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Synthesis and Biological Evaluation of Bicyclic Nucleosides as Inhibitors of *M. tuberculosis* Thymidylate Kinase

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Herein we describe the synthesis and conformational analysis of a series of bicyclic thymidine derivatives and their evaluation as inhibitors of thymidine monophosphate kinase from Mycobacterium tuberculosis *(TMPKmt), based on previously discovered bicyclic sugar nucleosides. With a* K_i *value of 2.3* μM*, 1-[3-aminomethyl-3,5-dideoxy-2-*O*,6-*N*-(thiocarbonyl)-*β*-*D*-ribofuranosyl]thymine emerged as the most potent TMPK inhibitor of this series. Moreover, this promising compound displays inhibitory potency against* Mycobacteria *cultures with an* IC_{99} *value of 100* $\mu g\,mL^{-1}$*, thus promoting TMPKmt for the first time as a validated target for further inhibitory design. Attempts to rationalise the observed structure–activity relationship (SAR) involving molecular modelling and conformational analysis are described.*

Introduction

After AIDS, tuberculosis (TB) is the leading cause of death among infectious diseases in the world. *Mycobacterium tuberculosis* kills two million people worldwide each year. One third of the population is infected with this bacillus, and the World Health Organisation (WHO) estimates that within the next 20 years nearly 30 million more people will become infected. The rising incidence is caused by the synergism between HIV and TB and the appearance of multidrug-resistant (MDR) strains.[1,2]

HIV infection is the most potent risk factor for converting latent TB into active transmissible TB, while TB bacteria assist in accelerating the progress of AIDS infection in the patient. As the combination of both diseases is more destructive than either alone, the TB and HIV epidemics form a vicious circle. TB is the leading cause of death among people who are HIV positive and it accounts for 13% of AIDS deaths worldwide.[1,2]

Additionally, infectious disease experts at the WHO estimate that globally there are 300000 new cases per year of MDR TB. MDR TB is defined as the disease caused by TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. The main reason for the spread of those MDR TB strains is long and expensive treatment. As MDR TB cannot be treated by the standard short-course therapy, treatment for up to two years is needed with "second line" drugs. These medications are often toxic and ineffective, as no new drug has been introduced in 40 years. As the poorer endemic countries do not have the money to pay for the long regimen of the current drugs, a shorter TB drug regimen will radically improve treatment and compliance.[1,2] The urgency to battle this disease makes TB a challenging and imminently important topic in current drug research.[3]

M. tuberculosis thymidine monophosphate kinase (TMPKmt) recently emerged as a potentially attractive target for the design of a novel class of antituberculosis agents.[4] TMPK catalyzes the conversion of dTMP to dTDP, using ATP as its preferred phosphoryl donor.[5] As part of the biosynthetic pathway for deoxythymidine triphosphate (dTTP), this enzyme is essential for providing the organism with dTTP. Its low sequence identity (22%) with the human isozyme (TMPKh) and the identification of AZTMP as a selective competitive inhibitor of TMPKmt make it an attractive target for the selective inhibition of mycobacterial DNA synthesis.[4]

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Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author: conformational analysis of compounds **6** and **9** (in solution) and **8** (modelled).

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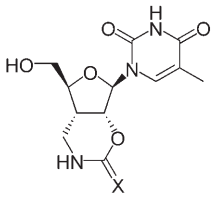
Recently, the X-ray crystal structure of TMPKmt in complex with dTMP was determined at a resolution of 1.95 Å,[6] thus allowing structure-based design of TMPKmt inhibitors.[7] Our research group recently reported the serendipitous discovery of two bicyclic nucleosides (compounds **1** and **2**, Figure 1) with promising TMPKmt inhibitory potency.[8] The present synthetic work was directed toward functional, structural, and conformational variations of these bicyclic derivatives to gain further insight into their binding mode and to improve inhibitory activity.



1: X = S
K_i (TMPKmt) = 3.5 μM
K_i (TMPKh) = 700 μM

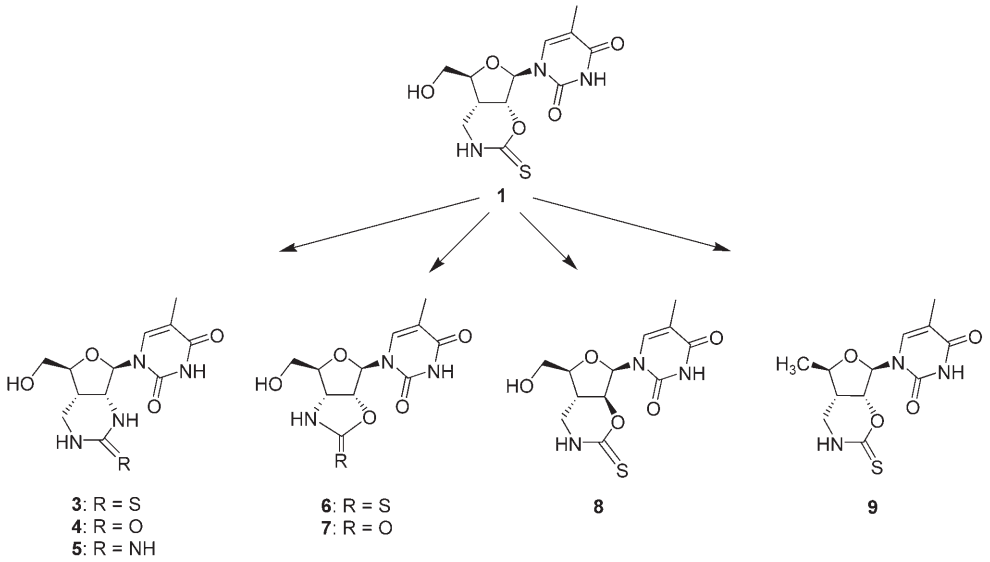
2: X=O
K_i (TMPKmt) = 13.5 μM
K_i (TMPKh) = 1100 μM

Figure 1. Bicyclic TMPKmt inhibitors used as leads in the current study.[8]

First, a series of bicyclic six-membered ring derivatives **3**, **4**, and **5** (Figure 2) was envisaged, in which the thiourethane moiety of **1** was replaced by thiourea, urea, and guanidine functions. The goal of introducing a guanidine functionality was to determine whether delocalisation of a positive charge might confer an optimal interaction with the carboxylate residue of Asp 9, which is one of the important amino acids in the active site of TMPKmt.[6] A second modification involved ring contraction to give five-membered ring bicyclic nucleosides **6** and **7**. To investigate the influence of a different sugar puckering on binding the enzyme, an analogue with inverted configuration at C2′ (compound **8**) was considered.

Despite the fact that compound **1** is a potent inhibitor of TMPKmt, it failed to inhibit the growth of the *M. tuberculosis* H37Rv strain. An important hurdle in drug development for TB is the very low permeability of the cell wall, a waxy coating primarily composed of mycolic acids. These lipids can compose up to 60% of the cell wall mass, making the penetration of molecules through this barrier difficult. Hence, a less polar derivative (compound **9**) was designed to penetrate the lipophilic bacterial cell wall more easily. This 5′-deoxy analogue also allows further estimates of the contribution the 5′-hydroxy group makes toward enzyme affinity. Munier-Lehmann et al.[9] already demonstrated that removal of the 5′-OH group of thymidine improved the affinity for TMPKmt.

The conformational behaviour of nucleosides is considered to be of great importance for their interactions with target proteins and thus for their biological activity.[10] The sugar puckering of natural ribo- and deoxyribonucleosides in solution appears as a dynamic equilibrium between two major conformers, the North (N) and the South (S), with a very low energy barrier between the two states. In the solid state, usually one of the two solution conformations is present, determined mainly by crystal packing forces. The activity toward the enzyme is determined particularly by the adopted sugar puckering of the nucleoside in solution. The more this puckering corresponds to the conformation that binds the enzyme, the easier the nucleoside fits into the active site. Next to this, other structural features of nucleosides, such as rotamers about the C1′–N1, C4′–C5′, and C5′–O5′ bonds also affect enzyme activity. Conformation–activity studies based exclusively on solid-state conformational parameters are often not very significant. This explains why the evaluation of conformationally restrained nucleosides can be very useful, as they adopt certain restricted geometrical shapes.[11] Kifli et al.[12] described the synthesis and conformational analysis of bicyclic nucleosides related to those reported herein.



