SYNTHESIS OF AMINOPROPYL PHOSPHONATE NUCLEOSIDES WITH PURINE AND PYRIMIDINE BASES

Ding ZHOU[1], Irene M. LAGOJA[2], Arthur VAN AERSCHOT[3] and Piet HERDEWIJN[4,*]

Laboratory of Medicinal Chemistry, Rega Institute, Minderbroedersstraat 10, B-3000 Leuven, Belgium; e-mail: [1] ding.zhou@rega.kuleuven.be, [2] irene.lagoja@rega.kuleuven.be, [3] arthur.vanaerschot@rega.kuleuven.be, [4] piet.herdewijn@rega.kuleuven.be

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The synthesis and antiviral evaluation of new acyclic phosphonate nucleosides related to HPMPC (Cidofovir) has been described. These aminopropyl phosphonate nucleosides **1**–**3** have an amino function within either the acyclic chain (series **2** and **3**) or as substituent (series **1**). Both purine and pyrimidine nucleotide analogues have been synthesized. In contrast to HPMPC the oxygen analogue of **2c**, only a weak antiherpes virus activity could be demonstrated for **2b** and **2c**.

Keywords: Acyclic nucleoside phosphonates; Acyclic nucleotide analogues; Aminopropyl phosphonic acids; Antiviral activity; Mitsunobu reaction.

Replication of human immunodeficiency virus type 1 can be reduced in HIV-infected patients using a combination of antiviral drugs targeted at the reverse transcriptase and protease[1]. Unfortunately, due to the high mutation rate of HIV, the treatment, even combination therapy, selects drug-resistant variants[2].

The acyclic nucleotide phosphonates (ANPs) are broad-spectrum antiviral agents with potent and selective antiviral activity *in vitro* and *in vivo*. They have shown to be effective against several DNA viruses and retroviruses including HIV [3]. These compounds were designed to circumvent the first phosphorylation step, necessary for the activation of normal nucleoside analogues[4]. Once inside the cells, they are converted into active diphosphorylated metabolites, which react with the viral DNA polymerase (of herpes viruses) or act as chain terminators in the HIV reverse transcriptase (RT) reaction[5]. (*S*)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]adenine, ((*S*)-HPMPA), an acyclic nucleotide analogue reported by Holý and De Clercq et al.[6], is a representative of this class which possesses broad-spectrum antiviral activity. (*S*)-1-[3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine ((*S*)-HPMPC, cidofovir) has been approved for the treatment of CMV-retinitis in AIDS patients.

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More recently, a series of purine and pyrimidine *N*-[2-(phosphonomethoxy)ethyl] derivatives bearing aminomethyl or azidomethyl groups were reported[7–9]. Among them, 9-[3-amino-2-(phosphonomethoxy)propyl]-adenine proved active against varicella-zoster virus (VZV) and cytomegalovirus (CMV). Following this interest, we describe here the synthesis and evaluation of the whole series of acyclic nucleotide analogues, with the natural purine (A, G) and pyrimidine (U, C) bases, bearing an amino function in the position 2 or 3 of the acyclic sugar moiety, as shown in Chart 1.

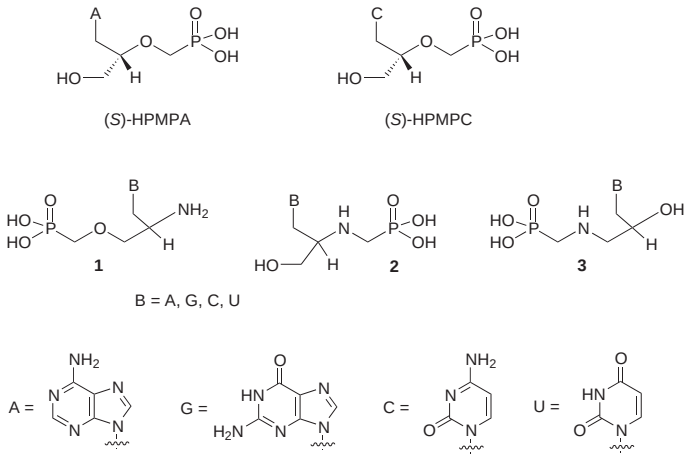


CHART 1

Compounds **1a**–**1d** are (phosphonomethoxy)propyl analogues with an amino substituent in the 2′-position. This amino group forms an internal salt with the phosphonate function, influencing the polarity and the conformational flexibility of the molecule. Compounds **2a**–**2d** are the (phosphonomethyl)amino congeners of HPMPA and HPMPC. The third series **3a**–**3d** have the inverse functionality compared with the first series **1a**–**1d**, and a secondary amino group instead of a primary amino function. The free secondary hydroxy group of **3a**–**3d** and the primary hydroxy group of **2a**–**2d** might mimic one of the hydroxy groups of a ribose unit (should this be important for the interaction with metabolic enzymes and/or with polymerases).

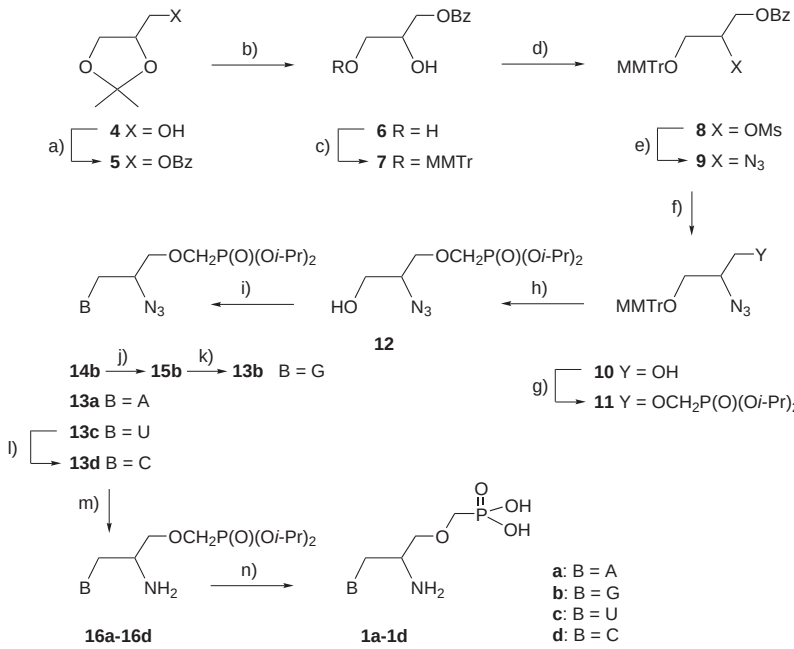
RESULTS AND DISCUSSION

Aminopropyl phosphonate nucleosides can be prepared by two different strategies. Either they are obtained by phosphonoylation of the acyclic nucleosides, as exemplified for aminopropyl phosphonic acids of type **2**

and **3**. Alternatively, the aglycon is introduced on an aminopropyl group, already functionalized with a protected phosphonomethyl ether group, as exemplified for aminopropyl phosphonate nucleosides of type **1**. A possible chiral synthesis was envisaged only if some of these phosphonates would display interesting antiviral activity.

*Synthesis of Aminopropyl Phosphonate Nucleosides **1a–1d***

A possible reaction pathway, starting from racemic 1,2-*O*-isopropylideneglycerol (**4**) is outlined in Scheme 1. Benzoylation of **4** followed by hydrolysis of the isopropylidene protecting group derives (±)-2,3-dihydroxypropyl

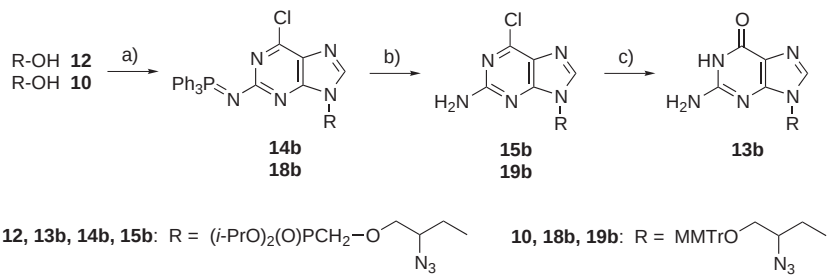


SCHEME 1

Synthesis of aminopropyl phosphonate nucleosides **1a**–**1d**

a) BzCl, Py (not isolated); b) TFA/H_2O (3:1) (49%, both steps); c) MTrCl, Py; d) MsCl, Py (77%, both steps); e) NaN_3, DMF, 100 °C; f) 1 M NaOH in MeOH (50%, 2 steps); g) NaH, $CF_3SO_3CH_2PO(Oi\text{-}Pr)_2$, –20 °C; h) 3% TFA in DCM (73%, 2 steps); i) **13a**: Ph_3P, DIAD, adenine, THF (53%), **14b**: Ph_3P, DIAD, 6-chloro-2-aminopurine THF (47%), **13c**: Ph_3P, DIAD, 3-benzoyluracil, THF, sat. NH_3 in MeOH (69%, 2 steps); j) **15b**: H_2O, reflux (85%); k) **13b**: TFA in H_2O (63%); l) MesCl, Et_3N, MeCN, 26% NH_4OH in H_2O (60%, 2 steps); m) H_2S, Py, Et_3N; n) Me_3SiBr, MeCN (**1a**: 49%, **1b**: 44%, **1c**: 51%, **1d**: 48%, 2 steps)

benzoate (**6**). The primary hydroxy function is selectively protected with a methoxytrityl group to obtain **7**. Reaction with mesyl chloride giving **8** introduces a good leaving group allowing nucleophilic substitution with sodium azide to yield **9**. The following debenzoylation to **10**, treatment with (diisopropoxyphosphoryl)methyl trifluoromethanesulfonate yields the phosphonate **11**, which in turn can be converted into the key intermediate of this synthetic pathway: (±) diisopropyl [(2-azido-3-hydroxypropoxy)-methyl]phosphonate **12**. Mitsunobu conditions[10] (triphenylphosphine, diisopropyl azodicarboxylate (DIAD) in THF at room temperature) were used to introduce the nucleobases onto the phosphonate **12**. Coupling of 3-benzoyluracil to **12**, followed by debenzoylation yields the 1-alkyluracil derivative **13c** in good yield (69%, both steps). However, no product formation could be observed using cytosine as nucleobase. Therefore, **13c** was smoothly converted to the cytosine derivative **13d** by treatment with NH_4OH, following activation with mesitylenesulfonyl chloride (MesCl) in Et_3N/acetonitrile (60%, both steps)[11]. An alternative method using activation with $POCl_3$/triazole[12] was less satisfactory. Mitsunobu conditions were applied to introduce the purine bases. Reaction of **12** with adenine yields **13a** in 53% yield. Guanine was not directly introduced by the Mitsunobu coupling due to the formation of an inseparable mixture of N7/N9 isomers (1:2). Therefore, the coupling was accomplished with 2-amino-6-chloropurine (N9 47%; N7/N9 1:4), yielding the triphenylphosphoranylidene derivative **14b**, which could be hydrolyzed to the 2-amino-6-chloropurine derivative **15b**. Treatment thereof with trifluoroacetic acid (TFA) finally affords the desired intermediate **13b** (Scheme 2).



