Synthesis and Antiviral Evaluation of Base-modified Deoxythreosyl Nucleoside Phosphonates

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ABSTRACT: L- α -2'-deoxythreosyl nucleoside phosphonates and their phosphonodiamidate prodrugs with a hypoxanthine, 2,6-diaminopurine, 2-amino-6-cyclopropylaminopurine, 7-deazaadenine, 5-fluorouracil and 5-methylcytosine heterocycle as nucleobase were synthesized and evaluated for their inhibitory activity against HIV and HBV. The 2,6-diaminopurine modified analogue **23a** displayed the most potent activity against HIV, with an EC₅₀ value of 11.17 μ M against HIV-1 (III_B) and an EC₅₀ value of 8.15 μ M against HIV-2 (ROD). The application of the prodrug strategy on nucleoside phosphonate **23a** led to a 200-fold boost in anti-HIV potency. None of the compounds showed any activity against HBV at the highest concentration tested.

Introduction

Nucleoside phosphonates are a key class of drugs for the treatment of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infected patients.¹ Examples of commonly prescribed nucleotide analogues are adefovir dipivoxil (a phosphonate nucleoside ester prodrug of adefovir), tenofovir disoproxil (an ester based prodrug of the nucleoside phosphonate tenofovir) and tenofovir alafenamide (a phosphonoamidate prodrug of tenofovir).

Fig. 1 Approved nucleoside phosphonate prodrugs.

Once inside the cell, nucleoside analogues undergo three consecutive phosphorylations yielding the biologically active triphosphate analogues, which can be incorporated in the viral genome, causing chain termination. The first phosphorylation step is very often inefficient and rate limiting in the conversion to the triphosphate. In theory, the administration of a nucleoside monophosphate analogue allows to circumvent this first phosphorylation. However, these nucleotides are potential substrates for phosphatases that cleave off the phosphate group. Replacement of this phosphate moiety by an isosteric and isoelectronic phosphonate group yields a chemically and metabolically stable nucleoside phosphonate that bypasses the initial enzymatic phosphorylation step. Moreover, nucleoside phosphonates have a long intracellular half-life, which allows infrequent dosing. In addition, the free phosphonate moiety allows to apply different prodrug strategies in order to boost antiviral potency and to optimize pharmacokinetics of the parent compound.

Structurally, nucleoside phosphonate analogues are divided in two major groups, the acyclic nucleoside phosphonates (ANPs) and the cyclic phosphonates (CNPs).² The pioneering work of Dr. Holý in the 80ies led to the synthesis and antiviral testing of a wide variety of ANPs. It ultimately gave rise to marketing of three ANPs, i.e. cidofovir, adefovir and tenofovir (the latter two as their orally bioavailable prodrug forms, Figure 1).³ On the other hand, biologically active CNPs are described to a much lesser extent. The best-known examples are d4AP⁴ and its

fluorinated counterpart 2'Fd4AP⁵, which are both endowed with promising anti-HIV activity (Figure 2). In previous studies, we reported the synthesis of series of L- α -2'-deoxythreosyl phosphonate nucleosides.^{6,7} Phosphonomethoxydeoxythreosyl adenine (PMDTA) and phosphonomethoxydeoxythreosyl thymine (PMDTT) (Figure 2) were the most potent congeners and they exhibited potent antiviral activity *in vitro* against HIV-1, HIV-2 and HBV. It has been demonstrated that PMDTA diphosphate is an efficient substrate for HIV-1 reverse transcriptase, whereas it is a poor substrate for human DNA polymerase α .⁶

Fig. 2 Selected examples of biologically active cyclic nucleoside phosphonates.

The promising antiviral activity of this class of compounds encouraged us to further study the structure-activity relationship (SAR) of deoxythreosyl nucleoside phosphonates as antiviral agents. Up to now, all structural modifications that have been endorsed encompass modifications of the sugar moiety and includes a 2'-fluoro-threose sugar,^{7,8} different stereochemistry of the phosphonomethoxygroup at position 3',⁹ insertion of different substituents at positions 2' and 4' of the threose sugar moiety⁹ and different linkers between the phosphonate moiety and the threose unit.¹⁰ None of these sugar modified L- α -2'-deoxythreosyl phosphonate nucleosides displayed any antiviral activity.

To date, base-modified L- α -2'-deoxythreosyl phosphonate nucleosides have not been explored. However, modification of the nucleobase moiety has been shown to be a successful strategy in the discovery of biologically active nucleosides (Figure 3). Didanosine (ddI) and abacavir (ABC) are both marketed anti-HIV drugs and bear a hypoxanthine and 2-amino-6-cyclopropylaminopurine as nucleobase, respectively.¹¹ Amdoxovir, with a 2,6-diaminopurine nucleobase, is a HIV reverse transcriptase inhibitor that underwent clinical trials.^{12,13} 5-Fluorouracil is the base moiety of the anticancer drug floxuridine, which is often used when colorectal cancer is advanced and has metastasized to the liver.¹⁴ Pyrrolo[2,3-d]pyrimidines (or 7-deaza-purines) have been extensively

investigated. 7-Deaza-2'-*C*-methyladenosine is an inhibitor of HCV replication,¹⁵⁻¹⁷ and the structurally similar 7-deaza-2'-*C*-ethynyladenosine is a potent inhibitor of dengue virus type 2.¹⁸ 2'-Deoxy-2'-fluoro- β -D-arabinofuranosyl-5-methylcytosine (FMAC) is endowed with promising antiviral activity against herpes simplex virus.^{19,20} Herein, we present the synthesis and antiviral evaluation of novel base-modified L- α -2'-deoxythreosyl nucleoside phosphonates with a hypoxanthine (Hx), 2,6-diaminopurine (DAP), 2-amino-6-cyclopropylaminopurine (cPrDAP), 7-deazaadenine (7-deazaA), 5-fluorouracil (5FU) or 5-methylcytosine (5mC) heterocycle as nucleobase.

Fig. 3 Selected examples of biologically active base-modified nucleosides.

The acidic phosphonate groups are ionized at physiological pH, and hence, nucleoside phosphonates hardly penetrate the lipid-rich cell membrane, which hampers their antiviral activity. Our laboratory previously demonstrated that the corresponding aryloxyphosphonoamidate and phosphonobisamidate ProTides of PMDTA and PMDTT are endowed with low nanomolar activity against HIV and HBV.⁷ Especially, the phosphonobisamidate prodrugs are attractive. In contrast with aryloxyphosphonoamidate prodrugs, phosphonobisamidate prodrugs avoid the formation of diastereomeric mixtures, as two identical amino acid esters are attached to the phosphorus centre. In addition, the byproducts of cleavage of this type of prodrug consist exclusively of non-toxic amino acids, whereas aryloxyphosphonoamidate prodrugs also release toxic phenol. Therefore, the base-modified L- α -2'-deoxythreosyl nucleoside phosphonates synthesized in this manuscript were converted to their corresponding phosphonobisamidate prodrug, using L-phenylalanine *n*-propyl

ester as the amino acid motif, as this was shown before to be beneficial for anti-HIV and anti-HBV activity.⁷

Results and discussion

Chemistry

Synthesis of a hypoxanthine L- α -2'-deoxythreosyl phosphonate

The synthesis of a hypoxanthine containing L- α -2'-deoxythreosyl phosphonate nucleoside is shown in Scheme 1. A Vorbrüggen-type coupling of 6-chloropurine with the known 1',2'-diacylated L-threose sugar **1**^{7,9} in the presence of TMSOTf and DBU afforded threoside **2** in 91% yield. Deprotection of the silyl protecting group with Et₃N·3HF gave nucleoside **3**, which was subjected to sodium hydride mediated phosphonomethylation reaction to afford phosphonate nucleoside **4**. Simultaneous deprotection of the 2'-benzoyl group and transformation of the 6-chloropurine to hypoxanthine was achieved by refluxing **4** with 2-mercaptoethanol and sodium methoxide. The 2'-deoxygenation of **5** was carried out under standard Barton deoxygenation conditions. Finally, Lewis acid mediated cleavage of the phosphonate esters of **6** afforded nucleoside phosphonic acid **7**. The bisamidate prodrug **8** was prepared from **7** using 2,2'-dithiodipyridine and triphenylphosphine as activating agents and L-phenylalanine *n*-propyl ester as a coupling partner.

Scheme 1 Synthesis of a hypoxanthine L- α -2'-deoxythreosyl phosphonate. Reagents and conditions: (a) 6-chloropurine, DBU, TMSOTf, 60 °C, 1.5 h, 91%; (b) Et₃N·3HF, THF, rt, overnight, 98%; (c) (iPrO)₂POCH₂OTf, NaH, THF, –5 to 0 °C, 30 min, 84%; (d) 2-mercaptoethanol, NaOMe, MeOH, reflux, 20 h, 78%; (e) (1) TCDI, DMAP, CH₂Cl₂, rt, overnight; (2) Bu₃SnH, AIBN, toluene, reflux, 20 min, 89%; (f) TMSBr, 2,6-lutidine, CH₃CN, rt, overnight, 40%; (g) L-phenylalanine-*O*-*n*-Pr ester hydrochloride, Et₃N, 2,2'-dithiodipyridine, PPh₃, pyridine, 60 °C, overnight, 60%.

Synthesis of a 7-deazaadenine L- α -2'-deoxythreosyl phosphonate

For the synthesis of a 7-deazaadenine containing L- α -2'-deoxythreosyl phosphonate nucleoside, a similar strategy as for the synthesis of the hypoxanthine congener was initially envisioned (Scheme 2). Surprisingly, coupling of 6-chloro-7-deazapurine with threose sugar **1** under Vorbrüggen glycosylation conditions failed to give the desired *N*-9 glycosylated nucleoside **9**. On the other hand, using 7-deaza-*N*⁶-benzoyladenine as nucleobase furnished mainly the *N*-1 glycosylated nucleoside **11**. The observation of HMBC correlations of H-1' with C-2 and C-6

indicated that N-1 was connected to the sugar moiety, and the observation of COSY correlations of the active hydrogen with H-8 and H-7 indicated that the active hydrogen was attached to N-9. Selective removal of the 2'-benzoyl group under basic conditions afforded nucleoside **12** in 79% yield. The structure of compound **12** was confirmed using the same approach as described for compound **11**. Due to the particular properties of 7-deazapurine system, the nitrogen in pyrrole ring is rather inert against Lewis acid catalyzed glycosylation. Therefore, 6-chloro-7-iodo-7-deazapurine (Scheme 3) was selected as a coupling partner as it has been often used for the synthesis of 6 and/or 7-substituted 7-deazapurine nucleoside analogues.²¹⁻²⁴

Scheme 2 Attempted synthesis of a 7-deazaadenine L- α -2'-deoxythreosyl phosphonate. Reagents and conditions: (a) 7-deaza-*N*⁶-benzoyladenine, BSA, TMSOTf, 95 °C, 1.5 h, 63%; (b) NaOH, THF/MeOH/H₂O, 0 °C, 4 h, 79%.

Scheme 3 Synthesis of a 7-deazaadenine L- α -2'-deoxythreosyl phosphonate. Reagents and conditions: (a) 6-chloro-7-iodo-7-deazapurine, DBU, TMSOTf, 60 °C, 1.5 h, 52%; (b) i PrMgCl·Cl, THF, –78 °C, 20 min; (c) 7 N NH₃ in MeOH, rt, overnight, 91% for 2 steps; (d) (1) TCDI, DMAP, CH₂Cl₂, rt, overnight; (2) Bu₃SnH, AIBN, toluene, reflux, 20 min, 80%; (e) Et₃N·3HF, THF, rt, overnight, 83%; (f) (i PrO)₂POCH₂OTf, NaH, THF, –5 to 0 °C, 30 min, 84%; (g) 7 N NH₃ in MeOH, 120 °C, 16 h, 70%; (h) TMSBr, 2,6-lutidine, CH₃CN, rt, overnight, 54%; (i) L-phenylalanine-*O*-*n*-Pr ester hydrochloride, Et₃N, 2,2'-dithiodipyridine, PPh₃, pyridine, 60 °C, overnight, 36%.

Vorbrüggen coupling of L-threose sugar **1** with 6-chloro-7-iodo-7-deazapurine afforded iodonucleoside **13** in 52% yield. Deiodination of nucleoside **13** via selective iodine/magnesium exchange followed by cleavage of the 2'-benzoyl group with ammonia at room temperature afforded nucleoside **14** in 91% yield. Removal of the 2' hydroxyl of **14** was accomplished under standard Barton deoxygenation conditions. Deprotection of the silyl group of **15**, was followed by introduction of the phosphonate moiety affording the nucleoside phosphonate analogue **17**. Treatment of **17** with 7 N NH₃ in methanol gave nucleoside **18**. Treatment of **18** with a Lewis acid (TMSBr or TMSI) led to cleavage of the phosphonate ester groups, but, surprisingly, a concomitant

unexpected sugar ring opening occurred, yielding an alkenyl type of acyclic nucleoside phosphonate **19** was formed. Based on the vicinal coupling constant ($^3J_{H-1',H-2'} = 14.4$ Hz), the geometry of the double bond was found to be (*E*). Finally, coupling of nucleoside **19** with L-phenylalanine *n*-propyl ester gave the cyclic phosphonamidates as a mixture of diastereoisomers (**20a** and **20b**) in a ratio of ca. 2:1. The two isomers were successfully separated by reversed phase HPLC.

