2.07 (m, 4H, H-3', H-4'), 2.80 (bs, 1H, OH), 3.50 (dd, 1H, $J_{2',\text{CH}_2\text{a}} = 4.4$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 11.8$ Hz, CH_2aOH), 3.74 (dd, 1H, $J_{2',\text{CH}_2\text{b}} = 3.0$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 11.8$ Hz, CH_2aOH), 3.93 (s, 3H, CH_3), 4.12 (m, 1H, H-2'), 4.33 (m, 2H, CH_2aOar , H-5'), 4.45 (m, 1H, CH_2bOar), 6.09 (s, 1H, H-5), 8.40 (s, 1H, H-2). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 26.7, 28.3 (C-3', C-4'), 54.3 (OCH_3), 64.6 (CH_2OH), 69.0 (CH_2Oar), 77.9 (C-5'), 80.8 (C-5), 91.0 (C-2'), 157.4 (C-2), 170.8 (C-6), 171.5 (C-4). HPLC (Agilent 1120, gradient 3): $t_R = 4.83$ min (97%). HRMS (ES+) m/z : Calcd for $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_4$ 240.1101. Found 240.1110.

4,6-Diamino-2-[(2'*RS*,5'*SR*)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy-1,3,5-triazine (47**):** The general procedure was followed with **21**^[34] (105 mg, 0.47 mmol) in dry DMF (6 mL) and 2-chloro-4,6-diamino-1,3,5-triazine (**45**) (96 mg, 0.66 mmol). The reaction was heated at 100 °C 3 h in the microwave, additional portions of 60% NaH (19 mg, 0.47 mmol) and triazine (**48** mg, 0.33 mmol) were added each hour. After cooling to room temperature, MeOH was added (to remove the excess of NaH) and evaporated to dryness. The crude was purified by reverse phase chromatography using HPFC in the Biotage (water/acetonitrile, 9:1) to give **47** (98 mg, 62%) as an amorphous white solid. $[\alpha]_D - 0.1^\circ$ (c 0.5, CH_3OH). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 1.65-1.94 (m, 4H, H-3', H-4'), 3.40 (dd, 1H, $J_{5',\text{CH}_2\text{a}} = 5.8$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.2$ Hz, CH_2aObn), 3.44 (dd, 1H, $J_{5',\text{CH}_2\text{b}} = 4.6$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.2$ Hz, CH_2bObn), 4.05 (m, 1H, H-5'), 4.16 (m, 1H, H-2'), 4.20 (dd, 1H, $J_{2',\text{CH}_2\text{a}} = 6.1$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.8$ Hz, CH_2aOar), 4.31 (dd, 1H, $J_{2',\text{CH}_2\text{b}} = 3.7$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.8$ Hz, CH_2bOar), 4.48 (m, 2H, CH_2Ph), 7.23-7.37 (m, 5H, Ar), 7.83 (bs, 4H, 2NH_2). $^{13}\text{C-RMN}$ (100 MHz, DMSO-d_6) δ : 27.2, 27.5 (C-3', C-4'), 69.6 (CH_2Oar), 72.2 (CH_2Ph), 72.5 (CH_2Obn), 76.3 (C-2'), 78.4 (C-5'), 127.3, 127.4, 128.2 (CH Ar), 138.5 (C Ar), 163.0 (C-4, C-6), 166.4 (C-2). HPLC (Agilent 1120, gradient 3): $t_R = 5.71$ min (93%). HPLC (Agilent 1120, gradient 3): $t_R = 5.72$ min (99%). HRMS (ES+) m/z : Calcd for $\text{C}_{16}\text{H}_{21}\text{N}_5\text{O}_3$ 331.1636. Found 331.1644.

2-Amino-6-[(2'*RS*,5'*SR*)-5'-(benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy-7H-purine (53**):** The general procedure was followed with **21**^[34] (50 mg, 0.22 mmol) in dry DMF (5 mL), and **51**^[39] (74 mg, 0.26 mmol). The

reaction was heated at 100 °C in a pressure tube overnight. After the work-up, the crude was purified by HPFC in the Biotage (dichloromethane/methanol, 9:1) to give **53** (23 mg, 30%) as an amorphous solid. $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 1.70-2.00 (m, 4H, H-3', H-4'), 3.42 (dd, 1H, $J_{5',\text{CH}_2\text{a}} = 5.6$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.1$ Hz, CH_2aObn), 3.46 (dd, 1H, $J_{5',\text{CH}_2\text{b}} = 4.5$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.1$ Hz, CH_2bObn), 4.07 (m, 1H, H-5'), 4.25 (m, 1H, H-2'), 4.32 (dd, 1H, $J_{2',\text{CH}_2\text{a}} = 6.4$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.9$ Hz, CH_2aOar), 4.41 (dd, 1H, $J_{2',\text{CH}_2\text{b}} = 3.9$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.9$ Hz, CH_2bOar), 4.48 (m, 2H, CH_2Ph), 6.25 (s, 2H, NH_2), 7.27-7.37 (m, 5H, Ar), 7.82 (s, 1H, H-8), 12.42 (s, 1H, NH). $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6) δ : 27.5, 27.9 (C-3', C-4'), 67.9 (CH_2Oar), 72.2 (CH_2Ph), 72.6 (CH_2Obn), 76.8 (C-2'), 78.3 (C-5'), 112.4 (C-5), 127.3, 127.4, 128.2 (CH Ar), 138.3 (C Ar), 138.5 (C-8), 155.8 (C-4), 159.6 (C-6), 159.7 (C -2). HPLC (Agilent 1120, gradient 3): $t_R = 5.62$ min (99%). HRMS (ES+) m/z : Calcd for $\text{C}_{18}\text{H}_{21}\text{N}_5\text{O}_3$ 355.1647. Found: 355.1644.