3: R = S
4: R = O
5: R = NH

6: R = S
7: R = O

8

9

Figure 2. Overview of the bicyclic nucleosides synthesized.

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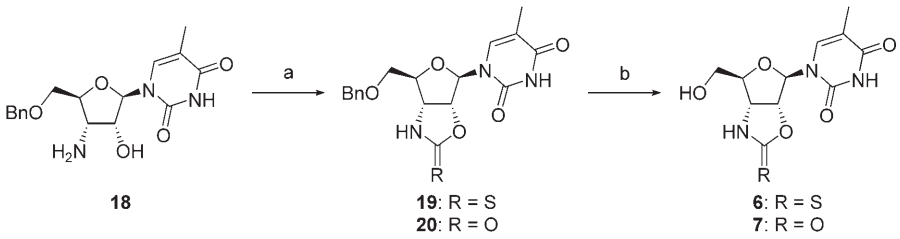
Results

Chemistry

The synthesis of compounds **3**, **4**, and **5** started from compound **10**, which was obtained in 10 steps from 1,2-*O*-isopropylidene-α-D-xylofuranose with an overall yield of 21% (Scheme 1).[13] The 5′-*O*-benzyl-protected compound was converted into anhydronucleoside **11** upon treatment with trifluoromethanesulfonyl chloride and DMAP. Opening of the anhydro ring with NaN_3 afforded the bis-azido derivative **12**. Reduction of both azido groups was carried out by catalytic hydrogenation (Pd/C), without loss of the 5′-benzyl group. Ring closure was realized by reaction of **13** with carbonyldiimidazole or its thiocarbonyl analogue in THF. Finally, deprotection with BCl_3 gave the desired bicyclic nucleosides **3** and **4**. Thiourea compound **14** also served as a starting point to prepare the guanidine analogue **5** by subsequent methylation using iodomethane, substitution with ammonia in MeOH at 100 °C, and removal of the benzyl group.

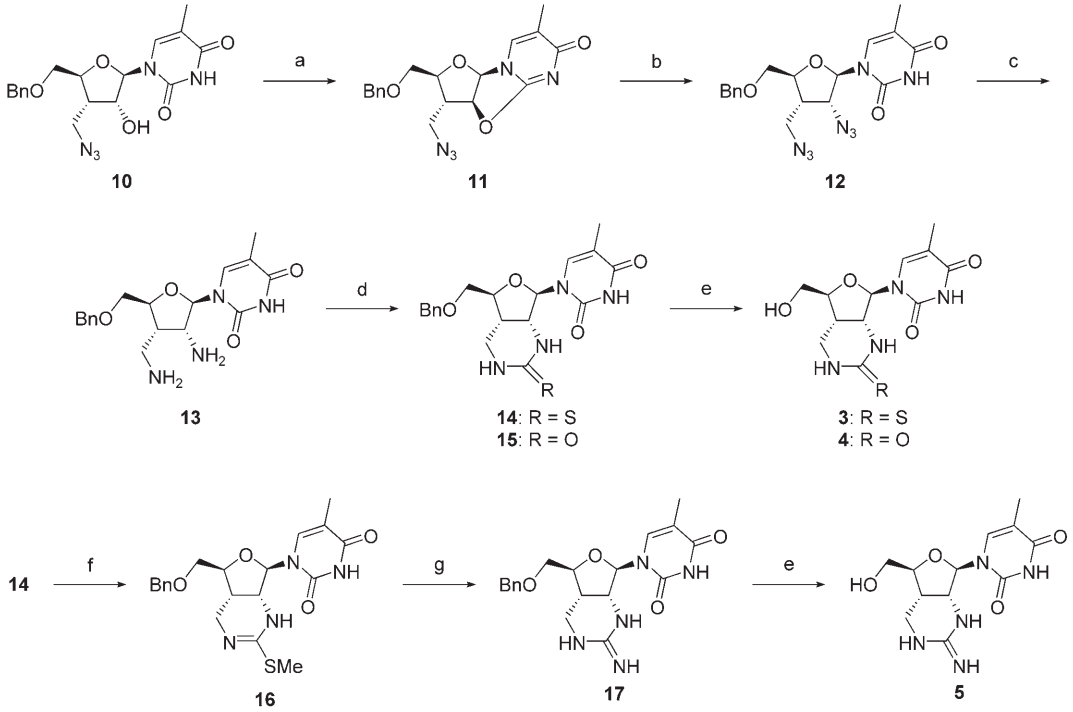
The synthesis of the five-membered bicyclic nucleosides (Scheme 2) started from **18** (obtained from 1,2-*O*-isopropylidene-α-D-xylofuranose in nine steps with an overall yield of 26%).[14] Ring closure and final deprotection were carried out by following the same procedures as described for the six-membered ring compounds.



Scheme 2. Reagents and conditions: a) for **19**: thiocarbonyldiimidazole, THF, 50%; for **20**: carbonyldiimidazole, THF, 72%; b) BCl_3, CH_2Cl_2, −78 °C, 70%.

The synthesis of compound **8**, with an altered configuration at the 2′ position, started from the previously used anhydronucleoside **11** (Scheme 3). The anhydro ring was opened under basic conditions to afford the arabino sugar. Reduction of the azide then gave compound **22**. Ring closure with thiocarbonyldiimidazole was sluggish, but gave access to the desired compound **8** after final debenzylation.

Several attempts to convert **1** to its 5′-deoxy analogue remained unsuccessful. We were therefore forced to perform the deoxygenation step before ring closure, as depicted in Scheme 4. Selective tosylation of the primary alcohol of **24**[15] followed by nucleophilic displacement of the tosylate with sodium iodide gave the 5′-iodo analogue **26**. Simultaneous reduction of the azide and the iodide using catalytic hydrogenation at atmospheric pressure led to compound **27**, which gave target compound **9** after ring closure.



Scheme 1. Reagents and conditions: a) TfCl, DMAP, CH_2Cl_2, 93%; b) NaN_3, benzoic acid, DMF, reflux, 74%; c) Pd/C, H_2, MeOH, 90%; d) for **14**: thiocarbonylimidazole, THF, 60%; for **15**: carbonyldiimidazole, THF, 60%; e) BCl_3, CH_2Cl_2, −78 °C, 47% (for **3**), 60% (for **4**) and 38% (for **5**); f) MeI, MeOH, 78%; g) NH_3, MeOH, 100 °C, 50%. DMAP = 4-dimethylaminopyridine; Tf = trifluoromethanesulfonyl.

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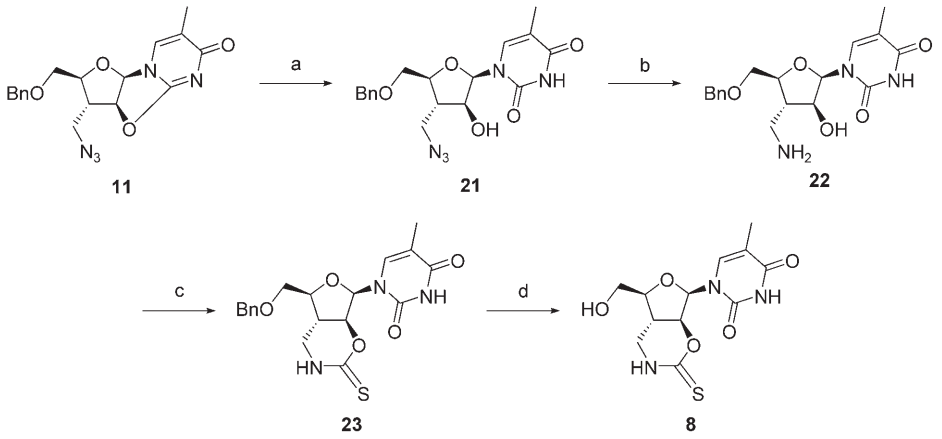
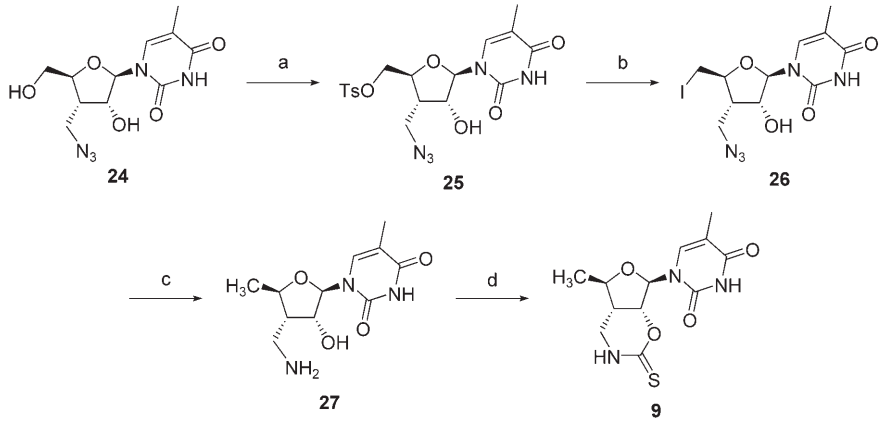


Scheme 3. Reagents and conditions: a) NaOH, dioxane, EtOH/$H_2$0, 92%; b) Pd/C, H_2, MeOH; c) thiocarbonyldiimidazole, THF, 43%; d) BCl_3, CH_2Cl_2, −78 °C, 38%.