Synthesis of a 2,6-diaminopurine and a 2-amino-6-cyclopropylaminopurine L- α -2'-deoxythreosyl phosphonate

The 2,6-diaminopurine modified derivatives **22a** and **22b** (Scheme 4) were prepared by treatment of nucleoside **21**⁸ with 7 N ammonia and cyclopropylamine, respectively. After cleavage of the isopropyl ester groups, the resulting nucleoside phosphonates **23a** and **23b** were condensed with the appropriate amino acid ester to afford the desired bisamidate prodrugs **24a** and **24b**.

Scheme 4 Synthesis of a 2,6-diaminopurine and a 2-amino-6-cyclopropylaminopurine L- α -2'-deoxythreosyl phosphonate. Reagents and conditions: (a) 7 N NH₃ in MeOH, 100 °C, 4 h, 70%; (b) cyclopropylamine, CH₃CN, 100 °C, 4 h, 91%; (c) TMSBr, 2,6-lutidine, CH₃CN, rt, overnight, 60% for **23a** and 62% for **23b**; (d) L-phenylalanine-*O*-*n*-Pr ester hydrochloride, Et₃N, 2,2'-dithiodipyridine, PPh₃, pyridine, 60 °C, overnight, 53% for **24a** and 67% for **24b**.

Synthesis of a 5-fluorouracil L- α -2'-deoxythreosyl phosphonate

The 2'-deoxy threose nucleoside **29** (Scheme 5) was synthesized following a similar procedure as described for nucleoside phosphonate **6**. While Lewis acid (TMSBr or TMSI) mediated hydrolysis of the phosphonate ester groups of **29** were unsuccessful, cleavage of the glycosidic bond did happen.

Scheme 5 Synthesis of a 5-fluorouracil L- α -2'-deoxythreosyl phosphonate. Reagents and conditions: (a) 5-fluorouracil, BSA, TMSOTf, 60 °C, 1 h, 88%; (b) Et₃N·3HF, THF, rt, overnight, 90%; (c) (iPrO)₂POCH₂OTf, NaH, THF, -5 to 0 °C, 40 min, 52%; (d) 2 N NH₃ in MeOH, rt, overnight, 94%; (e) (1) TCDI, DMAP, CH₂Cl₂, rt, overnight; (2) Bu₃SnH, AIBN, toluene, reflux, 20 min, 92%; (f) TMSBr or TMSI, 2,6-lutidine, CH₃CN, rt, overnight, no product.

In contrast, hydrolysis of the phosphonate ester functions of compound **28** afforded nucleoside phosphonate **31** (Scheme 6) from **28** under the standard Lewis acid conditions. Coupling of **31**

with L-phenylalanine *n*-propyl ester gave 39% of bisamidate prodrug **32** and ca.15% of a single diastereomer of 2',3'-diol cyclized phosphonamidate prodrug **33**. To avoid the formation of side product **33**, the 2'-hydroxyl group was protected as a *tert*-butyldimethylsilyl (TBS) group by treatment of nucleoside **28** with TBSCl in the presence of imidazole furnishing the silyl protected nucleoside **34** in 89% yield. Hydrolysis of the phosphonate esters, was followed by coupling with the amino acid ester and removal of the silyl protecting group, giving rise to nucleoside **32** in a higher yield. Finally, 2'-deoxygenation of **32** under standard Barton deoxygenation conditions afforded bisamidate prodrug **36**.

Scheme 6 Synthesis of a 5-fluorouracil L- α -2'-deoxythreosyl phosphonate. Reagents and conditions: (a) TMSBr, 2,6-lutidine, CH₃CN, rt, overnight, 68% for **31** and 67% for **35**; (b) L-phenylalanine-*O*-*n*-Pr ester hydrochloride, 2,2'-dithiodipyridine, PPh₃, pyridine, 60 °C, overnight, 39% for **32** and 15% for **33**; (c) TBSCl, imidazole, DMF, rt, overnight, 89%; (d) Et₃N·3HF, THF, rt, overnight, 69% from **35**; (e) (1) TCDI, DMAP, CH₂Cl₂, rt, overnight. (2) Bu₃SnH, AIBN, toluene, reflux, 20 min, 90%.

Synthesis of a 5-methylcytosine L- α -2'-deoxythreosyl phosphonate

The synthesis of this derivative starts from the known thymidine nucleoside phosphonate **37**.⁷ Treatment of **37** with *p*-toluenesulfonyl chloride (TsCl) in the presence of *N*-methylpyrrolidine

was followed by aqueous ammonia. Hydrolysis of the phosphonate esters of **38** was accomplished by treatment of **38** with TMSI in the presence of 2,6-lutidine to furnish target compound **39**, which was condensed with L-phenylalanine *n*-propyl ester to afford prodrug **40**.

Scheme 7 Synthesis of a 5-methylcytosine L- α -2'-deoxythreosyl phosphonate. Reagents and conditions: (a) (1) TsCl, *N*-Methylpyrrolidine, Et₃N, CH₂Cl₂, rt, 2 h. (2) aq. NH₄OH, rt, overnight, 75%; (b) TMSI, 2,6-lutidine, CH₃CN, rt, overnight, 51%; (c) L-phenylalanine-*O-n*-Pr ester hydrochloride, 2,2'-dithiodipyridine, PPh₃, pyridine, 60 °C, overnight, 30%.

Antiviral activity

The nucleoside phosphonates, as well as their phosphonodiamidate prodrugs were evaluated for anti-HIV activity using the MTT method in MT-4 cells infected with a wild-type HIV-1 strain III_B and a wild-type HIV-2 strain (ROD). AZT and Lamivudine were included as positive controls, whereas PMDTA and PMDTT were included as reference compounds. Cytotoxicity in the MT-4 cell line was determined simultaneously (Table 1).^{25, 26} Most compounds completely lacked anti-HIV activity, demonstrating that only the natural nucleobases adenine and thymine are tolerated for antiviral activity. The only exception is the 2,6-diaminopurine base modified analogue **23a**, which exhibited anti-HIV activity at micromolar concentrations (EC₅₀ of 11.2 μ M against HIV-1 and EC₅₀ of 8.15 μ M against HIV-2). This is an approximate two-fold drop in anti-HIV activity,

when compared to PMDTA. No cytotoxicity was observed at the highest tested concentration ($CC_{50} > 378 \mu\text{M}$). Substitution of the 6-amino group in nucleoside **23a** with a cyclopropylamino moiety afforded compound **23b**, which is completely devoid of antiviral activity. The application of a prodrug strategy led to a boost in anti-HIV potency. Compound **24a**, the bisamidate prodrug of **23a**, showed a 230-fold increased activity against HIV-1 and a 270-fold improvement against HIV-2, displaying EC_{50} values of 0.048 and 0.03 μM , respectively. Prodrug **24a** was about 6 times more cytotoxic than the parent phosphonate **23a**, but the potent antiviral activity still gives rise to favorable selectivity indexes (CC_{50}/EC_{50}) of 1264 (for HIV-1) and 2095 (for HIV-2). Analogously, although no activity against HIV was observed for nucleoside phosphonate **23b**, its prodrug (compound **24b**) exhibited low micromolar activity with an EC_{50} of 3.63 μM against HIV-1 and an EC_{50} of 3.99 μM against HIV-2. None of the other base modified analogues showed any activity against HIV. This lack of activity can be explained by either the fact that they cannot be converted to their diphosphate form or that once phosphorylated to the triphosphate analogue the compounds are poor substrates for HIV reverse transcriptase.

Table 1. *In Vitro* Anti-HIV activity and cytotoxicity of base-modified deoxythreosyl nucleoside phosphonates.

Cmpd	B	R	EC_{50}^a (μM)		CC_{50}^b (μM)	SI^c	
			HIV-1	HIV-2		HIV-1	HIV-2
			(III _B)	(ROD)			
7	Hx	OH	> 395	> 395	> 395		
8	Hx	Phe-nPr	> 95.1	> 95.1	95.1		
19	7-deazaA	OH	> 398	> 398	> 398		
20a	7-deazaA	Phe-nPr	> 66.0	> 66.0	66.0		
20b	7-deazaA	Phe-nPr	> 38.8	> 38.8	38.8		
23a	DAP	OH	11.17	8.15	> 378	> 34	> 47
24a	DAP	Phe-nPr	0.048	0.030	61.4	1264	2095

23b	cPrDAP	OH	> 338	> 338	>338		
24b	cPrDAP	Phe-nPr	3.63	3.99	60.1	17	15
36	5FU	Phe-nPr	> 64.0	> 64.0	64.0		
39	5mC	OH	> 410	> 410	> 410		
40	5mC	Phe-nPr	≥ 23.4	16.7	85.5	≤ 4	5
PMDTA ⁷	A	OH	4.69	5.23	≥ 315	≥ 67	≥ 60
PMDTT ⁷	T	OH	27.07	23.55	297	11	13
3TC			2.53	9.90	> 87.2	> 34	> 9
AZT			0.0075	0.0082	> 7.48	> 1004	> 925

^aEC₅₀: effective concentration required to achieve 50% protection of MT-4 cells against HIV-induced cytopathicity. ^bCC₅₀: cytotoxic concentration required to reduce the viability of mock-infected cells by 50%. ^cSI: selectivity index, CC₅₀/EC₅₀

Given the potent activity of PMDTA and PMDTT against HBV,⁷ the anti-HBV activity of the nucleobase modified compounds was assessed in HepG2 2.2.15 cell line, which is a stable human hepatoblastoma cell line that contains two copies of the HBV wild-type strain ayw1 genome and constitutively produces high levels of HBV.²⁷ A quantitative blot hybridization assay was used to measure extracellular HBV DNA copy number associated with virions released from HepG2 2.2.15 cell. A tetrazolium dye uptake assay was used to measure cell viability and to calculate CC₅₀ values.^{28, 29} Unfortunately, none of these compounds showed any activity against HBV at the highest tested concentration (100 μM).

Conclusion

In summary, a number of base-modified deoxythreosyl nucleoside phosphonates and their phosphonobisamidate prodrugs was prepared, and their anti-HIV and anti-HBV activities were evaluated. The 2,6-diaminopurine containing deoxythreosyl nucleoside phosphonate **23a** exhibited the most potent activity against HIV-1 and HIV-2, with EC₅₀ values of 11.2 μM and 8.15 μM, respectively. The synthesis of a prodrug of nucleoside phosphonate **23a** afforded phosphonobisamidate prodrug **24a** that displayed an improved anti-HIV activity (EC₅₀ = 48 nM for HIV-1 and 30 nM for HIV-2). None of the other base modified analogues exhibited potent activity against HIV and all studied deoxythreosyl nucleoside phosphonates completely lacked activity against HBV.

Experimental Section

General information

All reagents and solvents were purchased from commercial sources and used as received. Moisture sensitive reactions were performed under an argon or nitrogen atmosphere using oven-dried glassware. Reactions were monitored by thin-layer chromatography (TLC) on precoated aluminum foil (silica gel matrix, with fluorescence indicator 254 nm). ^1H , ^{13}C and ^{31}P NMR spectra were recorded on a 300, 500 or 600 MHz Bruker Advance spectrometer with tetramethylsilane as internal standard or referenced to the residual solvent signal, and 85% H_3PO_4 for ^{31}P NMR. Two-dimensional (2D) NMRs (H-COSY, HSQC, and HMBC) were used for the assignment of the intermediates and final compounds. Chemical shifts are reported in parts per million. Splitting patterns are described as singlet (s), broad singlet (brs), doublet (d), triplet (t), double of doublets (dd), and multiplet (m). High resolution mass spectra (HRMS) were recorded on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 $\mu\text{L}/\text{min}$, and spectra were obtained in positive or negative ionization mode with a resolution of 15000 (fwhm) using leucine enkephalin as lock mass. Preparative high performance liquid chromatography (HPLC) purifications were carried out on a Phenomenex Gemini 110A column (C18, 10 μm , 21.2 mm $\times$ 250 mm). Column chromatography was performed on silica gel 60 $\text{\AA}$, 0.035–0.070 mm (Acros Organics).

