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Design, Synthesis, and Biological Evaluation of Unconventional Aminopyrimidine, Aminopurine, and Amino-1,3,5-triazine Methyloxynucleosides

Gloria Fernández-Cureses,[a] Sonia de Castro,*[a] María-Luisa Jimeno,[b] Jan Balzarini,[c] and María-José Camarasa*[a]

Dedicated to Professor José Elguero Bertolini on the occasion of his 80th birthday

Herein we describe a class of unconventional nucleosides (methyloxynucleosides) that combine unconventional nucleobases such as substituted aminopyrimidines, aminopurines, or aminotriazines with unusual sugars in their structures. The allitollyl or altritollyl derivatives were pursued as ribonucleoside mimics, whereas the tetrahydrofuran analogues were pursued as their dideoxynucleoside analogues. The compounds showed poor, if any, activity against a broad range of RNA and DNA viruses, including human immunodeficiency virus (HIV). This inactivity may be due to lack of an efficient metabolic conversion into 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 cervical carcinoma HeLa cells. A few compounds were inhibitory to Moloney murine sarcoma virus (MSV) in C3H/3T3 cell cultures, with the 2,6-diaminotri-*O*-benzyl-D-allitolyl- and -D-altritolyl pyrimidine analogues being the most potent among them. This series of unconventional nucleosides may represent a novel family of potential antiproliferative agents.

Introduction

Nucleosides, the building blocks of nucleic acids, play crucial roles in many biological processes such as RNA and DNA synthesis, regulation of gene expression, and in immunomodulation. Naturally occurring nucleosides consist mainly 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 activities.[4–9] Nucleosides are key leads in drug development and have been in clinical use for more than 40 years in 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 the development of drug resistance, toxicity, and poor oral bioavailability.[6–10] This highlights 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/cytotoxic activity of nucleoside analogues than resting cells.[1]

Nucleosides have been extensively modified, in both the nucleobase and sugar portions.[11,12] In general, such modifications maintain the relative spatial disposition between the nucleobase and the hydroxymethyl group that is phosphorylated in the corresponding nucleotide (the biologically active form). Moreover, several of the modifications are directed to increase resistance against enzymatic degradation. On one hand, some nucleoside analogues incorporate unconventional nucleobases; examples include ribavirin, which bears a 1,2,4-triazole carboxamide as an adenine or guanine mimetic,[13] or BVDU, which incorporates a 5-bromovinyluracil nucleobase.[14] On the other hand, some nucleoside analogues incorporate unconventional sugar moieties: for example, methylene cyclopropane as in synadenol **1** and synguanol **2** (Figure 1), which 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**), which are active against HSV, HCMV, and HIV.[16,17] Among the various modifications in the sugar region, 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 prominent representatives of

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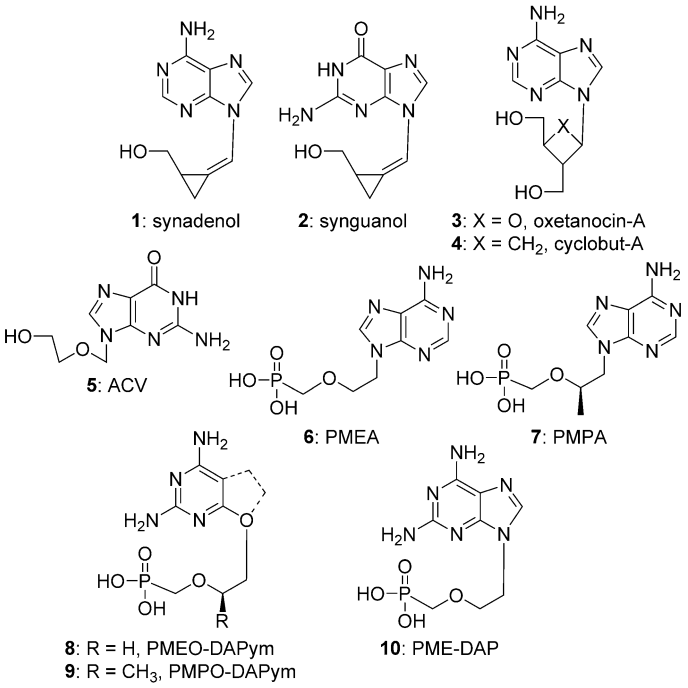


Figure 1. Examples of antiviral nucleoside analogues.

this class of nucleosides is acyclovir (ACV, **5**), a potent and selective antiherpetic drug[18] discovered by the 1988 Nobel Laureate, Gertrude B. 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]

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 hydrolyzable 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**),[10,23b,24] 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 group, via an ether bond, at the C6 position of the pyrimidine base instead of the N1 or N9 positions of the pyrimidine or purine base.[23b,25] A unique feature of these ANP 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 open-ring mimics of the PME-2,6-diaminopurine (PME-DAP, **10**) and guanine derivatives, being potent anti-HIV compounds.[23b,24–29] 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 is incorporated into the growing DNA chain as a purine nucleotide analogue instead of a pyrimidine nucleotide.[24] Interestingly, and in contrast to the PMEO-DAPyms, the pyrimidine derivatives of PME analogues are generally devoid of antiviral activity.[27]

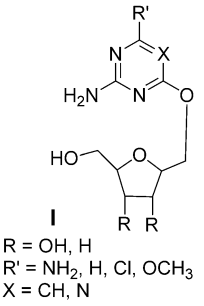


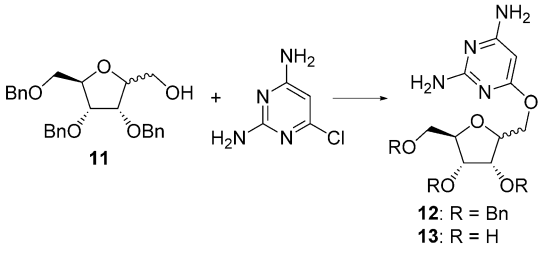
Figure 2. General structure **I** of target nucleosides.

Inspired by the uniqueness of PMEO-DAPyms and with an aim to discover new families of nucleoside analogues (of unknown structures), we designed a novel class of unconventional nucleosides, of general structure **I** (Figure 2). 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, conformational studies, and cell-based antiviral and cytostatic evaluations 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.

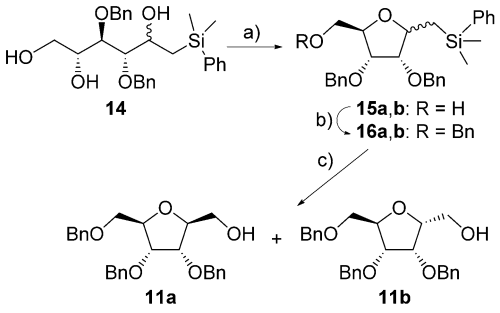
Results and Discussion

Chemistry

In an effort to discover new nucleoside analogues as potential antiviral or anticancer hits, we first pursued the tetrahydrofuran nucleosides of 2,6-diaminopyrimidines bearing hydroxy groups at the 3′ and 4′ positions (compound **13**), as ribofuranosyl nucleoside mimics. The procedure shown in Scheme 1 was devised for the synthesis of these key nucleosides. Coupling of key benzyl intermediate **11**[30] with 2,4-diamino-6-chloropyrimidine would give nucleoside **12**, which, after debenzylation, may provide the desired unprotected nucleoside **13**.



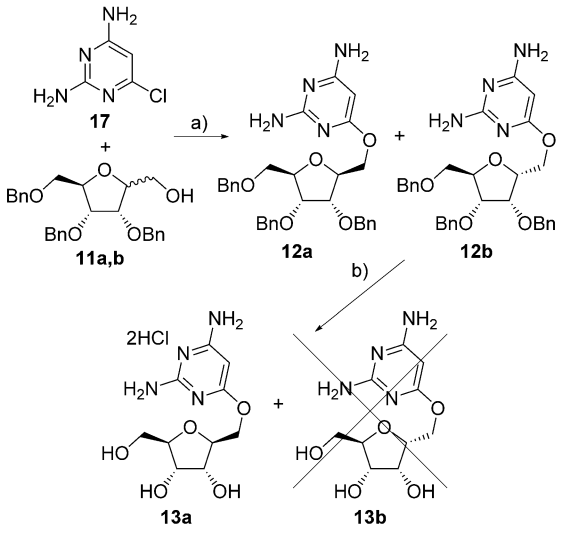
Scheme 1. General synthesis of key nucleosides **12** and **13**.



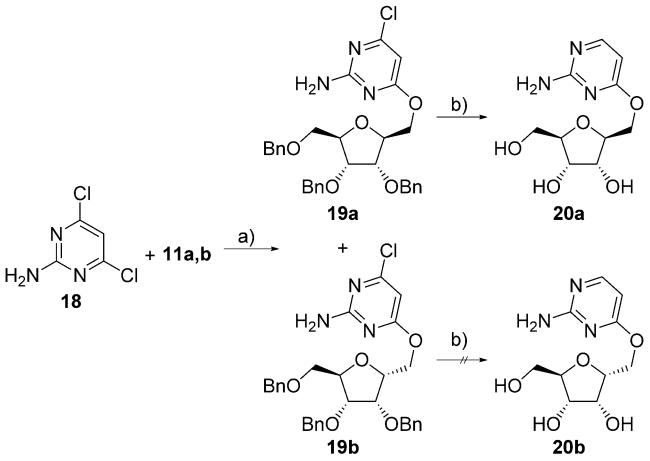
Scheme 2. *Reagents and conditions*: a) H_2SO_4, THF, 50 °C, 48 h; b) NaH 60%, BnBr, THF, RT, ~16 h; c) NaOAc, AcOH, KBr, AcOOH, 10 °C, 4 h.

