

Discovery of Bicyclic Thymidine Analogues as Selective and High-Affinity Inhibitors of *Mycobacterium tuberculosis* Thymidine Monophosphate Kinase

Veerle Vanheusden,[†] Hélène Munier-Lehmann,^{*,‡} Matheus Froeyen,[§] Roger Busson,[§] Jef Rozenski,[§] Piet Herdewijn,[§] and Serge Van Calenbergh^{*,†}

Laboratory for Medicinal Chemistry (FFW), Ghent University, Harelbekestraat 72, 9000 Gent, Belgium, Unité de Chimie Organique, Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France, and Laboratory for Medicinal Chemistry, Rega Institute, Catholic University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received May 24, 2004

Thymidine monophosphate kinase of *Mycobacterium tuberculosis* (TMPKmt) represents an attractive target for selectively blocking bacterial DNA synthesis. Hereby, we report on the discovery of a novel class of bicyclic nucleosides (**10** and **11**) and one dinucleoside (**12**), belonging to the most selective inhibitors of TMPKmt discovered so far.

Introduction

The appearance of multiple drug-resistant strains of *Mycobacterium tuberculosis* and the synergism between human immunodeficiency virus (HIV) and *M. tuberculosis* infection has caused a steeply rising incidence of tuberculosis during the past decades. This has made the development of new antituberculosis agents, preferably acting on novel targets, a research priority for many health organizations.¹

M. tuberculosis thymidine monophosphate kinase (TMPKmt) recently emerged as a potentially attractive target for the design of a novel class of antituberculosis agents.²

TMPK catalyzes the γ -phosphate transfer from ATP to thymidine monophosphate (dTMP) in the presence of Mg^{2+} , yielding thymidine diphosphate (dTDP) and ADP.³ Because TMPK is essential for thymidine triphosphate (dTTP) synthesis and in view of its low (22%) sequence identity with the human isozyme (TMPKh),² it represents an attractive target for selectively inhibiting mycobacterial DNA synthesis. Recently, the X-ray structure of TMPKmt was solved at 1.95 Å as a complex with dTMP,⁴ allowing structure-based design of TMPKmt ligands.

A series of 2', 3', and 5'-modified nucleosides and nucleotides was tested for their affinities with respect to TMPKmt.^{5–8} The results showed that, in general, nucleosides and their corresponding 5'-O-monophosphate esters were nearly equivalent inhibitors of TMPKmt. The low permeability of the cell wall for phosphorylated compounds (i.e., nucleotides) prompted us to focus on nucleosides as new drug leads. Recently, we reported a series of 3'-C-branched chain nucleosides and nucleotides that exhibit micromolar inhibitory activity against TMPKmt.⁹ The introduction of 3'-CH₂OH, 3'-CH₂NH₂, 3'-CH₂N₃, and 3'-CH₂F substituents was aimed at occupying a cavity in the enzyme near the 3'-

position. Biological results and modeling confirmed this hypothesis. Modeling also indicated that these nucleosides bind to TMPKmt in the N-type (northern, 2'-exo-3'-endo) conformation [dTMP, in contrast, binds to TMPKmt in the S-type (southern, 2'-endo, 3'-exo) conformation]. This N-type sugar pucker enables interactions between the 6'-substituent and Asp9.⁹ From this series of 3'-branched chain derivatives, 1-[3-C-(azidomethyl)-2,3-dideoxy- β -D-threo-pentofuranosyl]thymine (**1**) emerged as a promising lead for further research, since it combines a low K_i value with a favorable selectivity profile for TMPKmt versus TMPKh (Table 1). Moreover, conformational analysis of a series of 2'- and 3'-modified nucleosides showed that the presence of a halogen at the α -face of the 2'-position, especially a 2'-fluorine, biases the sugar pucker of thymidine analogues strongly toward the N-type conformation.¹⁰ On the basis of these results and the high affinities of 2'-halogen-substituted nucleotides for TMPKmt⁵ (**3** and **4**, Table 1), it was considered interesting to combine a 2'-chlorine or a 2'-fluorine substituent with a 3'-aminomethyl or a 3'-azidomethyl group (**5–8**).⁹ In an attempt to supersede the good affinities of the 3'-C-branched-chain nucleosides, we also further explored the enzyme cavity near the 3'-position with alternative nitrogen-containing substituents.

Results and Discussion

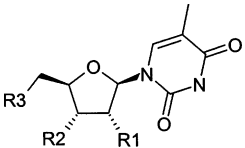
Chemistry. The synthesis of **5–9** started from **14** (obtained from 1,2-O-isopropylidene- α -D-xylofuranose in 11 steps¹¹) (Scheme 1). After protection of the 5'-hydroxyl group, **15** was converted into anhydronucleoside **16** upon treatment with trifluoromethanesulfonyl chloride and DMAP.¹² Opening of the anhydro ring with HCl in dioxane yielded **5**, the 2'- α -chloro analogue of **1**.¹³ The corresponding 2'-fluoro derivative **7** was obtained via formation of *ara*-nucleoside **17** from **16** with NaOH,¹⁴ followed by fluorination with DAST¹⁵ and removal of the trityl protective group. Reduction of **5** and **7** with Ph₃P and NH₃ yielded the corresponding 3'-amino analogues **6** and **8**, respectively.¹¹ Due to unwanted intramolecular attack of the 2-carbonyl of

* Address communication to either author: (S.V.C.) tel +32 (0)9 264 81 24; fax +32 (0)9 264 81 92; e-mail serge.vancalenbergh@ugent.be; (H.M.-L.) tel +33 (0)1 45 68 83 81; fax +33 (0)1 45 68 84 04; e-mail hmunier@pasteur.fr

[†] Ghent University.

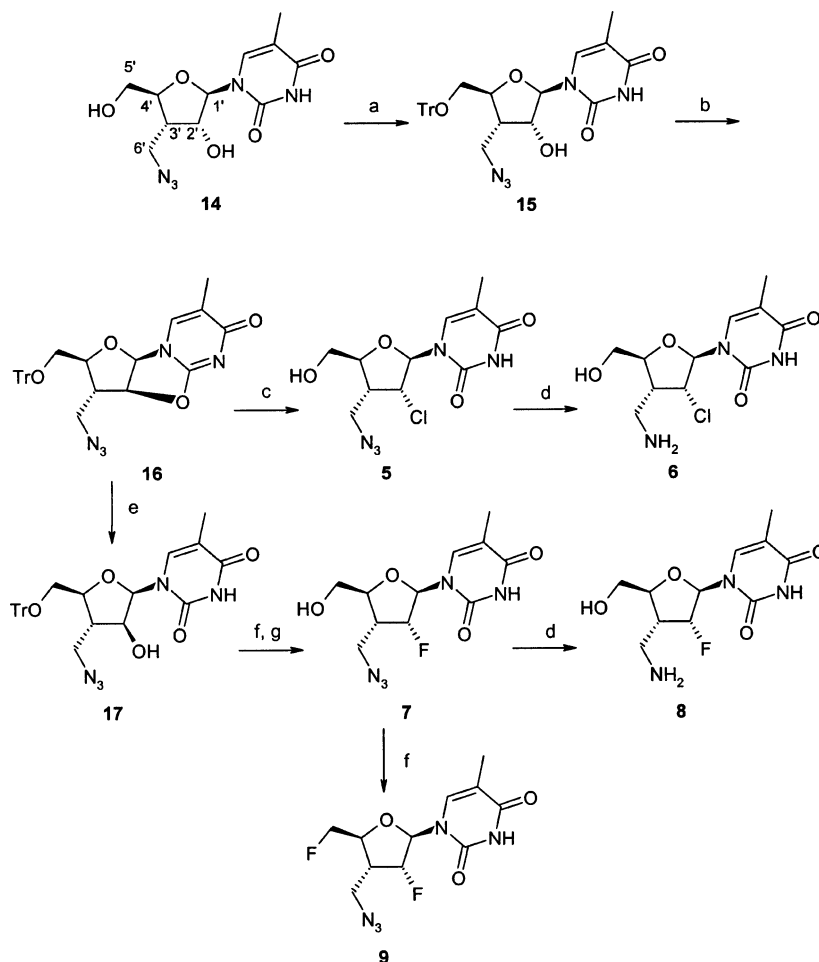
[‡] Institut Pasteur.

[§] Catholic University of Leuven.