1' α -(6-chloropurin-9-yl)-2'-O-benzoyl-3'-O-*tert*-butyldimethylsilyl-L-threose (2). A solution of threose sugar **1** (0.20 g, 0.53 mmol) and 6-chloropurine (90 mg, 0.58 mmol) in 6 mL of dry acetonitrile was treated with DBU (0.24 mL, 1.58 mmol) followed by TMSOTf (0.38 mL, 2.10 mmol) at 0 $^\circ\text{C}$. The resulting reaction mixture was stirred at 60 $^\circ\text{C}$ for 1.5 h. The reaction mixture was then cooled to rt and poured into an ice-cold stirred mixture of sat. aq. NaHCO_3 and AcOEt. The organic layer was separated and washed with brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography (20:1 to 10:1, $\text{CH}_2\text{Cl}_2/\text{EtOAc}$) to obtain **2** (0.23 g, 91% yield) as a white solid. ^1H NMR (500 MHz, CDCl_3): δ 8.76 (s, 1H, H-2), 8.58 (s, 1H, H-8), 8.09 (d, J = 8.2 Hz, 2H, ArH), 7.64 (t, J = 7.4 Hz, 1H, ArH), 7.50 (t, J = 7.4 Hz, 2H, ArH), 6.49 (s, 1H, H-1'), 5.60 (s, 1H, H-2'), 4.55 (d, J = 3.0 Hz, 1H, H-3'), 4.39 (dd, J = 9.9, 3.6 Hz, 1H, H-4a'), 4.34 (d, J = 9.9 Hz, H-4b'), 0.86 [s, 9H, $\text{C}(\text{CH}_3)_3$], 0.16 (s, 3H, SiCH_3), 0.06 (s, 3H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 165.0 (CO), 152.1 (C-2), 151.2

(C-4), 151.0 (C-6), 144.2 (C-8), 134.0 (Ar-C), 131.8 (C-5), 129.9, 128.7, 128.6 (Ar-C), 88.5 (C-1'), 82.6 (C-2'), 77.0 (C-4'), 75.0 (C-3'), 25.6 [C(CH₃)₃], 17.9 [C(CH₃)₃]. HRMS: [M+H]⁺ calcd for C₂₂H₂₈ClN₄O₄Si, 475.1563; found, 475.1560.

1'α-(6-chloropurin-9-yl)-2'-O-benzoyl-L-threose (3). To a solution of nucleoside **2** (0.16 g, 0.34 mmol) in 4 mL of anhydrous THF at rt was added triethylamine trihydrofluoride (0.11 mL, 0.67 mmol). The reaction mixture was stirred at rt for overnight. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and sat. aq. NaHCO₃. The organic layer was separated and washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography (5:1 to 3:1, CH₂Cl₂/EtOAc) to obtain **3** (0.12 g, 98% yield) as a white solid. ¹H NMR (600 MHz, CDCl₃): δ 8.79 (s, 1H, H-2), 8.40 (s, 1H, H-8), 8.05–8.03 (m, 2H, ArH), 7.66–7.63 (m, 1H, ArH), 7.51–7.48 (m, 2H, ArH), 6.12 (d, *J* = 1.7 Hz, 1H, H-1'), 5.96 (d, *J* = 9.4 Hz, 1H, OH), 5.56 (s, 1H, H-2'), 4.69–4.66 (m, 1H, H-3'), 4.40 (d, *J* = 10.3 Hz, 1H, H-4a'), 4.32 (dd, *J* = 10.3, 4.0 Hz, H-4b'). ¹³C NMR (150 MHz, CDCl₃): δ 165.9 (CO), 152.1 (C-6), 151.5 (C-2), 150.2 (C-4), 145.3 (C-8), 134.1 (Ar-C), 132.7 (C-5), 129.9, 128.7, 128.3 (Ar-C), 90.8 (C-1'), 84.3 (C-2'), 76.1 (C-4'), 74.6 (C-3'). HRMS: [M+H]⁺ calcd for C₁₆H₁₄ClN₄O₄, 361.0698; found, 361.0700.

1'α-(6-chloropurin-9-yl)-2'-O-benzoyl-3'-O-diisopropylphosphonomethyl-L-threose (4). To a solution of **3** (5.60 g, 15.5 mmol) and diisopropylphosphonomethyl trifluoromethanesulfonate (6.62 g, 20.2 mmol) in dry THF (150 mL) was added NaH (60% in oil, 0.75 g, 18.6 mmol) at –5 °C. The reaction mixture was stirred at 0 °C for 0.5 h. The reaction was quenched with 1 ml of acetic acid in EtOAc (100 mL). The residue was partitioned between water and EtOAc. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography (2:1 to 1:2, CH₂Cl₂/EtOAc) to afford **4** (7.0 g, 84% yield) as a white foam. ¹H NMR (500 MHz, CDCl₃): δ 8.75 (s, 1H, H-2), 8.57 (s, 1H, H-8), 8.08–8.08 (m, 2H, ArH), 7.64–7.63 (m, 1H, ArH), 7.52–7.48 (m, 2H, ArH), 6.52 (s, 1H, H-1'), 5.78 (s, 1H, H-2'), 4.79–4.71 [m, 2H, CH(CH₃)₂], 4.53 (d, *J* = 10.6 Hz, H-4a'), 4.49 (d, *J* = 4.0 Hz, 1H, H-3'), 4.39 (dd, *J* = 10.6, 4.2 Hz, 1H, H-4b'), 3.98–3.89 (m, 2H, PCH₂), 1.36–1.29 [m, 12H, CH(CH₃)₂]. ¹³C NMR (125 MHz, CDCl₃): δ 165.1 (CO), 152.1 (C-2), 151.4 (C-4), 151.1 (C-6), 144.1 (C-8), 134.1 (Ar-C), 131.7 (C-5), 129.9, 128.7, 128.4 (Ar-C), 88.3 (C-1'), 83.69 (d, ³*J*_{PC} = 9.5 Hz, C-3'), 80.1 (C-2'), 73.8 (C-4'), 71.5 [CH(CH₃)₂], 64.8 (d, ¹*J*_{PC} = 168.8 Hz, PCH₂), 24.0 [CH(CH₃)₂]. ³¹P NMR (121 MHz, CDCl₃): δ 17.5. HRMS: [M+H]⁺ calcd for C₂₃H₂₉ClN₄O₇P,

539.1457; found, 539.1455.

1' α -(Hypoxantin-9-yl)-3'-O-diisopropylphosphonomethyl-L-threose (5). A solution of **4** (40 mg, 0.074 mmol), 2-mercaptoethanol (23 mg, 0.30 mmol) and NaOMe (16 mg, 0.30 mmol) in 1.5 mL of dry MeOH was heated at 66 °C for 20 h. The reaction mixture was cooled to rt and concentrated under reduced pressure. The residue was purified by column chromatography (20:1 to 10:1, CH₂Cl₂/MeOH) to obtain **5** (24 mg, 78%) as a colorless oil. ¹H NMR (600 MHz, MeOD): δ 8.18 (s, 1H, H-8), 8.08 (s, 1H, H-2), 6.05 (d, J = 1.4 Hz, 1H, H-1'), 4.70–4.62 [m, 2H, CH(CH₃)₂], 4.64 (s, 1H, H-2'), 4.41 (d, J = 10.4 Hz, 1H, H-4a'), 4.28 (dd, J = 10.4, 4.1 Hz, H-4b'), 4.19–4.17 (m, 1H, H-3'), 3.95–3.84 (m, 2H, PCH₂), 1.33–1.26 [m, 12H, CH(CH₃)₂]. ¹³C NMR (150 MHz, MeOD): δ 158.9 (C-6), 149.6 (C-4), 146.8 (C-2), 140.5 (C-8), 125.3 (C-5), 92.4 (C-1'), 87.5 (d, ³ $J_{\text{P,C}}$ = 12.1 Hz, C-3'), 80.1 (C-2'), 74.0 (C-4'), 73.3 [CH(CH₃)₂], 64.9 (d, ¹ $J_{\text{P,C}}$ = 168.9 Hz, PCH₂), 24.3 [CH(CH₃)₂]. ³¹P NMR (121 MHz, MeOD): δ 19.2. HRMS: [M+H]⁺ calcd for C₁₆H₂₆N₄O₇P, 417.1533; found, 417.1531.

1' α -(Hypoxantin-9-yl)-2'-deoxy-3'-O-diisopropylphosphonomethyl-L-threose (6). To a solution of **5** (0.27 g, 0.65 mmol) and DMAP (24 mg, 0.19 mmol) in anhydrous CH₂Cl₂ (13 mL) was added 1,1'-thiocarbonyldiimidazole (TCDI) (0.23 g, 1.30 mmol) at rt. The reaction mixture was stirred at rt for overnight. The reaction mixture was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. To the residue in toluene (13 mL) was added azobisisobutyronitrile (AIBN) (43 mg, 0.26 mmol) and tributyltin hydride (0.70 mL, 2.59 mmol). The reaction mixture was refluxed for 20 min. The solvent was removed under reduced pressure and the residue was purified by column chromatography (20:1 to 10:1, CH₂Cl₂/MeOH) to afford **6** (0.23 g, 89% yield) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ 8.30 (s, 1H, H-8), 8.12 (s, 1H, H-2), 6.42 (dd, J = 7.7, 1.9 Hz, 1H, H-1'), 4.81–4.66 [m, 2H, CH(CH₃)₂], 4.46–4.43 (m, 1H, H-3'), 4.35 (d, J = 10.5 Hz, 1H, H-4a'), 4.05 (dd, J = 10.5, 4.1 Hz, H-4b'), 3.82–3.69 (m, 2H, PCH₂), 2.74–2.55 (m, 2H, H-2'), 1.34–1.29 [m, 12H, CH(CH₃)₂]. ¹³C NMR (75 MHz, CDCl₃): δ 158.6 (C-6), 148.5 (C-4), 145.2 (C-2), 139.0 (C-8), 124.4 (C-5), 83.6 (C-1'), 80.5 (d, ³ $J_{\text{P,C}}$ = 11.8 Hz, C-3'), 73.7 (C-4'), 71.7 [CH(CH₃)₂], 64.1 (d, ¹ $J_{\text{P,C}}$ = 169.6 Hz, PCH₂), 38.6 (C-2'), 23.9 [CH(CH₃)₂]. ³¹P NMR (121 MHz, CDCl₃): δ 18.8. HRMS: [M+H]⁺ calcd for C₁₆H₂₆N₄O₆P, 401.1584; found, 401.1585.

1' α -(Hypoxantin-9-yl)-2'-deoxy-3'-O-phosphonomethyl-L-threose (7). To a solution of **6** (0.23 g, 0.57 mmol) and 2,6-lutidine (0.54 mL, 4.60 mmol) in dry CH₃CN (12 mL) was added

bromotrimethylsilane (0.61 mL, 4.60 mmol) at 0 °C. The reaction mixture was stirred at rt for overnight and quenched with 1.0 M TEAB buffer (2 mL). The solvent was removed under reduced pressure. The residue was partitioned between water and EtOAc (1:1), the water layer was lyophilized and the residue was first purified by chromatography on a silica gel column (10:1:0 to 10:5:1, CH₂Cl₂/MeOH/1 M TEAB) to give the crude product. Further purification using preparative reverse phase HPLC with gradient CH₃CN in 0.05 M TEAB solution from 2% to 30% gave **7** (96 mg, 40%) as a white foam. ¹H NMR (300 MHz, D₂O): δ 8.55 (s, 1H, H-8), 8.19 (s, 1H, H-2), 6.42 (dd, *J* = 8.0, 2.3 Hz, 1H, H-1'), 4.55 (brs, 1H, H-3'), 4.36 (d, *J* = 10.0 Hz, 1H, H-4a'), 4.09 (dd, *J* = 10.0, 4.2 Hz, H-4b'), 3.55–3.51 (m, 2H, PCH₂), 2.85–2.65 (m, 2H, H-2'). ¹³C NMR (75 MHz, D₂O): δ 159.6 (C-6), 149.1 (C-4), 146.8 (C-2), 141.6 (C-8), 123.9 (C-5), 84.4 (C-1'), 80.0 (d, ³*J*_{P,C} = 10.4 Hz, C-3'), 74.4 (C-4'), 67.3 (d, ¹*J*_{P,C} = 150.7 Hz, PCH₂), 37.6 (C-2'). ³¹P NMR (121 MHz, D₂O): δ 13.4. HRMS: [M-H][−] calcd for C₁₀H₁₂N₄O₆P, 315.0500; found, 315.0486.

