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Authors: Toti, Kiran; Derudas, Marco; McGuigan, Christopher; Balzarini, Jan and Van Calenbergh, Serge

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Synthesis and antiviral evaluation of α-L-2'-deoxythreofuranosyl nucleosides

Kiran S. Toti,[a] Marco Derudas,[b] Christopher McGuigan,[b] Jan Balzarini,[c] and Serge Van Calenbergh[a,*]

[a] *Laboratory for Medicinal Chemistry (FFW), UGent, Harelbekestraat 72, 9000 Gent, Belgium*

[b] *Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3NB, UK*

[c] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, 3000 Leuven, Belgium*

Abstract

The synthesis of a series of α-L-2'-deoxythreofuranosyl nucleosides featuring the nucleobases A, T, C and U is described in seven steps from 1,2-O-isopropyledene-α-L-threose, involving a Vorbrüggen coupling and a Barton-McCombie deoxygenation protocol as the key steps. All analogues, including a phosphoramidate nucleoside phosphate prodrug of the T analogue, were evaluated against a broad panel of different viruses but found inactive, while also lacking notable cellular toxicity. The thymidine analogue showed inhibition to mitochondrial thymidine kinase-2 (TK-2), herpes simplex virus type 1 (HSV-1) TK, varicella-zoster virus (VZV) TK and *M. tuberculosis* thymidylate kinase.

* Corresponding author. Tel.: +32 9 264 81 24; fax: +32 9 264 81 46; e-mail: serge.vancalenbergh@ugent.be

Introduction

The human immunodeficiency virus (HIV) epidemic has fostered research on antiviral drugs both in academia and industry [1]. Although preventive vaccines are available for a number of viral diseases, the chances of successfully developing a safe and effective vaccine for HIV and hepatitis C virus (HCV) in the near future looks very uncertain [2]. Structural modifications on either the sugar or base moiety of natural nucleosides has resulted in a series of 24 nucleoside or nucleotide analogues licensed as drugs against various viral infections, such as herpes simplex virus (HSV), human cytomegalovirus (HCMV), varicella zoster virus (VZV), HIV-1, human hepatitis B virus (HBV) and HCV [3-5]. Despite this achievement, continued efforts toward the discovery of new nucleoside-based antivirals are required to overcome common problems in antiviral chemotherapy, such as toxicity, adverse effects, drug-drug interactions and, last but not least, the development of drug resistance.

Recently, Herdewijn and coworkers showed that selected L-2-deoxythreose nucleoside phosphonates (**I**; Figure 1) selectively inhibit HIV without affecting human DNA synthesis [6]. PMDTA (**Ia**) and PMDTT (**Ib**) lack a hydroxymethyl group at the 4'-position of the furanose ring, but instead have a phosphonomethyl ether moiety at the 3'-position, which mimicks the monophosphate ester of a 3’-hydroxymethyl substituent.

These nucleoside analogues require two additional phosphorylation steps by cellular kinases before they are incorporated in the viral genome and lead to chain termination of the proviral DNA strand during the reverse transcription process.

By synthesizing and evaluating the isomeric analogues of PMDTA, the importance of the relative (*cis*) and absolute orientation of the substituents at the 1' and 3'- positions has been demonstrated to be crucial for antiviral activity [6b]. Also, it was shown that homologation of the 3'-O-phosphonomethyl group of **Ia-b** to a phosphonoethyl was at the expense of the biological activity [6c]. Most likely, this loss of activity results from the poor acceptance of these homologues as substrates by kinases required for conversion to their metabolic diphosphate form. Noteworthy, in 2000 Eschenmoser and coworkers showed that (L)-α-threofuranosyl oligonucleotides (TNAs) containing vicinally connected (3'→2') phosphodiester bridges undergo informational base pairing in antiparallel strand orientation and are capable of cross-pairing with RNA and DNA [7].

Inspired by the promising anti-HIV-1/HIV-2 activity of **Ia-b**, we decided to explore the synthesis and the antiviral properties of the corresponding non-phosphonylated 2-deoxythreose-based nucleoside analogues **II**, characterized by a four-carbon-only carbohydrate part. We expected that due to the absence of the 4'-hydroxymethyl group, the secondary 3'-OH could become accessible for phosphorylation by cellular nucleoside kinases and anticipated **IIa-d** to have a superior bioavailability compared to the phosphonates. Remarkably, the synthesis of such α-L-2'-deoxythreofuranosyl analogues (exhibiting the 1'R, 3'R configuration) has not been reported before.

Results and Discussion

Chemical Synthesis

The synthesis of target compounds started from 1,2-*O*-isopropyledene-α-L-threose **1** (Scheme 1), which was synthesized in multi-gram scale following literature procedures[8,9]. The hydroxyl group of **1** was protected using either benzyl bromide or 4-methoxybenzyl (PMB) chloride to give compounds **2** and **3** in excellent yields. A benzyl group is a safe and versatile protecting group for carbohydrates, but may require harsh deprotection conditions. We also explored a more sensitive PMB protecting group, which may be removed under alternative conditions. Next, deprotection of the isopropylidene group upon treatment with 80% aq. acetic acid followed by acetylation afforded the key precursors **4** and **5** in good yield.

A Vorbrüggen coupling reaction of **4** and **5** with the preformed silyl-protected nucleobases exclusively gave the α-nucleosides **6-11** due to anchimeric assistance from the 2-O-acetyl group [10]. The benzyl-protected thymine- and uracil-nucleosides **6** and **7** were isolated in considerably better yields than the PMB protected analogues, due to instability of the PMB-protecting group under Vorbrüggen conditions. Moreover, the reaction between PMB-protected glycon **5** with silylated N^6-benzoyladenine and N^4-acetylcytosine failed to produce the desired coupling products in acceptable yields, which forced us to restrict further investigations to the benzyl-protected nucleosides. Under normal coupling conditions, the yield of the adenine coupling product **10** was also very low, possibly due to the formation of other isomers [11]. However, prolonged reaction time at elevated temperature (50 °C) afforded the thermodynamically favored isomer **10**

in an acceptable yield of 42%. Under the normal coupling conditions the cytidine analogue **11** was obtained in 70 % yield.

The 2'-*O*-acetyl group of **6** and **7** was removed by treatment with 7N ammonia in methanol. Similar treatment of **8** and **9** led to the formation of small amounts of 2',3'-dihydroxy analogues (10-15%). Lowering the ammonia concentration to 1N allowed to obtain the desired compounds **14** and **15** in high yields. Stirring compound **10** at room temperature in a 7N solution of ammonia in methanol for 2 days removed both the 2'-O-acetyl and the N(6)-benzoyl group to yield **16**. Likewise, **11** was deprotected to the dideacetylated compound **17**, which was selectively N-acylated to **18** with one equivalent of acetic anhydride in dimethylformamide [12].

Deoxygenation of the 2'-hydroxy group was realized following a two step Barton-McCombie procedure [13]. The xanthate was formed by reacting the 2'-OH group of **12-16** and **18** with p-tolyl chlorothionocarbonate in the presence of 4-dimethylaminopyridine. The intermediate formed was subjected to heating with tributyltinhydride and azoisobisbutyronitrile in toluene to give the 2'-deoxygenated compounds **19-24** in moderate to good yields (47-86%).

All target compounds were obtained after debenzylation. However, optimal debenzylation procedures were distinct for each analogue. The 2'-deoxythreose thymine and uracil analogues **25** and **26** could be obtained by catalytic hydrogenation of **19** and **20** in good albeit variable yields, possibly due to catalyst poisoning by the residual sulphur from the previous deoxygenation reaction. Desulphurization with hydrogen over Raney-Nickel prior to palladium-catalysed debenzylation improved the reproducibility of this step. Alternatively, CAN-mediated deprotection [14] of **21** and **22** afforded **25** and **26**,

respectively. The adenine analogue **27** was obtained by a palladium-catalysed hydrogenation reaction in moderate yields.

The cytosine analogue **29** was obtained via two different routes. After an unsuccessful attempt to hydrogenate **24**, the benzyl group was removed by treatment with excess of dichlorodicyanoquinone (DDQ) in dichloromethane at 50 °C in a sealed reaction vial for 3 days [15]. This method gave **28** in a disappointing yield of 11%. Hence, we also explored the transformation of the uridine analogue **26** to compound **29** (Scheme 2) [16]. This was realized by protecting the 3'-hydroxy group of **26** to the corresponding acetate **30**, which was subsequently treated with phosphorous oxychloride and 1,2,4-triazole, bubbling ammonia gas and a 7N solution of ammonia in methanol.

The coupling between the phenyldichlorophosphate (**31**) and L-alanine benzyl ester tosylate (**32**) has been performed in the presence of Et_3N (2 eq) giving the desired product (**33**) as an oil, which was used in the following step as a crude (Scheme 3). The final coupling of the nucleoside **25** has been performed using an excess of the phosphorochloridate (**33**) (3 eq) in the presence of ^{t}BuMgCl (3eq) following the procedure reported by Uchiyama [17] and extensively used for the synthesis of the ProTides [18]. The desired compound **34** was obtained as a mixture of two diastereoisomers confirmed by the presence of two peaks in the ^{31}P NMR (2.92, 2.27).

Pharmacological Activity

None of the final nucleoside analogues showed cytotoxicity at 100 µg/mL. No anti-HIV-1 and anti-HIV-2 activity was observed in human T-lymphocyte (CEM) cells (EC50 >100 µg/mL). Furthermore, no significant activity could be detected against the

following viruses: human cytomegalovirus (HEL), varicella-zoster virus (VZV) (HEL); influenza A (H1N1 &H3N2) and B virus (MDCK), feline corona & feline herpes virus (CRFK); herpes simplex virus type 1 (HSV-1), HSV-2, vesicular stomatitis virus (VSV) and vaccinia virus (HEL); Coxsackie virus B4 and respiratory syncytial virus (HeLa); para-Influenza virus-3, reovirus-3, Sindbis virus and Punta Toro virus (Vero). Since the lack of antiviral activity could be due to the inability of virally-induced and/or cellular enzymes to active the nucleoside analogues, a nucleoside monophosphate prodrug was prepared from compound **25** (Scheme 3). Such prodrug may intracellularly release the free monophosphorylated form according to a multistep process lined-out in Scheme 4. However, also the phosphoramidate **34** failed to show activity against HIV-1, HIV-2, HSV-1, HSV-2, vaccinia virus, vesicular stomatitis virus and HSV-1 (TK^-).

