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Authors: Van Poecke, Sara; Munier-Lehmann, Hélène; Helynck, Olivier; Froeyen, Matheus and Van Calenbergh, Serge

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Synthesis and inhibitory activity of thymidine analogues targeting *Mycobacterium tuberculosis* Thymidine Monophosphate Kinase

Sara Van Poecke,[a] Hélène Munier-Lehmann,[b,c] Olivier Helynck,[b,c] Matheus Froeyen,[d] and Serge Van Calenbergh[a,*]

[a]*Laboratory for Medicinal Chemistry,Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium,*

[b]*Institut Pasteur, Unité de Chimie et Biocatalyse, 28 rue du Dr Roux, 75724 Paris Cedex 15, France.*

[c]*CNRS URA2128, 28 rue du Dr Roux, France*

[d]*Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

Keywords: TMPKmt, thymidine analogues, α- and β-nucleosides, spectrophotometric binding assay, inhibitory activity

*correspondence to Prof. Serge Van Calenbergh, Ghent University, Faculty of Pharmaceutical Sciences, Laboratory for Medicinal Chemistry, Harelbekestraat 72, B-9000 Gent, Belgium, Tel 09 264 81 24, Fax 09 264 81 46.

Abstract

We report on *Mycobacterium tuberculosis* thymidine monophosphate kinase (TMPKmt) inhibitory activities of a series of new 3'- and 5'-modified thymidine analogues including α- and β-derivatives. In addition, several analogues were synthesized in which the 4-oxygen was replaced by a more lipophilic sulfur atom to probe the influence of this modification on TMPKmt inhibitory activity. Several compounds showed an inhibitory potency in the low micromolar range, with the 5'-arylthiourea 4-thio-α-thymidine analogue being the most active one (K_i = 0.17 μM). This compound was capable of inhibiting *mycobacteria growth a*t a concentration of 25 μg/mL.

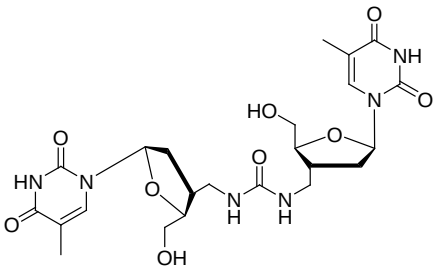
1. Introduction

Worldwide, tuberculosis (TB) remains one of the leading causes of death from infectious diseases. About one third of the world's population is infected with *Mycobacterium tuberculosis* that causes TB. On average 5-10% of these carriers become sick or infectious at some time during their life. Annually, more than 9 million new cases are reported and TB claims almost 2 million lives each year.[1]

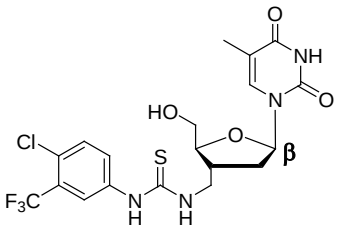
TB forms a lethal combination with HIV, each speeding the other's progress. TB is a leading cause of HIV-related deaths worldwide. In 2008, there were an estimated 1.4 million new cases of TB among persons with HIV infection and TB accounted for 23% of AIDS-related deaths. The global resurgence of TB due to HIV infection and the rapid emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of TB bacilli underscore the importance of developing new antimycobacterial drugs against TB.[2]

Recently, thymidine monophosphate kinase of *M. tuberculosis* (TMPKmt)[3] was put forward as an attractive target for new antituberculosis agents.[4] TMPK catalyzes the conversion of dTMP to dTDP using ATP as phosphate donor and is crucial for maintaining the thymidine triphosphate pools required for DNA synthesis and replication of bacteria. TMPK acts at the junction of the de novo and salvage pathways for the synthesis of deoxythymidine triphosphate (dTTP), which is indispensable for growth and survival. Therefore, TMPK represents a promising target for developing new TB drugs. Experiments with TMPK-deficient mutant of *Saccharomyces cerevisiae* underscore the criticality of this enzyme for DNA replication and cellular growth.[5]

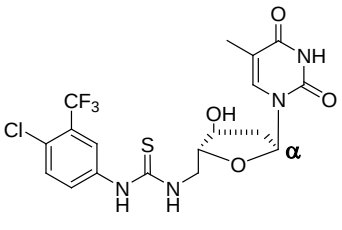
Although the global folding of TMPKmt is similar to that of other TMPKs, the configuration of its active site is unique. Compared to the human isozyme TMPKmt is peculiar in that it is competitively inhibited by AZT-MP (K_i = 10 μM), making the latter an attractive starting point for the design of selective inhibitors.[3,6]