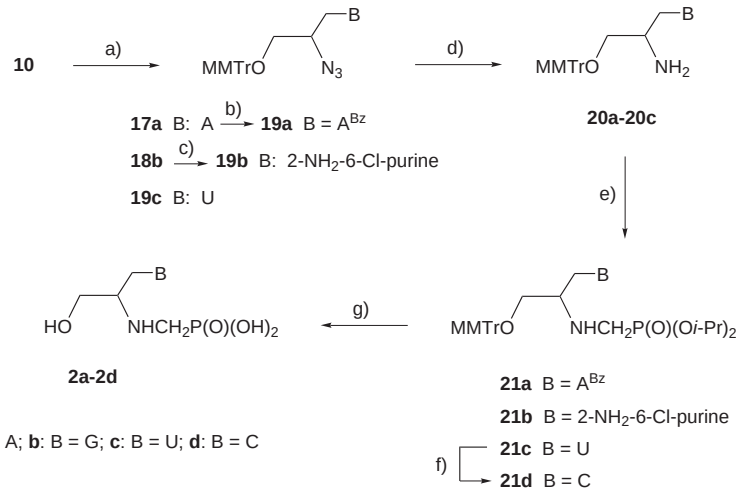
SCHEME 2
Detailed pathway for the synthesis of **13b** and **19b**
a) 2-NH_2-6-Cl-purine, Ph_3P, DIAD, THF; b) THF, H_2O, reflux; c) TFA, H_2O

As hydrogenation was precluded in view of the trityl moiety, reduction of the azide function was accomplished using H_2S in pyridine to obtain **16a**–**16d**. The desired aminopropyl phosphonate nucleosides **1a**–**1d** were obtained by deprotection of the phosphonate esters using bromotrimethylsilane.

*Synthesis of Aminopropyl Phosphonate Nucleosides **2a**–**2d***

Initially, the same strategy, i.e. introduction of the nucleobases to the phosphonoylated acyclic precursor, was applied to obtain aminopropyl phosphonate nucleosides **2**. However, the reaction failed, due to the favoured formation of an aziridine by-product under Mitsunobu conditions. To circumvent this problem, the base was introduced prior to the phosphonate moiety (Scheme 3).

Starting from intermediate **10**, the nucleobases were introduced under Mitsunobu conditions (**17a**, **19b**, **19c**). In the case of adenine, the 6-amino function had to be protected with a benzoyl group to give **19a**, before in-

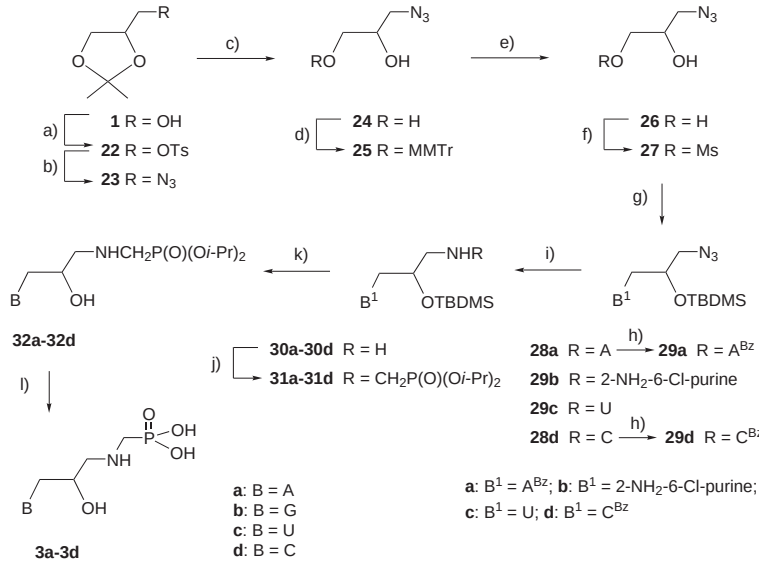


SCHEME 3
Synthesis of aminopropyl phosphonate nucleosides **2a**–**2d**
a) **17a**: A, Ph_3P, DIAD, THF (45%), **18b**: 2-amino-6-chloropurine, Ph_3P, DIAD, THF (45%), **19c**: U^{Bz}, Ph_3P, DIAD, THF, NH_3/MeOH (46%); b) BzCl, Py, 0 °C (85%); c) H_2O, THF, reflux (82%); d) Ph_3P, THF, H_2O, reflux (**20a**: 94%, **20b**: 91%, **20c**: 88%); e) $CF_3SO_3CH_2P(O)(O\textit{i}\text{-}Pr)_2$, Et_3N, THF (not isolated except **21c** (85%)); f) MesCl, Py, 26% NH_4OH (not isolated); g) **2a**: NH_3/MeOH, Me_3SiBr, DMF (45%, 3 steps), **2b**: 75% TFA, Me_3SiBr, DMF (42%, 3 steps), **2c**: Me_3SiBr, MeCN (68%), **2d**: Me_3SiBr, MeCN (40%, 2 steps)

troduction of the phosphonate moiety, whereas protection of the less reactive 2-amino function of 2-amino-6-chloropurine is not needed. Following reduction of the azide function to obtain **20a**–**20c**, the introduction of the phosphonate moiety and subsequent deprotection to yield the required aminopropyl phosphonate nucleosides **2a**–**2c** could be achieved without isolation and purification of the intermediates. Analogously to the procedure described in Scheme 1, the cytosine derivative **2d** was prepared by conversion of uracil analogue **21c**, followed by deprotection.

*Synthesis of Aminopropyl Phosphonate Nucleosides **3a**–**3d***

A synthetic pathway yielding aminopropyl phosphonate nucleosides of type **3** is outlined in Scheme 4. Again, the synthesis starts from racemic isopropylideneglycerol **4**. Following activation with a tosyl group, nucleo-



SCHEME 4

Synthesis of aminopropyl phosphonate nucleosides **3a**–**3d**

a) TsCl, Py (94%); b) NaN_3, DMF, 100 °C; c) TFA, H_2O (3:1) (33%, 2 steps); d) MTrCl, Py; e) TBDMSCl, imidazole, DMF, 4% TsOH in MeOH, CH_2Cl_2 (1:2) (37%, 3 steps); f) MsCl, Py (81%); g) **29a**: LiH, adenine, THF, 100 °C (47%), BzCl, Py, NH_3/MeOH (82%), **29b**: Cs_2CO_3, 2-amino-6-chloropurine, DMF, 90 °C (38%), **29c**: K_2CO_3, NaI, uracil, DMF, 90 °C (43%), **28d**: Cs_2CO_3, cytosine, DMF, 90 °C (45%); h) BzCl, Py (85%); i) Ph_3P, THF, H_2O, reflux (**30a**: 80%, **30b**: 90%, **30c**: 91%, **30d**: 81%); j) Et_3N, $CF_3SO_3CH_2P(O)(Oi\text{-}Pr)_2$, THF; k) **31a**: NH_3/MeOH, 1 M TBAF in THF, **31b**: 75% TFA in H_2O, 1 M TBAF in THF, **31c**: 1 M TBAF in THF, **31d**: NH_3/MeOH, 1 M TBAF in THF; l) Me_3SiBr, MeCN (3 steps: **3a**: 24%, **3b**: 23%, **3c**: 41%, **3d**: 24%)

philic substitution to introduce an azido function is carried out. Compound **23** is subsequently hydrolyzed to **24**. Protection of the secondary alcohol function to obtain **26** was achieved using a methoxytrityl group for intermediate protection of the primary hydroxy group. To avoid reduction of the azido function to an amino function under Mitsunobu conditions, the classical method of coupling purine and pyrimidine bases by nucleophilic substitution of alkyl sulfonates was applied[13]. Following the reaction with mesyl chloride to **27**, the pyrimidine and purine bases could be introduced to obtain **28a**, **29b**, **29c** and **28d**. In the case of adenine and cytosine, the exocyclic amino group was protected by benzoylation to obtain **29a** and **29d**, respectively. During the coupling reaction of cytosine to obtain **28d** (45%), the *O*-alkylated analogue (less polar) was formed as a by-product. The introduction of 2-amino-6-chloropurine to yield **29b** was accomplished with moderate yield (38%). However, the required N9 isomer was obtained almost exclusively. Having in hand the intermediates **29a**–**29d**, reduction of the azide to an amino function followed by alkylation with (diisopropoxyphosphoryl)methyl trifluoromethanesulfonate yielded compounds **31a**–**31d**. Conversion of the 2-amino-6-chloropurine to guanine, as well as deprotection of the adenine and cytosine base followed by hydrolysis of the silyl moiety, afforded the intermediates **32a**–**32d**. Treatment with bromotrimethylsilane yielded the desired aminopropyl phosphonic acids **3a**–**3d**. All phosphonates **1**, **2** and **3** were exchanged to their disodium salts. Purity of the different analogues was verified by analytic RP-HPLC with Bu_4NHSO_4 as the ionpairing agent (not shown).

BIOLOGICAL RESULTS

All acyclic nucleoside phosphonates **1**–**3**, in their sodium form, were evaluated *in vitro* for cytotoxicity and for their activity against a variety of viruses including HIV-1, HIV-2, HSV-1, HSV-2, thymidine kinase (TK)-deficient HSV-1/TK^- and vaccinia virus (VV), varicella-zoster virus (VZV TK^- and TK^+) and cytomegalovirus (CMV) replication in HEL cells. Brivudin, Ribavirin, Acyclovir, Ganciclovir were used as reference drugs. Compounds **1**–**3** did not show cytotoxicity and are inactive against HIV-1 and HIV-2. Marginal biological activity only could be observed for some of the aminopropyl phosphonate derivatives **2** and **3** (Table I).