Enzymatic study using carboxypeptidase Y enzyme.

The first step of the bioactivation of the phosphoramidate ProTide moiety involves the hydrolysis of the ester moiety which is supposed to be mediated by a carboxypeptidase-type enzyme (Scheme 4). An enzymatic study using carboxypeptidase Y enzyme has been performed in order to understand whether compound **34** may be metabolized under these conditions.

The experiment was performed by incubating compound **34** with carboxypeptidase in acetone-d_6 and trizma buffer (pH = 7.6) following its conversion by ^{31}P-NMR. The spectra (Figure 2) showed a fast conversion of one of the diasteroisomers **34** (δ_P = 2.38) to the compound **37** (δ_P = 6.25) through the intermediate **35** (δ_P = 3.66). The other

diastereoisomer (δ_P = 2.24) was slower converted and it was still detectable after 14 h. This experiment showed that **34** is partially converted to the metabolite **37**.

Interaction of test compounds with nucleoside kinases.

Interestingly, upon evaluation of their capacity to inhibit thymidine (1 µM) phosphorylation by recombinant purified human cytosolic TK1, human mitochondrial TK2, HSV-1 TK, VZV TK, and *Drosophila melanogaster* (Dm) dNK (Table 1), compound **25** proved inhibitory to TK-2, HSV-1 TK, and VZV TK at an IC_{50} ranging between 14 and 91 µg/mL and *M. tuberculosis* thymidylate kinase with a *K*i of 18 µM. Therefore, compound **25** was investigated more in detail. Whereas 75% of the natural substrate thymidine (100 µM) was converted to its 5'-mono- (and 5'-di)phosphate within 5 minutes upon exposure to HSV-1 TK, no signs of any conversion of compound **25** to its phosphorylated derivative was observed after 60 minutes of incubation with the enzyme. Therefore, we may assume that these nucleosides purely act as enzyme inhibitors, rather than acting as alternative substrates. Lineweaver-Burk kinetic analysis revealed a non-competitive mechanism of inhibitory action of compound **25** against HSV-1 TK using dThd as the natural substrate (Figure 3). The inhibitory potential of some of the test compounds opens perspectives for using this L-α-2'-deoxythreofuranosyl ring as a scaffold for optimizing these enzyme inhibitory activities.

In conclusion, we have developed a practical and straightforward procedure for the synthesis of a series of α-L-2'-deoxythreofuranosyl nucleosides containing four natural nucleobases. None of the analogues displayed significant antiviral activity or cytotoxicity. In an effort to bypass the first activation (phosphorylation) step of the nucleoside

analogues, a phosphoramidate nucleoside phosphate prodrug was synthesized for compound **25**, but this prodrug still lacked any measurable antiviral activity. However, the thymine analogue showed encouraging inhibitory activity towards a number of thymidine kinases, which may be a new starting point towards more potent and selective nucleoside kinase inhibitors.

Experimental Section

Chemical Synthesis

All reagents were from standard commercial sources and of analytic grade. Dry solvents were obtained directly from commercial sources and stored on molecular sieves. Moisture sensitive reactions were carried out under argon atmosphere. Precoated Merck silica gel F254 plates were used for TLC, spots were examined under ultraviolet light at 254 nm and further visualized by sulphuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (63-200 μm, 60 Å, Biosolve, Valkenswaard, The Netherlands). NMR spectra were determined using a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent signals or TMS as internal standard. Exact mass measurements were performed on a Waters LCT PremierXETMTime of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/water (1:1) mixture at 10 μL/min.

(3aR,6S,6aR)-6-(benzyloxy)-tetrahydro-2,2-dimethylfuro[2,3-d][1,3]dioxole (2). To a solution of compound **1** (2.3 g, 14.36 mmol) in dry DMF (50 mL) cooled at 0 °C under inert atmosphere, NaH (60% in mineral oil, 0.861 g, 21.54 mmol) was added portionwise

and the mixture was stirred for 10 minutes. Benzyl bromide (2.56 mL, 21.54 mmol) was added dropwise to the above suspension, which was allowed to come to room temperature and stirred for an additional 4h. The reaction mixture was quenched by addition of methanol (1 mL) and the volatile solvents were evaporated under reduced pressure. The residue was partitioned between water-ethyl acetate (1:3, 300 mL). The organic layer was separated, washed with water, brine and dried over anhydrous $MgSO_4$. After evaporation, the crude product was purified by silica-gel chromatography (15% EtOAc-hexanes) to afford compound **2** (3.1 g, 87%) as a colorless oil. ^{1}H NMR($CDCl_3$, 300 MHz): δ 1.32 (*s*, 3H), 1.47 (*s*, 3H), 4.01-4.05 (*m*, 3H), 4.57 (*s*, 2H), 4.61 (*d*, *J* = 3.77 Hz, 1H), 5.96 (*d*, *J* = 3.76 Hz, 1H), 7.27-7.39 (*m*, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.15, 26.71, 70.27, 71.09, 81.81, 82.84, 105.44, 111.46, 127.59, 127.80, 128.41, 137.32. ESI-HRMS for $[C_{14}H_{18}O_4 + NH_4]^+$ calcd, 268.1549; found, 268.1565.

(3aR,6S,6aR)-6-(4-methoxybenzyloxy)-tetrahydro-2,2-dimethylfuro[2,3-d][1,3]dioxole (3). Compound **1** (1.5 g, 9.4 mmol) was reacted with NaH (60% in mineral oil, 0.45 g, 11.24 mmol) and p-methoxybenzylchloride (1.5 mL, 11.24 mmol) as described for synthesis of **2** to afford compound **3** (2.2 g, 84%) as a white low melting solid. ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.31 (*s*, 3H), 1.46 (*s*, 3H), 3.78 (*s*, 3H), 3.98-4.02 (m, 3H), 4.48 (*s*, 3H), 4.58 (*d*, *J* = 3.76 Hz, 1H), 5.94 (*d*, *J* = 3.76 Hz, 1H), 6.87 (*d*, *J* = 8.72 Hz, 2H), 7.24 (*d*, *J* = 8.77 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.15, 26.71, 55.16, 70.28, 70.78, 81.45, 82.87, 105.42, 111.42, 113.82, 129.27, 129.34, 159, 30. ESI-HRMS for $[C_{15}H_{20}O_5 + K]^+$ calcd, 319.0942; found, 319.0937.

(3R,4S)-4-(benzyloxy)-tetrahydro-2, 3-furanyl diacetate (4). A solution of **2** (2.0 g, 8 mmol) in 80% aq. acetic acid (40 mL) was stirred at 80 °C for 8 h. The reaction mixture

was evaporated to give the crude intermediate as syrup. This syrup was dissolved in pyridine (30 mL) and treated with acetic anhydride (7.5 mL, 80 mmol). The solution was stirred at room temperature for 4h. The solvent was removed under vacuum and the resulting residue was purified by silica-gel column chromatography (20% EtOAc-hexanes) to yield **4** (1.9 g, 81%) as a low melting solid. α,β anomeric ratio 3:2. ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.98, 2.00, 2.02, 2.03 (s's, 6H), 3.78-4.29 (m, 3H), 4.43-4.71 (m, 2H), 5.13-5.19 (m, 1H), 6.06-6.32 (s & d, *J* = 4.51 Hz, 1H), 7.20-7.32 (m, 5H). ESI-HRMS for $[C_{15}H_{18}O_6 + K]^+$ calcd, 333.0735; found, 333.0738.

(3R,4S)-4-(4-methoxybenzyloxy)-tetrahydro-2,3-furanyl diacetate (5). Employing the procedure described above on 2.2 g of compound **3** resulted in 1.6 g (63% yield) of compound **5** as a white solid. α,β anomeric ratio 1:1. ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.04, 2.07, 2.08, 2.09 (s's, 6H), 3.79 (s, 3H), 3.82-4.32 (m, 3H), 4.42-4.68 (m, 2H), 5.17-5.24 (m, 1H), 6.11, 6.37 (2d's, *J* = 4.49, 2.39 Hz, 1H), 6.83-6.91 (m, 2H), 7.20-7.30 (m, 2H). ESI-HRMS for $[C_{16}H_{20}O_7 + Na]^+$ calcd, 347.1101; found, 347.1112.

General condition for Vorbrüggen coupling reaction: The nucleobase (protected in case of adenine and cytosine) (1.2 eq.) was suspended in hexamethyldisilazane (50 eq.) containing trimethylsilyl chloride (0.7 eq.) and pyridine (10 eq.). The mixture was heated at reflux overnight. After cooling, the reaction mixture was evaporated and dried under high vacuum. The silylated nucleobase and the diacetate compound **4** or **5** (1 eq.) were dissolved in dry 1,2-dichloroethane (7 mL/mmol), and trimethylsilyl triflate (1.2 eq) was added dropwise at 0 °C. The clear solution was stirred for 4h at room temperature. The reaction mixture was diluted with dichloromethane (50 mL/ mmol) and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and

evaporated. Purification of the residue by silica-gel column chromatography (1% MeOH-dichloromethane) afforded the pure coupling product as a white foam.