1: K_i (TMPKmt) = 37 μM



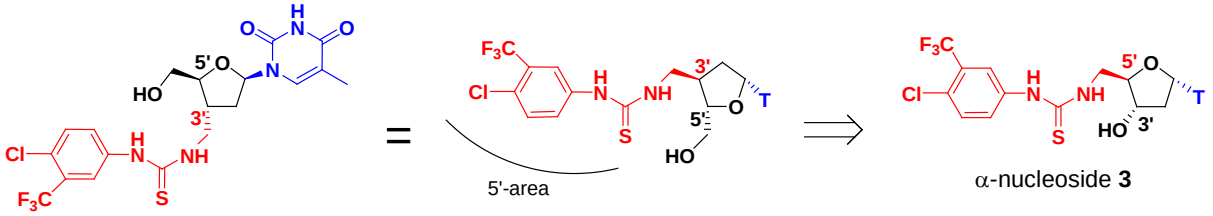
2: K_i (TMPKmt) = 5.0 μM



3: K_i (TMPKmt) = 0.6 μM

Chart 1

On the basis of the structure of a dinucleoside **1** (Chart 1), discovered by chance to produce significant inhibition of TMPKmt (K_i = 37 µM),[7] we have prepared a series of 3'-*C*-arylthiourea derivatives of β-D-thymidine, which led to the arylthiourea analogue **2** (K_i = 5.0 µM).[8] Modeling experiments suggested a binding mode for these 3'-*C*-arylthiourea analogues that differs from that of the natural substrate in that the sugar ring of the thymidine moiety is tilted over 180° compared to that of dTMP, thereby positioning the aromatic 3'-substituent into the phosphoryl donor binding area and the nucleobase below the sugar plane (Figure 1).



β-nucleoside **2**

α-nucleoside **3**

Figure 1. Suggested inverse sugar binding of 3'-C-arylthiourea-modified β-thymidine **2** and anticipated similar relative orientation of the colored moieties in 5'-deoxy-5'-arylthiourea modified α-thymidine **3**.

This unusual binding mode led us to explore if an alternative sugar scaffold could be used to impose a similar relative orientation of the thymine and the phenylthiourea moieties for TMPKmt inhibition. It was hypothesized that an α-nucleoside in which the 5'-position served as the thiourea anchor might fulfill this criterion. From a small library of easily accessible 5'-*N*-arylthiourea derivatives of α-thymidine, **3** emerged as one of the most potent TMPKmt inhibitors to date with a K_i of 0.6 µM, a selectivity index (versus TMPKh) of 600, and good inhibitory activity on the growing *M. bovis* (MIC_{99} 20 µg/mL) and *M. tuberculosis* (39% inhibition at 6.25 µg/mL) strains.[8] Next to the relative orientation between the aryl moiety and the nucleobase, structural exploration of the α-thymidine derivatives revealed the importance for aromatic residues at the 5'-position and the positive impact of electronic-withdrawing and lipophilic substituents on the aryl moiety for optimal inhibition of TMPKmt.

In this contribution we report on the TMPKmt inhibitory activities of a series of new thymidine analogues. Analogues **4** and **5** represent close analogues of 3'-*C*-arylthiourea **2**, in which the methylene group between C-3' and the (thio)urea group has been omitted.[9] Analogues **8**-**11**, derived from AZT (**6**), were selected to investigate if a 1,4-disubstituted 1,2,3-triazole motif can act as a bioisostere for the 3'-*C*-thiourea linker of **2** as previously found to be the case for TK-2 inhibition.[10] The aminotetrazole isomers **12** and **13** were recently synthesized in the context of TK-2 inhibition[11] and are also characterized by the presence of a heterocyclic linker to connect the aromatic moiety to position 3 of the 2'-deoxyribofuranose ring.