6-[(2'*RS*,5'*SR*)-5'-(Benzyloxymethyl)tetrahydrofuran-2'-yl]methoxy}-7H-

purine (54): Following the general procedure **21**^[34] (390 mg, 1.75 mmol) reacted with **52**³⁹ (650 mg, 2.62 mmol) in dry DMF (10 mL) at 100 °C in a pressure tube overnight. The crude was purified by HPFC in the Biotage (dichloromethane/methanol, 9:1) to give **54** (0.60 g, 53%) as an amorphous solid. $[\alpha]_D$ 0.4 ° (c 0.9, CHCl_3). $^1\text{H-NMR}$ (500 MHz, DMSO-d_6) δ : 1.75-2.01 (m, 4H, H-3', H-4'), 3.41 (dd, 1H, $J_{5',\text{CH}_2\text{a}} = 5.8$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.3$ Hz, CH_2aObn), 3.46 (dd, 1H, $J_{5',\text{CH}_2\text{b}} = 4.5$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 10.3$ Hz, CH_2bObn), 4.07 (m, 1H, H-5'), 4.30 (m, 1H, H-2'), 4.43 (dd, 1H, $J_{2',\text{CH}_2\text{a}} = 6.6$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 11.2$ Hz, CH_2aOar), 4.46 (m, 2H, CH_2Ph), 4.58 (dd, 1H, $J_{2',\text{CH}_2\text{b}} = 3.8$ Hz, $J_{\text{CH}_2\text{a},\text{CH}_2\text{b}} = 11.2$ Hz, CH_2bOar), 7.27-7.37 (m, 5H, Ar), 8.39 (s, 1H, H-2), 8.47 (s, 1H, H-8), 13.42 (bs, 1H, NH). $^{13}\text{C-NMR}$ (125 MHz, DMSO-d_6) δ : 27.4, 27.5 (C-3', C-4'), 68.7 (CH_2Oar), 72.2 (CH_2Ph), 72.6 (CH_2Obn), 76.7 (C-2'), 78.3 (C-5'), 120.2 (C-5), 127.3, 127.4, 128.1 (CH Ar), 138.6 (C Ar), 141.8 (C-8), 150.8 (C-2), 153.1 (C-4), 159.7 (C-6). HPLC (Agilent 1120, gradient 3): $t_R = 6.42$ min (99%). HRMS (ES+) m/z : Calcd for $\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_3$: 340.1531. Found 340.1535.

General procedure for the catalytic hydrogenation of benzyl protected compounds: A solution of the corresponding benzyl derivatives (0.30 mmol) in methanol (10 mL) and 37 % HCl (49 μL , 0.60 mmol) was hydrogenated

overnight at 30 °C y 40 psi, in the presence of 20% Pd(OH)₂/C (10 mg). The reaction was filtered, washed with methanol, evaporated to dryness and the residue purified. The purification method, solvents and yields are indicated in each case.

2,6-Diamino-4-(2',5'-anhydro-D-allitoly)pyrimidine dihydrochloride (13^a): A solution of **12a,b** (90 mg, 0.17 mmol) in methanol (10 mL) was hydrogenated overnight at 30 °C y 40 psi, in the presence of 20% Pd(OH)₂/C (10 mg). The reaction was filtered, washed with methanol and evaporated to dryness. The residue was purified by CCTLC in the chromatotron (dichloromethane/ methanol/ ammonium hydroxide, 4:1:0.1) to give **13a** (49 mg, 80%) as an oil. ¹H-NMR (400 MHz, DMSO-d₆) δ: 3.34 (dd, 1H, *J*_{5',6'a} = 5.0 Hz, *J*_{6'a,6'b} = 11.6 Hz, H-6'a), 3.42 (dd, 1H, *J*_{5',6'b} = 4.3 Hz, *J*_{6'a,6'b} = 11.6 Hz, H-6'b), 3.70 (m, 1H, H-5'), 3.74 (m, 1H, H-3'), 3.80 (dd, 1H, *J*_{3',4'} = 5.5 Hz, *J*_{4',5'} = 4.3 Hz, H-4'), 3.89 (m, 1H, H-2'), 4.15 (dd, 1H, *J*_{1'a,2'} = 6.1 Hz, *J*_{1'a,1'b} = 11.3 Hz, H-1'a), 4.35 (dd, 1H, *J*_{1'b,2'} = 3.4 Hz, *J*_{1'a,1'b} = 11.3 Hz, H-1'b), 5.35 (s, 1H, H-5). ¹³C-NMR (100 MHz, DMSO-d₆) δ: 61.9 (C-6'), 68.3 (C-1'), 71.3 (C-4'), 71.6 (C-3'), 75.8 (C-5), 79.8 (C-2'), 85.0 (C-5'), 154.9 (C-4), 157.1 (C-2), 170.8 (C-6). HPLC (Waters 600, gradient 4): *t_R* = 0.71 min (97%). HRMS (ES+) *m/z*: Calcd for C₁₀H₁₆N₄O₅ 272.1126. Found 272.1121.