First, it was necessary to prepare the key intermediate **11** as shown in Scheme 2. Thus, acid-mediated cyclization of **14**,[31] prepared in five steps from commercially available D-ribose diethyl dithioacetal, as reported,[30] in the presence of a catalytic amount of sulfuric acid at 50 °C gave **15a**,**b**[31] in 58% yield as an intractable mixture of 2,3-*cis*- and 2,3-*trans*-tetrahydrofuran isomers. Although van Delft et al.[31] reported that under these conditions the ratio of 2,3-*trans* versus 2,3-*cis* isomer was 20:1, in our hands all attempts resulted in a 2:1 ratio. Treatment of **15a**,**b**[31] with benzyl bromide in the presence of 60% sodium hydride, followed by Fleming–Tamao oxidation (KBr, AcOOH)[31] 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, which could not be separated. Next, removal of the benzyl groups of **12a**,**b** was attempted. Initial attempts of hydrogenolysis (H_2, 10% Pd/C) of the mixture **12a**,**b** failed to produce the desired unprotected nucleosides, rendering unreacted starting compound. However, if the mixture **12a**,**b** was hydrogenated in the presence of 20% $Pd(OH)_2$/C and a catalytic amount of hydrochloric acid, the deprotected nucleoside **13a** was obtained in 80% yield as the sole product. Unexpectedly, no traces of the deprotected **13b** isomer were detected.



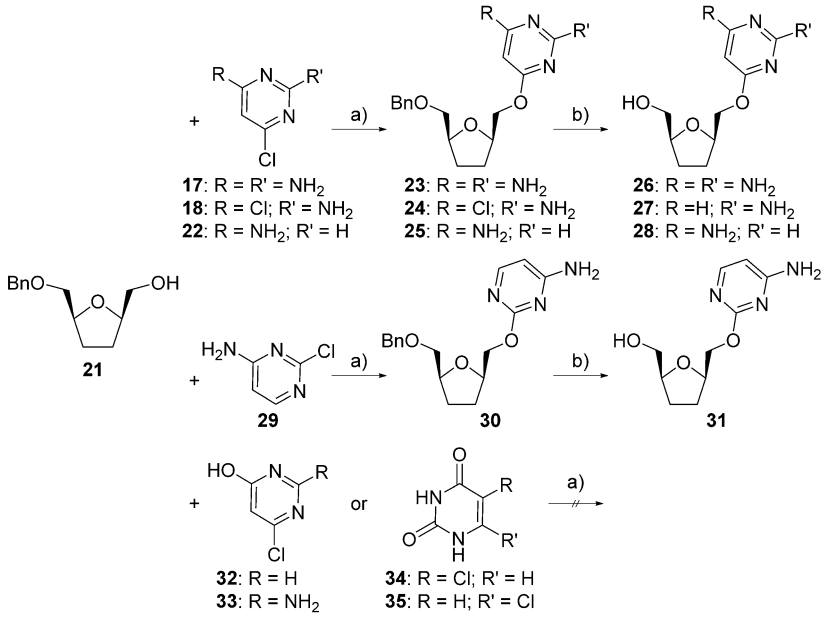
Scheme 3. *Reagents and conditions*: a) NaH 60%, dry THF, 90 °C, 16 h; b) H_2, $Pd(OH)_2$/C 20%, MeOH, HCl, 30 °C, 280 kPa, ~16 h.



Scheme 4. *Reagents and conditions*: a) NaH 60%, dry THF, 90 °C, 16 h; b) H_2, Pd(C) 10%, MeOH, 30 °C, 280 kPa, ~16 h.

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 as described above (NaH, THF, 90 °C), gave a 5:1 mixture of compounds **19a**,**b**, which was separated by preparative centrifugal circular thin-layer chromatography, to give the individual isomers **19a** and **19b** in 71 and 15% respective yields. The structures of **19a** and **19b** were unequivocally assigned on the basis of their corresponding spectroscopic data, using a combination of one- and two-dimensional ^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 de-halogenated derivative **20a** in 50% yield. However, all attempts to remove the benzyl groups of **19b** under various conditions (H_2, 10% Pd/C, 5% Pd/C or 20% $Pd(OH)_2$, in the presence or absence of catalytic amounts of HCl) rendered complex mixtures without formation of the desired derivative **20b**. 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 by following a procedure similar to that described for the synthesis of **13** and **20**. Thus, treatment of (2*RS*,5*SR*)-**21**[32] with two equivalents of the appropriate aminopyrimidine [2,4-diamino-



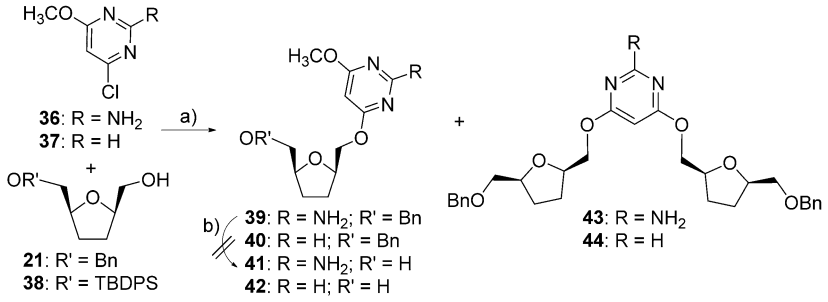
Scheme 5. *Reagents and conditions*: a) NaH 60%, DMF or THF, pressure tube, RT, ~16 h; b) H_2/$Pd(OH)_2$, 37% HCl, 30 °C, 280 kPa, ~16 h.

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% sodium hydride 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, 280 kPa] 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.

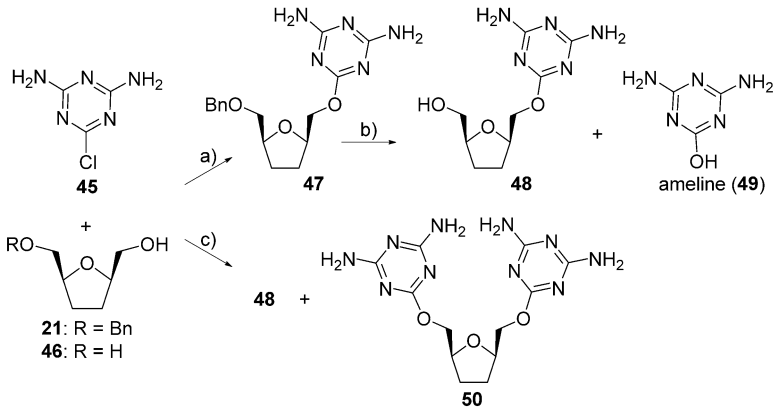
A similar alkylation of pyrimidines bearing C=O, or OH substituents (**32**–**35**) under various conditions (large excess of pyrimidines in the presence of 60% sodium hydride in acetonitrile or tetrahydrofuran at 70 or 100 °C in a pressure tube or under microwave irradiation at 100 °C) failed to produce 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% sodium hydride in a pressure tube at 100 °C), the desired nucleosides **39** and **40** were obtained in 54 and 45% yields, respectively. In both reactions the dimer compounds **43** and **44** (9 and 8% yield, respectively) were observed as byproducts (Scheme 6). These unexpected derivatives may result from transetherification by the alcoholate anion of **21**. Transetherifications by alcoholate anions have been described before.[33b,c] A similar transetherification of a methoxypyrimidine ring by a benzylate anion to give a benzyl ether was reported by Delacotte et al.[33] upon heating at 95 °C. Brown and Sugimoto[34] reported transetherifications in the presence of silver oxide as catalyst.

Hydrogenolysis of benzyl derivative **39** in the presence of catalytic amounts of $Pd(OH)_2$/C and HCl at 30 °C and 280 kPa resulted in a complex reaction mixture, and no desired deprotected nucleoside **41** was detected. Instead, analogous treatment of derivative **40** gave unprotected **42** (10%), although in low yield. To avoid the hydrogenolysis step, we replaced the benzyl group by a *tert*-butyldiphenylsilyl (TBDPS) protecting group, removable under acidic conditions, and used **38** as starting compound. Thus, treatment of **38**[35] with pyrimidines **36** or **37**, under the standard conditions, afforded the deprotected nucleosides **41** and **42** in 10 and 12% yield, respectively.

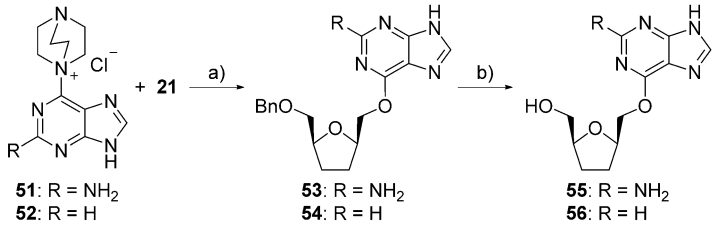
Next, we explored the synthesis of aza analogues of the nucleosides mentioned thus far. Disubstituted triazines are much less reactive, and substitution of the chlorine atom requires stronger conditions.[36] Thus, alkylation of 2-chloro-4,6-diamino-1,3,5-triazine (**45**) with **21** (Scheme 7) was carried out by treatment with 60% sodium hydride under microwave irradiation at 100 °C for 3 h to give **47** in 62% yield. Hydrogenolysis of **47** (H_2, 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 formation of ameline, 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 treated with 2-chloro-4,6-diamino-1,3,5-triazine (**45**) to give the de-



Scheme 6. *Reagents and conditions*: a) NaH 60%, DMF or THF pressure tube, 100 °C, ~16 h; b) H_2/$Pd(OH)_2$, 37% HCl, 30 °C, 280 kPa, ~16 h.