1'α-(Hypoxanthin-9-yl)-2'-deoxy-3'-O-[N,N'-bis(*n*-propyl-L-phenylalaninate)]methylphosphonobisamidate]-L-threose (8). To a mixture of **7** (30 mg, 0.072 mmol, Et₃N salt) and (*S*)-phenylalanine-*O*-*n*-Pr ester hydrochloride (105 mg, 0.43 mmol) in anhydrous pyridine (2 mL) was added Et₃N (0.1 mL). The resultant mixture was stirred at 60 °C under argon. In a separate flask, 2,2'-dithiodipyridine (111 mg, 0.50 mmol) and PPh₃ (132 mg, 0.50 mmol) were dissolved in anhydrous pyridine (1 mL) and stirred at rt for 15 min to give a light yellow solution, this reagent solution was then added to the solution of **7** in one portion. The reaction mixture was stirred at 60 °C for overnight under argon and then concentrated under reduced pressure. The resultant residue was first purified by chromatography on a silica gel column (20:1 to 10:1, DCM/MeOH) and then by preparative reverse phase HPLC (linear gradient, 30–95% CH₃CN in water to give **8** as a white solid (30 mg, 60%). ¹H NMR (300 MHz, acetone-*d*₆): δ 8.26 (s, 1H, H-8), 8.06 (s, 1H, H-2), 7.33–7.15 (m, 10H, ArH), 6.36 (dd, *J* = 7.8, 1.8 Hz, 1H, H-1'), 4.37–4.31 (m, 2H, H-4a' and H-3'), 4.30–4.19 (m, 1H, Phe-CH), 4.10–3.93 (m, 6H, H-4b', Phe-CH and OCH₂CH₂CH₃), 3.82 (t, *J* = 11.2 Hz, 1H, NH), 3.56–3.31 (m, 3H, PCH₂ and NH), 3.16–2.76 (m, 4H, Phe-CH₂), 2.76–2.67 (m, 1H, H-2a'), 2.45 (d, *J* = 14.7 Hz, 1H, H-2b'), 1.68–1.54 (m, 4H, OCH₂CH₂CH₃), 0.93–0.86 (m, 6H, OCH₂CH₂CH₃). ¹³C NMR (75 MHz, acetone-*d*₆): 174.2, 173.5 (Phe-CO), 157.3 (C-6), 148.9 (C-4), 146.1 (C-2), 139.6 (C-8), 138.5, 138.1, 130.64, 130.61, 129.2, 129.0, 127.41, 127.35 (Ar-C), 125.7 (C-5), 85.0 (C-1'), 81.3 (d, ³*J*_{P,C} = 12.1 Hz, C-3'), 74.3 (C-4'), 67.1 (OCH₂CH₂CH₃), 66.8 (d, ¹*J*_{P,C} = 136.3 Hz, PCH₂), 55.4, 54.8 (Phe-CH), 41.2,

41.1 (Phe-CH₂), 39.6 (C-2'), 22.6 (OCH₂CH₂CH₃), 10.7 (OCH₂CH₂CH₃). ³¹P NMR (121 MHz, acetone-*d*₆): δ 19.7. HRMS: [M+H]⁺ calcd for C₃₄H₄₄N₆O₈P, 695.2953; found, 695.2966.

4-Benzoylamino-3-(2'-*O*-benzoyl-3'-*O*-*tert*-butyldimethylsilyl- α -L-threofuranosyl)-7*H*-

pyrrolo[2,3-*d*]pyrimidine (11). A solution of **1** (130 mg, 0.34 mmol) and 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-benzamide (90 mg, 0.38 mmol) in 2 mL of dry toluene was treated with BSA (0.18 mL, 0.75 mmol). The reaction mixture was stirred at rt for 0.5 h, and then TMSOTf (0.10 mL, 0.51 mmol) was added. The resulting reaction mixture was stirred at 95 °C for 1.5 h. The reaction mixture was cooled to rt and poured into an ice-cold stirred mixture of sat. aq. NaHCO₃ and AcOEt. The organic layer was separated and washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography (5:1 CH₂Cl₂/EtOAc) to obtain **11** (120 mg, 63% yield) as a white foam. ¹H NMR (500 MHz, CDCl₃): δ 10.85 (s, 1H, NH), 8.80 (s, 1H, H-2), 8.35–8.34 (m, 4H, ArH), 7.87–7.31 (m, 6H, ArH), 7.22 (s, 1H, H-6), 7.06 (s, 1H, H-1'), 7.04 (s, 1H, H-5), 5.90 (s, 1H, H-2'), 4.75–4.65 (m, 3H, H-3' and H-4'), 1.07 [s, 9H, C(CH₃)₃], 0.43 (s, 3H, SiCH₃), 0.28 (s, 3H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 174.3 (CO), 165.2 (CO), 152.0 (C-4), 147.3 (C-7a), 142.2 (C-2), 137.3, 133.6, 131.2, 129.9, 129.3, 129.0, 128.5, 127.7 (Ar-C), 120.8 (C-6), 107.0 (C-5), 105.3 (C-4a), 92.2 (C-1'), 82.7 (C-2'), 77.6 (C-4'), 74.6 (C-3'), 25.5 [C(CH₃)₃], 17.8 [C(CH₃)₃], -4.9, -5.5 (SiCH₃). HRMS: [M+H]⁺ calcd for C₃₀H₃₅N₄O₅Si, 559.2371; found, 559.2388.

4-Benzoylamino-3-(3'-*O*-*tert*-butyldimethylsilyl- α -L-threofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (12).

To a solution of **11** (100 mg, 0.18 mmol) in 3.6 mL of THF/MeOH/H₂O (5:4:1) was added 2 M aq. NaOH (0.18 mL, 0.36 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 4 h. The reaction mixture was neutralized with acetic acid and the solvent was removed under reduced pressure. The residue was then portioned between water and ethyl acetate. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (5:1 to 5:2, CH₂Cl₂/EtOAc) to give **12** (64 mg, 79%) as a white powder. ¹H NMR (600 MHz, CDCl₃): δ 10.58 (s, 1H, NH), 8.44 (s, 1H, H-2), 8.20–8.17 (m, 2H, ArH), 7.51–7.48 (m, 1H, ArH), 7.45–7.41 (m, 2H, ArH), 6.93 (dd, *J* = 3.5, 2.4 Hz, 1H, H-6), 6.62 (dd, *J* = 3.5, 2.2 Hz, 1H, H-5), 6.25 (d, *J* = 1.0 Hz, 1H, H-1'), 4.39 (brs, 1H, H-2'), 4.37 (dd, *J* = 9.5, 3.6 Hz, 1H, H-4a'), 4.33–4.31 (m, 1H, H-3'), 4.24 (d, *J* = 9.5 Hz, 1H, H-4b'), 0.78 [s, 9H, C(CH₃)₃], 0.08 (s, 3H, SiCH₃), -0.03 (s, 3H, SiCH₃). ¹³C NMR (150 MHz, CDCl₃): δ 175.0 (CO), 151.7 (C-4), 147.1 (C-7a), 141.4 (C-2), 136.8, 131.8, 129.4, 128.2 (Ar-C), 121.1 (C-6), 106.3 (C-5), 105.2

(C-4a), 95.6 (C-1'), 82.8 (C-2'), 76.7 (C-4'), 76.4 (C-3'), 25.5 [C(CH₃)₃], 17.8 [C(CH₃)₃], -5.0, -5.2 (SiCH₃). HRMS: [M+H]⁺ calcd for C₂₃H₃₁N₄O₄Si, 455.2109; found, 455.2100.

4-Chloro-5-iodo-7-(2'-*O*-benzoyl-3'-*O*-*tert*-butyldimethylsilyl- α -L-threofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (13). This compound was prepared as described for **2**. Obtained from sugar **1** (0.25 g, 0.66 mmol) and 4-chloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine 6-chloropurine (202 mg, 0.72 mmol) as a white solid. Yield 52% (0.20 g).

¹H NMR (600 MHz, CDCl₃): δ 8.60 (s, 1H, H-2), 8.09–8.06 (m, 2 H, ArH), 8.00 (s, 1H, H-6), 7.64–7.60 (m, 1H, ArH), 7.50–7.46 (m, 2H, ArH), 6.67 (d, *J* = 0.7 Hz, 1H, H-1'), 5.43 (d, *J* = 0.8 Hz, 1H, H-2'), 4.51–4.49 (m, 1H, H-3'), 4.30 (dd, *J* = 10.0, 3.6 Hz, 1H, H-4a'), 4.24 (d, *J* = 9.8 Hz, 1H, H-4b'), 0.95 [s, 9H, C(CH₃)₃], 0.19 (s, 3H, SiCH₃), 0.14 (s, 3H, SiCH₃). ¹³C NMR (150 MHz, CDCl₃): δ 165.0 (CO), 152.5 (C-4), 150.9 (C-2), 150.5 (C-7a), 133.8 (Ar-C), 133.3 (C-6), 129.9, 128.7, 128.6 (Ar-C), 117.2 (C-4a), 88.1 (C-1'), 83.1 (C-2'), 76.5 (C-4'), 75.2 (C-3'), 52.0 (C-5), 25.7 [C(CH₃)₃], 18.0 [C(CH₃)₃], -4.9, -5.2 (SiCH₃). HRMS: [M+H]⁺ calcd for C₂₃H₂₈ClIN₃O₄Si, 600.0579; found, 600.0602.

4-Chloro-7-(3'-*O*-*tert*-butyldimethylsilyl- α -L-threofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (14). A solution of idonucleoside **13** (3.80 g, 6.33 mmol) in 40 mL of anhydrous THF was dropwise added *i*PrMgCl·LiCl (1.3 M in THF, 6.1 mL, 7.92 mmol) at -78 °C and the reaction mixture was stirred at the same temperature for 20 min. The reaction was quenched by dropwise addition of isopropanol (0.68 mL, 8.87 mmol). The reaction mixture was poured into an ice-cold mixture of AcOEt and sat. aq. NH₄Cl. The organic layer was separated and washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. To the residue, 100 mL of 7 N NH₃ in MeOH was added and the resultant mixture was stirred at rt for overnight. The volatiles were evaporated and the residue was purified by column chromatography (2:1 Hexane/EtOAc) to obtain **14** (2.13 g, 91% yield) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ 8.58 (s, 1H, H-2), 7.63 (d, *J* = 3.7 Hz, 1H, H-6), 6.57 (d, *J* = 3.7 Hz, 1H, H-5), 6.25 (s, 1H, H-1'), 4.68 (brs, 1H, OH), 4.38–4.36 (m, 2H, H-2' and H-3'), 4.32 (dd, *J* = 9.5, 4.0 Hz, 1H, H-4a'), 4.16 (d, *J* = 9.5 Hz, 1H, H-4b'), 0.83 [s, 9H, C(CH₃)₃], 0.10 (s, 3H, SiCH₃), 0.01 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 152.1 (C-4), 150.2 (C-2), 150.0 (C-7a), 127.7 (C-6), 118.4 (C-4a), 99.4 (C-5), 91.6 (C-1'), 82.4 (C-2'), 76.8 (C-3'), 75.8 (C-4'), 25.6 [C(CH₃)₃], 17.9 [C(CH₃)₃], -4.9, -5.0 (SiCH₃). HRMS: [M+H]⁺ calcd for C₁₆H₂₅ClN₃O₃Si, 370.1348; found, 370.1349.

4-Chloro-7-(2'-deoxy-3'-*O*-*tert*-butyldimethylsilyl- α -L-threofuranosyl)-7*H*-pyrrolo[2,3-

***d*]pyrimidine (15).** This compound was prepared as described for **6**. Obtained from **14** (2.23 g, 6.03 mmol) as a colorless oil. Yield 80% (1.70 g). ¹H NMR (300 MHz, CDCl₃): δ 8.59 (s, 1H, H-2), 7.85 (d, *J* = 3.8 Hz, 1H, H-6), 6.69 (dd, *J* = 8.2, 2.2 Hz, 1H, H-1'), 6.56 (d, *J* = 3.8 Hz, 1H, H-5), 4.61–4.56 (m, 1H, H-3'), 4.07–3.98 (m, 2H, H-4'), 2.72–2.62 (m, 1H, H-2a'), 2.27–2.21 (m, 1H, H-2b'), 0.87 [s, 9H, C(CH₃)₃], 0.08 (s, 3H, SiCH₃), 0.03 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 151.7 (C-4), 150.9 (C-7a), 150.5 (C-2), 127.8 (C-6), 117.9 (C-4a), 99.6 (C-5), 83.6 (C-1'), 77.0 (C-4'), 71.3 (C-3'), 41.6 (C-2'), 25.6 [C(CH₃)₃], 17.9 [C(CH₃)₃], –5.00, –5.03 (SiCH₃). HRMS: [M+H]⁺ calcd for C₁₆H₂₅ClN₃O₂Si, 354.1399; found, 354.1396.

4-Chloro-7-(2'-deoxy-α-L-threofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (16). This compound was prepared as described for **3**. Obtained from **15** (1.70 g, 4.80 mmol) as a white solid. Yield 83% (0.95 g). ¹H NMR (300 MHz, CDCl₃): δ 8.62 (s, 1H, H-2), 7.59 (d, *J* = 3.7 Hz, 1H, H-6), 6.61 (d, *J* = 3.7 Hz, 1H, H-5), 6.25 (dd, *J* = 9.1, 2.9 Hz, 1H, H-1'), 4.64–4.61 (m, 1H, H-3'), 4.21–4.17 (m, 1H, H-4a'), 3.94 (dd, *J* = 9.9, 3.8 Hz, 1H, H-4b'), 2.87–2.77 (m, 1H, H-2a'), 2.55–2.48 (m, 1H, H-2b'). ¹³C NMR (75 MHz, CDCl₃): δ 152.5 (C-4), 149.7 (C-2), 149.4 (C-7a), 129.7 (C-6), 119.2 (C-4a), 99.7 (C-5), 86.3 (C-1'), 76.9 (C-4'), 70.6 (C-3'), 40.5 (C-2'). HRMS: [M+H]⁺ calcd for C₁₀H₁₁ClN₃O₂, 240.0534; found, 240.0532.