2-Amino-4-(2',5'-anhydro-D-allitoly)pyrimidine (20^a): A solution of **19a** (80 mg, 0.14 mmol) in methanol (10 mL) was hydrogenated overnight at 30 °C y 40 psi, in the presence of 10% Pd/C (10 mg). The reaction was filtered, washed with methanol and evaporated to dryness. The residue was purified by CCTLC in the chromatotron (dichloromethane/ methanol/ ammonium hydroxide, 4:1:0.1) to give **20a** (18 mg, 50%) as an oil. ¹H-NMR (400 MHz, DMSO-d₆) δ: 3.33 (dd, 1H, *J*_{5',6'a} = 5.0 Hz, *J*_{6'a,6'b} = 11.5 Hz, H-6'a), 3.41 (dd, 1H, *J*_{5',6'b} = 4.2 Hz, *J*_{6'a,6'b} = 11.5 Hz, H-6'b), 3.68 (dt, 1H, *J*_{4',5'} = 4.3 Hz, *J*_{5',6'a} = 5.0 Hz, *J*_{5',6'b} = 4.2 Hz, H-5'), 3.73 (dd, 1H, *J*_{2',3'} = 6.6 Hz, *J*_{3',4'} = 5.4 Hz, H-3'), 3.78 (dd, 1H, *J*_{3',4'} = 5.4 Hz, *J*_{4',5'} = 4.3 Hz, H-4'), 3.89 (td, 1H, *J*_{2',3'} = 6.6 Hz, *J*_{1'a,2'} = 6.7 Hz, *J*_{1'b,2'} = 3.2 Hz, H-2'), 4.13 (dd, 1H, *J*_{1'a,2'} = 6.7 Hz, *J*_{1'a,1'b} = 11.4 Hz, H-1'a), 4.33 (dd, 1H, *J*_{1'b,2'} = 3.2 Hz, *J*_{1'a,1'b} = 11.4 Hz, H-1'b), 4.69 (bs, 1H, OH), 4.85 (bs, 1H, OH), 4.92 (bs, 1H,

OH), 6.00 (d, 1H, $J_{5,6} = 5.7$ Hz, H-5), 6.58 (bs, 2H, NH₂), 7.94 (d, 1H, $J_{5,6} = 5.7$ Hz, H-6). ¹³C-NMR (100 MHz, DMSO-d₆) δ: 62.1 (C-6'), 66.5 (C-1'), 71.3 (C-4'), 71.8 (C-3'), 80.1 (C-2'), 84.9 (C-5'), 96.4 (C-5), 158.4 (C-6), 163.2 (C-2), 169.4 (C-4). HPLC (Agilent 1120, gradient 4): $t_R = 2.55$ min (98%). HRMS (ES+) m/z : Calcd for C₁₀H₁₅N₃O₅ 257.1014. Found 257.1012.

2,6-Diamino-4-[(2'*RS*,5'*SR*)-5'-(hydroxymethyl)tetrahydrofuran-2'-

yl]methoxy}pyrimidine dihydrochloride (26): A solution of **23** (100 mg, 0.30 mmol) in methanol (10 mL) and 37 % HCl (49 µL, 0.60 mmol) was hydrogenated overnight at 30 °C y 40 psi, in the presence of 20% Pd(OH)₂/C (10 mg). The reaction was filtered, washed with methanol and evaporated to dryness. The residue was purified by CCTLC in the chromatotron (dichloromethane/methanol/ammonium hydroxide, 4:1:0.1) to give **26** (48 mg, 51%) as an oil. ¹H-NMR (400 MHz, DMSO-d₆) δ: 1.60-1.86 (m, 4H, H-3', H-4'), 3.33 (m, 2H, CH₂OH), 3.85 (m, 1H, H-5'), 4.04 (m, 3H, H-2' y CH₂Oar), 4.66 (bs, 1H, OH), 5.02 (s, 1H, H-5), 5.90 (s, 2H, NH₂), 6.03 (s, 2H, NH₂). ¹³C-NMR (100 MHz, DMSO-d₆) δ: 27.2, 27.4 (C-3', C-4'), 63.9 (CH₂OH), 67.4 (CH₂Oar), 76.2 (C-5), 76.9 (C-2'), 80.2 (C-5'), 162.8 (C-6), 166.0 (C-2), 169.9 (C-4). HPLC (Agilent 1120, gradient 4): $t_R = 3.16$ min (97%). HRMS (ES+) m/z : Calcd for C₁₀H₁₆N₄O₃ 240.1231. Found 240.1222.