(2R,3R,4S)-4-(benzyloxy)-tetrahydro-2-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)furan-3-yl acetate (6). Following the general reaction conditions a Vorbrüggen coupling between **4** and thymine afforded the title compound (1.07 g, 87%) ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.72 (d, *J*= 1.19 Hz, 3H), 2.12 (s, 3H), 4.00-4.1 (m, 2H), 4.32 (app-dm, *J*= 8.75 Hz, 1H), 4.63 (app- dd, *J*= 14.68, 11.48 Hz, 2H), 5.22 (s, 1H), 6.08 (d, *J*= 1.36 Hz, 1H), 7.22-7.34 (m, 5H), 7.37 (d, *J*= 1.22 Hz, 1H), 9.42 (*brs*, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.57, 21.03, 71.81, 73.74, 79.93, 80.88, 89.41, 110.94, 128.17, 128.47, 128.83, 136.41, 136.89, 150.69, 164.23, 169.90. ESI-HRMS for $[C_{18}H_{20}N_2O_6 + H]^+$ calcd, 361.1400; found, 361.1399.

(2R,3R,4S)-4-(benzyloxy)-tetrahydro-2-(3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)furan-3-yl acetate (7). Vorbrüggen coupling between **4** (500 mg, 1.7 mmol) and uracil (230 mg, 2.04 mmol) afforded 481 mg of the uridine analogue **7** (82 % yield). ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.07 (s, 3H), 3.96 (d, *J*= 3.78 Hz, 1H), 4.02 (dd, *J*= 10.25, 3.81 Hz, 1H), 4.24 (d, *J*= 10.28 Hz, 1H), 4.56 (app-q, *J*= 11.76 Hz, 2H), 5.17 (s, 1H), 5.55 (dd, *J*= 8.19, 1.77 Hz, 1H), 5.96 (d, *J*= 1.18 Hz, 1H), 7.17-7.32 (m, 5H), 7.48 (d, *J*= 8.20 Hz, 1H), 8.93 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.03, 71.88, 74.27, 79.72, 80.52, 89.82, 102.23, 128.28, 128.54, 128.87, 136.80, 140.58, 150.39, 163.39, 169.79. ESI-HRMS for $[C_{17}H_{18}N_2O_6 + H]^+$ calcd, 347.1238; found 347.1246.

(2R,3R,4S)-4-(4-methoxybenzyloxy)-tetrahydro-2-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)furan-3-yl acetate (8). Vorbrüggen coupling between **5** (600

mg, 1.85 mmol) and thymine yielded 420 mg of the title compound (58 %). ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.68 (d, *J*= 1.21 Hz, 3H), 2.07 (s, 3H), 3.73 (s, 3H), 3.98 (dd, *J*= 14.17, 3.72 Hz, 2H), 4.14-4.25 (m, 1H), 4.50 (app-q, *J*= 14.14 Hz, 2H), 5.15 (m, 1H), 6.00 (d, *J*= 1.34 Hz, 1H), 6.80 (d, *J*= 8.72 Hz, 2H), 7.14 (d, *J*= 8.78 Hz, 2H), 7.31 (d, *J*= 1.24 Hz, 1H), 8.76 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.60, 21.05, 55.54, 71.48, 73.85, 79.87, 80.53, 89.45, 110.83, 114.21, 128.95, 129.88, 136.50, 150.48, 159.84, 163.97, 169.87. ESI-HRMS for $[C_{19}H_{22}N_2O_7 + H]^+$ calcd, 391.15; found, 391.1521.

(2R,3R,4S)-4-(4-methoxybenzyloxy)-tetrahydro-2-(3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)furan-3-yl acetate (9). Vorbrüggen coupling between **5** (325 mg, 1.0 mmol) and uracil yielded compound **9** (180 mg, 47%). ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.12 (s, 3H), 3.79 (s, 3H), 4.00 (d, *J*= 3.73 Hz, 1H), 4.07 (dd, *J*= 10.24, 3.81 Hz, 1H), 4.27 (d, *J*= 10.23 Hz, 1H), 4.55 (app-q, *J*= 12.73 Hz, 2H), 5.23 (s, 1H), 5.62 (dd, *J*= 8.21, 1.47 Hz, 1H), 6.02 (d, *J*= 1.03 Hz, 1H), 6.86 (d, *J*= 8.81 Hz, 2H), 7.19 (d, *J*= 8.79 Hz, 2H), 7.54 (d, *J*= 8.21 Hz, 1H), 9.36 (brm, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.00, 55.50, 71.42, 74.32, 79.61, 80.15, 89.76, 102.16, 114.20, 128.91, 129.99, 140.68, 150.71, 159.80, 164.03, 169.85. ESI-HRMS for $[C_{18}H_{20}N_2O_7 + H]^+$ calcd, 377.1343; found 377.1369.

(2R,3R,4S)-2-(6-(benzamido)-9H-purin-9-yl)-4-(benzyloxy)-tetrahydrofuran-3-yl acetate (10). To a suspension of N^6-benzoyladenine (1.0 g, 4.08 mmol) in hexamethyldisilazane (35 mL, 0.17 mol) was added trimethylsilyl chloride (0.3 mL, 2.38 mmol) and pyridine (2.7 mL, 34 mmol). The mixture was heated at reflux overnight. After cooling it was evaporated and dried under high vacuum. The silylated nucleobase and the diacetate **4** (1.0 g, 3.4 mmol) were dissolved in dry 1,2-dichloroethane (20 mL) and trimethylsilyl triflate (0.74 mL, 4.08 mmol) was added dropwise at 0 °C. The clear

solution was stirred at 50 °C for 24h. After dilution with dichloromethane (250 mL), the reaction mixture was washed with saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. purification of the residue by silica-gel flash column chromatography (60 % EtOAc – hexanes) afforded pure **10** (0.68 g, 42%) as white foam.^{1}H NMR ($CDCl_3$, 300MHz): δ 2.08 (s, 3H), 4.04 (d, *J*= 3.76 Hz, 1H), 4.31 (dd, *J*= 10.34, 3.89 Hz, 1H), 4.4 - 4.52 (m, 3H), 5.48 (s, 1H), 6.71 (s, 1H), 7.00 - 7.10 (m, 5H), 7.31 – 7.50 (m, 3H), 8.08 (s, 1H), 8.12 – 8.19 (m, 2H), 8.59 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.03, 72.12, 76.07, 79.94, 80.49, 93.07, 127.81, 128.08, 128.16, 128.22, 128.56, 128.74, 129.89, 132.32, 136.84, 137.81, 142.01, 143.61, 148.71, 169.68, 175.13. ESI-HRMS for $[C_{25}H_{23}N_5O_5 + H]^+$ calcd, 474.1772; found, 474.1698.

(2R,3R,4S)-4-(benzyloxy)-tetrahydro-2-(4-(acetylamino)-2-oxopyrimidin-1(2H)-yl)furan-3-yl acetate (11). A Vorbrüggen coupling reaction between N^4-acetylcytosine and compound **4** (295 mg, 1.0 mmol) yielded compound **11** (270 mg, 70%). ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.11 (s, 3H), 2.28 (s, 3H), 4.01 (d, *J*= 3.77 Hz, 1H), 4.20 (dd, *J*= 10.31, 3.86 Hz, 1H), 4.35 (d, *J*= 10.25 Hz, 1H), 4.97 (app-q, *J*= 13.94 Hz, 2H), 5.28 (s, 1H), 5.34 (s, 1H), 6.05 (s, 1H), 7.14 - 7.20 (m, 2H), 7.25 – 7.32 (m, 3H), 7.34 (d, *J*= 7.61 Hz, 1H), 7.89 (d, *J*= 7.57 Hz, 1H), 10.34 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.10, 25.10, 71.67, 75.25, 79.15, 80.15, 91.22, 96.48, 128.21, 128.37, 128.77, 136.84, 145.07, 155.25, 163.53, 169.70, 171.44. ESI-HRMS for $[C_{19}H_{21}N_3O_6 + H]^+$ calcd, 388.1503; found, 388.1509

1-((2R,3R,4S)-4-(benzyloxy)-tetrahydro-3-hydroxyfuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (12). The 2'-*O*-acetylated nucleoside **6** (580 mg, 1.6 mmol) was treated with 7N methanolic ammonia solution (15 mL) at room temperature overnight.

Evaporation yielded a residue which was purified by column chromatography (2% MeOH-CH_2Cl_2) to afford compound **12** (420 mg, 82 %) as a white foam.^{1}H NMR ($CDCl_3$, 300MHz): δ 1.67 (d, *J*= 0.75 Hz, 3H), 4.07 (s, 1H), 4.30 (s, 2H), 4.42 (s, 3H), 5.59 (brs, 1H), 7.78 (s, 1H), 7.06-7.24 (m, 5H), 7.30 (d, *J*= 1.14 Hz, 1H), 10.60 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.24, 71.45, 74.97, 78.10, 82.20, 93.54, 109.38, 127.59, 127.96, 128.45, 136.75, 136.92, 150.96, 164.73. ESI-HRMS for $[C_{16}H_{18}N_2O_5 - H]^-$ calcd, 317.1143; found 317.1147.

1-((2R,3R,4S)-4-(benzyloxy)-tetrahydro-3-hydroxyfuran-2-yl)pyrimidine-2,4(1H,3H)-dione (13). Following the procedure described for **12**, compound **7** (475 mg, 1.37 mmol) was hydrolysed to afford **13** (340 mg, 81%) as a white foam. ^{1}H NMR ($CDCl_3$, 300MHz): δ 4.05 (s, 1H), 4.28 (s, 2H), 4.41 (s, 1H), 4.43 (s, 2H), 5.54 (dd, *J*= 8.10, 1.81 Hz, 1H), 5.73 (s, 1H), 7.10-7.30 (m, 5H), 7.48 (d, *J*= 8.16 Hz, 1H), 10.52 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 71.85, 75.31, 82.29, 94.00, 101.32, 128.01, 128.37, 128.79, 137.11, 140.94, 151.30, 164.38. ESI-HRMS for $[C_{15}H_{16}N_2O_5 + H]^+$ calcd, 305.1132; found, 305.1132.