Table 1. Kinetic Parameters of TMPKmt and TMPKh with Compounds 1–13


compound	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 ⁷	180 ⁸	6.6
1	H	CH ₂ N ₃	OH	40 ⁹	1040 ⁹	26.0
2	H	CH ₂ NH ₂	OH	57 ⁹	220 ⁹	3.9
3	F	OH	OPO ₃ ²⁻	43 ⁵	nd ^b	nd
4	Cl	OH	OPO ₃ ²⁻	19 ⁵	nd	nd
5	Cl	CH ₂ N ₃	OH	180	nd	nd
6	Cl	CH ₂ NH ₂	OH	390	nd	nd
7	F	CH ₂ N ₃	OH	165	nd	nd
8	F	CH ₂ NH ₂	OH	ni at 1 mM	nd	nd
9	F	CH ₂ N ₃	F	80	nd	nd
10	2'-OC(S)NHCH ₂ -3'		OH	3.5	700	200.0
11	2'-OC(O)NHCH ₂ -3'		OH	13.5	1100	81.5
12	Scheme 2		OH	37	ni at 1mM	nd
13^c	H	CH ₂ N ₃	OH	29	ni at 2 mM	nd

^a K_m value. ^b nd, not determined; ni, no inhibition. ^c Thymine is oriented at the α -side of the sugar ring.

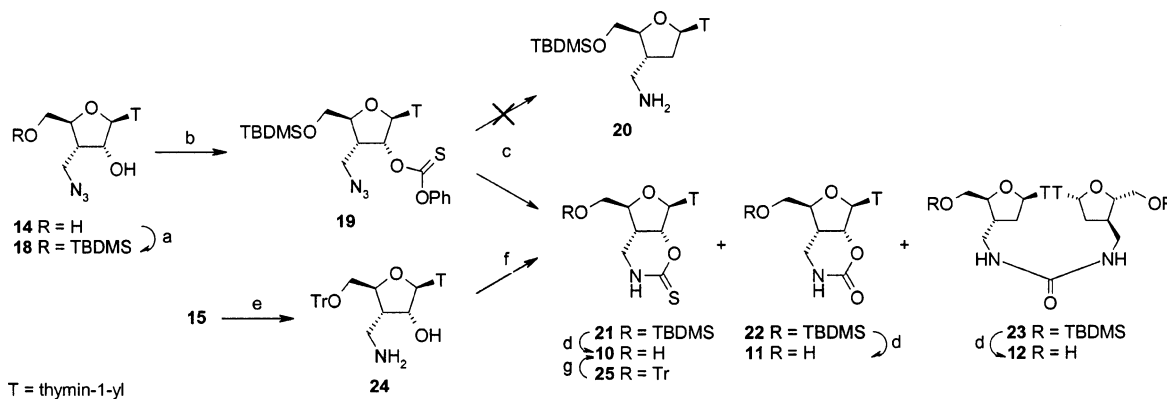
Scheme 1^a

^a Reagents: (a) trityl chloride, DMAP, pyridine; (b) trifluoromethanesulfonyl chloride, DMAP, CH₂Cl₂; (c) HCl, dioxane; (d) Ph₃P, NH₄OH, pyridine; (e) NaOH, EtOH, H₂O; (f) DAST, toluene, pyridine; (g) 80% HOAc in H₂O.

thymine on the 5'-*O*-diethylaminosulfur difluoride intermediate, reaction of **7** with DAST afforded **9** in low yield.

In an attempt to prepare intermediate **20** for further derivatization, the 5'-hydroxyl group of **14** was first

protected as *tert*-butyldimethylsilyl ether **18** (Scheme 2). After esterification of the 2'-hydroxyl with phenylchlorothionocarbonate (\rightarrow **19**),¹¹ however, attempted simultaneous reduction of the azido function and Barton deoxygenation at the 2'-position to give **20** failed.

Scheme 2^a

^a Reagents: (a) *tert*-butyldimethylsilyl chloride, AgNO₃, pyridine, THF; (b) phenyl chlorothionocarbonate, DMAP, CH₃CN; (c) AIBN, Bu₃SnH, toluene; (d) *n*-Bu₄NF, THF; (e) Ph₃P, NH₄OH, pyridine; (f) 1,1-thiocarbonyldiimidazole, DMF; (g) 80% HOAc in H₂O.

Instead, three peculiar nucleoside analogues **21**, **22**, and **23** were formed. In a similar attempt, Robins et al.¹⁶ found that, apart from a low yield of the desired aminonucleoside, Barton deoxygenation of an azide in the presence of a phenoxythiocarbonyl ester led to some uncharacterized byproducts. Compounds **21** and **22** represent a novel class of bicyclic nucleoside analogues.

The formation of carbamates through reaction of thiocarbonyl esters with azides has recently been studied by Shang-guan et al.¹⁷ A mechanism is proposed in which the three nitrogens of the azide take part in formation of the carbamate. However, since access to the thiocarbamate cannot be explained this way, it is suggested that a radical intermediate of the reduction of the azide interacts with a radical resulting from attack of a tributyltin radical on the thioester.

Another surprising byproduct of this radical-mediated reduction is dinucleoside **23**. Most probably, the ureido moiety of this dimer is formed through attack of the 6'-amines (resulting from reduction of the 6'-azide of **19**) on the carboxysulfide, arising from decomposition of the 2'-phenylthiocarbonate ester.¹⁸

The *tert*-butyldimethylsilyl (TBDMS) protective groups of **21**, **22**, and **23** were removed with TBAF in tetrahydrofuran (THF) and the free nucleosides (**10**, **11**, and **12**) were tested for their affinity for TMPKmt.

In view of the interesting biological properties of **10** (see below), a more efficient way for its synthesis was desirable. Tritylation of the 5'-hydroxyl of **14**, followed by reduction of the 6'-azido group (\rightarrow **24**), treatment with thiocarbonyldiimidazole, and subsequent acidic removal of the 5'-trityl group afforded **10** in 62% overall yield. Likewise, **11** can be obtained by use of carbonyldiimidazole.

Conformational Analysis. Conformational analysis was performed for compounds **6** and **7**. On the basis of the large vicinal "pseudoaxial-axial" coupling between 3'-H and 4'-H ($^3J_{3',4'} = 10.5$ Hz) and the very small coupling between 2'-H and 1'-H ($^3J_{1',2'} \approx 0.0$ Hz), we can conclude that in solution both compounds exist almost completely in the N-conformation (3'-endo-2'-exo-twist conformation). Indeed, it is accepted that the percentage of N- or S-conformer can be estimated by multiplying $^3J_{3',4'}$ and $^3J_{1',2'}$ by a factor of 10, respectively.¹⁹ The finding that the 3'- α -CH₂X group apparently drives the pseudorotational N \rightleftharpoons S equilibrium toward the N-conformation, even with a chlorine or fluorine substitu-

ent at the 2'-position, is completely in agreement with the observation of Plavec et al.²⁰ They also found a predominant N-conformation for their branched-chain 3'-CH₂OH-2',3'-dideoxy-D-*erythro*-cytidine derivatives.

Biological Activity. All nucleosides were tested for their affinity for TMPKmt (Table 1) via a reported spectrometric assay.²¹ The affinities of most 2'-halogen-substituted nucleosides were not as expected. In **5**–**8**, the introduction of the 2'-halogen led to a drastic decrease in affinity compared to the corresponding 2'-deoxynucleosides. Due to the presence of the 2'-fluorine, **8** showed no inhibition of the enzyme at 1 mM. Docking suggested that instead of enhancing the N-conformation, necessary for optimal interaction between Asp9 and the 3'-substituent, the 2'-halogens compete with the 3'-substituents for the same binding pocket,⁵ thereby abolishing the affinity for the enzyme. Remarkably, introduction of a 5'-fluorine slightly increases the affinity of **7** (K_i of **9** = 80 μ M).