Scheme 7. *Reagents and conditions*: a) NaH 60%, dry DMF, 100 °C, microwave, 3 h; b) H_2/Pd(OH)$_2$, 37% HCl, 30 °C, 280 kPa, ~16 h; c) NaH 60%, dry DMF, pressure tube, 100 °C, 48 h.



Scheme 8. *Reagents and conditions*: a) NaH 60%, DMF, pressure tube, 100 °C, ~16 h; b) H_2/Pd(OH)$_2$, 37% HCl, 30 °C, 280 kPa, ~16 h.

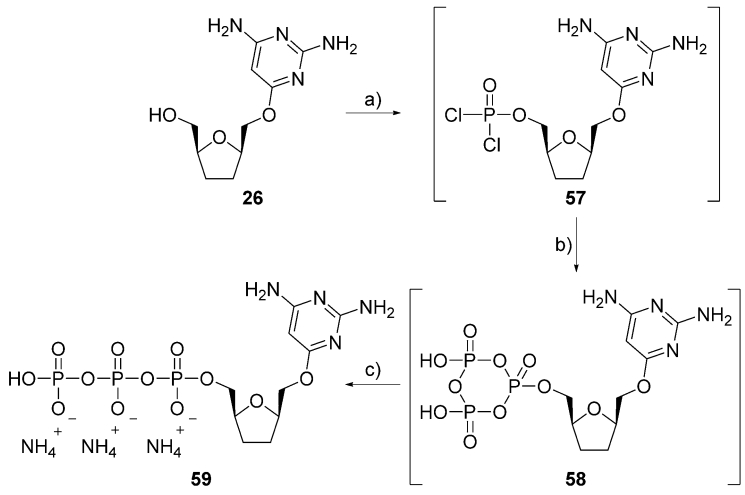
protected derivative **48** in moderate yield (42%) together with the disubstituted **50** (14%) as minor compound.

The results obtained in the synthesis of aminopyrimidine nucleosides were taken into consideration to expand the synthetic application to purine methyloxynucleoside analogues. 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 C6.[37] 6-DABCO purines show a higher rate of displacement than chlorine or quinuclidine.[36] Thus, treatment of DABCO purines **51** or **52**[37] (Scheme 8) with the sodium alkoxide of **21**, generated in situ upon treatment with 60% sodium hydride at 100 °C overnight, gave the protected derivatives **53** and **54** in moderate yields (30 and 53%, respectively). Subsequently, hydrogenolysis (20% Pd(OH)$_2$/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 were interested in studying the capacity of an example of this new kind of methyloxynucleoside to interact with DNA polymerases (i.e., the DNA polymerase activity of HIV RT). Therefore, we prepared compound **59** (the triphosphate derivative of **26**; Scheme 9). For the synthesis of the target triphosphate we used methodology reported by Yoshikawa et al.,[38] which involves phosphorylation of unprotected nucleoside **26** with phosphorous oxychloride in the presence of 1,8-bis(dimethylamino)naphthalene (Proton Sponge™) as base, and trimethyl phosphate as solvent. This was followed by in situ reaction of the phosphorodichlorate intermediate **57** with pyrophosphate to yield the cyclic triphosphate **58**, which was finally hydrolyzed to **59** (8% yield) upon treatment with a 1 M solution of triethylammonium bicarbonate (TEAB).

Conformational studies

With the aim to determine whether the conformational freedom of the furanose ring is restricted in these nucleosides, we carried out solution-state conformational studies of compounds **20a** and **27**. For this type of nucleoside, three conformational features must be considered: furanose ring pucker, the conformation of the exocyclic bonds C4′–C5′ and C1′–C7, and the conformation around the glycosidic bond. Puckering of the furanose ring in solution can be determined by using the concept of pseudorotation,[39] 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, a two-state North⇄South conformational equilibrium is often accepted as model. Therefore, from the experimental three-bond proton–proton coupling constant values ($^3J_{H,H}$), and by using the PSEUROT program,[40] information about the geometry and



Scheme 9. *Reagents and conditions*: a) 1,8-Bis(dimethylamino)naphthalene (Proton Sponge™), PO(OCH$_3$)$_3$, POCl$_3$, 5 °C, 16 h; b) *n*Bu$_3$N, tributylammonium pyrophosphate, DMF, 0 °C, 30 min; c) triethylammonium trimethyl phosphate (0.2 M), RT, 15 min.

population of both North- and South-type conformers can be obtained. Five parameters are required 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; therefore, two variables must be constrained in the calculation. The results obtained for both compounds are listed in Table 1. The conformational parameters for compound **20a**—$P_N = 43.0$, $P_S = 133.5$, $X_n = 0.34$—indicate that the furanose ring adopts an equilibrium between $_4$E and $_1$E shifted to the South conformer. For compound **27**, the conformational parameters ($P_N = 8.1$, $P_S = 158.5$) indicate an equilibrium in the usual ranges for dideoxynucleosides ($P_N = 0$–36 and $P_S = 144$–180).[41]

Table 1. Pseudorotational parameter values for **20a** and **27** obtained by PSEUROT[40] software.

Parameter	20a		27	
	Exptl[a]	Calcd	Exptl[a,b]	Calcd
$J_{(H2',H3'a)}$	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
τ_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
RMS[d]		0.13		0.16

[a] Measured in DMSO at 400 MHz; coupling constant (J) values in Hz. [b] Values obtained from the spectrum simulation. [c] Constrained values. [d] Root mean square value in Hz.

The conformation around the 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 with 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 constant values $^3J_{H1'a,H2'} = 3.7$ and $^3J_{H1'b,H2'} = 6.7$ Hz indicate that g+ (43%) and t (50%) rotamers are also preferred (Figure 3).

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, whereas 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 small correlation was observed with H6′a. These data point to a major *anti* conformation for the glycosidic bond in both compounds.

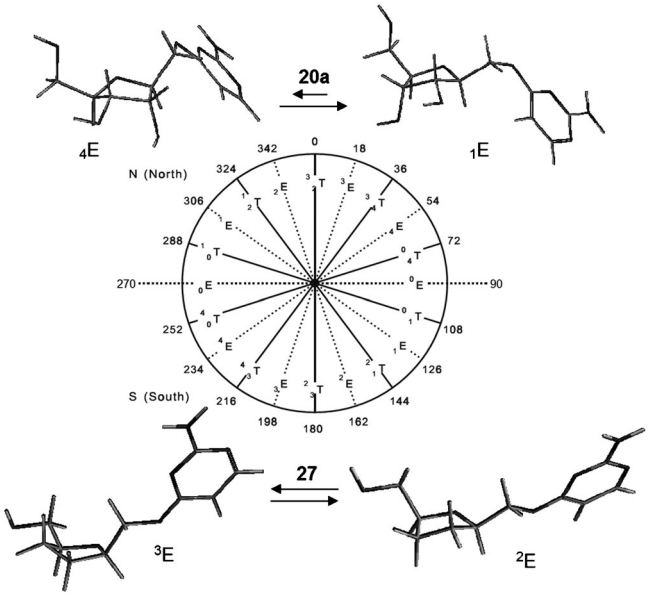


Figure 3. Molecular modeling conformers of compounds **20a** and **27** as determined by PSEUROT[40] software.

Biological evaluations

None of the compounds were significantly effective as inhibitors against a variety of DNA and RNA viruses. Given the structural similarity of several compounds with antiretroviral 2′,3′-dideoxynucleoside analogues (NRTI), the compounds were also evaluated for their inhibitory activity against HIV-1 and HIV-2 in CD_4^+ T-lymphocytic CEM cell cultures (Table 2). Unfortunately, they were also devoid of anti-HIV activity at sub-toxic concentrations.

Although none of the compounds showed anti-HIV activity at sub-toxic concentrations, it could not be excluded that the antiviral inactivity was due to a lack of metabolic conversion into the corresponding 5′-triphosphates. Therefore, compound **59**, the triphosphate derivative of compound **26**, was synthesized and evaluated for its inhibitory activity against HIV-1 RT. PMEO-DAPym-pp was included as a positive control. As shown in Table 3, **59** affects HIV-1 RT only with the poly(rU:dA) template/primer (as with PMEO-DAPym-pp). Because HIV-1 RT is inhibited by compound **59** solely in the presence of this template/primer (as was previously demonstrated to be the case for PMEO-DAPym-pp as well), it is tempting to assume that this compound—like PMEO-DAPym-pp—acts as an adenine nucleotide mimic. However, it should be recognized that the IC_{50} value for **59** was quite high in the presence of poly(rU:dA) and dATP; therefore, more research is 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 CD_4^+ T-lympho-

Table 2. Anti-HIV-1 and -HIV-2 activity and cytostatic properties of test compounds in human T-lymphocytes (CEM).

Compd	EC_{50} [μM][a]		CC_{50} [μM][b]
	HIV-1	HIV-2	
12a,b	>10	>10	23±2.1
13a	>100	>100	>250
19a	>10	>10	35±8.5
19b	>250	>250	>250
20a	>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; data are the mean ±SD of two independent experiments. [b] 50% Cytostatic concentration; data are the mean ±SD of 2–3 independent experiments.

Table 3. Inhibitory activity of compound **59** and DAPym-pp against HIV-1 RT with various template/primers and radiolabeled substrates.

