3′-*C*-Branched-Chain-Substituted Nucleosides and Nucleotides as Potent Inhibitors of *Mycobacterium tuberculosis* Thymidine Monophosphate Kinase

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Thymidine monophosphate kinase (TMPK) of *Mycobacterium tuberculosis* (TMPKmt) represents an attractive target for blocking the bacterial DNA synthesis. In an attempt to find high-affinity inhibitors of TMPKmt, a cavity in the enzyme at the 3′-position was explored via the introduction of various substituents at the 3′-position of the thymidine monophosphate (dTMP) scaffold. Various 3′-*C*-branched chain substituted nucleotides in the 2′-deoxyribo (**3**−**6**) and ribo series (**7**, **8**) were synthesized from one key intermediate (**23**). 2′-Deoxy analogues proved to be potent inhibitors of TMPKmt: 3′-CH_2NH_2 (**4**), 3′-CH_2N_3 (**3**), and 3′-CH_2F (**5**) nucleotides exhibit the highest affinities within this series, with K_i values of 10.5, 12, and 15 μM, respectively. These results show that TMPKmt tolerates the introduction of sterically demanding substituents at the 3′-position. Ribo analogues experience a significant affinity decrease, which is probably due to steric hindrance of Tyr103 in close vicinity of the 2′-position. Although the 5′-*O*-phosphorylated compounds have somewhat higher affinities for the enzyme, the parent nucleosides generally exhibit affinities for TMPKmt in the same order of magnitude and display a superior selectivity profile versus human TMPK. This series of inhibitors holds promise for the development of a new class of antituberculosis agents.

Introduction

Mycobacterium tuberculosis is responsible for at least 2 million deaths per year worldwide, and 30 million people are at risk of dying from tuberculosis (TB) in the next 10 years.[1] Due to demographic factors, socio-economic trends, neglected tuberculosis control in many countries, and HIV infection, this epidemic has been able to adopt such proportions. Efforts to stop this frightening trend are hampered by the lack of financial resources in developing countries, the appearance of multi-drug-resistant strains of *M. tuberculosis*, and bad therapy compliance. As a current strategy in the battle against TB, directly observed treatment short-course (DOTS) is preferred. In DOTS, TB patients are subjected to standardized treatment regimens (generally consisting of combinations of the most powerful anti-TB drugs), which last 6−8 months under direct observation of drug intake. As a consequence, the duration of illness, the risk of death, and the appearance of resistant strains are reduced. For those that are or will be infected with the multi-drug-resistant bacteria or that develop resistance after a previous drug treatment (50−80% of previously treated cases), however, more effective drugs acting on novel targets are eagerly awaited.[2]

Thymidine monophosphate kinase (TMPK) catalyzes the conversion of dTMP to dTDP. This enzyme is ubiquitous in all living organisms. It lies at the junction of the de novo and salvage pathways for thymidine triphosphate (dTTP) synthesis. These enzymes are well characterized, both at biochemical[3] and structural[4] levels. Li de la Sierra et al. recently determined the structure of *M. tuberculosis* TMPK (TMPKmt) using X-ray diffraction.[5] Because its crucial role in thymidine metabolism and in view of its low (22%) sequence identity with the human isozyme (TMPKh), it represents an attractive target for blocking mycobacterial DNA synthesis. The main interactions between dTMP and the enzyme are depicted in Figure 1. The most important catalytic regions are the following:

(i) the P-loop (amino acid residues 6−17), which binds and positions the α- and β-phosphoryl groups of the phosphate donor (ATP); it is unique among all known TMPK sequences in that it does not have an arginine at the position where it is found in the yeast and human enzymes; instead, it possesses a distant arginine residue four amino acids further;

(ii) the DRY/H-motif (residues 94−96), which acts as a clip that favorably orients the phosphate donor and acceptor;

(iii) the LID region (residues 144−154) that leans over the phosphoryl donor, when dTMP binds the enzyme; it is very similar to *E. coli* TMPK, having three arginines.

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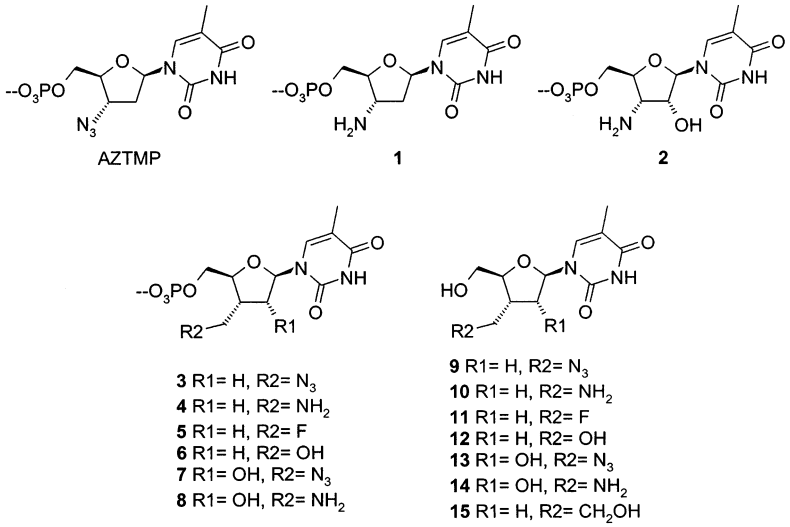
Chart 1



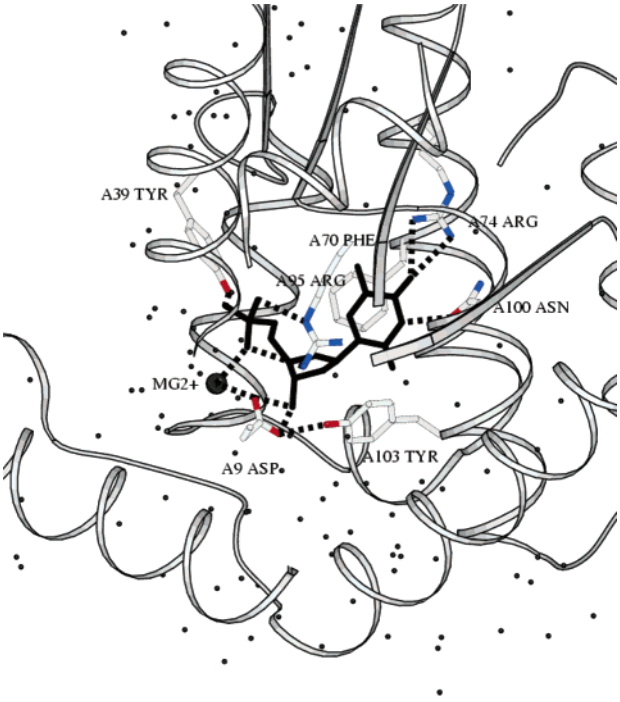


Figure 1. Schematic representation of the most important amino acid residues of TMPKmt interacting with dTMP (in black). The main bonding forces between dTMP and the enzyme are (i) a stacking interaction between the pyrimidine ring and Phe70, (ii) a hydrogen bond between *O*4 of thymine and the Arg74 side chain, which results in a preference for thymine over cytosine, (iii) a hydrogen bond between Asn100 and *N*3 of the thymine ring, (iv) a hydrogen bond between the 3′-hydroxyl of dTMP and the terminal carboxyl of Asp9, that in its turn interacts with the magnesium ion that is responsible for positioning the phosphate oxygen of dTMP, and (v) hydrogen bonds and an ionic interaction between the 5′-*O*-phosphoryl and Tyr39, Arg95, and Mg^{2+}, respectively. The presence of Tyr-103 close to the 2′-position is believed to render the enzyme catalytically selective for 2′-deoxy nucleotides versus ribo nucleotides.

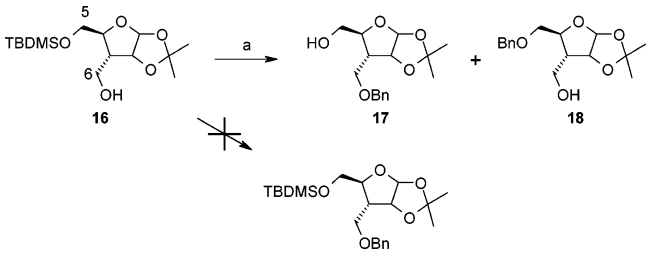
Moreover, 3′-azido-3′-deoxythymidine monophosphate (AZTMP) was identified as a competitive inhibitor of TMPKmt with a K_i of 10 μM.[3d] TMPKmt represents the first reported TMPK that does not phosphorylate AZTMP, a feature that could be exploited in the search for other selective inhibitors of TMPKmt. Lavie et al.[4a] postulated that, in yeast TMPK, the azido group interacts via its terminal nitrogen with the side-chain carboxyl of Asp9, resulting in a P-loop displacement. Thus, Arg15 binds ATP less efficiently, slowing down catalysis. A similar mechanism may account for the lack of phosphorylation of AZTMP by TMPKmt. Li de la Sierra et al.[5] suggested that the azido group displaces the magnesium ion in the active site of TMPKmt (responsible for positioning one oxygen of the phosphate and Asp9), thereby abolishing catalysis. Despite the uncertainty of the mechanism, it should be interesting to explore changes at position 3′ of the dTMP-scaffold for finding more potent inhibitors of TMPKmt. The X-ray structure of TMPK complexed with dTMP reveals that relatively bulky substituents at the 3′-position could be accommodated.[5] Therefore, we decided to synthesize a series of 3′-*C*-branched-chain nucleotides, namely, the 3′-*C*-azidomethyl, 3′-*C*-aminomethyl, 3′-*C*-fluoromethyl, and 3′-*C*-hydroxymethyl derivatives of dTMP (**3**−**6**) (Chart 1). To delineate the most appropriate length of the spacer between the 3′-carbon and the introduced functionality, 3′-deoxy-3′-hydroxyethylthymidine (**15**) was also investigated for its affinity for TMPKmt.

