CHEMISTRY &
BIODIVERSITY

Accepted Article

Title: Synthesis and anti-HIV activity of guanine modified fluorinated acyclic nucleoside phosphonates derivatives

Authors: Min Luo, Elisabetta Groaz, Steven De Jonghe, Dominique Schols, and Piet Herdewijn

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Chem. Biodiversity* 10.1002/cbdv.201800532

Link to VoR: <http://dx.doi.org/10.1002/cbdv.201800532>

Synthesis and anti-HIV activity of guanine modified fluorinated acyclic nucleoside phosphonates derivatives

Min Luo,^a Elisabetta Groaz,^a Steven De Jonghe,^b Dominique Schols,^b and Piet Herdewijn^{*a}

^aMedicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Herestraat 49, 3000 Leuven, Belgium, e-mail: piet.herdewijn@kuleuven.be

^bLaboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Herestraat 49 bus 1043, 3000 Leuven, Belgium

Abstract

The preparation of an unprecedented series of nucleobase modified 3-fluoro-2-(phosphonomethoxy)propyl (FPMP) acyclic nucleosides in both their (*R*) and (*S*) enantiomerically pure forms is described. The synthesis focuses on a Mitsunobu alkylation reaction to construct the C-N9 bond between a chiral fluorinated side-chain residue and 6- or 7-modified guanine analogues. Prodrugs of FPMP-7-deazaguanine were also synthesized by derivatization of the corresponding phosphonic acid functionality with (bis)diamyl aspartate amidate groups, leading to moderate activity against human immunodeficiency virus type 1 (HIV-1).

Keywords

acyclic nucleoside phosphonates, Mitsunobu reaction, prodrugs, HIV, antiviral activity

Introduction

Nucleoside analogues belonging to the family of acyclic nucleoside phosphonates (ANPs) have proven to be a particularly valuable source of potent antiviral agents with a broad spectrum antiviral activity, especially against DNA viruses and retroviruses.^[1] The chemical structure common to all ANPs includes a nucleobase attached to an aliphatic side chain enclosing a phosphonmethoxy (P-C-O) residue. The presence of such motif imparts a greater metabolic stability to the resulting molecules compared to the naturally occurring isosteric phosphoxymethyl functionality (P-O-C).^[2] Indeed, contrary to phosphates, the phosphonate moiety is not susceptible to phosphodiesterase and phosphatase hydrolysis, but can likewise undergo enzymatic phosphorylation.

3-Fluoro-2-(phosphonmethoxy)propyl (FPMP) derivatives are a subclass of ANPs characterized by the presence of a fluorine atom in the acyclic side chain,^[3] which exhibited moderate antiviral activity limited to HIV and HBV.^[4, 5] Up to now, limited efforts have been done on the synthesis and antiviral screening of FPMP derivatives with non-canonical nucleobases, with the exception of a FPMP analogue bearing a 2,6-diaminopurine (DAP) moiety (*Figure 1*).^[4]

Figure 1. Examples of purine modified acyclic nucleoside analogues relevant to this study.

Here, we describe an unprecedented combination of structural variations obtained by reacting under Mitsunobu conditions a chiral fluorinated acyclic pseudosugar with 6- and 7-modified guanine analogues. The target functionalized purine moieties were chosen based on the knowledge available in the literature from structure-activity studies. For instance, 6-arylthio substituted guanine containing acyclic nucleoside analogues have been described as potent antiviral agents against HBV (*Figure 1*).^[6] The presence of a 7-deazapurine nucleobase (systematic name: pyrrolo[2,3-d]pyrimidine) often confers antiviral activity to cyclic nucleosides,^[7-12] and a 7-deazaguanine acyclic nucleoside that inhibits HIV replication ($IC_{50} = 3 \mu M$) has been reported (*Figure 1*).^[13]

Thus, 2-amino-6-arylthiopurine and 7-deazaguanine were selected as prime nucleobase candidates for the synthesis and anti-HIV evaluation of base modified FPMPs. Additional 7-deazaguanine analogues bearing an electron-withdrawing substituent at the C7 position were also investigated in order to determine the effect of such modification on the antiviral activity of the resulting FPMP derivatives as well as the efficiency of the key Mitsunobu alkylation.

Results and discussion

Chemistry

The chiral pool strategy has been frequently applied in the synthesis of ANPs.^[14] Various types of reactions are possible (alkylation, epoxide ring opening, Mitsunobu condensation) between the heterocyclic base moiety and a chiral side-chain residue, which is preferably prefunctionalized with an esterified phosphonate functionality. Recently, we and others have

employed phosphonate esters of fluorohydrin precursors **2a/b** (Scheme 1) as convenient chiral starting materials in the synthesis of enantiomerically pure FPMPs bearing natural nucleobases.^[15, 16] Likewise, 6-chloropurine **1** was reacted under Mitsunobu conditions (Ph_3P , DIAD) with precursors **2a/b** to provide compounds **3a/b** along with the concomitant formation of the corresponding triphenylphosphine adducts. However, by refluxing the crude reaction mixtures for 24 h in water, the undesired adducts were successfully hydrolyzed leading to the isolation of compounds **3a/b** in excellent yields over two steps. The replacement of the chlorine atom at the 6-position of the nucleobase with an arylthio group was achieved by treatment of **3a/b** with 4-methoxythiophenol in the presence of triethylamine in DMF, affording **4a/b** in 87 and 67% yield, respectively. TMSBr-promoted hydrolysis of the phosphonate diester group furnished target 2-amino-6-methoxyphenylthio purine containing acyclic nucleoside phosphonates **5a/b** in good yields (Scheme 1).

Scheme 1. Synthesis of (*R*)/(*S*)-fluorinated acyclic phosphonate acids **5a/b**. Reagents and conditions: (a) Ph_3P , DIAD, THF, rt, 24 h; (b) THF/ H_2O , reflux, 24 h; (c) Et_3N , DMF, 100 °C, 4 h; (d) TMSBr, 2,6-lutidine, CH_3CN , rt, 12 h.

Subsequent efforts were directed towards the synthesis of FPMPs bearing 7-deazaguanine and its 7-substituted structural analogues. Commercially available 2-amino-6-chloro-7-deazapurine **6** was protected as a pivaloyl amide to afford compound **7**,^[17] which in turn served as substrate for the preparation of 4-chloro-5-fluoro-2-pivaloylamino-7H-pyrrolo[2,3-d]pyrimidine **8**, as illustrated in *Scheme 2*. Specifically, Selectfluor was preferred as fluorinating reagent based on a method described by Chittepu *et al.*^[18] Under the previously established condensation conditions (Ph₃P, DIAD), nucleobases **7** and **8** were coupled with **2a/b** to provide two enantiomeric sets consisting of nucleoside phosphonate diethyl esters **9a/b** and **12a/b**. The presence of fluorine at the 7-position led to lower yields of isolated products (25-33%) compared to those of their unsubstituted counterparts **9a/b** (78-79%). Next, compounds **10a/b** and **13a/b** were obtained from **9a/b** and **12a/b**, respectively, after the one-pot sequential displacement of the chlorine atom by a hydroxyl group in the presence of DABCO/K₂CO₃, followed by the removal of the pivaloyl group upon methanolic ammonia treatment. After standard removal of the phosphonate ester groups, the fluorinated acyclic phosphonate acids **11a/b** and **14a/b** were obtained in 50-70% yields.

Scheme 2. Synthesis of (*R*)/(*S*)-fluorinated acyclic phosphonate acids **11a/b** and **14a/b**.

Reagents and conditions: (a) PivCl, Pyr, rt, 3 h; (b) Selectfluor, MeCN, 50 °C, 30 min; (c) Ph₃P, DIAD, **2a/b**, THF, rt, 24 h; (d) (i) DABCO, K₂CO₃, dioxane/H₂O, 90 °C, 3 h; (ii) NH₃/MeOH, rt, 12 h; (e) TMSBr, 2,6-lutidine, CH₃CN, rt, 12 h.

The heterocyclic building block **19** was available in four steps from chloroacetonitrile **15** according to a literature procedure (*Scheme 3*).^[19] Formylation of **15** with methyl formate resulted in the formation of the unstable chloroaldehyde **16**, which was immediately used in the next step without further purification to afford the cyclocondensation product **17**. Then, the exocyclic amino group of compound **17** was protected using pivaloyl chloride, while the subsequent chlorination was accomplished with POCl₃ in the presence of a phase transfer catalyst to give the desired chloro-intermediate **19**. Starting from **2a/b**, the base condensation under Mitsunobu conditions occurred smoothly to yield nucleoside phosphonates **20a/20b**. Similar to the low yield observed for **13a/b**, the catalytic hydrolysis of **20a** to **21a** in the presence of DABCO/K₂CO₃ proceeded also in poor yield,^[20] however this step could be improved by switching to DABCO/NaOAc,^[21] which allowed to access the desired products **21a/b** in good yield (60% over 2 steps). Cleavage of the phosphonate esters in the presence of the TMSBr afforded **22a/b** in 70 and 75% yield, respectively.