2-Amino-4-[(2'*RS*,5'*SR*)-5'-(hydroxymethyl)tetrahydrofuran-2'-

yl]methoxy}pyrimidine hydrochloride (27): Following the general procedure compound **24** (46 mg, 0.13 mmol) was hydrogenated. After the work-up the crude was purified by reverse phase chromatography using SPE cartridges (dichloromethane/methanol, 9:1) to give **27** (25 mg, 74%) as an oil. ¹H-NMR (400 MHz, DMSO-d₆) δ: 1.66-1.95 (m, 4H, H-3', H-4'), 3.34 (m, 2H, CH₂OH), 3.88 (m, 1H, H-5'), 4.17 (m, 2H, H-2'), 4.26 (dd, 1H, $J_{2',CH2a} = 6.8$ Hz, $J_{CH2a,CH2b} = 11.1$ Hz, CH_{2a}Oar), 4.39 (dd, 1H, $J_{2',CH2b} = 3.6$ Hz, $J_{CH2a,CH2b} = 11.1$ Hz, CH_{2b}Oar), 6.43 (d, 1H, $J_{5,6} = 6.9$ Hz, H-5), 8.18 (d, 1H, $J_{5,6} = 6.9$ Hz, H-6). ¹³C-NMR (100 MHz, DMSO-d₆) δ: 27.1, 27.3 (C-3', C-4'), 63.7 (CH₂OH), 70.1 (CH₂Oar), 76.0 (C-2'), 80.5 (C-5'), 98.9 (C-5), 147.0 (C-6), 156.8 (C-4), 171.3 (C-2). HPLC (Agilent 1120, gradient 3): $t_R = 3.76$ min (98%). HRMS (ES+) m/z : Calcd for C₁₀H₁₅N₃O₃ 225.1115. Found 225.1113.

6-Amino-4-[(2'*RS*,5'*SR*)-5'-(hydroxymethyl)tetrahydrofuran-2'-

yl]methoxy}pyrimidine hydrochloride (28): Following the general procedure compound **25** (57 mg, 0.18 mmol) was hydrogenated overnight. After the work-up the crude was purified by CCTLC in the chromatotron (dichloromethane/methanol, 9:1) to give **28** (10 mg, 20%) as an oil (hydrochloride). ¹H-NMR (400 MHz, DMSO-d₆) δ: 1.62-1.90 (m, 4H, H-3', H-4'), 3.35 (m, 2H, CH₂OH), 3.86 (m, 1H, H-5'), 4.09 (m, 1H, H-2'), 4.09 y 4.18 (m, 2H, CH₂Oar), 4.64 (t, 1H, *J* = 5.7 Hz, OH), 5.67 (s, 1H, H-5), 6.62 (s, 2H, NH₂), 8.06 (s, 1H, H-2). ¹³C-NMR (100 MHz, DMSO-d₆) δ: 27.4, 27.5 (C-3', C-4'), 64.1 (CH₂OH), 68.1 (CH₂Oar), 76.9 (C-2'), 80.3 (C-5'), 85.8 (C-5), 157.9 (C-2), 165.5 (C-6), 168.9 (C-4). HPLC (Waters 600, gradient 2): 0.58 min (97 %) HRMS (ES+) *m/z*: Calcd for C₁₀H₁₅N₃O₃ 225.1119. Found 225.1113.

4-Amino-2-[(2'*RS*,5'*SR*)-5'-(hydroxymethyl)tetrahydrofuran-2'-

yl]methoxy}pyrimidine hydrochloride (31): Compound **30** (34 mg, 0.11 mmol) was hydrogenated according to the general procedure. The residue was purified by reverse phase chromatography using SPE cartridges (dichloromethane/methanol, 9:1) to give **31** (8 mg, 28%) as an oil. ¹H-RMN (500 MHz, DMSO-d₆) δ: 1.65-1.92 (m, 4H, H-3', H-4'), 3.33 (m, 2H, CH₂OH), 3.86 (m, 1H, H-5'), 4.10 (m, 3H, CH₂Oar, H-2'), 4.63 (bs, 1H, OH), 6.06 (d, 1H, *J*_{5,6} = 5.7 Hz, H-5), 6.84 (bs, 2H, NH₂), 7.83 (d, 1H, *J*_{5,6} = 5.7 Hz, H-6). ¹³C-RMN (125 MHz, DMSO-d₆) δ: 27.2, 27.5 (C-3', C-4'), 63.9 (CH₂OH), 68.5 (CH₂Oar), 76.7 (C-2'), 80.2 (C-5'), 99.4 (C-5), 156.1 (C-6), 164.7 (C-4), 165.4 (C-2). HPLC (Agilent 1120, gradient 4): *t_R* = 4.94 min (99%). HRMS (ES+) *m/z*: Calcd for C₁₀H₁₆N₃O₃ 226.1197. Found 226.1192.