We have already reported that a ribo analogue exhibits a higher affinity for TMPKmt than its corresponding 2′-deoxy analogue, i.e., the ribo analogue of 3′-amino-3′-deoxy-5′-*O*-phosphorylthymidine (**2**) with a K_i of 27 μM versus 235 μM for **1**.[6a] To gain detailed insight in the postulated steric hindrance caused by Tyr103 at the 2′-position, the ribo analogues of the azido and amino compounds were also synthesized (**7**−**8**). The nonphosphorylated analogues **9**−**14** of the above-mentioned compounds were included in this report as well.

Results and Discussion

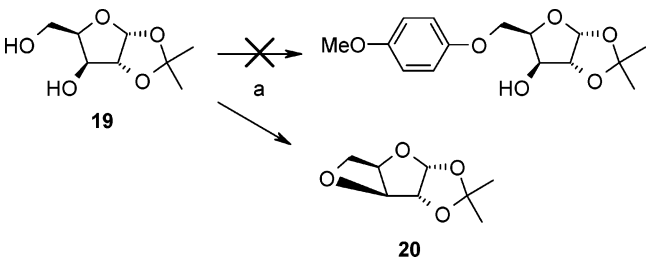
Chemistry. Although an efficient synthetic route for the synthesis of 3′-*C*-branched-chain 2′,3′-deoxy substituted thymidine has been established,[7] we developed a route that gives access to both 2′-deoxy and ribo analogues and allows easier conversion to the corresponding monophosphates. The synthesis of the parent 3′-*C*-branched-chain substituted nucleosides **9**−**14** was described by Lin et al.[8] In this procedure, all 3′-*C*-

Scheme 1[a]



[a] Reagents: (a) BnCl, NaH, DMF.

Scheme 2[a]



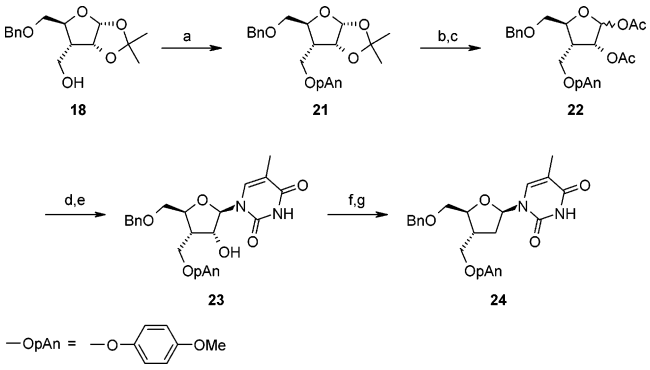
[a] Reagents: (a) *p*-methoxyphenol, DEAD, Ph_3P, THF.

modifications are introduced on the sugar moiety before coupling with thymine. In our approach, the 3′-*C*-modifications are effected following sugar-base coupling, which allows synthesizing all derivatives from a unique key intermediate. This approach requires a judicious choice of 5′- and 6′-*O*-protective groups, which must be stable under various reaction conditions and be chemoselectively removed to permit conversion of the key compound to the 3′-hydroxymethylnucleotide on one hand and to the 3′-azidomethyl-, 3′-aminomethyl-, and 3′-fluoromethyl-derivatives on the other hand.

First, we chose to combine 5-*O*-(*tert*-butyldimethyl)-silyl and a 6-*O*-benzyl protecting groups (Scheme 1). However, introduction of the 6-*O*-protective group in **16** (prepared in three steps from 1,2-*O*-isopropylidene-α-D-xylofuranose[8]) using BnCl and NaH in DMF[9] was cumbersome. The 5-*O*-(*tert*-buthyldimethyl)silyl group was cleaved thereby furnishing a mixture of 3-deoxy-3-(benzyloxymethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (**17**) (17%) and 5-*O*-benzyl-3-deoxy-3-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (68%) (**18**) resulting from silyl ether cleavage and/or migration under basic conditions.[10] Therefore, we decided to take the 5-*O*-*p*-anisyl protecting group instead of the 5-*O*-(*tert*-butyldimethyl)silyl, as migration during introduction of the benzyl would be obviated under the given reaction conditions (Scheme 2). Unfortunately, attempts to selectively protect the primary hydroxyl of 1,2-*O*-isopropylidene-α-D-xylofuranose (**19**) with *p*-methoxyphenol under Mitsunobu conditions[11] did not afford the desired 5′-*O*-protected derivative. Instead, 3,5-anhydro-1,2-*O*-isopropylidene-α-D-xylofuranose[12] (**20**) was formed via intramolecular dehydration.

To circumvent this problem, we protected the 5-hydroxyl as a benzyl ether and the 6-hydroxyl with a *p*-anisyl group (Scheme 3). 5-*O*-Benzyl-3-deoxy-3-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (**18**) was prepared using the method of Tino et al.[12] The 3-hydroxymethyl group was protected as a *p*-anisyl ether under Mitsunobu conditions,[11] affording the fully protected sugar **21**. Acidic cleavage of the 1,2-*O*-isopropylidene group, followed by acetylation, provided **22**. Vorbrüggen-type coupling[13] of this acetylated sugar with silylated thymine, followed by alkaline removal of the 2′-*O*-acetyl protecting group, yielded **23** (78% in 2 steps), a convenient key intermediate for further derivatization. Removal of the 2′-hydroxyl function was performed by reduction of its phenoxythiocarbonate ester with AIBN and nBu_3SnH affording **24** (40% yield from **18**). Selective deprotection of the 5′-*O*-benzyl group of **24** by catalytic hydrogenolysis, followed by 5′-*O*-phosphorylation and removal of the *p*-anisyl group, gave the desired 2′,3′-dideoxy-3′-*C*-hydroxymethylthymidine-5′-*O*-monophosphate (**6**) (Scheme 4). To obtain the corresponding free nucleoside **12**, **25** was treated with ceric ammonium nitrate. Besides the removal of the 6′-*O*-anisyl group, oxidation of the 5-methyl group occurred during workup of the aqueous layer (possibly by residual ceric ammonium nitrate), thereby leading to the undesired 5-hydroxymethylthymine analogue **27**. The 5-hydroxymethyl was identified by COSY (an extra hydroxymethyl was present that did not show COSY contacts with any other proton) and gHMBC experiments (^3J contact between 5-CH_2OH and H-6 and between 5-CH_2OH and C-4 and C-6; ^2J contact between 5-CH_2OH and C-5). Consequently, we interchanged the deprotection steps c and a described in Scheme 4 and obtained **12** via **28** (68% yield from **24**).

Scheme 3[a]



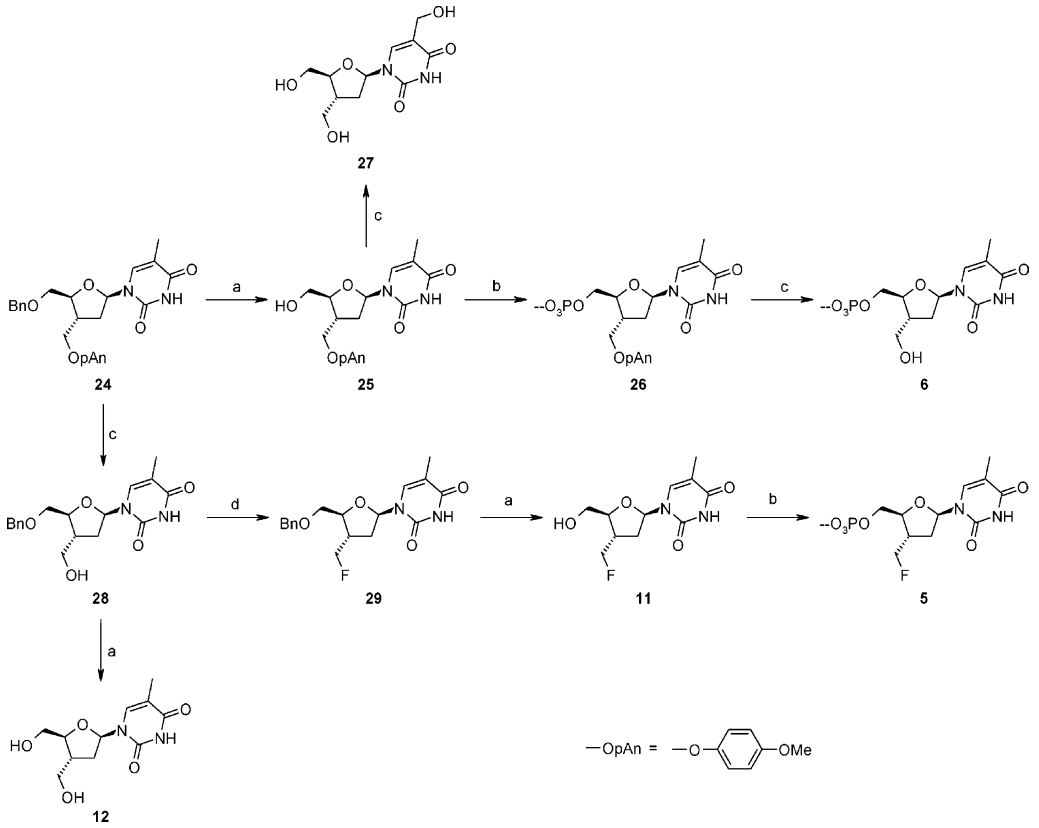
[a] Reagents: (a) *p*-methoxyphenol, DEAD, Ph_3P, THF, 80 °C; (b) HOAc 75%, 80 °C; (c) Ac_2O, pyridine; (d) 5-methyl-2,4-bis[(trimethylsilyl)oxy]pyrimidine, $(CH_3)_3SiOSO_2CF_3$, $Cl_2(CH_2)_2$; (e) NH_3, MeOH; (f) phenylchlorothionocarbonate, DMAP, CH_3CN; (g) AIBN, nBu_3SnH, toluene.