Scheme 3. Synthesis of (*R*)/(*S*)-fluorinated acyclic phosphonate acids **22a/b**. Reagents and conditions: (a) methyl formate, NaH, THF, 0 °C, 5 h; (b) 2,6-diamino-6-hydroxypyrimidine, NaOAc, H₂O, 100 °C, 24 h; (c) PivCl, Pyr, 85 °C, 2 h; (d) POCl₃, DMA, BnEt₃NCl, MeCN, 90 °C, 1 h; (e) Ph₃P, DIAD, **2a/b**, THF, rt, 24 h; (f) (i) DABCO, K₂CO₃, dioxane/H₂O, 90 °C, 3 h or DABCO, NaOAc, DMF, rt, 48 h; (ii) NH₃/MeOH, rt, 12 h; (g) TMSBr, 2,6-lutidine, CH₃CN, rt, 12 h.

Recently, we disclosed that the prodrug derivatization of the phosphonic acid functionality of FPMPs with a diamyl aspartate phenoxyamidate group not only produced a dramatic improvement in antiretroviral potency but also led to compounds endowed with an unprecedented spectrum of activity against DNA viruses.^[15] Therefore, the amyl aspartate phosphonoamidate and phosphonobisamidate prodrugs **23** and **24** were prepared from the parent 7-deazaguanine derived FPMP nucleoside (*R*)-**11a** in moderate yields using a common procedure, which utilizes 2,2'-dithiodipyridine and triphenylphosphine as activating agents (*Scheme 4*). Compound **23** was isolated as a diastereomeric mixture (ratio: 1/1.4), which was biologically tested as such.

Scheme 4. Synthesis of (R)-fluorinated acyclic phosphonamidates **23** and **24**. Reagents and conditions: (a) L-aspartic acid amyl ester HCl salt, PhOH, Et₃N, 2,2'-dithiodipyridine, PPh₃, Pyr, 60 °C, 12 h; (b) L-aspartic acid amyl ester HCl salt, Et₃N, 2,2'-dithiodipyridine, PPh₃, Pyr, 60 °C, 12 h.

Anti-HIV activity

All base modified FPMPs **5a/b**, **11a/b**, **14a/b**, and **22a/b** as well as two prodrugs, compounds **23** and **24**, were evaluated for antiviral activity by luciferase assay in TZM-bl cells^[22] infected with the wild type HIV-1 strains NL4.3 (CXCR4-tropic HIV-1 strain) and BaL (CCR5-tropic HIV-1 strain). In parallel, their possible toxic effects on the same cell line were also assessed. Tenofovir (PMPA), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) were included as positive controls. Disappointingly, all synthesized base-modified FPMPs completely lacked anti-HIV activity, as indicated by their EC₅₀ values higher than 100 μM. However, the fluorinated acyclic aryloxyphosphonomonoamidate **23** and the phosphonodiamidate prodrug **24** displayed a significant improvement of the anti-HIV activity

with EC₅₀ values in the 2-10 µM range compared to the parent (*R*)-FPMP-7-deazaguanine nucleoside phosphonate **11** (Table 1), most likely due to their increased lipophilicity and consequential improved cellular permeability. Such prodrugs were also tested for anti-HIV activity in peripheral blood mononuclear cells (PBMCs), exhibiting moderate anti-HIV-1 activity against CXCR4-using (X4) NL4.3 with IC₅₀ values of 4.80 and 1.81 µM, respectively, whereas being endowed with weak antiviral activity against the CCR5-using (R5) BaL strain. However, for both cell lines, the increased cytotoxicity of such prodrugs in combination with their weak antiviral activity gave rise to unfavorable selectivity indexes.

Table 1. Anti-HIV-1 activity and cytotoxicity of prodrugs **23** and **24** in TZM-bl cells and (PHA)-stimulated PBMCs.

Compound	EC ₅₀ ^a (µM) in TZM-bl		CC ₅₀ ^b (µM)	EC ₅₀ ^a (µM) in PBMCs		CC ₅₀ ^b (µM)
	HIV-1 X4 (NL4.3)	HIV-1 R5 (BaL)		HIV-1 X4 (NL4.3)	HIV-1 R5 (BaL)	
23	9.28	7.95	44.35	4.80	> 20	31.8
24	> 4	2.62	8.94	1.81	> 4	6.86
PMPA	2.38	3.13	>100	0.49	1.69	> 25
TDF	0.027	0.02	39.97	0.0023	0.068	> 5
TAF	0.003	0.0033	82.75	0.0007	0.0024	3.27

^[a] Compound concentration required to reduce HIV-induced cytopathicity by 50%. ^[b]

Compound concentration required to reduce cell viability by 50%.

Conclusions

Both enantiomeric series of 3-fluoro-2-(phosphonmethoxy)propyl acyclic nucleoside phosphonates bearing modified nucleobases including 2-amino-6-arylthiopurine, 7-deazaguanine and its analogues were synthesized. The presence of an electron-withdrawing substituent at the C7 position of 7-deazaguanine was found to reduce the efficiency of the Mitsunobu alkylation. In addition, two representative L-aspartic acid (di)amyl ester

phosphonoamidate prodrugs of (*R*)-FPMP-7-deazaguanine were also prepared. All compounds were tested for their antiviral activity against HIV-1. While the parent phosphonates were completely devoid of anti-HIV activity, prodrugs **23** and **24** were endowed with moderate antiviral activity, with EC₅₀ values in the low micromolar range against HIV-1 in both TZM-bl cells and (PHA)-stimulated PBMCs.

Experimental Section

Unless otherwise specified, all reagents and solvents were obtained from commercial sources and used as received. NMR spectra were measured on a Bruker Avance 300, 500, or 600 MHz spectrometer using tetramethylsilane as internal standard or the residual solvent as internal standard, and 85% H₃PO₄ for ³¹P NMR experiments. The numbering system utilized in the assignment of the NMR peaks is illustrated in *Figure 2*. High resolution mass spectra (HRMS) were recorded on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Reactions were monitored by thin-layer chromatography on silica gel (pre-coated aluminum sheets, 254 nm). Column chromatography was performed on silica gel (60 Å, 0.035–0.070 mm, Acros Organics). Preparative reverse phase HPLC purification was performed on a Phenomenex Gemini 110A column (C18, 10 µm, 21.2 mm × 250 mm, 5 or 8 mL/min) using H₂O/CH₃CN containing 50 mmol TEAB or H₂O/CH₃CN as eluent gradient. The purity of all final compounds was determined to be >95% by analytic HPLC analysis.

Figure 2. General numbering system for the assignment of NMR peaks.

(*R*)-2-Amino-6-chloro-9-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}purine (3a).

To a solution of **1** (120 mg, 0.74 mmol), **2a** (150 mg, 0.61 mmol), and Ph₃P (300 mg, 1.20 mmol) in anhydrous THF (5 mL) was added DIAD (0.24 mL, 1.20 mmol) dropwise at room temperature. The reaction mixture was stirred for 24 h, then H₂O (5 mL) was added. The resulting mixture was refluxed for 24 h in order to remove the triphenylphosphine adducts. The solvent was removed under reduced pressure, and the crude residue was purified by silica gel chromatography (DCM/MeOH, 50:1 to 25:1, v/v) to give **3a** (200 mg). Colorless oil. Yield: 83%
¹H NMR (300 MHz, CDCl₃): 7.91 (s, H-C(8)), 5.50 (s, NH₂), 4.71-4.35 (m, H-C(3'), H_a-C(1')), 4.26-3.92 (m, H_b-C(1'), H-C(2'), 2 x CH₂CH₃, H_a-C(4')), 3.83 (dd, *J* = 13.9, 8.8, H_b-C(4')), 1.35-1.26 (m, 2 x CH₃); ¹³C NMR (75 MHz, CDCl₃): 159.5 (C(2)), 154.1 (C(4)), 151.5 (C(6)), 143.5 (C(8)), 125.1 (C(5)), 82.0 (d, *J* = 173.9, C(3')), 78.4 (dd, *J* = 19.5, 9.6, C(2')), 64.7 (d, *J* = 167.1 Hz, C(4')), 62.9, 62.8 (CH₂CH₃), 43.8 (d, *J* = 8.0 Hz, C(1')), 16.7, 16.6 (CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.5; HR-MS: 396.1002 ([M+H]⁺, C₁₃H₂₁ClFN₅O₄P⁺, calc. 396.0998).

(*S*)-2-Amino-6-chloro-9-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}purine (3b).

Compound **3b** was synthesized according to the procedure used for the preparation of **3a**, starting from **1** (250 mg, 1.47 mmol), **2b** (300 mg, 1.23 mmol), Ph₃P (640 mg, 2.46 mmol), and DIAD (0.48 mL, 2.46 mmol) in anhydrous THF (10 mL). The crude residue was purified by

silica gel chromatography (DCM/MeOH, 50:1 to 25:1, v/v). Colorless oil. Yield: 70%. ^1H NMR (300 MHz, CDCl_3): 7.90 (s, H-C(8)), 5.40 (s, NH_2), 4.71-4.34 (m, H-C(3'), H_a -C(1')), 4.25-3.79 (m, H_b -C(1'), H-C(2'), 2 x CH_2CH_3 , H-C(4')), 1.35-1.24 (m, 2 x CH_3); ^{13}C NMR (75 MHz, CDCl_3): 159.5 (C(2)), 154.2 (C(4)), 151.5 (C(6)), 143.6 (C(8)), 125.1 (C(5)), 82.0 (d, $J = 173.7$, C(3')), 78.4 (dd, $J = 19.7$, 9.5, C(2')), 64.8 (d, $J = 167.0$, C(4')), 62.9, 62.8 (CH_2CH_3), 43.8 (d, $J = 7.8$, C(1')), 16.7, 16.6 (CH_3); ^{31}P NMR (121 MHz, CDCl_3): 20.5; HR-MS: 396.0998 ($[\text{M}+\text{H}]^+$, $\text{C}_{13}\text{H}_{21}\text{ClFN}_5\text{O}_4\text{P}^+$, calc. 396.0998).

