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DISCOVERY OF BICYCLIC THYMIDINE ANALOGUES AS SELECTIVE AND HIGH AFFINITY INHIBITORS OF *MYCOBACTERIUM TUBERCULOSIS* THYMIDINE MONOPHOSPHATE KINASE

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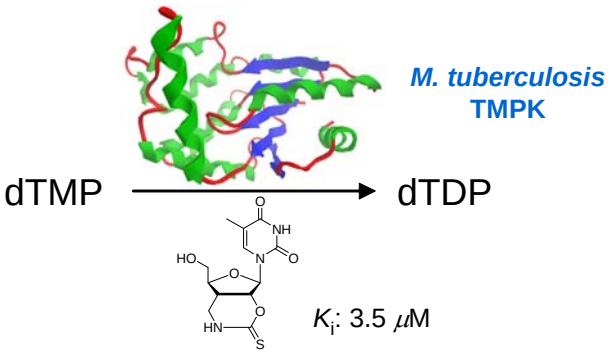

Abstract

Thymidine monophosphate kinase of *Mycobacterium tuberculosis* represents an attractive target for selectively blocking the 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 HIV and *M. tuberculosis* infection has caused a steeply rising incidence of tuberculosis during the last decades. This has made the development of new anti-tuberculosis 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 anti-tuberculosis agents.[2] TMPK catalyses 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 1.95Å 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.[5,6,7,8] 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'-CH_2OH, 3'-CH_2NH_2, 3'-CH_2N_3 and 3'-CH_2F 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*) (northern, 2'-*exo*-3'-*endo*) conformation [dTMP, in contrast, binds to TMPKmt in the (*S*) (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 favourable 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 towards the (*N*) conformation.[10] Based on these results and the high affinities of 2'-halogeno substituted nucleotides for TMPKmt[5] (**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 steps[11]) (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 DAST[15] and removal of the trityl protective group. Reduction of **5** and **7** with Ph_3P and NH_3 yielded the corresponding 3'-amino analogues **6** and **8**, respectively.[11] Due to unwanted intramolecular attack of the 2-carbonyl of thymine on the 5'-*O*-diethylaminosulphur difluoride intermediate, reaction of **7** with DAST afforded **9** in low yield.

In an attempt to prepare intermediate **20** for further derivatisation, the 5'-hydroxyl group of **14** was first protected as *t*-butyldimethylsilyl ether **18** (Scheme 2). After esterification of the 2'-hydroxyl with phenylchlorothionocarbonate (→**19**),[11] however, attempted simultaneous reduction of the azido function and Barton deoxygenation at the 2'-position to give **20** failed. Instead three peculiar nucleoside analogues **21**, **22** and **23** were formed. In a similar attempt, Robins *et al.* 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 uncharacterised byproducts.[16] Compounds **21** and **22** represent a novel class of bicyclic nucleoside analogues.

The formation of carbamates through reaction of thioacids with azides has recently been studied by Shangguan *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 carbonoxysulfide, arising from decomposition of the 2'-phenylthionocarbonate ester.[18]

The TBDMS-protective groups of **21**, **22** and **23** were removed with TBAF in 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 (→**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 using carbonyldiimidazole.

Conformational analysis

Conformational analysis was performed for compounds **6** and **7**. Based on the large vicinal 'pseudoaxial-axial' coupling between 3'-H and 4'-H ($^3J_{3',4'}$ = 10.5 Hz) and on 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 N- or S-conformer can be estimated by multiplying $^3J_{3',4}$ and $^3J_{1',2'}$ by a factor 10 respectively.[19] The finding that the 3'-α-CH_2X group apparently drives the pseudorotational N⇆S equilibrium towards the *N*-conformation even with a chlorine or fluorine substituent 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'-CH_2OH-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'-halo substitued 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 μM).

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 μM) was most unexpected. A modeling experiment in GOLD showed that the sugar ring of the first monomer **I** binds 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 towards 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 few hydrophobic interactions with Phe36, Ala35 and Tyr39. Comparison with the binding positions of ATP[4] and bisubstrate inhibitor Ap_5T[22] 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 not only a competitive inhibitor 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 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 interaction 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 towards the 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 modelled into the crystal structure of TMPKmt (Figure 3).[4] The six-membered ring, fused to the 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 sulphur atom, in its turn, undergoes hydrophobic interactions with Tyr103 and Tyr165 residues. When the sulphur 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 towards 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 μg/ml. This is not surprising since this concentration is lower then 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 towards 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 μM and a SI of 200, which will be used as starting compound to increase further affinity for TMPKmt.

Experimental Section

(1) Spectrophotometric binding assay.