Compound **28** also served as precursor for the synthesis of the 3′-CH_2N_3, 3′-CH_2NH_2 and 3′-CH_2F derivatives. Fluorination of **28** with (diethylamino)sulfur trifluoride (DAST), followed by reductive debenzylation, afforded the desired 2′,3′-dideoxy-3′-fluoromethylnucleoside **11**. Phosphorylation of the 5′-hydroxyl group led to the corresponding nucleotide **5** (Scheme 4).

Mesylation of **28** followed by treatment with NaN_3 afforded azide **30** in 79% yield (2 steps) (Scheme 5). Debenzylation (BCl_3 in CH_2Cl_2), followed by 5′-phosphorylation, furnished 3′-*C*-azidomethyl nucleotide **3**. Reduction of the azido group in **3** with Ph_3P and NH_4OH in pyridine led to the 3′-CH_2NH_2 derivative **4**. Likewise, **9** was converted to **10**.

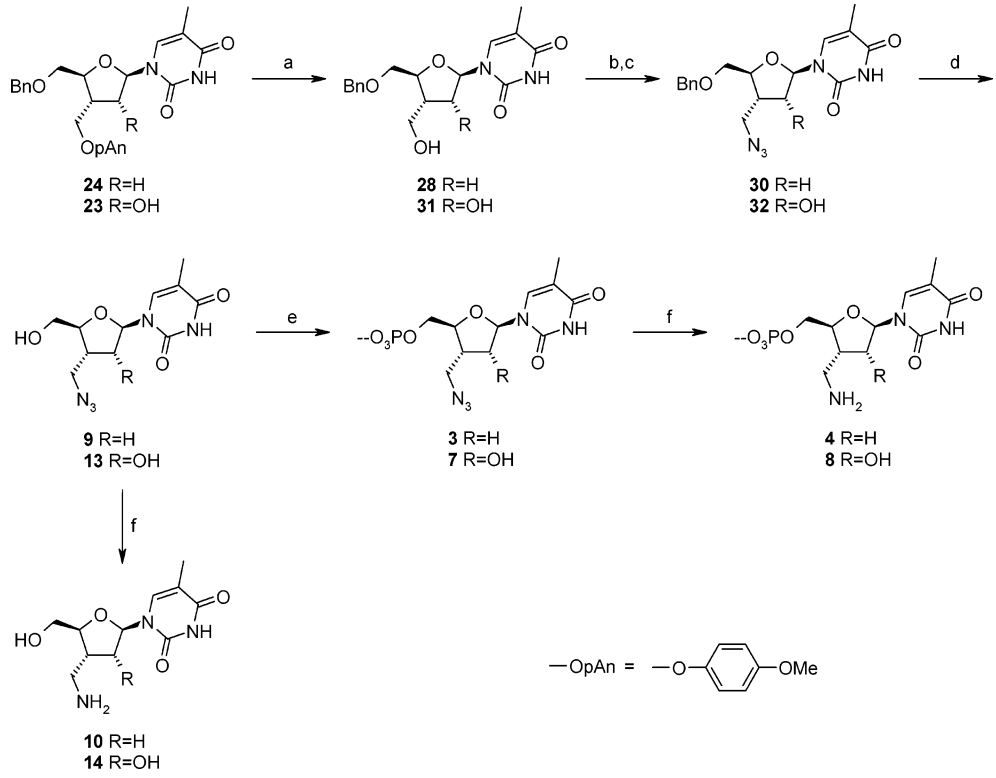
The synthesis of the 3′-*C*-aminomethyl and 3′-*C*-azidomethyl ribo analogues from **23** was performed in a similar way as for the 2′-deoxy analogues. After selective removal of the *p*-anisyl group of **23**, the 6′-azido

Scheme 4[a]



[a] Reagents: (a) H_2, 10% Pd/C, MeOH; (b) $POCl_3$, $(MeO)_3PO$; (c) ceric ammonium nitrate, CH_3CN, H_2O; (d) DAST, CH_2Cl_2.

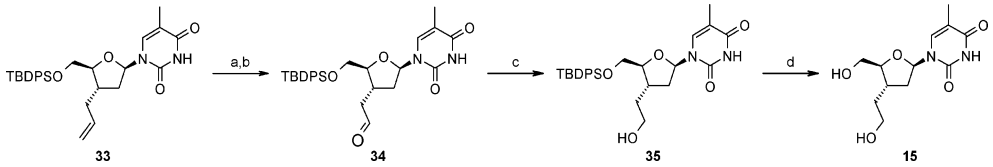
Scheme 5[a]



[a] Reagents: (a) ceric ammonium nitrate, CH_3CN, H_2O; (b) methanesulfonyl chloride, pyridine; (c) NaN_3, DMF, 95 °C; (d) BCl_3, CH_2Cl_2, −78 °C; (e) $POCl_3$, $(MeO)_3PO$; (f) Ph_3P, NH_4OH, pyridine.

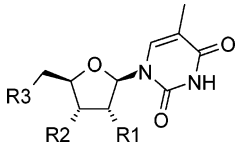
moiety was introduced via mesylation and treatment with NaN_3 to yield nucleoside **13** after deprotection. Phosphorylation of **13** led to analogue **7** and subsequent reduction of the 6′-azido group afforded analogue **8**. Similarly, reduction of the azido group of **13** afforded **14**.

Scheme 6[a]



[a] Reagents: (a) OsO_4, 4-methylmorpholine *N*-oxide, dioxane; (b) $NaIO_4$; (c) $NaBH_4$, EtOH, H_2O; (d) TBAF, THF.

Table 1. Kinetic Parameters of TMPKmt and TMPKh with the 3′-Deoxy-3′-*C*-Branched-Chain-Substituted Nucleosides and Nucleotides



compound code	R1	R2	R3	K_i (μM) TMPKmt	K_i (μM) TMPKh	SI (K_i TMPKh/ K_i TMPKmt
dTMP	H	OH	OPO_3^{2-}	4.5[a]	5.0[a]	1.1[a]
thymidine	H	OH	OH	27[6b]	n.d.	n.d.
AZTMP	H	N3	OPO_3^{2-}	10[3d]	12[a,4e]	1.2[a]
AZT	H	N3	OH	28[6b]	n.d.	n.d.
1	H	NH2	OPO_3^{2-}	235[6a]	n.d.	n.d.
2	OH	NH2	OPO_3^{2-}	27[6a]	n.d.	n.d.
3	H	CH2N3	OPO_3^{2-}	12	32	2.7
4	H	CH_2NH_2	OPO_3^{2-}	10.5	4.7	0.4
5	H	CH_2F	OPO_3^{2-}	15	40	2.7
6	H	CH_2OH	OPO_3^{2-}	29	43.5	1.5
7	OH	CH_2N_3	OPO_3^{2-}	116	n.i.[b]	–
8	OH	CH_2NH_2	OPO_3^{2-}	315	280	0.9
9	H	CH_2N_3	OH	40	1040	26
10	H	CH_2NH_2	OH	57	220	3.9
11	H	CH_2F	OH	45	970	21.6
12	H	CH_2OH	OH	41	420	10
13	OH	CH_2N_3	OH	770	n.i.[c]	–
14	OH	CH_2NH_2	OH	393	110	0.3
15	H	$(CH_2)_2OH$	OH	156	n.d.	n.d.

[a] K_m-value. [b] No inhibition at 1000 μM. [c] No inhibition at 4000 μM.

Compound **15** was obtained according to reported procedures with slight modifications (Scheme 6). An allyl group was introduced at the 3′-position of 5′-silylated thymidine by a free-radical coupling described by C. K. Chu[14] to give nucleoside **33**. Aldehyde **34** was obtained from **33** by a two-step one-pot procedure involving *cis*-hydroxylation with osmium tetraoxide in the presence of 4-methylmorpholine *N*-oxide and sodium periodate cleavage of the diol.[15] Reduction of **34** with sodium borohydride in aqueous ethanol, followed by 5′-desilylation of the alcohol **35**, afforded the free nucleoside **15** in 24% yield from **33**.[16]

Binding Assay. All compounds were tested as described in the Experimental Section[17] and are presented in Table 1. Clearly, 2′-deoxy nucleotides **3**−**6** are potent inhibitors of TMPKmt. The 3′-CH_2NH_2 nucleotide **4**, its 3′-CH_2N_3 precursor **3**, and the 3′-CH_2F nucleotide **5** exhibit the highest affinities within this series with K_i values of 10.5, 12, and 15 μM, respectively. A modeling experiment (in GOLD) showed that **4** adopts a 2′-*exo*-3′-*endo* conformation (dTMP, in contrast, is in the crystal in the 2′-*endo*-3′-*exo* conformation). Consequently, the amino group occupies the same space as the 3′-hydroxyl group of dTMP; hence it is in an appropriate position to interact with Asp9 (Figure 2).

These results indicate that TMPKmt can accommodate sterically more demanding substituents at the 3′-position. Since **5** binds the enzyme as efficiently as **3**

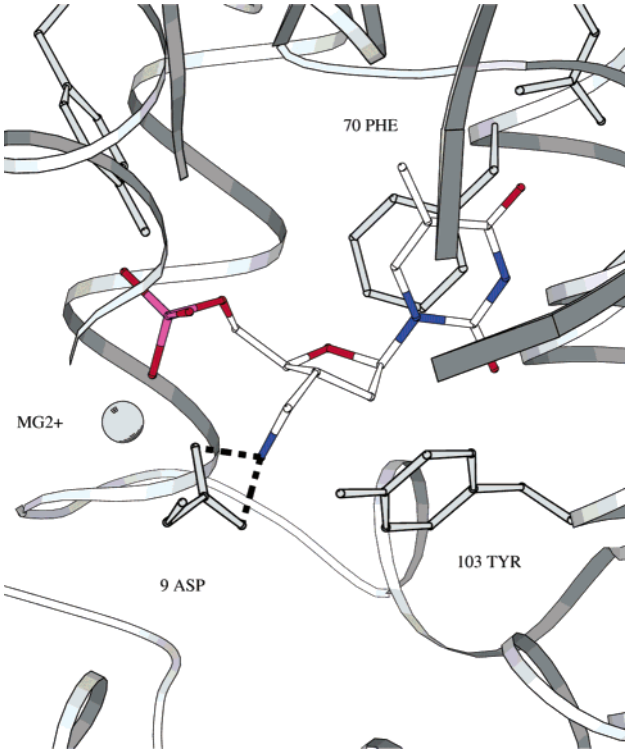


Figure 2. Predicted binding mode of **4** with TMPKmt. The sugar ring is in the 2′-*exo*-3′-*endo* conformation, and the 6′-amine lies at 2.64 Å of the carboxylic oxygen of Asp9.

and **4**, ionic interaction with Asp9 seems not a prerequisite for effective affinity. Interestingly, deletion of the phosphate moiety typically results in a modest (at most 6-fold) affinity loss, while this effect is negligible in the case of the 3′-CH_2OH substituent. The largest discrepancy between the K_i values of the free and phosphorylated forms is found for the 3′-CH_2NH_2 couple. The positively charged ammonium ion may well interact with the negatively charged phosphate moiety, thereby forcing the nucleotide in a favorable conformation for interaction with TMPKmt. In the absence of the phosphoryl group, this conformation is no longer preferred and the conformational mobility of the 3′-CH_2NH_2 may account for the loss of affinity.