4-Chloro-7-(2'-deoxy-3'-*O*-diisopropylphosphonomethyl-α-L-threofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (17). This compound was prepared as described for **4**. Obtained from **16** (0.45 g, 1.88 mmol) as a colorless oil. Yield 84% (0.66 g). ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.64 (s, 1H, H-2), 7.93 (d, *J* = 3.7 Hz, 1H, H-6), 6.68–6.62 (m, 2H, H-5 and H-1'), 4.71–4.55 [m, 2H, CH(CH₃)₂], 4.42–4.38 (m, 1H, H-3'), 4.24 (d, *J* = 10.4 Hz, 1H, H-4a'), 3.94–3.78 (m, 3H, H-4b' and PCH₂), 2.84–2.74 (m, 1H, H-2a'), 2.35 (d, *J* = 15.1 Hz, 1H, H-2b'), 1.28–1.23 [m, 12H, CH(CH₃)₂]. ¹³C NMR (75 MHz, DMSO-*d*₆): δ 150.7 (C-4), 150.6 (C-7a), 150.5 (C-2), 128.8 (C-6), 117.0 (C-4a), 99.4 (C-5), 82.9 (C-1'), 80.3 (d, ³*J*_{P,C} = 13.5 Hz, C-3'), 72.8 (C-4'), 70.3, 70.2 [CH(CH₃)₂], 63.3 (d, ¹*J*_{P,C} = 166.2 Hz, PCH₂), 37.8 (C-2'), 23.8 [CH(CH₃)₂]. ³¹P NMR (121 MHz, DMSO-*d*₆): δ 19.3. HRMS: [M+H]⁺ calcd for C₁₇H₂₆ClN₃O₅P, 418.1293; found, 418.1292.

4-Amino-7-(2'-deoxy-3'-*O*-diisopropylphosphonomethyl-α-L-threofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (18). A mixture of **17** (0.60 g, 1.44 mmol) in 50 mL of 7 N NH₃ in MeOH was stirred in a pressure flask at 120 °C for 16 h. After cooling to rt, the volatiles were evaporated under reduced pressure. The residue was purified by column chromatography (20:1 to 10:1, CH₂Cl₂/MeOH) to afford **18** (0.40 g, 70% yield) as a white foam. ¹H NMR (600 MHz,

DMSO-*d*₆): δ 8.23 (s, 1H, H-2), 7.46 (d, J = 3.7 Hz, 1H, H-6), 7.35 (brs, 2H, NH₂), 6.65 (d, J = 3.7 Hz, 1H, H-5), 6.52 (dd, J = 8.5, 2.7 Hz, 1H, H-1'), 4.68–4.61 [m, 2H, CH(CH₃)₂], 4.36–4.33 (m, 1H, H-3'), 4.15 (d, J = 10.3 Hz, 1H, H-4a'), 3.90–3.80 (m, 3H, H-4b' and PCH₂), 2.74–2.69 (m, 1H, H-2a'), 2.22 (d, J = 14.9 Hz, 1H, H-2b'), 1.29–1.26 [m, 12H, CH(CH₃)₂]. ¹³C NMR (150 MHz, DMSO-*d*₆): δ 156.3 (C-4), 150.1 (C-2), 149.5 (C-7a), 122.5 (C-6), 102.2 (C-4a), 100.4 (C-5), 82.0 (C-1'), 80.5 (d, ³*J*_{P,C} = 14.0 Hz, C-3'), 72.2 (C-4'), 70.3 [CH(CH₃)₂], 63.2 (d, ¹*J*_{P,C} = 166.3 Hz, PCH₂), 37.7 (C-2'), 23.8 [CH(CH₃)₂]. ³¹P NMR (121 MHz, CDCl₃): δ 19.1. HRMS: [M+H]⁺ calcd for C₁₇H₂₈N₄O₅P, 399.1792; found, 399.1789.

(4*R*,1*E*)-4-Amino-7-(3-phosphonmethoxy-4-hydroxy-1-buten-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (19). This compound was prepared as described for **7**. Obtained from **18** (Et₃N salt; 200 mg, 0.50 mmol) as a white foam. Yield 54% (112 mg). ¹H NMR (600 MHz, D₂O): δ 7.88 (s, 1H, H-2), 7.15 (d, J = 3.8 Hz, 1H, H-6), 7.09 (d, J = 14.5 Hz, 1H, H-1'), 6.30 (d, J = 3.7 Hz, 1H, H-5), 5.77 (dd, J = 14.4, 8.0 Hz, 1H, H-2'), 4.21–4.17 (m, 1H, H-3'), 3.83–3.63 (m, 3H, H-4' and PCH₂). ¹³C NMR (150 MHz, D₂O): δ 157.9 (C-4), 151.7 (C-2), 149.5 (C-7a), 128.0 (C-1'), 124.6 (C-6), 115.8 (C-2'), 105.4 (C-4a), 104.4 (C-5), 83.9 (d, ³*J*_{P,C} = 12.1 Hz, C-3'), 68.1 (d, ²*J*_{P,C} = 156.3 Hz, PCH₂), 66.8 (C-4'). ³¹P NMR (121 MHz, D₂O): δ 15.8. HRMS: [M-H][−] calcd for C₁₁H₁₄N₄O₅P, 313.0707; found, 313.0710.

Propyl ((5*R*)-5-((*E*)-2-(4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)vinyl)-2-oxido-1,4,2-dioxaphosphinan-2-yl)-L-phenylalaninate (20a and 20b). This compound was prepared as described for **8**. Obtained from **19** (28 mg, 0.067 mmol) as a white solid and a mixture of diastereoisomers with a ratio of ca. 2:1 (fast elute/slow elute). Yield 24% (8 mg, **20a**) and 12% (4 mg, **20b**). The two isomers showed similar NMR characteristics. Major isomer **20a**: ¹H NMR (600 MHz, MeOD): δ 8.14 (s, 1H, H-2), 7.58 (dd, J = 14.6, 1.1 Hz, 1H, H-1'), 7.43 (d, J = 3.8 Hz, 1H, H-6), 7.31–7.20 (m, 5H, ArH), 6.71 (d, J = 3.7 Hz, 1H, H-5), 5.98 (dd, J = 14.5, 6.0 Hz, 1H, H-2'), 4.44–4.40 (m, 1H, H-3'), 4.37–4.30 (m, 2H, H-4'), 4.24–4.20 (m, 1H, Phe-CH), 4.04 (t, J = 6.6 Hz, 2H, OCH₂CH₂CH₃), 3.77–3.71 (m, 2H, PCH₂), 3.18–3.14 (m, 1H, Phe-CH₂), 2.94 (dd, J = 13.6, 8.8 Hz, 1H, Phe-CH₂), 1.63–1.58 (m, 2H, OCH₂CH₂CH₃), 0.88 (t, J = 7.4 Hz, 3H, OCH₂CH₂CH₃). ¹³C NMR (150 MHz, MeOD): δ 174.4 (CO), 159.1 (C-4), 153.2 (C-2), 150.6 (C-7a), 138.5, 130.6, 129.5, 127.9 (Ar-C), 127.3 (C-1'), 121.8 (C-6), 110.0 (C-2'), 104.8 (C-4a), 103.0 (C-5), 75.4 (d, ³*J*_{P,C} = 4.6 Hz, C-3'), 73.6 (d, ²*J*_{P,C} = 7.8 Hz, C-4'), 68.0 (OCH₂CH₂CH₃), 65.3 (d, ¹*J*_{P,C} = 134.1 Hz, PCH₂), 56.2 (Phe-CH), 41.2 (Phe-CH₂), 23.0 (OCH₂CH₂CH₃), 10.7

(OCH₂CH₂CH₃). ³¹P NMR (121 MHz, MeOD): δ 16.8. HRMS: [M+H]⁺ calcd for C₂₃H₂₉N₅O₅P, 486.1901; found, 486.1904.

1'α-(2,6-diamino-purin-9-yl)-2'-deoxy-3'-O-diisopropylphosphonomethyl-L-threose (22a). A solution of **21** (170 mg, 0.39 mmol) in 7 N NH₃ in MeOH (30 mL) was placed in a reaction bomb and heated to 100 °C for 4 h. The reaction mixture was cooled to rt and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (20:1 to 10:1, CH₂Cl₂/MeOH) to give **22a** (113 mg, 70%) as a white foam. ¹H NMR (600 MHz, DMSO-*d*₆): δ 7.86 (s, 1H, H-8), 6.68 (brs, 2H, NH₂), 6.12 (dd, *J* = 8.4, 2.4 Hz, 1H, H-1'), 5.79 (d, *J* = 6.3 Hz, 2H, NH₂), 4.66–4.58 [m, 2H, CH(CH₃)₂], 4.37–4.33 (m, 1H, H-3'), 4.16 (d, *J* = 10.2 Hz, 1H, H-4a'), 3.90–3.81 (m, 3H, H-4b' and PCH₂), 2.70–2.65 (m, 1H, H-2a'), 2.34 (d, *J* = 14.8 Hz, H-2b'), 1.27–1.24 [m, 12H, CH(CH₃)₂]. ¹³C NMR (150 MHz, DMSO-*d*₆): δ 160.4 (C-2), 156.1 (C-6), 151.7 (C-4), 135.6 (C-8), 112.9 (C-5), 81.7 (C-1'), 80.3 (d, ³*J*_{P,C} = 14.1 Hz, C-3'), 72.4 (C-4'), 70.4 [CH(CH₃)₂], 63.2 (d, ¹*J*_{P,C} = 165.8 Hz, PCH₂), 37.6 (C-2'), 23.8 [CH(CH₃)₂]. ³¹P NMR (121 MHz, DMSO-*d*₆): δ 19.2. HRMS: [M+H]⁺ calcd for C₁₆H₂₈N₆O₅P, 415.1853; found, 415.1853.

1'α-(2-amino-6-cyclopropylamino-purin-9-yl)-2'-deoxy-3'-O-diisopropylphosphonomethyl-L-threose (22b). A mixture of **21** (200 mg, 0.46 mmol) and cyclopropylamine (80 mg, 1.40 mmol) in CH₃CN (4 mL) was placed in a reaction bomb and heated to 100 °C for 4 h. The reaction mixture was cooled to rt and concentrated. The residue was purified by column chromatography (40:1 to 20:1, CH₂Cl₂/MeOH) to give **22b** (190 mg, 91%) as white foam. ¹H NMR (300 MHz, CDCl₃): δ 7.91 (s, 1H, H-8), 6.27 (dd, *J* = 7.6, 2.1 Hz, 1H, H-1'), 5.77 (brs, 1H, NH), 4.88 (brs, 2H, NH₂), 4.69–4.82 [m, 2H, CH(CH₃)₂], 4.46 (brs, 1H, H-3'), 4.27 (d, *J* = 10.4 Hz, 1H, H-4a'), 4.00 (dd, *J* = 10.4, 4.3 Hz, 1H, H-4b'), 3.74 (d, *J* = 8.9 Hz, 2H, PCH₂), 2.99 [brs, 1H, CH(CH₂)₂], 2.68–2.50 (m, 2H, H-2), 1.30–1.35 [m, 12H, CH(CH₃)₂], 0.80–0.87 [m, 2H, CH(CH₂)₂], 0.57–0.62 [m, 2H, CH(CH₂)₂]. ¹³C NMR (75 MHz, CDCl₃): δ 160.0 (C-2), 156.2 (C-6), 151.0 (C-4), 136.3 (C-8), 114.5 (C-5), 82.7 (C-1'), 80.4 (³*J*_{P,C} = 10.4 Hz, C-3'), 73.1 (C-4'), 71.3 [CH(CH₃)₂], 64.0 (¹*J*_{P,C} = 168.3 Hz, PCH₂), 38.1 (C-2'), 24.0 [CH(CH₃)₂], 23.6 [CH(CH₂)₂], 7.4 [CH(CH₂)₂]. ³¹P NMR (121 MHz, CDCl₃): δ 18.3. HRMS: [M+H]⁺ calcd for C₁₉H₃₂N₆O₅P, 455.2166; found, 455.2165.

1'α-(2,6-diamino-purin-9-yl)-2'-deoxy-3'-O-phosphonomethyl-L-threose (23a). This compound was prepared as described for compound **7**. Obtained from **22a** (113 mg, 0.27 mmol) as a white foam. Yield 60% (70 mg). ¹H NMR (300 MHz, D₂O): δ 8.18 (s, 1H, H-8), 6.11 (dd, *J* = 8.1, 1.8 Hz, 1H, H-1'), 4.42 (brs, 1H, H-3'), 4.20 (d, *J* = 10.2 Hz, 1H, H-4a'), 3.94 (dd, *J* = 10.2,

4.1 Hz, 1H, H-4b'), 4.20 (d, $J = 8.8$ Hz, 2H, PCH₂). 2.48–2.60 (m, 2H, H-2'). ¹³C NMR (75 MHz, D₂O): δ 159.8 (C-2), 155.8 (C-6), 150.6 (C-4), 138.6 (C-8), 112.6 (C-5), 82.5 (C-1'), 79.1 (d, $^3J_{P,C} = 10.5$ Hz, C-3'), 73.0 (C-4'), 66.7 (d, $^1J_{P,C} = 150.5$ Hz, PCH₂), 36.5 (C-2'). ³¹P NMR (121 MHz, D₂O): δ 13.1. HRMS: [M-H][−] calcd for C₁₀H₁₄N₆O₅P, 329.0769; found, 329.0769.