TMPKmt and TMPKh activities were determined using 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 $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate and 2 units each of lactate dehydrogenase, pyruvate kinase and nucleoside diphosphate kinase. The concentrations of ATP and dTMP were kept constant at 0.5 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 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Assay (MABA).[23]

(3) Modeling: docking experiments using 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 sulphate counter ions were removed. The Mg^{2+} was considered as being part of the enzyme. Explicit hydrogen atoms were added to the enzyme and inhibitor structures using Reduce.[24] The inhibitor structures **10**, **12** and **13** were created using Macromodel 5.0,[25] based on the dTMP substrate from pdb entry 1G3U. Their geometry was optimized in the AM1 force field using Mopac6.0.[26] PDB files were then converted to mol2 files using 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 1G3U [4] and 1MRN.[22] Criteria for selection were the same position (but not necessarily the same orientation) of the base, the

nonbonded interactions as calculated by Ligplot[30] and HBPLUS.[31] Figures 1-3 were generated using Molscript.[32]

(4) Synthesis. General. NMR spectra were obtained with a Varian Mercury 300 or 500 spectrometer. Chemical shifts are given in ppm (δ) relative to residual solvent peak of DMSO-d_6 (2.5 ppm). All signals assigned to amino and hydroxyl groups were exchangeable with D_2O. Mass spectra and exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, UK) equipped with a standard electrospray ionisation (ESI) interface. Samples were infused in a 2-propanol:water (1:1) mixture at 3 μL/min. Precoated Merck silica gel F_{254} plates were used for TLC and spots were examined under UV light at 254 nm and revealed by sulfuric acid-anisaldehyde spray. 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) trityl chloride (786 mg, 2.82 mmol) was added in different portions. The mixture was heated to 65 °C and stirred overnight. Then it was diluted with CH_2Cl_2 (50 mL), washed with saturated aqueous $NaHCO_3$ (50 mL), and dried over anhydrous $MgSO_4$. The solvent was removed under diminished pressure and the resulting residue was purified by column chromatography (CH_2Cl_2-MeOH, 98:2) affording **15** (1.15 g, 92%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.42 (3H, d, J = 1.2 Hz, 5-CH_3), 2.50 (1H, m, H-3'), 3.13-3.22 (2H, m, H-5' and H-6'), 3.37 (1H, dd, J = 1.8 Hz 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 $C_{30}H_{29}N_5O_5Na$ $[M + Na]^+$: 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 CH_2Cl_2 (17 mL) was stirred at room temperature. After stirring for 30 minutes trifluoromethanesulfonyl chloride (0.37 mL, 3.50 mmol) was added to the cooled (4 °C) solution. After further stirring for 3h at the same temperature, the reaction was quenched with water (20 mL) and extracted with CH_2Cl_2 (20 mL). The organic layer was successively washed with an aqueous saturated solution of Na_2CO_3 (20 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The obtained residue was purified by column chromatography (CH_2Cl_2-MeOH, 97:3) to yield **16** (743 mg, 82 %) as a white foam.^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76 (3H, d, 5-CH_3), 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 $C_{30}H_{28}N_5O_4$ $[M + H]^+$: 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 purified by column chromatography (CH_2Cl_2-MeOH, 97:3), yielding **5** (90 mg, 71%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.74 (3H, s, 5-CH_3), 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-CH_3), 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 $C_{11}H_{14}ClN_5O_4Na$ $[M + Na]^+$: found, 338.0615; calcd, 338.0632. Anal. ($C_{11}H_{14}ClN_5O_4$) 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 1h, concentrated NH_4OH (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 (CH_2Cl_2-MeOH-7N NH_3 in MeOH, 90:7.5:2.5) to yield **6** (42 mg, 72 %) as a white foam. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.75 (3H, d, J = 1.2 Hz, 5-CH_3), 2.80 (1H, app sext, J = 5.4 Hz (5x), H-3'), 2.97 (2H, d, $J_{6'A/B,3'}$ = 6.6 Hz, H-6'$_{A+B}$), 3.31 (br, 5'-OH, NH_2, N^3-H), 3.73 (1H, dd, $J_{5'B,4'}$ = 2.0 Hz, $J_{5'B,5'A}$ = 13.2 Hz, H-5'$_B$), 3.89 (1H, dd, $J_{5'A,4'}$ = 2.4 Hz, $J_{5'A,5'B}$ = 13.2 Hz, H-5'$_A$), 4.04 (1H, dt, $J_{4',3'}$ = 10.5 Hz, $J_{4',5'}$ = 2.2 Hz (2x), 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) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.93 (5-CH_3), 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-4), 164.47 (C-2); HRMS (ESI-MS) for $C_{11}H_{17}ClN_3O_4$ $[M + H]^+$: found, 290.0911; calcd, 290.0907. Anal. ($C_{11}H_{16}ClN_3O_4$**.**$2H_2O$) 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), 1N NaOH (3.2 mL), dioxane (45 mL) and EtOH-H_2O (1:1, 45 mL) was stirred at room temperature for 2h. The solution was neutralized with HOAc/EtOH (1:1, v/v) to pH 7. The resulting mixture was extracted with CH_2Cl_2 (100 mL) and the organic layer was dried over anhydrous $MgSO_4$, evaporated under reduced pressure and purified by column