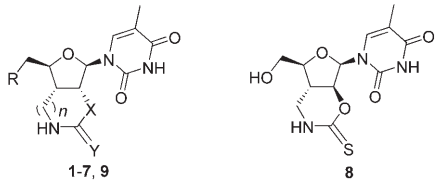
Scheme 4. Reagents and conditions: a) *p*-toluenesulfonyl chloride, pyridine, 46%; b) NaI, acetone, TBAI, 60 °C, 100%; c) Pd/C, H_2, MeOH, TEA, 69%; d) thiocarbonyldiimidazole, THF, 43%. TBAI = tetra-*n*-butylammonium iodide.

Biological evaluation and structure–activity relationship (SAR)

All compounds were tested for TMPKmt inhibition as described in the experimental section. This series of bicyclic thymidine derivatives revealed several potent inhibitors (Table 1). The rationalisation of the observed structure–activity relationship involving molecular modelling and conformational analysis is described in the following section.

The compounds with the highest inhibitory activity toward the enzyme (i.e. **6** and **9**) were evaluated for their in vitro inhibitory activity against *Mycobacterium bovis* BCG. While compound **6** showed 100% inhibition of bacterial growth at 500 μg mL^{-1}, derivative **9** displayed stronger inhibitory activity: no growth was observed at 100 μg mL^{-1}. Neither compound demonstrated toxicity against VERO cell lines in a concentration range of 0–500 μg mL^{-1}. As compound **1** failed to show any inhibition of bacterial growth at 64 μg mL^{-1}, the activity of **9** represents significant progress in our search for new antituberculosis agents.

Table 1. Inhibition of TMPKmt with bicyclic sugar derivatives **1**–**9**.



1-7, 9 8

Compd	X	Y	R	*n*	K_i [μM]	IC_{99} [μg mL^{-1}][a]
1	O	S	OH	1	3.5[b]	
2	O	O	OH	1	13.5[b]	
3	NH	S	OH	1	21	
4	NH	O	OH	1	59	
5	NH	NH	OH	1	46	
6	O	S	OH	0	8	500
7	O	O	OH	0	27	
8 (arabino)	O	S	OH	1	150	
9	O	S	H	1	2.3	100

[a] Against *M. Bovis*. [b] Reference [8].

Model building

Compared with the bicyclic nucleosides **1** and **2**, isosteres **3** and **4**, in which the 2′ oxygen atom is replaced by a 2′-NH group, showed a significant drop in affinity. Modelling indicates that this substitution would lead to repulsion between 2′-NH and the aromatic ring of Tyr 103.

The five-membered bicyclic derivatives **6** and **7** are twofold less active than the corresponding leads **1** and **2**. This result confirms earlier observations that a 3′ branching enhances interaction with the enzyme.[8] Upon superimposition of the five- and six-membered bicyclic (thio)urethane derivatives, differences in binding TMPKmt become perceptible, that is, the H-bond between 3′-NH and Asp 9 is weaker in the five-membered ring derivative. In both series, the distance between NH and the O atoms of Asp 9 is very small (< 3.5 Å), which is a requirement for strong H bonding. In the five-membered ring derivatives, however, the angle of the H bond is too large. For a strong H bond, the angle between the N–H bond and the O–H bond should be smaller than 35 de-

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grees.[16] For compound **1** this angle is 23 degrees, for compound **6**, this angle is 139 degrees, thus explaining the weakness of the H bond in compounds **6** and **7** (Figure 3).

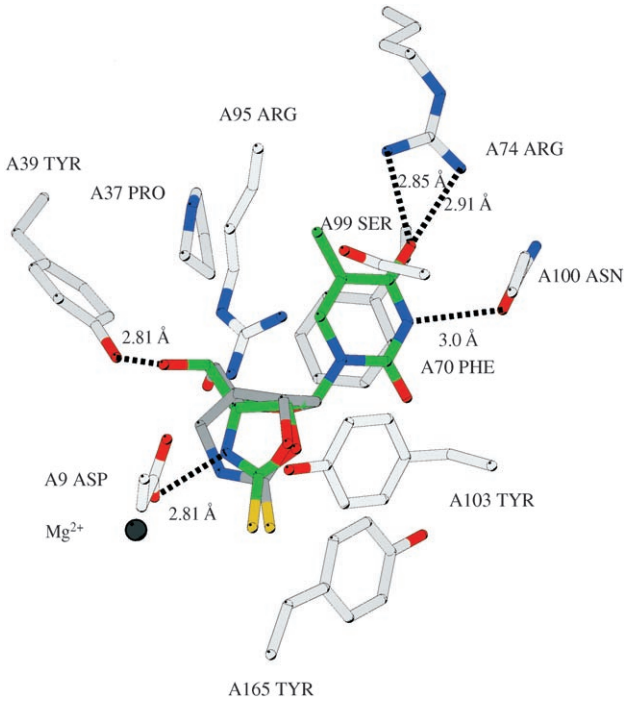

Figure 3. Superimposition of compounds **1** and **6** in their predicted binding modes to the enzyme. (Carbon atoms of **6** are shown in green.)

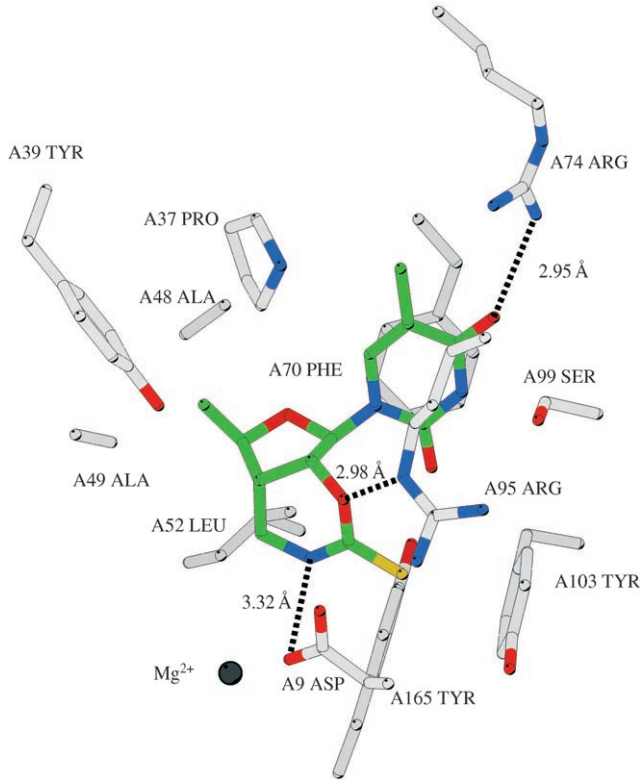

Figure 4. The modelled binding mode of compound **9** to TMPKmt.

Comparison of all final compounds shows that the sulfur derivatives are consistently more active than the corresponding oxygen derivatives. Presumably, the large lipophilic sulfur atom gives a stronger hydrophobic interaction with the enzyme.

With a K_i value of 150 μM, compound **8** showed low binding affinity. Modelling indicates that the anti-bonded bicyclic six-membered ring strongly reduces the flexibility of the system into a conformation between C4′ *exo* and C3′ *endo* (see Supporting Information).