Template/Primer	Substrate		IC_{50} [μM][a]	
			59	DAPym-pp
poly(rC:dG)	dGTP	$MgCl_2$	>500	>100
poly(rC:dG)	dGTP	$MnCl_2$	>500	14±2
poly(rA:dT)	dTTP	$MgCl_2$	>500	>100
poly(rI:dC)	dCTP	$MgCl_2$	>500	>100
poly(rU:dA)	dATP	$MnCl_2$	352	0.75±0.66

[a] 50% Inhibitory concentration; data are the mean ±SD of at least 1–4 independent experiments.

cytic 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 cervical carcinoma HeLa cells. There was a trend toward preferential cytostatic activity against leukemia and lymphoma tumor cell types versus the solid carcinoma tumor cell types (Table 4). 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), particularly compound **12a,b** (EC_{50}: 13 μM; Table 5).

Conclusions

We have described the first series of unconventional aminopyrimidine, aminopurine, and amino-1,3,5-triazine methyloxynucleosides of hitherto unknown structure. These unconventional nucleosides were 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-hydroxymethyltetrahydrofuran. The allitollyl or altritollyl derivatives were pursued as unusual ribonucleoside mimics, whereas the tetrahydrofuran analogues can be considered as dideoxynucleoside analogues. The tetrahydrofuran ring in the polyhydroxylated nucleosides adopts an equilibrium between $_4$E and $_1$E shifted to the South conformer, while in the corresponding dideoxy analogues, the equilibrium is in the usual ranges for dideoxynucleosides (between ^{3}E and ^{2}E).

None of the compounds showed specific antiviral (including anti-HIV) activity at sub-toxic concentrations in cell culture. This might be due to lack of efficient metabolic conversion into 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), with **12a,b** (EC_{50}: 13 μM) being superior to the others. In summary, this series of unconventional methyloxynucleosides may be considered a novel family of candidate antiproliferative agents that warrant further investigation for structural optimization.

Experimental Section

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 24 h. THF was distilled from Na/benzophenone and stored over molecular sieves (4 Å). Anhydrous DMF was purchased from Sigma–Aldrich and stored over molecular sieves (4 Å). Microwave reactions were performed in a Biotage Initiator 2.0 single-mode cavity instrument 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 detected under UV light (λ 254 nm) and/or by heating after treatment with a 5% solution of H_2SO_4 in EtOH. Preparative TLC was performed on pre-coated silica gel 20×20 cm glass plates (Altech 02013, Silica Gel GF with UV 254, 1000 μm 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 PF_{254} gipshaltig, Merck), with a layer thickness of 1 or 2 mm and a flow rate of 4 or 8 mL min^{-1}, respectively. Flash

Table 4. Inhibitory effects of test compounds on the proliferation of murine leukemia (L1210) cells, human T-lymphocytes (CEM), human cervical carcinoma (HeLa) cells, human prostate carcinoma (PC3) cells, and human kidney carcinoma (CAKI-1) cells.

Compd	IC_{50} [μM][a]				
	L1210	CEM	HeLa	PC3	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[b]	0.33±0.17	18±5	0.54±0.12	1.6±1.0	2.6±0.4
5-FUd[c]	0.014±0.000	–	0.014±0.002	–	0.046±0.016

[a] 50% Inhibitory concentration; data are the mean ±SD of at least two to three independent experiments. [b] 5-Fluorouracil. [c] 5-Fluorouridine.

Table 5. Inhibitory effect of test compounds against MSV-induced transformation of C3H/3T3 embryo murine fibroblasts in vitro.

Compd	EC_{50} [μM][a]	MIC [μM][b]
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 concentration. [b] Minimal inhibitory concentration. Data are the mean ±SD of at least two to three independent experiments.

chromatography was performed by HPFC using a force flow Isolera One (Biotage) with flash silica gel SNAP cartridges KP-Sil of 50 g (39×81 mm) or 100 g (39×157 mm). Reversed-phase chromatography was performed by: a) HPFC in an Isolera One (Biotage) using SNAP 12 g KP-C_{18}-HS cartridges, or b) solid-phase extraction chromatography (SPE) under vacuum in the manifold using DSC-Si 1 g (Supelco) cartridges. Samples were lyophilized using mixtures of H_2O/CH_3CN on a Telstar 8-80 instrument.

The purity of the compounds was determined by analytical RP-HPLC on: a) a Waters 2690 instrument equipped with a diode array and a C_{18} Sunfire column (4.6 mm×50 mm, 3.5 μm), with A:B mixtures used as mobile phase, where A = CH_3CN (0.04% TFA) and B = H_2O (0.05% TFA); b) an Agilent Technologies 1120 Compact LC instrument equipped with a diode array and an ACE 5 C_{18} 300 column (4.6 mm×150 mm, 3.5 μm), using as mobile phase A/B mixtures in which A = CH_3CN and B = H_2O (0.05% TFA). In all cases, the flow rate was 1 mL min^{-1}, and the samples were analyzed at λ 217 and 254 nm in gradient mode. Gradients used: a) *gradient 1*: from A:B mixtures 80:20→100:0 in 5 min; b) *gradient 2*: A:B mixtures 40:60→70:30 in 8 min; c) *gradient 3*: A:B mixtures 10:90→100:0 in 10 min; d) *gradient 4*: A:B mixtures 2:98→30:70 in 10 min; e) *gradient 5*: A:B mixtures 50:50→100:0 in 10 min. HPLC–MS was performed on an 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×50 mm, 3.5 μm), and the flow rate was 1 mL min^{-1}. Solvents used were mixtures of A: CH_3CN (0.08% formic acid) and B: H_2O (0.1% formic acid). The gradient used was from 10% A to 100% A in 10 min. Detection was at λ 214 and 254 nm. Triphosphate **59** was analyzed by HPLC (Waters 600, UV/Vis detection and ion-exchange column [Waters Spherisorb SAX, 4.6 mm×250 mm, 10.0 μm]). Mobile phase: 0.5 M KH_2PO_4 solution in isocratic mode for 15 min at a flow rate of 1.5 mL min^{-1}, with detection at λ 220 and 254 nm.

Optical rotations were measured in a PerkinElmer 241 polarimeter at 23±2 °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 on an Agilent 6520 Accurate-Mass Q-TOF LC–MS spectrometer using MeOH/H_2O (0.1% AcOH). NMR spectra were recorded with Varian Inova-300, Varian Inova-400 or Varian System-500 spectrometers operating at 300, 400, or 500 MHz for ^{1}H 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 (δ) are reported in parts per million. Coupling constants (*J*) are reported in Hz, and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad signal). Complex ^{1}H and ^{13}C NMR spectra were assigned by homonuclear ^{1}H–^{1}H COSY and NOESY experiments and by heteronuclear ^{1}H–^{13}C HSQC and HMBC experiments.

2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-dimethylphenylsilyl-D-allitol and 2,5-anhydro-3,4-di-*O*-benzyl-1-deoxy-1-dimethylphenylsilyl-D-altritol (**15a** and **15b**):[31] To a solution of 3,4-di-*O*-benzyl-1-

deoxy-1-dimethylphenylsilyl-D-allitol and 3,4-di-*O*-benzyl-1-deoxy-1-dimethylphenylsilyl-D-altritol[31] (960 mg, 1.99 mmol) in dry THF (7 mL), concentrated H_2SO_4 (49.5 μL) was added and stirred at 50 °C for 48 h, diluted with EtOAc (15 mL), and washed with saturated $NaHCO_3$ (3×10 mL). The layers were separated, and the organic phase dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by HPFC (Biotage; hexane/EtOAc, 7:3). The fastest-moving fractions gave **15 a** and **15 b** (532 mg, 58%) as an intractable mixture (ratio 2,3-*trans* (**1 a**) and 2,3-*cis* (**1 b**), 2:1), as determined by ^{1}H NMR, after purification. Analytical and spectroscopic data (NMR) are similar to those described previously.[31] ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.30$, 0.33 (s, 6H, CH_3, $SiCH_{3trans}$), 0.31, 0.33 (s, 6H, CH_3, $SiCH_{3cis}$), 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, $CH_2Ph_{trans,cis}$), 7.25–7.53 ppm (m, 30H, Ar$_{trans,cis}$); HPLC (Agilent 1120, gradient 3): $t_R = 10.84$ min (95%); HRMS (ES+) *m/z*: calcd for $C_{28}H_{34}O_4Si$ 462.2224, found 462.2226.