chromatography (CH_2Cl_2-MeOH, 90:10), yielding **17** (530 mg, 87 %) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.53 (3H, s, 5-CH_3), 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), DAST (0.59 mL, 4.42 mmol) was added 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 CH_2Cl_2 (50 mL). The organic layer was dried over anhydrous $MgSO_4$, evaporated under reduced pressure and purified by column chromatography (CH_2Cl_2-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. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.45 (3H, s, 5-CH_3), 2.85 (1H, m, $J_{3',F}$ = 33.0 Hz, H-3'), 3.20 (4H, m, H-5'/5" and H-6'/6"), 4.11 (1H, app d, $J_{4',F}$ = 9.6 Hz, H-4'), 5.40 (1H, dd, $J_{2',3'}$ = 3.9 Hz, $J_{2',F}$ = 52.2 Hz, H-2'), 5.87 (1H, d, $J_{1',F}$ = 21.6 Hz, H-1'), 7.32 (15H, m, arom H), 7.55 (1H, s, H-6); HRMS (ESI-MS) for $C_{30}H_{28}FN_5O_4Na$ $[M + Na]^+$: found, 564.2020; calcd, 564.2023.

The obtained foam was dissolved in 80% HOAc in H_2O (7 mL). The mixture was heated to 90°C during 1h. The solvent was removed under reduced pressure and the residue purified by column chromatography (CH_2Cl_2-MeOH, 90:10), yielding **10** (221 mg, 62% from **17**) as a white foam. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.75 (3H, d, J = 1.2 Hz, 5-CH_3), 2.64 (1H, ddddd, , $J_{3',F}$ = 33.1 Hz, $J_{3',2'}$ = 4.4 Hz, $J_{3',6'B}$ = 5.9 Hz, $J_{3',6'A}$ = 8.7 Hz, $J_{3',4'}$ = 10.5 Hz, H-3'), 3.50 (1H, ddd, $J_{6'B,F}$ = 1.4 Hz, $J_{6'B,3'}$ = 6.0 Hz, $J_{6'B,6'A}$ = 12.7 Hz, H-6'$_B$,), 3.57 (1H, dd, $J_{6'A,3'}$ = 8.7 Hz, $J_{6'A,6'B}$ = 12.7 Hz, H-6'$_A$), 3.61 (1H, ddd, $J_{5'B,4'}$ = 3.0 Hz, $J_{5'B,OH}$ = 4.9 Hz, $J_{5'B,5'A}$ = 12.7 Hz, H-5'$_B$), 3.84 (1H, ddd, H-5'$_A$ = 2.5 Hz, $J_{5'A,OH}$ = 5.6 Hz, $J_{5'A,5'B}$ = 12.7 Hz, $J_{5'A,4'}$), 3.99 (1H,

dt, $J_{4',3'}$ = 10.5 Hz, $J_{4',5'A/B}$ = 2.7 Hz (2x), H-4'), 5.30 (1H, t, $J_{OH,5'A/B}$ = 5.25 Hz, 5'-OH), 5.32 (1H, dd, $J_{2',3'}$ = 4.4 Hz, $J_{2',F}$ = 52.5 Hz, H-2'), 5.88 (1H, d, $J_{1',F}$ = 19.3 Hz, H-1'), 7.88 (1H, q, J = 1.2 Hz, H-6), 11.34 (1H, s, N^3-H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 12.29 (5-CH_3), 40.53 (d, C-3', $^2J_{3',F}$ = 18.5 Hz), 46.13 (d, $^3J_{6',F}$ = 7.8 Hz, C-6'), 59.76 (C-5'), 82.83 (C-4'), 88.80 (d, $^2J_{1',F}$ = 36.3 Hz, C-1'), 96.84 (d, $^1J_{2',F}$ = 181.6 Hz, C-2'), 108.73 (C-5), 136.03 (C-6), 150.29 (C-2), 163.94 (C-4) ppm; ^{19}F NMR (300 MHz, D_2O): δ -195.046; HRMS (ESI-MS) for $C_{11}H_{14}FN_5O_4Na$ $[M + Na]^+$: found, 322.0908; calcd, 322.092. Anal. ($C_{11}H_{14}FN_5O_4$) 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) using the procedure described for the synthesis of **6**, yielding 54 mg (77 %) of amine **8** as a foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.73 (3H, s, 5-CH_3), 2.30 (1H, m, $J_{3',F}$ = 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, $J_{4',F}$ = 10.2 Hz, H-4'), 5.03 (1H, br s, 5'-OH), 5.22 (1H, d, $J_{2',F}$ = 51.9 Hz, H-2'), 5.82 (1H, d, $J_{1',F}$ = 18.3 Hz, H-1'), 7.85 (1H, s, H-6); ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.95 (5-CH_3), 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 (C-1', $J_{1',F}$ = 36.3 Hz), 97.89 (C-2', $J_{2',F}$ = 179.4 Hz), 109.21 (C-5), 136.43 (C-6), 150.79 (C-2), 164.51 (C-4); ^{19}F NMR (300 MHz, D_2O): δ −195.70; HRMS (ESI-MS) for $C_{11}H_{17}FN_3O_4$ $[M + H]^+$: found, 274.1191; calcd, 274.1202. Anal. ($C_{11}H_{16}FN_3O_4$**.**1/2H_2O) 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) using the procedure described for the synthesis of **7**, to yield 12 mg (18 %) of **9**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (3H, s, 5-CH_3), 2.67 (1H, m, $J_{3',F}$ = 24.0 Hz, H-3'), 3.60 (2H, m, H-6'/6''), 4.61 (1H, ddd, $J_{5',4'}$ = 4.2 Hz, $J_{5',5''}$ = 10.8 Hz, $J_{5',F}$ = 49.5 Hz, H-5'), 4.80 (1H, dd, H-5''), 5.41 (1H, dd, $J_{2',3'}$ = 4.8 Hz, $J_{2',F}$ = 52.5 Hz, H-2'), 5.88 (1H, d, $J_{1',F}$ = 21.3 Hz, H-1'), 7.40 (1H, s, H-6); ^{13}C NMR (75 MHz,