Removal of 5′-OH, the only hydroxy function in compound **1**, strongly decreases polarity. As deoxygenation comes with the loss of strong H bonding between 5′-OH and Tyr 39, a lower binding affinity for this compound was expected. Surprisingly, this 5′-deoxy derivative **9** was almost twice as active as compound **1** toward inhibition of TMPKmt. The same phenomenon was observed for the 5′-methyl derivative of dT accompanied by a sixfold increase in binding affinity.[9] This higher activity can be explained by a higher hydrophobic interaction between 5′-CH_3 and Pro 37 (See Figure 4 for a modelled binding mode of compound **9** and the LIGPLOT interaction map in Figure 5).

Conformational analysis of compounds 6 and 9

The complete definition of the conformation of a nucleoside involves the determination of three principal structural parameters: 1) the glycosyl torsion angle χ, which determines the relative position of the base to the sugar moiety (*syn* or *anti*); 2) the torsion angle γ, which determines the orientation of the 5′-OH group with respect to C3′; and 3) the puckering of the furanose ring (pseudorotation *P*) and its deviation from planarity (maximum out-of-plane pucker ν_{max}).[17] In the present study, the most important determinant is the ring puckering because it is able to influence the χ and γ angles. It is established that TMPKmt prefers to bind its substrate and related analogues with the thymine moiety in the *anti* position and prefers a specific rotamer (γ angle) around the C4′–C5′ bond upon binding. By convention, a pseudorotation angle at $P = 0^\circ$ corresponds to the absolute Northern conformation (C2′ *exo*, C3′ *endo*), whereas the Southern conformation (C2′ *endo*, C3′ *exo*) is present when $P = 180^\circ$.[18]

The solution-state conformation of compounds **6** and **9** was analyzed using Pseurot 6.2.[19] To this end, vicinal proton–proton coupling constants ($^3J_{1',2'}$, $^3J_{2',3'}$ and $^3J_{3',4'}$) of **6** and **9** were accurately measured at 500 MHz in D_2O using the selective refocussing (SERF) technique[20] at five different temperatures ranging from 293 to 325 K. In the case of compound **6**, the *J* values were virtually unaffected within the temperature interval studied. This observation can be explained by assuming that the sugar adopts one particular conformation independent of temperature. Alternatively, an equilibrium might exist between two conformations, which are already present in their T_∞ ratio at the lowest temperature, indicating a small conformational energy barrier. The latter scenario proved to be the only satisfactory one, with the following conformations

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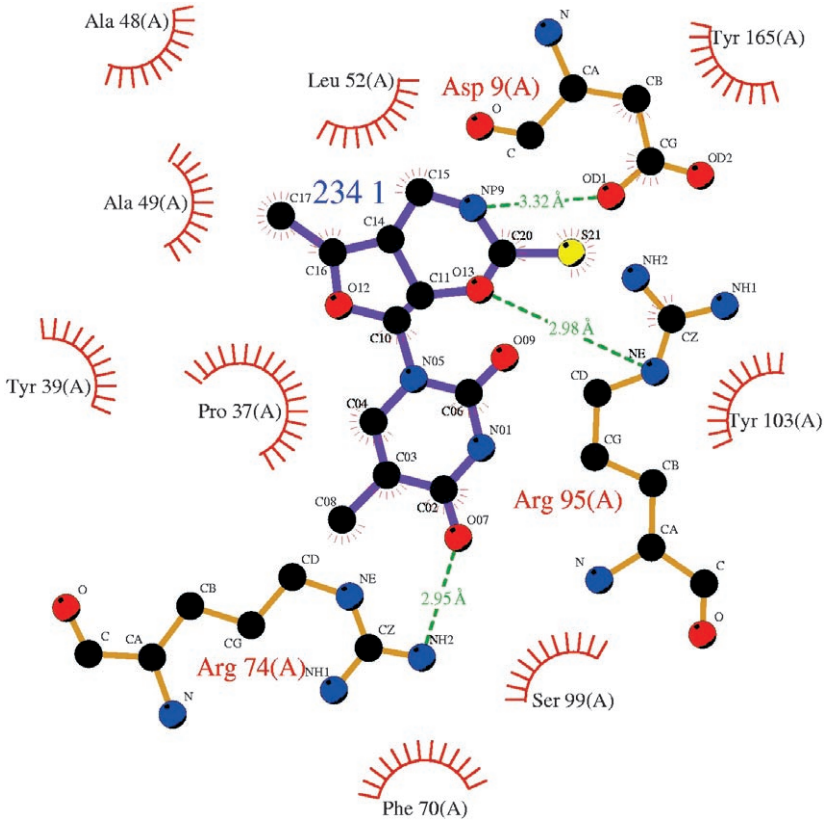

Figure 5. LIGPLOT interaction map of compound **9**.

found: one with $P=77^{\circ}$ and $\nu_{max}\approx18^{\circ}$ (O4′ *endo*) and the other with $P=-88^{\circ}$ and $\nu_{max}\approx17^{\circ}$ (O4′ *exo*). These results extend a previously reported conformational analysis of the thymine congener of **6** using Macromodel potential energy calculations, which only pointed toward a single O4′ *endo* C4′ *exo* puckering.[12] Pseurot analysis of **9** reveals a predominant sugar puckering with $P=40^{\circ}$ and $\nu_{max}=39^{\circ}$, corresponding to a puckering between C4′ *exo* and C3′ *endo*. Although this puckering is very similar to the one deduced from modelling of compound **8**, the inverted configuration at C2′ probably excludes a favourable interaction of the fused ring of **8** with TMPKmt (see Supporting Information).

Because the X-ray crystallographic analysis of the dTMP–TMPKmt complex indicates that the substrate adopts a Southern conformation with the base in an equatorial orientation,[6] one would expect that nucleoside analogues that exhibit a similar S-type conformation would have an optimal affinity for TMPKmt.[14] In the present case, however, the extra ring fused to the sugar portion through 2′ and 3′ seems to exclude such conformation. The established rigidification in compound **9** seems to contribute to its enhanced affinity for TMPKmt by allowing favourable interactions with Asp 9 and Arg 95.

Conclusions

This paper describes the synthesis and biological and conformational analysis of a series of bicyclic sugar nucleosides as inhibitors of TMPKmt. Most compounds showed an inhibitory potency in the low micromolar range, with compound **9** being the most active ($K_i=2.3$ μM). With an IC_{99} value of 100 μg mL^{-1}, this compound gives a strong indication toward the capacity to inhibit the growth of *M. tuberculosis*, promoting TMPKmt as an attractive target for further inhibitor design.

Experimental Section

Spectrophotometric binding assay: In vitro tests were performed on recombinant TMPKmt overexpressed in *E. coli*. TMPKmt activity was determined using the coupled spectrophotometric assay described by Blondin et al.[21] at $\lambda=334$ nm in an Eppendorf ECOM 6122 spectrophotometer. 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 kinase 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 0.02 and 1.5 mM.

General synthesis procedures: NMR spectra were obtained with a Varian Mercury 300 spectrometer (Varian, Palo Alto, CA, USA). Chemical shifts are given in ppm (δ) relative to residual solvent peak (for [D_6]DMSO, $\delta=2.54$ ppm for ^{1}H and $\delta=40.5$ ppm for ^{13}C; for $CDCl_3$, $\delta=7.26$ ppm for ^{1}H and $\delta=77.4$ ppm for ^{13}C). All signals assigned to hydroxy groups were exchangeable with D_2O. MS data and exact mass measurements were performed on a quadrupole/orthogonal acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTOF 2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL min^{-1}. Precoated Merck silica gel F254 plates were used for TLC, and spots were examined under UV light at $\lambda=254$ nm and revealed by sulfuric acid–anisaldehyde spray. Column chromatography was performed on ICN silica gel (63–200 μm, ICN, Asse Relegem, Belgium).

2,2′-Anhydro-1-(3-azidomethyl-3-deoxy-5-*O*-benzyl-β-D-arabinofuranosyl)thymine (11): A solution of **10** (3.00 g, 7.74 mmol) and DMAP (3.78 g, 30.96 mmol) in dichloromethane (77 mL) was stirred at room temperature. After 30 min the mixture was cooled to 0 °C and trifluoromethanesulfonyl chloride (1.65 mL, 15.50 mmol) was added. After being stirred for 2 h at room temperature, the reaction mixture was quenched by the addition of water. The mixture was partitioned between CH_2Cl_2 and a saturated solution of $NaHCO_3$. The aqueous layer was washed with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatogra-

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phy (CH_2Cl_2/MeOH 98:2), affording **11** (2.86 g, 92.5%) as a white foam.