(R)-2-Amino-6-(4-methoxyphenylthio)-9-{3-fluoro-2-

[(diethylphosphoryl)methoxy]propyl}purine (4a). 4-Methoxythiophenol (0.12 mL, 0.96 mmol) was added to a mixture of **3a** (190 mg, 0.48 mmol) and triethylamine (0.07 mL, 0.48 mmol) in anhydrous DMF (10 mL) at room temperature, and the mixture was stirred at 100 °C for another 4 h. The solution was diluted with EtOAc (100 mL) and washed with saturated aq. NaHCO_3 (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was then purified by silica gel chromatography (DCM/MeOH, 60:1 to 40:1, v/v) to give **4a** (210 mg). Colorless oil. Yield: 87%. ^1H NMR (600 MHz, CDCl_3): 7.76 (s, H-C(8)), 7.52 (d, $J = 8.9$, 2 arom. H), 6.94 (d, $J = 8.9$, 2 arom. H), 4.88 (s, NH_2), 4.64-4.38 (m, H-C(3')), 4.33-4.28 (m, H_a -C(1')), 4.19-4.04 (m, H_b -C(1'), H-C(2'), 2 x CH_2CH_3), 3.91 (dd, $J = 13.9$, 8.7, 1H, H_a -C(4')), 3.85-3.77 (m, OCH_3 , H_b -C(4')), 1.34-1.27 (m, 2 x CH_3); ^{13}C NMR (150 MHz, CDCl_3): 161.7 (C(2)), 160.4 (arom. C), 159.1 (C(6)), 150.9 (C(4)), 141.0 (C(8)), 137.1 (arom. C), 124.6 (C(5)), 118.0 (arom. C), 114.5 (arom. C), 82.0 (d, $J = 173.6$, C(3')), 78.3 (dd, $J = 19.2$, 9.9, C(2')), 64.5 (d, $J = 166.9$, C(4')), 62.5, 62.4 (d, $J = 6.4$, CH_2CH_3), 55.3 (OCH_3), 43.1 (d, $J = 7.9$, C(1')), 16.4, 16.3 (CH_3); ^{31}P NMR (121 MHz, CDCl_3): 19.9; HR-MS:

500.1526 ($[M+H]^+$, $C_{20}H_{28}FN_5O_5PS^+$, calc. 500.1527).

(S)-2-Amino-6-(4-methoxyphenylthio)-9-[3-fluoro-2-

[(diethylphosphoryl)methoxy]propyl]purine (4b). Compound **4b** was synthesized according to the procedure used for the preparation of **4a**, starting from **3b** (190 mg, 0.48 mmol), 4-methoxythiophenol (0.12 mL, 0.96 mmol), and triethylamine (0.07 mL, 0.48 mmol) in anhydrous DMF (10 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 60:1 to 40:1, v/v). Colorless oil. Yield: 67%. 1H NMR (300 MHz, $CDCl_3$): 7.77 (s, H-C(8)), 7.53 (d, $J = 8.8$, 2 arom. H), 6.95 (d, $J = 8.8$, 2 arom. H), 5.03 (s, 1H, NH_2), 4.70-4.30 (m, H-C(3'), H_a -C(1')), 4.22-3.98 (m, H_b -C(1'), H-C(2'), 2 x CH_2CH_3), 3.93-3.77 (m, H-C(4'), OCH_3), 1.35-1.26 (m, 2 x CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 162.1 (C(2)), 160.9 (arom. C), 159.0 (C(6)), 151.0 (C(4)), 141.7 (C(8)), 137.5 (arom. C), 124.9 (C(5)), 118.0 (arom. C), 115.0 (arom. C), 82.3 (d, $J = 173.7$, C(3')), 78.7 (dd, $J = 19.5$, 9.9, C(2')), 64.9 (d, $J = 166.9$, C(4')), 62.9, 62.8 (CH_2CH_3), 55.6 (OCH_3), 43.6 (d, $J = 8.1$, C(1')), 16.8, 16.7 (CH_3); ^{31}P NMR (121 MHz, $CDCl_3$): 20.5; HR-MS: 500.1518 ($[M+H]^+$, $C_{20}H_{28}FN_5O_5PS^+$, calc. 500.1527).

(R)-2-Amino-6-(4-methoxyphenylthio)-9-[3-fluoro-2-(phosphonomethoxy)propyl]

purine (5a). To a solution of **4a** (200 mg, 0.40 mmol) and 2,6-lutidine (0.74 mL, 6.40 mmol) in anhydrous acetonitrile (10 mL) was added bromotrimethylsilane (0.43 mL, 3.20 mmol) dropwise at 0 °C. The reaction mixture was then set aside in the dark for 12 h at room temperature. The reaction was quenched with 1.0 M TEAB buffer (1 mL), and the solvent was removed under reduced pressure. The residue was first purified by silica gel chromatography (acetone/ Et_3N/H_2O , 6:1:1, v/v/v), followed by further preparative reverse phase HPLC (linear gradient, 2-98% CH_3CN in a 0.05 M TEAB solution) to give **5a** (120 mg). White foam. Yield:

70%. ^1H NMR (300 MHz, D_2O): 7.93 (s, H-C(8)), 7.19 (d, $J = 8.8$, 2 arom. H), 6.73-6.70 (m, 2 arom. H), 4.60-4.21 (m, H-C(3')), 4.18-4.08 (m, H-C(1')), 3.89-3.81 (m, H-(2')), 3.61 (s, OCH_3), 3.59-3.41 (m, H-(4')); ^{13}C NMR (75 MHz, D_2O): 161.1 (C(2)), 160.0 (arom. C), 159.3 (C(6)), 150.1 (C-4), 142.8 (C-8), 136.8 (arom. C), 123.0 (C(5)), 116.8 (arom. C), 114.8 (arom. C), 82.1 (d, $J = 168.0$, C(3')), 77.4 (dd, $J = 18.9$, 11.2, C(2')), 66.9 (d, $J = 154.8$, C(4')), 55.3 (OCH_3), 42.7 (d, $J = 7.1$, C(1')); ^{31}P NMR (121 MHz, D_2O): 14.1; HR-MS: 442.0752 ($[\text{M}-\text{H}]^-$, $\text{C}_{16}\text{H}_{18}\text{FN}_5\text{O}_5\text{PS}^-$, calc. 442.0756).

(S)-2-Amino-6-(4-methoxyphenylthio)-9-[3-fluoro-2-(phosphonomethoxy)propyl]

purine (5b). Compound **5b** was synthesized according to the procedure used for the preparation of **5a**, starting from compound **4b** (150 mg, 0.30 mmol), bromotrimethylsilane (0.32 mL, 2.40 mmol), and 2,6-lutidine (0.56 mL, 4.80 mmol) in anhydrous acetonitrile (10 mL). The crude residue was purified by silica gel chromatography (acetone/ $\text{Et}_3\text{N}/\text{H}_2\text{O}$, 6:1:1, v/v/v). White foam. Yield: 65%. ^1H NMR (300 MHz, D_2O): 7.95 (s, H-C(8)), 7.22 (d, $J = 8.4$, 2 arom. H), 6.82-6.65 (m, 2 arom. H), 4.63-4.24 (m, H-C(3')), 4.20-4.10 (m, H-C(1')), 3.93-3.85 (m, H-(2')), 3.64 (s, OCH_3), 3.61-3.45 (m, H-C(4')); ^{13}C NMR (75 MHz, D_2O): 161.1 (C(2)), 160.0 (arom. C), 159.3 (C(6)), 150.1 (C(4)), 142.8 (C-8), 136.8 (arom. C), 123.0 (C(5)), 116.8 (arom. C), 114.8 (arom. C), 82.1 (d, $J = 168.1$ Hz, C(3')), 77.5 (dd, $J = 18.9$, 11.1, C(2')), 66.7 (d, $J = 155.5$, C(4')), 55.3 (OCH_3), 42.8 (d, $J = 7.1$, C(1')); ^{31}P NMR (121 MHz, D_2O): 14.3; HR-MS: 442.0734 ($[\text{M}-\text{H}]^-$, $\text{C}_{16}\text{H}_{18}\text{FN}_5\text{O}_5\text{PS}^-$, calc. 442.0756).

(R)-4-Chloro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-2-pivaloylamino-

7H-pyrrolo[2,3-d]pyrimidine (9a). DIAD (0.24 mL, 1.20 mmol) was added dropwise to a mixture of **7**^[17] (180 mg, 0.74 mmol), **2a** (150 mg, 0.61 mmol), and Ph_3P (300 mg, 1.20 mmol)

in anhydrous THF (10 mL) at room temperature. After stirring for 24 h, the solvent was removed under reduced pressure. The resultant residue was purified by silica gel column chromatography (DCM/MeOH, 60:1 to 30:1, v/v) to give **9a** (220 mg). Colorless oil. Yield: 78%. ¹H NMR (300 MHz, CDCl₃): 8.16 (s, NHCO), 7.29 (dd, *J* = 0.9, H-C(6)), 6.53 (dd, *J* = 3.6, 0.9, H-C(5)), 4.74-4.31 (m, H-C(3'), H-C(1')), 4.18-3.86 (m, H-C(2'), 2 x CH₂CH₃, H-C(4')), 1.36-1.27 (m, (CH₃)₃C, 2 x CH₂CH₃); ¹³C NMR (150 MHz, CDCl₃): 175.7 (NHCO), 152.3 (C(4), C(7a)), 151.6 (C(2)), 130.1 (C(6)), 114.2 (C(4a)), 100.1 (C(5)), 82.9 (d, *J* = 173.2, C(3')), 79.4 (dd, *J* = 19.1, 10.9, C(2')), 64.9 (d, *J* = 166.9, C(4')), 62.7, 62.6 (CH₂CH₃), 44.9 (d, *J* = 8.5, C(1')), 40.5 (C(CH₃)₃), 27.7 (C(CH₃)₃), 16.7, 16.6 (CH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.9; HR-MS: 479.1617 ([M+H]⁺, C₁₉H₃₀ClFN₄O₅P⁺, calc. 479.1621).