DMSO-d_6): δ 12.87 (5-CH_3), under DMSO signal (C-3'), 46.68 (C-6'), 81.10 (C-4', $J_{4',F}$ = 18.1 Hz), 83.02 (C-5', $J_{5',F}$ = 170.0 Hz), 90.60 (C-1', $J_{1',F}$ = 38.0 Hz), 96.90 (C-2', $J_{2',F}$ = 181.2 Hz), 110.01 (C-5), 136.81 (C-6), 150.87 (C-2), 164.52 (C-4); ^{19}F NMR (300 MHz, D_2O): δ –194.04 and –228.74; HRMS (ESI-MS) for $C_{11}H_{13}F_2N_5O_3Na$ $[M + Na]^+$: found, 324.0881; calcd, 324.0884. Anal. ($C_{11}H_{13}F_2N_5O_3$) C, H, N.

1-[3-Aminomethyl-3-deoxy-5-*O*-(*tert*-butyldimethyl)silyl-2-*O*,6-*N*-(thiocarbonyl)-β-D-ribofuranosyl]thymine (21), 1-(3-aminomethyl-2-*O*,6-*N*-carbonyl-3-deoxy-5-*O*-(*tert*-butyldimethyl)silyl-β-D-ribofuranosyl)thymine (22) and 1,3-bis[(3*R*)-(3'-deoxy-5'-*O*-(*tert*-butyldimethyl)silyl-thymidin-3'-yl)methyl]urea (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 N_2. The reaction mixture was stirred at 95-100 °C for 5h. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography (CH_2Cl_2-MeOH, 99:1 $\rightarrow$ 95:5), affording produce **21** (0.16 mmol, 20 %), **22** (17 %) and **23** (19 %) as white solids. **21**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.00 (6H, s, $(CH_3)_2Si$), 0.80 (9H, s, $C(CH_3)_3$), 1.70 (3H, s, 5-CH_3), 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 $C_{18}H_{30}N_3O_5SSi$ $[M + H^+]$: found, 428.1675; calcd, 428.1675.

22: ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.00 (6H, s, $(CH_3)_2Si$), 0.81 (9H, s, $C(CH_3)_3$), 1.70 (3H, s, 5-CH_3), 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 $C_{18}H_{30}N_3O_6Si$ $[M + H]^+$: found, 412.1897; calcd, 412.1903.

23: ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.15 (12H, s, $(CH_3)_2Si$), 0.83 (18H, s, $(CH_3)_3C$), 1.73 (6H, s, 5-CH_3), 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 $C_{35}H_{60}N_6O_9Si_2Na$ $[M + Na]^+$: 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 equivalents TBAF in THF. After 1 hour the mixture was evaporated to dryness and purified by column chromatography (CH_2Cl_2-MeOH, 95:5), yielding **10** as a white powder (47 mg, 92%).^{1}H NMR (300 MHz, DMSO-d_6): δ 1.73 (3H, s, 5-CH_3), 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); ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.89 (5-CH_3), 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 $C_{12}H_{15}N_3O_5SNa$ $[M + Na]^+$: found, 336.0636; calcd, 336.0630. Anal. ($C_{12}H_{15}N_3O_5S$) C, H, N.

1-(3-Aminomethyl-2-*O*,6-*N*-carbonyl-3-deoxy-β-D-ribofuranosyl)thymine (11)

11 was prepared from **22** (60 mg, 0.15 mmol) using the procedure as described for the synthesis of **10**, yielding **11** (39 mg, 90 %) as a white powder.^{1}H NMR (300 MHz, DMSO-d_6): δ 1.74 (3H, s, 5-CH_3), 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); ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.88 (5-CH_3), 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 $C_{12}H_{15}N_3O_6Na$ $[M + Na]^+$: found, 320.0856; calcd, 320.0858. Anal. ($C_{12}H_{15}N_3O_6$) C, H, N.