TABLE I
Summary of biological activities found for aminopropyl phosphonate nucleosides **1**–**3**

Compound	HSV, μg/ml			VZV, μM				CMV, μg/ml		
	HSV-1 (F)	HSV-2 (Lyons)	MCC[a]	TK^+ VZV	TK^- VZV	MCC	CC_{50}[b]	AD-169 strain	MCC	CC_{50}
2b	48	48	>400	72	23	>100	>50	45	>100	>50
2c	80	n.a	>400	n.a	n.a	>100	>50	n.a	>100	>50
3c	n.a	n.a	>400	>20	>20	100	>50	n.a	>100	>50

[a] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology; [b] cytotoxic concentration required to reduce cell growth by 50%; n.a., not active at the highest concentration tested.

CONCLUSION

The synthesis of aminopropyl phosphonate nucleosides **1**–**3** has been described. Compounds of type **2** and **3** are obtained by phosphonoylation of the acyclic nucleosides. The aglycon is introduced on the aminopropyl group already functionalized with a protected phosphonomethyl ether group in the case of aminopropyl phosphonate nucleosides **1**. Compounds **2a**–**2d** are the (phosphonomethyl)amino congeners of HPMPA and HPMPC. Only in this series marginal activity against HSV and VZV could be demonstrated. Replacement of the oxygen atom in the acyclic chain of HPMPC with a nitrogen function results in a large reduction in biological activity.

EXPERIMENTAL

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (100 °C) under nitrogen atmosphere. ^{1}H NMR was determined using a Varian Unity 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. A 200 MHz Varian Gemini apparatus was used for ^{13}C NMR determination in DMSO-d_6 (39.6 ppm) or $CDCl_3$ (76.9 ppm) and using the solvent peak as reference. Chemical shifts (δ) are given in ppm, coupling constants (*J*) in Hz. Where needed COSY and DEPT were used to assign proton and carbon resonances. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, U.K.) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in *i*-PrOH/H_2O 1:1 at 3 μl/min. TLC was performed with TLC aluminum sheets (Merck, Silica gel 60 F_{254}). The spots were examined with UV light, or sprayed with sulfuric acid/anisaldehyde or 1% potassium permanganate solution. Column chromatography was performed on ICN silica gel 60–200 60A. The names of the compounds were made in accord with IUPAC rules.

The synthesis of the enantiomerically pure compounds **22**–**27**, **29a** has been published recently, starting from (*S*)-1,2-*O*-isopropylideneglycerol[14]. The racemic congeners used here were prepared in analogous fashion.

(±)-2,3-Dihydroxypropyl Benzoate (**6**)

Under ice-cooling, benzoyl chloride (20.00 ml, 0.17 mmol) was added slowly to a solution of 1,2-*O*-isopropylideneglycerol **4** (15.00 g, 0.11 mol) in dry pyridine (100 ml). The reaction was then allowed to warm to room temperature and stirred for 5 h. After the solvent was evaporated, the residual oil was co-evaporated with toluene (2 × 20 ml), dissolved in CH_2Cl_2 (40 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 25 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was treated with 75% TFA in water (50 ml) for 4 h, concentrated in vacuo and purified by column chromatography (CH_2Cl_2/MeOH 95:5 to 92:8). Yield 10.91 g (49%); R_F 0.60 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): 3.58–3.61 (d, 2 H, H-3′, $J_{3',2'}$ = 5.8), 3.90–3.97 (m, 1 H, H-2′), 4.24–4.33 (dd, 1 H, H-1′A, $J_{1'A,2'}$ = 6.0, J_{gem} = 11.2), 4.36–4.44 (dd, 1 H, H-1′B, $J_{1'B,2'}$ = 4.4, J_{gem} = 11.2), 7.43–7.63 (m, 3 H, 3,4,5-H-Ar), 7.80–8.04 (d, 2 H, 2,6-H-Ar, J = 8.4). ^{13}C NMR ($CDCl_3$): 63.2 (C-3′), 66.7 (C-2′), 70.0 (C-1′), 129.0 (C-3,5-Ar), 129.8 (C-2,6-Ar), 133.6 (C-4-Ar), 166.5 (C=O). ES-MS calculated for $C_{10}H_{13}O_4$ $[M + H]^+$: 197.0814; found: 197.0809.

(±)-2-(Mesyloxy)-3-[(4-methoxytrityl)oxy]propyl Benzoate (**8**)

Compound **6** (10.00 g, 51.0 mmol) was co-evaporated with dry pyridine (2 × 20 ml) and dissolved in pyridine (40 ml). MTrCl (17.30 g, 56.1 mmol) was added and the reaction mixture was stirred for 5 h. Mesyl chloride (6.41 g, 56.1 mmol) was added and stirring was continued for an additional 2 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (30 ml) and extracted with CH_2Cl_2 (4 × 30 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2). Yield 21.43 g (77%); R_F 0.54 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 3.04 (s, 3 H, CH_3), 3.45–3.53 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 5.6, J_{gem} =11.4), 3.55–3.62 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 4.4, J_{gem} = 11.4), 3.80 (s, 3 H, CH_3), 4.59–4.62 (m, 2 H, H-1′), 5.09–5.19 (m, 1 H, H-2′), 6.84–6.92 (d, 2 H, H-MTr), 7.31–7.64 (m, 15 H, H-MTr, 3,4,5-H-Bz), 8.00–8.05 (d, 2 H, 2,6-H-Ar). ^{13}C NMR ($CDCl_3$): 38.5 (CH_3 Ms), 55.0 (O-CH_3), 62.4 (C-3′), 63.3 (C-1′), 78.4 (C-2′), 87.0 (C-MTr), 113.1 (C-MTr), 127.1, 127.8, 128.0, 128.2, 128.4, 129.3, 129.8, 130.4 (C-MTr), 129.6 (C-3,5-Bz), 130.2 (C-2,6-Bz), 133.3 (C-4-Bz), 134.6, 143.6, 158.7 (C-MTr), 165.9 (C=O). ES-MS calculated for $C_{31}H_{30}NaO_7S$ $[M + Na]^+$: 569.1609; found: 569.1605.

(±)-2-Azido-3-[(4-methoxytrityl)oxy]propan-1-ol (**10**)

A mixture of compound **8** (21.00 g, 38.5 mmol) and NaN_3 (3.74 g, 54.9 mmol) was dissolved in DMF (30 ml) and stirred at 100 °C overnight. The reaction was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 ml) and washed with water (2 × 20 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residual oil was treated with 1 M NaOH in MeOH (40 ml) overnight. After concentrating the mixture, the residue was dissolved in H_2O (20 ml) and extracted with CH_2Cl_2 (2 × 30 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography (CH_2Cl_2/MeOH 99:1). Yield 7.44 g (50%); R_F 0.25 (CH_2Cl_2/MeOH 99:1). ^{1}H NMR ($CDCl_3$): 3.29–3.32 (m, 2 H, H-3′), 3.58–3.70 (m, 3 H, H-2′, H-1′), 3.80 (s, 3 H, CH_3),

6.82–6.87 (d, 2 H, H-MTr), 7.23–7.54 (m, 12 H, H-MTr). ^{13}C NMR ($CDCl_3$): 55.2 (CH_3), 62.8 (C-3′), 63.3 (C-2′), 63.5 (C-1′), 87.1 (C-MTr), 113.3 (C-MTr), 127.1, 128.0, 128.4, 130.4, 135.1, 144.0, 158.8 (C-MTr). ES-MS calculated for $C_{23}H_{23}N_3NaO_3$ $[M + Na]^+$: 412.1637; found: 412.1643.

(±) Diisopropyl [(2-Azido-3-hydroxypropoxy)methyl]phosphonate (**12**)

A mixture of **10** (3.40 g, 8.7 mmol) and $CF_3SO_3CH_2P(O)(O\textit{i}\text{-}Pr)_2$ (3.90 g, 10.4 mmol) was dissolved in THF (30 ml) and cooled to –30 °C. NaH (0.34 g, 11.2 mmol) was added under stirring, the reaction mixture was allowed to warm to 0 °C. Stirring at 0 °C was continued for 1 h. Following addition of MeOH, the solvent was evaporated, the residue was dissolved in CH_2Cl_2 (30 ml) and extracted with saturated aqueous $NaHCO_3$ (2 × 30 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated and the residue was treated with 3% TFA in CH_2Cl_2 (25 ml) over a period of 2 h. Then a saturated aqueous $NaHCO_3$ solution (25 ml) was added and the mixture was extracted with CH_2Cl_2 (2 × 30 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (EtOAc, ethyl acetate). Yield 2.02 g (73%); R_F 0.20 (EtOAc). ^{1}H NMR ($CDCl_3$): 1.31–1.34 (d, 12 H, 4 × CH_3), 3.55–3.88 (m, 7 H, H-3′, H-2′, H-1′, O-CH_2-P), 4.68–4.78 (m, 2 H, 2 × O-C**H**-$(CH_3)_2$). ^{13}C NMR ($CDCl_3$): 23.8 (4 × CH_3), 61.3 (C-2′), 61.9 (C-3′), 64.3, 67.6 (O-CH_2-P), 71.5 (d, 2 × O-C**H**-$(CH_3)_2$, $J_{C,P}$ = 5.2), 72.0 (d, C-1′, $J_{1',P}$ = 5.2). FAB-MS calculated for $C_{10}H_{22}N_3NaO_5P$ $[M + Na]^+$: 318.3; found: 318.0.

Synthesis of Compounds **13** and **14b** by Mitsunobu Reaction. General Procedure

A mixture of **12** (0.60 g, 2.03 mmol), a nucleobase [**a** adenine (0.56 g, 4.06 mmol), **b** 2-amino-6-chloropurine (0.69 g, 4.07 mmol), **c** 3-benzoyluracil (0.68 g, 3.05 mmol)] and triphenylphosphine (for **a**, **b** 1.07 g, 4.07 mmol, for **c** 0.81 g, 3.05 mmol) were dissolved in dry THF (10 ml) and cooled to 0 °C in an ice bath. Then DIAD (for **a**, **b**, **c** 0.51 g, 2.54 mmol) in THF (2 ml) was added dropwise over 30 min. The reaction was allowed to warm to room temperature and stirring was continued overnight. The reaction mixture was concentrated; the residue was dissolved in CH_2Cl_2 (20 ml) and washed with water (2 × 15 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography.