4,6-Diamino-2-[(2'*RS*,5'*SR*)-5'-(hydroxymethyl)tetrahydrofuran-2'-

yl]methoxy}-1,3,5-triazine dihydrochloride (48): *Method A:* To a solution of **47** (98 mg, 0.29 mmol) in methanol (10 mL) and 37 % HCl (48 μL, 0.58 mmol) in the presence of Pd(OH)₂/C 20% (10 mg) was hydrogenated at 30 °C and 40 psi overnight. After filtering, the residue was washed with methanol and evaporated to dryness. The residue was triturated with ethyl ether/dichloromethane/methanol, successively washed with several portions of

cold ethyl ether, and evaporated to dryness to give 21 mg of (2:1) mixture of **48** and ameline (**49**) determined by HPLC.

Method B: The general procedure was followed with **(2R,5S)-2,5-bis(hydroxymethyl)tetrahydrofuran (46)** (150 mg, 1.13 mmol), in dry DMF (5 mL) and 2-chloro-4,6-diamino-1,3,5-triazine (**45**) (329 mg, 2.26 mmol). The reaction was heated at 100 °C in a pressure tube for 48 h. The crude was purified by preparative TLC (dichloromethane/methanol/acetic acid, 9:1:0.1). From the fastest moving band compound **48** (114 mg, 42%) was isolated as an amorphous white solid. ¹H-NMR (400 MHz, DMSO-d₆) δ: 1.65-1.92 (m, 4H, H-3', H-4'), 3.35 (m, 2H, CH₂OH), 3.88 (m, 1H, H-5'), 4.13 (m, 1H, H-2'), 4.23 (dd, 1H, *J*_{2',CH2a} = 6.3 Hz, *J*_{CH2a,CH2b} = 11.0 Hz, CH_{2a}Oar), 4.33 (dd, 1H, *J*_{2',CH2b} = 3.9 Hz, *J*_{CH2a,CH2b} = 11.0 Hz, CH_{2b}Oar), 8.30 (bs, 4H, NH₂). ¹³C-NMR (100 MHz, DMSO-d₆) δ: 27.1, 27.2 (C-3', C-4'), 63.9 (CH₂OH), 70.2 (CH₂Oar), 75.9 (C-2'), 80.4 (C-5'), 161.0 (C-4, C-6), 165.1 (C-2). HPLC (Agilent 1120, gradient 4): *t_R* = 4.95 min (98%). HRMS (ES, +) *m/z*: Calcd for C₉H₁₅N₅O₃ 241.1179. Found 241.1175.

From the slowest moving band **(2R,5S)-2,5-bis[(4',6'-diamino-1',3',5'-triazin-2'-yl)oxymethyl]tetrahydrofuran (50)** (55 mg, 14%) was isolated, as an amorphous white solid. ¹H-NMR (400 MHz, DMSO-d₆ + HCOOH) δ: 1.69-1.96 (m, 4H, H-3, H-4), 4.12 (m, 6H, H-2, H-5, 2CH₂Oar), 6.63 (bs, 8H, 4NH₂); ¹³C-NMR (100 MHz, DMSO-d₆ + HCOOH) δ: 27.6 (C-3, C-4), 68.0 (2CH₂Oar), 77.0 (C-2, C-5), 168.3 (C-4', C-6'), 170.6 (C-2'). HPLC (Agilent 1120, gradient 4): *t_R* = 5.46 min (95%). HRMS (ES+) *m/z*: Calcd for C₁₂H₁₈N₁₀O₃ 350.1571. Found 350.1563.

2-Amino-6-[(2'RS,5'SR)-5'-(hydroxymethyl)tetrahydrofuran-2'-yl]methoxy}-7H-purine hydrochloride (55):

Compound **53** (52 mg, 0.15 mmol) was hydrogenated following the general procedure. The crude was purified by HPFC in the Biotage (dichloromethane/methanol, 9:1) to give **55** (25 mg, 41%) as an oil. ¹H-NMR (500 MHz, DMSO-d₆) δ: 1.68-1.98 (m, 4H, H-3', H-4'), 3.35 (m, 2H, CH₂OH), 3.89 (m, 1H, H-5'), 4.21 (m, 1H, H-2'), 4.30 (dd, 1H, *J*_{2',CH2a} = 6.6 Hz, *J*_{CH2a,CH2b} = 11.0 Hz, CH_{2a}Oar), 4.40 (dd, 1H, *J*_{2',CH2b} = 4.2 Hz, *J*_{CH2a,CH2b} = 11.0 Hz, CH_{2b}Oar), 4.70 (bs, 1H, OH), 6.25 (s, 2H, NH₂), 7.84 (s, 1H, H-8), 12.45 (bs, 1H, NH). ¹³C-NMR (125 MHz, DMSO-d₆) δ: 27.2, 27.6 (C-

3', C-4'), 63.9 (CH₂OH), 68.2 (CH₂Oar), 76.7 (C-2'), 80.4 (C-5'), 113.4 (C-5), 137.7 (C-8), 155.0 (C-4), 159.6 (C-6), 172.2 (C-2). HPLC (Agilent 1120, gradient 4): t_R = 5.61 min (99%). HRMS (ES+) m/z : Calcd for C₁₁H₁₅N₅O₃ 265.1162. Found 265.1175.