1-((2R,3R,4S)-4-(4-methoxybenzyloxy)-tetrahydro-3-hydroxyfuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (14). Compound **8** (400 mg, 1.02 mmol) was treated with 1N methanolic ammonia solution (10 mL) at room temperature for 6h. Evaporation yielded a residue which was purified by column chromatography (2% MeOH-CH_2Cl_2) to afford compound **14** (280 mg, 78 %) as a white foam.^{1}H NMR ($CDCl_3$, 300MHz): δ 1.69 (d, *J*= 1.1 Hz, 3H), 3.71 (s, 3H), 4.03 (m, 1H), 4.27 (d, *J*= 1.84 Hz, 2H), 4.35 (s, 2H), 4.40 (s, 1H), 5.76 (s, 1H), 6.76 (d, *J*= 8.72 Hz, 2H), 7.03 (d, *J*= 8.70 Hz, 2H), 7.29 (d, *J*= 1.21 Hz, 1H),10.47 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ

12.57, 55.54, 71.41, 75.15, 78.48, 82.24, 93.77, 109.64, 114.15, 129.30, 129.57, 137.02, 151.20, 159.67, 164.92. ESI-HRMS for $[C_{17}H_{20}N_2O_6 + H]^+$ calcd, 349.1394; found, 349.1389.

1-((2R,3R,4S)-4-(4-methoxybenzyloxy)-tetrahydro-3-hydroxyfuran-2-yl)pyrimidine-2,4(1H,3H)-dione (15). The ester moiety of **9** (180 mg, 0.48 mmol) was hydrolysed as described in the preparation of **14** to give compound **15** (145 mg, 90%) as white foam. ^{1}H NMR ($CDCl_3$, 300MHz): δ 3.77 (s, 3H), 4.08-4.12 (m, 1H), 4.26-4.36 (m, 2H), 4.42 (s, 2H), 4.46 (s, 1H), 5.55 (brs, 1H), 5.60 (dd, *J*= 8.14, 1.74 Hz, 1H), 5.79 (s, 1H), 6.83 (d, *J*= 8.77 Hz, 2H), 7.11 (d, *J*= 8.65 Hz, 2H), 7.53 (d, *J*= 8.19 Hz, 1H), 10.74 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 55.52, 71.50, 75.44, 78.24, 82.05, 93.93, 101.26, 114.17, 129.28, 129.69, 141.05, 151.40, 159.71, 164.51. ESI-HRMS for $[C_{16}H_{18}N_2O_6 + H]^+$ calcd, 335.1238; found, 335.1255.

(2R,3R,4S)-2-(6-amino-9H-purin-9-yl)-4-(benzyloxy)-tetrahydrofuran-3-ol (16). Nucleoside **10** (680 mg, 1.43 mmol) was treated with 7N methanolic ammonia solution (20 mL) at room temperature for 2 days. Evaporation yielded a residue which was purified by column chromatography (4% MeOH-CH_2Cl_2) to afford compound **16** (450 mg, 95 %) as a white foam. ^{1}H NMR (DMSO-D_6, 300MHz): δ 4.10 – 4.15 (m, 1H), 4.19 (dd, *J*= 9.75, 4.72 Hz, 1H), 4.29 (dd, *J*= 9.72, 2.61 Hz, 1H), 4.57 (s, 2H), 4.66 – 4.71 (m, 1H), 5.95 (d, *J*= 2.28 Hz, 1H), 6.02 (d, *J*= 4.52 Hz, 1H), 7.20 – 7.40 (m, 7H), 8.16 (s, 2H). ^{13}C NMR (DMSO-D_6, 75 MHz): δ 75.82, 78.18, 82.47, 87.32, 95.55, 123.17, 131.76, 131.96, 132.43, 141.20, 143.53, 152.62, 156.47, 159.74. ESI-HRMS for $[C_{16}H_{18}N_5O_3 + H]^+$ calcd, 328.1404; found, 328.1419.

4-amino-1-((2R,3R,4S)-4-(benzyloxy)-tetrahydro-3-hydroxyfuran-2-yl)pyrimidin-2(1H)-one (17). The 2'-*O*-acetylated nucleoside **11** (210 mg, 0.54 mmol) was treated with 7N methanolic ammonia solution (15 mL) at room temperature overnight. Evaporation yielded a residue which was purified by column chromatography (2% MeOH-CH_2Cl_2) to afford 140 mg of compound **17** (85% yield) as a white foam. ^{1}H NMR (DMSO-D_6, 300MHz): δ 3.94 (app-d, *J*= 3.98 Hz, 1H), 4.10 (dd, *J*= 10.08, 4.06 Hz, 1H), 4.18 (d, *J*= 4.28 Hz, 1H), 4.28 (d, *J*= 10.08 Hz, 1H), 4.47 (app-q, *J*= 11.70 Hz, 2H), 5.60 (d, *J*= 7.44 Hz, 1H), 5.68 (d, *J*= 1.08 Hz, 1H), 5.75 (s, 1H), 5.80 (d, *J*= 4.35 Hz, 1H), 7.00 (brd, *J*= 23.36 Hz, 2H), 7.19 – 7.26 (m, 2H), 7.26 – 7.35 (m, 3H), 7.53 (d, *J*= 7.41 Hz, 1H). ^{13}C NMR (DMSO-D_6, 75 MHz): δ 55.60, 70.91, 73.60, 78.12, 83.64, 93.06, 93.53, 128.18, 128.34, 128.86, 138.44, 142.12, 155.94, 166.44. ESI-HRMS for $[C_{15}H_{17}N_3O_4 + H]^+$ calcd, 304.1292; found, 304.1294.

N-(1-((2R,3R,4S)-4-(benzyloxy)-tetrahydro-3-hydroxyfuran-2-yl)-1,2-dihydro-2-oxopyrimidin-4-yl)acetamide (18). To a solution of compound **17** (180 mg, 0.59 mmol) in dry DMF (1.5 mL) was added acetic anhydride (56 µL, 0.59 mmol) and the mixture was stirred at room temperature for 24h. The residue obtained after distillation of the volatiles under reduced pressure, was purified by silica-gel column chromatography to give **18** (160 mg, 78%) as a white foam. ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.2 (s, 3H), 4.00 – 4.04 (m, 1H), 4.23 (dd, *J*= 9.79, 1.48 Hz, 1H), 4.29 (dd, *J*= 9.93, 3.88 Hz, 1H), 4.39 (s, 1H), 4.42 (s, 1H), 4.85 (brs, 1H), 5.73 (d, *J*= 0.75 Hz, 1H), 7.05 – 7.11 (m, 2H), 7.17 – 7.25 (m, 3H), 7.31 (d, *J*= 7.50 Hz, 1H), 7.88 (d, *J*= 7.50 Hz, 1H), 9.36 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.14, 71.68, 74.99, 78.65, 82.67, 94.89, 96.26, 127.96,

128.17, 128.69, 137.25, 145.33, 156.17, 163.04, 171.17, 177.08. ESI-HRMS for $[C_{17}H_{19}N_3O_5 + H]^+$ calcd, 346.1397; found, 346.1400.

Barton – McCombie deoxygenation procedure for the synthesis of compounds 19 - 24. To an ice-cold solution of compound **12 - 18** (1 eq.) and DMAP (2 eq.) in CH_3CN (25 mL/mmol) was gradually added p-tolylchlorothionocarbonate (1.2 equivalents) at 0 °C and stirring was continued at room temperature. After 2-4h the solvent was removed in vacuo and the residue was dissolved in ethyl acetate. The solution was washed twice with water, dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo to give the corresponding xanthate as yellow solid/syrup. The latter was dissolved in toluene (50 mL/mmol), to which azobisisobutyronitrile (AIBN, 2 equivalent) was added. Tri-n-butyltinhydride (2.5 equivalent) was added to this mixture at 60-70 °C, which was further stirred for 2-4 h at 95-100 °C. The solvent was removed in vacuo and the residue was purified by column chromatography (30-40 % EtOAc - hexanes) to yield the 2'-deoxy compound **19 – 24** as a foam.

1-((2R,4R)-4-(benzyloxy)-tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (19). Deoxygenation of **12** (420 mg, 1.32 mmol) rendered 330 mg of compound **19** in 82% yield. ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.69 (d, *J*= 1.19 Hz, 3H), 2.19 (ddd, *J*= 15.07, 1.19, 0.88 Hz, 1H), (ddd, *J*= 15.11, 7.94, 5.45 Hz, 1H), 3.82 (dd, *J*= 10.27, 3.60 Hz, 1H), 4.19 (dd, *J*= 5.47, 3.66 Hz, 1H), 4.30 (dd, *J*= 10.33, 1.50 Hz, 1H), 4.42 (app-dd, *J*= 15.15, 11.27 Hz, 2H), 6.17 (*J*= 7.88, 2.17 Hz, 1H), 7.16-7.32 (m, 5H), 7.47 (d, *J*= 1.22 Hz, 1H), 8.67 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.60, 38.49, 71.41, 74.51, 77.58, 85.48, 110.35, 127.90, 128.32, 128.82, 136.98, 137.28, 150.76, 164.10. ESI-HRMS for $[C_{16}H_{18}N_2O_4 + H]^+$ calcd, 303.1339; found, 303.1342.

1-((2R,4R)-4-(benzyloxy)-tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (20). Deoxygenation of **13** (306 mg, 1.0 mmol) rendered 250 mg of compound **20** in 86% yield. ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.23 (dm, *J*= 15.11 Hz, 1H), 2.39 (ddd, *J*= 15.11, 7.56, 5.31 Hz, 1H), 3.85 (dd, *J*= 10.25, 3.67 Hz, 1H), 4.17 (app-t, *J*= 4.40 Hz, 1H), 4.28 (dd, *J*= 10.24, 1.83 Hz, 1H), 4.41 (app-dd, *J*= 13.75, 11.63 Hz, 2H), 5.55 (dd, *J*= 8.16, 1.73 Hz, 1H), 6.12 (dd, *J*= 7.54, 2.00 Hz, 1H), 7.14-7.34 (m, 5H), 7.61 (d, *J*= 8.16 Hz, 1H), 8.86 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 38.56, 71.39, 74.99, 77.28, 86.15, 101.69, 127.96, 128.36, 128,85, 137.20, 141. 09, 150.70, 163.63. ESI-HRMS for $[C_{15}H_{16}N_2O_4 + H]^+$ calcd, 289.1183; found, 289.1183.