1,3-Bis[(3*R*)-(3'-deoxythymidin-3'-yl)methyl]urea (12)

12 was prepared from **23** (120 mg, 0.16 mmol) using the procedure as described for the synthesis of **10**, yielding **12** (80 mg, 93 %) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (6H, s, 5-CH_3), 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); ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.96 (5-CH_3), 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 (NH*CO*NH), 164.47 (C-4); HRMS (ESI-MS) for $C_{23}H_{32}N_6O_9Na$ $[M + Na]^+$: found, 559.2121; calcd, 559.2128. Anal. ($C_{23}H_{32}N_6O_9$) 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 1h concentrated NH_4OH (8.5 mL) was added, and the solution was allowed to stir for an additional 2h. 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 NH_3 in CH_2Cl_2-MeOH, 90:10) to yield the title compound (415 mg, 97 %) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.37 (3H, s, 5-CH_3), 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 H_2O 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 $C_{30}H_{31}N_3O_5Na$ $[M + Na]^+$: 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 (CH_2Cl_2-MeOH, 95:5) to yield **25** as a white powder (162 mg, 75 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.44 (3H, d, J = 1.2 Hz, 5-CH_3), 2.90 (1H, d, $J_{6', 6''}$ = 13.8 Hz, H-6'), 3.02 (1H, m, H-3'), H-5' hidden by residual H_2O 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 $C_{31}H_{29}N_3O_5SNa$ $[M + Na]^+$: found, 578.1825; calcd, 578.1725.

1-[3-Aminomethyl-3-deoxy-2-*O*,*N*-(thiocarbonyl)-β-D-ribofuranosyl]thymine (10) from 25. Compound **24** (148 mg, 0.26 mmol) was dissolved in 80% HOAc in H_2O (6mL). The mixture was heated to 90 °C during 1h. The solvent was removed under reduced pressure and the residue purified by column chromatography (CH_2Cl_2-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**.[9] ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76 (3H, d, 5-CH_3), 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), ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.95 (5-CH_3), 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 $C_{11}H_{16}N_5O_4$ $[M + H]^+$: found, 282.1205; calcd, 282.1202. Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

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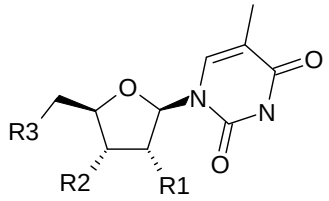
Table 1. Kinetic parameters of TMPKmt and TMPKh with compounds **1-13**.



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_2N_3	OH	40[9]	1040[9]	26.0
2	H	CH_2NH_2	OH	57[9]	220[9]	3.9
3	F	OH	OPO_3^{2-}	43[5]	n. d.	n. d.
4	Cl	OH	OPO_3^{2-}	19[5]	n. d.	n. d.
5	Cl	CH_2N_3	OH	180	n. d.	n. d.
6	Cl	CH_2NH_2	OH	390	n. d.	n. d.
7	F	CH_2N_3	OH	165	n. d.	n. d.
8	F	CH_2NH_2	OH	n.i. at 1 mM	n. d.	n. d.
9	F	CH_2N_3	F	80	n. d.	n. d.
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	n.i. at 1mM	n. d.
13[b]	H	CH_2N_3	OH	29	n.i.at 2 mM	n. d.

[a] K_m-value; [b] Thymine is oriented at the α-side of the sugar ring.

n.i. no inhibition; n.d. not determined

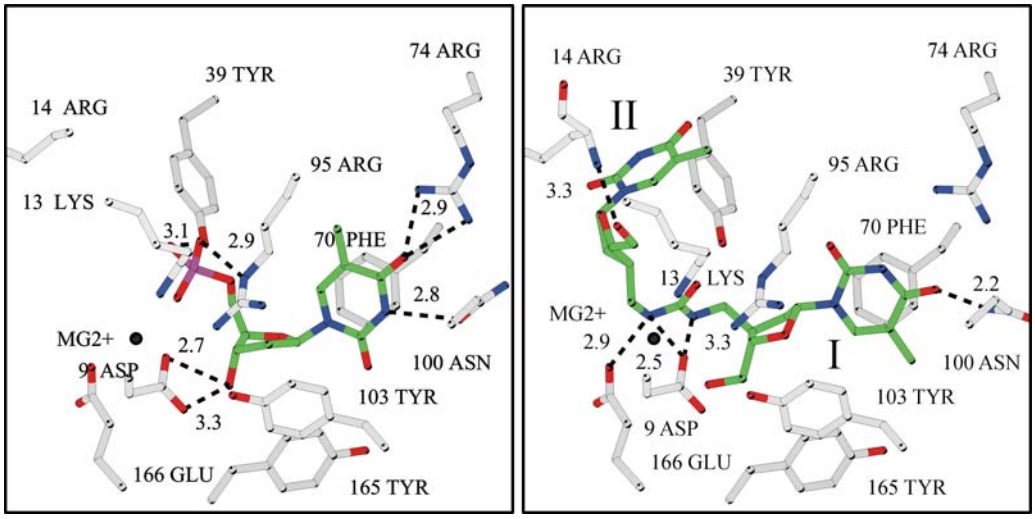
FIGURE LEGENDS

Figure 1. Comparison of the binding mode of dTMP with the binding mode of compound **12**. a) 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 of which atoms make hydrophobic contact with **12**, based on the 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 of which atoms make hydrophobic contact with **13**, based on a ligplot analysis, are Leu52, phe70, Arg95, Tyr103 and Tyr165.