Because nucleotide **2** has a much higher affinity than the corresponding 2′-deoxyribo analogue **1**, compound **8** was expected to be active. However, introduction of a methylene spacer between the NH_2-group and C-3′ of **2**, leads to **8** with a 30-fold lower affinity than the deoxyribo analogue **4**. The affinities of the other ribo analogues were weak, the smallest decrease (6-fold) in affinity upon introduction of the 2′-hydroxyl being found for **14**. Apparently, the presence of Tyr103 close to the 2′-position impedes favorable binding characteristics of these ribo analogues. Thus, compound **2** is the only ribo analogue with a higher affinity than its deoxy counterpart.

To probe the influence of the arm length at position 3′, **15** was synthesized. Its K_i value was 5.8 and 3.8 times higher than that of dT ($n = 0$) and **12** ($n = 1$), respectively.

The selectivity of all target compounds for TMPKmt versus TMPKh was evaluated. All are inhibitors of TMPKh except the 3′-hydroxymethylnucleotide **6,** which behaves as a weak substrate. Most nucleotides (**3**, **5**, **6** and **8**) show affinities for TMPKh that lie in the same order of magnitude as those for TMPKmt. The nonphosphorylated 3′-*C*-branched-chain nucleosides **9**, **11**, and **12**, on the other hand, exhibit very low affinities for TMPKh, which results in selectivity indexes between 10 and 26. Somehow a 3′-CH_2N_3 functionality combined with a 2′-hydroxyl seems to introduce a TMPKmt versus TMPKh selectivity in these 3′-*C*-branched-chain analogues, whereas an opposite tendency is observed upon reduction of the 3′-CH_2N_3 group. To conclude, nucleosides **9**, **11** and **12** emerge as the inhibitors that combine a low K_i value with a favorable SI ratio and are therefore put forward as promising leads for further research.

Conclusions

In the search for more potent drugs to treat tuberculosis, the discovery of new leads with a novel mechanism of action is of utmost importance. From this point of view, TMPKmt represents an attractive potential target for the rational design of inhibitors, mainly because of its crucial positioning in the metabolic activation of thymidine and because of the different structures between the enzymes of the bacterium and the host.

This work describes the synthesis of a number of 3′-*C*-branched-chain-substituted substrate analogues of dTMP in the ribo and 2′-deoxyribo series.

Several 3′-deoxy 3′-*C*-branched-chain-substituted dTMP analogues (**3**−**5**) emerged as promising leads for the design of novel antituberculosis agents. These molecules have a high diversity at the 3′-substitution site: the fluorine atom in **5** is sterically much smaller than an azido group in **3**, while the amino group of **4** is positively charged. The K_i values of these compounds for TMPKmt are very similar. This suggests that they bind in a different way involving (most probably) Asp9 and Tyr103 and the Mg^{2+} ion present near the 5′-*O*-phosphate group. As these nucleosides have a 3′-C substituent, they lack the gauche effect that might stabilize 2′-deoxynucleosides in the South type conformation. In a cooperative manner, a voluminous 3′-substituent should preferably occupy a pseudoequatorial position, which is achieved in the North type conformation. On the other hand, the steric interactions between the 3′−H and the thymine base should theoretically drive their pseudorotational equilibria to the South type sugar conformation. This conformational mobility, together with the flexibility of the amino acid side chain in the active site, renders prediction of binding affinities of nucleoside analogues difficult.

Importantly, it should be noted that introduction of a hydroxyl group at the 2′-position leads to an increase in affinity for the 3′-amino analogues **1** and **2**, which is a remarkable feature for an enzyme that metabolizes only 2′-deoxynucleotides. However, protonation of the 3′-amino group may affect the conformation of the nucleotide, as well as its interaction with the enzyme. The most important observation refers to the small differences in binding affinities of the 5′-*O*-phosphorylated and nonphosphorylated analogues, as adequately shown in the very similar activities of **6** and **12**. This result facilitates further elaboration, as it is generally accepted that 5′-*O*-phosphorylated nucleosides are hardly taken up by cells. Originally, our objective was to synthesize modified nucleoside 5′-O-phosphates that are not prone to further phosphorylation in order to avoid cell toxicity. However, we confirmed that nonphosphorylated analogues are as active as their phosphorylated analogues.[6b] In addition, affinity tests on TMPKh indicated that nucleosides have a significantly better selectivity profile vis à vis TMPKh. Therefore, we will pursue in this area. Further research will be directed to (i) exploration of other 3′-substituents of 2′,3′-dideoxythymidine with increased affinities and (ii) investigation of the most appropriate 5′-substituent devoid of phosphorylation capacity and, thus, associated to a low risk of toxicity.

Experimental Section

A. Spectrophotometric Binding Assay. The in vitro tests were done on recombinant enzymes overexpressed in *E. coli*: TMPKmt[3d] and TMPKh (will be described elsewhere by Pochet et al.). TMPKmt and TMPKh activities were determined using the coupled spectrophotometric assay described by Blondin et al.[17] at 334 nm in an Eppendorf ECOM 6122 photometer. The reaction medium (0.5 mL final volume) contained 50 mM Tris-HCl pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate, and 2 units each of lactate dehydrogenase, pyruvate kinase, and nucleoside diphosphate kinase. The concentrations of ATP and dTMP were kept constant at 0.5 and 0.05 mM, respectively, whereas the concentrations of analogues varied between 0.01 and 4 mM.

B. Modeling. The X-ray structure of TMPKmt published by de la Sierra et al.[5] (pdb entry 1G3U) was used in all docking experiments. Water molecules and sulfate counterions were

removed. The Mg^{2+} was considered as being part of the enzyme. Explicit hydrogen atoms were added to the enzyme and inhibitor structures using Reduce.[18] PDB files were then converted to mol2 files using Babel.[19] The position of atom C1′ in the dTMP ligand in the pdb file 1G3U was used as the center of a 20 Å docking sphere. Default settings were used in Gold for all dockings.[20,21] The structures in the top 25 of the docking scores were retained for visual inspection and analysis. Figures 1 and 2 were generated using Molscript.[22]

C. Synthesis. General. NMR spectra were obtained with a Varian Mercury 300 or a Unity 500 spectrometer. Chemical shifts are given in parts per million (δ) relative to residual solvent peak in the case of DMSO-d_6 (2.5 ppm). All signals assigned to amino and hydroxyl groups were exchangeable with D_2O. Mass spectra and exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol:water (1:1) mixture at 3 μL/min. The final nucleoside 5′-*O*-monophosphates were ultimately purified using a Gilson HPLC system with a Gilson 322 pump, a UV/VIS-156 detector on a C18 column (10 μM; Altech; Altima; 250 × 22 mm). The final nucleosides were purified by HPLC on a Perkin-Elmer system with a diode-array ultraviolet absorption detector. A Kromasil reverse phase column (C18, 250 × 4.6 mm) was used with a linear gradient of acetonitrile (A) in 10 mM triethylammonium acetate buffer at pH 7.5 (B) over 20 min at a flow rate of 5.5 mL/min. The purity of all target compounds was assessed by HPLC using either a reverse phase column (Kromasil C18) with a linear gradient of A in B at a flow rate of 1 mL/min over 20 min (method A) or an anion-exchange column (Mono Q HR5/5) with a linear gradient of 1 M triethylammonium bicarbonate buffer at pH 7.8 in water (method B).

Precoated Merck silica gel F_{254} plates were used for TLC and spots were examined under UV light at 254 nm and revealed by sulfuric acid-anisaldehyde spray or phosphomolybdic acid (0.5% in EtOH) solution. Column chromatography was performed on Uetikon silica (0.2−0.06 mm).

Anhydrous solvents were obtained as follows: THF was distilled from sodium/benzophenone; pyridine was refluxed overnight over potassium hydroxide and distilled; dichloromethane, dichloroethane, and toluene were stored over calcium hydride, refluxed, and distilled; DMF was stored over Linde 4 Å molecular sieves, followed by distillation under reduced pressure.

3-[(*p*-Anisyloxy)methyl]-5-*O*-benzyl-3-deoxy-1,2-*O*-(isopropylidene)-α-D-ribofuranose (21). To a solution of **18**[12] (612 mg, 2.08 mmol), Ph_3P (708 mg, 2.7 mmol) and *p*-methoxyphenol (775 mg, 6.24 mmol) in THF (6 mL) was added dropwise diethyl azodicarboxylate (0.42 mL, 2.7 mmol). The reaction mixture was heated to 80 °C and stirred for 1.5 h. The mixture was diluted with ether (150 mL), and the organic layer was washed with water (150 mL), dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography (pentane−EtOAc, 9:1), yielding **21** (724 mg, 87%) as a foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 and 1.43 (6 H, 2s, CMe_2), 2.38 (1H, m, H-3), 3.63 (1H, dd, H-5B), 3.78 (3H, s, OCH_3), 3.83 (1H, dd, $J_{5A,\ 5B} = -10.8$ Hz, H-5A), 3.97 and 4.25 (2H, 2dd, 6-H), 4.15 (1H, m, H-4), 4.54 and 4.66 (2H, 2d, C*H*$_2$Ph), 4.79 (1H, app t, H-2,) 5.89 (1H, d, $J = 3.7$ Hz, H-1), 6.80 (4H, m, 4-MeO*Ph*), 7.34 (5H, m, CH_2*Ph*); HRMS (ESI-MS) for $C_{23}H_{28}O_6Na$ [M + Na]$^+$: found, 423.1813; calcd, 423.1783.