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.79$ (3H, d, $J = 1.2$ Hz, 5-CH_3), 2.66 (1H, m, H-3′), 3.19 (1H, dd, $J = 6.6$ and 10.2 Hz, H-5′), 3.35 (1H, dd, $J = 3.9$ and 10.5 Hz, H-5″), 3.63 (2H, m, H-6′/H-6″), 4.21 (1H, m, H-4′), 4.31 (2H, s, *CH*$_2$Ph), 5.28 (1H, dd, $J = 2.1$ and 5.4 Hz, H-2′), 6.20 (1H, d, $J = 6.0$ Hz, H-1′), 7.26 (5H, m, CH_2*Ph*), 7.74 ppm (1H, d, $J = 1.5$ Hz, H-6); HRMS (ESI MS) for $C_{18}H_{20}N_5O_4$ $[M+H]^+$ found, 370.1512; calcd, 370.1515.

1-(2-Azido-3-azidomethyl-2,3-dideoxy-5-*O*-benzyl-β-D-ribofuranosyl)thymine (12): A solution of **11** (2.46 g, 6.66 mmol), NaN_3 (2.16 g, 33 mmol) and benzoic acid (0.813 g, 6.66 mmol) in DMF (40 mL) was heated at 150 °C. The reaction mixture was held at reflux for 7 h and evaporated to dryness. The residue was partitioned between water and CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and evaporated. Further purification by column chromatography (CH_2Cl_2/MeOH 99:1) yielded **12** (2.01 g, 74%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.40$ (3H, d, $J = 1.2$ Hz, 5-CH_3), 2.63 (1H, m, H-3′), 3.43 (1H, dd, $J = 6.6$ and 12.6 Hz, H-6′), 3.60 (2H, m, H-6″ and H-5′), 3.88 (1H, dd, $J = 2.1$ and 11.4 Hz, H-5″), 4.01 (1H, m, H-4′), 4.57 (2H, s, *CH*$_2$Ph), 4.55 (1H, d, $J = 1.5$ Hz, H-2′), 5.79 (1H, d, $J = 1.8$ Hz, H-1′), 7.32 (5H, m, CH_2*Ph*), 7.62 (1H, d, $J = 0.9$ Hz, H-6), 11.34 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{18}H_{20}N_8O_4Na$ $[M+Na]^+$ found, 435.1508; calcd, 435.1505.

1-(2-Amino-3-aminomethyl-2,3-dideoxy-5-*O*-benzyl-β-D-ribofuranosyl)thymine (13): A solution of **12** (200 mg, 0.48 mmol) in methanol (12 mL) was hydrogenated at atmospheric pressure for 5 h in the presence of 10% Pd/C (20 mg). The catalyst was removed by filtration through celite and the filtrate was evaporated. The residue was purified by column chromatography (CH_2Cl_2/MeOH 85:15) to give compound **13** (155 mg, 90%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.45$ (3H, s, 5-CH_3), 2.14 (1H, m, H-3′), 2.56 (1H, dd, $J = 6.0$ and 12.6 Hz, H-6′), 3.71 (1H, dd, $J = 7.5$ and 12.3 Hz, H-6″), 3.41 (1H, dd, $J = 3.6$ and 6.9 Hz, H-2′), 3.56 (1H, dd, $J = 3.6$ and 11.1 Hz, H-5′), 3.79 (1H, dd, $J = 2.4$ and 11.4 Hz, H-5″), 4.07 (1H, m, H-4′), 4.55 (2H, s, *CH*$_2$Ph), 5.55 (1H, d, $J = 3.6$ Hz, H-1′), 7.32 (5H, m, CH_2*Ph*), 7.64 ppm (1H, d, $J = 1.2$ Hz, H-6); HRMS (ESI MS) for $C_{18}H_{25}N_4O_4$ $[M+H]^+$ found, 361.1871; calcd, 361.1875.

1-[2-Amino-3-aminomethyl-2,3-dideoxy-2-*N*,6-*N*-(thiocarbonyl)-5-*O*-benzyl-β-D-ribofuranosyl]thymine (14): A solution of **13** (200 mg, 0.56 mmol) and thiocarbonyldiimidazole (109 mg, 0.61 mmol) in THF (6 mL) was stirred at room temperature for 1.5 h (TLC monitoring). The mixture was evaporated to dryness and purified by column chromatography (CH_2Cl_2/MeOH 95:5) to give compound **14** (135 mg) in 60% yield.

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.50$ (3H, d, $J = 0.9$ Hz, 5-CH_3), 2.48 (1H, m, H-3′), 3.06 (1H, dd, $J = 9.3$ and 12.0 Hz, H-6′), 3.32 (1H, m, H-6″), 3.63 (1H, dd, $J = 3.3$ and 10.5 Hz, H-5′), 3.75 (1H, dd, $J = 3.0$ Hz and 11.1 Hz, H-5″), 3.86 (1H, m, H-2′), 4.03 (1H, dd, $J = 3.0$ and 6.3 Hz, H-4′), 4.56 (2H, s, *CH*$_2$Ph), 5.79 (1H, d, $J = 6.3$ Hz, H-1′), 7.33 (5H, m, CH_2*Ph*), 7.47 (1H, d, $J = 1.2$ Hz, H-6), 8.33 (2H, m, N(2′)H and N(6′)H), 11.27 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{19}H_{23}N_4O_4S$ $[M+H]^+$ found, 403.1447; calcd, 403.1439.

1-[2-amino-3-aminomethyl-2,3-dideoxy-2-*N*,6-*N*-(carbonyl)-5-*O*-benzyl-β-D-ribofuranosyl]thymine (15): A solution of **13** (150 mg, 0.41 mmol) and carbonyldiimidazole (73 mg, 0.45 mmol) in THF (4 mL) was stirred at room temperature for 3 h (TLC monitoring). The mixture was evaporated to dryness and purified by column chromatography (CH_2Cl_2/MeOH 95:5) to give compound **15** (95 mg) in 60% yield.

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.50$ (3H, d, $J = 0.9$ Hz, 5-CH_3), 2.52 (1H, m, H-3′), 3.07 (1H, m, H-6′), 3.21 (1H, m, H-6″), 3.63 (1H, dd, $J = 3.3$ and 10.5 Hz, H-5′), 3.75 (1H, dd, $J = 3.0$ Hz and 11.1 Hz, H-5″), 3.86 (1H, m, H-2′), 4.06 (1H, m, H-4′), 4.56 (2H, s, *CH*$_2$Ph), 5.74 (1H, d, $J = 5.4$ Hz, H-1′), 6.45 (1H, brs, N(2′)H), 6.55 (1H, brs, N(6′)H), 7.33 (5H, m, CH_2*Ph*), 7.51 (1H, d, $J = 1.2$ Hz, H-6), 11.29 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{19}H_{22}N_4O_5Na$ $[M+Na]^+$ found, 409.1488; calcd, 409.1498.

1-[2-Amino-3-aminomethyl-2,3-dideoxy-2-*N*,6-*N*-(thiocarbonyl)-β-D-ribofuranosyl]thymine (3) and 1-[2-amino-3-aminomethyl-2,3-dideoxy-2-*N*,6-*N*-(carbonyl)-β-D-ribofuranosyl]thymine (4): A 1 M BCl_3 solution in CH_2Cl_2 was added (4 equiv) to a solution of **14** or **15** (1 equiv) in dry CH_2Cl_2 (40 mL mmol^{-1}) at −78 °C. After 15 min the reaction was quenched with methanol, the mixture was evaporated and the residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5) to yield final compounds **3** (47%) and **4** (60%).