To assess the inhibitory activity of anomeric variants of **3**, its β-anomer **14**, as well as two heterocyclic analogues **15** and **16** are included in this study. Compounds **18**-**20** are derived from 5'-azido-5'-deoxy-α-D-thymidine (**17**) and synthesized in an effort to improve the activity of compound **3**. To further

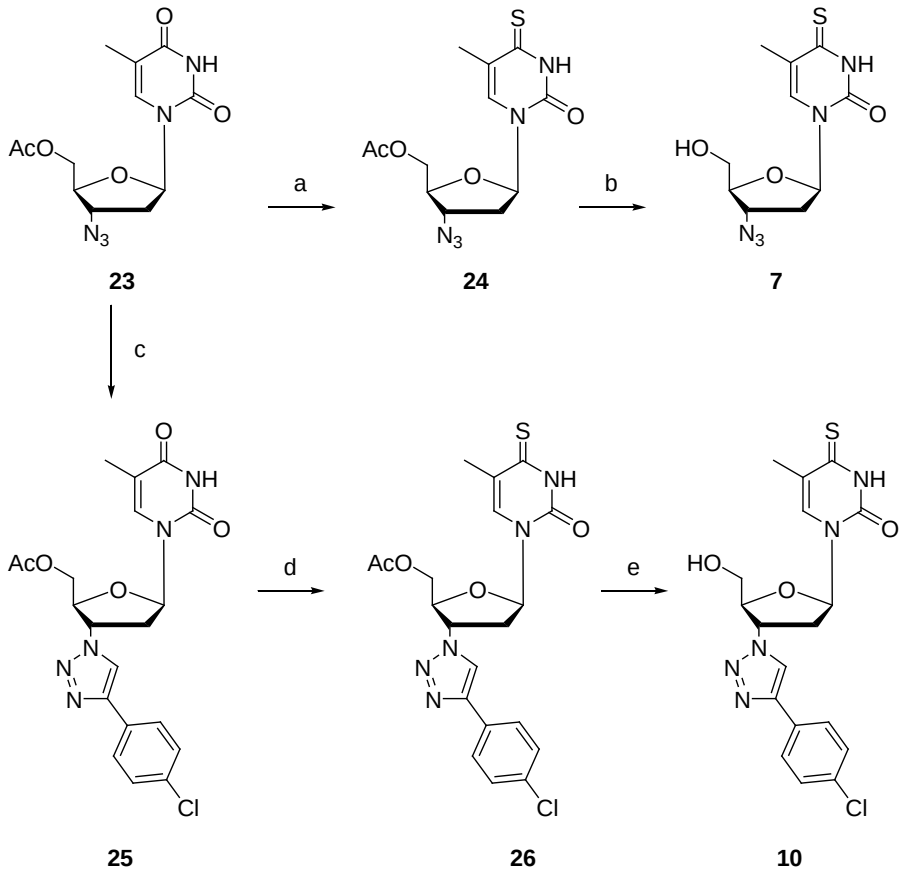
investigate the influence of the relative orientation between the aryl moiety and the nucleobases, compound **21**, which is the α-analogue of **9**, was synthesized and evaluated.

Based on earlier reports of 4-thiothymidine analogues showing promising antimycobacterial potency against *M. bovis* and *M. tuberculosis* in vitro and thus capable of entering the bacillus,[12] several analogues were synthesized in which the 4-oxygen of the thymine moiety was replaced by a more lipophilic sulfur atom (e.g., **7**, **10, 19** and **20**) to probe the influence of this modification on TMPKmt inhibitory activitity.