(S)-4-Chloro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-2-pivaloylamino-7H-pyrrolo[2,3-*d*]pyrimidine (9b). Compound **9b** was synthesized according to the procedure used for the preparation of **9a**, starting from **7**^[17] (180 mg, 0.74 mmol), **2b** (150 mg, 0.61 mmol), Ph₃P (300 mg, 1.20 mmol), and DIAD (0.24 mL, 1.20 mmol) in anhydrous THF (10 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 60:1 to 30:1, v/v). Colorless oil. Yield: 79%. ¹H NMR (600 MHz, CDCl₃): 8.13 (s, NHCO), 7.30 (dd, *J* = 3.6, 2.0, H-C(6)), 6.53 (dd, *J* = 3.6, 2.5, H-C(5)), 4.69-4.59 (m, H_a-C(3')), 4.55-4.51 (m, H_a-C(1')), 4.46-4.33 (m, H_b-C(3'), H_b-C(1')), 4.15-4.08 (m, H-C(2'), 2 x CH₂CH₃), 4.00-3.96 (m, H_a-C(4')), 3.92-3.88 (m, H_b-C(4')), 1.35-1.29 (m, (CH₃)₃C, 2 x CH₂CH₃); ¹³C NMR (150 MHz, CDCl₃): 175.5 (NHCO), 152.1 (C(4), C(7a)), 151.3 (C(2)), 129.8 (C(6)), 113.9 (C(4a)), 99.8 (C(5)), 82.5 (d, *J* = 173.0, C(3')), 79.1 (dd, *J* = 19.0, 11.1, C(2')), 64.5 (d, *J* = 167.2, C(4')), 62.5, 62.4 (CH₂CH₃), 44.5 (d, *J* = 8.5, C(1')), 40.2 (C(CH₃)₃), 27.4 (C(CH₃)₃), 16.4 (CH₂CH₃); ³¹P NMR

(121 MHz, CDCl₃): 20.9; HR-MS: 479.1624 ([M+H]⁺, C₁₉H₃₀ClFN₄O₅P⁺, calc. 479.1621).

(R)-2-Amino-3,4-dihydro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (10a). A solution of **9a** (200 mg, 0.42 mmol), DABCO (46 mg, 0.42 mmol), and K₂CO₃ (58 mg, 0.42 mmol) in a mixture of dioxane and H₂O (6 mL, 5:1, v/v) was stirred at 90 °C for 3 h. The solvent was then removed under reduced pressure to give crude residue that was redissolved in satd NH₃ in MeOH (20 mL) and stirred at room temperature for 12 h. After removal of all the volatiles, the residue was purified by silica gel chromatography (DCM/MeOH, 20:1 to 10:1, v/v) to give **10a** (100 mg). Colorless oil. Yield: 64%. ¹H NMR (300 MHz, CDCl₃): 11.01 (s, NHCO), 6.59 (dd, *J* = 3.5, 0.9, H-C(6)), 6.50 (dd, *J* = 3.5, 0.9, H-C(5)), 6.23 (s, NH₂), 4.67-4.28 (m, H-C(3')), 4.23-4.10 (m, H-C(1')), 2 x CH₂CH₃, 4.05-3.91 (m, H-C(2')), H_a-C(4')), 3.85-3.77 (m, H_b-C(4')), 1.37-1.31 (m, 2 x CH₃); ¹³C NMR (75 MHz, CDCl₃): 160.7 (C(4)), 152.8 (C(2)), 151.1 (C(7a)), 121.6 (C(6)), 102.6 (C(4a)), 101.3 (C(5)), 82.2 (d, *J* = 173.4, C(3')), 79.6 (dd, *J* = 18.7, 11.5, C(2')), 64.7 (d, *J* = 168.3, C(4')), 62.3, 62.2 (CH₂CH₃), 44.5 (d, *J* = 8.0, C(1')), 16.7, 16.6 (CH₃); ³¹P NMR (121 MHz, CDCl₃): 21.0; HR-MS: 377.1387 ([M+H]⁺, C₁₄H₂₃FN₄O₅P⁺, calc. 377.1384).

(S)-2-Amino-3,4-dihydro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (10b). Compound **10b** was synthesized according to the procedure used for the preparation of **10a**, starting from **9b** (100 mg, 0.21 mmol), DABCO (23 mg, 0.21 mmol), and K₂CO₃ (29 mg, 0.21 mmol) in a mixture of dioxane and H₂O (6 mL, 5:1, v/v), followed by treatment with NH₃/MeOH (20 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 20:1 to 10:1, v/v). Colorless oil. Yield: 64%. ¹H NMR (300 MHz, CDCl₃): δ 11.04 (s, NHCO), 6.60 (d, *J* = 3.4, H-C(6)), 6.49 (d, *J* = 3.5, H-C(5)), 6.21 (s,

NH_2), 4.67-4.29 (m, H-C(3')), 4.22-4.11 (m, H-C(1')), 2 x CH_2CH_3), 4.04-3.93 (m, H-C(2')), H_a -C(4')), 3.86-3.78 (m, H_b -C(4')), 1.36-1.30 (m, 2 x CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 160.6 (C(4)), 152.7 (C(2)), 150.7 (C(7a)), 121.6 (C(6)), 102.6 (C(4a)), 101.2 (C(5)), 82.2 (d, $J = 173.6$, C(3')), 79.6 (C(2')), 64.7 (d, $J = 168.3$, C(4')), 62.3, 62.2 (CH_2CH_3), 44.6 (d, $J = 8.0$ Hz, C(1')), 16.7, 16.6 (CH_3); ^{31}P NMR (121 MHz, $CDCl_3$): 21.0; HR-MS: 377.1384 ($[M+H]^+$, $C_{14}H_{23}FN_4O_5P^+$, calc. 377.1384).

(R)-2-Amino-3,4-dihydro-7-[3-fluoro-2-(phosphonomethoxy)propyl]-7H-pyrrolo[2,3-d]pyrimidin-4-one (11a). Compound **11a** was synthesized according to the procedure used for the preparation of **5a**, starting from compound **10a** (100 mg, 0.26 mmol), bromotrimethylsilane (0.28 mL, 2.12 mmol), and 2,6-lutidine (0.50 mL, 4.24 mmol) in anhydrous acetonitrile (10 mL). The crude residue was purified by silica gel chromatography (acetone/ Et_3N/H_2O , 4:1:1, v/v/v). White foam. Yield: 70%. 1H NMR (300 MHz, D_2O): 6.65 (d, $J = 3.7$, H-C(6)), 6.17 (d, $J = 3.6$, H-C(5)), 4.50-4.04 (m, H-C(3')), 4.95-3.93 (m, H-C(1')), 3.83-3.70 (m, H-C(2')), 3.41 (d, $J = 9.2$, H-C(4')); ^{13}C NMR (75 MHz, D_2O): 160.8 (C(4)), 152.0 (C(2)), 150.3 (C(7a)), 123.1 (C(6)), 100.9 (C(5)), 99.5 (C(4a)), 82.3 (d, $J = 167.3$, C(3')), 78.0 (dd, $J = 18.4$, 10.7, C(2')), 67.6 (d, $J = 151.9$, C(4')), 43.4 (d, $J = 7.3$, C(1')); ^{31}P NMR (121 MHz, D_2O): 12.3; HR-MS: 319.0611 ($[M-H]^-$, $C_{10}H_{13}FN_4O_5P^-$, calc. 319.0612).

(S)-2-Amino-3,4-dihydro-7-[3-fluoro-2-(phosphonomethoxy)propyl]-7H-pyrrolo[2,3-d]pyrimidin-4-one (11b). Compound **11b** was synthesized according to the procedure used for the preparation of **5a**, starting from compound **10b** (50 mg, 0.13 mmol), bromotrimethylsilane (0.14 mL, 1.06 mmol), and 2,6-lutidine (0.25 mL, 2.12 mmol) in anhydrous acetonitrile (5 mL). The crude residue was purified by silica gel chromatography on (acetone/ Et_3N/H_2O , 4:1:1,

v/v/v). White foam. Yield: 70%. ^1H NMR (300 MHz, D_2O): δ 6.73 (d, $J = 3.5$, H-C(6)), 6.25 (d, $J = 3.5$, H-C(5)), 4.56-4.11 (m, H-C(3')), 4.09-3.98 (m, H-C(1')), 3.90-3.78 (m, H-C(2')), 3.42 (d, $J = 9.2$, H-C(4')); ^{13}C NMR (75 MHz, D_2O): 166.0 (C(4)), 156.5 (C(2)), 151.1 (C(7a)), 122.3 (C(6)), 100.6 (C(4a), C(5)), 82.4 (d, $J = 167.0$, C(3')), 78.0 (dd, $J = 18.2$, 10.4, C(2')), 68.1 (d, $J = 150.0$, C(4')), 43.2 (d, $J = 7.2$, C(1')); ^{31}P NMR (121 MHz, D_2O): 12.6; HR-MS: 319.0598 ($[\text{M}-\text{H}]^-$, $\text{C}_{10}\text{H}_{13}\text{FN}_4\text{O}_5\text{P}^-$, calc. 319.0612).