1,2-Di-*O*-Acetyl-3-[(*p*-anisyloxy)methyl]-5-*O*-benzyl-3-deoxy-α-D-ribofuranose (22). A solution of **21** (7.812 g, 19.51 mmol) in 75% HOAc (200 mL) was stirred at 80 °C for 8 h. The reaction mixture was evaporated to give crude 5-*O*-benzyl-3-deoxy-3-[(*p*-anisyloxy)methyl]-α-D-ribofuranose as a syrup. The obtained residue was dissolved in pyridine (150 mL), and acetic anhydride (23 mL, 246 mmol) was added. The solution was stirred at room temperature for 3 h. The solvent was removed under vacuum, and the resulting residue was purified by column chromatography (pentane-EtOAc, 8:2) to yield **22** (8.039 g, 93%) as a foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.96 and 2.05 (6H, 2s, 2 $COCH_3$), 2.96 (1H, m, H-3), 3.57−3.73 and 4.00−4.14 (4H, 2m, H-5 and H-6), 3.78 (3H, s, OCH_3), 4.33 (1H, m, H-4), 4.59 (2H, app d, C*H*$_2$Ph), 5.40 (1H, m, H-2), 6.12 and 6.46 (1H, s and d, H-1α and H-1β), 6.81 (4H, m, 4-MeO*Ph*), 7.34 (5H, m, CH_2*Ph*); HRMS (ESI-MS) for $C_{24}H_{28}O_8Na$ [M + Na]$^+$: found, 467.1707; calcd, 467.1682.

1-{3-[(*p*-Anisyloxy)methyl]-5-*O*-benzyl-3-deoxy-β-D-ribofuranosyl}thymine (23). Thymine (225 mg, 1.78 mmol) was suspended in hexamethyldisilazane (20 mL, 95 mmol) containing trimethylsilyl chloride (0.16 mL, 1.26 mmol) and pyridine (1.6 mL). The mixture was heated at reflux overnight. The reaction mixture was evaporated and coevaporated with toluene. The resulting residue and **22** (720 mg, 1.62 mmol) were dissolved in dry 1,2-dichloroethane (12 mL), and trimethylsilyl triflate (0.34 mL, 1.88 mmol) was added dropwise. The clear solution was stirred 5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The resulting crude 1-[2-*O*-acetyl-3-[(*p*-anisyloxy)methyl]-5-*O*-benzyl-3-deoxy-β-D-ribofuranosyl]thymine was treated with a 7N methanolic ammonia solution (19 mL) at room temperature for 15 h. Evaporation yielded a residue which was purified by column chromatography (CH_2Cl_2−MeOH, 98:2) to afford **23** (592 mg, 78%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.39 (3 H, s, 5-CH_3), 2.68 (1H, m, H-3′), 3.69 (3H, s, OCH_3), 3.73 (1H, app dd, H-2′), 3.95 (2H, m, H-5′), 4.17−4.31 (3H, m, H-4′ and H-6′), 4.59 (2H, 2 app d, C*H*$_2$Ph), 5.72 (1H, app s, H-1′), 5.88 (1H, d, 2′-OH), 6.85 (4H, s, 4-MeO*Ph*), 7.33 (5H, m, CH_2*Ph*), 7.74 (1H, d, $J_{6,\ 5-CH3} = 1.0$, H-6); HRMS (ESI-MS) for $C_{25}H_{28}N_2O_7Na$ [M + Na]$^+$: found, 491.1820; calcd, 491.1794.

1-{3-[(*p*-Anisyloxy)methyl]-5-*O*-benzyl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl}thymine (24). To an ice-cold solution of **23** (6.55 g, 14.0 mmol) and DMAP (3.42 g, 28.0 mmol) in CH_3CN (290 mL) was gradually added phenyl chlorothionocarbonate (2.6 mL, 18.8 mmol). The mixture was stirred at 0 °C for 1.5 h. The solvent was removed in vacuo, and the residue was dissolved in EtOAc (500 mL). The solution was washed with water (2 × 500 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo to give 1-[3-[(*p*-anisyloxy)-methyl]-5-*O*-benzyl-3-deoxy-2-*O*-(phenoxycarbonothioyl)-β-D-ribofuranosyl]thymine as a syrup. The syrup was dissolved in toluene (290 mL), to which 2,2′-azobis(2-methyl-propionitrile) (AIBN, 5.89 g, 35.87 mmol) and tri-*n*-butyltinhydride (8.00 g, 27.5 mmol) were added at 50−60 °C under N_2. The reaction mixture was stirred at 95−100 °C for 1 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (pentane-EtOAc, 2:1) to yield **24** (3.98 g, 63%) as a foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.52 (3 H, s, 5-CH_3), 2.24−2.29 (2H, m, H-2′), 2.80 (1H, m, H-3′), 3.69 (3 H, s, OCH_3), 3.71 (1H, m, H-5′B), 3.87 (1H, dd, $J_{5'A,\ 5'B} = -10.9$ Hz, H-5′A), 3.94−4.06 (3H, m, H-4′ and H-6′), 4.53 and 4.57 (2H, 2d, C*H*$_2$Ph), 6.1 (1H, dd, $J = 7.0$ Hz, H-1′), 6.87 (4H, s, 4-MeO*Ph*), 7.28−7.36 (5H, m, CH_2*Ph*), 7.67 (1H, br s, H-6); MS (ESI) *m*/*z* (%) 475.2 ([M + Na]$^+$, 100), 453.2 ([M + H]$^+$, 47).

1-{3-[(*p*-Anisyloxy)methyl]-2,3-dideoxy-β-D-*erythro*-pentofuranosyl}thymine (25). A solution of **24** (730 mg, 1.61 mmol) in MeOH (40 mL) was hydrogenated at atmospheric pressure for 5h in the presence of 10% Pd/C (390 mg). The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2−MeOH, 98:2) to give 496 mg (85%) of the debenzylated intermediate **25** as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.78 (3 H, s, 5-CH_3), 2.13−2.28 (2H, m, H-2′), 2.70 (1H, m, H-3′), 3.64 (1H, m, H-5′B), 3.69 (3H, s, OCH_3) 3.78 (1H, m, H-5′A), 3.88 (1H, m, H-4′), 3.96 (2H, d, H-6′), 5.13 (1H, t, $J = 5.3$ Hz, 5′-OH), 6.09 (1H, dd, H-1′), 6.87 (4H, m, 4-MeO*Ph*), 7.88 (1H, d, $J = 1.2$ Hz, H-6); HRMS (ESI-MS) for $C_{18}H_{22}N_2O_6Na$ [M + Na]$^+$: found, 385.1413; calcd, 385.1375.

1-(2,3-Dideoxy-3-hydroxymethyl-β-D-*erythro*-pentofuranosyl)-5-hydroxymethylthymine (27). To a solution of **25** (124 mg, 0.34 mmol) in CH_3CN-H_2O (4:1) (5.5 mL) was added ceric ammonium nitrate (940 mg, 1.70 mmol). After 5 min the mixture was diluted with water (20 mL), washed with $CHCl_3$ (20 mL), and the aqueous layer was evaporated. The residue was purified by column chromatography (CH_2Cl_2-MeOH, 95:5 → 90:10) to give **27** (36 mg, 39%) as a foam. ^1H NMR (500 MHz, DMSO-d_6): δ 2.17 (ddd, $J_{1',2'} = 4.6$ Hz, H-2′), 2.00 (1H, dt, $J_{2',2''} = 13.7$ Hz, $J_{1',2''} = 7.2$ Hz, H-2″), 2.31 (1H, m, H-3′), 3.44 (2H, m, H-6′), 3.67 and 3.55 (2H, 2m, H-5′), 3.78 (1H, dt, H-4′), 4.13 (2H, s, 5-CH_2), 4.76 (1H, t, 6′-OH), 4.86 (1H, t, 5-CH_2OH), 4.94 (1H, t, 5′-CH_2OH), 6.01 (1H, dd, $J_{1',2'} = 4.4$ and $J_{1',2''} = 6.9$ Hz, H-1′), 7.81 (1H, s, H-6), 11.24 (1H, s, H-3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.04 (C-2′), 40.82 (C-3′), 56.07 (5-CH_2), 61.59 (C-6′), 62.62 (C-5′), 83.47 (C-4′), 84.26 (C-1′), 113.7 (C-5′), 137.01 (C-6), 150.33 (C-2), 162.98 (C-4); MS (ESI) *m*/*z* (%) 295.1 ([M + Na]$^+$, 70).

General Procedure for the Selective Phosphorylation of the 5′-Hydroxyl Group. To a cooled (0 °C) solution of the nucleoside (0.42 mmol) in trimethyl phosphate (10 mL/mmol) was added $POCl_3$ (6.5 eq) dropwise, and the mixture was stirred for 5 h at 0 °C and for 30 min at room temperature, after which it was poured into crushed ice−water (10 mL), neutralized with concentrated NH_4OH, and evaporated to dryness. The resulting residue was purified by column chromatography (^{i}PrOH−NH_4OH-H_2O, 77.5:15:2.5 → 60:30:5). Further purification of the white powder by HPLC (C-18, CH_3CN−MeOH−0.05% HCOOH in H_2O 45:45:10, 3 mL/min) and lyophilisation of the appropriate fractions provided the nucleotide as a white powder.