1-((2R,4R)-4-(4-methoxybenzyloxy)-tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (21). Deoxygenation reaction of **14** (280 mg, 0.81 mmol) rendered 200 mg of compound **21** in 75% yield. ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.70 (d, *J*= 1.20 Hz, 3H), 2.16 (ddd, *J*= 15.09, 2.04, 1.05 Hz, 1H), 2.40 (ddd, *J*= 15.1, 7.93, 5.52 Hz, 1H), 3.73 (s, 3H), 3.80 (dd, *J*= 10.22, 3.66 Hz, 1H), 4.17 (app-t, *J*= 4.46 Hz, 1H), 4.28 (dd, *J*= 10.26, 1.34 Hz, 1H), 4.35 (dd, *J*= 14.60, 11.00 Hz, 2H), 6.16 (dd, *J*= 7.90, 2.20 Hz, 1H), 6.80 (d, *J*= 8.74 Hz, 2H), 7.12 (d, *J*= 8.74 Hz, 2H), 7.46 (d, *J*= 1.23 Hz, 1H), 8.69 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.65, 38.44, 55.55, 71.06, 74.57, 85.50, 110.28, 114.21, 129.37, 129.56, 137.04, 150.79, 159.71, 164.16. ESI-HRMS for $[C_{17}H_{20}N_2O_5 + H]^+$ calcd, 333.1445; found, 333.1461.

1-((2R,4R)-4-(4-methoxybenzyloxy)-tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (22). Deoxygenation of **15** (100 mg, 0.3 mmol) rendered 70 mg of compound **22** in 74% yield. ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.21 (app-dm, *J*= 15.15 Hz, 1H), 2.37 (ddd, *J*= 15.03, 7.47, 5.28 Hz, 1H), 3.73 (s, 3H), 3.83 (dd, *J*= 10.20, 3.66 Hz, 1H), 4.25 (dd, *J*=

10.14, 1.17 Hz, 1H), 4.33 (d, *J*= 1.40 Hz, 2H), 5.56 (d, *J*= 8.13 Hz, 1H), 6.11 (dd, *J*= 7.54, 1.96 Hz, 1H), 6.80 (d, *J*= 8.70 Hz, 2H), 7.09 (d, *J*= 8.70 Hz, 2H), 7.61 (d, *J*= 8.13 Hz, 1H), 9.21 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 38.46, 55.53, 71.00, 75.06, 76.97, 86.18, 101.67, 114.22, 129.28, 129.63, 141,24, 150.96, 159.70. ESI-HRMS for $[C_{16}H_{18}N_2O_5 + H]^+$ calcd, 319.1294; found, 319.1295.

9-((2R,4R)-4-(benzyloxy)-tetrahydrofuran-2-yl)-9H-purin-6-amine (23). Deoxygenation of **16** (450 mg, 1.38 mmol) rendered 200 mg of compound **23** in 47% yield. ^{1}H NMR (DMSO-D_6, 300MHz): δ 2.59 (dm, *J*= 14.60 Hz, 1H), 2.70 (ddd, *J*= 14.60, 7.61, 5.84 Hz, 1H), 4.00 (dd, *J*= 9.88, 4.45 Hz, 1H), 4.25 (app-dt, *J*= 10.05, 1.30 Hz, 1H), 4.41 (m, 1H), 4.54 (app-d, *J*= 2.42, 2H), 6.34 (dd, *J*= 7.55, 2.64 Hz, 1H), 7.24 (brs, 2H), 7.26 – 7.38 (m, 5H), 8.24 (s, 1H), 8.22 (s, 1H). ^{13}C NMR (DMSO-D_6, 75 MHz): δ 37.32, 70.30, 73.21, 77.47, 82.78, 118.52, 127.43, 127.53, 128.18, 137.87, 138.79, 149. 04, 152. 47, 155.85. ESI-HRMS for $[C_{16}H_{17}N_5O_2 + H]^+$ calcd, 312.1455; found, 312.1469.

N-(1-((2R,4R)-4-(benzyloxy)-tetrahydrofuran-2-yl)-1,2-dihydro-2-oxopyrimidin-4-yl)acetamide (24). Deoxygenation of **18** (190 mg, 0.55 mmol) rendered 100 mg of compound **24** in 56% yield. ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.20 (s, 3H), 2.35 – 2.44 (m, 2H), 3.98 (dd, *J*= 10.12, 3.72 Hz, 1H), 4.13 - 4.18 (m, 1H), 4.31 (s, 2H), 4.34 (dd, *J*= 10.20, 0.96 Hz, 1H), 6.09 (dd, *J*= 6.08, 2.31 Hz, 1H), 7.06 – 7.12 (m, 2H), 7.21 (d, *J*= 8.44 Hz, 1H), 7.20 – 7.28 (m, 3H), 7.91 (d, *J*= 7.50 Hz, 1H), 9.46 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.06, 38.46, 71.08, 75.89, 77.20, 88.21, 96.20, 127.92, 128.19, 128.76, 137.23, 145.37, 155.54, 163.35, 171.57. ESI-HRMS for $[C_{17}H_{19}N_3O_4 + H]^+$ calcd, 330.1448; found, 330.1463.

Deprotection method A. A solution of benzyl protected nucleoside in MeOH (40 mL/mmol) was hydrogenated at atmospheric pressure for 5-24h in the presence of 10% Pd/C (150 mg/mmol). The catalyst was removed by filtration over a Celite path and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (3% MeOH-CH_2Cl_2) to isolate the desired product as a white amorphous solid.

Deprotection method – B. To a solution of benzyl protected nucleoside (1 eq.) in an acetonitrile-water (5:1) mixture (20 mL/mmol) at 0 °C ceric ammonium nitrate (CAN, 2.5 equivalents) was added and stirring was continued for 1h. The reaction mixture was treated with solid $NaHCO_3$ (2.0 g/mmol) followed by 25% MeOH-CH_2Cl_2 (50 mL/mmol). The mixture was filtered over celite and washed with 25% MeOH-CH_2Cl_2 (100 mL/mmol). The filtrate was concentrated in vacuum, and the residue was purified by column chromatography (3% MeOH-CH_2Cl_2) to isolate the final product as a white amorphous solid.

1-((2R,4R)-tetrahydro-4-hydroxyfuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (25).

Employing deprotection method A, 30 mg of title compound was obtained in 75 % yield from **19** (57 mg, 0.19 mmol), while method B afforded 80 mg of the same compound from **21** (150 mg, 0.45 mmol) in 84 % yield. ^{1}H NMR (DMSO-D_6, 300 MHz): δ 1.77 (d, *J*= 1.16 Hz, 3H), 1.87 (ddd, *J*= 14.49, 2.89, 1.52 Hz, 1H), 2.46 (ddd, *J*= 14.05, 8.07, 5.59 Hz, 1H), 3.77 (dd, *J*= 9.37, 3.72 Hz, 1H), 3.98 (dt, *J*= 9.36, 1.41 Hz, 1H), 4.36 (brm, 1H), 5.29 (d, *J*= 2.68 Hz, 1H), 6.05 (dd, *J*= 8.13, 2.72 Hz, 1H), 7.76 (d, *J*= 1.21 Hz, 1H), 11.23 (brs, 1H). ^{13}C NMR (DMSO-D_6, 75 MHz): δ 13.06, 40.75, 69.54, 77.04, 85.32, 109.20,

137.79, 151.21, 164.54. ESI-HRMS for $[C_9H_{12}N_2O_4 - H]^-$ calcd, 211.0724; found, 211.0732.

1-((2R,4R)-tetrahydro-4-hydroxyfuran-2-yl)pyrimidine-2,4(1H,3H)-dione (26). Employing deprotection method A, 43 mg of the title compound was obtained in 89% yield from **20** (70 mg, 0.24 mmol), while compound **22** (70 mg, 0.22 mmol) was converted to 34 mg of the title compound (78 % yield) using method B. ^{1}H NMR (DMSO-D_6, 300 MHz): δ 1.87 (ddd, , *J*= 14.53, 2.91, 1.88 Hz, 1H), 2.43 (ddd, *J*= 14.45, 8.00, 5.48 Hz, 1H), 3.78 (dd, *J*= 9.43, 3.66 Hz, 1H), 3.97 (app-dt, *J*= 9.46, 1.25 Hz, 1H), 4.34 (t, *J*= 4.23 Hz, 1H), 5.27 (brs, 1H), 5.60 (d, *J*= 8.05 Hz, 1H), 6.01 (dd, *J*= 8.00, 2.23 Hz, 1H), 7.85 (d, *J*= 8.11 Hz, 1H), 11.19 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 41.64, 70.08, 78.56, 87.97, 100.61, 141.97, 150.95, 165.38. ESI-HRMS for $[C_8H_{10}N_2O_4 + H]^+$ calcd, 199.0713; found, 199.072.

(3R,5R)-5-(6-amino-9H-purin-9-yl)-tetrahydrofuran-3-ol (27). Using method A with catalytic amount of acetic acid compound **23** (100 mg, 0.32 mmol) was converted to 29 mg of compound **27** in 40 % yield. ^{1}H NMR (DMSO-D_6, 300MHz): δ 2.29 (ddd, *J*= 14.44, 2.70, 1.54 Hz, 1H), 2.68 (ddd, *J*= 14.42, 8.24, 6.10 Hz, 1H), 3.91 (dd, *J*= 9.30, 4.10 Hz, 1H), 3.97 (ddd, *J*= 9.32, 2.10, 1.06 Hz, 1H), 4.48 (m, 1H), 5.79 (d, *J*= 4.35 Hz, 1H), 6.28 (dd, *J*= 8.27, 2.56 Hz, 1H), 7.28 (s, 2H), 8.15 (s, 1H), 8.36 (s, 1H). ^{13}C NMR (DMSO-D_6, 75 MHz): δ 40.86, 69.95, 76.98, 83.83, 119.53, 140.40, 149.53, 153.06, 156.73. ESI-HRMS for $[C_9H_{11}N_5O_2 + H]^+$ calcd, 222.0986; found, 222.0938.