Compounds **10**, **11**, and **12** were likewise assayed for their affinity for TMPKmt. Unexpectedly, these compounds are among the highest affinity inhibitors for TMPKmt found so far. Due to the lack of a binding site for a second nucleoside at the 3'-position, the K_i value of **12** (37 μ M) was most unexpected. A modeling experiment in GOLD showed that the sugar ring of the first monomer **I** binds in the dTMP pocket upside down, which enables hydrogen-bonding of its 5'-hydroxyl with Asp9 (Figure 1). This binding mode permits the sugar ring of the second monomer (**II**) to be directed toward the outside of the enzyme, where normally the phosphoryl donor binds. This orientation is the only feasible conformer to have **12** accommodated by the enzyme. The thymine base of **II** undergoes only a few hydrophobic interactions with Phe36, Ala35, and Tyr39. Comparison with the binding positions of ATP⁴ and bisubstrate inhibitor Ap₅T²² showed that nucleoside **II** does not bind to the pocket where adenosine normally resides. Instead, it is buried deeper in the enzyme. Competition experiments, performed with dTMP and ATP, showed that this dinucleoside is a competitive inhibitor not only for dTMP but also for ATP (at 0.2 mM dTMP, **12** is a competitive inhibitor for ATP with a K_i value of 0.65 mM). These results are in agreement with the binding mode proposed in Figure 1. The fact that these two nucleosides in **12** are bound via their 3'-positions makes it a unique example of a bisubstrate inhibitor, thereby

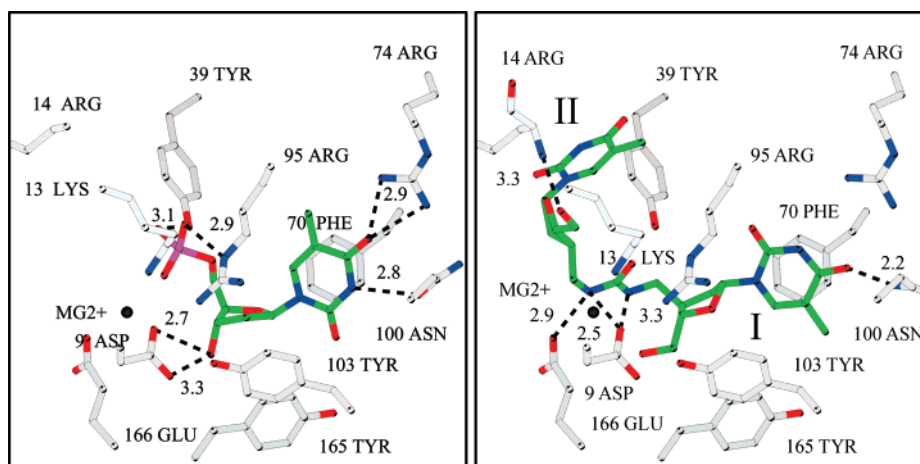


Figure 1. Comparison of the binding mode of dTMP with the binding mode of compound **12**. (a, left panel) dTMP as observed in the X-ray structure with TMPKmt.⁴ (b) Predicted binding mode of **12** with TMPKmt. Hydrogen bonds (based on distance) are drawn as dashed lines from **12** to residues Asp9, Arg14, Asn100, and Glu166. Residues whose atoms make hydrophobic contact with **12**, on the basis of a Ligplot analysis, are Lys13, Ala35, Tyr39, Phe36, Phe70, Arg95, Asn100, Tyr103, and Tyr165.

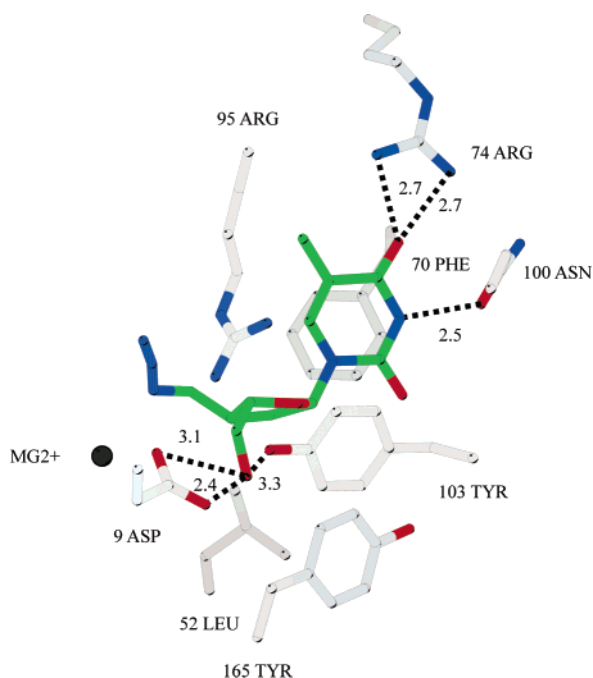


Figure 2. Predicted binding mode of **13** with TMPKmt. Possible hydrogen bonds are drawn as dashed lines with residues Asp9, Arg74, and Asn100. Residues whose atoms make hydrophobic contact with **13**, on the basis of a Ligplot analysis, are Leu52, Phe70, Arg95, Tyr103, and Tyr165.

paving the way for the design of much larger molecules as inhibitors for TMPKmt than explored thus far.

The observed affinity of α -nucleoside **13** (an undesired anomer formed during the synthesis of **1**⁹) could further confirm the postulated binding mode of monomer I. A similar sugar orientation would position the thymine base ideally for stacking with Phe70. Indeed, with a K_i value of 29 μM , this compound proves to be a good inhibitor of TMPKmt. Docking in GOLD showed a similar binding mode of the sugar ring, rendering stacking interactions with Phe70 possible (Figure 2). The 5'-hydroxyl interacts through hydrogen-bonding with Asp9, and the azido group, lying in the cavity where normally the 5'-phosphoryl of dTMP resides, is engaged in a polar interaction with Arg95 and Asp9. This exceptional flexibility of TMPKmt toward the

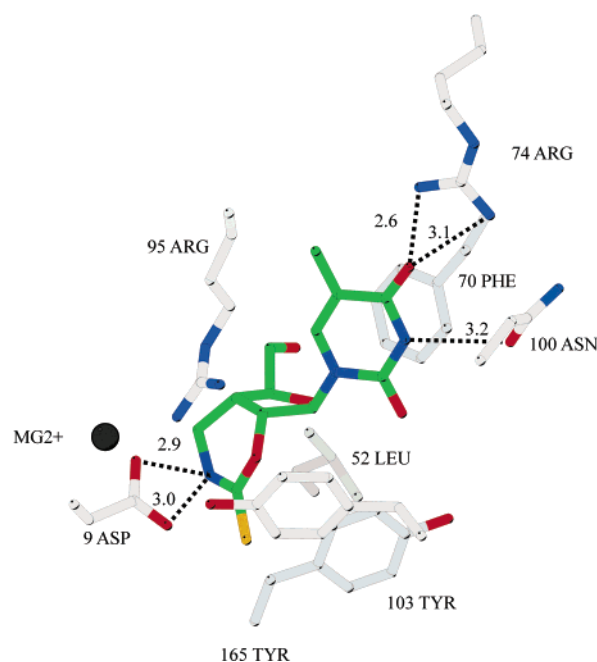


Figure 3. Predicted binding mode of **10** with TMPKmt. Hydrogen bonds are drawn as dashed lines involving residues Asp9, Asn100, and Arg74. Residues whose atoms make hydrophobic contact with **10**, on the basis of a Ligplot analysis, are Phe36, Pro37, Leu52, Phe70, Arg95, Tyr103, and Tyr165.

orientation of the sugar ring is of great interest for further inhibitor design.

Bicyclic nucleosides **10** and **11** showed excellent binding affinities. The K_i value of 3.5 μM of **10** is lower than the K_m value of the natural substrate (4.5 μM). Compound **10** was modeled into the crystal structure of TMPKmt (Figure 3).⁴ The six-membered ring, fused to the C-2', C-3' bond of the sugar, apparently forces the 6'-nitrogen into the most appropriate position for interaction with the Asp9 residue in the enzyme cavity. The sulfur atom, in its turn, undergoes hydrophobic interactions with Tyr103 and Tyr165 residues. When the sulfur atom in **10** is replaced by a smaller oxygen in **11**, the cavity near the 3'-position is filled less efficiently, which is reflected in the somewhat lower affinity of **11**. The functionalities of the extra ring

convincingly contribute to the high-affinity interactions with the biological target.

The highest affinity inhibitors of this series were tested for their affinity for TMPKh. The low affinities of **12** and **13** for the human enzyme indicate that the flexibility toward the orientation of the sugar ring is unique for TMPKmt. Most interesting, however, is compound **10**, with a selectivity index (K_i TMPKh/ K_i TMPKmt) of 200, superseding **1** not only in affinity but also in selectivity.

Compounds **1** and **2**, which were described earlier,⁹ did not show any significant inhibition of *M. tuberculosis* at a concentration of 6.25 μ g/mL. This is not surprising since this concentration is lower than the K_i values of these inhibitors for TMPKmt. Therefore, inhibitors with superior K_i values are indispensable to provide novel antimycobacterial leads. The discovery of the bicyclic analogues **10** and **11** is an important step toward this goal.