1-{3-[(*p*-Anisyloxy)methyl]-2,3-dideoxy-5-*O*-phosphoryl-β-D-*erythro*-pentofuranosyl}thymine (26). 25 was phosphorylated as described in the general procedure and nucleotide **26** isolated in 52% yield. ^1H NMR (300 MHz, D_2O): δ 1.94 (3 H, s, 5-CH_3), 2.28−2.44 (2H, m, H-2′), 2.86 (1H, m, H-3′), 3.81 (3H, s, OCH_3), 4.05−4.26 (5H, m, H-6′, H-4′ and H-5′), 6.22 (1H, dd, $J = 5.2$ and 6.6 Hz, H-1′), 6.98 (4H, m, 4-MeO*Ph*), 7.80 (1H, d, $J = 0.9$ Hz, H-6); ^{13}C NMR (125 MHz, D_2O): δ 14.26 (5-CH_3), 37.21 (C-2′), 40.77 (C-3′), 58.51 (OCH_3), 68.36 (C-5′, $^2J_{C,P} = 19.6$ Hz), 72.19 (C-6′), 84.83 (C-4′, $^3J_{C,P} = 8.8$ Hz), 88.15 (C-1′), 113.86, 117.71 and 118.76 (C-5 and arom H), 140.13 (C-6), 154.18, 155.28, 156.10 (C-2 and arom H), 169 (C-4); ^{31}P NMR (500 MHz, D_2O): δ 1.03; HRMS (ESI-MS) for $C_{18}H_{24}N_2O_9P$ [M + H]$^+$: found, 443.1231; calcd, 443.1219.

1-[2,3-Dideoxy-3-(hydroxymethyl)-5-*O*-phosphoryl-β-D-*erythro*-pentofuranosyl]thymine (6). To a solution of **26** (106 mg, 0.23 mmol) in H_2O (2 mL) was added ceric ammonium nitrate (162 mg, 0.29 mmol). After 15 min ^{i}PrOH−NH_4OH-H_2O (60:30:5) (20 mL) was added, the resulting suspension was filtered, and the precipitate was washed with water. The combined filtrates were evaporated under reduced pressure, and the residue was purified by column chromatography (^{i}PrOH−NH_4OH-H_2O, 77.5:15:2.5 → 60:30:5). The resulting white powder was further purified by HPLC (C-18, CH_3CN−MeOH−0.05% HCOOH in H_2O, 45:45:10, 3 mL/min), and the appropriate fractions were lyophilized yielding **6** (62 mg, 76%) as a white powder. ^1H NMR (300 MHz, D_2O): δ 1.93 (3 H, d, 5-CH_3), 2.30 (2H, m, H-2′), 2.62 (1H, m, H-3′), 3.70 (2H, d, $J_{6',3'} = 6.1$ Hz, H-6′), 4.01 (1H, m, H-4′), 4.14 (2H, m, H-5′), 6.16 (1H, dd, $J = 5.0$ and 6.7 Hz, H-1′), 7.83 (1H, d, $J = 1.2$ Hz, H-6); ^{13}C NMR (125 MHz, D_2O): δ 14.28 (5-CH_3), 37.10 (C-2′), 43.04 (C-3′), 64.75 (C-6′), 67.88 (C-5′), 85.21 (C-4′), 88.01 (C-1′), 140.42 (C-6); ^{31}P NMR (500 MHz, D_2O): δ 1.03; HRMS (ESI-MS) for $C_{11}H_{18}N_2O_8P$ [M + H]$^+$: found, 337.0817; calcd, 337.0800.

1-[5-*O*-Benzyl-2,3-dideoxy-3-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]thymine (28). To a solution of **24** (3.3 g, 7.29 mmol) in CH_3CN-H_2O (4:1) (120 mL) was added ceric ammonium nitrate (19.89 g, 36.68 mmol). After 5 min the mixture was diluted with $CHCl_3$ (300 mL), washed with brine (200 mL) and a saturated solution of $Na_2S_2O_5$ (200 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (CH_2Cl_2-MeOH, 97:3) to give the expected product **28** (1.810 g, 72%) as a foam. ^1H NMR (300 MHz, DMSO-d_6): δ 1.52 (3 H, d, 5-CH_3), 2.01−2.19 (2H, m, H-2′), 2.44 (1H, m, H-3′), 3.46 (2H, app t, H-6′), 3.64 (1H, dd, H-5′B), 3.79 (1H, dd, $J_{5'A,5'B} = -10.8$ Hz, H-5′A), 3.95 (1H, m, H-4′), 4.56 (2H, app s, CH_2Ph), 4.82 (1H, t, 6′-CH_2OH), 6.01 (1H, dd, $J = 7.0$ and 4.5 Hz, H-1′), 7.36 (5H, m, CH_2Ph), 7.64 (1H, d, $J = 1.1$ Hz, H-6); HRMS (ESI-MS) for $C_{18}H_{22}N_2O_5Na$ [M + Na]$^+$: found, 369.1455; calcd, 369.1426.

1-[2,3-Dideoxy-3-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]thymine (12). Hydrogenation of **28** (62.0 mg, 0.179 mmol) was accomplished at atmospheric pressure of H_2 at room temperature for 45 min using 10% Pd/C (50 mg) in MeOH (5 mL). Column chromatography (CH_2Cl_2-MeOH, 95:5) yielded **12** (43.4 mg, 95%) as a white foam. ^1H NMR (300 MHz, DMSO-d_6): δ 1.78 (3 H, d, $J = 1.0$ Hz, 5-CH_3), 1.97−2.04 (2H, m, H-2′), 2.36 (1H, m, H-3′), 3.44 (2H, app d, $J_{6',3'} = 5.7$ Hz, 6′-H), 3.56 (1H, dd, H-5′B) 3.69 (1H, dd, $J_{5'A,5'B} = -11.9$ Hz, H-5′A), 3.78 (1H, m, H-4′), 4.76 and 5.03 (2H, 2t, 5′-OH and 3′-OH), 5.98 (1H, dd, $J = 4.6$ and 6.9 Hz, H-1′), 7.84 (1H, br s, H-6); HRMS (ESI-MS) for $C_{11}H_{16}N_2O_5Na$ [M + Na]$^+$: found, 279.0953; calcd, 279.0957. Anal. ($C_{11}H_{16}N_2O_5$) C, H, N.

1-[5-*O*-Benzyl-2,3-dideoxy-3-(fluoromethyl)-β-D-*erythro*-pentofuranosyl]thymine (29). DAST (0.280 mL, 2.12 mmol) was dissolved in CH_2Cl_2 (5 mL) and pyridine (0.36 mL) at 0 °C under nitrogen. Nucleoside **28** (619 mg, 1.79 mmol) was added in several portions, and the solution was allowed to warm to room temperature. After 7 h, the solvent was removed under vacuum to give a syrup which was purified by column chromatography (CH_2Cl_2-MeOH, 99:1) to yield **29** (185 mg, 30%) as a white foam. ^1H NMR (300 MHz, DMSO-d_6): δ 1.54 (3 H, s, 5-CH_3), 2.10−2.23 (2H, m, H-2′), 2.69−2.80 (1H, m, H-3′), 3.65 (1H, dd, H-5′B), 3.80 (1H, dd, $J_{5'A,5'B} = -10.9$ Hz, H-5′A), 4.02 (1H, m, H-4′), 4.45−4.61 (4H, m, H-6′ and CH_2-Ph), 6.05 (1H, dd, $J = 4.7$ and 7.0 Hz, H-1′), 7.35 (5H, m, CH_2Ph), 7.62 (1H, d, $J = 1.1$ Hz, H-6); HRMS (ESI-MS) for $C_{18}H_{22}FN_2O_4$ [M + H]$^+$: found, 349.1586; calcd 349.1563.

1-[2,3-Dideoxy-3-(fluoromethyl)-β-D-*erythro*-pentofuranosyl]thymine (11). A solution of compound **29** (185 mg, 0.531 mmol) in MeOH (8 mL) was hydrogenated at atmospheric pressure in the presence of Pd/C 10% (107 mg) for 5 h. The catalyst was removed by filtration over a Celite path, the solvent evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2-MeOH, 95:5), yielding **11** (134 mg, 98%) as a white foam. ^1H NMR (300 MHz, DMSO-d_6): δ 1.78 (3 H, s, 5-CH_3), 2.06−2.21 (2H, m, H-2′), 2.60−2.71 (1H, m, H-3′), 3.56 (1H, dd, H-5′B), 3.71 (1H, dd, $J_{5'A,5'B} = -12.0$ Hz, H-5′A), 3.84 (1H, m, H-4′), 4.44−4.57 (2H, dd, $J_{6',F} = 47.3$ Hz, H-6′), 5.14 (1H, br s, 5′-OH), 6.02 (1H, dd, $J = 4.9$ and 6.7, H-1′), 7.82 (1H, s, H-6); ^{13}C NMR (75 MHz, DMSO-d_6): δ 62.19 (C-5′), 83.04 (C-4′, $J_{4',F} = 4.5$ Hz), 84.50 (C-1′), 85.64 (C-6′, $J_{6',F} = 179$ Hz), 109.64 (C-6), 136.96 (C-5), 151.06 (C-4), 164.50 (C-2), under DMSO-signal (C-3′), 34.50 (C-2′, $J_{2',F} = 5.3$ Hz), 12.95 (5-CH_3); ^{19}F NMR (300 MHz, DMSO-d_6): δ −222.73; HRMS (ESI-MS) for $C_{11}H_{16}FN_2O_4$ [M + H]$^+$: found, 259.1119; calcd, 259.1093. Anal. ($C_{11}H_{15}FN_2O_4$) C, H, N.