6-[(2'*RS*,5'*SR*)-5'-(Hydroxymethyl)tetrahydrofuran-2'-yl]methoxy}-7H-

purine hydrochloride (56): Following the general procedure, **54** (59 mg, 0.17 mmol) was hydrogenated overnight. After the work-up the crude was purified by HPFC in the Biotage (dichloromethane/methanol, 9:1) to give **56** (17 mg, 35%) as an oil. ¹H-NMR (500 MHz, DMSO-d₆) δ : 1.73-1.99 (m, 4H, H-3', H-4'), 3.36 (m, 2H, CH₂OH), 3.90 (m, 1H, H-5'), 4.27 (m, 1H, H-2'), 4.45 (dd, 1H, $J_{2',CH2a}$ = 6.8 Hz, $J_{CH2a,CH2b}$ = 11.2 Hz, CH_{2a}Oar), 4.60 (dd, 1H, $J_{2',CH2b}$ = 3.8 Hz, $J_{CH2a,CH2b}$ = 11.2 Hz, CH_{2b}Oar), 8.56 (s, 1H, H-2), 8.70 (s, 1H, H-8). ¹³C-NMR (125 MHz, DMSO-d₆) δ : 27.2, 27.4 (C-3', C-4'), 63.8 (CH₂OH), 69.2 (CH₂Oar), 76.5 (C-2'), 80.4 (C-5'), 116.4 (C-5), 142.8 (C-8), 151.8 (C-2), 154.0 (C-4), 158.6 (C-6). HPLC (Agilent 1120, gradient 4): t_R = 3.89 min (91%). HRMS (ES+) m/z : Calcd for C₁₁H₁₄N₄O₃ 250.1054. Found 250.1066.

2,6-Diamino-4-[(2'*RS*,5'*SR*)-5'-(triphosphoxymetyl)tetrahydrofuran-2'-

yl]methoxy}pyrimidine triammonium salt (59): To a cooled (0 °C) solution of compound **26** (150 mg, 0.54 mmol) and Proton Sponge (196 mg, 0.92 mmol), previously dried under vacuum for 5h, in trimethyl phosphate (2 mL), phosphorous oxychloride (0.14 mL, 1.46 mmol) was slowly added. The reaction was stirred at 5 °C overnight. A solution of tributylammonium pyrophosphate (1.6 g, 2.92 mmol) and tributylamine (0.56 mL, 2.38 mmol) in dry DMF (2.7 mL) was added and stirring was continued at 0 °C for 20 min. Then, 1M triethylammonium bicarbonate (TEAB) solution (6.7 mL) was added, and the reaction mixture was stirred at rt for 15 min and evaporated at a reduced pressure and the residue was lyophilized. The crude was purified by Sephadex-DEAE A-25 resin ion-exchange column chromatography (500 mL, linear gradient, 0-0.5 M NH₄HCO₃) to obtain **59** (23 mg, 8%) as a white solid after lyophilization. ¹H-NMR (400 MHz, D₂O) δ : 1.72-1.92 (m, 4H, H-3', H-4'), 3.53-4.14 (m, 6H, CH₂OP, CH₂Oar, H-2', H-5'), 5.56 (s, 1H, H-5). ³¹P NMR (162 MHz, D₂O) δ : 1.16, -8.49, -21.31. HPLC (Waters 600, isocratic conditions 0.5 M

KH_2PO_4): $t_R = 6.49$ min (98%). HRMS (ES+) m/z : Calcd for $\text{C}_{10}\text{H}_{18}\text{N}_4\text{O}_{12}\text{P}_3$ 480.0216. Found 480.0212.

4.2. Biological methods

Antiviral activity assays other than HIV: The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strains Lyons and G, varicella-zoster virus (VZV) strain Oka, TK^- VZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, Sindbis, reovirus-1, Punta Toro, human immunodeficiency virus type 1 strain III_B and human immunodeficiency virus type 2 strain ROD. The antiviral, other than anti-HIV, assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa) or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 CCID₅₀ of virus (1 CCID₅₀ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (VZV) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC₅₀ or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

Anti-HIV-1 and HIV-2 activity assay: Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing $\sim 3 \times 10^5$ CEM cells/mL infected with 100 CCID₅₀ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4–5 days of incubation at 37 °C in a CO₂-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The EC₅₀ (50% effective concentration) was defined as

the compound concentration required to inhibit HIV-induced giant cell formation by 50%.

HIV-1 RT Assays in the Presence of Artificial Template/Primer: To prepare the template/primers for the RT experiments, 0.15 mM poly(U), poly(A), poly(C) and poly(I) were mixed with an equal volume of 0.0375 mM oligo(dA), oligo(dT), oligo(dG) and oligo(dC), respectively. The final concentrations of the templates in the RT reaction mixture were 0.015 mM. The reaction mixture (50 μ l) contained 50 mM Tris.HCl (pH 7.8), 5 mM dithiothreitol, 300 mM glutathione, 500 μ M EDTA, 150 mM KCl, 5 mM MgCl₂, 1.25 μ g of bovine serum albumin, an appropriate concentration of labeled (tritiated) substrate dTTP, dCTP, dGTP or dATP (2 μ Ci/assay), a fixed concentration of the template/primer poly(A).oligo(dT) (0.015 mM), poly(I).oligo(dC) (0.015 mM), poly(C).oligo(dG) (0.015 mM) and poly(U).oligo(dA) (0.015 mM), 0.06% Triton X-100, 10 μ l of inhibitor solution (containing various concentrations of the compounds), and 1 μ l of the RT preparation. The reaction mixtures were incubated at 37°C for 30 min, at which time 100 μ l of yeast RNA (1 mg/ml) and 1 ml of Na₄P₂O₇ (0.02 M) in trichloroacetic acid (5% v/v) were added. The solutions were kept on ice for 30 min, after which the acid-insoluble material was washed and analyzed for radioactivity.