Conclusions

In inhibitor design, the binding pocket of the nucleoside in nucleoside- and nucleotide-metabolizing enzymes is generally explored by introducing small modifications to the substrate. In the present example, however, a variety of sugar-modified thymidine analogues have been discovered with apparently different binding modes to TMPKmt involving Asp9, Tyr103, and Phe70 as common amino acids to anchor the inhibitors. These molecules may function as leads for further drug design, increasing considerably the variety of nucleoside analogues that may be considered for further synthesis. Of particular importance is the bicyclic nucleoside **10** with a K_i for TMPKmt of 3.5 μ M and an SI of 200, which will be used as a starting compound to increase further affinity for TMPKmt.

Experimental Section

(1) Spectrophotometric Binding Assay. TMPKmt and TMPKh activities were determined by the coupled spectrophotometric assay described by Blondin et al.²¹ 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 MgCl₂, 0.2 mM NADH, 1 mM phosphoenol pyruvate, and 2 units each lactate dehydrogenase, pyruvate kinase, and nucleoside diphosphate kinase. The concentrations of ATP and dTMP were kept constant at 0.5 mM and 0.05 mM, respectively, whereas the concentrations of analogues varied between 0.1 and 2.5 mM.

(2) Inhibition Assay of *M. tuberculosis*. The screening was conducted at 6.25 μ g/mL against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium by a broth microdilution assay, the microplate assay (MABA).²³

(3) Modeling: Docking Experiments with GOLD. The X-ray structure published by de la Sierra et al.⁴ (PDB entry 1G3U) was used in all docking experiments. Water molecules and sulfate counterions were removed. The Mg²⁺ was considered as being part of the enzyme. Explicit hydrogen atoms were added to the enzyme and inhibitor structures by use of Reduce.²⁴ The inhibitor structures **10**, **12**, and **13** were created with MacroModel 5.0,²⁵ based on the dTMP substrate from PDB entry 1G3U. Their geometry was optimized in the AM1 force field with Mopac6.0.²⁶ PDB files were then converted to mol2 files by use of Babel.²⁷ 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.^{28,29} The structures in the top 50 of the docking scores were retained for visual inspection and comparison with

published X-ray structures 1G3U⁴ and 1MRN.²² Criteria for selection were the same position (but not necessarily the same orientation) of the base and the nonbonded interactions as calculated by Ligplot³⁰ and HBPLUS.³¹ Figures 1–3 were generated by use of Molscript.³²

(4) Synthesis. General. NMR spectra were obtained with a Varian Mercury 300 or 500 spectrometer. Chemical shifts are given in parts per million (ppm, δ) relative to the residual solvent peak of DMSO-*d*₆ (2.5 ppm). All signals assigned to amino and hydroxyl groups were exchangeable with D₂O. 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 μ L/min. Precoated Merck silica gel F₂₅₄ plates were used for TLC, and spots were examined under UV light at 254 nm and revealed by sulfuric acid–anisaldehyde spray. 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.

1-[3-Azidomethyl-3-deoxy-5-O-trityl- β -D-ribofuranosyl]thymine (15). To a solution of **14**¹¹ (700 mg, 2.35 mmol) in pyridine (6 mL) containing DMAP (345 mg, 2.82 mmol) was added trityl chloride (786 mg, 2.82 mmol) in different portions. The mixture was heated to 65 °C and stirred overnight. Then it was diluted with CH₂Cl₂ (50 mL), washed with saturated aqueous NaHCO₃ (50 mL), and dried over anhydrous MgSO₄. The solvent was removed under diminished pressure and the resulting residue was purified by column chromatography (CH₂Cl₂/MeOH 98:2), affording **15** (1.15 g, 92%) as a white foam. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.42 (3H, d, J = 1.2 Hz, 5-CH₃), 2.50 (1H, m, H-3'), 3.13–3.22 (2H, m, H-5' and H-6'), 3.37 (1H, dd, J = 1.8 and 10.8 Hz, H-5''), 3.60 (1H, dd, J = 6.9 and 12.6 Hz, H-6''), 4.03 (1H, ddd, J = 2.1, 3.9, and 9.0 Hz, H-4'), 4.32 (1H, dd, J = 2.1 and 6.0 Hz, H-2'), 5.68 (1H, d, J = 2.4 Hz, 2'-OH), 5.86 (1H, d, J = 5.4 Hz, H-1'), 7.35 (15H, m, arom H), 7.55 (1H, d, H-6); HRMS (ESI-MS) for C₃₀H₂₉N₅O₅Na [M + Na]⁺ found, 562.2067; calcd, 562.2066.

2,2'-Anhydro-1-(3-azidomethyl-3-deoxy-5-O-trityl- β -D-arabinofuranosyl)thymine (16). A solution of **15** (940 mg, 1.74 mmol) and DMAP (854 mg, 7.00 mmol) in CH₂Cl₂ (17 mL) was stirred at room temperature. After 30 min of stirring, trifluoromethanesulfonyl chloride (0.37 mL, 3.50 mmol) was added to the cooled (4 °C) solution. After further stirring for 3 h at the same temperature, the reaction was quenched with water (20 mL) and extracted with CH₂Cl₂ (20 mL). The organic layer was successively washed with an aqueous saturated solution of Na₂CO₃ (20 mL), dried over anhydrous MgSO₄, and evaporated under reduced pressure. The obtained residue was purified by column chromatography (CH₂Cl₂/MeOH 97:3) to yield **16** (743 mg, 82%) as a white foam. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.76 (3H, d, 5-CH₃), 2.66 (1H, m, H-3'), 2.82 (1H, dd, J = 6.6 and 10.2 Hz, H-5'), 3.01 (1H, dd, J = 3.9 and 10.5 Hz, H-5''), 5.57 (2H, m, H-6'/6''), 5.21 (1H, m, H-4'), 5.27 (1H, dd, J = 2.1 and 5.4 Hz, H-2'), 6.20 (1H, d, J = 6.0 Hz, H-1'), 7.22 (15H, m, arom H), 7.80 (1H, d, J = 1.5 Hz, H-6); HRMS (ESI-MS) for C₃₀H₂₈N₅O₄ [M + H]⁺ found, 522.2138; calcd, 522.2141.

1-(3-Azidomethyl-2-chloro-2,3-dideoxy- β -D-ribofuranosyl)thymine (5). An ice-cooled suspension of **16** (220 mg, 0.42 mmol) in dry dioxane (50 mL) was saturated with anhydrous hydrogen chloride. The mixture was heated in a sealed tube at 75–80 °C for 24 h. After cooling, the solution was concentrated and the obtained residue was purified by column chromatography (CH₂Cl₂/MeOH 97:3), yielding **5** (90 mg, 71%) as a white foam. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.74 (3H, s, 5-CH₃), 2.72 (1H, m, H-3'), 3.57 (3H, m, H-5' and

H-6''/6'''), 3.84 (1H, m, H-5''), 4.01 (1H, app d, $J_{3',4'} = 9.9$ Hz, H-4'), 4.81 (1H, app d, $J_{2',3'} = 5.4$ Hz, H-2'), 5.36 (1H, t, $J = 4.8$ Hz, 5'-OH), 5.89 (1H, d, $J = 1.5$ Hz, H-1'), 8.01 (1H, d, H-6), 11.30 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO- d_6) δ 12.96 (5-CH₃), C-3' hidden by DMSO signal, 48.73 (C-6'), 60.15 (C-5'), 65.32 (C-2'), 83.57 (C-4'), 91.36 (C-1'), 109.07 (C-5), 136.06 (C-6), 151.04 (C-2), 162.12 (C-4); HRMS (ESI-MS) for C₁₁H₁₄ClN₅O₄Na [M + Na]⁺ found, 338.0615; calcd, 338.0632. Anal. (C₁₁H₁₄ClN₅O₄) C, H, N.