(R)-4-Chloro-5-fluoro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-2-pivaloylamino-7H-pyrrolo[2,3-d]pyrimidine (12a). Compound **12a** was synthesized according to the procedure used for the preparation of **9a**, starting from **8** (200 mg, 0.74 mmol), **2a** (150 mg, 0.61 mmol), Ph_3P (300 mg, 1.20 mmol), and DIAD (0.24 mL, 1.20 mmol) in anhydrous THF (10 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 80:1 to 40:1, v/v). Colorless oil. Yield: 25%. ^1H NMR (300 MHz, CDCl_3): 8.13 (s, NHCO), 7.10 (d, $J = 2.6$, H-C(6)), 4.72-3.96 (m, H-C(3'), H-C(1'), H-C(2')), 2 x CH_2CH_3 , 4.00 (dd, $J = 13.8$, 8.7, $\text{H}_a\text{-C}(4')$), 3.88 (dd, $J = 13.8$, 9.2, $\text{H}_b\text{-C}(4')$), 1.34-1.28 (m, $(\text{CH}_3)_3\text{C}$, 2 x CH_2CH_3); ^{13}C NMR (125 MHz, CDCl_3): 175.6 (NHCO), 152.0 (C(2)), 150.9 (d, $J = 4.2$, C(4)), 147.9 (C(7a)), 141.6 (d, $J = 252.5$, C(5)), 112.5 (d, $J = 26.5$, C(6)), 103.6 (d, $J = 14.9$, C(4a)), 82.6 (d, $J = 173.3$, C(3')), 79.5, 79.4, 79.2, 79.1 (C(2')), 64.8 (d, $J = 166.9$, C(4')), 62.7, 62.6 (CH_2CH_3), 44.4 (d, $J = 8.5$, C(1')), 40.5 ($\text{C}(\text{CH}_3)_3$), 27.6 ($\text{C}(\text{CH}_3)_3$), 16.6, 16.5 (CH_2CH_3); ^{31}P NMR (121 MHz, CDCl_3): 20.9; HR-MS: 497.1523 ($[\text{M}+\text{H}]^+$, $\text{C}_{19}\text{H}_{29}\text{ClF}_2\text{N}_4\text{O}_5\text{P}^+$, calc. 497.1527).

(S)-4-Chloro-5-fluoro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-2-pivaloylamino-7H-pyrrolo[2,3-d]pyrimidine (12b). Compound **12b** was synthesized

according to the procedure used for the preparation of **9a**, starting from **8** (200 mg, 0.74 mmol), **2b** (150 mg, 0.61 mmol), Ph₃P (300 mg, 1.20 mmol), and DIAD (0.24 mL, 1.20 mmol) in anhydrous THF (10 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 80:1 to 40:1, v/v). Colorless oil. Yield: 33%. ¹H NMR (600 MHz, CDCl₃): δ 8.13 (s, NHCO), 7.10 (d, *J* = 2.5, H-C(6)), 4.67-4.25 (m, H-C(3'), H-C(1')), 4.18-4.02 (m, H-C(2'), 2 x CH₂CH₃), 4.00 (dd, *J* = 13.8, 8.7, H_a-C(4')), 3.89 (dd, *J* = 13.8, 9.2, H_b-C(4')), 1.34-1.30 (m, (CH₃)₃C, 2 x CH₂CH₃); ¹³C NMR (125 MHz, CDCl₃): 175.5 (NHCO), 151.8 (C(2)), 150.7 (C(4)), 147.6 (C(7a)), 141.3 (d, *J* = 252.3, C(5)), 112.4 (d, *J* = 26.3, C(6)), 103.4 (d, *J* = 14.9, C(4a)), 82.4 (d, *J* = 173.3, C(3')), 79.2, 79.0 (C(2')), 64.5 (d, *J* = 167.0, C(4')), 62.5 (CH₂CH₃), 44.1 (d, *J* = 8.5, C(1')), 40.3 (C(CH₃)₃), 27.4 (C(CH₃)₃), 16.4 (CH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.8; HR-MS: 497.1521 ([M+H]⁺, C₁₉H₂₉ClF₂N₄O₅P⁺, calc. 497.1527).

(R)-2-Amino-5-fluoro-3,4-dihydro-7-{3-fluoro-2-

[(diethylphosphoryl)methoxy]propyl]-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (**13a**).

Compound **13a** was synthesized according to the procedure used for the preparation of **10a**, starting from **12a** (60 mg, 0.12 mmol), DABCO (14 mg, 0.12 mmol) and K₂CO₃ (17 mg, 0.12 mmol) in a mixture of dioxane and H₂O (6 mL, 5:1, v/v), followed by treatment with NH₃/MeOH (20 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 18:1 to 10:1, v/v). Colorless oil. Yield: 30%. ¹H NMR (300 MHz, CDCl₃): 6.42 (d, *J* = 2.3, H-C(6)), 5.99 (s, NH₂), 4.66-3.83 (m, H-C(3'), H-C(1'), H-C(2'), 2 x CH₂CH₃, H-C(4')), 1.37-1.32 (m, 2 x CH₃); ¹³C NMR (75 MHz, CDCl₃): 158.4 (d, *J* = 2.6, C(4)), 152.3 (C(2)), 146.7 (C(7a)), 145.4 (d, *J* = 248.6, C(5)), 103.6 (d, *J* = 26.7, C(6)), 90.6 (d, *J* = 13.9, C(4a)), 81.9 (d, *J* = 173.2, C(3')), 79.0 (dd, *J* = 19.0, 10.8, C(2')), 64.2 (d, *J* = 167.5, C(4')),

62.4, 62.3 (CH₂CH₃), 43.5 (d, $J = 7.9$, C(1')), 16.1, 16.0 (CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.3; HR-MS: 395.1284 ([M+H]⁺, C₁₄H₂₂F₂N₄O₅P⁺, calc. 395.1290).

(S)-2-Amino-5-fluoro-3,4-dihydro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (13b).

Compound **13b** was synthesized according to the procedure used for the preparation of **10a**, starting from **12b** (90 mg, 0.18 mmol), DABCO (20 mg, 0.18 mmol), and K₂CO₃ (25 mg, 0.18 mmol) in a mixture of dioxane and H₂O (6 mL, 5:1, v/v), followed by treatment with NH₃/MeOH (20 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 18:1 to 10:1, v/v). Colorless oil. Yield: 35%. ¹H NMR (300 MHz, CDCl₃): δ 6.41 (d, $J = 2.4$, H-C(6)), 6.00 (s, NH₂), 4.66-3.82 (m, H-C(3'), H-C(1'), H-C(2')), 2 x CH₂CH₃, H-C(4')), 1.36-1.31 (m, 2 x CH₃); ¹³C NMR (75 MHz, CDCl₃): 159.0 (C(4)), 152.9 (C(2)), 147.3 (C(7a)), 146.1 (d, $J = 248.6$, C(5)), 104.2 (d, $J = 26.5$, C(6)), 91.2 (d, $J = 13.7$, C(4a)), 82.5 (d, $J = 173.1$, C(3')), 79.6 (dd, $J = 19.2, 11.1$, C(2')), 64.8 (d, $J = 167.6$, C(4')), 63.0, 62.9 (CH₂CH₃), 44.2 (d, $J = 7.9$, C(1')), 16.7, 16.6 (CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.9; HR-MS: 395.1286 ([M+H]⁺, C₁₄H₂₂F₂N₄O₅P⁺, calc. 395.1290).

(R)-2-Amino-5-fluoro-3,4-dihydro-7-[3-fluoro-2-(phosphonomethoxy)propyl]-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (14a). Compound **14a** was synthesized according to the procedure used for the preparation of **5a**, starting from compound **13a** (20 mg, 0.05 mmol), bromotrimethylsilane (0.05 mL, 0.40 mmol), and 2,6-lutidine (0.09 mL, 0.81 mmol) in anhydrous acetonitrile (5 mL). The crude residue was purified by silica gel chromatography acetone/Et₃N/H₂O, 4:1:1, v/v/v). White foam. Yield: 50%. ¹H NMR (300 MHz, D₂O): 6.56 (d, $J = 2.1$, H-C(6)), 4.59-4.16 (m, H-C(3')), 4.00-3.98 (m, H-C(1')), 3.90-3.81 (m, H-C(2')), 3.49

(d, $J = 9.1$, H-C(4')); ^{13}C NMR (75 MHz, D_2O): 159.5 (d, $J = 2.8$, C(4)), 152.3 (C(2)), 146.0 (d, $J = 3.0$, C(7a)), 144.6 (d, $J = 246.3$, C(5)), 105.1 (d, $J = 26.6$, C(6)), 90.0 (d, $J = 14.4$, C(4a)), 82.4 (d, $J = 167.2$, C(3')), 78.1 (dd, $J = 18.3$, 10.4, C(2')), 67.2 (d, $J = 153.2$, C(4')), 43.2 (d, $J = 7.4$, C(1')); ^{31}P NMR (121 MHz, D_2O): 13.8; HR-MS: 337.0503 ($[\text{M}-\text{H}]^-$, $\text{C}_{10}\text{H}_{12}\text{F}_2\text{N}_4\text{O}_5\text{P}^-$, calc. 337.0519).