The slowest-moving fractions gave **3,4-di-*O*-benzyl-1,2-dideoxi-D-ribo-hex-1-enitol**[31] (260 mg, 40%). MS (ES+) *m/z*: 351 $[M]^+$; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.10$ (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, = 6.1 Hz, H-3), 4.52 (d, 2H, $J = 11.8$ Hz, CH_2Ph), 4.62 (d, 2H, $J = 11.8$ Hz, CH_2Ph), 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 ppm (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 (**16 a** and **16 b**): To a mixture of **15 a,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 for ~16 h, diluted with Et_2O (15 mL), and then a saturated solution of NH_4Cl (5 mL) was slowly added. The layers were separated, and the organic phase was washed with saturated NaCl (2×10 mL), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by HPFC in the Biotage (hexane/EtOAc, 9:1) to give a mixture of **16 a** and **16 b**; yield 1.02 g (82%) as an oil. Ratio 2,3-*trans*/2,3-*cis*, 4:1 (by ^{1}H NMR and HPLC); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.31$, 0.32 (s, 6H, CH_3, $SiCH_{3trans}$), 0.32, 0.33 (s, 6H, CH_3, $SiCH_{3cis}$), 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, $CH_2Ph_{trans,cis}$), 7.24–7.58 ppm (m, 40H, Ar$_{trans,cis}$); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -2.3$, −1.5 (CH_3, $SiCH_{3trans}$), −2.2, −1.8 (CH_3, $SiCH_{3cis}$), 17.1 (C1$_{cis}$), 21.6 (C1$_{trans}$), 70.5 (C6$_{cis}$), 70.9 (C6$_{trans}$), 71.9, 72.1, 73.5 (CH_2Ph_{trans}), 72.6, 73.0, 73.5 (CH_2Ph_{cis}), 77.6 (C4$_{trans}$), 78.8 (C4$_{cis}$, C3$_{cis}$), 79.0 (C2$_{trans}$), 79.4 (C2$_{cis}$), 80.5 (C5$_{cis}$), 81.3 (C5$_{trans}$), 83.6 (C3$_{trans}$), 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 ppm (C Ar$_{cis}$); HPLC (Waters 2690, gradient 1): $t_R = 4.58$ min (21%), 7.74 (78%); HRMS (ES+) *m/z*: calcd for $C_{35}H_{40}O_4Si$ 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 (**11 a** and **11 b**): To a solution of NaOAc (1.97 g, 24.05 mmol) in AcOH (9 mL) the phenylsilane mixture **16 a,b** (1.02 g, 1.85 mmol) was added. KBr (0.26 g, 2.22 mmol) was added, and the reaction was 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 EtOAc (20 mL) and poured into a cooled (0 °C) solution of saturated $Na_2S_2O_3$ (20 mL). The organic phase was separated and neutralized with $NaHCO_3$, until the complete stop of gas liberation. The organic phase was washed with H_2O (2×50 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by HPFC in the Biotage (hexane/EtOAc, 3:2) to give the mixture **11 a** and **11 b** (632 mg, 77%) as an oil. Ratio 2,3-*trans* (**11 a**) and 2,3-*cis* (**11 b**) (6:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.70$ (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, CH_2Ph), 4.47 (d, 1H, $J = 11.8$ Hz, CH_2Ph), 4.53 (d, 1H, $J = 11.9$ Hz, CH_2Ph), (d, 1H, $J = 11.8$ Hz, CH_2Ph), 4.61 (s, 2H, CH_2Ph), 7.22–7.37 ppm (m, 15H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 62.2$ (C1$_{cis}$), 63.4 (C1$_{trans}$), 69.7 (C6$_{trans}$), 70.3 (C6$_{cis}$), 72.7, 73.2, 73.6 (CH_2Ph_{cis}), 72.2, 72.4, 73.6 (CH_2Ph_{trans}), 78.0, 78.1 (C3$_{trans}$, C4$_{trans}$), 78.2, 78.7 (C3$_{cis}$, C4$_{cis}$), 81.0 (C2$_{trans}$), 79.7 (C2$_{cis}$), 80.9 (C5$_{cis}$), 83.0 (C5$_{trans}$), 127.8–128.7 (CH Ar$_{trans,cis}$), 137.6, 137.9, 138.0 (C Ar$_{trans}$), 137.7, 137.9, 138.1 ppm (C Ar$_{cis}$); HPLC (Agilent 1120, gradient 3): $t_R = 9.52$ min (95%); HRMS (ES+) *m/z*: calcd For $C_{27}H_{30}O_5$ 434.2108, found 434.2093.

General procedure for the synthesis of D-allitolyl-, D-altritolyl-, and tetrahydrofuran-2′-yl pyrimidine/purine derivatives: To a cooled (0 °C) solution of **11 a,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 1 h; the corresponding chloropyrimidine/purine (0.92 mmol) was then added, and the reaction was stirred at 90–100 °C, either in a pressure tube or in the microwave, for 3–16 h. After cooling to room temperature, MeOH was added (to remove excess NaH) and evaporated to dryness. The residue was dissolved in EtOAc (10 mL), washed with 1 N HCl (3×5 mL) and the organic phase dried (Na_2SO_4), 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-allitolyl)pyrimidine and 2,6-diamino-4-(2′,5′-anhydro-3′,4′,6′-tri-*O*-benzyl-D-altritolyl)pyrimidine (**12 a** and **12 b**): Following the general procedure **11 a,b** (50 mg, 0.12 mmol) was treated with 2,4-diamino-6-chloropyrimidine (**17**) (34 mg, 0.24 mmol), for ~16 h to give, after purification by CCTLC on the chromatotron (CH_2Cl_2/MeOH, 9:1), a 4:1 mixture of **12 a** and **12 b** (54 mg, 82%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.52$ (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, $CH_2Ph_{trans,cis}$), 5.12 (s, 1H, H-5$_{trans}$), 5.25 (s, 1H, H-5$_{cis}$), 7.27–7.37 ppm (m, 15H, Ar$_{trans,cis}$); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 64.1$ (C1′$_{cis}$), 65.6 (C1′$_{trans}$), 70.0 (C6′$_{cis}$), 70.3 (C6′$_{trans}$), 71.9, 72.0, 73.5 (CH_2Ph_{trans}), 72.6, 73.4 (CH_2Ph_{cis}), 77.5 (C4′$_{trans}$), 77.7 (C3′$_{trans}$), 78.2 (C4′$_{cis}$), 78.5 (C3′$_{cis}$), 78.8 (C5$_{trans}$), 79.2 (C2′$_{cis}$), 79.8 (C2′$_{trans}$), 80.1 (C5′$_{cis}$), 81.4 (C5′$_{trans}$), 127.5–128.5 (CH Ar$_{trans,cis}$), 137.8–138.4 (C Ar$_{trans,cis}$), 162.6 (C2$_{trans}$), 162.7 (C2$_{cis}$), 165.3 (C6$_{trans}$), 165.4 (C6$_{cis}$), 171.0 (C4$_{rans}$), 171.1 ppm (C4$_{cis}$); HPLC (Waters 2690, gradient 2): $t_R = 3.51$ min (79%) and 3.93 min (19%); HRMS (ES+) *m/z*: calcd for $C_{31}H_{34}N_4O_5$ 542.2527, found 542.2529.

2-Amino-4-(2′,5′-anhydro-3′,4′,6′-tri-*O*-benzyl-D-allitolyl)-6-chloropyrimidine and 2-Amino-4-(2′,5′-anhydro-3′,4′,6′-tri-*O*-benzyl-D-altritolyl)-6-chloropyrimidine (**19a** and **19b**): Following the general procedure **11a,b** (200 mg, 0.46 mmol) was treated with 2-amino-4,6-dichloropyrimidine (**18**) (133 mg, 0.92 mmol) in a pressure tube for 6 h. Workup and purification by CCTLC in the chromatotron (hexane/Et_2O, 1:1), gave from the fastest-moving band compound **19b** (40 mg, 15%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.51$ (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 ppm (m, 15H, Ar); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 65.9$ (C1′), 70.1 (C6′), 72.7, 73.4, 73.6 (CH_2Ph), 77.6 (C3′), 77.7 (C2′), 79.1 (C4′), 80.5 (C5′), 97.4 (C5), 127.5–128.5 (CH Ar), 137.9, 138.1, 138.2 (C Ar), 160.8 (C2), 162.2 (C6), 171.1 ppm (C4); HPLC (Agilent 1120, gradient 3): $t_R =$ 10.63 min (98%); HRMS (ES+) *m/z*: calcd for $C_{31}H_{32}ClN_3O_5$ 561.2051, found 561.2030.