For the experiments in which the 50% inhibitory concentration (IC₅₀) of the test compounds was determined, fixed concentrations of 1.25 μ M [³H]dTTP, 1.75 μ M [³H]dATP, 2.5 μ M [³H]dGTP or 2.5 μ M [³H]dCTP were used.

Cytostatic Activity Assays: All assays were performed in 96-well microtiter plates. To each well were added (5–7.5) $\times 10^4$ tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and human cervix carcinoma HeLa cells) or 96 h (human kidney carcinoma Caki-1 and human liver hepatoma Huh-7) at 37 °C in a humidified CO₂-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC₅₀ (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

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Supplementary data

Supplementary data related with this article can be found, in the online version, at <http://>

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Table 1. Pseudorotational parameter values for 20a and 27 obtained by PSEUROT program.				
20a			27	
	Exp. ^[a]	Calc.	Exp. ^[a,b]	Calc.
$J_{(H2',H3'a)}$	6.58	6.68	5.30	5.18
$J_{(H2',H3'b)}$	---		6.20	6.34
$J_{(H3'a,H4'a)}$	5.35	5.17	7.00	6.89
$J_{(H3'a,H4'b)}$	---		6.50	6.51
$J_{(H3'b,H4'a)}$			6.50	6.33
$J_{(H3'b,H4'b)}$	---		7.10	6.89
$J_{(H4'a,H5')}$	4.32	4.42	5.80	5.97
$J_{(H4'b,H5')}$			6.00	6.24
P_N		43.0		8.1
T_N		40.0 ^c		39.4
P_S		133.5		158.5
T_S		40.0 ^c		36.9
X_n		0.32		0.47
r.m.s. ^d		0.13		0.16

[a] Measured in DMSO at 400 MHz. Coupling constants values in Hertz.

[b] Values obtained from the spectrum simulation.

[c] Constrained values.

[d] Root mean square in Hertz.

Table 2. Anti-HIV-1 and –HIV-2 activity and cytostatic properties of the compounds, in human T-lymphocyte (CEM) cells

Comp.	EC ₅₀ ^[a] (μM)		CC ₅₀ ^[b] (μM)
	HIV-1	HIV-2	
12^{a,b}	> 10	> 10	23 ± 2.1
13^a	> 100	> 100	> 250
19^a	> 10	> 10	35± 8.5
19b	> 250	> 250	> 250
20^a	> 100	> 100	> 250
23	> 250	> 250	> 250
24	> 50	> 50	110± 4.9
25	> 50	> 50	129± 7.1
26	> 250	> 250	> 250
27	> 250	> 250	> 250
28	> 250	> 250	> 250
30	> 250	> 250	> 250
31	> 250	> 250	> 250
39	> 10	> 10	52± 2.8
40	> 10	> 10	50± 2.8
41	> 50	> 50	> 50
42	> 50	> 50	> 50
43	> 10	> 10	28± 4.2
44	> 10	> 10	21± 3.5
47	> 250	> 250	> 250
48	> 250	> 250	> 250
50	> 50	> 50	> 50
53	> 50	> 50	191± 16
54	> 2	> 2	13± 0.71
55	> 250	> 250	> 250
56	> 250	> 250	> 250
(R) PMPA	1.1 ± 0.7	1.3 ± 1.2	>250
PMEO-DAPym	2.7 ± 1.2	1.9 ± 0.6	33

[a] 50% effective concentration

[b] 50% cytostatic concentration

Table 3. Inhibitory effects of the test compounds on the proliferation of murine Leukemia cells (L1210), human T-lymphocyte cells (CEM), Human cervix carcinoma cells (HeLa), Human prostate carcinoma cells (PC-3) and human kidney carcinoma cells (CAKI-1)