(S)-2-Amino-5-fluoro-3,4-dihydro-7-[3-fluoro-2-(phosphonomethoxy)propyl]-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (14b). Compound **14b** was synthesized according to the procedure used for the preparation of **5a**, starting from compound **13b** (20 mg, 0.05 mmol), bromotrimethylsilane (0.05 mL, 0.40 mmol), and 2,6-lutidine (0.09 mL, 0.81 mmol) in anhydrous acetonitrile (5 mL). The crude residue was purified by silica gel chromatography (acetone/ $\text{Et}_3\text{N}/\text{H}_2\text{O}$, 4:1:1, v/v/v). White foam. Yield: 60%. ^1H NMR (300 MHz, D_2O): 6.52 (d, $J = 2.2$, H-C(6)), 4.57-4.09 (m, H-C(3')), 4.07-3.92 (m, H-C(1')), 3.89-3.77 (m, H-C(2')), 3.43 (d, $J = 9.1$, H-C(4')); ^{13}C NMR (75 MHz, D_2O): 167.1 (C(4)), 159.1 (C(2)), 147.4 (C(7a)), 144.6 (d, $J = 244.3$, C(5)), 103.5 (d, $J = 27.2$, C(6)), 91.2 (d, $J = 14.4$, C(4a)), 82.4 (d, $J = 166.9$, C(3')), 77.9 (dd, $J = 18.2$, 10.3, C(2')), 68.1 (d, $J = 150.1$, C(4')), 42.7 (d, $J = 7.2$, C(1')); ^{31}P NMR (121 MHz, D_2O): 12.7; HR-MS: 337.0522 ($[\text{M}-\text{H}]^-$, $\text{C}_{10}\text{H}_{12}\text{F}_2\text{N}_4\text{O}_5\text{P}^-$, calc. 337.0519).

(R)-4-Chloro-5-cyano-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-2-pivaloylamino-7H-pyrrolo[2,3-*d*]pyrimidine (20a).

Compound **20a** was synthesized according to the procedure used for the preparation of **9a**, starting from **19** (150 mg, 0.54 mmol), **2a** (120 mg, 0.49 mmol), Ph_3P (260 mg, 0.98 mmol), and DIAD (0.19 mL, 0.98 mmol) in anhydrous THF (10 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 50:1 to 30:1, v/v). Colorless oil. Yield: 80%. ^1H

NMR (300 MHz, CDCl₃): 8.24 (s, NHCO), 7.91 (s, H-C(6)), 4.74-4.29 (m, H-C(3'), H-C(1')), 4.17-3.96 (m, H-C(2'), 2 x CH₂CH₃, H_a-C(4')), 3.81 (dd, *J* = 13.9, 9.0, H_b-C(4')), 1.34-1.25 (m, (CH₃)₃C, 2 x CH₂CH₃); ¹³C NMR (75 MHz, CDCl₃): 175.8 (NHCO), 153.4, 153.2, 152.3 (C(4), C(7a), C(2)), 138.2 (C(6)), 113.5 (C(5)), 112.1 (C(4a)), 86.0 (CN), 82.3 (d, *J* = 174.0, C(3')), 78.7 (dd, *J* = 19.2, 9.8, C(2')), 64.7 (d, *J* = 167.1, C(4')), 62.9, 62.8 (CH₂CH₃), 45.6 (d, *J* = 8.4, C(1')), 40.6 (C(CH₃)₃), 27.6 (C(CH₃)₃), 16.7 (CH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.7; HR-MS: 504.1567 ([M+H]⁺, C₂₀H₂₉ClFN₅O₅P⁺, calc. 504.1573).

(S)-4-Chloro-5-cyano-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-2-pivaloylamino-7H-pyrrolo[2,3-*d*]pyrimidine (20b).

Compound **20b** was synthesized according to the procedure used for the preparation of **9a**, starting from **19** (150 mg, 0.54 mmol), **2b** (120 mg, 0.49 mmol), Ph₃P (260 mg, 0.98 mmol), and DIAD (0.19 mL, 0.98 mmol) in anhydrous THF (10 mL). The crude residue was purified by silica gel chromatography (DCM/MeOH, 50:1 to 30:1, v/v). Colorless oil. Yield: 60%. ¹H NMR (300 MHz, CDCl₃): 8.24 (s, NHCO), 7.92 (s, H-C(6)), 4.74-4.30 (m, H-C(3'), H-C(1')), 4.15-3.96 (m, H-C(2'), 2 x CH₂CH₃, H_a-C(4')), 3.81 (dd, *J* = 13.9, 9.0, H_b-C(4')), 1.35-1.26 (m, (CH₃)₃C, 2 x CH₂CH₃); ¹³C NMR (75 MHz, CDCl₃): 175.7 (NHCO), 153.2, 153.0, 152.1 (C(4), C(7a), C(2)), 138.1 (C(6)), 113.4 (C(5)), 111.9 (C(4a)), 85.8 (CN), 82.1 (d, *J* = 174.0, C(3')), 78.5 (dd, *J* = 19.2, 9.9, C(2')), 64.5 (d, *J* = 167.0, C(4')), 62.8-62.6 (CH₂CH₃), 45.4 (d, *J* = 8.6, C(1')), 40.5 (C(CH₃)₃), 27.5 (C(CH₃)₃), 16.6, 16.5 (CH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.7; HR-MS: 504.1578 ([M+H]⁺, C₂₀H₂₉ClFN₅O₅P⁺, calc. 504.1573).

(R)-2-Amino-5-cyano-3,4-dihydro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (21a).

Compound **21a** was synthesized according to the procedure used for the preparation of **10a**, starting from **20a** (220 mg, 0.44 mmol), DABCO (33 mg, 0.30 mmol) and K_2CO_3 (61 mg, 0.44 mmol) in a mixture of dioxane and H_2O (6 mL, 5:1, v/v), followed by treatment with $NH_3/MeOH$ (20 mL). The residue was purified by silica gel chromatography (DCM/MeOH, 20:1 to 10:1, v/v). Colorless oil. Yield: 30%. 1H NMR (300 MHz, $CDCl_3$): 10.89 (s, $NHCO$), 7.30 (d, $J = 1.1$, H-C(6)), 6.16 (s, NH_2), 4.71-4.29 (m, H-C(3'), H_a -C(1')), 4.21-3.93 (m, H_b -C(1'), H-C(2'), 2 x CH_2CH_3 , H_a -C(4')), 3.83 (dd, $J = 13.8, 9.1$, H_b -C(4')), 1.38-1.32 (m, 2 x CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 159.2 (C(4)), 154.0 (C(2)), 152.2 (C(7a)), 130.9 (C(6)), 115.5 (C(5)), 99.6 (C(4a)), 86.7 (CN), 82.4 (d, $J = 173.6$, C(3')), 79.1 (dd, $J = 19.1, 10.4$, C(2')), 64.8 (d, $J = 167.7$, C(4')), 62.9, 62.8 (CH_2CH_3), 45.2 (d, $J = 8.1$, C(1')), 16.8, 16.7 (CH_3); ^{31}P NMR (121 MHz, $CDCl_3$): 20.7; HR-MS: 402.1324 ($[M+H]^+$, $C_{15}H_{22}FN_5O_3P^+$, calc. 402.1336).

(S)-2-Amino-5-cyano-3,4-dihydro-7-{3-fluoro-2-[(diethylphosphoryl)methoxy]propyl}-7H-pyrrolo[2,3-d]pyrimidin-4-one (21b). A mixture of **20b** (150 mg, 0.30 mmol), Et_3N (0.12 mL, 0.89 mmol), NaOAc (73 mg, 0.89 mmol), and DABCO (33 mg, 0.30 mmol) in DMF (5 mL) was stirred at room temperature for 40 h. Subsequently, H_2O (5 mL) was added and the reaction mixture was stirred for another 1 h. The mixture was extracted with EtOAc (2 x 25 mL). The combined organic phase was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give a crude product. The residue was then resolved in satd NH_3 in MeOH (20 mL) and stirred at room temperature for 12 h. After removal of all the volatiles, the residue was purified by silica gel chromatography (DCM/MeOH, 20:1 to 10:1, v/v) to give **21b** (80 mg). Colorless oil. Yield: 67%. 1H NMR (300 MHz, $CDCl_3$): 10.89 (s, $NHCO$), 7.30 (d, $J = 1.1$, H-C(6)), 4.71-4.28 (m, H-C(3'), H_a -C(1')), 4.21-3.93 (m, H_b -C(1'), H-C(2'), 2 x CH_2CH_3 , H_a -

C(4')), 3.87-3.80 (m, H_b-C(4')), 1.38-1.32 (m, 2 x CH₃); ¹³C NMR (75 MHz, CDCl₃): 159.2 (C(4)), 154.0 (C(2)), 152.2 (C(7a)), 130.9 (C(6)), 115.5 (C(5)), 99.5 (C(4a)), 86.6 (CN), 82.4 (d, *J* = 173.5, C(3')), 79.1 (dd, *J* = 19.2, 10.4, C(2')), 64.8 (d, *J* = 167.2, C(4')), 63.0, 62.9 (CH₂CH₃), 45.2 (d, *J* = 8.2, C(1')), 16.8, 16.7 (CH₃); ³¹P NMR (121 MHz, CDCl₃): 20.7; HR-MS: 402.1329 ([M+H]⁺, C₁₅H₂₂FN₅O₅P⁺, calc. 402.1336).