3: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.75$ (3H, d, $J = 1.2$ Hz, 5-CH_3), 2.43 (1H, m, H-3′), 3.04 (1H, dd, $J = 9.6$ and 12.3 Hz, H-6′), 3.25 (1H, m, H-6″), 3.60 (2H, m, H-5′ and H-5″), 3.85 (2H, m, H-4′ and H-2′), 5.18 (1H, t, $J = 5.1$ Hz, 5′-OH), 5.76 (1H, d, $J = 6.3$ Hz, H-1′), 7.71 (1H, d, $J = 1.2$ Hz, H-6), 8.31 (2H, brs, N(6′)H and N(2′)H), 11.27 ppm (1H, brs, N(3)H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 12.94$ (5-CH_3), 33.14 (C-3′), 37.58 (C-6′), 57.28 (C-5′), 62.71 (C-2′), 82.50 (C-4′), 89.08 (C-1′), 110.12 (C-5), 137.04 (C-6), 151.40 (C-4), 164.64 (C-2), 177.18 ppm (C=S); HRMS (ESI MS) for $C_{12}H_{17}N_4O_4S$ $[M+H]^+$ found, 313.0986; calcd, 313.0970; Anal. ($C_{12}H_{16}N_4O_4S$) C, H, N, S.

4: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.75$ (3H, d, $J = 0.9$ Hz, 5-CH_3), 2.45 (1H, m, H-3′), 3.05 (1H, m, H-6′), 3.19 (1H, m, H-6″), 3.54 (1H, m, H-5′), 3.65 (1H, m, H-5″), 3.85 (2H, m, H-4′ and H-2′), 5.18 (1H, t, $J = 5.1$ Hz, 5′-OH), 5.71 (1H, d, $J = 5.7$ Hz, H-1′), 6.42 (1H, brs, N(6′)H), 6.55 (1H, brs, N(2′)H), 7.74 (1H, d, $J = 0.9$ Hz, H-6), 11.29 ppm (1H, brs, N(3)H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 12.93$ (5-CH_3), 34.96 (C-3′), hidden by DMSO signal (C-6′), 57.51 (C-5′), 62.57 (C-2′), 82.22 (C-4′), 90.02 (C-1′), 109.90 (C-5), 137.02 (C-6), 151.39 (C-4), 156.49 (C=O), 164.56 ppm (C-2); HRMS (ESI MS) for $C_{12}H_{16}N_4O_5Na$ $[M+Na]^+$ found, 319.1015; calcd, 319.1018; Anal. ($C_{12}H_{16}N_4O_5$) C, H, N.

1-[2-Amino-3-aminomethyl-2,3-dideoxy-2-*N*,6-*N*-(methylthiomethylidyne)-5-*O*-benzyl-β-D-ribofuranosyl]thymine (16): MeI (168 μL, 2.71 mmol) was added to a solution of compound **14** (778 mg, 1.93 mmol) in methanol (60 mL), and the solution was stirred for 8 h. The reaction mixture was evaporated to dryness in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/MeOH 98:2) to yield **16** (630 mg, 78%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.46$ (3H, s, 5-CH_3), 2.61 (1H, m, H-3′), 3.18 (1H, m, H-6′), 3.41 (1H, dd, $J = 4.8$ and 12.9 Hz, H-6″), 3.66 (3H, s, S-CH_3), 3.79 (1H, d, $J = 2.4$ Hz, H-5′), 3.83 (1H, d, $J = 2.7$ Hz, H-5″), 3.96 (1H, m, H-2′), 4.06 (1H, m, H-4′), 4.58 (2H, s, *CH*$_2$Ph), 5.74 and 5.81 (1H, 2 tautomers, d, $J = 2.7$ and 4.8 Hz, H-1′), 7.32 (5H, m, CH_2*Ph*), 7.61 and 7.65 (1H, 2 tautomers, s, H-6), 11.32 and 11.36 ppm (1H, 2 tautomers, s, N(3)H); HRMS (ESI MS) for $C_{20}H_{25}N_4O_4S$ $[M+H]^+$ found, 417.1589; calcd, 417.1596.

1-[2-Amino-3-aminomethyl-2,3-dideoxy-2-*N*,6-*N*-(imine)-5-*O*-benzyl-β-D-ribofuranosyl]thymine (17): Compound **16** (189 mg, 0.45 mmol) was dissolved in 7 N NH_3 in methanol (20 mL). The solution was allowed to stir overnight at 100 °C. The reaction mixture was evaporated to dryness. The residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5), affording **17** (86 mg, 50%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.47$ (3H, d, $J = 1.2$ Hz, 5-CH_3), 2.71 (1H, m, H-3′), 3.22 (1H, dd, $J = 7.5$ and 12.9 Hz, H-6′), 3.43 (1H, dd, $J = 5.4$ and 13.2 Hz, H-6″), 3.67 (1H, dd, $J = 3.0$ and 11.1 Hz, H-

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5′), 3.82 (1H, dd, $J=2.7$ and 10.8 Hz, H-5″), 4.04 (1H, t, $J=5.1$ Hz, H-2′), 4.12 (1H, m, H-4′), 4.58 (2H, s, CH_2Ph), 5.77 (1H, d, $J=4.5$ Hz, H-1′), 7.04 (1H, brs, NH), 7.32 (5H, m, CH_2Ph), 7.57 (1H, d, $J=1.2$ Hz, H-6), 7.99 (1H, brs, N(6′)H), 8.24 (1H, brs, N(2′)H), 11.40 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{20}H_{24}N_4O_4$ $[M+H]^+$ found, 386.1817; calcd, 386.1953.

1-[2-Amino-3-aminomethyl-2,3-dideoxy-2-*N*,6-*N*-(imine)-β-D-ribofuranosyl]thymine (5): The title compound was synthesized from **17** (72 mg, 0.19 mmol) using the same procedure as described for the deprotection of **14**, in a yield of 21 mg or 38%.

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.76$ (3H, s, 5-CH_3), 2.65 (1H, m, H-3′), 3.22 (1H, dd, $J=6.9$ and 13.2 Hz, H-6′), 3.41 (1H, dd, $J=5.1$ and 13.5 Hz, H-6″), 3.61 (1H, d, $J=11.7$ Hz, H-5′), 3.72 (1H, d, $J=11.7$ Hz, H-5″), 3.93 (1H, m, H-4′), 4.03 (1H, m, H-2′), 5.41 (1H, s, 5′-OH), 5.74 (1H, d, $J=3.6$ Hz, H-1′), 7.23 (1H, brs, C=NH), 7.75 (2H, brs, N(6′)H and N(2′)H), 7.88 ppm (1H, s, H-6); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=12.97$ (5-CH_3), 33.19 (C-6′), 36.89 (C-3′), 56.42 (C-2′), 61.50 (C-5′), 82.58 (C-4′), 89.46 (C-1′), 109.76 (C-5), 136.58 (C-6), 151.29 (C-4), 154.31 (C=NH), 164.52 ppm (C-2); HRMS (ESI MS) for $C_{13}H_{18}N_4O_4$ $[M+H]^+$ found, 296.1366; calcd, 296.1358; Anal. ($C_{13}H_{17}N_4O_4\cdot H_2O$) C, H, N.

1-(3-Amino-3-deoxy-2-*O*,3-*N*-(thiocarbonyl)-5-*O*-benzyl-β-D-ribofuranosyl)thymine (19): Compound **18** (410 mg, 1.10 mmol) and thiocarbonyldiimidazole (215 mg, 1.21 mmol) in THF (18 mL) were stirred at 50 °C overnight. The reaction mixture was evaporated to dryness, and the residue was purified by column chromatography (CH_2Cl_2/MeOH 97:3) to yield **19** (228 mg, 50%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.65$ (3H, d, $J=1.2$ Hz, 5-CH_3), 3.59 (1H, dd, $J=5.4$ and 10.8 Hz, H-5′), 3.67 (1H, dd, $J=3.9$ and 11.1 Hz, H-5″), 4.21 (1H, m, H-4′), 4.50 (2H, d, CH_2Ph), 4.54 (1H, d, $J=3.9$ Hz, H-3′), 5.59 (1H, dd, $J=3.0$ and 8.7 Hz, H-2′), 5.92 (1H, d, $J=2.7$, H-1′), 7.31 (5H, m, CH_2Ph), 7.51 (1H, d, $J=1.2$ Hz, H-6), 10.56 (1H, brs, N(3′)H), 11.39 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{18}H_{20}N_3O_5S$ $[M+H]^+$ found, 390.1123; calcd, 390.1123.