(±) Diisopropyl {[3-(6-amino-9H-purin-9-yl)-2-azidopropoxy]methyl}phosphonate (**13a**): Yield 0.44 g (53%); R_F 0.28 (CH_2Cl_2/MeOH 95: 5). ^{1}H NMR ($CDCl_3$): 1.30, 1.33 (d, 12 H, 4 × CH_3), 3.61–4.43 (m, 7 H, H-3′, H-2′, H-1′, O-CH_2-P), 4.67–4.83 (m, 2 H, 2 × O-C**H**-$(CH_3)_2$), 6.54 (s, 2 H, NH_2), 7.88 (s, 1 H, H-2), 8.30 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 22.5 (4 × CH_3), 43.8 (C-1′), 59.8 (C-2′), 64.6, 67.9 (O-CH_2-P), 69.8–69.9 (d, 2 × O-C**H**-$(CH_3)_2$, $J_{C,P}$ = 6.1), 71.1–71.4 (d, C-3′, $J_{3',P}$ = 10.7), 119.3 (C-5), 141.0 (C-8), 150.0 (C-4), 153.1 (C-2), 155.9 (C-6). ES-MS calculated for $C_{15}H_{26}N_8O_4P$ $[M + H]^+$: 413.1814; found: 413.1819.

(±) Diisopropyl {[3-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-2-azidopropoxy]methyl}phosphonate (**13b**): The phosphoranylidene derivative (**14b**) (0.60 g, 0.85 mmol) obtained after Mitsunobu reaction [yield 0.68 g (47%); R_F 0.40 (CH_2Cl_2/MeOH 95:5); FAB-MS $[M + H]^+$: 707.1] was dissolved in THF (10 ml), water (3 ml) was added and the mixture was heated under reflux for two days. The solvent was removed in vacuo and the residue **15b** was purified by column chromatography (CH_2Cl_2/MeOH 95:5) [yield 0.32 g (85%); R_F 0.50 (CH_2Cl_2/MeOH 95:5); FAB-MS $[M + H]^+$: 447.1]. Compound **15b** (0.30 g, 0.75 mmol) was treated further with 75% TFA in water (10 ml) at room temperature overnight. The solvent

was evaporated and the residue **13b** was purified by column chromatography (CH_2Cl_2/MeOH 9:1). Yield 0.20 g (63%); R_F 0.45 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR ($CDCl_3$): 1.20, 1.23 (d, 12 H, 4 × CH_3), 3.47–4.16 (m, 7 H, H-3′, H-2′, H-1′, O-CH_2-P), 4.51–4.63 (m, 2 H, 2 × O-C**H**-$(CH_3)_2$), 6.52 (s, 2 H, NH_2), 7.71 (s, 1 H, H-8), 10.67 (s, 1 H, NH). ^{13}C NMR ($CDCl_3$): 23.6 (4 × CH_3), 43.0 (C-1′), 59.7 (C-2′), 63.4, 66.6 (O-CH_2-P), 70.1–70.2 (d, 2 × O-C**H**-$(CH_3)_2$, $J_{C,P}$ = 6.1), 72.1–72.3 (d, C-1′, $J_{1',P}$ = 10.7), 116.6 (C-5), 137.6 (C-8), 151.8 (C-2), 153.7 (C-4), 156.9 (C-6). ES-MS calculated for $C_{15}H_{26}N_8O_5P$ $[M + H]^+$: 429.1764; found: 429.1760.

(±) Diisopropyl {[2-azido-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)propoxy]methyl}phosphonate (**13c**): Yield 0.69 g (69%); R_F 0.22 (EtOAc). ^{1}H NMR ($CDCl_3$): 1.31, 1.34 (d, 12 H, 4 × CH_3), 3.56–4.05 (m, 7 H, H-3′, H-2′, H-1′, O-CH_2-P), 4.70–4.80 (m, 2 H, 2 × O-C**H**-$(CH_3)_2$), 5.68–5.72 (d, 1 H, H-5, $J_{5,6}$ = 8.0), 7.27–7.31 (d, 1 H, H-6, $J_{5,6}$ = 8.0), 10.2 (br, 1 H, NH). ^{13}C NMR ($CDCl_3$): 22.5 (4 × CH_3), 47.7 (C-1′), 57.9 (C-2′), 63.1, 66.5 (O-CH_2-P), 69.9–70.0 (d, 2 × O-C**H**-$(CH_3)_2$, $J_{C,P}$ = 6.1), 71.0–71.2 (d, C-1′, $J_{1',P}$ = 10.7), 100.8 (C-5), 144.0 (C-6), 149.8 (C-2), 162.8 (C-4). ES-MS calculated for $C_{14}H_{25}N_5O_6P$ $[M + H]^+$: 390.1543; found: 390.1548.

(±) Diisopropyl {[3-(4-amino-2-oxopyrimidin-1(2H)-yl)-2-azidopropoxy]methyl}phosphonate (**13d**): Et_3N (350 µl) was added dropwise to a solution of **13c** (0.30 g, 0.77 mmol) and mesitylenesulfonyl chloride (0.51 g, 2.31 mmol) in MeCN (10 ml) at 0 °C under nitrogen. After stirring at room temperature for 2 h, 26% NH_4OH in water (5 ml) was added and the mixture was stirred for another 3 h. The resulting mixture was extracted with ethyl acetate (2 × 15 ml). The organic layer was washed with water (15 ml), dried over anhydrous Na_2SO_4 and concentrated under high vacuum. The residue was purified by column chromatography (CH_2Cl_2/MeOH 9:1). Yield 0.18 g (60%); R_F 0.48 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR ($CDCl_3$): 1.33, 1.36 (d, 12 H, 4 × CH_3), 3.43–4.10 (m, 7 H, H-3′, H-2′, H-1′, O-CH_2-P), 4.68–4.82 (m, 2 H, 2 × O-C**H**-$(CH_3)_2$), 5.89–5.93 (d, 1 H, H-5, $J_{5,6}$ = 6.8), 7.26–7.30 (d, 1 H, H-6, $J_{5,6}$ = 6.8). ^{13}C NMR ($CDCl_3$): 23.9 (4 × CH_3), 50.4 (C-1′), 59.6 (C-2′), 64.5, 67.8 (O-CH_2-P), 71.2–71.4 (d, 2 × O-C**H**-$(CH_3)_2$, $J_{C,P}$ = 7.6), 72.8–73.0 (d, C-1′, $J_{1',P}$ = 10.7), 95.0 (C-5), 146.0 (C-6), 156.7 (C-2), 166.6 (C-4). ES-MS calculated for $C_{14}H_{26}N_6O_5P$ $[M + H]^+$: 389.1702; found: 389.1704.

Synthesis of Aminopropyl Phosphonate Nucleosides **1**. General Procedure

Under ice-cooling, H_2S gas was bubbled through a solution of compound **13a**–**13d** (**a** 0.21 g, 0.51 mmol; **b** 0.13 g, 0.30 mmol; **c** 0.25 g, 0.64 mmol; **d** 0.15 g, 0.39 mmol) in pyridine/triethylamine 4:1 (10 ml) for 20 min, followed by stirring for another 1 h. The solvent was evaporated and the residue was co-evaporated with toluene (2 × 10 ml). The residue (**16a**–**16d**) was dissolved in MeCN (10 ml) and Me_3SiBr (0.24 g, 15.3 mmol) was added. Stirring was continued overnight. The reaction mixture was concentrated and was purified by column chromatography (CH_2Cl_2/MeOH 9:1, CH_2Cl_2/MeOH/H_2O 5:4:1). All compounds **1a**–**1d** were analysed as disodium salts.

(±)-{[2-Amino-3-(6-amino-9H-purin-9-yl)propoxy]methyl}phosphonic acid (**1a**): Yield 75 mg (49%); R_F 0.24 (MeOH). ^{1}H NMR (D_2O): 3.45–3.79 (m, 5 H, CH_2-P, H-1′, H-2′), 4.42 (m, 2 H, H-3′), 7.95 (s, 1 H, H-2), 8.02 (s, 1 H, H-8). ^{13}C NMR (D_2O): 45.5 (C-1′), 52.3 (C-2′), 69.9, 72.9 (CH_2-P), 72.8–72.1 (d, C-3′, $J_{C\text{-}3',P}$ = 11.2), 120.2 (C-5), 144.5 (C-8), 150.9 (C-4), 154.6 (C-2), 157.3 (C-6). ES-MS calculated for $C_9H_{14}N_6O_4P$ $[M - H]^-$: 301.0814; found: 301.0809.

(±)-{[2-Amino-3-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)propoxy]methyl}phosphonic acid (**1b**): Yield 42 mg (44%); R_F 0.33 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 3.47–3.60 (m,

5 H, CH_2-P, H-1′, H-2′), 4.19 (m, 2 H, H-3′), 7.71 (s, 1 H, H-8). ^{13}C NMR (D_2O): 46.8 (C-1′), 52.3 (C-2′), 70.1, 73.0 (CH_2-P), 73.8–74.1 (d, C-3′, $J_{C\text{-}3',P}$ = 12.2), 118.0 (C-5), 142.2 (C-8), 153.9 (C-4), 156.4 (C-2), 161.6 (C-6). ES-MS calculated for $C_9H_{14}N_6O_5P$ $[M - H]^-$: 317.0763; found: 317.0770.

(±)-{[2-Amino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)propoxy]methyl}phosphonic acid (**1c**): Yield 90 mg (51%); R_F 0.32 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 3.44–3.48 (d, 2 H, CH_2-P, $J_{H,P}$ = 8.8), 3.56–3.71 (m, 3 H, H-1′, H-2′), 3.91–4.12 (m, 2 H, H-3′), 5.78–5.82 (d, 1 H, H-5, $J_{5,6}$ = 7.8), 7.60–7.64 (d, 1 H, H-6, $J_{5,6}$ = 7.8). ^{13}C NMR (D_2O): 50.6 (C-1′), 51.8 (C-2′), 69.9, 72.9 (CH_2-P), 72.0–72.2 (d, C-3′, $J_{C\text{-}3',P}$ = 12.2), 104.3 (C-5), 149.5 (C-6), 155.2 (C-2), 169.5 (C-4). ES-MS calculated for $C_8H_{13}N_3O_6P$ $[M - H]^-$: 278.0542; found: 278.0547.