(*R*)-2-Amino-5-cyano-3,4-dihydro-7-[3-fluoro-2-(phosphonomethoxy)propyl]-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (22a). Compound **22a** was synthesized according to the procedure used for the preparation of **5a**, starting from compound **21a** (50 mg, 0.13 mmol), bromotrimethylsilane (0.13 mL, 1.00 mmol), and 2,6-lutidine (0.23 mL, 2.00 mmol) in anhydrous acetonitrile (5 mL). The crude residue was purified by silica gel chromatography (acetone/Et₃N/H₂O, 5:1:1, v/v/v). White foam. Yield: 70%. ¹H NMR (300 MHz, D₂O): 7.51 (s, H-C(6)), 4.60-4.21 (m, H-C(3')), 4.10-4.08 (m, H-C(1')), 3.90-3.83 (m, H-C(2')), 3.44 (d, *J* = 9.1, H-C(4')); ¹³C NMR (75 MHz, D₂O): 162.0 (C(4)), 155.6 (C(2)), 151.1 (C(7a)), 132.6 (C(6)), 116.2 (C(5)), 99.3 (C(4a)), 84.3 (CN), 82.2 (d, *J* = 167.1, C(3')), 77.3 (dd, *J* = 18.6, 10.5, C(2')), 68.0 (d, *J* = 149.9, C(4')), 44.1 (d, *J* = 7.0, C(1')); ³¹P NMR (121 MHz, D₂O): 12.5; HR-MS: 344.0573 ([M-H]⁻, C₁₁H₁₂FN₅O₅P⁻, calc. 344.0565).

(*S*)-2-Amino-5-cyano-3,4-dihydro-7-[3-fluoro-2-(phosphonomethoxy)propyl]-7H-pyrrolo[2,3-*d*]pyrimidin-4-one (22b). Compound **22b** was synthesized according to the procedure used for the preparation of **5a**, starting from compound **21b** (80 mg, 0.20 mmol), bromotrimethylsilane (0.21 mL, 1.60 mmol), and 2,6-lutidine (0.37 mL, 3.20 mmol) in anhydrous acetonitrile (5 mL). The crude residue was purified by silica gel chromatography (acetone/Et₃N/H₂O, 5:1:1, v/v/v). White foam. Yield: 75%. ¹H NMR (300 MHz, D₂O): 7.40 (d,

$J = 1.1$ Hz, H-C(6)), 4.70-4.20 (m, H-C(3')), 4.08-3.93 (m, H-C(1')), 3.90-3.78 (m, H-C(2')), 3.58-3.42 (m, H-C(4')); ^{13}C NMR (75 MHz, D_2O): 159.0 (C(4)), 153.3 (C(2)), 150.7 (C(7a)), 132.7 (C(6)), 115.7 (C(5)), 98.7 (C(4a)), 84.6 (CN), 82.2 (d, $J = 167.7$, C(3')), 77.7 (dd, $J = 18.5$, 11.3, C(2')), 66.7 (d, $J = 155.2$, C(4')), 44.3 (d, $J = 7.6$, C(1')); ^{31}P NMR (121 MHz, D_2O): 14.3; HR-MS: 344.0557 ($[\text{M}-\text{H}]^-$, $\text{C}_{11}\text{H}_{12}\text{FN}_5\text{O}_5\text{P}^-$, calc. 344.0565).

(*R*)-2-Amino-3,4-dihydro-7-{3-fluoro-2-[phenyloxy-bis(*n*-amyl-L-aspartyl)phosphoryl]methoxy]propyl}-7H-pyrrolo[2,3-d]pyrimidin-4-one (23).

Anhydrous Et_3N (0.13 mL, 0.95 mmol) was added to a solution of compound **11a** (40 mg, 0.09 mmol, Et_3N salt), L-aspartic acid amyl diester HCl salt (50 mg, 0.16 mmol), and phenol (40 mg, 0.42 mmol) in anhydrous pyridine (5 mL). The mixture was stirred at 60 °C under a nitrogen atmosphere. In a separate flask, 2,2'-dithiodipyridine (150 mg, 0.66 mmol) was mixed with PPh_3 (180 mg, 0.66 mmol) in anhydrous pyridine (2 mL) and the resultant mixture was stirred for 10-15 min. This light yellow solution was then added to the solution of **11a**. The combined mixture was stirred at 60 °C for 12 h and then concentrated under reduced pressure. The resultant residue was first purified by silica gel chromatography (DCM/MeOH, 30:1, v/v; 20:1, v/v) and then purified by RP-HPLC (linear gradient, 5-95% CH_3CN in water) to afford compound **23** (60 mg). White powder. Yield: 40%. ^1H NMR (300 MHz, CD_3CN): δ 10.08 (s, NHCO), 7.37-7.08 (m, 5 arom. H), 6.72-6.69 (m, H-C(6)), 6.40-6.38 (m, H-C(5)), 5.80, 5.78 (s, NH_2), 4.70-4.25 (m, H-C(3'), NHPO , H- α -Asp), 4.16-3.89 (m, H-C(1'), H-C(2'), 2 x $\text{OCH}_2(\text{CH}_2)_3\text{CH}_3$, H-C(4')), 2.84-2.57 (m, H- β -Asp), 1.58-1.52 (m, 2 x $\text{OCH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_3$), 1.32-1.24 (m, 2 x $\text{O}(\text{CH}_2)_2(\text{CH}_2)_2\text{CH}_3$), 0.91-0.84 (m, 2 x $\text{O}(\text{CH}_2)_4\text{CH}_3$); ^{13}C NMR (75 MHz, CD_3CN): 172.9, 172.8, 172.7 (CO- α), 171.6 (CO- β), 160.5 (C(4)), 153.5, 153.4 (C(2)), 152.0,

151.9 (C(7a)), 130.7 (arom. C), 125.9 (arom. C), 122.4 (C(6)), 121.9, 121.8 (arom. C), 102.7 (C(5)), 101.7 (C(4a)), 83.6 (d, $J = 170.3$, C(3')), 83.5 (d, $J = 170.0$, C(3')), 80.7, 80.5, 80.4, 80.3, 80.1 (C(2')), 66.8 (d, $J = 156.2$, C(4')), 66.6 (d, $J = 154.2$ Hz, C(4')), 66.6, 65.9, 65.8 (OCH₂(CH₂)₃CH₃), 51.7, 51.6 (C- α -Asp), 45.1, 45.0, 44.9 (C(1')), 39.9 (d, $J = 3.9$, C- β -Asp), 39.7 (d, $J = 3.9$, C- β -Asp), 29.1, 29.0, 28.8, 28.7 (OCH₂(CH₂)₂CH₂CH₃), 23.1, 23.0 (O(CH₂)₃CH₂CH₃), 14.3 (O(CH₂)₄CH₃); ³¹P NMR (121 MHz, CD₃CN): 23.0, 22.2; HR-MS: 652.2923 ([M+H]⁺, C₃₁H₄₃FN₅O₈P⁺, calc. 652.2905).

(R)-2-Amino-3,4-dihydro-7-{3-fluoro-2-[[N,N'-bis(*n*-amyl-L-aspartyl)phosphoryl]methoxy]propyl}-7H-pyrrolo[2,3-d]pyrimidin-4-one (24).

Anhydrous Et₃N (0.04 mL, 0.40 mmol) was added to a solution of compound **11a** (15 mg, 0.04 mmol, Et₃N salt) and L-aspartic acid amyl diester HCl salt (44 mg, 0.14 mmol) in anhydrous pyridine (3 mL). The mixture then was stirred at 60 °C under a nitrogen atmosphere. In a separate flask, 2,2'-dithiodipyridine (55 mg, 0.25 mmol) was mixed with PPh₃ (65 mg, 0.25 mmol) in anhydrous pyridine (2 mL) and the resultant mixture was stirred for 10-15 min. This light yellow solution was added to the solution of **11a**. The combined mixture was stirred at 60 °C for 12 h and then concentrated under reduced pressure. The resultant residue was first purified by silica gel chromatography (DCM/MeOH, 30:1, v/v; 20:1, v/v) and then purified by RP-HPLC (linear gradient, 5-95% CH₃CN in water) to afford compound **24** (5 mg). White powder. Yield: 20%. ¹H NMR (300 MHz, CD₃CN): 9.87 (s, NHCO), 6.63 (d, $J = 3.5$, H-C(6)), 6.32 (d, $J = 3.5$, H-C(5)), 6.06 (s, NH₂), 4.68, 3.74 (m, H-C(3')), 2 x NHPO, 2 x H- α -Asp, H-C(1'), H-C(2'), 4 x OCH₂(CH₂)₃CH₃, H-C(4')), 2.89-2.73 (m, 2 x H- β -Asp), 1.61-1.53 (m, 4 x OCH₂CH₂(CH₂)₂CH₃), 1.33-1.24 (m, 4 x O(CH₂)₂(CH₂)₂CH₃), 0.91-0.84 (m, 4 x O(CH₂)₄CH₃);

^{13}C NMR (75 MHz, CD_3CN): 173.3, 173.2 (CO- α), 171.9, 171.8 (CO- β), 160.0 (C(4)), 153.7 (C(2)), 151.6 (C(7a)), 122.1 (C(6)), 102.8 (C(5)), 101.8 (C(4a)), 83.1 (d, $J = 170.2$, C(3')), 80.13 (dd, $J = 18.7$, 11.5, C(2')), 68.1 (d, $J = 136.9$, C(4')), 66.6, 66.5, 65.8 ($\text{OCH}_2(\text{CH}_2)_3\text{CH}_3$), 51.1, 50.5 (C- α -Asp), 44.86 (d, $J = 8.1$, C(1')), 40.0, 39.9, 39.8 (d, $J = 3.9$, C- β -Asp), 29.1, 29.0, 28.8 ($\text{OCH}_2(\text{CH}_2)_2\text{CH}_2\text{CH}_3$), 23.1, 23.0 ($\text{O}(\text{CH}_2)_3\text{CH}_2\text{CH}_3$), 14.3 ($\text{O}(\text{CH}_2)_4\text{CH}_3$); ^{31}P NMR (121 MHz, CD_3CN): 21.5; HR-MS: 829.4298 ($[\text{M}-\text{H}]^-$, $\text{C}_{38}\text{H}_{63}\text{FN}_6\text{O}_{11}\text{P}^-$, calc.829.4282).