From the slowest-moving band, compound **19a** (184 mg, 71%) was isolated as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.49$ (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 ppm (m, 15H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 66.3$ (C1′), 70.3 (C6′), 72.0, 72.2, 73.6 (CH_2Ph), 77.2 (C4′), 77.5 (C3′), 79.2 (C2′), 81.7 (C5′), 97.4 (C5), 127.6–128.6 (CH Ar), 137.7, 137.8, 138.2 (C Ar), 160.8 (C2), 162.1 (C6), 170.9 ppm (C4); HPLC (Agilent 1120, gradient 3): $t_R = 10.47$ min (98%); HRMS (ES+) *m/z*: calcd for $C_{31}H_{32}ClN_3O_5$ 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**[32] (850 mg, 3.82 mmol) was treated with 2,4-diamino-6-chloropyrimidine (**17**) (828 mg, 5.73 mmol) in a pressure tube for ~16 h. After workup, the residue was purified by reversed-phase chromatography using HPFC in the Biotage (H_2O/CH_3CN, 1:1) to give **23** (693 mg, 55%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.75$–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, CH_2Ph), 4.68 (bs, 2H, NH_2), 4.86 (bs, 2H, NH_2), 5.22 (s, 1H, H-5), 7.27–7.37 ppm (m, 5H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 27.9$ (C3′, C4′), 68.2 (CH_2Obn), 73.0 (CH_2Oar), 73.3 (CH_2Ph), 77.7 (C5), 78.4 (C2′), 78.8 (C5′), 127.5, 127.7, 128.4 (CH Ar), 138.5 (C Ar), 162.2 (C6), 164.9 (C2), 171.3 ppm (C4); HPLC (Waters 2690, gradient 2): $t_R = 3.51$ min (79%); HRMS (ES+) *m/z*: calcd for $C_{17}H_{22}N_4O_3$ 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**[32] (259 mg, 1.16 mmol) was treated with 2-amino-4,6-dichloropyrimidine (**18**) (228 mg, 1.39 mmol) in dry DMF (5 mL) in a pressure tube for ~16 h. Workup and purification by reversed-phase chromatography using HPFC in the Biotage (H_2O/CH_3CN, 1:1) gave **24** (321 mg, 79%) as an oil. ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 1.64$–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, CH_2Ph), 6.07 (s, 1H, H-5), 7.08 (bs, 2H, NH_2), 7.27–7.37 ppm (m, 5H, Ar); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 27.3$, 27.5 (C3′, C4′), 68.5 (CH_2Oar), 72.2 (CH_2Ph), 72.5 (CH_2Obn), 76.5 (C2′), 78.3 (C5′), 94.3 (C5), 127.3, 127.4, 128.3 (CH Ar), 138.5 (C Ar), 160.0 (C2), 162.8 (C6), 170.5 ppm (C4); HPLC (Agilent 1120, gradient 3): $t_R = 8.43$ min (98%); HRMS (ES+) *m/z*: calcd for $C_{17}H_2ClN_3O_3$ 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**[32] (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 (EtOAc/MeOH, 9:1) to give **25** (69 mg, 58%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.78$–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, CH_2Ph), 4.58 (d, 1H, $J = 12.2$ Hz, CH_2Ph),4.83 (bs, 2H, NH_2), 5.77 (s, 1H, H-5), 7.27–7.37 (m, 5H, Ar), 8.23 ppm (s, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 27.9$, 28.0 (C3′, C4′), 68.7 (CH_2Oar), 72.9 (CH_2Obn), 73.5 (CH_2Ph), 77.7 (C2′), 79.1 (C5′), 87.7 (C5), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 158.0 (C2), 164.3 (C6), 170.0 ppm (C4); HPLC (Waters 2690, gradient 3): $t_R = 3.17$ min (99%); HRMS (ES+) *m/z*: calcd for $C_{17}H_{21}N_3O_3$ 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**[32] (111 mg, 0.50 mmol) was treated with 4-amino-2-chloropyrimidine (**29**) (78 mg, 0.60 mmol). The crude was purified by reversed-phase chromatography using HPFC in the Biotage (H_2O/CH_3CN, 1:1) to give **30** (59 mg, 37%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.76$–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′, CH_2Oar), 4.53 (d, 1H, $J = 12.1$ Hz, CH_2Ph), 4.58 (d, 1H, $J = 12.1$ Hz, CH_2Ph), 5.04 (bs, 2H, NH_2), 6.06 (d, 1H, $J_{5,6} = 5.7$ Hz, H-5), 7.27–7.37 (m, 5H, Ar), 7.99 ppm (d, 1H, $J_{5,6} = 5.7$ Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 28.1$, 28.3 (C3′, C4′), 69.1 (CH_2Oar), 73.1 (CH_2Obn), 73.5 (CH_2Ph), 77.5 (C2′), 78.9 (C5′), 99.6 (C5), 127.7, 127.8, 128.5 (CH Ar), 138.5 (C Ar), 157.3 (C6), 164.8 (C4), 165.1 ppm (C2); HPLC (Agilent 1120, gradient 3): $t_R = 5.74$ min (99%); HRMS (ES+) *m/z*: calcd for $C_{17}H_{21}N_3O_3$ 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**[32] (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 for ~16 h. After the workup the crude was purified by reversed-phase chromatography using HPFC in the Biotage (H_2O/CH_3CN, 3:2). From the fastest-moving bands **39** (168 mg, 54%) was isolated as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.80$–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, $J_{CH2a,CH2b} = 10.1$ Hz, $CH_{2b}Obn$), 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 ppm (m, 5H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 27.8$, 27.9 (C3′, C4′), 56.2 (CH_3), 70.9 (CH_2Oar), 72.7 (CH_2Obn), 73.4 (CH_2Ph), 77.0 (C2′), 78.3 (C5), 79.3 (C5′), 127.3, 127.9, 128.4 (CH Ar), 138.3 (C Ar), 157.8 (C2), 169.2 (C6), 171.1 ppm (C4); HPLC (Agilent 1120, gradient 3): $t_R = 6.92$ min (97%); HRMS (ES+) *m/z*: calcd for $C_{18}H_{23}N_3O_4$ 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. $[\alpha]_D = 0.9^\circ$ ($c = 0.25$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.80–2.06$ (m, 8H, H-3′, H-4′) 3.48 (dd, 2H, $J_{5',CH2a} = 4.8$ Hz, $J_{CH2a,CH2b} = 10.0$ Hz, CH_{2a}Obn), 3.51 (dd, 2H, $J_{5',CH2b} = 5.4$ Hz, $J_{CH2a,CH2b} = 10.0$ Hz, CH_{2b}Obn), 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 ppm (m, 10H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 27.9$, 28.1 (C3′, C4′), 71.3 (CH_2Oar), 72.8 (CH_2Obn), 73.5 (CH_2Ph), 77.2 (C2′), 78.9 (C5), 79.5 (C5′), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 157.5 (C2′), 169.7 ppm (C4′, C6′); HPLC (Agilent 1120, gradient 3): $t_R = 9.06$ min (99%); HRMS (ES+) *m/z*: calcd for $C_{30}H_{37}N_3O_6$ 535.2683, found 535.2682.

6-Methoxy-4-{[(2′*RS*,5′*SR*)-5′-(benzyloxymethyl)tetrahydrofuran-2′-yl]mehtoxy}pyrimidine (**40**): The general procedure was followed with **21**[32] (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 for ~16 h. After workup, the crude was purified by reversed-phase chromatography using HPFC in the Biotage (H_2O/CH_3CN, 1:2). From the fastest-moving fractions **40** (133 mg, 45%) was isolated as an oil. $[\alpha]_D = 0.1^\circ$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.78–2.03$ (m, 4H, H-3′, H-4′), 3.48 (dd, 1H, $J_{5',CH2a} = 4.9$ Hz, $J_{CH2a,CH2b} = 10.0$ Hz, CH_{2a}Obn), 3.52 (dd, 1H, $J_{5',CH2b} = 5.3$ Hz, $J_{CH2a,CH2b} = 10.0$ Hz, CH_{2b}Obn), 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 ppm (s, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 27.9$, 28.1 (C3′, C4′), 54.2 (CH_3), 69.1 (CH_2Oar), 72.8 (CH_2Obn), 73.5 (CH_2Ph), 77.6 (C2′), 79.1 (C5′), 90.8 (C5), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 157.5 (C2), 170.9 (C4), 171.3 ppm (C6); 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. $[\alpha]_D = 0.8^\circ$ ($c = 0.25$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.77–2.03$ (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 ppm (s, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 27.9$, 28.1 (C3′, C4′), 69.1 (CH_2Oar), 72.8 (CH_2Obn), 73.5 (CH_2Ph), 77.6 (C2′), 79.1 (C5′), 91.3 (C5), 127.7, 127.8, 128.4 (CH Ar), 138.4 (C Ar), 157.3 (C2), 170.9 ppm (C4, C6); 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**[35] (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 for ~16 h. The crude was purified by CCTLC in the chromatotron (EtOAc/MeOH, 10:1) to give **41** (16 mg, 10%) as an oil. ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 1.62–1.94$ (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 ppm (bs, 2H, NH_2); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 27.2$, 27.5 (C3′, C4′), 53.2 (OCH_3), 63.9 (CH_2OH), 68.1 (CH_2Oar), 76.7 (C5′), 78.1 (C5), 80.3 (C2′), 162.7 (C2), 171.2 (C6), 171.7 ppm (C4); 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**[35] (120 mg, 0.32 mmol) and 4-cloro-6-methoxypyrimidine (**37**) (56 mg, 0.38 mmol). The reaction was heated at 100 °C in dry DMF (5 mL) in a pressure tube for ~16 h. The crude was purified by CCTLC in the chromatotron (EtOAc/MeOH, 10:1) **42** (18 mg, 12%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.79–2.07$ (m, 4H, H-3′, H-4′), 2.80 (bs, 1H, OH), 3.50 (dd, 1H, $J_{2',CH2a} = 4.4$ Hz, $J_{CH2a,CH2b} = 11.8$ Hz, CH_{2a}OH), 3.74 (dd, 1H, $J_{2',CH2b} = 3.0$ Hz, $J_{CH2a,CH2b} = 11.8$ Hz, CH_{2a}OH), 3.93 (s, 3H, CH_3), 4.12 (m, 1H, H-2′), 4.33 (m, 2H, CH_{2a}Oar, H-5′), 4.45 (m, 1H, CH_{2b}Oar), 6.09 (s, 1H, H-5), 8.40 ppm (s, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 26.7$, 28.3 (C3′, C4′), 54.3 (OCH_3), 64.6 (CH_2OH), 69.0 (CH_2Oar), 77.9 (C5′), 80.8 (C5), 91.0 (C2′), 157.4 (C2), 170.8 (C6), 171.5 ppm (C4); HPLC (Agilent 1120, gradient 3): $t_R = 4.83$ min (97%); HRMS (ES+) *m/z*: calcd for $C_{11}H_{16}N_2O_4$ 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**[32] (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 excess NaH) and evaporated to dryness. The crude was purified by reversed-phase chromatography using HPFC in the Biotage (H_2O/CH_3CN, 9:1) to give **47** (98 mg, 62%) as an amorphous white solid. $[\alpha]_D = 0.1^\circ$ ($c = 0.5$, MeOH); ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 1.65–1.94$ (m, 4H, H-3′, H-4′), 3.40 (dd, 1H, $J_{5',CH2a} = 5.8$ Hz, $J_{CH2a,CH2b} = 10.2$ Hz, CH_{2a}Obn), 3.44 (dd, 1H, $J_{5',CH2b} = 4.6$ Hz, $J_{CH2a,CH2b} = 10.2$ Hz, CH_{2b}Obn), 4.05 (m, 1H, H-5′), 4.16 (m, 1H, H-2′), 4.20 (dd, 1H, $J_{2',CH2a} = 6.1$ Hz, $J_{CH2a,CH2b} = 10.8$ Hz, CH_{2a}Oar), 4.31 (dd, 1H, $J_{2',CH2b} = 3.7$ Hz, $J_{CH2a,CH2b} = 10.8$ Hz, CH_{2b}Oar), 4.48 (m, 2H, CH_2Ph), 7.23–7.37 (m, 5H, Ar), 7.83 ppm (bs, 4H, $2NH_2$); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 27.2$, 27.5 (C3′, C4′), 69.6 (CH_2Oar), 72.2 (CH_2Ph), 72.5 (CH_2Obn), 76.3 (C2′), 78.4 (C5′), 127.3, 127.4, 128.2 (CH Ar), 138.5 (C Ar), 163.0 (C4, C6), 166.4 ppm (C2); 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 $C_{16}H_{21}N_5O_3$ 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**[32] (50 mg, 0.22 mmol) in dry DMF (5 mL), and **51**[37] (74 mg, 0.26 mmol). The reaction was heated at 100 °C in a pressure tube for ~16 h. After the workup, the crude was purified by HPFC in the Biotage (CH_2Cl_2/MeOH, 9:1) to give **53** (23 mg, 30%) as an amorphous solid. ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 1.70–2.00$ (m, 4H, H-3′, H-4′), 3.42 (dd, 1H, $J_{5',CH2a} = 5.6$ Hz, $J_{CH2a,CH2b} = 10.1$ Hz, CH_{2a}Obn), 3.46 (dd, 1H, $J_{5',CH2b} = 4.5$ Hz, $J_{CH2a,CH2b} = 10.1$ Hz, CH_{2b}Obn), 4.07 (m, 1H, H-5′), 4.25 (m, 1H, H-2′), 4.32 (dd, 1H, $J_{2',CH2a} = 6.4$ Hz, $J_{CH2a,CH2b} = 10.9$ Hz, CH_{2a}Oar), 4.41 (dd, 1H, $J_{2',CH2b} = 3.9$ Hz, $J_{CH2a,CH2b} = 10.9$ Hz, CH_{2b}Oar), 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 ppm (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 27.5$, 27.9 (C3′, C4′), 67.9 (CH_2Oar), 72.2 (CH_2Ph), 72.6 (CH_2Obn), 76.8 (C2′), 78.3 (C5′), 112.4 (C5), 127.3, 127.4, 128.2 (CH Ar), 138.3 (C Ar), 138.5 (C8), 155.8(C4), 159.6 (C6), 159.7 ppm (C −2); HPLC (Agilent 1120, gradient 3): $t_R = 5.62$ min (99%); HRMS (ES+) *m/z*: calcd for $C_{18}H_{21}N_5O_3$ 355.1647, found: 355.1644.