(±)-{[2-Amino-3-(4-amino-2-oxopyrimidin-1(2H)-yl)propoxy]methyl}phosphonic acid (**1d**): Yield 52 mg (48%); R_F 0.25 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 3.43–3.73 (m, 5 H, CH_2-P, H-1′, H-2′), 3.90–4.13 (m, 2 H, H-3′), 5.92–5.96 (d, 1 H, H-5, $J_{5,6}$ = 7.2), 7.53–7.56 (d, 1 H, H-6, $J_{5,6}$ = 7.2). ^{13}C NMR (D_2O): 51.2 (C-2′), 52.1 (C-1′), 69.9, 72.9 (CH_2-P, J = 151), 72.3–72.5 (d, C-3′, $J_{C,P}$ = 13.7), 98.4 (C-5), 149.3 (C-6), 160.8 (C-2), 168.9 (C-4). ES-MS calculated for $C_8H_{14}N_4O_5P$ $[M - H]^-$: 277.0702; found: 277.0703.

Synthesis of Compounds **19a**–**19c** by Mitsunobu Reaction. General Procedure

Compound **10** (1.50 g, 3.86 mol), a nucleobase [**a** adenine (1.05 g, 7.71 mmol), **b** 2-amino-6-chloropurine (1.31 g, 7.63 mmol), **c** 3-benzoyluracil (1.30 g, 5.78 mmol)] and triphenylphosphine (2.02 g, 7.71 mmol) were dissolved in dry THF (10 ml) and cooled to 0 °C. DIAD (1.53 ml, 7.71 mmol) in THF (1 ml) was added dropwise over 30 min. The reaction was allowed to warm to room temperature and stirring was continued overnight. The reaction mixture was concentrated; the residue was dissolved in CH_2Cl_2 (30 ml) and washed with water (2 × 20 ml). The organic layer was dried over anhydrous Na_2SO_4, the solvent was evaporated and the residue was purified by column chromatography.

(±)-N-(9-{2-Azido-3-[(4-methoxytrityl)oxy]propyl}-9H-purin-6-yl)benzamide (**19a**): Following the Mitsunobu reaction, **17a** was obtained [yield 0.88 g (45%); R_F 0.21 (CH_2Cl_2/MeOH 95:5); ES-MS $[M + H]^+$: 507.2257]. Under ice-cooling, benzoyl chloride (4.43 ml, 4.73 mmol) was added to a solution of compound **17a** (0.80 g, 1.58 mmol) in pyridine (15 ml). The reaction was stirred for 1 h and allowed to warm to room temperature. After stirring overnight, the reaction was quenched with methanol and the solvent was evaporated. The residue was cooled to 0 °C and treated with saturated NH_3 in MeOH (20 ml) for 60 min. Following evaporation of the solvent, the remaining mixture was dissolved in CH_2Cl_2 (20 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 10 ml). The organic layer was dried over anhydrous Na_2SO_4, evaporated and purified by column chromatography to obtain **19a** (CH_2Cl_2/MeOH 97:3). Yield 0.89 g (85%); R_F 0.81 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$): 3.25–3.33 (dd, 1 H, H-1′A, $J_{1'A,2'}$ = 6.0, J_{gem} = 10.0), 3.41–3.48 (dd, 1 H, H-1′B, $J_{1'B,2'}$ = 4.0, J_{gem} = 10.0), 3.79 (s, 3 H, O-CH_3), 3.96–4.03 (m, 1 H, H-2′), 4.12–4.23 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 8.2, J_{gem} = 12.2), 4.28–4.37 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 4.2, J_{gem} = 12.2), 6.83–6.87 (d, 2 H, H-Ar), 7.20–7.64 (m, 15 H, H-Ar), 7.97–8.05 (m, 3 H, H-Ar), 7.98 (s, 1 H, H-2), 8.75 (s, 1 H, H-8), 9.19 (br, 1 H, NH). ^{13}C NMR ($CDCl_3$): 44.5 (C-1′), 55.1 (O-CH_3), 60.7 (C-2′), 63.7 (C-3′), 87.3 (C-MTr), 122.8 (C-5), 113.3, 127.2, 127.9, 128.2, 128.8, 130.3, 132.8, 133.7, 134.7, 143.5 (C-Ar), 143.7 (C-8), 149.6 (C-4), 152.7 (C-2), 155.9 (C-6), 158.8 (C-Ar), 164.7 (C=O). ES-MS calculated for $C_{35}H_{36}N_8O_3$ $[M + H]^+$: 661.2910; found: 661.2520.

(±)-9-{2-Azido-3-[(4-methoxytrityl)oxy]propyl}-6-chloro-9H-purin-2-amine (**19b**): Following the Mitsunobu reaction, **18b** was obtained [yield 1.39 g (45%); R_F 0.67 (CH_2Cl_2/MeOH 94:6); FAB-MS $[M + H]^+$: 801.2]. To a solution of compound **18b** (0.90 mg, 1.25 mmol) in THF (20 ml), water (5 ml) was added and the mixture was refluxed for two days. The solvent was removed under vacuum and the residue **19b** was purified by column chromatography (CH_2Cl_2/MeOH 95:5). Yield 0.56 g (82%); R_F 0.65 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$): 3.20–3.28 (dd, 1 H, H-1′A, $J_{1'A,2'}$ = 6.0, J_{gem} = 10.2), 3.32–3.40 (dd, 1 H, H-1′B, $J_{1'B,2'}$ = 4.0, J_{gem} = 10.2), 3.78 (s, 3 H, O-CH_3), 3.88–3.97 (m, 1 H, H-2′), 4.04–4.09 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 4.0, J_{gem} = 12.0), 4.28–4.37 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 5.2, J_{gem} = 12.0), 5.37 (s, 2 H, NH_2), 6.81–6.88 (d, 2 H, H-Ar), 7.19–7.35 (m, 8 H, H-Ar), 7.43–7.48 (d, 2 H, H-Ar), 7.69 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 44.0 (C-1′), 55.2 (O-CH_3), 60.4 (C-2′), 63.5 (C-3′), 87.2 (C-MTr), 124.9 (C-5), 113.2, 127.2, 127.9, 128.2, 130.2, 134.6 (C-Ar), 142.6 (C-8), 143.7 (C-Ar), 151.3 (C-2), 153.7 (C-4), 158.8 (C-Ar), 159.1 (C-6). ES-MS calculated for $C_{28}H_{26}ClN_8O_2P$ $[M + H]^+$: 541.1862; found: 541.1868.

(±)-1-{2-Azido-3-[(4-methoxytrityl)oxy]propyl}pyrimidine-2,4(1H,3H)-dione (**19c**): Yield 1.76 g (46%); R_F 0.40 (CH_2Cl_2/MeOH 99:1). ^{1}H NMR ($CDCl_3$): 3.15–3.23 (dd, 1 H, H-1′A, $J_{1'A,2'}$ = 5.68, J_{gem} = 10.7), 3.36–3.45 (dd, 1 H, H-1′B, $J_{1'B,2'}$ = 3.7, J_{gem} = 10.4), 3.41–3.52 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 8.24, J_{gem} = 13.1), 3.79 (s, 3 H, O-CH_3), 3.84–3.93 (m, 1 H, H-2′), 3.97–4.05 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 4.2, J_{gem} = 13.1), 5.58–5.62 (d, 1 H, H-5, $J_{5,6}$ = 7.6), 6.83–6.87 (d, 2 H, H-Ar), 7.07–7.11 (d, 1 H, H-6, $J_{5,6}$ = 7.6), 7.27–7.56 (m, 8 H, H-Ar), 7.80–7.85 (d, 4 H, H-Ar), 10.04 (br, 1 H, NH). ^{13}C NMR ($CDCl_3$): 49.0 (C-1′), 55.2 (O-CH_3), 60.1 (C-2′), 63.2 (C-3′), 87.1 (C-MTr), 102.0 (C-5), 113.2, 127.3, 128.0, 128.6, 130.3, 134.7, 143.7 (C-Ar), 145.2 (C-6), 151.0 (C-4), 158.8 (C-Ar), 164.1 (C-2). ES-MS calculated for $C_{27}H_{24}N_5O_4$ $[M - H]^-$: 482.1828; found: 482.1831.

Reduction to Compounds **20a**–**20c**. General Procedure

Triphenylphosphine (0.39 g, 1.5 mmol) was added to a solution of **19a** (0.61 g, 1.0 mmol), **19b** (0.53 g, 1.0 mmol) and **19c** (0.49 g, 1.0 mmol), respectively, in dry THF (10 ml). The reaction was stirred overnight. Then water (10 ml) was added and the reaction was refluxed for another 3 h. The reaction mixture was concentrated to a small volume and purified by column chromatography.

(±)-N-(9-{2-Amino-3-[(4-methoxytrityl)oxy]propyl}-9H-purin-6-yl)benzamide (**20a**): Yield 0.55 g (94%); R_F 0.31 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$): 3.15–3.17 (m, 2 H, H-1′), 3.31–3.40 (m, 1 H, H-2′), 3.79 (s, 3 H, O-CH_3), 4.16-4.26 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 7.6, J_{gem} = 14.0), 4.18–4.28 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 4.4, J_{gem} = 14.0), 6.82–6.86 (d, 2 H, H-Ar), 7.27–7.61 (m, 15 H, H-Ar), 7.97 (s, 1 H, H-2), 8.00–8.06 (d, 2 H, H-Ar), 8.77 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 47.9 (C-1′), 51.3 (C-2′), 55.2 (O-CH_3), 65.7 (C-3′), 86.6 (C-MTr), 113.3, 127.1, 128.0, 128.2, 128.9, 130.3, 132.7, 133.8, 135.1 (C-Ar), 143.9 (C-8), 144.1 (C-Ar), 149.4 (C-4), 152.5 (C-2), 158.8 (C-Ar), 164.7 (C=O). ES-MS calculated for $C_{35}H_{33}N_6O_3$ $[M + H]^+$: 585.2614; found: 585.2601.