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

Figure 1


74 ARG
39 TYR
14 ARG
95 ARG
13 LYS
3.1
2.9
70 PHE
2.9
2.8
MG2+
2.7
9 ASP
100 ASN
103 TYR
3.3
166 GLU
165 TYR
14 ARG
II
39 TYR
74 ARG
3.3
95 ARG
70 PHE
13 LYS
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2.9
2.5
3.3
I
100 ASN
103 TYR
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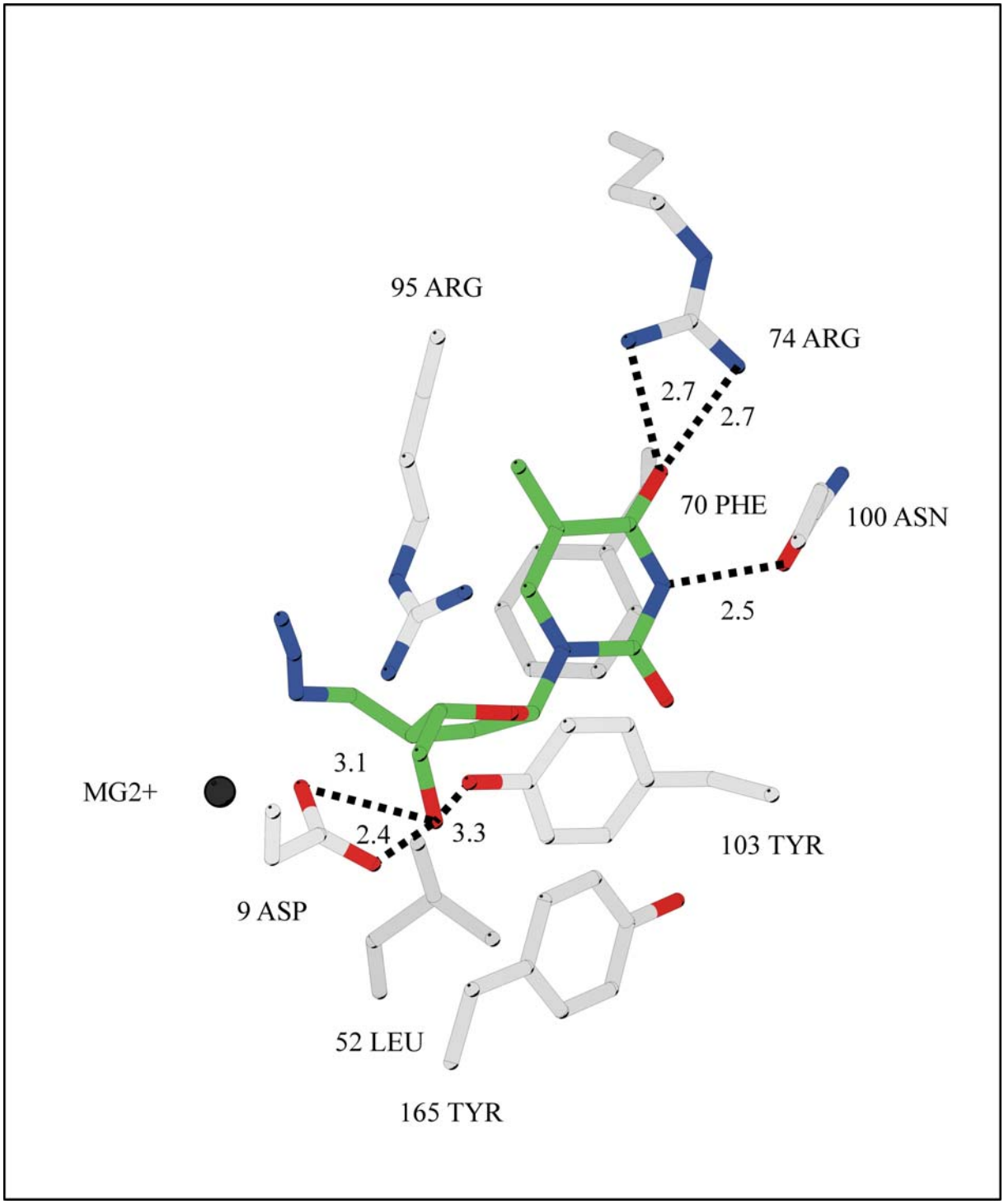
166 GLU
165 TYR