N-(1,2-dihydro-1-((2R,4R)-tetrahydro-4-hydroxyfuran-2-yl)-2-oxopyrimidin-4-yl)acetamide (28). 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ, 770 mg, 3.4 mmol)

was added to a stirring solution of compound **24** (112 mg, 0.34 mmol) in dry dichloromethane (5.0 mL). The mixture was heated to 50 °C for 2 days in a sealed tube. Then it was sequentially treated with $NaHCO_3$ (2.0 g), water (0.25 mL) and 20 mL of 1:1 CH_2Cl_2-MeOH. After addition of anhydrous $MgSO_4$ the resulting slurry was filtered. The filtrate was concentrated and purified by column chromatography (4-6% MeOH-CH_2Cl_2) to afford 9 mg of compound **28** (11 % yield). ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.14 (s, 3H), 2.37 (ddd, *J*= 14.90, 7.02, 4.53 Hz, 1H), 2.61 (d, *J*= 14.90 Hz, 1H), 4.02 (dd, *J*= 9.80, 3.46 Hz, 1H), 4.28 (dd, *J*= 9.78, 1.14 Hz, 1H), 4.52 (t, *J*= 3.86 Hz, 1H), 5.94 (d, *J*= 6.56 Hz, 1H), 7.32 (d, *J*= 7.50 Hz, 1H), 8.03 (d, *J*= 7.48 Hz, 1H), 9.39 (brs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.12, 41.49, 70.28, 78.87, 89.48, 95.73, 146.47, 155.43, 162.11, 170.95. ESI-HRMS for $[C_{10}H_{13}N_3O_4 + H]^+$ calcd, 240.0979; found, 240.0982 & $[2M + H]^+$ 479.1936.

4-amino-1-((2R,4R)-tetrahydro-4-hydroxyfuran-2-yl)pyrimidin-2(1H)-one (29).

Method 1. Applying the reaction conditions described for **12** on compound **28** (18 mg, 75 µmol) gave 10 mg of compound **29** (67% yield) as an amorphous solid.

Method – 2. To a stirring mixture of 1,2,4-triazole (223 mg, 3.24 mmol) in 5 mL of pyridine $POCl_3$ (75 µL, 0.81 mmol) was added dropwise at room temperature. After 10 minutes compound **30** (65 mg, 0.27 mmol) was added and stirring was continued for an additional 3h. After that time the reaction was bubbled with ammonia gas for 2h. Since TLC indicated that the deacetylation was not completed, the volatiles were removed and the residue treated with 7N methanolic ammonia (5 mL) for 5h. After evaporation the residue was purified by flash column chromatography (6% MeOH-CH_2Cl_2) to give 19

mg of the title compound **29** in 36% yield. ^{1}H NMR (DMSO-$D_{6,}$ 300MHz): δ 1.82 (ddd, *J*= 14.28, 1.40, 1.10 Hz, 1H), 2.40 (ddd, *J*= 14.06, 7.87, 5.59 Hz, 1H), 3.81 (dd, *J*= 9.35, 3.74Hz, 1H), 3.97 (dt, *J*= 9.31, 1.28 Hz, 1H), 4.33 (m, 1H), 5.16 (d, *J*= 2.76 Hz, 1H), 5.69 (d, *J*= 7.42 Hz, 1H), 5.99 (dd, *J*= 7.83, 2.43 Hz, 1H), 7.08 (brd, *J*= 15.32 Hz, 2H), 7.75 (d, *J*= 7.41 Hz, 1H). ^{13}C NMR (DMSO-$D_{6,}$ 75 MHz): δ 41.48, 69.70, 77.26, 86.52, 93.85, 142.46, 155.98, 166.37. ESI-HRMS for $[C_8H_{11}N_3O_3 + H]^+$ calcd, 198.0873; found, 198.0876.

(3R,5R)-tetrahydro-5-(3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)furan-3-yl acetate (30). To a stirring solution of compound **26** (55 mg, 0.277 mmol) and 4-dimethyl aminopyridene (DMAP, 50 mg, 0.41 mmol) in 2 mL of DMF acetic anhydride (31 μL, 0.33 mmol) was added. After stirring for 24h, the solvent was evaporated under vacuum and the residue was purified by column chromatography to isolate compound **30** (65 mg, 97%). ^{1}H NMR ($CDCl_{3,}$ 300MHz): δ 1.96 (s, 3H), 2.22 (ddd, *J*= 15.48, 2.74, 1.76 Hz, 1H), 2.58 (ddd, *J*= 15.49, 7.24, 5.81 Hz, 1H), 4.04 (dd, *J*= 10.95, 3.87 Hz, 1H), 4.21 (ddd, *J*= 10.95, 1.60, 0.69 Hz, 1H), 5.31 (app-tt, *J*= 4.80, 0.84 Hz, 1H), 5.68 (d, *J*= 8.18 Hz, 1H), 6.07 (dd, *J*= 7.21, 1.94 Hz, 1H), 7.45 (d, *J*= 8.18 Hz, 1H), 9.20 (brs, 1H). ^{13}C NMR ($CDCl_{3,}$ 75 MHz): δ 21.24, 39.13, 72.88, 75.39, 86.61, 101.63, 139.77, 150.53, 163.72, 170.19. ESI-HRMS for $[C_{10}H_{12}N_2O_5 + H]^+$ calcd, 241.0819; found, 241.0830.

Phenyl-(benzoxy-L-alaninyl)-phosphorochloridate (33). To a stirred solution of phenyldichlorophosphate **31** (0.30 mL, 2.00 mmol), L-alanine benzyl ester tosylate **32** (0.43 g, 2.00 mmol) in anhydrous DCM (15 mL), was added dropwise under an Ar atmosphere at -78 ^{o}C anhydrous TEA (0.56 mL, 4.00 mmol). Following the addition the reaction mixture was stirred at -78 ^{o}C for 30 min, then at room temperature for 2 h.

Formation of the desired compound was monitored by ^{31}P NMR. After this period the solvent was removed under reduced pressure and the residue triturated with anhydrous diethyl ether. The precipitate was filtered under nitrogen and the solution was concentrated to give a yellow oil (87%, 0.62 g). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.33-7.28 (10H, m, PhO, OCH_2Ph), 5.15-5.13 (2H, m, OCH_2Ph), 4.18-4.13 (1H, m, CHNH), 1.46-1.44 (3H, m, CH_3). ^{31}P-NMR ($CDCl_3$, 202 MHz): δ 7.86, 7.52.

1-((2R,4R)-tetrahydro-4-hydroxyfuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione - [phenyl-(benzoxy-L-alaninyl)] phosphate (34). To a solution of **25** (0.093 g, 0.44 mmol) in anhydrous THF (10 mL) was added 1.0M solution of *tert*-butyl magnesium chloride in THF (0.88 mL, 0.88 mmol) and the reaction mixture was stirred under an Ar atmosphere for 30 min. After this period, a solution of **33** (0.31 g, 0.88 mmol) in anhydrous THF (5 mL) was added dropwise and the reaction mixture was stirred at room temperature for 18 h. Then, 1.0M solution of *tert*-butyl magnesium chloride in THF (0.44 mL, 0.44 mmol) and a solution of 32 (0.15 g, 0.44 mmol) in anhydrous THF (3 mL) was added and the stirring was continued for further 5 h. After this period, the solvent was removed and the residue was purified by column chromatography, gradient elution of DCM/MeOH = 98/2 then 96/4 to give a white solid (33%, 0.077 g). ^{1}H-NMR (MeOD, 500 MHz): δ 7.52 (0.5H, d, J = 1.2 Hz, H-6 of one diastereoisomer), 7.47 (0.5H, d, J = 1.2 Hz, H-6 of one diastereoisomer), 7.38-7.30 (7H, m, PhO, OCH_2Ph), 7.22-7.16 (2H, m, PhO, OCH_2Ph), 7.12-7.10 (1H, m, PhO, OCH_2Ph), 6.10 (0.5H, dd, J = 7.8 Hz, 2.3 Hz, H-1’), 6.06 (0.5H, dd, J = 7.5 Hz, 1.8 Hz, H-1’), 5.21-5.17 (1H, m, H-3’), 5.16, 5.15 (2H, 2s, OCH_2Ph), 4.45 (0.5H, dd, J = 10.8 Hz, 1.7 Hz, H-4’ of one diastereoisomer), 4.37 (0.5H, dd, J = 10.8 Hz, 1.7 Hz, H-4’ of one diastereoisomer), 4.03-3.92 (2H, m, H-4’, $CHCH_3$),

2.63-2.55 (1H, m, H-2'), 2.32 (0.5H, d, J = 15.5 Hz, H-2' of one diastereoisomer), 2.24 (0.5H, d, J = 15.5 Hz, H-2' of one diastereoisomer), 1.83 (1.5H, d, J = 1.1 Hz, 5-CH_3 of one diastereoisomer), 1.82 (1.5H, d, J = 1.1 Hz, 5-CH_3 of one diastereoisomer), 1.36 (1.5H, d, J = 0.9 Hz, CHC*H*$_3$ of one diastereoisomer), 1.34 (1.5H, d, J = 1.0 Hz, CHC*H*$_3$ of one diastereoisomer). ^{13}C-NMR (MeOD, 125 MHz): δ 12.71 (5-CH_3), 20.16 (d, J_{C-P} = 7.5 Hz, CH*C*H$_3$), 20.33 (d, J_{C-P} = 6.5 Hz, CH*C*H$_3$), 40.51 (d, J_{C-P} = 5.6 Hz, C-2'), 40.73 (d, J_{C-P} = 3.9 Hz, C-2'), 51.60, 51.84 (2s, *C*HCH$_3$), 68.00, 68.04 (2s, O*C*H$_2$Ph), 76.34 (d, J_{C-P} = 4.0 Hz, C-4'), 76.79 (d, J_{C-P} = 5.5 Hz, C-4'), 77.95 (d, J_{C-P} = 5.2 Hz, C-3'), 77.99 (d, J_{C-P} = 4.9 Hz, C-3'), 86.95, 87.47 (2s, C-1'), 110.70, 111.03 (2s, C-5), 121.09, 121.14, 121.18, 126.26, 126.29, 129.41, 129.43, 129.64, 129.65, 130.82, 130.92 (PhO, OCH$_2$*Ph*), 137.29 ('ipso' OCH$_2$P*h*), 137.77, 137.80 (2s, C-6), 152.03, 152.07, 152.12, 152.22, 152.31 (C-2, ('ipso' OP*h*), 166.49, 166.55 (2s, C-4), 174.49 (d, J_{C-P} = 4.6, *C*OOCH$_2$Ph), 174.91 (d, J_{C-P} = 4.1, *C*OOCH$_2$Ph). ^{31}P-NMR (MeOD, 202 MHz): δ 2.92, 2.27. ES- MS= 528.16 (M run on negative mode). HPLC = H_2O/AcCN from 95/5 to 0/100 in 30 min = retention time 13.87, 14.19 min.