Acknowledgments

Min Luo greatly acknowledges the China Scholarship Council (CSC) for funding. We thank Eric Fonteyn, Sandra Claes and Evelyne Van Kerckhove for excellent technical assistance in the generation of the anti-HIV-1 data.

Author Contribution Statement

M. L. synthesized the compounds. E. G. wrote the article. P. H. conceived the study. S. D. J., and D. S. contributed to the biological evaluation.

References

- [1] E. De Clercq, A. Holý, 'Acyclic nucleoside phosphonates: a key class of antiviral drugs', *Nat. Rev. Drug Discovery* **2005**, *4*, 928-940.
- [2] U. Pradere, E. C. Garnier-Amblard, S. J. Coats, F. Amblard, R. F. Schinazi, 'Synthesis of nucleoside phosphate and phosphonate prodrugs', *Chem. Rev.* **2014**, *114*, 9154-9218.
- [3] J. Jindřich, A. Holý, H. Dvořáková, 'Synthesis of *N*-(3-fluoro-2-phosphonomethoxypropyl)(FPMP) derivatives of heterocyclic bases', *Collect. Czechoslov. Chem. Commun.* **1993**, *58*, 1645-1667.

- [4] J. Balzarini, A. Holy, J. Jindrich, L. Naesens, R. Snoeck, D. Schols, E. De Clercq, 'Differential antiherpesvirus and antiretrovirus effects of the (*S*) and (*R*) enantiomers of acyclic nucleoside phosphonates: potent and selective *in vitro* and *in vivo* antiretrovirus activities of (*R*)-9-(2-phosphonomethoxypropyl)-2, 6-diaminopurine', *Antimicrob. Agents Chemother.* **1993**, 37, 332-338.
- [5] R. Heijntink, J. Kruining, G. De Wilde, J. Balzarini, E. De Clercq, S. Schalm, 'Inhibitory effects of acyclic nucleoside phosphonates on human hepatitis B virus and duck hepatitis B virus infections in tissue culture', *Antimicrob. Agents Chemother.* **1994**, 38, 2180-2182.
- [6] K. Sekiya, H. Takashima, N. Ueda, N. Kamiya, S. Yuasa, Y. Fujimura, M. Ubasawa, '2-Amino-6-arylthio-9-[2-(phosphonomethoxy) ethyl] purine bis (2, 2, 2-trifluoroethyl) esters as novel HBV-specific antiviral reagents', *J. Med. Chem.* **2002**, 45, 3138-3142.
- [7]. A. B. Eldrup, M. Prhavc, J. Brooks, B. Bhat, T. P. Prakash, Q. Song, S. Bera, N. Bhat, P. Dande, P. D. Cook, 'Structure-activity relationship of heterobase-modified 2'-C-methyl ribonucleosides as inhibitors of hepatitis C virus RNA replication', *J. Med. Chem.* **2004**, 47, 5284-5297.
- [8] M. M. Bio, F. Xu, M. Waters, J. M. Williams, K. A. Savary, C. J. Cowden, C. Yang, E. Buck, Z. J. Song, D. M. Tschaen, 'Practical synthesis of a potent hepatitis C virus RNA replication inhibitor', *J.Org.Chem.* **2004**, 69, 6257-6266.
- [9] S. S. Carroll, S. Ludmerer, L. Handt, K. Koeplinger, N. R. Zhang, D. Graham, M.-E. Davies, M. MacCoss, D. Hazuda, D. B. Olsen, 'Robust antiviral efficacy upon administration of a nucleoside analog to hepatitis C virus-infected chimpanzees', *Antimicrob. Agents Chemother.* **2009**, 53, 926-934.

- [10] Y.-L. Chen, Z. Yin, J. Duraiswamy, W. Schul, C. C. Lim, B. Liu, H. Y. Xu, M. Qing, A. Yip, G. Wang, 'Inhibition of dengue virus RNA synthesis by an adenosine nucleoside', *Antimicrob. Agents Chemother.* **2010**, *54*, 2932-2939.
- [11] B. K. Bhattacharya, J. O. Ojwang, R. F. Rando, J. H. Huffman, G. R. Revankar, 'Synthesis and anti-dna viral activities *in vitro* of certain 2, 4-disubstituted-7-(2-deoxy-2-fluoro-. beta.-D-arabinofuranosyl) pyrrolo [2, 3-*d*] pyrimidine nucleosides', *J. Med. Chem.* **1995**, *38*, 3957-3966.
- [12] D. B. Olsen, A. B. Eldrup, L. Bartholomew, B. Bhat, M. R. Bosserman, A. Ceccacci, L. F. Colwell, J. F. Fay, O. A. Flores, K. L. Getty, 'A 7-deaza-adenosine analog is a potent and selective inhibitor of hepatitis C virus replication with excellent pharmacokinetic properties', *Antimicrob. Agents Chemother.* **2004**, *48*, 3944-3953.
- [13] S. M. Bennett, N. B. Nghe, K. K. Ogilvie, 'Synthesis and antiviral activity of some acyclic and C-acyclic pyrrolo [2, 3-*d*] pyrimidine nucleoside analogs', *J. Med. Chem.* **1990**, *33*, 2162-2173.
- [14] M.-S. Xie, H.-Y. Niu, G.-R. Qu, H.-M. Guo, 'The development for the synthesis of chiral acyclic nucleosides and their phosphonates', *Tetrahedron Lett.* **2014**, *55*, 7156-7166.
- [15] M. Luo, E. Groaz, G. Andrei, R. Snoeck, R. Kalkeri, R. G. Ptak, T. Hartman, R. W. Buckheit Jr, D. Schols, S. De Jonghe, 'Expanding the antiviral spectrum of 3-fluoro-2-(phosphonomethoxy) propyl acyclic nucleoside phosphonates: diamyl aspartate amidate prodrugs', *J. Med. Chem.* **2017**, *60*, 6220-6238.
- [16] O. Baszczyński, P. Jansa, M. Dračinský, B. Klepetářová, A. Holý, I. Votruba, E. De Clercq, J. Balzarini, Z. Janeba, 'Synthesis and antiviral activity of *N*⁹-[3-fluoro-2-

- (phosphonomethoxy) propyl] analogues derived from N^6 -substituted adenines and 2, 6-diaminopurines', *Biorg. Med. Chem.* **2011**, *19*, 2114-2124.
- [17] F. Seela, X. Peng, 'Regioselective syntheses of 7-halogenated 7-deazapurine nucleosides related to 2-amino-7-deaza-2'-deoxyadenosine and 7-deaza-2'-deoxyisoguanosine', *Synthesis* **2004**, *2004*, 1203-1210.
- [18] F. Seela, K. Xu, P. Chittep, 'Fluorinated pyrrolo [2, 3-*d*] pyrimidine nucleosides: 7-fluoro-7-deazapurine 2'-deoxyribofuranosides and 2'-deoxy-2'-fluoroarabinofuranosyl derivatives', *Synthesis* **2006**, *2006*, 2005-2012.
- [19] S. Llona-Minguez, S. P. Mackay, 'Stereoselective synthesis of carbocyclic analogues of the nucleoside Q precursor (PreQ₀)', *Beilstein J. Org. Chem.* **2014**, *10*, 1333.
- [20] M. M. Kaiser, D. Hocková, T. H. Wang, M. Dračinský, L. Poštová-Slavětínská, E. Procházková, M. D. Edstein, M. Chavchich, D. T. Keough, L. W. Guddat, 'Synthesis and evaluation of novel acyclic nucleoside phosphonates as inhibitors of plasmodium falciparum and human 6 - oxopurine phosphoribosyltransferases', *ChemMedChem* **2015**, *10*, 1707-1723.
- [21] T. Brückl, F. Klepper, K. Gutsmedl, T. Carell, 'A short and efficient synthesis of the tRNA nucleosides PreQ₀ and archaeosine', *Org. Biomol. Chem.* **2007**, *5*, 3821-3825.
- [22] D. C. Montefiori, 'Measuring HIV neutralization in a luciferase reporter gene assay', *Methods Mol Biol.* **2009**, 395-405.

Table of Contents