1-[2,3-Dideoxy-3-(fluoromethyl)-5-*O*-phosphoryl-β-D-*erythro*-pentofuranosyl]thymine (5). Phosphorylation of **11** was performed as described in the general procedure and the title compound isolated in 49% yield. ^1H NMR (300 MHz, D_2O): δ 1.94 (3 H, s, 5-CH_3), 2.29−2.44 (2H, m, H-2′), 2.77−2.89 (1H, m, H-3′), 4.00−4.22 (2H, m, H-5′), 4.25 (1H, m, H-4′), 4.53−4.70 (2H, m, H-6′), 6.16 (1H, dd, $J = 5.0$ and 7.0 Hz, H-1′), 7.84 (1H, d, $J = 1.2$ Hz, H-6); ^{13}C NMR (75 MHz, D_2O): δ 11.69 (5-CH_3), 33.45 (C-2′, $J_{2',F} = 6.0$ Hz), 65.30 (C-5′), 38.66 (C-3′, $J_{3',F} = 19.6$ Hz), 81.50 (C-4′, $J_{4',F} = 6.7$ Hz), 84.10 (C-6′, $J_{6',F} = 165.2$ Hz), 85.43 (C-1′), 111.40 (C-6), 137.69 (C-5), 151.80 (C-4), 166.80 (C-2); ^{19}F NMR (300 MHz, D_2O): δ *−224.50;* ^{31}P NMR (500 MHz, D_2O): δ 1.12; HRMS (ESI-MS) for $C_{11}H_{17}FN_2O_7P$ [M + H]$^+$: found, 339.0806; calcd, 339.0757.

1-[3-(Azidomethyl)-5-*O*-benzyl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (30). Methanesulfonyl chloride (0.180 mL, 2.33 mmol) was added to a solution of compound **28** (306 mg, 0.883 mmol) in pyridine (3 mL) at 0 °C. The reaction mixture was stirred at 0 °C during 1 h. Then it was

diluted with CH_2Cl_2 (50 mL), washed with saturated aqueous $NaHCO_3$ (50 mL), and dried over anhydrous $MgSO_4$. The solvent was removed under diminished pressure to give the crude mesylate. The obtained residue was dissolved in DMF (16 mL) and treated with NaN_3 (574 mg 8.83 mmol) at 95 °C for 1.5 h to complete the reaction. The reaction mixture was evaporated to dryness in vacuo, and the residue was dissolved in CH_2Cl_2 (50 mL). The organic layer was washed with water (50 mL), dried over anhydrous $MgSO_4$, and evaporated to give a sirup which was purified by column chromatography (CH_2Cl_2−MeOH, 99:1), yielding **30** (260 mg, 79%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.52 (3 H, d, 5-CH_3), 2.50 (2H, m, H-2′), 2.60 (1H, m, H-3′), 3.46−3.59 (2H, 2dd, H-6′), 3.66 (1H, dd, H-5′B), 3.83 (1H, dd, $J_{5'A,5'B} = -10.4$ Hz, H-5′A), 3.93 (1H, m, H-4′), 4.57 (2H, app s, CH_2Ph), 6.04 (1H, app t, J = 5.9 Hz, H-1′), 7.31 (5H, m, CH_2Ph), 7.62 (1H, br s, H-6); HRMS (ESI-MS) for $C_{18}H_{21}N_5O_4Na$ [M + Na]$^+$: found, 394.1489; calcd, 394.1491.

1-[3-(Azidomethyl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (9). To a solution of **30** (1.49 g, 4.01 mmol) in CH_2Cl_2 (160 mL) at −78 °C was slowly added a 1 M solution of BCl_3 in CH_2Cl_2 (16.1 mL, 16.1 mmol). After 15 min MeOH (5 mL) was added, and the reaction mixture was evaporated to dryness under reduced pressure. The resulting residue was purified by column chromatography (CH_2Cl_2−MeOH, 90:10), affording **9** (1.00 g, 89%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.78 (3 H, d, 5-CH_3), 2.13 (2H, m, H-2′), 2.51 (1H, m, H-3′), 3.51 (2H, app d, H-6′), 3.55−3.61 (1H, m, H-4′), 3.69−3.74 (2H, m, H-5′), 5.13 (1H, app t, 5′-OH), 6.02 (1H, dd, J = 5.0 and 6.3 Hz, H-1′), 7.82 (1H, d, J = 1.1 Hz, H-6); ^{13}C NMR (125 MHz, DMSO-d_6): δ 12.96 (5-CH_3), 36.36 (C-2′), 38.07 (C-3′), 52.71 (C-6′), 61.90 (C-5′), 84.45 and 84.13 (C-1′ and C-4′), 109.56 (C-6), 136.97 (C-5), 151.05 (C-4), 164.51 (C-2); HRMS (ESI-MS) for $C_{11}H_{15}N_5O_4Na$ [M + Na]$^+$: found, 304.1033; calcd, 304.1021. Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

1-[3-(Aminomethyl)-2,3-dideoxy-β-D-*erythro*-pentofuran-osyl]thymine (10). Compound **9** (169 mg, 0.60 mmol) and triphenylphosphine (252 mg, 0.96 mmol) were dissolved in pyridine (7 mL) and stirred at room temperature. After 1 h, concentrated NH_4OH (6 mL) was added, and the solution was allowed to stir for an additional 2 h. Pyridine was removed under reduced pressure, water (8 mL) was added, and the unreacted triphenylphosphine and triphenylphosphine oxide were removed by filtration. The filtrate was extracted with toluene, and the water layer was evaporated under reduced pressure to give a syrup. The syrup was purified by column chromatography (0.175 N NH_3 in CH_2Cl_2−MeOH, 80:20) to yield amine **10** (112 mg, 73%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.78 (3 H, s, 5-CH_3), 2.3−2.14 (2H, m, H-2′), 2.24 (1H, m, H-3′), 2.55−2.65 (2H, m, H-6′), 3.55−3.71 (3H, m, H-5′ and H-4′), 5.95 (1H, dd, J = 3.9 and 6.9 Hz, H-1′), 7.8 (1H, d, J = 1.1 Hz, H-6); ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.98 (5-CH_3), 43.38, 41.60 and 37.05 (C-6′, C-3′ and C-2′), 62.27 (C-5′), 85.49 (C-4′), 85.24 (C-1′), 109.37 (C-6), 136.95 (C-5), 151.04 (C-4), 164.51 (C-2); HRMS (ESI-MS) for $C_{11}H_{18}N_3O_4$ [M + H]$^+$: found, 256.1293; calcd, 256,1296. Anal. ($C_{11}H_{17}N_3O_4$) C, H, N.

1-[3-(Azidomethyl)-2,3-dideoxy-5-*O*-phosphoryl-β-D-*erythro*-pentofuranosyl]thymine (3). 9 was phosphorylated as described in the general procedure to yield 53% of the title compound. ^{1}H NMR (300 MHz, D_2O): δ 1.91 (3 H, s, 5-CH_3), 2.33 (2H, app dd, H-2′), 2.68 (1H, m, H-3′), 3.54 (2H, app d, $J_{6',3'}$ = 6.3 Hz, H-6′), 4.00−4.19 (3H, m, H-5′ and H-4′), 6.16 (1H, app t, J = 5.8 Hz, H-1′), 7.80 (1H, d, J = 1.1 Hz, 6-H); ^{13}C NMR (75 MHz, D_2O): δ 11.75 (5-CH_3), 35.36 (C-2′), 37.71 (C-3′), 52.15 (C-6′), 65.13 (C-5′), 82.62 (C-4′), 85.36 (C-1′), 111.36 (C-6), 137.71 (C-5), 151.79 (C-4), 166.79 (C-2); ^{31}P NMR (500 MHz, D_2O): δ 1.14; HRMS (ESI-MS) for $C_{11}H_{16}N_5O_7P$ [M + H]$^+$: found, 362.0889; calcd, 362.0865.

1-[3-(Aminomethyl)-2,3-dideoxy-5-*O*-phosphoryl-β-D-*erythro*-pentofuranosyl]thymine (4). Compound **3** (476 mg, 1.26 mmol) and triphenylphosphine (528 mg, 2.01 mmol) were dissolved in pyridine (15 mL) and stirred at room temperature. After 1 h, concentrated NH_4OH (12.6 mL) was added, and the solution was allowed to stir for an additional 6.5 h. Pyridine was removed at reduced pressure, water (200 mL) was added, and the unreacted triphenylphosphine and triphenylphosphine oxide were removed by filtration. The filtrate was extracted with toluene, and the water layer was evaporated under reduced pressure to give a syrup. The syrup was purified by column chromatography (^{i}PrOH−NH_4OH−H_2O, 77.5:15:2.5 → 60:30:5). The resulting white powder was further purified by HPLC (C-18, CH_3CN-MeOH-0.05% HCOOH in H_2O, 45:45:10, 3 mL/min), and the fractions containing the title compound were lyophilized to yield **4** (306 mg, 69%) as a white powder. ^{1}H NMR (300 MHz, D_2O): δ 1.92 (3 H, s, 5-CH_3), 2.32−2.50 (2H, m, H-2′), 2.74−2.82 (1H, m, H-3′), 3.12−3.26 (2H, 2dd, H-6′), 4.05−4.12 (3H, m, H-4′ and H-5′), 6.17 (1H, dd, J = 3.7 and 7.3 Hz, H-1′), 7.78 (1H, d, J = 1.2 Hz, H-6); ^{13}C NMR (75 MHz, D_2O): δ 11.76 (5-CH_3), 166.79 (C-2), 151.74 (C-4), 137.70 (C-5), 111.40 (C-6), 85.29 (C-1′), 82.72 (C-4′), 63.94 (C-5′), 40.80 (C-6′), 36.88 (C-3′), 35.95 (C-2′); ^{31}P NMR (500 MHz, D_2O): δ 2.82; HRMS (ESI-MS) for $C_{11}H_{19}N_3O_7PNa$ [M + Na]$^+$: found, 336.0980; calcd, 336.0960.