1' α -(2-amino-6-cyclopropylamino-purin-9-yl)-2'-deoxy-3'-O-phosphonomethyl-L-threose (23b). This compound was prepared as described for **7**. Obtained from **22b** (170 mg, 0.37 mmol) as a white foam. Yield 62% (109 mg). ¹H NMR (300 MHz, D₂O): δ 8.04 (s, 1H, H-8), 5.99 (d, $J = 5.9$ Hz, 1H, H-1'), 4.35 (brs, 1H, H-3'), 4.10 (d, $J = 10.2$ Hz, 1H, H-4a'), 3.86 (dd, $J = 10.2$, 4.1 Hz, 1H, H-4b'), 3.35 (d, $J = 8.9$ Hz, 2H, PCH₂), 2.62–2.54 [m, 2H, CH(CH₂)₂ and H-2a'], 2.40 (d, $J = 14.8$ Hz, H-2b'), 0.68 [d, $J = 6.8$ Hz, 2H, CH(CH₂)₂], 0.44 [brs, 2H, CH(CH₂)₂]. ¹³C NMR (75 MHz, D₂O): δ 159.7 (C-2), 155.7 (C-6), 149.6 (C-4), 137.7 (C-8), 112.6 (C-5), 82.3 (C-1'), 79.03 (d, $^3J_{P,C} = 10.3$ Hz, C-3'), 72.9 (C-4'), 66.7 (d, $^1J_{P,C} = 150.0$ Hz, PCH₂), 36.5 (C-2'), 22.9 [CH(CH₂)₂], 6.4 [CH(CH₂)₂]. ³¹P NMR (121 MHz, D₂O): δ 13.0. HRMS: [M-H][−] calcd for C₁₃H₁₈N₆O₅P, 369.1082; found, 369.1071.

1' α -(2,6-diamino-purin-9-yl)-2'-deoxy-3'-O-[N,N'-bis(*n*-propyl-L-phenylalaninate)]methylphosphonobisamidate]-L-threose (24a). This compound was prepared as described for **8**. Obtained from **23a** (23 mg, 0.055 mmol, Et₃N salt) as a white foam. Yield 53% (20 mg). ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.89 (s, 1H, H-8), 7.29–7.12 (m, 10H, ArH), 6.66 (brs, 2H, NH₂), 6.09 (dd, $J = 7.9$, 2.7 Hz, 1H, H-1'), 5.81 (brs, 2H, NH₂), 4.55 (t, $J = 10.9$ Hz, 1H, NH), 4.24–4.16 (m, 2H, NH and H-3'), 4.08–3.77 (m, 8H, H-4', Phe-CH and OCH₂CH₂CH₃), 3.37–3.19 (m, 2H, PCH₂), 3.98–2.76 (m, 4H, Phe-CH₂), 2.65–2.56 (m, 1H, H-2a'), 2.21 (d, $J = 13.8$ Hz, H-2b'), 1.58–1.40 (m, 4H, OCH₂CH₂CH₃), 0.84–0.75 (m, 6H, OCH₂CH₂CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): 173.0, 172.9 (Phe-CO), 160.4 (C-2), 156.2 (C-6), 151.7 (C-4), 137.3, 137.2 (Ar-C), 135.9 (C-8), 129.5, 128.2, 126.6 (Ar-C), 113.0 (C-5), 81.9 (C-1'), 79.9 (d, $^3J_{P,C} = 12.2$ Hz, C-3'), 72.4 (C-4'), 66.02, 65.96 (OCH₂CH₂CH₃), 65.8 (d, $^1J_{P,C} = 134.9$ Hz, PCH₂), 54.2, 54.1 (Phe-CH), 39.7 (Phe-CH₂, overlap with DMSO-*d*₆), 37.5 (C-2'), 21.53, 21.48 (OCH₂CH₂CH₃), 10.33, 10.29 (OCH₂CH₂CH₃). ³¹P NMR (121 MHz, DMSO-*d*₆): δ 20.3. HRMS: [M+H]⁺ calcd for C₃₄H₄₆N₈O₇P, 709.3221; found, 709.3244.

1' α -(2-amino-6-cyclopropylamino-purin-9-yl)-2'-deoxy-3'-O-[N,N'-bis(*n*-propyl-L-phenylalaninate)]methylphosphonobisamidate]-L-threose (24b). This compound was prepared as described for **8**. Obtained from **23b** (23 mg, 0.049 mmol) as a white solid. Yield 67% (24 mg).

^1H NMR (300 MHz, DMSO- d_6): δ 7.91 (s, 1H, H-8), 7.28–7.11 (m, 10H, ArH), 6.10 (dd, J = 7.9, 2.5 Hz, 1H, H-1'), 5.92 (brs, 2H, NH₂), 4.55 (t, J = 11.3 Hz, 1H, NH), 4.23–4.16 (m, 2H, NH and H-3'), 4.07–3.77 (m, 8H, H-4', Phe-CH and OCH₂CH₂CH₃), 3.37–3.16 (m, 2H, PCH₂), 3.05 [brs, 1H, CH(CH₂)₂], 2.93–2.75 (m, 4H, Phe-CH₂), 2.66–2.56 (m, 1H, H-2a'), 2.21 (d, J = 14.1 Hz, 1H, H-2b'), 1.57–1.39 (m, 4H, OCH₂CH₂CH₃), 0.83–0.73 (m, 6H, OCH₂CH₂CH₃), 0.66–0.53 [m, 4H, CH(CH₂)₂]. ^{13}C NMR (75 MHz, DMSO- d_6): 173.0, 172.9 (Phe-CO), 160.0 (C-2), 155.8 (C-6), 151.4 (C-4), 137.3, 137.2 (Ar-C), 135.8 (C-8), 129.5, 128.2, 126.6 (Ar-C), 113.2 (C-5), 81.9 (C-1'), 80.0 (d, $^3J_{\text{P,C}}$ = 11.7 Hz, C-3'), 72.4 (C-4'), 66.0, 65.9 (OCH₂CH₂CH₃), 65.8 (d, $^1J_{\text{P,C}}$ = 135.9 Hz, PCH₂), 54.2, 54.0 (Phe-CH), 39.7 (Phe-CH₂, overlap with DMSO- d_6), 37.6 (C-2'), 24.2 [CH(CH₂)₂], 21.53, 21.47 (OCH₂CH₂CH₃), 10.32, 10.27 (OCH₂CH₂CH₃), 6.6 [CH(CH₂)₂]. ^{31}P NMR (121 MHz, DMSO- d_6): δ 20.3. HRMS: $[\text{M}+\text{H}]^+$ calcd for C₃₇H₅₀N₈O₇P, 749.3534; found, 749.3547.

1' α -(5-fluorouracil-1-yl)-2'-O-benzoyl-3'-O-tert-butylidimethylsilyl-L-threose (25). A solution of **1** (0.20 g, 0.53 mmol) and 5-fluorouracil (68 mg, 0.53 mmol) in 2 mL of dry acetonitrile was treated with BSA (0.26 mL, 1.05 mmol) and stirred at 60 °C for 1 h. TMSOTf (0.28 mL, 1.58 mmol) was then added and the heating was continued for another 1 h. The reaction mixture was cooled to rt and poured into an ice-cold stirred mixture of sat. aq. NaHCO₃ and AcOEt. The organic layer was separated and washed with brine, dried over Na₂SO₄, and concentrated. The residue was purified by column chromatography (3:1 to 2:1, hexane/EtOAc) to obtain **25** (0.21 g, 88% yield) as a white foam. ^1H NMR (500 MHz, CDCl₃): δ 9.39 (brs, 1H, NH), 8.10–8.08 (m, 2H, ArH), 7.91 (d, J = 6.4 Hz, 1H, H-6), 7.66–7.63 (m, 1H, ArH), 7.53–7.49 (m, 2H, ArH), 6.17 (s, 1H, H-1'), 5.28 (d, J = 0.8 Hz, 1H, H-2'), 4.42 (d, J = 2.9 Hz, 1H, H-3'), 4.28 (d, J = 9.8 Hz, 1H, H-4a'), 4.24 (dd, J = 9.8, 2.9 Hz, 1H, H-4b'), 0.96 [s, 9H, C(CH₃)₃], 0.22 (s, 3H, SiCH₃), 0.21 (s, 3H, SiCH₃). ^{13}C NMR (125 MHz, CDCl₃): δ 164.9 (PhCO), 156.9 (d, $^2J_{\text{F,C}}$ = 26.9 Hz, C-4), 148.7 (C-2), 140.1 (d, $^1J_{\text{F,C}}$ = 236.7 Hz, C-5), 133.8, 129.9, 128.7, 128.6 (Ar-C), 125.3 (d, $^2J_{\text{F,C}}$ = 34.8 Hz, C-6), 89.8 (C-1'), 82.4 (C-2'), 76.8 (C-4'), 74.4 (C-3'), 25.5 [C(CH₃)₃], 17.9 [C(CH₃)₃], –4.9, –5.3 (SiCH₃). HRMS: $[\text{M}+\text{H}]^+$ calcd for C₂₁H₂₈FN₂O₆Si, 451.1695; found, 451.1695.

1' α -(5-fluorouracil-1-yl)-2'-O-benzoyl-L-threose (26). This compound was prepared as described for **3**. Obtained from compound **25** (5.90 g, 13.1 mmol) as a white solid. Yield 90% (3.96 g). ^1H NMR (300 MHz, DMSO- d_6): δ 11.9 (brs, 1H, NH), 8.06–8.00 (m, 3H, ArH and H-6), 7.73–7.68 (m, 1H, ArH), 7.59–7.54 (m, 2H, ArH), 5.98 (s, 2H, H-1' and OH), 5.33 (s, 1H, H-2'),

4.38–4.37 (m, 1H, H-3'), 4.25 (d, $J = 9.8$ Hz, 1H, H-4a'), 4.24 (dd, $J = 9.8, 3.7$ Hz, 1H, H-4b'). ^{13}C NMR (75 MHz, DMSO- d_6): δ 164.6 (PhCO), 157.1 (d, $^2J_{\text{F,C}} = 26.1$ Hz, C-4), 148.9 (C-2), 139.7 (d, $^1J_{\text{F,C}} = 231.0$ Hz, C-5), 133.9, 129.5, 128.9, 128.8 (Ar-C), 125.4 (d, $^2J_{\text{F,C}} = 35.0$ Hz, C-6), 89.2 (C-1'), 81.7 (C-2'), 75.4 (C-4'), 72.7 (C-3'). HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{14}\text{FN}_2\text{O}_6$, 337.0830; found, 337.0824.

1' α -(5-fluorouracil-1-yl)-2'-O-benzoyl-3'-O-diisopropylphosphonomethyl-L-threose (27).

This compound was prepared as described for **4**. Obtained from **26** (0.27 g, 0.80 mmol), diisopropylphosphonomethyl trifluoromethanesulfonate (0.31 g, 0.96 mmol) and NaH (60% in oil, 96 mg, 2.40 mmol) as a white foam. Yield 52% (0.22 g). ^1H NMR (300 MHz, CDCl_3): δ 9.35 (d, $J = 4.2$ Hz, 1H, NH), 8.03 (d, $J = 7.5$ Hz, 2H, ArH), 7.72 (d, $J = 6.3$ Hz, 1H, H-6), 7.61 (t, $J = 7.5$ Hz, 1H, ArH), 7.46 (t, $J = 7.5$ Hz, 2H, Ar-H), 6.20 (s, 1H, H-1'), 5.43 (s, 1H, H-2'), 4.82–4.72 [m, 2H, $\text{CH}(\text{CH}_3)_2$], 4.44 (d, $J = 10.8$ Hz, 1H, H-4a'), 4.26 (d, $J = 3.4$ Hz, 1H, H-3'), 4.16 (dd, $J = 10.8, 3.7$ Hz, 1H, H-4b'), 4.06–3.88 (m, 2H, PCH_2), 1.35–1.32 [m, 12H, $\text{CH}(\text{CH}_3)_2$]. ^{13}C NMR (75 MHz, CDCl_3): δ 165.1 (PhCO), 156.8 (d, $^2J_{\text{F,C}} = 27.3$ Hz, C-4), 148.7 (C-2), 140.4 (d, $^1J_{\text{F,C}} = 236.4$ Hz, C-5), 133.9, 129.9, 128.6, 128.5 (Ar-C), 124.6 (d, $^2J_{\text{F,C}} = 34.9$ Hz, C-6), 89.7 (C-1'), 83.4 (d, $^3J_{\text{P,C}} = 10.3$ Hz, C-3'), 79.9 (C-2'), 73.5 (C-4'), 71.6 [$\text{CH}(\text{CH}_3)_2$], 64.7 (d, $^1J_{\text{P,C}} = 168.6$ Hz, PCH_2), 23.9 [$\text{CH}(\text{CH}_3)_2$]. ^{31}P NMR (121 MHz, CDCl_3): δ 17.7. HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{29}\text{FN}_2\text{O}_9\text{P}$, 515.1589; found, 515.1583.