Comp.	IC ₅₀ ^[a] (μM)				
	L1210	CEM	HeLa	PC-3	CAKI-1
12a,b	18±1	23±2	22±1	22±2	22±1
13a	175±21	> 250	> 250	> 250	137±53
19a	33±0	35±8	104±14	45±7	76±17
19b	> 250	> 250	> 250	> 250	> 250
20a	> 250	> 250	> 250	> 250	202±68
23	> 250	> 250	> 250	130±12	144±1
24	132±1	110±5	179±7	115±7	120±34
25	182±16	129±7	208±60	136±9	127±7
26	> 250	> 250	> 250	> 250	> 250
27	> 250	> 250	> 250	> 250	156±81
28	> 250	> 250	> 250	> 250	154±35
30	≥ 250	≥ 250	≥ 250	≥ 250	≥ 250
31	≥ 250	> 250	230±5	≥ 250	202±16
39	70±4	52±3	171±6	69±6	107±21
40	83±1	50±3	67±17	90±10	90±4
41	> 250	> 250	> 250	> 250	240±14
42	196±35	> 250	≥ 250	≥ 250	150±39
43	25±1	28±4	65±16	20±0	40±13
44	20±3	21±4	175±68	17±0	33±7
47	235±20	> 250	> 250	> 250	134±2
48	> 250	> 250	> 250	> 250	210±27
50	> 250	> 250	> 250	> 250	≥ 250
53	140±6	191±16	203±6	189±2	184±13
54	29±6	13±1	220±42	84±35	139±8
55	≥ 250	> 250	> 250	> 250	183±10
56	> 250	> 250	> 250	> 250	≥ 250
5-FU	0.33 ±0.17	18 ± 5	0.54 ± 0.12	1.6 ± 1.0	2.6 ± 0.4
5-F-Uridine	0.014 ±0.000	—	0.014 ± 0.002	—	0.046 ± 0.016

[a] 50% inhibitory concentration.

Data are the mean of ± SD of at least two to three independent experiments.

Table 4. Inhibitory effect of the test compounds against MSV-induced transformation of C3H/3T3 embryo murine fibroblasts <i>in vitro</i>		
Compound	EC₅₀^a (μM)	MIC^b (μM)
12a,b	13 ± 0	100 (> 20)
19a	> 100	> 100
19b	> 100	> 100
23	> 100	> 100
24	> 100	> 100
25	> 100	> 100
27	> 100	> 100
28	> 100	> 100
30	> 100	> 100
31	63 ± 13	> 100
39	> 20	100 (> 20)
40	60 ± 8	> 100
43	> 20	100 (> 20)
53	> 100	> 100
54	49 ± 4	≥ 100
55	> 100	> 100
56	> 100	> 100
(R) PMPA	4.2 ± 2.7	> 100
PMEO-DAPym	0.48 ± 0.12	> 100

[a] 50% Effective dose.

[b] Minimal inhibitory concentration.

Data are the mean of ± SD of at least two to three independent experiments.

Table 5. Inhibitory activity of compound **59** against HIV-1 RT using different template/primers and radiolabeled substrates

			IC₅₀^a (μM)	IC₅₀^a (μM)
Template/primer	Substrate		59	DAPym-pp
PolyrC.dG	dGTP	MgCl ₂	> 500	> 100
PolyrC.dG	dGTP	MnCl ₂	> 500	16.07
PolyrA.dT	dTTP	MgCl ₂	> 500	> 100
PolyrI.dC	dCTP	MgCl ₂	> 500	> 100
PolyrU.dA	dATP	MnCl ₂	352	1.34

[^a] 50% inhibitory concentration.

Figures and Schemes legends

Figure 1

Figure 2. General structure of target nucleosides

Figure 3. MM conformers, as determined by PSEUROT program, of compounds **20a** and **27**

Scheme 1. Synthesis of key nucleosides

Scheme 2. Reagents and conditions: a) H_2SO_4 THF; b) NaH 60 %, BnBr, THF; c) NaOAc, AcOH, KBr, AcOOH, 10°C, 4h

Scheme 3. Reagents and conditions: a) NaH 60%, dry THF, 90 °C, 16h; b) H_2 , Pd(OH)₂/C 20%, MeOH, HCl, 30 °C, 40 psi

Scheme 4. Reagents and conditions: a) NaH 60%, dry THF, 90 °C, 16h ; b) H_2 , Pd(C) 10%, MeOH, 30 °C, 40 psi

Scheme 5. Reagents and conditions: a) NaH 60 %, DMF or THF, pressure tube; b) H_2 / Pd(OH)₂, 37% HCl, 30 °C, 40 psi

Scheme 6. Reagents and conditions: a) NaH 60 %, DMF or THF pressure tube; b) H_2 / Pd(OH)₂, 37% HCl, 30 °C, 40psi.

Scheme 7. Reagents and conditions: a) NaH 60 %, dry DMF, 100 °C, microwave; b) H_2 / Pd(OH)₂, 37% HCl, 30 °C, 40psi; c) NaH 60 %, dry DMF 100°C, pressure tube.

Scheme 8. Reagents and conditions: a) NaH 60 %, DMF, pressure tube; b) H_2 / Pd(OH)₂, 37% HCl, 30 °C, 40 psi

Scheme 9. Reagents and conditions: a) Proton sponge, trimethyl phosphate, POCl₃, 5°C, 16 h; b) NBU₃, tributylammonium pyrophosphate, DMF, 0°C, 30 min; c) 0.2M triethylammonium trimethyl phosphate solution, r.t.

Table of Contents

Unconventional nucleosides combining in their structure methyloxy-bound-pyrimidines or purines, with D-allitoyl, D-altritoyl as unusual ribofuranosyl mimics or 2,5-bis-hydroxymethyl tetrahydrofurane as their dideoxyanalogues, are described. The pyrimidine nucleotides act as adenine nucleotide mimics. This class of unconventional nucleosides may be considered as a novel family of potential antiproliferatives.