Figure 2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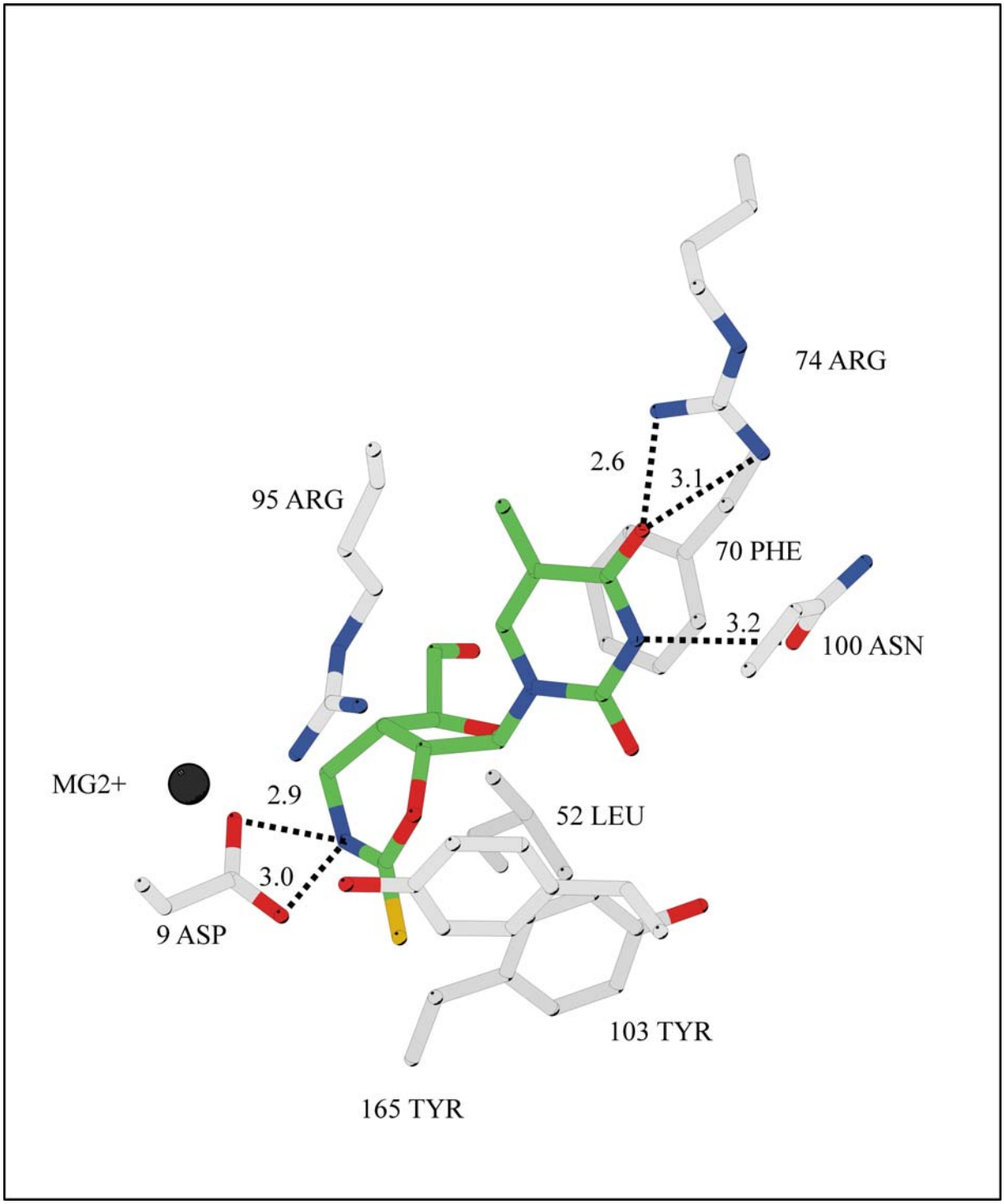
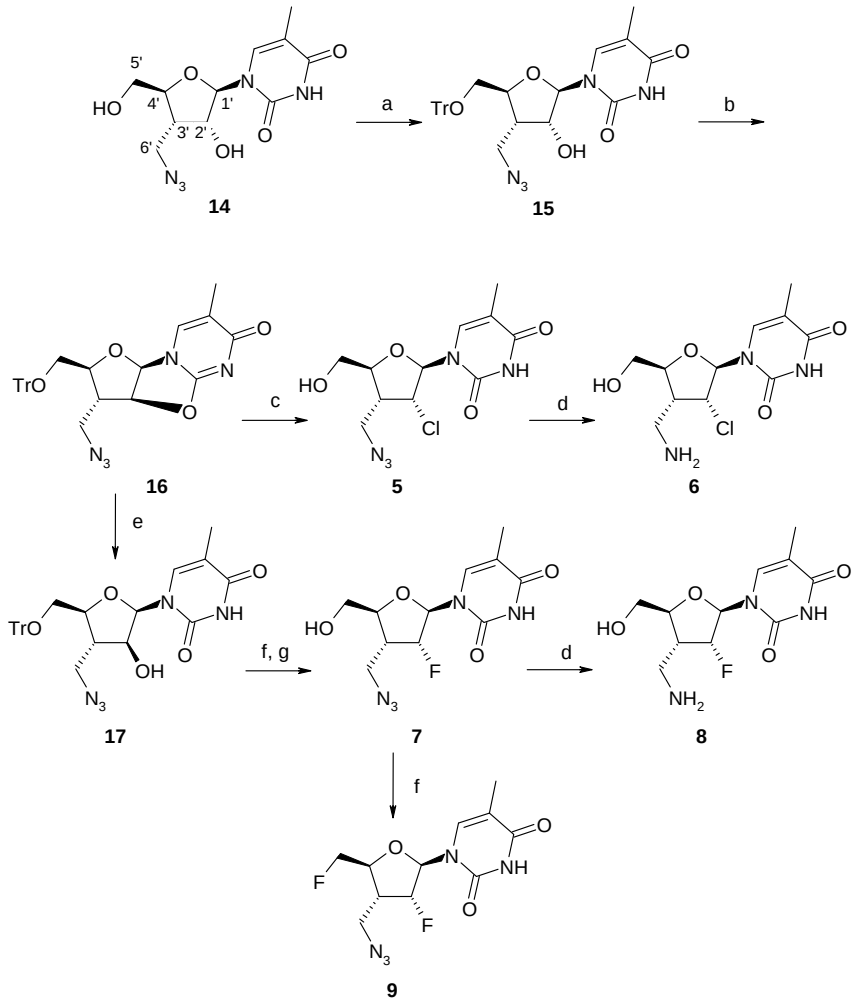
74 ARG
2.7
2.7
70 PHE
100 ASN
2.5
3.1
MG2+
2.4
3.3
103 TYR
9 ASP
52 LEU
165 TYR