1-[5-*O*-Benzyl-3-deoxy-3-(hydroxymethyl)-β-D-ribofuranosyl]thymine (31). This compound was synthesized from **23** (346 mg, 0.74 mmol) using the procedure described for **28** to yield 130 mg (49%) of the title compound as a foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.42 (3 H, s, 5-CH_3), 2.31 (1H, m, $J_{3',6'}$ = 6.6 Hz, H-3′), 3.49 and 3.68 (2H, 2m, 6′-H), 3.64 (1H, m, H-5′B), 3.98 (1H, m, $J_{5'A,5'B} = -11.1$ Hz, H-5′A), 4.13 (1H, m, H-4′), 4.19 (1H, m, H-2′), 4.58 (3H, m, CH_2Ph and 6′-OH), 5.60 (1H, d, J = 5.0 Hz, 2′-OH), 5.67 (1H, d, J = 1.9, H-1′), 7.37 (5H, m, CH_2Ph), 7.70 (1H, br s, 6-H); HRMS (ESI-MS) for $C_{18}H_{22}N_2O_6Na$ [M + Na]$^+$: found, 385.1386; calcd, 385.1375.

1-[3-Azidomethyl-5-*O*-benzyl-3-deoxy-β-D-ribofuranosyl]thymine (32). This compound was synthesized from **31** (130 mg, 0.36 mmol) using the same procedure as described for the synthesis of **30** to yield 123 mg (89%, 2 steps) of the title compound as a foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.42 (3 H, s, 5-CH_3), 2.46 (1H, m, H-3′), 3.35−3.90 (4H, m, H-5′ and H-6′), 4.10 (1H, m, H-4′), 4.22 (1H, m, H-2′), 4.59 (2H, s, CH_2Ph), 5.67 (1H, d, J = 1.6 Hz, H-1′), 5.93 (1H, d, J = 5.1 Hz, 2′-OH), 7.37 (CH_2Ph), 7.67 (1H, br s, H-6); HRMS (ESI-MS) for $C_{18}H_{21}N_5O_5Na$ [M + Na]$^+$: found, 410.1504; calcd, 410.144.

1-[3-Azidomethyl-3-deoxy-β-D-ribofuranosyl]thymine (13). Debenzylation of **32** (346 mg, 0.89 mmol) was performed as described for the synthesis of **9** to yield 210 mg (79%) of the title compound as a foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76 (3 H, s, 5-CH_3), 2.37 (1H, m, H-3′), 3.35−3.78 4H, m, H-5′ and H-6′), 3.91 (1H, m, H-4′), 4.20 (1H, app d, H-2′), 5.24 (1H, br s, 5′-OH), 5.66 (1H, d, $J_{1',2'}$ = 1.9 Hz, H-1′), 5.94 (1H, br s, 2′-OH), 7.95 (1H, br s, H-6). The assignment, done by a cosy-experiment, differs from that reported in ref 8. ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.95 (5-CH_3), 41.35 (C-3′), 47.90 (C-6′), 61.14 (C-5′), 75.36 (C-2′), 82.90 (C-4′), 91.26 (C-1′), 164.53 (C-2), 151.11 (C-4), 136.93 (C-5), 109.10 (C-6); HRMS (ESI-MS) for $C_{11}H_{15}N_5O_5Na$ [M + Na]$^+$: found, 320.0975; calcd, 320.0971. Anal. ($C_{11}H_{15}N_5O_5$) C, H, N.

1-[3-Aminomethyl-3-deoxy-β-D-ribofuranosyl]thymine (14). Reduction of azide **13** (173 mg, 0.58 mmol) was accomplished using the procedure described for the synthesis of **11** to yield 123 mg (73%) of amine **14** as a foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76 (3 H, d, 5-CH_3), 2.16 (1H, m, H-3′), 2.63−2.89 (2H, 2dd, H-6′), 3.59 (1H, dd, H-5′B), 3.76 (1H, dd, $J_{5'A,5'B} = -12.3$ Hz, H-5′A), 3.92 (1H, m, H-4′), 4.21 (1H, app d, H-2′), 5.63 (1H, d, J = 1.4 Hz, H-1′), 7.94 (1H, d, J = 1.1 Hz, H-6); HRMS (ESI-MS) for $C_{11}H_{18}N_3O_5$ [M + H]$^+$: found, 272.1245; calcd, 272.1246. Anal. ($C_{11}H_{17}N_3O_5$) C, H, N.

1-[3-Azidomethyl-3-deoxy-5-*O*-phosphoryl-β-D-ribofuranosyl]thymine (7). 13 was phosphorylated as described in the general procedure to yield 51% of nucleotide **7**. ^{1}H NMR (300 MHz, D_2O): δ 1.92 (3 H, d, 5-CH_3), 2.59 (1H, m, H-3′), 3.56 and 3.66 (2H, 2dd, $J_{6',3'}$ = 5.8 and 8.1 Hz, H-6′), 4.04 (1H, m, H-5′B), 4.26 (1H, m, H-5′A), 4.32 (1H, app d, H-4′), 4.52 (1H, dd, H-2′), 5.86 (1H, d, J = 2.0 Hz, H-1′), 7.89 (1H, d, J = 1.0 Hz, H-6); ^{13}C NMR (125 MHz, D_2O): δ 14.28 (5-CH_3), 43.40

(C-3′), 49.71 (C-6′), 66.68 (C-5′, $^2J_{C,P}$ = 36.0 Hz), 77.95 (C-2′), 84.52 (C-4′, $^3J_{C,P}$ = 8.9 Hz), 93.83 (C-1′), 113.62 (C-5), 139.84 (C-6), 154.25 (C-2), 169.33 (C-4); ^{31}P NMR (500 MHz, D_2O): δ 1.27; HRMS (ESI-MS) for $C_{11}H_{17}N_5O_8P$ $[M + H]^+$: found, 378.0813; calcd, 378.0814.

1-[3-Aminomethyl-3-deoxy-5-*O*-phosphoryl-*β*-D-ribofuranosyl]thymine (8). Azide **7** (346 mg, 0.74 mmol) was reduced to the corresponding amine following the procedure described for the preparation of **4** to yield 110 mg (65%) of the amino-nucleotide **8** as a foam. ^{1}H NMR (300 MHz, D_2O): δ 1.92 (3 H, s, 5-CH_3), 2.69 (1H, m, H-3′), 3.17 and 3.38 (2H, 2dd, J = 5.2 and 10.2 Hz, H-6′), 4.07 and 4.17 (2H, 2m, H-5′), 4.30 (1H, app d, H-4′), 4.56 (1H, dd, H-2′), 5.84 (1H, d, J = 1.8 Hz, H-1′), 7.86 (1H, d, J = 1.1 Hz, H-6); ^{13}C NMR (125 MHz, D_2O): δ 14.30 (5-CH_3), 38.26 (C-6′), 42.22 (C-3′), 65.74 (C-5′, $^2J_{C,P}$ = 4.9 Hz), 77.88 (C-2′), 84.43 (C-4′, $^3J_{C,P}$ = 7.8 Hz), 94.17 (C-1′), 113.72 (C-5), 139.95 (C-6), 155.51 (C-2), 169.32 (C-4); ^{31}P NMR (500 MHz, D_2O): δ 3.45; HRMS (ESI-MS) for $C_{11}H_{19}N_3O_8P$ $[M + H]^+$: found, 352.0902; calcd, 352.0909.

2′,3′-Dideoxy-3′−C−(2-hydroxyethyl)thymine (15). To a mixture of 5′-*O*-*tert*-butyldiphenylsilyl-3′−C-allyl-2′,3′-dideoxythymidine (**33**)[14] (0.80 g, 1.58 mmol) and 4-methylmorpholine *N*-oxide (0,26 g, 1.91 mmol) in dioxane (10 mL) was added an aqueous solution of OsO_4 (0.09 mmol, 1% in water). After stirring 3 h at room temperature under light protection, the reaction mixture was complete (TLC); then sodium periodate (0.53 g, 2.49 mmol) was added in small portions. After completion of the reaction (2 h), the mixture was diluted with ethyl acetate, filtered through Celite, and solids washed with ethyl acetate. The combined filtrates were washed with saturated aqueous NaCl, dried, and evaporated under vacuum. To the crude aldehyde **34** in ethanol/water (6/2 mL) was added dropwise $NaBH_4$ (0.18 g, 4.90 mmol) in ethanol (30 mL). After 2 h at room temperature, the reaction was complete. The mixture was diluted in ethyl acetate and washed with water, and the organic layer was dried and concentrated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH), compound **35** (0.24 g, 0.48 mmol) was isolated in 30% yield from **33** as a white foam. Compound **35** (50 mg, 0.098 mmol) was treated with a 1M solution of TBAF in THF (0.5 mL). After stirring 1 h at room temperature, solvent was evaporated and the residue was purified by HPLC on a C18 reverse phase column (5−25% linear gradient of acetonitrile in 10 mM TEAA) to give compound **15** (22 mg, 81%). Rt = 8.98 min. ^{1}H NMR (400 MHz, D_2O): δ 1.50 (1H, m, H-6′), 1.75 (1H, m, H-6"), 1.81 (3 H, d, $J_{6,5-CH3}$ = 1.1 Hz, 5-CH_3), 2.15 (1H, m, H-2′), 2.25 (2H, m, H-2" and H-3′), 3.58 (2H, m, H-7′ and H-7"), 3.70 (1H, dd, J = 8.6, J = 4.4, H-5′), 3.79 (1H, m, H-4′), 3.86 (1H, dd, J = 12.7 Hz, J = 2.5 Hz, H-5′′), 6.06 (1H, dd, J = 7.2 Hz, J = 2.1 Hz, H-1′), 7.71 (1H, d, $J_{6,5-CH3}$ = 1.1 Hz, H-6). ^{13}C NMR (100 MHz, D_2O) δ: 11.92 (CH_3), 33.83 (C6′), 34.46 (C3′), 37.90 (C2′), 60.51 (C7′), 61.34 (C5′), 86.96 (C1′), 85.61 (C4′), 111.19 (C5), 138.17 (C6), 152.09 (C2), 167.00 (C4); HRMS (ESI-MS) for $C_{12}H_{19}N_2O_5$ $[M + H]^+$: found, 271.1288; calcd, 271.1293. Anal. ($C_{12}H_{18}N_2O_5$) C, H, N.

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Supporting Information Available: HPLC retention times of all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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