(±)-9-{2-Amino-3-[(4-methoxytrityl)oxy]propyl}-6-chloro-9H-purin-2-amine (**20b**): Yield 0.47 g (91%); R_F 0.73 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$): 3.08–3.11 (m, 2 H, H-1′), 3.31–3.40 (m, 1 H, H-2′), 3.79 (s, 3 H, O-CH_3), 3.98–4.08 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 7.0, J_{gem} = 14.0), 4.18–4.28 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 5.2, J_{gem} = 14.0), 5.14 (s, 2 H, NH_2), 6.80–6.85 (d, 2 H, H-Ar), 7.19–7.34 (m, 15 H, H-Ar), 7.40–7.45 (m, 4 H, H-Ar), 7.70 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 47.6 (C-1′), 51.1 (C-2′), 55.2 (O-CH_3), 65.6 (C-3′), 86.6 (C-MTr), 125.2 (C-5), 113.3,

127.1, 127.9, 128.3, 130.3, 135.1 (C-Ar), 143.2 (C-8), 144.3 (C-Ar), 151.3 (C-2), 154.2 (C-4), 158.8 (C-Ar), 159.0 (C-6). ES-MS calculated for $C_{28}H_{28}ClN_6O_2$ $[M + H]^+$: 515.1962; found: 515.1958.

(±)-1-{2-Amino-3-[(4-methoxytrityl)oxy]propyl}pyrimidine-2,4(1H,3H)-dione (**20c**): Yield 0.41 g (88%); R_F 0.35 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$): 3.10–3.30 (m, 3 H, H-1′, H-2′), 3.60–3.67 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 6.96, J_{gem} = 13.6), 3.79 (s, 3 H, O-CH_3), 3.84–3.94 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 5.40, J_{gem} = 13.6), 5.43–5.48 (d, 1 H, H-5, $J_{5,6}$ = 8.0), 6.81–6.86 (d, 2 H, H-Ar), 6.99–7.03 (d, 1 H, H-6, $J_{5,6}$ = 8.0), 7.15–7.51 (m, 12 H, H-Ar). ^{13}C NMR ($CDCl_3$): 5.06 (C-1′), 52.0 (C-2′), 55.2 (O-CH_3), 64.9 (C-3′), 86.4 (C-MTr), 101.5 (C-5), 113.2, 127.2, 128.0, 128.3, 130.3, 135.1, 143.7 (C-Ar), 145.5 (C-6), 151.3 (C-4), 158.8 (C-Ar), 163.9 (C-2). ES-MS calculated for $C_{27}H_{27}N_3NaO_4$ $[M + Na]^+$: 480.1899; found: 480.1917.

Synthesis of Aminopropyl Phosphonate Nucleosides **2a**, **2b**

Compound **20a** (0.53 g, 0.91 mmol) or **20b** (0.18 g, 0.35 mmol) was dissolved in THF (10 ml) and cooled to 0 °C in an ice bath. Then Et_3N (2 ml) was added dropwise followed by the addition of $CF_3SO_3CH_2P(O)(O\textit{i}\text{-Pr})_2$ (for **a** 0.45 g, 1.36 mmol, for **b** 0.22 g, 0.64 mmol). Stirring was continued at 0 °C for 5 h and MeOH was added. The mixture was concentrated, dissolved in CH_2Cl_2 (15 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. In the synthesis of **2a**, the residue was treated with saturated NH_3 in MeOH (10 ml) at room temperature overnight and the reaction was concentrated and co-evaporated with MeCN (3 × 5 ml), whereas in that of **2b**, the mixture was treated with 75% TFA in water (10 ml) for two days followed by concentration and co-evaporation with toluene (3 × 10 ml). In both cases, the obtained residue was dissolved in DMF (10 ml) and Me_3SiBr (for **a** 0.29 g, 1.89 mmol, for **b** 0.16 g, 1.05 mmol) was added. The reaction was stirred for 3 days, concentrated and purified by column chromatography (CH_2Cl_2/MeOH 9:1, CH_2Cl_2/MeOH/H_2O 5:4:1). Compounds **2a**, **2b** were analysed as disodium salts.

(±)-({[2-(6-Amino-9H-purin-9-yl)-1-(hydroxymethyl)ethyl]amino}methyl)phosphonic acid (**2a**): Yield 0.12 g (45%); R_F 0.50 (MeOH + 1% EtN_3). ^{1}H NMR (D_2O): 2.98–3.05 (d, 2 H, CH_2-P, $J_{H,P}$ = 12.6), 3.53–3.86 (m, 3 H, H-1′, H-2′), 4.46–4.49 (m, 2 H, H-3′), 8.01 (s, 2 H, H-2), 8.03 (s, 1 H, H-8). ^{13}C NMR (D_2O): 44.1, 46.7 (CH_2-P, $J_{C,P}$ = 131.2), 51.0 (C-1′), 59.0 (C-3′), 61.0–61.1 (d, C-2′, $J_{C\text{-}2',P}$ = 6.1), 120.1 (C-5), 144.5 (C-8), 151.0 (C-4), 154.7 (C-2), 157.4 (C-6). ES-MS calculated for $C_9H_{14}N_6O_4P$ $[M - H]^-$: 301.0814; found: 301.0807.

(±)-({[2-(2-Amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-1-(hydroxymethyl)ethyl]amino}methyl)-phosphonic acid (**2b**): Yield 46 mg (42%); R_F 0.25 (MeOH). ^{1}H NMR (D_2O): 2.59–2.65 (d, 2 H, CH_2-P, $J_{H,P}$ = 12.0), 3.11 (m, 1 H, H-2′), 3.38–3.60 (m, 2 H, H-1′), 4.09 (m, 2 H, H-3′), 7.70 (s, 1 H, H-8). ^{13}C NMR (D_2O): 46.1 (C-1′), 47.4, 48.5 (CH_2-P, $J_{C,P}$ = 131.2), 51.6 (C-3′), 62.0–62.4 (d, C-2′, $J_{C\text{-}2',P}$ = 6.1), 119.7 (C-5), 142.2 (C-8), 154.6 (C-4), 161.5 (C-2), 167.2 (C-6). ES-MS calculated for $C_9H_{14}N_6O_5P$ $[M - H]^-$: 317.0763; found: 317.0764.

(±) Diisopropyl {[(2-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-1-{[(4-methoxytrityl)oxy]methyl}ethyl)amino]methyl}phosphonate (**21c**)

Compound **20c** (1.00 g, 2.07 mmol) was dissolved in THF (20 ml) and cooled to 0 °C. EtN_3 (3 ml) was added dropwise followed by the addition of $CF_3SO_3CH_2P(O)(O\textit{i}\text{-Pr})_2$ (1.02 g, 3.10 mmol). Stirring was continued at 0 °C for 5 h. MeOH was added, the mixture was con-

centrated, dissolved in CH_2Cl_2 (25 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 15 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5). Yield 1.12 g (85%); R_F 0.35 (CH_2Cl_2/MeOH 96:4). ^{1}H NMR ($CDCl_3$): 1.33, 1.36 (d, 12 H, 4 × CH_3), 2.71–3.31 (m, 5 H, H-1′, H-3′, H-2′), 3.79 (s, 3 H, O-CH_3), 3.85–3.98 (m, 2 H, NH-CH_2-P), 4.65–4.84 (m, 2 H, O-C**H**-$(CH_3)_2$), 5.43–5.47 (d, 1 H, H-5, $J_{5,6}$ = 7.6), 6.82–6.87 (d, 2 H, H-MTr), 7.12–1.16 (d, 1 H, H-6, $J_{5,6}$ = 7.6), 7.19–7.47 (m, 12 H, H-Ar), 9.19 (br, 1 H, NH). ^{13}C NMR ($CDCl_3$): 23.9 (CH_3), 42.1, 45.2 (NH-CH_2-P), 49.9 (C-1′), 55.1 (O-CH_3), 57.7–58.0 (d, C-2′, $J_{C\text{-}3',P}$ = 16.7), 60.3 (C-3′), 68.9–69.1 (d, 2 × O-CH-$(CH_3)_2$, $J_{C,P}$ = 6.1), 86.5 (C-MTr), 101.1 (C-5), 113.2, 127.1, 127.9, 128.2, 130.2, 134.9, 143.9, 145.9 (C-Ar), 150.1 (C-6), 158.8 (C-2), 163.9 (C-4). ES-MS calculated for $C_{34}H_{42}N_3NaO_7P$ [M + Na]$^+$: 658.2658; found: 658.2651.

(±)-({[2-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-1-(hydroxymethyl)ethyl]amino}methyl)phosphonic Acid (**2c**)

Me_3SiBr (0.22 g, 1.42 mmol) was added to a solution of **21c** (0.30 g, 0.47 mmol) in MeCN (10 ml). Stirring was continued overnight and the mixture was concentrated and purified by column chromatography (CH_2Cl_2/MeOH 9:1, CH_2Cl_2/MeOH/H_2O 5:4:1). **2c** was analysed as disodium salt. Yield 88 mg (68%); R_F 0.42 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 3.03–3.08 (d, 2 H, CH_2-P, $J_{H,P}$ = 10.2), 3.58–3.74 (m, 2 H, H-1′), 3.90 (m, 1 H, H-2′), 4.12 (m, 2 H, H-3′), 5.78–5.82 (d, 1 H, H-5, $J_{5,6}$ = 7.8), 7.59–7.63 (d, 1 H, H-6, $J_{5,6}$ = 7.8). ^{13}C NMR (D_2O): 43.9, 46.5 (CH_2-P, $J_{C,P}$ = 128.2), 49.0 (C-1′), 58.8 (C-3′), 61.9 (C-2′), 104.4 (C-5), 149.4 (C-6), 155.0 (C-2), 168.9 (C-4). ES-MS calculated for $C_8H_{13}N_3O_6P$ [M – H]$^-$: 278.0542; found: 278.0552.

(±)-({[2-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-1-(hydroxymethyl)ethyl]amino}methyl)phosphonic Acid (**2d**)

Compound **21c** (0.50 g, 0.79 mmol) was co-evaporated with dry pyridine (2 × 10 ml) and dissolved in dry pyridine (10 ml). Mesitylenesulfonyl chloride (0.52 g, 2.36 mmol) was added. Stirring was continued for 4 h, after which 26% NH_4OH in water (5 ml) was added and the mixture was stirred overnight. The reaction mixture was concentrated, dissolved in CH_2Cl_2 (15 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was co-evaporated with toluene (2 × 10 ml) and dissolved in MeCN (10 ml). Me_3SiBr (0.36 g, 2.36 mmol) was added and stirring was continued overnight. The mixture was concentrated and further purified by column chromatography (CH_2Cl_2/MeOH 9:1, CH_2Cl_2/MeOH/H_2O 5:4:1). **2d** was analysed as disodium salt. Yield 87 mg (40%); R_F 0.32 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 2.96 (d, 2 H, CH_2-P), 3.61–3.84 (m, 3 H, H-1′, H-2′), 4.05 (m, 2 H, H-3′), 5.95–5.99 (d, 1 H, H-5, $J_{5,6}$ = 7.1), 7.55–7.58 (d, 1 H, H-6, $J_{5,6}$ = 7.1). ^{13}C NMR (D_2O): 44.7, 47.3 (CH_2-P, $J_{C,P}$ = 131.2), 50.8 (C-1′), 59.6 (C-3′), 61.3 (C-2′), 98.3 (C-5), 149.4 (C-6), 161.1 (C-2), 168.9 (C-4). ES-MS calculated for $C_8H_{14}N_4O_5P$ [M – H]$^-$: 277.0702; found: 277.0705.