1-[3-Aminomethyl-2-chloro-2,3-dideoxy- β -D-ribofuranosyl]thymine (6). Compound **5** (60 mg, 0.20 mmol) and triphenylphosphine (87 mg, 0.33 mmol) were dissolved in pyridine (3 mL) and stirred at room temperature. After 1 h, concentrated NH₄OH (2 mL) was added and the solution was allowed to stir for an additional 6.5 h. Pyridine was removed at reduced pressure, water (50 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, which was purified by column chromatography (CH₂Cl₂/MeOH/7 N NH₃ in MeOH, 90:7.5:2.5) to yield **6** (42 mg, 72%) as a white foam. ^1H NMR (500 MHz, DMSO- d_6) δ 1.75 (3H, d, $J = 1.2$ Hz, 5-CH₃), 2.80 (1H, app sextet, $J = 5.4$ Hz (5 \times), H-3'), 2.97 (2H, d, $J_{6',\text{AB},3'} = 6.6$ Hz, H-6'_{A+B}), 3.31 (br, 5'-OH, NH₂, N³-H), 3.73 (1H, dd, $J_{5',\text{B},4'} = 2.0$ Hz, $J_{5',\text{A},4'} = 13.2$ Hz, H-5'_B), 3.89 (1H, dd, $J_{5',\text{A},4'} = 2.4$ Hz, $J_{5',\text{A},5'\text{B}} = 13.2$ Hz, H-5'_A), 4.04 (1H, dt, $J_{4',3'} = 10.5$ Hz, $J_{4',5'} = 2.2$ Hz (2 \times), H-4'), 4.90 (1H, d, $J_{2',3'} = 4.6$ Hz, H-2'), 5.87 (1H, s, H-1'), 8.14 (1H, q, $J = 1.2$ Hz, H-6); ^{13}C NMR (75 MHz, DMSO- d_6) δ 12.93 (5-CH₃), 38.06 (C-3'), 42.90 (C-6'), 60.04 (C-5'), 66.39 (C-2'), 84.06 (C-4'), 91.53 (C-1'), 108.90 (C-5), 136.06 (C-6), 150.89 (C-2), 164.47 (C-4); HRMS (ESI-MS) for C₁₁H₁₇ClN₅O₄ [M + H]⁺ found, 290.0911; calcd, 290.0907. Anal. (C₁₁H₁₆ClN₅O₄·2H₂O) C, H, N; N calcd, 12.90; found, 11.81.

1-(3-Azidomethyl-3-deoxy-5-O-trityl- β -D-arabinofuranosyl)thymine (17). A mixture of **16** (590 mg, 1.13 mmol), 1 N NaOH (3.2 mL), dioxane (45 mL), and EtOH-H₂O (1:1, 45 mL) was stirred at room temperature for 2 h. The solution was neutralized with HOAc/EtOH (1:1 v/v) to pH 7. The resulting mixture was extracted with CH₂Cl₂ (100 mL) and the organic layer was dried over anhydrous MgSO₄, evaporated under reduced pressure, and purified by column chromatography (CH₂Cl₂/MeOH 90:10), yielding **17** (530 mg, 87%) as a white foam. ^1H NMR (300 MHz, DMSO- d_6) δ 1.53 (3H, s, 5-CH₃), 2.32 (1H, m, H-3'), 3.21–3.43 (3H, m, H-5'/5'', H-6'), 3.56 (1H, dd, $J = 5.7$ and 12.6 Hz, H-6''), 3.80 (1H, m, H-4'), 4.11 (1H, t, $J = 5.4$ Hz, H-2'), 5.60 (1H, d, $J = 5.1$ Hz, 2'-OH), 5.97 (1H, d, $J = 5.7$ Hz, H-1'), 7.32 (15H, m, arom H).

1-(3-Azidomethyl-2,3-dideoxy-2-fluoro- β -D-ribofuranosyl)thymine (7). To a solution of **17** (640 mg, 1.19 mmol) in toluene (12 mL) containing pyridine (1.2 mL) was added DAST (0.59 mL, 4.42 mmol), and the mixture was stirred for 2 h at room temperature and then for 3 h at 50 °C. The reaction was quenched with ice-water (50 mL) and extracted with CH₂Cl₂ (50 mL). The organic layer was dried over anhydrous MgSO₄, evaporated under reduced pressure, and purified by column chromatography (CH₂Cl₂/MeOH 97:3), yielding 1-[3-azidomethyl-2,3-dideoxy-2-fluoro-5-O-trityl- β -D-ribofuranosyl]thymine (460 mg, 72%) as a white foam. ^1H NMR (300 MHz, DMSO- d_6) δ 1.45 (3H, s, 5-CH₃), 2.85 (1H, m, $J_{3',\text{F}} = 33.0$ Hz, H-3'), 3.20 (4H, m, H-5'/5'' and H-6'/6''), 4.11 (1H, app d, $J_{4',\text{F}} = 9.6$ Hz, H-4'), 5.40 (1H, dd, $J_{2',3'} = 3.9$ Hz, $J_{2',\text{F}} = 52.2$ Hz, H-2'), 5.87 (1H, d, $J_{1',\text{F}} = 21.6$ Hz, H-1'), 7.32 (15H, m, arom H), 7.55 (1H, s, H-6); HRMS (ESI-MS) for C₃₀H₂₈FN₅O₄Na [M + Na]⁺ found, 564.2020; calcd, 564.2023.

The obtained foam was dissolved in 80% HOAc in H₂O (7 mL). The mixture was heated to 90 °C during 1 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (CH₂Cl₂/MeOH 90:10), yielding **7** (221 mg, 62% from **17**) as a white foam. ^1H NMR (500 MHz, DMSO- d_6) δ 1.75 (3H, d, $J = 1.2$ Hz, 5-CH₃), 2.64 (1H, dddd, $J_{3',\text{F}} = 33.1$ Hz, $J_{3',2'} = 4.4$ Hz, $J_{3',6'\text{B}} = 5.9$ Hz, $J_{3',6'\text{A}} = 8.7$ Hz, $J_{3',4'} = 10.5$ Hz, H-3'), 3.50 (1H, ddd, $J_{6'\text{B},\text{F}} = 1.4$ Hz, $J_{6'\text{B},3'} = 6.0$ Hz, $J_{6'\text{B},6'\text{A}} = 12.7$ Hz, H-6'_B), 3.57 (1H, dd,

$J_{6'\text{A},3'} = 8.7$ Hz, $J_{6'\text{A},6'\text{B}} = 12.7$ Hz, H-6'_A), 3.61 (1H, ddd, $J_{5',\text{B},4'} = 3.0$ Hz, $J_{5',\text{B},\text{OH}} = 4.9$ Hz, $J_{5',\text{B},5'\text{A}} = 12.7$ Hz, H-5'_B), 3.84 (1H, ddd, $J_{5',\text{A},4'} = 2.5$ Hz, $J_{5',\text{A},\text{OH}} = 5.6$ Hz, $J_{5',\text{A},5'\text{B}} = 12.7$ Hz, H-5'_A), 3.99 (1H, dt, $J_{4',3'} = 10.5$ Hz, $J_{4',5'\text{A}/\text{B}} = 2.7$ Hz (2 \times), H-4'), 5.30 (1H, t, $J_{\text{OH},5'\text{A}/\text{B}} = 5.25$ Hz, 5'-OH), 5.32 (1H, dd, $J_{2',3'} = 4.4$ Hz, $J_{2',\text{F}} = 52.5$ Hz, H-2'), 5.88 (1H, d, $J_{1',\text{F}} = 19.3$ Hz, H-1'), 7.88 (1H, q, $J = 1.2$ Hz, H-6), 11.34 (1H, s, N³-H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 12.29 (5-CH₃), 40.53 (d, $^2J_{3',\text{F}} = 18.5$ Hz, C-3'), 46.13 (d, $^3J_{6',\text{F}} = 7.8$ Hz, C-6'), 59.76 (C-5'), 82.83 (C-4'), 88.80 (d, $^2J_{1',\text{F}} = 36.3$ Hz, C-1'), 96.84 (d, $^1J_{2',\text{F}} = 181.6$ Hz, C-2'), 108.73 (C-5), 136.03 (C-6), 150.29 (C-2), 163.94 (C-4); ^{19}F NMR (300 MHz, D₂O) δ -195.046; HRMS (ESI-MS) for C₁₁H₁₄FN₅O₄Na [M + Na]⁺ found, 322.0908; calcd, 322.092. Anal. (C₁₁H₁₄FN₅O₄) C, H, N.