1' α -(5-fluorouracil-1-yl)-3'-O-diisopropylphosphonomethyl-L-threose (28). A solution of **27** (2.00 g, 3.89 mmol) in 2 M ammonia in MeOH (65 mL) was stirred at rt for overnight. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (30:1 to 20:1, $\text{CH}_2\text{Cl}_2/\text{MeOH}$) to give compound **28** (1.50 g, 94% yield) as a white foam. ^1H NMR (300 MHz, CDCl_3): δ 10.84 (brs, 1H, NH), 7.62 (d, $J = 6.3$ Hz, 1H, H-6), 5.80 (s, 1H, H-1'), 5.42 (brs, 1H, OH), 4.78–4.67 [m, 2H, $\text{CH}(\text{CH}_3)_2$], 4.45 (s, 1H, H-2'), 4.33 (d, $J = 10.3$ Hz, 1H, H-4a'), 4.26 (dd, $J = 10.3, 3.2$ Hz, 1H, H-4b'), 4.13 (d, $J = 2.8$ Hz, 1H, H-3'), 3.81 (d, $J = 9.1$ Hz, 2H, PCH_2), 1.33–1.28 [m, 12H, $\text{CH}(\text{CH}_3)_2$]. ^{13}C NMR (75 MHz, CDCl_3): δ 157.6 (d, $^2J_{\text{F,C}} = 27.3$ Hz, C-4), 149.5 (C-2), 140.2 (d, $^1J_{\text{F,C}} = 233.9$ Hz, C-5), 124.9 (d, $^2J_{\text{F,C}} = 34.5$ Hz, C-6), 93.0 (C-1'), 85.1 (d, $^3J_{\text{P,C}} = 10.3$ Hz, C-3'), 78.1 (C-2'), 74.1 (C-4'), 71.6 [$\text{CH}(\text{CH}_3)_2$], 64.2 (d, $^1J_{\text{P,C}} = 168.2$ Hz, PCH_2), 23.9 [$\text{CH}(\text{CH}_3)_2$]. ^{31}P NMR (121 MHz, CDCl_3): δ 18.3. HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{25}\text{FN}_2\text{O}_8\text{P}$, 411.1327; found, 411.1334.

1' α -(5-fluorouracil-1-yl)-2'-deoxy-3'-O-diisopropylphosphonomethyl-L-threose (29). This compound was prepared as described for **6**. Obtained from **28** (0.31 g, 0.76 mmol) as a white foam. Yield 92% (275 mg). ^1H NMR (300 MHz, CDCl_3): δ 10.37 (brs, 1H, *NH*), 7.81 (d, $J = 6.3$ Hz, 1H, H-6), 6.22 (d, $J = 7.6$ Hz, 1H, H-1'), 4.83–4.71 [m, 2H, $\text{CH}(\text{CH}_3)_2$], 4.38 (d, $J = 10.5$ Hz, 1H, H-4a'), 4.33 (brs, 1H, H-3'), 3.86 (dd, $J = 10.5, 2.8$ Hz, 1H, H-4b'), 3.75 (d, $J = 9.1$ Hz, 2H, PCH_2), 2.59–2.49 (m, 1H, H-2a'), 2.23 (d, $J = 15.3$ Hz, 1H, H-2b'), 1.34–1.31 [m, 12H, $\text{CH}(\text{CH}_3)_2$]. ^{13}C NMR (75 MHz, CDCl_3): δ 157.2 (d, $^2J_{\text{F,C}} = 26.4$ Hz, C-4), 149.2 (C-2), 140.1 (d, $^1J_{\text{F,C}} = 235.8$ Hz, C-5), 124.8 (d, $^2J_{\text{F,C}} = 34.4$ Hz, C-6), 85.5 (C-1'), 80.0 (d, $^3J_{\text{P,C}} = 11.0$ Hz, C-3'), 73.8 (C-4'), 71.3 [$\text{CH}(\text{CH}_3)_2$], 63.8 (d, $^1J_{\text{P,C}} = 169.3$ Hz, PCH_2), 38.2 (C-2'), 23.8 [$\text{CH}(\text{CH}_3)_2$]. ^{31}P NMR (121 MHz, CDCl_3): δ 18.2. HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{25}\text{FN}_2\text{O}_7\text{P}$, 395.1378; found, 395.1390.

1' α -(5-fluorouracil-1-yl)-3'-O-phosphonomethyl-L-threose (31). This compound was prepared as described for **7**. Obtained from **28** (0.46 g, 1.12 mmol) and bromotrimethylsilane (1.18 mL, 8.97 mmol) as a white foam. Yield 68% (0.33 g, Et_3N salt). ^1H NMR (300 MHz, D_2O): δ 7.79 (d, $J = 6.3$ Hz, 1H, H-6), 5.76 (s, 1H, H-1'), 4.34 (s, 1H, H-2'), 4.28 (d, $J = 9.2$ Hz, 1H, H-4a'), 4.14–4.10 (m, 2H, H-3' and H-4b'), 3.44 (d, $J = 8.5$ Hz, 2H, PCH_2). ^{13}C NMR (75 MHz, D_2O): δ 164.8 (d, $^2J_{\text{F,C}} = 20.0$ Hz, C-4), 154.5 (C-2), 141.1 (d, $^1J_{\text{F,C}} = 236.2$ Hz, C-5), 125.1 (d, $^2J_{\text{F,C}} = 35.3$ Hz, C-6), 91.3 (C-1'), 83.9 (d, $^3J_{\text{P,C}} = 8.4$ Hz, C-3'), 77.5 (C-2'), 72.4 (C-4'), 66.5 (d, $^1J_{\text{P,C}} = 150.3$ Hz, PCH_2). ^{31}P NMR (121 MHz, D_2O): δ 13.2. HRMS: $[\text{M}-\text{H}]^-$ calcd for $\text{C}_9\text{H}_{11}\text{FN}_2\text{O}_8\text{P}$, 325.0242; found, 325.0247.

1' α -(5-fluorouracil-1-yl)-3'-O-[*N,N'*-bis(*n*-propyl-L-phenylalaninate)]methylphosphonobisamidate]-L-threose (32).

This compound was prepared as described for **8**. Obtained from **31** (70 mg, 0.16 mmol) as a white foam. Yield (39%, 45 mg) and major isomer of **33** (15%, 12 mg). Or obtained from **35** (185 mg, 0.34 mmol). Yield (69% for two steps, 165 mg). ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 11.85 (s, 1H, *NH*), 7.82 (d, $J = 6.8$ Hz, 1H, H-6), 7.26–7.11 (m, 10H, *Ar-H*), 5.96 (s, 1H, *OH*), 5.62 (s, 1H, H-1'), 4.49 (t, $J = 11.5$ Hz, 1H, *NH*), 4.26–4.21 (m, 2H, *NH* and H-4a'), 4.05 (s, 1H, H-2'), 3.99–3.85 (m, 7H, H-4b', *Phe-CH* and $\text{OCH}_2\text{CH}_2\text{CH}_3$), 3.82 (s, 1H, H-3'), 3.35–3.19 (m, 2H, PCH_2), 2.90–2.74 (m, 4H, *Phe-CH}_2), 1.52–1.43 (m, 4H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 0.83–0.75 (m, 6H, $\text{OCH}_2\text{CH}_2\text{CH}_3$). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): 173.0, 172.8 (*Phe-CO*), 157.2 (d, $^2J_{\text{F,C}} = 26.1$ Hz, C-4), 149.1 (C-2), 139.6 (d, $^1J_{\text{F,C}} = 230.3$ Hz, C-5), 137.19, 137.15, 129.5, 129.4, 128.2, 126.59, 126.57 (*Ar-C*), 125.5 (d, $^2J_{\text{F,C}} = 34.7$ Hz, C-6), 91.9 (C-1'), 84.8 (d, $^3J_{\text{P,C}} = 10.7$ Hz, C-3'), 77.7 (C-2'), 72.3 (C-*

4'), 66.0, 65.9 (OCH₂CH₂CH₃), 65.8 (d, ¹J_{P,C} = 135.3 Hz, PCH₂), 54.2, 53.9 (Phe-CH), 39.6 (Phe-CH₂, overlap with DMSO-*d*₆), 21.5, 21.4 (OCH₂CH₂CH₃), 10.3, 10.2 (OCH₂CH₂CH₃). ³¹P NMR (121 MHz, DMSO-*d*₆): δ 20.0. HRMS: [M+H]⁺ calcd for C₃₃H₄₃FN₄O₁₀P, 705.2695; found, 705.2671.

1'α-(5-fluorouracil-1-yl)-3'-O-methyl-2'-O-[N-*n*-propyl-L-phenylalaninate-

cyclophosphonamidate]-L-threose (33). ¹H NMR (500 MHz, DMSO-*d*₆): δ 11.96 (brs, 1H, NH), 8.42 (d, *J* = 7.1 Hz, 1H, H-6), 7.31–7.20 (m, 5H, ArH), 6.00–5.93 (m, 2H, H-1' and NH), 4.99–4.96 (m, 1H, H-2'), 4.23–4.18 (m, 1H, H-3'), 4.15–4.07 (m, 1H, Phe-CH), 4.06–3.97 (m, 4H, H-4' and OCH₂CH₂CH₃), 3.51–3.21 (m, 2H, PCH₂), 3.07–2.69 (m, 2H, Phe-CH₂), 1.58–1.51 (m, 2H, OCH₂CH₂CH₃), 0.84 (t, *J* = 7.4 Hz, 3H, OCH₂CH₂CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): 172.4 (d, ³J_{P,C} = 3.1 Hz, Phe-CO), 156.9 (d, ²J_{F,C} = 26.5 Hz, C-4), 149.5 (C-2), 140.7 (d, ¹J_{F,C} = 232.8 Hz, C-5), 137.2, 129.6, 128.3, 126.7 (Ar-C), 124.9 (d, ²J_{F,C} = 34.6 Hz, C-6), 82.1 (d, ³J_{P,C} = 8.1 Hz, C-1'), 77.9 (d, ²J_{P,C} = 3.4 Hz, C-2'), 75.8 (d, ³J_{P,C} = 5.4 Hz, C-3'), 66.3 (OCH₂CH₂CH₃), 65.5 (C-4'), 65.4 (d, ¹J_{P,C} = 132.1 Hz, PCH₂), 54.5 (Phe-CH), 39.6 (Phe-CH₂, overlap with DMSO-*d*₆), 21.5 (OCH₂CH₂CH₃), 10.2 (OCH₂CH₂CH₃). ³¹P NMR (121 MHz, DMSO-*d*₆): δ 18.1. HRMS: [M+H]⁺ calcd for C₂₁H₂₆FN₃O₈P, 498.1436; found, 498.1441.

1'α-(5-fluorouracil-1-yl)-2'-O-*tert*-butyldimethylsilyl-3'-O-diisopropylphosphonomethyl-L-

threose (34). To a stirred solution of **28** (0.22 g, 0.54 mmol) and imidazole (110 mg, 1.61 mmol) in 5 mL of anhydrous DMF was added TBSCl (242 mg, 0.80 mmol) at 0 °C. The solution was stirred at rt overnight. The reaction mixture was diluted with ethyl acetate and washed with 1 M HCl solution and brine. The organic layer was separated, dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (60:1 to 30:1, DCM/MeOH) to give **34** (0.25 g, 89%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ 10.84 (d, *J* = 4.2 Hz, 1H, NH), 7.57 (d, *J* = 6.5 Hz, 1H, H-6), 5.67 (s, 1H, H-1'), 4.79–4.62 [m, 2H, CH(CH₃)₂], 4.33 (d, *J* = 10.3 Hz, 1H, H-4a'), 4.27 (s, 1H, H-2'), 4.15 (dd, *J* = 10.3, 3.1 Hz, 1H, H-4b'), 3.90 (d, *J* = 3.0 Hz, 1H, H-3'), 3.69 (d, *J* = 9.1 Hz, 2H, PCH₂), 1.31–1.25 [m, 12H, CH(CH₃)₂], 0.86 [s, 9H, C(CH₃)₃], 0.17 (s, 3H, SiCH₃), 0.12 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 157.3 (d, ²J_{F,C} = 26.5 Hz, C-4), 149.0 (C-2), 139.8 (d, ¹J_{F,C} = 235.1 Hz, C-5), 124.9 (d, ²J_{F,C} = 35.2 Hz, C-6), 92.8 (C-1'), 85.7 (d, ³J_{P,C} = 9.7 Hz, C-3'), 78.8 (C-2'), 73.6 (C-4'), 71.4 [CH(CH₃)₂], 64.3 (d, ¹J_{P,C} = 169.0 Hz, PCH₂), 25.5 [C(CH₃)₃], 23.8 [CH(CH₃)₂], 17.8 [C(CH₃)₃], –4.8, –5.3 (SiCH₃). ³¹P NMR (121 MHz, CDCl₃): δ 18.4. HRMS: [M+H]⁺ calcd for C₂₁H₃₉FN₂O₈PSi, 525.2192; found, 525.2201.