(±)-9-(3-Azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-6-chloro-9*H*-purin-2-amine (**29b**)

A mixture of compound **27** (0.50 g, 1.62 mmol), 2-amino-6-chloropurine (0.36 g, 2.11 mmol) and cesium carbonate (0.79 g, 2.43 mmol) in dry DMF (20 ml) was gently heated to 95 °C under nitrogen atmosphere for 5 h, after which the reaction mixture was cooled to room

temperature and filtered. The solvent of the filtrate was removed under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/MeOH 99:1). Yield 0.23 g (38%); R_F 0.67 (CH_2Cl_2/MeOH 97:3). ^{1}H NMR ($CDCl_3$): –0.23, –0.01 (s, 6 H, 2 × Si-CH_3), 0.84 (s, 9 H, 3 × CH_3), 3.15–3.47 (m, 2 H, H-3′), 4.10–4.15 (m, 3 H, H-1′,2′), 5.43 (s, 2 H, NH_2), 7.75 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): –5.6, –5.1 (2 × Si-CH_3), 17.6 (Si-C), 25.5 (3 × C-CH_3), 46.9 (C-1′), 53.9 (C-3′), 69.2 (C-2′), 124.9 (C-5), 143.7 (C-8), 151.3 (C-2), 154.0 (C-4), 159.3 (C-6). ES-MS calculated for $C_{14}H_{24}ClN_8OSi$ $[M + H]^+$: 383.1530; found: 383.1536.

(±)-1-(3-Azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)pyrimidine-2,4(1*H*,3*H*)-dione (**29c**)

A mixture of **27** (0.80 g, 2.58 mmol), uracil (0.35 g, 3.11 mmol), NaI (0.47 g, 3.11 mmol) and potassium carbonate (0.79 g, 2.43 mmol) in dry DMF (20 ml) was gently heated to 90 °C under nitrogen atmosphere overnight and then allowed to cool to room temperature. Following filtration, the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (20 ml) and washed with saturated $NaHCO_3$ (2 × 10 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated and further purified by column chromatography (CH_2Cl_2/MeOH 98:2). Yield 0.36 g (43%); R_F 0.49 (CH_2Cl_2/MeOH 97:3). ^{1}H NMR ($CDCl_3$): –0.12, 0.10 (2 × s, 6 H, 2 × Si-CH_3), 0.90 (s, 9 H, 3 × CH_3), 3.15–3.22 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 3.6, J_{gem} = 13.2), 3.40–3.48 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 4.0, J_{gem} = 13.2), 3.53–3.64 (dd, 1 H, H-1′A, $J_{1'A,2'}$ = 8.0, J_{gem} = 13.6), 3.91–4.00 (dd, 1 H, H-1′B, $J_{1'B,2'}$ = 3.8, J_{gem} = 13.6), 4.12–4.22 (m, 1 H, H-2′), 5.67–5.71 (d, 1 H, H-5, $J_{5,6}$ = 8.0), 7.18–7.22 (d, 1 H, H-6, $J_{5,6}$ = 8.0). ^{13}C NMR ($CDCl_3$): –5.1, –4.9 (2 × Si-CH_3), 17.7 (Si-C), 25.6 (3 × C-CH_3), 52.6 (C-1′), 54.2 (C-3′), 68.8 (C-2′), 101.6 (C-5), 146.3 (C-6), 151.0 (C-2), 164.0 (C-4). ES-MS calculated for $C_{13}H_{24}N_5O_3Si$ $[M + H]^+$: 326.1648; found: 326.1645.

(±)-*N*-[1-(3-Azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-2-oxo-1,2-dihydropyrimidin-4-yl]benzamide (**29d**)

A mixture of **27** (0.50 mg, 1.62 mmol), cytosine (0.23 mg, 2.10 mmol) and cesium carbonate (1.06 g, 3.24 mmol) in dry DMF (20 ml) was gently heated to 90 °C under nitrogen atmosphere overnight, then allowed to cool to room temperature and filtered. The solvent was removed under reduced pressure. The residue **28d** was purified by column chromatography (CH_2Cl_2/MeOH 95:5, CH_2Cl_2/MeOH 9:1) [yield 0.24 g (45%); R_F 0.52 (CH_2Cl_2/MeOH 9:1); ES-MS $[M + H]^+$: 325.1814]. To a solution of compound **28d** (0.22 g, 0.68 mmol) in pyridine (5 ml) benzoyl chloride (0.24 ml, 2.04 mmol) was added. After stirring for 3 h, the reaction was quenched with water (1 ml) and the reaction mixture was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (10 ml) and washed with water (3 × 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH 98:2). Yield 0.25 g (85%); R_F 0.42 (CH_2Cl_2/MeOH 98:2). ^{1}H NMR ($CDCl_3$): –0.13, 0.07 (2 × s, 6 H, 2 × Si-CH_3), 0.89 (s, 9 H, 3 × CH_3), 3.15–3.23 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 3.2, J_{gem} = 13.2), 3.46–3.55 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 3.8, J_{gem} = 13.2), 3.64–3.74 (dd, 1 H, H-1′A, $J_{1'A,2'}$ = 8.2, J_{gem} = 13.0), 4.17–4.25 (dd, 1 H, H-1′B, $J_{1'B,2'}$ = 3.0, J_{gem} = 13.2), 4.31–4.38 (m, 1 H, H-2′), 7.40–7.68 (m, 4 H, H-Ar, H-5), 7.80–7.84 (d, 1 H, H-6, $J_{5,6}$ = 7.2), 7.94–7.97 (d, 1 H, H-Ar). ^{13}C NMR ($CDCl_3$): –5.5, –5.0 (2 × Si-CH_3), 17.7 (Si-C), 25.7 (3 × C-CH_3), 54.5 (C-1′), 54.7 (C-3′), 68.2 (C-2′), 96.3 (C-5), 127.8, 129.0, 131.9, 133.2 (C-Ar), 150.7 (C-6), 156.0 (C-2), 162.8 (C-4). ES-MS calculated for $C_{20}H_{29}N_6O_3Si$ $[M + H]^+$: 429.2070; found: 429.2075.

Reduction to Compounds **30a**–**30d**. General Procedure

To a solution of compound **29a** (0.50 g, 1.11 mmol), **29b** (0.22 g, 0.58 mmol), **29c** (0.35 g, 1.07 mmol) and **29d** (0.20 g, 0.47 mmol), respectively, in dry THF (15 ml) triphenylphosphine (0.58 g, 2.22 mmol) (for **b** and **d** 0.29 g, 1.11 mmol) was added and the mixture was stirred overnight. Then water was added and the reaction was stirred for another 10 h. The reaction mixture was concentrated to a small volume and purified by column chromatography (CH_2Cl_2/MeOH 9:1).

(±)-N-[9-(3-Amino-2-{[tert-butyl(dimethyl)silyl]oxy}propyl)-9H-purin-6-yl]benzamide (**30a**): Yield 0.47 g (80%); R_F 0.25 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (DMSO-d_6): –0.43, –0.10 (2 × s, 6 H, 2 × Si-CH_3), 0.73 (s, 9 H, 3 × CH_3), 2.55–2.69 (m, 2 H, H-3′), 3.36 (s, br, NH_2), 4.05 (m, 1 H, H-2′), 4.26 (m, 2 H, H-1′), 7.51–7.68 (m, 3 H, 3,4,5 H-Bz), 8.03–8.07 (d, 2 H, 2,6 H-Bz, J = 7.0), 8.36 (s, 1 H, H-8), 8.37 (s, 1 H, H-2). ^{13}C NMR (DMSO-d_6): –5.2, –4.4 (2 × Si-CH_3), 18.0 (Si-C), 26.1 (3 × CH_3), 45.6 (C-3′), 48.5 (C-1′), 72.2 (C-2′), 125.2 (C-5), 129.1 (3,5CH-Bz), 129.4 (2,6CH-Bz), 133.4 (4CH-Bz), 134.1 (1C-Bz), 146.3 (C-8), 150.5 (C-4), 152.2 (C-2), 153.4 (C-6), 166.8 (C=O). ES-MS calculated for $C_{21}H_{29}N_8O_2Si$ $[M + H]^+$: 427.2278; found: 427.2279.

(±)-9-(3-Amino-2-{[tert-butyl(dimethyl)silyl]oxy}propyl)-6-chloro-9H-purin-2-amine (**30b**): Yield 0.19 g (90%); R_F 0.18 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$): –0.16, –0.00 (2 × s, 6 H, 2 × Si-CH_3), 0.86 (s, 9 H, 3 × CH_3), 1.81 (s, 2 H, NH_2), 2.64–2.68 (m, 2 H, H-3′), 4.02–4.26 (m, 3 H, H-2′, H-1′), 5.34 (s, 2 H, NH_2), 7.80 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): –5.3, –4.9 (2 × Si-CH_3), 17.8 (Si-C), 25.6 (3 × CH_3), 44.6 (C-3′), 46.6 (C-1′), 71.3 (C-2′), 143.8 (C-8), 151.4 (C-2), 154.1 (C-4), 159.2 (C-6). ES-MS calculated for $C_{14}H_{26}ClN_6OPSi$ $[M + H]^+$: 357.16262; found: 357.1629.