Antiviral Assays

The antiviral assays [except anti-human immunodeficiency virus (HIV) assays] were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus and vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), or HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 cell culture inhibitory dose-50 ($CCID_{50}$) of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, … μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/cm^3) cells were infected with 100 $CCID_{50}$ of HIV-1(III_B) or HIV-2(ROD)/mL and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

Enzymatic Assay

Compound **34** (5.5 mg) was dissolved in d_6-acetone (150 μL), and Trizma buffer (pH 7.6) (300 μL) was added. The resulting cloudy solution was placed in a NMR tube and a ^{31}P-NMR experiment at 25 °C was recorded as the blank experiment. Then a solution of carboxypeptidase Y (0.1 mg) in Trizma buffer (150 μL) was added and a ^{31}P-NMR

experiment was performed recording the experiment every 5 min.

Nucleoside Kinase Assay Using [CH_3-^{3}H]dThd as the Natural Substrate

The activity of recombinant thymidine kinase 1 (TK-1), TK-2, herpes simplex virus-1 (HSV-1) TK, varicella zoster virus (VZV) TK, and the multifunctional deoxynucleoside kinase (dNK) of *Drosophila melanogaster* and the 50% inhibitory concentration of the test compounds were assayed in a 50 μL reaction mixture containing 50 mM Tris/HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM CHAPS, 3 mg/mL bovine serum albumin, 2.5 mM ATP, 1 μM [methyl-^{3}H]dThd, and enzyme. The samples were incubated at 37 °C for 30 min in the presence or absence of different concentrations (5-fold dilutions) of the test compounds. At this time point, the enzyme reaction still proceeded linearly. Aliquots of 45 μL of the reaction mixtures were spotted on Whatman DE-81 filter paper disks (Whatman, Clifton, NJ). The filters were washed three times for 5 min each in 1 mM ammonium formate, once for 1 min in water, and once for 5 min in ethanol. The radioactivity was determined by scintillation counting.

Supplementary Material: NMR spectra of the final compounds are available.

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Table 1. Nucleoside kinase activity (IC_{50} in μg/mL)

	TK-1	TK-2	HSV-1 TK	VZV TK	Dm dNK
25	>200	91 ± 40	14 ± 3	26 ± 9	≥ 200
26	>200	60 ± 41	>200	>200	>200
27	>200	>200	>200	>200	>200
29	>200	>200	>200	>200	>200

Figure and Scheme Legends

Scheme 1. Synthesis of α-L-2'-deoxythreofuranosyl nucleoside analogues **25-27** and **29**. *Reagents and conditions*: (a) benzyl bromide, NaH, DMF, 0 °C→RT, 4h, 87%; (b) p-methoxybenzyl chloride, NaH, DMF, -20 °C→RT, 4h, 84%; (c) i. 80% aq. CH_3COOH, 80 °C, 8h; ii. Ac_2O, pyridine, RT, 4h, 63-81%; (d) i. nucleobase, pyridine, TMSCl, HMDS, 135 °C, overnight; ii. silylated base, **4**/**5**, TMSOTf, 1,2-dichloroethane, 0 °C→RT, 3-5h, 45-87%; (e, for 10) i. nucleobase, pyridine, TMSCl, HMDS, 135 °C, overnight; ii. silylated base, 4/5, TMSOTf, 1,2-dichloroethane, 50 °C, 24h, 42%; (f) 7N NH_3-MeOH, RT, 4-6h, 81-95%; (g, for 14-15) 1N NH_3-MeOH, RT, 5h, 78-90%; (h) Ac_2O, DMF, RT, 24h, 78%; (i) i. DMAP, O-(p-tolyl) chlorothionoformate, CH_3CN, 0 °C →RT, 4h; ii. Bu_3SnH, AIBN, toluene, 100 °C, 2-4h, 47-86%; (j) Pd-C/H_2, MeOH, RT, 3-8h, 40-89%; (k, on **21** and **22**) CAN, CH_3CN :H_2O, 0 °C →RT, 1h, 78-84%; (l, for **28**) DDQ, CH_2Cl_2, 50 °C, 72h, 11%.

Scheme 2. An improved synthesis of the cytosine analogue **29**. *Reagents and conditions*: (a) Acetic anhydride, DMAP, DMF, RT, 24h, 97%; (b) i. 1,2,4-triazole, $POCl_3$, pyridine, RT, 4h; ii. NH_3, 2h; iii. NH_3-MeOH, RT, 5h, 36%.

Scheme 3. Synthesis of the ProTide of **25**. *Reagents and conditions*: (a) anhydrous TEA, anhydrous DCM, -78 ^{o}C for 30 min, rt, 2 h; (b) 1.0M *tert*-butylmagnesium chloride in THF, anhydrous THF, rt, 24 h.

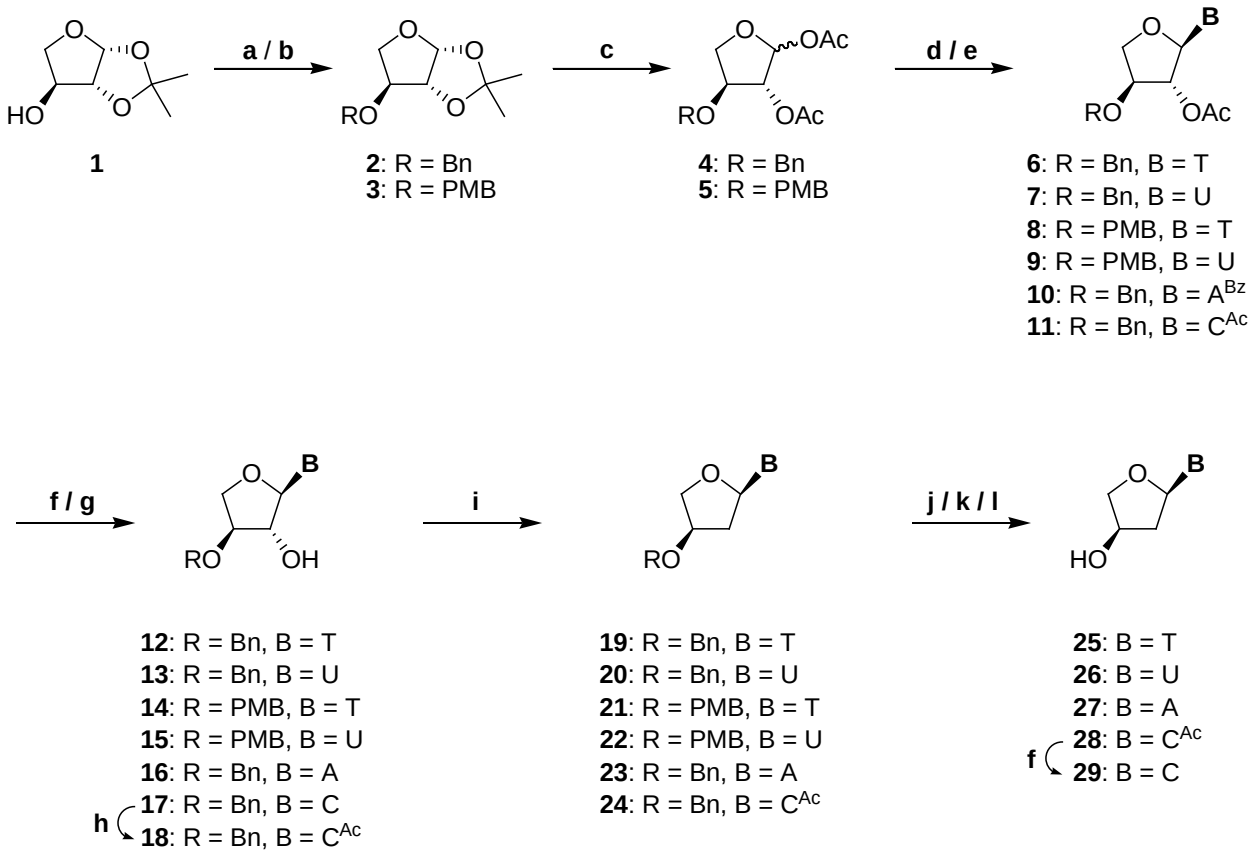
Scheme 4. Putative mechanism of bioactivation of the ProTide.

Figure 1. Structures of active L-2-deoxythreose nucleoside phosphonates (**I**) and the nucleoside surrogates of this study (**II**).

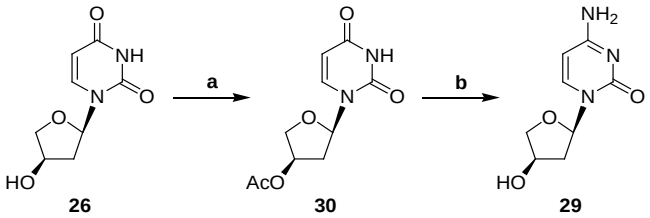
Figure 2. Carboxypeptidase-mediated cleavage of compound **34**, monitorated by ^{31}P-NMR.