1-(3-Aminomethyl-2,3-dideoxy-2-fluoro- β -D-ribofuranosyl)thymine (8). This compound was synthesized from **7** (80 mg, 0.26 mmol) by the procedure described for the synthesis of **6**, yielding 54 mg (77%) of amine **8** as a foam. ^1H NMR (300 MHz, DMSO- d_6) δ 1.73 (3H, s, 5-CH₃), 2.30 (1H, m, $J_{3',\text{F}} = 34.2$ Hz, H-3'), 2.57 (1H, m, H-6'), 2.76 (1H, m, H-6''), 3.62 (1H, d, $J = 12.0$ Hz, H-5'), 3.79 (1H, d, H-5''), 3.89 (1H, app d, $J_{4',\text{F}} = 10.2$ Hz, H-4'), 5.03 (1H, br s, 5'-OH), 5.22 (1H, d, $J_{2',\text{F}} = 51.9$ Hz, H-2'), 5.82 (1H, d, $J_{1',\text{F}} = 18.3$ Hz, H-1'), 7.85 (1H, s, H-6); ^{13}C NMR (75 MHz, DMSO- d_6) δ 12.95 (5-CH₃), 36.95 ($J = 7.7$ Hz) and 44.76 ($J = 18$ Hz) (C-3' and C-6'), 60.63 (C-5'), 84.41 (C-4'), 89.07 ($J_{1',\text{F}} = 36.3$ Hz, C-1'), 97.89 ($J_{2',\text{F}} = 179.4$ Hz, C-2'), 109.21 (C-5), 136.43 (C-6), 150.79 (C-2), 164.51 (C-4); ^{19}F NMR (300 MHz, D₂O) δ -195.70; HRMS (ESI-MS) for C₁₁H₁₇FN₃O₄ [M + H]⁺ found, 274.1191; calcd, 274.1202. Anal. (C₁₁H₁₆FN₃O₄·1/2H₂O) C, H, N; N calcd, 14.89; found, 13.61.

1-(3-Azidomethyl-2,5-difluoro-2,3,5-trideoxy- β -D-ribofuranosyl)thymine (9). This compound was synthesized from **7** (66 mg, 0.22 mmol) by the procedure described for the synthesis of **7**, to yield 12 mg (18%) of **9**. ^1H NMR (300 MHz, DMSO- d_6) δ 1.75 (3H, s, 5-CH₃), 2.67 (1H, m, $J_{3',\text{F}} = 24.0$ Hz, H-3'), 3.60 (2H, m, H-6'/6''), 4.61 (1H, dd, $J_{5',4'} = 4.2$ Hz, $J_{5',5''} = 10.8$ Hz, $J_{5',\text{F}} = 49.5$ Hz, H-5'), 4.80 (1H, dd, H-5''), 5.41 (1H, dd, $J_{2',3'} = 4.8$ Hz, $J_{2',\text{F}} = 52.5$ Hz, H-2'), 5.88 (1H, d, $J_{1',\text{F}} = 21.3$ Hz, H-1'), 7.40 (1H, s, H-6); ^{13}C NMR (75 MHz, DMSO- d_6) δ 12.87 (5-CH₃), under DMSO signal (C-3'), 46.68 (C-6'), 81.10 ($J_{4',\text{F}} = 18.1$ Hz, C-4'), 83.02 ($J_{5',\text{F}} = 170.0$ Hz, C-5'), 90.60 ($J_{1',\text{F}} = 38.0$ Hz, C-1'), 96.90 ($J_{2',\text{F}} = 181.2$ Hz, C-2'), 110.01 (C-5), 136.81 (C-6), 150.87 (C-2), 164.52 (C-4); ^{19}F NMR (300 MHz, D₂O) δ -194.04 and -228.74; HRMS (ESI-MS) for C₁₁H₁₃F₂N₅O₃Na [M + Na]⁺ found, 324.0881; calcd, 324.0884. Anal. (C₁₁H₁₃F₂N₅O₃) C, H, N.

1-[3-Aminomethyl-3-deoxy-5-O-(tert-butyl)dimethylsilyl-2-O-,6-N-(thiocarbonyl)- β -D-ribofuranosyl]thymine (21), 1-(3-Aminomethyl-2-O-,6-N-carbonyl-3-deoxy-5-O-(tert-butyl)dimethylsilyl- β -D-ribofuranosyl)thymine (22), and 1,3-Bis[(3R)-[3'-deoxy-5'-O-(tert-butyl)dimethylsilyl]methylurea (23). To a solution of **19**¹¹ (460 mg, 0.84 mmol) in toluene was added 2,2'-azobis(2-methylpropionitrile) (354 mg, 2.1 mmol) and tri-*n*-butyltinhydride (0.48 g, 1.65 mmol) at 50–60 °C under N₂. The reaction mixture was stirred at 95–100 °C for 5 h. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography (CH₂Cl₂/MeOH 99:1 → 95:5), affording products **21** (0.16 mmol, 20%), **22** (17%), and **23** (19%) as white solids.

21: ^1H NMR (300 MHz, DMSO- d_6) δ 0.00 [6H, s, (CH₃)₂Si], 0.80 [9H, s, C(CH₃)₃], 1.70 (3H, s, 5-CH₃), 2.76 (1H, m, H-3'), 3.11 (1H, d, H-6'), 3.39 (1H, dd, $J_{6',3'} = 5.70$ Hz, $J_{6',6''} = 13.5$ Hz, H-6''), 3.86 (3H, m, H-4' and H-5'/5''), 4.95 (1H, d, $J_{2',3'} = 5.1$ Hz, H-2'), 5.75 (1H, s, H-1'), 7.45 (1H, s, H-6), 9.81 (1H, s, 6'-NH), 11.36 [1H, s, N(3)H]; HRMS (ESI-MS) for C₁₈H₃₀N₃O₅SSi [M + H]⁺ found, 428.1675; calcd, 428.1675.

22: ^1H NMR (300 MHz, DMSO- d_6) δ 0.00 [6H, s, (CH₃)₂Si], 0.81 [9H, s, C(CH₃)₃], 1.70 (3H, s, 5-CH₃), 2.61 (1H, m, H-3'), 3.07 (1H, br d, H-6'), 3.37 (1H, dd, $J_{6',3'} = 5.7$ Hz, $J_{6',6''} = 12.3$ Hz, H-6''), 3.78 (1H, dd, $J_{5',4'} = 3.6$ Hz, $J_{5',5''} = 12.0$ Hz, H-5'), 3.91 (1H, dd, H-5''), 3.98 (1H, dt, $J = 2.7$ and 9.6 Hz, H-4'), 4.90 (1H, br d, $J_{2',3'} = 5.7$ Hz, H-2'), 5.70 (1H, d, $J = 1.2$ Hz,

H-1'), 7.37 (1H, br s, 6'-NH), 7.44 (1H, d = 1.2 Hz, H-6); HRMS (ESI-MS) for C₁₈H₃₀N₃O₆Si [M + H]⁺ found, 412.1897; calcd, 412.1903.

23: ¹H NMR (300 MHz, DMSO-*d*₆) δ 0.15 [12H, s, (CH₃)₂-Si], 0.83 [18H, s, (CH₃)₃C], 1.73 (6H, s, 5-CH₃), 2.03 (4H, m, H-2'), 2.30 (2H, m, H-3'), 3.05 (4H, m, H-6'/6''), 3.71 (6H, m, H-4' and H-5'/5''), 5.94 (2H, t, *J* = 5.4 Hz, H-1'), 6.01 (2H, t, 6'-NH), 7.46 (2H, d, *J* = 1.2 Hz, H-6); HRMS (ESI-MS) for C₃₅H₆₀N₆O₉Si₂Na [M + Na]⁺ found, 787.3970; calcd, 787.3858.

1-[3-Aminomethyl-3-deoxy-2-O-,6-N-(thiocarbonyl)-β-D-ribofuranosyl]thymine (10). A solution of **21** (70 mg, 0.16 mmol) was treated with 5 equiv of TBAF in THF. After 1 h the mixture was evaporated to dryness and purified by column chromatography (CH₂Cl₂/MeOH 95:5), yielding **10** as a white powder (47 mg, 92%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.73 (3H, s, 5-CH₃), 2.80 (1H, m, H-3'), 3.45 (1H, d, H-6'), 3.42 (1H, dd, *J*_{6',3'} = 6.0 Hz, *J*_{6',6''} = 14.1 Hz, H-6''), 3.66 (1H, br d, H-5'), 3.85 (1H, br d, *J*_{5',5''} = 12.6 Hz, H-5''), 3.92 (1H, m, H-4'), 4.95 (1H, d, *J*_{2',3'} = 5.0 Hz, H-2'), 5.34 (1H, t, 5'-OH), 5.82 (1H, s, H-1'), 7.97 (1H, s, H-6), 9.83 (1H, br s, 6'-NH), 11.35 [1H, br s, N(3)H]; ¹³C NMR (75 MHz, DMSO-*d*₆) δ 12.89 (5-CH₃), 31.54 (C-3'), 37.18 (C-6'), 59.65 (C-5'), 82.57 (C-4'), 83.98 (C-2'), 89.28 (C-1'), 109.30 (C-5), 136.84 (C-6), 150.84 (C-2), 164.43 (C-4), 184.48 (C=S); HRMS (ESI-MS) for C₁₂H₁₅N₃O₅SNa [M + Na]⁺ found, 336.0636; calcd, 336.0630. Anal. (C₁₂H₁₅N₃O₅S) C, H, N.