1-(3-Amino-3-deoxy-2-*O*,3-*N*-(carbonyl)-5-*O*-benzyl-β-D-ribofuranosyl)thymine (20): Compound **18** (325 mg, 0.87 mmol) and carbonyldiimidazole (156 mg, 0.96 mmol) in THF (14 mL) were stirred at 50 °C overnight. The reaction mixture was evaporated to dryness, and the residue was purified by column chromatography (CH_2Cl_2/MeOH 97:3) to yield **20** (250 mg, 72%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.65$ (3H, d, $J=1.2$ Hz, 5-CH_3), 3.59 (1H, dd, $J=5.4$ and 10.8 Hz, H-5′), 3.67 (1H, dd, $J=3.6$ and 10.8 Hz, H-5″), 4.13 (1H, dd, $J=5.4$ and 9.3 Hz, H-4′), 4.25 (1H, dd, $J=4.8$ and 8.4 Hz, H-3′), 4.51 (2H, d, CH_2Ph), 5.20 (1H, dd, $J=3.3$ and 8.7 Hz, H-2′), 5.90 (1H, d, $J=3.3$, H-1′), 7.32 (5H, m, CH_2Ph), 7.49 (1H, d, $J=1.2$ Hz, H-6), 8.27 (1H, brs, N(3′)H), 11.42 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{18}H_{20}N_3O_6$ $[M+H]^+$ found, 374.1354; calcd, 374.1351.

1-(3-Amino-3-deoxy-2-*O*,3-*N*-(thiocarbonyl)-β-D-ribofuranosyl)thymine (6) and 1-(3-Amino-3-deoxy-2-*O*,3-*N*-(carbonyl)-β-D-ribofuranosyl)thymine (7): These compounds were synthesized from **19** (250 mg, 0.67 mmol) and **20** (215 mg, 0.52 mmol) using the same procedure as described for the deprotection of **14**, to yield **6** (103 mg, 70%) and **7** (140 mg, 70%).

6: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.74$ (3H, d, $J=0.9$ Hz, 5-CH_3), 3.54 (2H, m, H-5′ and H-5″), 3.97 (1H, dd, $J=4.5$ and 9.3 Hz, H-4′), 4.45 (1H, dd, $J=4.5$ and 8.7 Hz, H-3′), 5.09 (1H, t, $J=5.1$ Hz, 5′-OH), 5.35 (1H, dd, $J=3.0$ and 8.7 Hz, H-2′), 5.90 (1H, d, $J=3.0$, H-1′), 7.58 ppm (1H, d, $J=0.9$ Hz, H-6); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=$ 12.75 (5-CH_3), 56.60 (C-3′), 61.71 (C-5′), 87.96 (C-4′), 88.85 (C-2′), 91.87 (C-1′), 110.34 (C-5), 138.25 (C-6), 150.99 (C-4), 164.51 (C-2), 187.56 ppm (C=S); HRMS (ESI MS) for $C_{11}H_{14}N_3O_5S$ $[M+H]^+$ found, 300.0659; calcd, 300.0654; Anal. ($C_{11}H_{13}N_3O_5S$) C, H, N, S.

7: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.74$ (3H, d, $J=0.6$ Hz, 5-CH_3), 3.56 (2H, m, H-5′ and H-5″), 3.90 (1H, dd, $J=4.5$ and 9.3 Hz, H-4′), 4.18 (1H, dd, $J=4.8$ and 8.7 Hz, H-3′), 5.08 (1H, s, 5′-OH), 5.15 (1H, dd, $J=3.6$ and 8.7 Hz, H-2′), 5.87 (1H, d, $J=3.3$, H-1′), 7.56 (1H, d, $J=0.9$ Hz, H-6), 8.24 (1H, brs, N(3′)H), 11.42 ppm (1H, brs, N(3)H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=12.79$ (5-CH_3), 56.85 (C-3′), 61.69 (C-5′), 82.24 (C-4′), 88.08 (C-2′), 91.27 (C-1′), 110.41 (C-5), 138.06 (C-6), 151.01 (C-4), 157.90 (C=O), 164.48 ppm (C-2); HRMS (ESI MS) for $C_{11}H_{13}N_3O_6Na$ $[M+Na]^+$ found, 306.0709; calcd, 306.0702; Anal. ($C_{11}H_{13}N_3O_6$) C, H, N.

1-(3-Azidomethyl-3-deoxy-5-*O*-benzyl-β-D-arabinofuranosyl)thymine (21): A solution of **11** (530 mg, 1.43 mmol), 1 M NaOH (4.24 mL), dioxane (58 mL) and EtOH/H_2O 1:1 (58 mL) was allowed to stir for 3 h at room temperature. The resulting mixture was extracted with CH_2Cl_2 (3×100 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated, and the crude material was purified by column chromatography (CH_2Cl_2/MeOH 98:2) to yield **21** (509 mg, 92%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.46$ (3H, d, $J=0.9$ Hz, 5-CH_3), 2.27 (1H, m, H-3′), 3.48 (1H, dd, $J=6.9$ and 12.6 Hz, H-6′), 3.66 (2H, m, H-5′ and H-6″), 3.82 (2H, m, H-4′ and H-5″), 4.16 (1H, dd, $J=6.0$ and 12.6 Hz, H-2′), 4.55 (2H, s, CH_2Ph), 5.62 (1H, d, $J=5.4$ Hz, 2′-OH), 5.95 (1H, d, $J=5.7$ Hz, H-1′), 7.33 (5H, m, CH_2Ph), 7.47 (1H, d, $J=1.2$ Hz, H-6), 11.22 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{18}H_{21}N_4O_5Na$ $[M+Na]^+$ found, 435.1508; calcd, 435.1505.

1-(3-Aminomethyl-3-deoxy-5-*O*-benzyl-β-D-arabinofuranosyl)thymine (22): A solution of **21** (195 mg, 0.50 mmol) in methanol (12 mL) was hydrogenated at atmospheric pressure for 5 h in the presence of 10% Pd/C (20 mg). The catalyst was removed by filtration through celite and the filtrate was evaporated. The residue was used in the next step without further purification.

1-(3-Aminomethyl-3-deoxy-2-*O*,6-*N*-(thiocarbonyl)-5-*O*-benzyl-β-D-arabinofuranosyl)thymine (23): Compound **22** (43 mg, 0.12 mmol) was dissolved in THF (6 mL), and the resulting solution was slowly added to a solution of thiocarbonyldiimidazole (64 mg, 0.36 mmol) in THF (2 mL). After 1 hour, 5 mL THF was added, and the solution was allowed to stir another 2 h. Evaporation of the solvent and purification of the residue by column chromatography (CH_2Cl_2/MeOH 97:3) gave **23** (20 mg, 43%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.43$ (3H, d, $J=0.6$ Hz, 5-CH_3), 2.40 (1H, m, H-3′), 3.70 (1H, dd, $J=3.9$ and 11.3 Hz, H-6′), 3.81 (1H, dd, $J=3.3$ and 11.1 Hz, H-6″), 3.88 (3H, m, H-4′, H-5′ and H-5″), 4.19 (1H, m, H-2′), 4.55 (2H, s, CH_2Ph), 5.98 (1H, d, $J=6.3$ Hz, H-1′), 7.32 (5H, m, CH_2Ph), 7.45 (1H, d, $J=1.2$ Hz, H-6), 11.23 ppm (1H, brs, N(3)H); HRMS (ESI MS) for $C_{19}H_{21}N_3O_5SNa$ $[M+Na]^+$ found, 426.1093; calcd, 426.1099.

1-(3-Aminomethyl-3-deoxy-2-*O*,6-*N*-(thiocarbonyl)-β-D-arabinofuranosyl)thymine (8): This compound was synthesized from **23** using the same procedure as described for the deprotection of **14**, in a yield of 38% (15 mg).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.72$ (3H, d, $J=0.9$ Hz, 5-CH_3), 2.99 (1H, m, H-3′), 3.59 (1H, m, H-6′), 2.67 (2H, m, H-5′ and H-6″), 3.88 (2H, m, H-4′ and H-5″), 4.17 (1H, m, H-2′), 5.18 (1H, apps, 5′-OH), 5.66 (1H, d, $J=6.0$ Hz, H-1′), 7.67 (1H, d, $J=0.9$ Hz, H-6), 11.22 ppm (1H, brs, N(3)H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=12.99$ (5-CH_3), 31.38 (C-3′), 33.31 (C-6′), 60.04 (C-5′), 79.82 (C-4′), 80.14 (C-2′), 81.07 (C-1′), 109.16 (C-5), 136.63 (C-6), 150.90 (C-4), 164.38 (C-2), 186.61 ppm (C=S); HRMS (ESI MS) for $C_{12}H_{15}N_3O_5SNa$ $[M+Na]^+$ found, 336.0630; calcd, 336.0636; Anal. ($C_{12}H_{15}N_3O_5S$) C, H, N.