Figure 3

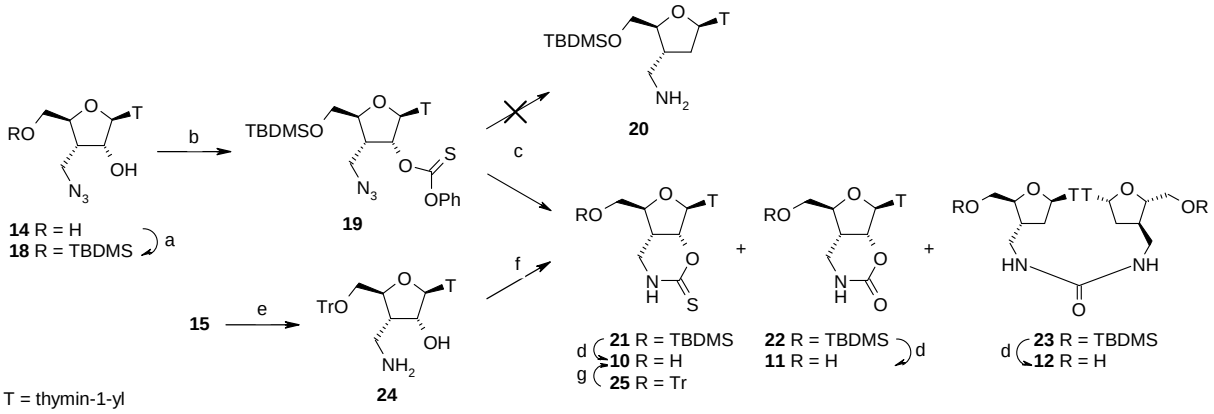

74 ARG
2.6
3.1
95 ARG
70 PHE
3.2
100 ASN
MG2+
2.9
52 LEU
3.0
9 ASP
103 TYR
165 TYR

Scheme 1



Reagents: (a) trityl chloride, DMAP, pyridine; (b) trifluoromethanesulfonyl chloride, DMAP, CH_2Cl_2; (c) HCl, dioxane; (d) Ph_3P, NH_4OH, pyridine; (e) NaOH, EtOH, H_2O; (f) DAST, toluene, pyridine; (g) 80% HOAc in H_2O.

Scheme 2



Reagents: (a) *t*-butyldimethylsilyl chloride, $AgNO_3$, pyridine, THF; (b) phenyl chlorothionocarbonate, DMAP, CH_3CN; (c) AIBN, Bu_3SnH, toluene; (d) n-Bu_4NF, THF; (e) Ph_3P, NH_4OH, pyridine; (f) 1,1-thiocarbonyldiimidazole, DMF; (g) 80% HOAc in H_2O.

Elemental analysis:

	Calculated			**Found**		
	C	**H**	**N**	**C**	**H**	**N**
5	41.85	4.47	22.18	41.78	4.81	21.74
6 + H_2O	42.93	5.90	13.65	42.68	5.98	13.31
7	44.15	4.72	23.40	44.14	4.96	23.04
8 + H_2O	45.36	6.23	14.43	45.39	6.01	14.10
9	46.00	4.83	13.41	45.67	4.84	13.21
10	43.86	4.35	23.25	43.74	4.32	23.16
11	48.49	5.09	14.14	48.61	5.31	13.92
12	51.49	6.01	15.66	51.48	5.78	15.34
13	46.97	5.38	24.90	46.95	5.24	24.63