1-(3-Aminomethyl-2-O-,6-N-carbonyl-3-deoxy-β-D-ribofuranosyl)thymine (11). Compound **11** was prepared from **22** (60 mg, 0.15 mmol) by the procedure described for the synthesis of **10**, yielding **11** (39 mg, 90%) as a white powder. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.74 (3H, s, 5-CH₃), 2.66 (1H, m, H-3'), 3.13 (1H, m, H-6'), 3.42 (1H, dd, *J*_{6',3'} = 5.4 Hz, *J*_{6',6''} = 12.9 Hz, H-6''), 3.64 (1H, m, H-5'), 3.82 (1H, m, H-5''), 4.02 (1H, dt, *J* = 2.7 and 10.2 Hz, H-4'), 4.90 (1H, d, *J*_{2',3'} = 5.4 Hz, H-2'), 5.31 (1H, t, *J* = 5.1 Hz, 5'-OH), 5.72 (1H, s, H-1'), 7.39 (1H, d, *J* = 3.0 Hz, H-6), 7.97 (1H, s, 6'-NH), 11.29 [1H, br s, N(3)H]; ¹³C NMR (75 MHz, DMSO-*d*₆) δ 12.88 (5-CH₃), 32.44 (C-3'), 36.73 (C-6'), 59.94 (C-5'), 82.50 (C-4'), 83.31 (C-2'), 89.83 (C-1'), 109.28 (C-5), 136.62 (C-6), 150.86 (C=O), 151.84 (C-2), 164.44 (C-4); HRMS (ESI-MS) for C₁₂H₁₅N₃O₆Na [M + Na]⁺ found, 320.0856; calcd, 320.0858. Anal. (C₁₂H₁₅N₃O₆) C, H, N.

1,3-Bis[(3R)-(3'-deoxythymidin-3'-yl)methyl]urea (12). Compound **12** was prepared from **23** (120 mg, 0.16 mmol) by the procedure described for the synthesis of **10**, yielding **12** (80 mg, 93%) as a white powder. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.75 (6H, s, 5-CH₃), 2.04 (4H, m, H-2'), 2.33 (2H, m, H-3'), 3.08 (4H, m, H-6'/6''), 3.52 (2H, dd, *J* = 4.5 and 12.6 Hz, H-5'), 3.66 (4H, m, H-4' and H-5''), 5.03 (2H, t, *J* = 5.1 Hz, 5'-OH), 5.94 (2H, t, *J* = 6.6 Hz, H-1'), 6.00 (2H, t, *J* = 6.0 Hz, 6'-NH), 7.84 (2H, s, H-6); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 12.96 (5-CH₃), 36.74 (C-2'), 38.97 and hidden by DMSO signal (C-3' and C-6'), 61.78 (C-5'), 84.58 (C-1' and C-4'), 109.30 (C-5), 137.05 (C-6), 150.97 (C-2), 158.82 (NHCONH), 164.47 (C-4); HRMS (ESI-MS) for C₂₃H₃₂N₆O₉Na [M + Na]⁺ found, 559.2121; calcd, 559.2128. Anal. (C₂₃H₃₂N₆O₉) C, H, N.

1-(3-Aminomethyl-3-deoxy-5-O-trityl-β-D-ribofuranosyl)thymine (24). Compound **15** (450 mg, 0.83 mmol) and triphenylphosphine (356 mg, 1.36 mmol) were dissolved in pyridine (10 mL) and stirred at room temperature. After 1 h concentrated NH₄OH (8.5 mL) was added, and the solution was allowed to stir for an additional 2 h. Pyridine was removed under reduced pressure, water (5 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 NH₃ in CH₂Cl₂/MeOH 90:10) to yield the title compound (415 mg, 97%) as a white foam. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.37 (3H, s, 5-CH₃), 2.25 (1H, m, H-3'), under DMSO signal (1H, H-5'), 2.67 (1H, dd, *J* = 7.8 and 12.6 Hz, H-5''), 3.18 (1H, dd, *J* = 4.5 and 10.5 Hz, H-6'), under H₂O signal (1H, H-6''), 4.04 (1H, d, *J* = 7.8 Hz, H-4'), 4.30 (1H, d, *J* = 5.4 Hz, H-2'), 5.64 (1H, s, H-1'), 7.33 (15H, m, arom H),

7.54 (1H, s, H-6); HRMS (ESI-MS) for C₃₀H₃₁N₃O₅Na [M + Na]⁺ found, 536.2162; calcd, 536.2161.

1-[3-Aminomethyl-3-deoxy-2-O-,6-N-(thiocarbonyl)-5-O-trityl-β-D-ribofuranosyl]thymine (25). A solution of amine **24** (200 mg, 0.39 mmol) and thiocarbonyldiimidazole (78 mg, 0.43 mg) in THF (6 mL) was stirred overnight. The solvent was removed under reduced pressure and the obtained residue was purified by column chromatography (CH₂Cl₂/MeOH 95:5) to yield **25** as a white powder (162 mg, 75%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.44 (3H, d, *J* = 1.2 Hz, 5-CH₃), 2.90 (1H, d, *J*_{6',6''} = 13.8 Hz, H-6'), 3.02 (1H, m, H-3'), H-5' hidden by residual H₂O signal, 3.38 (1H, dd, *J*_{5',4'} = 3.0 Hz, *J*_{5',5''} = 11.7 Hz, H-5''), 3.46 (1H, dd, *J*_{6',3'} = 5.4 Hz, *J*_{6',6''} = 13.8 Hz, H-6''), 3.99 (1H, dt, *J* = 4.2 and 10.5 Hz, H-4'), 5.07 (1H, d, *J*_{2',3'} = 5.7 Hz, H-2'), 5.80 (1H, d, *J* = 0.9 Hz, H-1'), 7.33 (15H, m, arom H), 7.53 (1H, s, H-6), 9.83 (1H, s, 6'-NH), 11.48 (1H, s, N(3)H); HRMS (ESI-MS) for C₃₁H₂₉N₃O₅SNa [M + Na]⁺ found, 578.1825; calcd, 578.1725.

1-[3-Aminomethyl-3-deoxy-2-O-,6-N-(thiocarbonyl)-β-D-ribofuranosyl]thymine (10) from 25. Compound **25** (148 mg, 0.26 mmol) was dissolved in 80% HOAc in H₂O (6 mL). The mixture was heated to 90 °C during 1 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (CH₂Cl₂/MeOH 90:10), yielding **10** (69 mg, 85%) as a white powder. For characterization data, see above.

1-[3-(Azidomethyl)-2,3-dideoxy-α-D-erythro-pentofuranosyl]thymine (13). α-Anomer **13** was a minor unreported byproduct from the synthesis of **1**. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.76 (3H, d, 5-CH₃), 1.80 (1H, m, H-2'), 2.25 (1H, ddd, *J* = 5.6, 7.0, and 12.3 Hz, H-2''), 2.73 (1H, m, H-3'), 3.50–3.67 (4H, m, H-5'/5'' and H-6'/6''), 4.02 (1H, dt, *J* = 3.9 and 8.1 Hz, H-4'), 5.06 (1H, t, 5'-OH), 5.99 (1H, dd, *J* = 5.7 and 8.7 Hz, H-1'), 7.83 (1H, d, H-6); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 12.95 (5-CH₃), 35.46 (C-2'), 39.43 (C-3'), 51.03 (C-6'), 61.68 (C-5'), 80.00 and 84.23 (C-1' and C-4'), 109.98 (C-5), 136.77 (C-6), 151.17 (C-2), 164.39 (C-4); HRMS (ESI-MS) for C₁₁H₁₆N₅O₄ [M + H]⁺ found, 282.1205; calcd, 282.1202. Anal. (C₁₁H₁₆N₅O₄) C, H, N.