1'- α -(5-fluorouracil-1-yl)-2'-O-tert-butyltrimethylsilyl-3'-O-phosphonomethyl-L-threose (35).

This compound was prepared as described for **7**, obtained from **34** (230 mg, 0.44 mmol) as a white foam. Yield 67% (160 mg, Et₃N salt). ¹H NMR (300 MHz, D₂O): δ 7.87 (d, J = 6.6 Hz, 1H, H-6), 5.70 (s, 1H, H-1'), 4.42 (s, 1H, H-2'), 4.38 (d, J = 10.7 Hz, 1H, H-4a'), 4.17 (dd, J = 10.7, 3.5 Hz, 1H, H-4b'), 4.01 (d, J = 3.1 Hz, 1H, H-3'), 3.52 (d, J = 9.6 Hz, 2H, PCH₂), 0.83 [s, 9H, C(CH₃)₃], 0.13 (s, 3H, SiCH₃), 0.11 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, D₂O): δ 160.0 (d, ² J_{FC} = 25.1 Hz, C-4), 150.1 (C-2), 140.0 (d, ¹ J_{FC} = 231.1 Hz, C-5), 126.3 (d, ² J_{FC} = 35.1 Hz, C-6), 92.4 (C-1'), 84.6 (d, ³ J_{PC} = 11.9 Hz, C-3'), 78.2 (C-2'), 73.6 (C-4'), 65.4 (d, ¹ J_{PC} = 156.3 Hz, PCH₂), 24.7 [C(CH₃)₃], 17.0 [C(CH₃)₃], -5.9 (SiCH₃). ³¹P NMR (121 MHz, D₂O): δ 14.5. HRMS: [M-H]⁻ calcd for C₁₅H₂₅FN₂O₈PSi, 439.1107; found, 439.1114.

1'- α -(5-fluorouracil-1-yl)-2'-deoxy-3'-O-[N,N'-bis(*n*-propyl-L-phenylalaninate)]methylphosphonobisamidate]-L-threose (36). This compound was prepared as described for **6**. Obtained from **32** (100 mg, 0.14 mmol) as a white foam. Yield 90% (88 mg).

¹H NMR (500 MHz, acetone-*d*₆): δ 10.62 (brs, 1H, NH), 7.94 (d, J = 7.0 Hz, 1H, H-6), 7.31–7.19 (m, 10H, ArH), 6.11–6.08 (m, 1H, H-1'), 4.33 (dd, J = 10.4, 1.5 Hz, 1H, H-4a'), 4.29–4.22 (m, 2H, H-3' and Phe-CH), 4.11–4.98 (m, 5H, Phe-CH and OCH₂CH₂CH₃), 3.83–3.72 (m, 2H, H-4b' and NH), 3.56–3.38 (m, 3H, NH and PCH₂), 3.10–2.83 (m, 4H, Phe-CH₂), 2.58–2.51 (m, 1H, H-2a'), 2.10 (d, J = 15.0 Hz, 1H, H-2b'), 1.65–1.55 (m, 4H, OCH₂CH₂CH₃), 0.92–0.86 (m, 6H, OCH₂CH₂CH₃). ¹³C NMR (125 MHz, acetone-*d*₆): 174.0, 173.5 (Phe-CO), 157.8 (d, ² J_{FC} = 26.7 Hz, C-4), 149.9 (C-2), 141.0 (d, ¹ J_{FC} = 230.4 Hz, C-5), 138.2, 138.0, 130.5, 130.4, 129.1, 129.0, 127.43, 127.41 (Ar-C), 125.9 (d, ² J_{FC} = 34.6 Hz, C-6), 86.5 (C-1'), 80.8 (d, ³ J_{PC} = 11.1 Hz, C-3'), 74.3 (C-4'), 61.1 (OCH₂CH₂CH₃), 66.6 (d, ¹ J_{PC} = 136.6 Hz, PCH₂), 55.1, 54.8 (Phe-CH), 41.3, 41.2 (Phe-CH₂), 39.0 (C-2'), 22.6, 22.5 (OCH₂CH₂CH₃), 10.64, 10.59 (OCH₂CH₂CH₃). ³¹P NMR (121 MHz, acetone-*d*₆): δ 19.9. HRMS: [M+H]⁺ calcd for C₃₃H₄₃FN₄O₉P, 689.2746; found, 689.2745.

1'- α -(5-methylcytosin-1-yl)-2'-deoxy-3'-O-diisopropylphosphonomethyl-L-threose (38). To a solution of **37** (0.20 g, 0.51 mmol), Et₃N (0.22 mL, 1.54 mmol) and *N*-Methylpyrrolidine (0.11 mL, 1.02 mmol) in 3 mL of CH₂Cl₂ was added TsCl (0.20 g, 1.02 mmol) at 0 °C. After stirring at rt for 2 h, 10 mL of 35% aq. NH₃ was added. The reaction mixture was stirred at rt for overnight and concentrated. The residue was purified by column chromatography (20:1 to 10:1, DCM/MeOH) to give product **38** (0.15 g, 75% yield) as white foam. ¹H NMR (300 MHz, DMSO-

d_6): δ 7.47 (s, 1H, H-6), 7.21 (brs, 1H, NH_2), 6.74 (brs, 1H, NH_2), 6.09 (dd, $J = 8.0, 2.3$ Hz, 1H, H-1'), 4.66–4.50 [m, 2H, $\text{CH}(\text{CH}_3)_2$], 4.28–4.17 (m, 2H, H-3' and H-4a'), 3.87–3.68 (m, 3H, PCH_2 and H-4b'), 2.55–2.40 (m, 1H, H-2a', overlap with DMSO), 1.89 (d, $J = 15.2$ Hz, 1H, H-2b'), 1.85 (s, 3H, Me-5), 1.18–1.26 [m, 12H, $\text{CH}(\text{CH}_3)_2$]. ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 165.4 (C-4), 155.2 (C-2), 138.6 (C-6), 100.9 (C-5), 84.7 (C-1'), 80.2 (d, $^3J_{\text{P,C}} = 13.4$ Hz, C-3'), 72.5 (C-4'), 70.2 [$\text{CH}(\text{CH}_3)_2$], 63.0 (d, $^1J_{\text{P,C}} = 166.0$ Hz, PCH_2), 38.0 (C-2'), 23.7 [$\text{CH}(\text{CH}_3)_2$], 13.3 (Me-5). ^{31}P NMR (121 MHz, $\text{DMSO}-d_6$): δ 19.3. HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{29}\text{N}_3\text{O}_6\text{P}$, 390.1788; found, 390.1789.

1' α -(5-methylcytosin-1-yl)-2'-deoxy-3'-O-phosphonomethyl-L-threose (39). This compound was prepared as described for **7**. Obtained from **38** (150 mg, 0.39 mmol) and iodotrimethylsilane (0.44 mL, 3.08 mmol) as a white foam. Yield 51% (80 mg). ^1H NMR (300 MHz, D_2O): δ 7.75 (s, 1H, H-6), 6.18 (d, $J = 7.8$ Hz, 1H, H-1'), 4.45–4.37 (m, 2H, H-3' and H-4a'), 3.98 (d, $J = 10.3$ Hz, 1H, H-4b'), 3.62–3.48 (m, 2H, PCH_2), 2.65–2.52 (m, 1H, H-2a'), 2.26 (d, $J = 15.2$ Hz, 1H, H-2b'), 1.99 (s, 3H, Me-5), ^{13}C NMR (75 MHz, D_2O): δ 165.7 (C-4), 157.2 (C-2), 139.5 (C-6), 104.0 (C-5), 86.0 (C-1'), 79.6 (d, $^3J_{\text{P,C}} = 11.6$ Hz, C-3'), 73.6 (C-4'), 64.7 (d, $^1J_{\text{P,C}} = 159.2$ Hz, PCH_2), 36.9 (C-2'), 12.2 (Me-5). ^{31}P NMR (121 MHz, D_2O): δ 15.2. HRMS: $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_6\text{P}$, 304.0704; found, 304.0705.

1' α -(5-methylcytosin-1-yl)-2'-deoxy-3'-O-[*N,N'*-bis(*n*-propyl-L-phenylalaninate)]methylphosphonobisamidate]-L-threose (40). This compound was prepared as described for **8**. Obtained from **39** (20 mg, 0.049 mmol) as a white solid. Yield 30% (10 mg). ^1H NMR (600 MHz, $\text{acetone}-d_6$): δ 7.61 (s, 1H, H-6), 7.30–7.19 (m, 10H, ArH), 6.17 (dd, $J = 8.0, 2.6$ Hz, 1H, H-1'), 4.26–4.95 (m, 8H, Phe-CH, $\text{OCH}_2\text{CH}_2\text{CH}_3$, H-3' and H-4a'), 3.78 (d, $J = 8.0$ Hz, 1H, H-4b'), 3.35–3.27 (m, 2H, PCH_2), 3.08–2.79 (m, 4H, Phe- CH_2), 2.50–2.49 (m, 1H, H-2a'), 1.99 (1H, H-2b', overlap with acetone), 1.65–1.54 (m, 4H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 0.92–0.85 (m, 6H, $\text{OCH}_2\text{CH}_2\text{CH}_3$). ^{13}C NMR (150 MHz, $\text{acetone}-d_6$): 173.8, 173.6 (Phe-CO), 166.5 (C-4), 156.2 (C-2), 140.1 (C-6), 138.2, 138.1, 130.6, 130.5, 129.1, 127.5 (Ar-C), 101.9 (C-5), 86.4 (C-1'), 81.3 (d, $^3J_{\text{P,C}} = 12.1$ Hz, C-3'), 73.8 (C-4'), 67.1 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 66.7 (d, $^1J_{\text{P,C}} = 137.1$ Hz, PCH_2), 55.2, 54.9 (Phe-CH), 41.4, 41.2 (Phe- CH_2), 39.0 (C-2'), 22.6, 22.5 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 13.6 (Me-5), 10.7, 10.6 ($\text{OCH}_2\text{CH}_2\text{CH}_3$). ^{31}P NMR (121 MHz, $\text{acetone}-d_6$): δ 19.8. HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{47}\text{N}_5\text{O}_8\text{P}$, 684.3157; found, 684.3148.

HIV Antiviral Assay. Evaluation of the antiviral activity of the compounds against HIV-1 strain III_B and HIV-2 strain ROD in MT-4 cells was performed using the MTT assay as previously described.^{25,26} Stock solutions (10× final concentration) of test compounds were added in 25 µL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated HIV- and mock-infected cell samples were included as controls. HIV-1(III_B) stock (50 µL) at 100–300 CCID₅₀ (50% cell culture infectious doses) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effects of test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 g, and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50 µL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock-and HIV-infected cells was examined spectrophotometrically using the MTT assay. The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics) by mitochondrial dehydrogenase activity in metabolically active cells to a blue–purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Infinite M1000, Tecan) at two wavelengths (540 and 690 nm). All data were calculated using the median absorbance value of three wells. The 50% cytotoxic concentration (CC₅₀) was defined as the concentration of the test compound that reduced the absorbance (OD₅₄₀) of the mock-infected control sample by 50%. The concentration achieving 50% protection against the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC₅₀).

HBV Antiviral Assay. The primary anti-HBV assay was performed as previously described^{28,29} with modifications to use a quantitative blot hybridization assay to measure extracellular HBV DNA copy number associated with virions released from HepG2 2.2.15 cells. The HepG2 2.2.15 cell line is a stable human hepatoblastoma cell line that contains two copies of the HBV wild-type strain ayw1 genome and constitutively produces high levels of HBV.²⁷ Briefly, HepG2 2.2.15 cells were plated in 96-well microtiter plates at 1.5×10^4 cells/well in Dulbecco's Modified Eagle's Medium supplemented with 2% FBS, 380 µg/mL G418, 2.0 mM L glutamine, 100 units/mL

penicillin, 100 µg/mL streptomycin, and 0.1 mM nonessential amino acids. Only the interior wells are utilized to reduce “edge effects” observed during cell culture; the exterior wells are filled with complete medium to help minimize sample evaporation. After 16–24 h, the confluent monolayer of HepG2 2.2.15 cells was washed and the medium was replaced with complete medium containing various concentrations of a test compound in triplicate. Lamivudine (3TC) was used as the positive control, while media alone was added to cells as a negative control (virus control, VC). Three days later, the culture medium is replaced with fresh medium containing the appropriately diluted test compounds. Six days following the initial administration of the test compound, the cell culture supernatant was collected, treated with Pronase and then used in a quantitative blot hybridization assay. The PCR-amplified HBV DNA was detected in real-time by monitoring increases in fluorescent signal that result from the exonucleolytic degradation of a quenched fluorescent probe molecule that hybridized to the amplified HBV DNA. For each PCR amplification, a standard curve was simultaneously generated using dilutions of purified HBV DNA. Antiviral activity was calculated from the reduction in HBV DNA levels (EC_{50} and EC_{90} values determined). A tetrazolium dye (MTS; 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; CellTiter96 Reagent, Promega) uptake assay was then employed to measure cell viability, which was used to calculate toxicity (CC_{50}).

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†Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR spectra of all novel compounds.

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