Figure 3. Lineweaver-Burk plot for compound **25**.

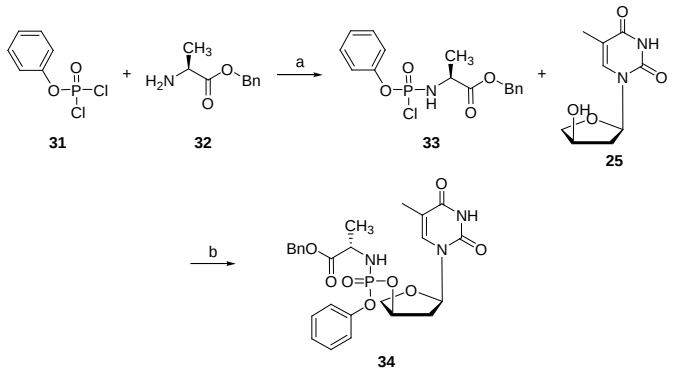
Scheme 1



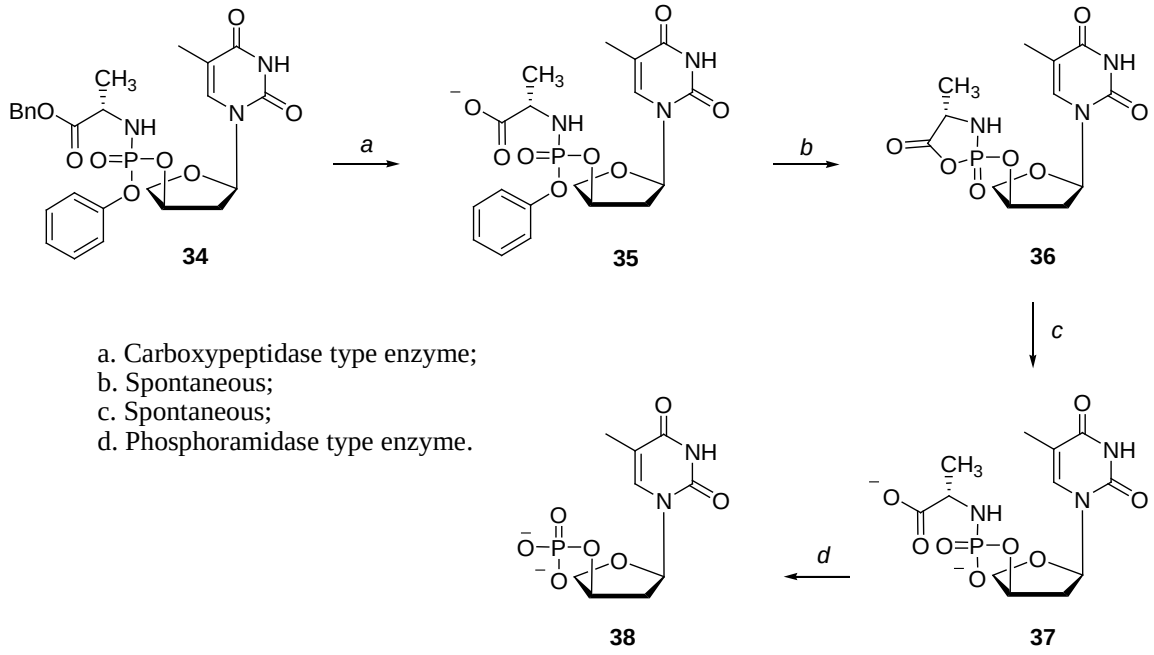
Scheme 2



Scheme 3

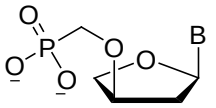


Scheme 4

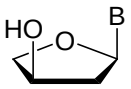


a. Carboxypeptidase type enzyme;
b. Spontaneous;
c. Spontaneous;
d. Phosphoramidase type enzyme.

Figure 1.



Ia B = adenin-9-yl (PMDTA)
Ib B = thymin-1-yl (PMDTT)



IIa B = adenin-9-yl (**25**)
IIb B = thymin-1-yl (**26**)
IIc B = uracil-1-yl (**27**)
IIa B = cytosin-1-yl (**29**)

Figure 2.

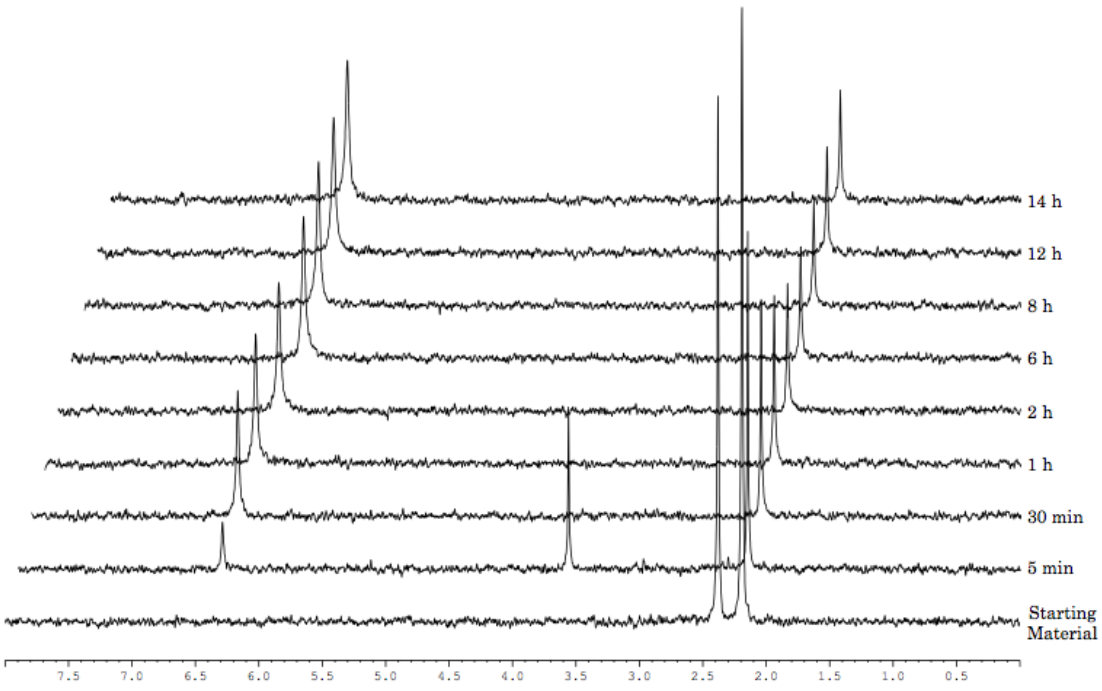
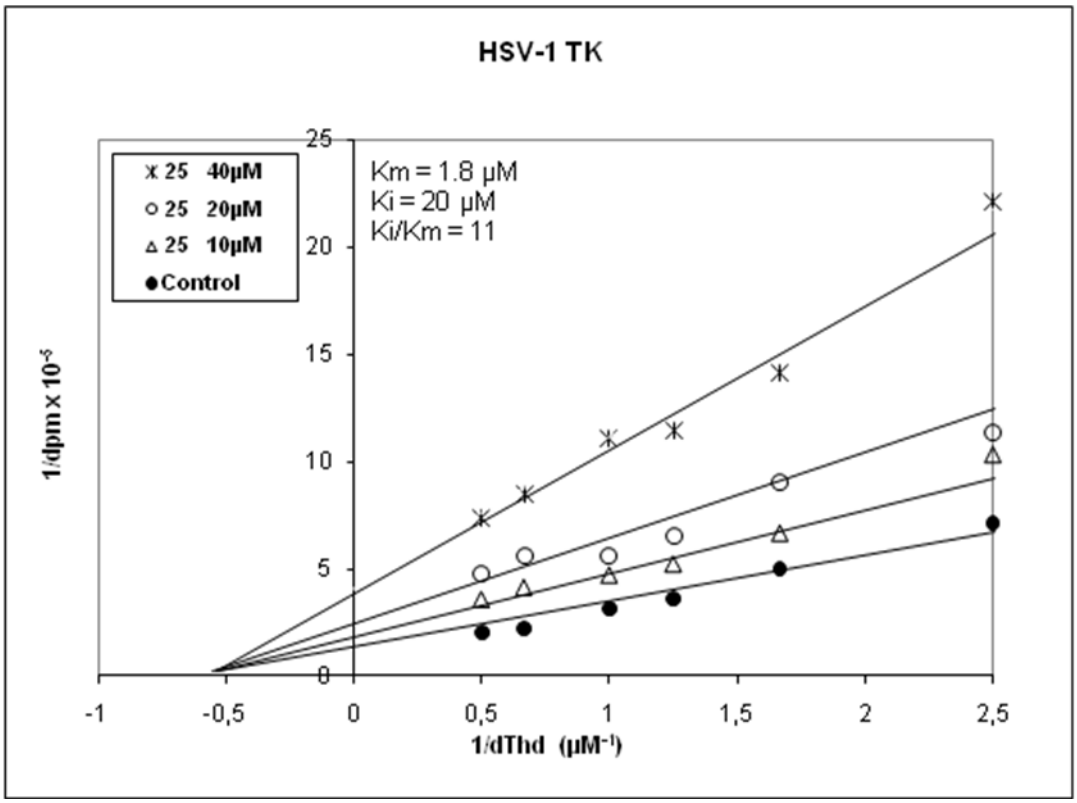


Figure 3.


HSV-1 TK
25 40μM
25 20μM
25 10μM
Control
Km = 1.8 μM
Ki = 20 μM
Ki/Km = 11
1/dpm x 10⁻⁵
1/dThd (μM⁻¹)
25
20
15
10
5
0
-1
-0,5
0
0,5
1
1,5
2
2,5