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1-(3-Azidomethyl-3-deoxy-5-*O*-tosyl-β-D-ribofuranosyl)thymine (25): *p*-Toluenesulfonyl chloride (307 mg, 1.61 mmol) was added to a solution of **24** (398 mg, 1.34 mmol) in pyridine (3.2 mL) cooled at 0 °C. The reaction mixture was stirred at room temperature over 2 days, then evaporated in vacuo to remove pyridine. The residue was partitioned between CH_2Cl_2 (10 mL) and H_2O (10 mL), separated, and the aqueous layer was extracted twice more with 15 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (CH_2Cl_2/MeOH 97:3) to yield compound **25** (280 mg, 46%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.77$ (3H, d, $J = 1.2$ Hz, 5-CH_3), 2.29 (1H, m, H-3′), 2.41 (3H, s, PhCH_3), 3.31 (1H, m, H-6′), 3.58 (1H, dd, $J = 5.7$ and 12.3, H-6″), 4.06 (1H, m, H-4′), 4.20 (2H, m, H-5′ and H-2′), 4.36 (1H, dd, $J = 2.1$ and 11.1, H-5″), 5.62 (1H, d, $J = 2.4$ Hz, H-1′), 5.90 (1H, d, $J = 5.4$ Hz, 2′-OH), 7.36 (1H, d, $J = 1.5$, H-6), 7.45 (2H, m, arom H), 7.77 (2H, m, arom H), 11.31 ppm (1H, br s, N(3)H); HRMS (ESI MS) for $C_{18}H_{22}N_5O_7S$ $[M+H]^+$ found, 452.1240; calcd, 452.1239.

1-(3-Azidomethyl-3,5-dideoxy-5-iodo-β-D-ribofuranosyl)thymine (26): A mixture of **25** (342 mg, 0.76 mmol), NaI (557 mg, 3.71 mmol) and a catalytic amount of tetrabutylammonium iodide (5 mg) in acetone (30 mL) was held at reflux (60 °C) overnight. Ice-cold water (5 mL) was poured into the reaction mixture. This mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH: 97:3) to yield compound **26** (311 mg, 100%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.79$ (3H, d, $J = 1.8$ Hz, 5-CH_3), 2.26 (1H, m, H-3′), 3.45 (2H, m, H-6′ and H-5′), 3.62 (2H, m, H-6″ and H-5″), 3.80 (1H, m, H-4′), 4.35 (1H, m, H-2′), 5.43 (1H, d, $J = 3.6$ Hz, H-1′), 5.88 (1H, d, $J = 5.1$ Hz, 2′-OH), 7.48 (1H, d, $J = 1.2$, H-6), 11.34 ppm (1H, br s, N(3)H); HRMS (ESI MS) for $C_{11}H_{14}N_5O_7INa$ $[M+Na]^+$ found, 429.9999; calcd, 429.9990.

1-(3-Aminomethyl-3,5-dideoxy-β-D-ribofuranosyl)thymine (27): A mixture of **26** (311 mg, 0.764 mmol) and triethylamine (250 μL) in methanol (20 mL) was hydrogenated at atmospheric pressure overnight in the presence of 10% Pd/C (95 mg). The catalyst was removed by filtration through celite, and the filtrate was evaporated. The residue was purified by column chromatography (CH_2Cl_2/MeOH 85:15 to 80:20) to give compound **27** (134 mg, 69%).

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.32$ (3H, d, $J = 6.0$ Hz, 5′-CH_3), 1.79 (3H, d, $J = 1.2$ Hz, 5-CH_3), 2.03 (1H, m, H-3′), 2.76 (1H, dd, $J = 5.1$ and 12.6, H-6′), 2.95 (1H, m, H-6″), 3.96 (1H, m, H-4′), 4.29 (1H, dd, $J = 2.1$ and 6.0 Hz, H-2′), 5.60 (1H, d, $J = 2.1$ Hz, H-1′), 7.32 ppm (1H, d, $J = 1.2$ Hz, H-6); HRMS (ESI MS) for $C_{11}H_{18}N_3O_4$ $[M+H]^+$ found, 256.1294; calcd, 256.1297.

1-(3-Aminomethyl-3,5-dideoxy-2-*O*,6-*N*-(thiocarbonyl)-β-D-ribofuranosyl)thymine (9): A solution of **27** (127 mg, 0.497 mmol) and thiocarbonyldiimidazole (97.47 mg, 0.547 mmol) in THF (7 mL) was stirred at room temperature for 5 h. The mixture was evaporated to dryness and the residue was purified by column chromatography (CH_2Cl_2/MeOH 97:3) to give compound **9** (64 mg, 43%) as a white solid.

^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 1.40$ (3H, d, $J = 6.3$ Hz, 5′-CH_3), 1.80 (3H, s, 5-CH_3), 2.61 (1H, m, H-3′), 3.16 (1H, d, $J = 4.2$ Hz, H-6′), 3.46 (1H, dd, $J = 5.7$ and 13.8 Hz, H-6″), 3.96 (1H, m, H-4′), 5.02 (1H, d, $J = 5.7$, H-2′), 5.80 (1H, s, H-1′), 7.37 (1H, s, H-6), 9.85 (1H, br s, N(6′)H), 11.40 ppm (1H, br s, N(3)H); ^{13}C NMR (300 MHz, [D$_6$]DMSO): $\delta = 12.83$ (5-CH_3), 18.67 (C-5′), 37.18 (C-6′), 38.52 (C-3′), 77.83 (C-4′), 84.14 (C-2′), 90.36 (C-1′), 110.24 (C-5), 136.54 (C-6), 150.88 (C-2), 164.49 (C-4), 184.70 ppm (6-NHCS); HRMS (ESI MS) for $C_{12}H_{16}N_3O_4S$ $[M+H]^+$ found, 298.0858; calcd, 298.0861; Anal. ($C_{12}H_{15}N_3O_4S$) C, H, N.

Modelling: The models (Figure 3 and 4) were created using Amber 8.0 software.[22] Force-field parameters for the ligands were created by the Antechamber program.[23] The inhibitors were positioned in the active site onto the TMP substrate[6] (the thymine bases and sugar rings were superimposed by fitting atoms of the new molecules onto corresponding atoms in the TMP molecule by least squares). The TMP molecule was then removed. The final complexes were energy minimized (enzyme residues were also allowed to move) using the Sander program in the Amber software. Because the inhibitors bind deep into the interior of the enzyme, no solvent was included in the calculations. Finally, to examine the interactions between the new inhibitors and the enzyme, LIGPLOT interaction maps were calculated.[24]

Biological assays on *Mycobacterium bovis* (BCG): The different compounds were assayed for their inhibitory potency toward *Mycobacterium bovis* (variant BCG) growth in vitro.[25] A micro-method of culture was performed in 7H9 Middlebrook broth medium containing 0.2% glycerol, 0.5% Tween 80, and supplemented with oleic acid, albumin, dextrose, and catalase (Becton-Dickinson). Serial twofold dilutions of each compound were prepared directly in 96-well plates. The bacterial inoculum was prepared previously at a concentration in the range of 10^7 bacteria (*M. bovis* BCG 1173P2) in 7H9 medium and stored at −80 °C until used. The bacteria, adjusted at 10^5 cells mL^{-1}, were delivered under a volume of 100 μL well^{-1}. The covered plates were sealed with Parafilm and incubated at 37 °C in plastic boxes containing humidified normal atmosphere. At day 8 of incubation, 30 μL of a resazurin (Sigma) solution at 0.01% (*w/v*) in water were added to each well. After storage overnight at 37 °C, the plates were assessed for colour development using the optical density difference at $\lambda = 570$ and 630 nm on an ELISA reader. The change from blue to pink indicates reduction of resazurin and therefore bacterial growth. The lowest compound concentration that prevented the colour change determined the MIC value for the given compound assayed.

Keywords: conformational analysis · *Mycobacterium tuberculosis* · thymidine analogues · TMPK inhibitors

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