Acknowledgment. V.V. is indebted to the "Fonds voor Wetenschappelijk Onderzoek—Vlaanderen" for a position as Aspirant. The FWO, EEC (BIO98 CT-0354), Pasteur Institute, the Centre National de la Recherche Scientifique (URA 2185 and 2128), the Ministère de la Recherche (ACI), and the Institut National de la Santé et de la Recherche Médicale are thanked for financial support.

References

- Duncan, K. Towards the next generation of drugs and vaccines for tuberculosis. *Chem. Ind.* **1997**, 861–865.
- Munier-Lehmann, H.; Chafotte, A.; Pochet, S.; Labesse, G. Thymidylate kinase of *Mycobacterium tuberculosis*: a chimera sharing properties common to eukaryotic and bacterial enzymes. *Protein Sci.* **2001**, *10*, 1195–1205.
- Anderson, E. P. In *The Enzymes*; Boyer, P. D., Ed.; Academic: New York, 1973; Vol. 9, pp 49–96.
- Li de la Sierra, I.; Munier-Lehmann, H.; Gilles, A. M.; Bärzu, O.; Delarue, M. X-ray structure of TMP kinase from *Mycobacterium tuberculosis* complexed with TMP at 1.95 Å resolution. *J. Mol. Biol.* **2001**, *311*, 87–100.
- Vanheusden, V.; Munier-Lehmann, H.; Pochet, S.; Herdewijn, P.; Van Calenbergh, S. Synthesis and evaluation of thymidine-5'-O-monophosphate analogues as inhibitors of *Mycobacterium tuberculosis* thymidylate kinase. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2695–2698.
- Vanheusden, V.; Van Rompaey, P.; Munier-Lehmann, H.; Pochet, S.; Herdewijn, P.; Van Calenbergh, S. Thymidine and thymidine-5'-O-monophosphate analogues as inhibitors of *Mycobacterium tuberculosis* thymidylate kinase. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3045–3048.
- Pochet, S.; Dugué, L.; Douguet, D.; Labesse, G.; Munier-Lehmann, H. Nucleoside analogues as inhibitors of thymidylate kinases: possible therapeutic applications. *ChemBioChem* **2002**, *3*, 108–110.

- (8) Pochet, S.; Dugué, L.; Labesse, G.; Delepiere, M.; Munier-Lehmann, H. Comparative study of purine and pyrimidine nucleoside analogues acting on the thymidylate kinases of *Mycobacterium tuberculosis* and of humans. *ChemBioChem* **2003**, *4*, 742–747.
- (9) Vanheusden, V.; Munier-Lehmann, H.; Froeyen, M.; Dugué, L.; Heyerick, A.; De Keukeleire, D.; Pochet, S.; Busson, R.; Herdewijn, P.; Van Calenbergh, S. 3'-C-Branched-chain-substituted nucleosides and nucleotides as inhibitors of *Mycobacterium tuberculosis* thymidine monophosphate kinase. *J. Med. Chem.* **2003**, *46*, 3811–3821.
- (10) Van Rompaey, P.; Nauwelaerts, K.; Vanheusden, V.; Rozenski, J.; Munier-Lehmann, H.; Herdewijn, P.; Van Calenbergh, S. *Mycobacterium tuberculosis* thymidine monophosphate kinase inhibitors: biological evaluation and conformational analysis of 2'- and 3'-modified thymidine analogues. *Eur. J. Org. Chem.* **2003**, 2911–2918.
- (11) Lin, T. S.; Zhu, J.-L.; Dutschman, G. E.; Cheng, Y.-C.; Prusoff, W. H. Syntheses and biological evaluations of 3'-deoxy-3'-C-branched-chain-substituted nucleosides. *J. Med. Chem.* **1993**, *36*, 353–362.
- (12) Aoyama, Y.; Sekine, T.; Iwamoto, Y.; Kawashima, E.; Ishido, Y. Efficient synthesis of 2'-bromo-2'-deoxy-3',5'-O-TPDS-pyrimidine nucleosides by boron trifluoride catalyzed reaction of O², 2'-anhydro-(1-β-D-arabinofuranosyl)pyrimidine nucleosides with lithium bromide. *Nucleosides Nucleotides* **1996**, *15*, 733–738.
- (13) Codington J. F.; Doerr I. L.; Fox J. J. Nucleosides VIII. Synthesis of 2'-fluorothymidine, 2'-fluorodeoxyuridine and other 2'-halogeno-2'-deoxy nucleosides. *J. Org. Chem.* **1964**, *29*, 558–564.
- (14) Lin, T.-S.; Yang, J.-H.; Liu, M.-C.; Shen, Z.-Y.; Cheng, Y.-C.; Prusoff, W. Birnbaum, G. I.; Giziewicz, J.; Ghazzouli, I.; Brankovan, V.; Feng, J. S.; Hsiung, G.-D. Synthesis and anticancer activity of various pyrimidine nucleoside analogues and crystal structure of 1-(3-deoxy-β-D-threo-pentofuranosyl)cytosine. *J. Med. Chem.* **1991**, *34*, 693–701.
- (15) Huang, J.-T.; Chen, L.-C.; Wang, L.; Kim, M.-H.; Warshaw, J. A.; Armstrong, D.; Zhu, Q.-Y.; Chou, T.-C.; Watanabe, K. A.; Matulic-Adamic, J.; Su, T.-L.; Fox, J. J.; Polsky, B.; Baron, P. A.; Gold, J. W. M.; Hardy, W. D.; Zuckerman, E. Fluorinated sugar analogues of potential anti-HIV-1 nucleosides. *J. Med. Chem.* **1991**, *34*, 1640–1646.
- (16) Robins, M. J.; Wnuk, S. F.; Hernández-Thirring, A. E.; Samano, M. C. Nucleic Acid Related Compounds. 91 Biomimetic reactions are in harmony with loss of 2'-substituents as free radicals (not anions) during mechanism-based inactivation of ribonucleotide reductases. Differential interactions of azide, halogen, and alkylthio groups with tributylstannane and triphenylsilane. *J. Am. Chem. Soc.* **1996**, *118*, 11341–11348.
- (17) Shangguan, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. The reaction of thio acids with azides: a new mechanism and new synthetic applications. *J. Am. Chem. Soc.* **2003**, *125*, 7754–7755.
- (18) Barton, D. H.; McCombie, S. A new method for the deoxygenation of secondary alcohols. *J. Chem. Soc., Perkin Trans I* **1975**, *16*, 1574–1585.
- (19) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; p 48.
- (20) Plavec, J.; Garg, N.; Chattopadhyaya, J. How does the steric effect drive the sugar conformation in the 3'-C-branched nucleosides. *J. Chem. Soc., Chem. Commun.* **1993**, *12*, 1011–1014.
- (21) Blondin, C.; Serina, L.; Wiesmüller, L.; Gilles, A. M.; Bärzu, O. Improved spectrophotometric assay of nucleoside monophosphate kinase activity using the pyruvate kinase/lactate dehydrogenase coupling system. *Anal. Biochem.* **1994**, *220*, 219–222.
- (22) Haouz, A.; Vanheusden, V.; Munier-Lehmann, H.; Froeyen, M.; Herdewijn, P.; Van Calenbergh, S.; Delarue, M. Enzymatic and structural analysis of inhibitors designed against *Mycobacterium tuberculosis* thymidylate kinase. New insights into the phosphoryl transfer mechanism. *J. Biol. Chem.* **2003**, *278*, 4963–4971.
- (23) Collins, L.; Franzblau, S. G. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.
- (24) Word, J. M.; Lovell, S. C.; Richardson, J. S.; Richardson, D. C. Asparagine and glutamine: using hydrogen atom contacts in the choice of side chain amide orientation. *J. Mol. Biol.* **1999**, *285*, 1733–1747.
- (25) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel- An integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.
- (26) MOPAC6.0 QCPE #455 by J. J. P. Stewart.
- (27) (a) Shah, A.; Walters, P.; Stahl, M. Babel: chemical format conversion program. (b) Walters, W. P.; Stahl, M. T.; Shah, A. V.; Dolata, D. P. *Abstr. Pap.-Am. Chem. Soc.* **1994**, *220*, 219–222.
- (28) Jones, G.; Willett, P.; Glen, R. C. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *J. Mol. Biol.* **1995**, *245*, 43–53.
- (29) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727–748.
- (30) Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.* **1995**, *8*, 127–134.
- (31) McDonald, I. K.; Thornton, J. M. Satisfying hydrogen bonding potential in proteins. *J. Mol. Biol.* **1994**, *238*, 777–793.
- (32) Kraulis, P. J. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **1991**, *24*, 946–950.

JM040847W