6-{[(2′*RS*,5′*SR*)-5′-(Benzyloxymetyl)tetrahydrofuran-2′-yl]methoxy}-7H-purine (**54**): Following the general procedure **21**[32] (390 mg, 1.75 mmol) was treated with **52**[37] (650 mg, 2.62 mmol) in dry DMF (10 mL) at 100 °C in a pressure tube for ~16 h. The crude was purified by HPFC in the Biotage (CH_2Cl_2/MeOH, 9:1) to give **54**

(0.60 g, 53%) as an amorphous solid. $[\alpha]_D = 0.4^\circ$ ($c = 0.9$, $CHCl_3$); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.75$–2.01 (m, 4H, H-3′, H-4′), 3.41 (dd, 1H, $J_{5',CH2a} = 5.8$ Hz, $J_{CH2a,CH2b} = 10.3$ Hz, CH_{2a}Obn), 3.46 (dd, 1H, $J_{5',CH2b} = 4.5$ Hz, $J_{CH2a,CH2b} = 10.3$ Hz, CH_{2b}Obn), 4.07 (m, 1H, H-5′), 4.30 (m, 1H, H-2′), 4.43 (dd, 1H, $J_{2',CH2a} = 6.6$ Hz, $J_{CH2a,CH2b} = 11.2$ Hz, CH_{2a}Oar), 4.46 (m, 2H, CH_2Ph), 4.58 (dd, 1H, $J_{2',CH2b} = 3.8$ Hz, $J_{CH2a,CH2b} = 11.2$ Hz, CH_{2b}Oar), 7.27–7.37 (m, 5H, Ar), 8.39 (s, 1H, H-2), 8.47 (s, 1H, H-8), 13.42 ppm (bs, 1H, NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 27.4$, 27.5 (C3′, C4′), 68.7 (CH_2Oar), 72.2 (CH_2Ph), 72.6 (CH_2Obn), 76.7 (C2′), 78.3 (C5′), 120.2 (C5), 127.3, 127.4, 128.1 (CH Ar), 138.6 (C Ar), 141.8 (C8), 150.8 (C2), 153.1 (C4), 159.7 ppm (C6); HPLC (Agilent 1120, gradient 3): $t_R = 6.42$ min (99%); HRMS (ES+) *m/z*: calcd for $C_{18}H_{20}N_4O_3$: 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 MeOH (10 mL) and 37% HCl (49 μL, 0.60 mmol) was hydrogenated for ~16 h at 30 °C and 280 kPa, in the presence of 20% Pd(OH)$_2$/C (10 mg). The reaction was filtered, washed with MeOH, 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-allitolyl)pyrimidine dihydrochloride (**13a**): A solution of **12a,b** (90 mg, 0.17 mmol) in MeOH (10 mL) was hydrogenated for ~16 h at 30 °C and 280 kPa, in the presence of 20% Pd(OH)$_2$/C (10 mg). The reaction was filtered, washed with MeOH, and evaporated to dryness. The residue was purified by CCTLC in the chromatotron (CH_2Cl_2/MeOH/NH_4OH, 4:1:0.1) to give **13a** (49 mg, 80%) as an oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 3.34$ (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 ppm (s, 1H, H-5); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 61.9$ (C6′), 68.3 (C1′), 71.3 (C4′), 71.6 (C3′), 75.8 (C5), 79.8 (C2′), 85.0 (C5′),154.9 (C4), 157.1 (C2), 170.8 ppm (C6); HPLC (Waters 600, gradient 4): $t_R = 0.71$ min (97%); HRMS (ES+) *m/z*: calcd for $C_{10}H_{16}N_4O_5$ 272.1126, found 272.1121.

2-Amino-4-(2′,5′-anhydro-D-allitolyl)pyrimidine (**20a**): A solution of **19a** (80 mg, 0.14 mmol) in MeOH (10 mL) was hydrogenated for ~16 h at 30 °C and 280 kPa, in the presence of 10% Pd/C (10 mg). The reaction was filtered, washed with MeOH and evaporated to dryness. The residue was purified by CCTLC in the chromatotron (CH_2Cl_2/MeOH/NH_4OH, 4:1:0.1) to give **20a** (18 mg, 50%) as an oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 3.33$ (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, $J_{6'a,'6b} = 11.5$ 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, NH_2), 7.94 ppm (d, 1H, $J_{5,6} = 5.7$ Hz, H-6); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 62.1$ (C6′), 66.5 (C1′), 71.3 (C4′), 71.8 (C3′), 80.1 (C2′), 84.9 (C5′), 96.4 (C5), 158.4 (C6), 163.2 (C2), 169.4 ppm (C4); HPLC (Agilent 1120, gradient 4): $t_R = 2.55$ min (98%); HRMS (ES+) *m/z*: calcd for $C_{10}H_{15}N_3O_5$ 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 MeOH (10 mL) and 37% HCl (49 μL, 0.60 mmol) was hydrogenated for ~16 h at 30 °C and 280 kPa, in the presence of 20% Pd(OH)$_2$/C (10 mg). The reaction was filtered, washed with MeOH and evaporated to dryness. The residue was purified by CCTLC in the chromatotron (CH_2Cl_2/MeOH/NH_4OH, 4:1:0.1) to give **26** (48 mg, 51%) as an oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 1.60$–1.86 (m, 4H, H-3′, H-4′), 3.33 (m, 2H, CH_2OH), 3.85 (m, 1H, H-5′), 4.04 (m, 3H, H-2′ and CH_2Oar), 4.66 (bs, 1H, OH), 5.02 (s, 1H, H-5), 5.90 (s, 2H, NH_2), 6.03 ppm (s, 2H, NH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 27.2$, 27.4 (C3′, C4′), 63.9 (CH_2OH), 67.4 (CH_2Oar), 76.2 (C5), 76.9 (C2′), 80.2 (C5′), 162.8 (C6), 166.0 (C2), 169.9 ppm (C4); HPLC (Agilent 1120, gradient 4): $t_R = 3.16$ min (97%); HRMS (ES+) *m/z*: calcd for $C_{10}H_{16}N_4O_3$ 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 workup the crude was purified by reversed-phase chromatography using SPE cartridges (CH_2Cl_2/MeOH, 9:1) to give **27** (25 mg, 74%) as an oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 1.66$–1.95 (m, 4H, H-3′, H-4′), 3.34 (m, 2H, CH_2OH), 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 ppm (d, 1H, $J_{5,6} = 6.9$ Hz, H-6); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 27.1$, 27.3 (C3′, C4′), 63,7 (CH_2OH), 70.1 (CH_2Oar), 76.0 (C2′), 80.5 (C5′), 98.9 (C5), 147.0 (C6), 156.8 (C4), 171.3 ppm (C2); HPLC (Agilent 1120, gradient 3): $t_R = 3.76$ min (98%); HRMS (ES+) *m/z*: calcd for $C_{10}H_{15}N_3O_3$ 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 for ~16 h. After the workup the crude was purified by CCTLC in the chromatotron (CH_2Cl_2/MeOH, 9:1) to give **28** (10 mg, 20%) as an oil (hydrochloride); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 1.62$–1.90 (m, 4H, H-3′, H-4′), 3.35 (m, 2H, CH_2OH), 3.86 (m, 1H, H-5′), 4.09 (m, 1H, H-2′), 4.09 and 4.18 (m, 2H, CH_2Oar), 4.64 (t, 1H, $J = 5.7$ Hz, OH), 5.67 (s, 1H, H-5), 6.62 (s, 2H, NH_2), 8.06 ppm (s, 1H, H-2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 27.4$, 27.5 (C3′, C4′), 64.1 (CH_2OH), 68.1 (CH_2Oar), 76.9 (C2′), 80.3 (C5′), 85.8 (C5), 157.9 (C2), 165.5 (C6), 168.9 ppm (C4); HPLC (Waters 600, gradient 2): 0.58 min (97%) HRMS (ES+) *m/z*: calcd for $C_{10}H_{15}N_3O_3$ 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 reversed-phase chromatography using SPE cartridges (CH_2Cl_2/MeOH, 9:1) to give **31** (8 mg, 28%) as an oil. ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.65$–1.92 (m, 4H, H-3′, H-4′), 3.33 (m, 2H, CH_2OH), 3.86 (m, 1H, H-5′), 4.10 (m, 3H, CH_2Oar, H-2′), 4.63 (bs, 1H, OH), 6.06 (d, 1H, $J_{5,6} = 5.7$ Hz, H-5), 6.84 (bs, 2H, NH_2), 7.83 ppm (d, 1H, $J_{5,6} = 5.7$ Hz, H-6); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 27.2$, 27.5 (C3′, C4′), 63.9 (CH_2OH), 68.5 (CH_2Oar), 76.7 (C2′), 80.2 (C5′), 99.4 (C5), 156.1 (C6), 164.7 (C4), 165.4 ppm (C2); HPLC (Agilent 1120, gradient 4): $t_R = 4.94$ min (99%); HRMS (ES+) *m/z*: calcd for $C_{10}H_{16}N_3O_3$ 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 MeOH (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 280 kPa for ~16 h. After filtering, the residue was washed with MeOH and evaporated to dryness. The