(±)-1-(3-Amino-2-{[tert-butyl(dimethyl)silyl]oxy}propyl)pyrimidine-2,4(1H,3H)-dione (**30c**): Yield 0.29 g (91%); R_F 0.18 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$): –0.16, –0.00 (2 × s, 6 H, 2 × Si-CH_3), 0.86 (s, 9 H, 3 × CH_3), 2.71–2.80 (dd, 1 H, H-3′A, $J_{3'A,2'}$ = 3.8, J_{gem} = 13.0), 2.84–2.93 (dd, 1 H, H-3′B, $J_{3'B,2'}$ = 5.2, J_{gem} = 13.0), 3.75–4.14 (m, 2 H, H-1′), 4.32 (m, 1 H, H-2′), 5.68–5.72 (d, 1 H, H-5, $J_{5,6}$ = 8.0), 7.27–7.30 (d, 1 H, H-5, $J_{5,6}$ = 8.0). ^{13}C NMR ($CDCl_3$): –5.2, –4.9 (2 × Si-CH_3), 17.7 (Si-C), 25.7 (3 × CH_3), 44.7 (C-3′), 51.9 (C-1′), 70.6 (C-2′), 101.2 (C-5), 146.5 (C-6), 151.3 (C-2), 164.1 (C-4). ES-MS calculated for $C_{13}H_{26}N_3O_3Si$ $[M + H]^+$: 300.1743; found: 300.1748.

(±)-N-[1-(3-Amino-2-{[tert-butyl(dimethyl)silyl]oxy}propyl)-2-oxo-1,2-dihydropyrimidin-4-yl]-benzamide (**30d**): Yield 0.15 g (81%); R_F 0.49 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR ($CDCl_3$): 0.04, 0.10 (2 × s, 6 H, 2 × Si-CH_3), 0.90 (s, 9 H, 3 × CH_3), 2.71–2.80 (m, 2 H, H-3′), 3.85–3.94 (m, 2 H, H-1′), 4.13–4.20 (m, 1 H, H-2′), 7.47–7.61 (m, 4 H, H-Ar, H-5), 7.61–7.71 (d, 1 H, H-6, $J_{5,6}$ = 7.0), 7.93–7.96 (d, 1 H, H-Ar). ^{13}C NMR ($CDCl_3$): –5.3, –4.9 (2 × Si-CH_3), 17.8 (Si-C), 25.6 (3 × C-CH_3), 44.8 (C-3′), 54.0 (C-1′), 70.1 (C-2′), 96.1 (C-5), 127.7, 129.0, 131.9, 133.1 (C-Ar), 150.9 (C-6), 156.2 (C-2), 162.6 (C-4). ES-MS calculated for $C_{20}H_{31}N_4O_3Si$ $[M + H]^+$: 403.2165; found: 403.2138.

Synthesis of Aminopropyl Phosphonate Nucleosides **31**. General Procedure

To a solution of **30a** (0.35 g, 0.82 mmol) or **30b** (0.18 g, 0.51 mmol) or **30c** (0.29 g, 0.95 mmol) or **30d** (0.15 mg, 0.35 mmol), Et_3N (10 µl) under cooling and $CF_3SO_3CH_2P(O)(O$*i*$-Pr)_2$ (for **a** 0.40 g, 1.23 mmol, for **b** 0.25 g, 0.76 mmol, for **c** 0.47 g, 1.42 mmol, for **d** 0.22 mg, 0.67 mmol) in THF (10 ml) were added. Stirring was continued for 30 min and the intermediates **31a**–**31d** were obtained.

In the case of **31a** and **31d**, the solvent was removed under high vacuum, the residue was dissolved in CH_2Cl_2 (10 ml) and washed with H_2O (2 × 10 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated and the residue was treated with saturated NH_3 in MeOH (10 ml) overnight. The solvent was removed to obtain the residue to be treated with tetrabutylammonium fluoride (TBAF, vide infra).

In the case of **31b**, the residue was treated with 75% TFA in aqueous solution (10 ml) for two days. Then saturated aqueous $NaHCO_3$ (15 ml) was added to quench the reaction. The mixture was extracted with CH_2Cl_2 (2 × 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain the residue of **32b** to be treated with TBAF (vide infra).

In the case of **31c**, the residue was dissolved in CH_2Cl_2 (10 ml) and washed with H_2O (2 × 20 ml). The organic layer was dried and concentrated to be treated further with TBAF.

General Deprotection Procedure

The respective residues **31** were co-evaporated with THF (2 × 10 ml) and then treated with 1 M TBAF in THF (10 ml) overnight to obtain **32a**–**32d**. The solvent was removed and the residue was co-evaporated with toluene (2 × 10 ml) and dissolved in MeCN (10 ml). Me_3SiBr (for **a**, **d** 0.38 g, 2.46 mmol, for **b** 0.23 g, 1.53 mmol, for **c** 0.44 g, 2.86 mmol) was added and the reaction mixture was stirred at room temperature for two days. The solvent was evaporated and the residue was purified by column chromatography (CH_2Cl_2/MeOH 9:1, CH_2Cl_2/MeOH/H_2O 5:4:1). All compounds **3a**–**3d** were analysed as disodium salts.

(±)-({[3-(6-Amino-9H-purin-9-yl)-2-hydroxypropyl]amino}methyl)phosphonic acid (**3a**): Yield 70 mg (24%); R_F 0.50 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 2.92–2.98 (d, 2 H, CH_2-P, $J_{H,P}$ = 11.6), 3.13–3.37 (m, 2 H, H-1′), 4.10–4.39 (m, 3 H, H-3′, H-2′), 8.08 (2 × s, 2 H, H-2, H-8). ^{13}C NMR (D_2O): 48.1, 49.1 (CH_2-P), 51.6 (C-1′), 54.1 (C-3′), 68.1 (C-2′), 121.0 (C-5), 145.5 (C-8), 151.7 (C-4), 155.2 (C-2), 158.1 (C-6). ES-MS calculated for $C_9H_{14}N_6O_4P$ [M – H]$^-$: 301.0814; found: 301.0813.

(±)-({[3-(2-Amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-2-hydroxypropyl]amino}methyl)phosphonic acid (**3b**): Yield 38 mg (23%); R_F 0.30 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 2.96–3.04 (d, 2 H, CH_2-P, $J_{H,P}$ = 11.6), 3.18 (m, 2 H, H-1′), 4.18 (m, 2 H, H-3′), 4.34 (m, 1 H, H-2′), 7.71 (s, 1 H, H-8). ^{13}C NMR (D_2O): 47.0, 49.6 (CH_2-P, $J_{C,P}$ = 128.2), 47.9 (C-1′), 52.7–52.9 (d, C-3′, $J_{C\text{-}3',P}$ = 6.1), 67.3 (C-2′), 117.4 (C-5), 142.5 (C-8), 153.9 (C-4), 155.3 (C-2). ES-MS calculated for $C_9H_{14}N_6O_5P$ [M – H]$^-$: 317.0763; found: 317.0667.

(±)-({[3-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-hydroxypropyl]amino}methyl)phosphonic acid (**3c**): Yield 115 mg (41%); R_F 0.46 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 2.91–2.99 (d, 2 H, CH_2-P, $J_{H,P}$ = 11.6), 3.07–3.37 (m, 2 H, H-1′), 3.65–3.76 (dd, 1 H, H-3′A, $J_{3A',2'}$ = 8.4, J_{gem} = 14.4), 3.98–4.06 (dd, 1 H, H-3′B, $J_{3B',2'}$ = 3.2, J_{gem} = 14.4), 4.26 (m, 1 H, H-2′), 5.77–5.81 (d, 1 H, H-5, $J_{5,6}$ = 7.6), 7.56–7.60 (d, 1 H, H-6, $J_{5,6}$ = 7.6). ^{13}C NMR (D_2O): 46.6, 49.1 (CH_2-P, $J_{C,P}$ = 126.7), 53.3–53.4 (d, C-3′, $J_{C\text{-}3',P}$ = 4.6), 53.8 (C-1′), 67.0 (C-2′), 103.6 (C-5), 150.4 (C-6), 154.8 (C-2), 169.4 (C-4). ES-MS calculated for $C_8H_{13}N_3O_6P$ [M – H]$^-$: 278.0542; found: 278.0547.

(±)-({[3-(4-Amino-2-oxopyrimidin-1(2H)-yl)-2-hydroxypropyl]amino}methyl)phosphonic acid (**3d**): Yield 23 mg (24%); R_F 0.33 (CH_2Cl_2/MeOH/H_2O 5:4:1). ^{1}H NMR (D_2O): 2.91–2.97 (d, 2 H, CH_2-P, $J_{H,P}$ = 11.6), 3.04–3.27 (m, 2 H, H-1′), 3.57–3.68 (dd, 1 H, H-3′A, $J_{3A',2'}$ = 9.3, J_{gem} = 14.0), 3.98–4.06 (dd, 1 H, H-3′B, $J_{3B',2'}$ = 3.0, J_{gem} = 14.0), 4.23 (m, 1 H, H-2′), 5.93–5.96 (d, 1 H, H-5, $J_{5,6}$ = 7.3), 7.50–7.54 (d, 1 H, H-6, $J_{5,6}$ = 7.3). ^{13}C NMR (D_2O): 46.8, 49.3 (CH_2-P, $J_{C,P}$ = 128.2), 53.5–53.6 (d, C-3′, $J_{C\text{-}3',P}$ = 6.1), 55.1 (C-1′), 67.3 (C-2′), 97.7 (C-5),

149.9 (C-6), 160.7 (C-2), 168.9 (C-4). ES-MS calculated for $C_8H_{14}N_4O_5$ [M – H]$^-$: 277.0702; found: 277.0702.

Preparation of Disodium Salts of Aminopropyl Phosphonate Nucleosides **1**–**3**. General Procedure

All phosphonates **1a**–**1d**, **2a**–**2d**, **3a**–**3d** were purified by ion exchange chromatography (Sephadex-DEAE A-25 resin). A gradient of triethylammonium bicarbonate (TEAB)/water (0.01 M TEAB to 0.5 M TEAB in 1 h, 0.5 M TEAB in 30 min) was used. The phosphonic acids were obtained as gums, they were dissolved in water, passed through a column of Dowex 50WX8 (Na^+) resin and eluted with water to afford the sodium salts as white solids.

HPLC Analysis of Disodium Salts of Aminopropyl Phosphonate Nucleosides **1**–**3**

The phosphonates **1a**, **1b**, **1c**, **2a**, **2d**, **3a**, **3b**, **3d** were analysed by reversed-phase analytic HPLC on an Alltima HP C_{18} HL 5μ column with 50 mM $NH_4H_2PO_4$ and 10 mM Bu_4NHSO_4 buffered at pH 5.0. The derivatives **1d**, **2b**, **2c**, **3c** proved still too polar for this column, but could be analysed on a platinum EPS C_{18} 100A 5μ column with a pH 6.0 buffer of 25 mM $NH_4H_2PO_4$ and 10 mM Bu_4NHSO_4. Both the columns were run at 0.65 cm^3/min.

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