residue was triturated with Et_2O/CH_2Cl_2/MeOH, successively washed with several portions of cold Et_2O, and evaporated to dryness to give 21 mg of (2:1) mixture of **48** and ameline (**49**) as determined by HPLC.

Method B: The general procedure was followed with **(2*R*,5*S*)-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 (CH_2Cl_2/MeOH/AcOH, 9:1:0.1). From the fastest-moving band compound **48** (114 mg, 42%) was isolated as an amorphous white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 1.65–1.92$ (m, 4H, H-3′, H-4′), 3.35 (m, 2H, CH_2OH), 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 ppm (bs, 4H, NH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 27.1$, 27.2 (C3′, C4′), 63.9 (CH_2OH), 70.2 (CH_2Oar), 75.9 (C2′), 80.4 (C5′), 161.0 (C4, C6), 165.1 ppm (C2); HPLC (Agilent 1120, gradient 4): $t_R = 4.95$ min (98%); HRMS (ES, +) *m/z*: calcd for $C_9H_{15}N_5O_3$ 241.1179, found 241.1175.

From the slowest-moving band **(2*R*,5*S*)-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. ^{1}H NMR (400 MHz, [D$_6$]DMSO + HCOOH): $\delta = 1.69–1.96$ (m, 4H, H-3, H-4), 4.12 (m, 6H, H-2, H-5, $2CH_2Oar$), 6.63 ppm (bs, 8H, $4NH_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO + HCOOH): $\delta = 27.6$ (C3, C4), 68.0 ($2CH_2Oar$), 77.0 (C2, C5), 168.3 (C4′, C6′), 170.6 ppm (C2′); HPLC (Agilent 1120, gradient 4): $t_R = 5.46$ min (95%); HRMS (ES+) *m/z*: calcd for $C_{12}H_{18}N_{10}O_3$ 350.1571, found 350.1563.

2-Amino-6-{[(2′*RS*,5′*SR*)-5′-(hydroxymethyl)tetrahydrofuran-2′-yl]methoxy}-7*H*-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 (CH_2Cl_2/MeOH, 9:1) to give **55** (25 mg, 41%) as an oil. ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.68–1.98$ (m, 4H, H-3′, H-4′), 3.35 (m, 2H, CH_2OH), 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, NH_2), 7.84 (s, 1H, H-8), 12.45 ppm (bs, 1H, NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 27.2$, 27.6 (C3′, C4′), 63.9 (CH_2OH), 68.2 (CH_2Oar), 76.7 (C2′), 80.4 (C5′), 113.4 (C5), 137.7 (C8), 155.0 (C4), 159.6 (C6), 172.2 ppm (C2); HPLC (Agilent 1120, gradient 4): $t_R = 5.61$ min (99%); HRMS (ES+) *m/z*: calcd for $C_{11}H_{15}N_5O_3$ 265.1162, found 265.1175.

6-{[(2′*RS*,5′*SR*)-5′-(Hydroxymethyl)tetrahydrofuran-2′-yl]methoxy}-7*H*-purine hydrochloride (**56**): Following the general procedure, **54** (59 mg, 0.17 mmol) was hydrogenated for ~16 h. After the workup the crude was purified by HPFC in the Biotage (CH_2Cl_2/MeOH, 9:1) to give **56** (17 mg, 35%) as an oil. ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.73–1.99$ (m, 4H, H-3′, H-4′), 3.36 (m, 2H, CH_2OH), 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 ppm (s, 1H, H-8); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 27.2$, 27.4 (C3′, C4′), 63.8 (CH_2OH), 69.2 (CH_2Oar), 76.5 (C2′), 80.4 (C5′), 116.4 (C5), 142.8 (C8), 151.8 (C2), 154.0 (C4), 158.6 ppm (C6); HPLC (Agilent 1120, gradient 4): $t_R = 3.89$ min (91%); HRMS (ES+) *m/z*: calcd for $C_{11}H_{14}N_4O_3$ 250.1054, found 250.1066.

2,6-Diamino-4-{[(2′*RS*,5′*SR*)-5′-(triphosphooxymetyl)tetrahydrofuran-2′-yl]methoxy}pyrimidine triammonium salt (**59**): To a cooled (0 °C) solution of compound **26** (150 mg, 0.54 mmol) and 1,8-bis(dimethylamino)naphthalene (*Proton Sponge*™; 196 mg, 0.92 mmol), previously dried under vacuum for 5 h, in trimethyl phosphate (2 mL), phosphorous oxychloride (0.14 mL, 1.46 mmol) was slowly added. The reaction was stirred at 5 °C for ~16 h. 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, 1 M triethylammonium bicarbonate (TEAB) solution (6.7 mL) was added, and the reaction mixture was stirred at room temperature 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 NH_4HCO_3) to obtain **59** (23 mg, 8%) as an amorphous white solid after lyophilization. ^{1}H NMR (400 MHz, D_2O): $\delta = 1.72–1.92$ (m, 4H, H-3′, H-4′), 3.53–4.14 (m, 6H, CH_2OP, CH_2Oar, H-2′, H-5′), 5.56 ppm (s, 1H, H-5); ^{31}P NMR (162 MHz, D_2O): $\delta = 1.16$, −8.49, −21.31 ppm; HPLC (Waters 600, isocratic conditions 0.5 M KH_2PO_4): $t_R = 6.49$ min (98%); HRMS (ES+) *m/z*: calcd for $C_{10}H_{18}N_4O_{12}P_3$ 480.0216, found 480.0212.

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 assays (other than anti-HIV) 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 $100 \times CCID_{50}$ of virus (with $1 \times CCID_{50}$ 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 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 test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to decrease virus-induced cytopathicity or viral plaque formation by 50%.

Anti-HIV-1 and HIV-2 activity assays: Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing $\approx 3 \times 10^5$ CEM cells per mL infected with $100 \times CCID_{50}$ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4–5 days of incubation at 37 °C in a CO_2-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} (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 μL) con-

tained 50 mM Tris·HCl (pH 7.8), 5 mM dithiothreitol, 300 mM glutathione, 500 μM EDTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 μg bovine serum albumin, an appropriate concentration of labeled (tritiated) substrate dTTP, dCTP, dGTP, or dATP (2 μCi per 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 μL of inhibitor solution (containing various concentrations of the compounds), and 1 μL of the RT preparation. The reaction mixtures were incubated at 37 °C for 30 min, at which time 100 μL yeast RNA (1 mg mL^{-1}) and 1 mL $Na_4P_2O_7$ (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 (IC_{50}) of the test compounds was determined, fixed concentrations of 1.25 μM [^{3}H]dTTP, 1.75 μM [^{3}H]dATP, 2.5 μM [^{3}H]dGTP, or 2.5 μM [^{3}H]dCTP were used.

Cytostatic activity assays: All assays were performed in 96-well microtiter plates. To each well were added (5–7.5)×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), 72 h (human lymphocytic CEM and human cervical carcinoma HeLa cells), or 96 h (human kidney carcinoma CAKI-1 and human liver hepatoma Huh-7) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

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