2. Results and discussion

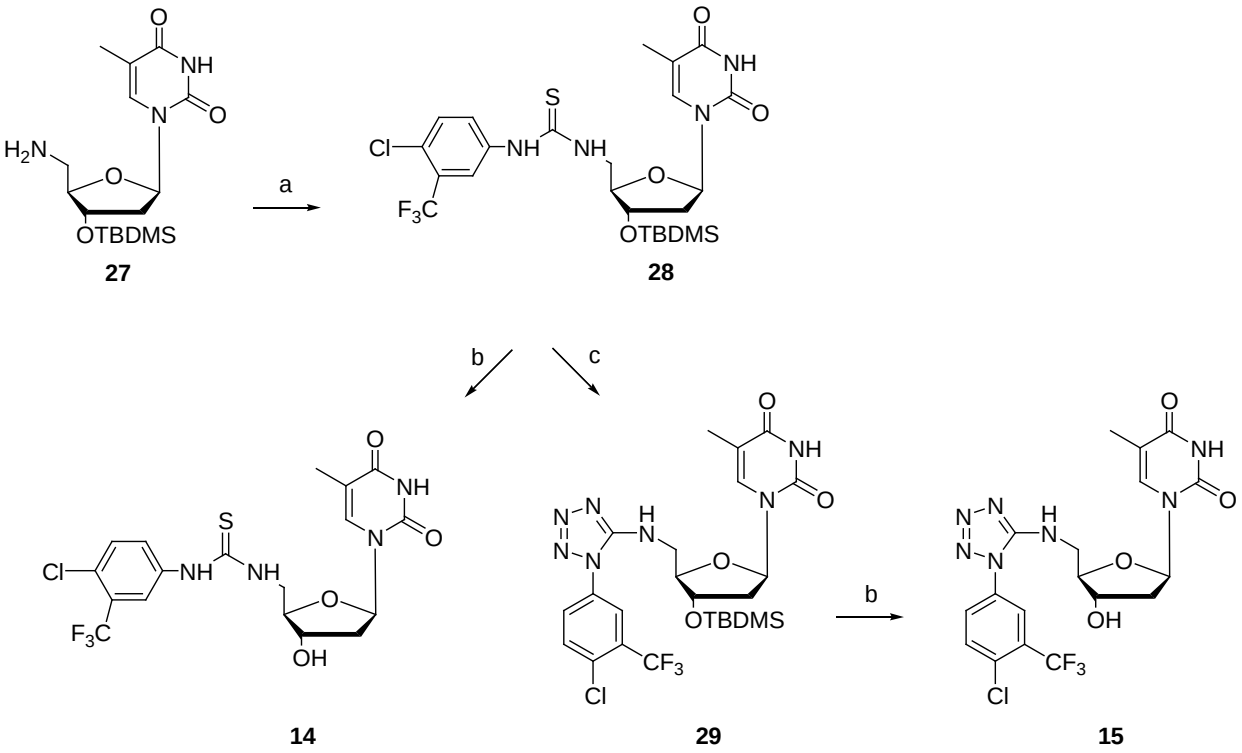
2.1. Chemistry

With the exception of compounds **7**, **10, 14-16, 18-20 and 22**, the chemical synthesis of all other final compounds has been reported before.[9,10,11] For the preparation of 4-thio-AZT (**7**), 5'-*O*-acetylated AZT **23**[13] was treated with Lawesson's reagent to generate the corresponding 4-thio pyrimidine **24**, followed by hydrolysis of the acetate ester (Scheme 1). A CuAAC reaction[14,15] between **23** and 1-chloro-4-ethynylbenzene, followed by thionation of the resulting 1,4-disubstituted 1,2,3-triazole **25** gave analogue **10** after final deprotection.



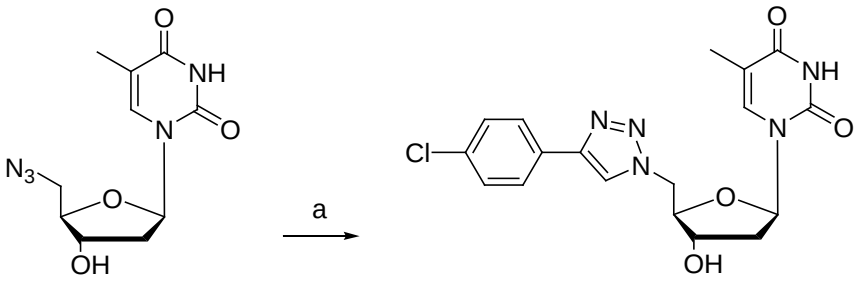
Scheme 1. Reagents and conditions. (a) Lawesson's reagent, toluene, 80 °C, overnight, 20%; (b) 7N NH_3 in MeOH, rt, 6 h, 42%, (c) 1-chloro-4-ethynylbenzene, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*-BuOH 2:1, rt, 24 h, 41%; (d) Lawesson's reagent, toluene, 80 °C, overnight, 49%; (e) 7N NH_3 in MeOH, rt, 6 h, 66%.

Coupling of amine **27**[16] with 4-chloro-3-(trifluoromethyl)phenyl isothiocyanate gave thiourea derivative **28** which was deprotected using TBAF in THF. A mercury(II)-promoted reaction of **28** with NaN_3 and Et_3N gave access to aminotetrazole **15** after final desilylation (Scheme 2).[11]



Scheme 2. Reagents and conditions: (a) 4-chloro-3-(trifluoromethyl)phenyl isothiocyanate, DMF, 0 °C → rt, 1 h, 76%; (b) 1M TBAF in THF, THF, rt, 1 h, 53-60%; (c) NaN_3, $HgCl_2$, Et_3N, DMF, 0 °C → rt, overnight, 83%.

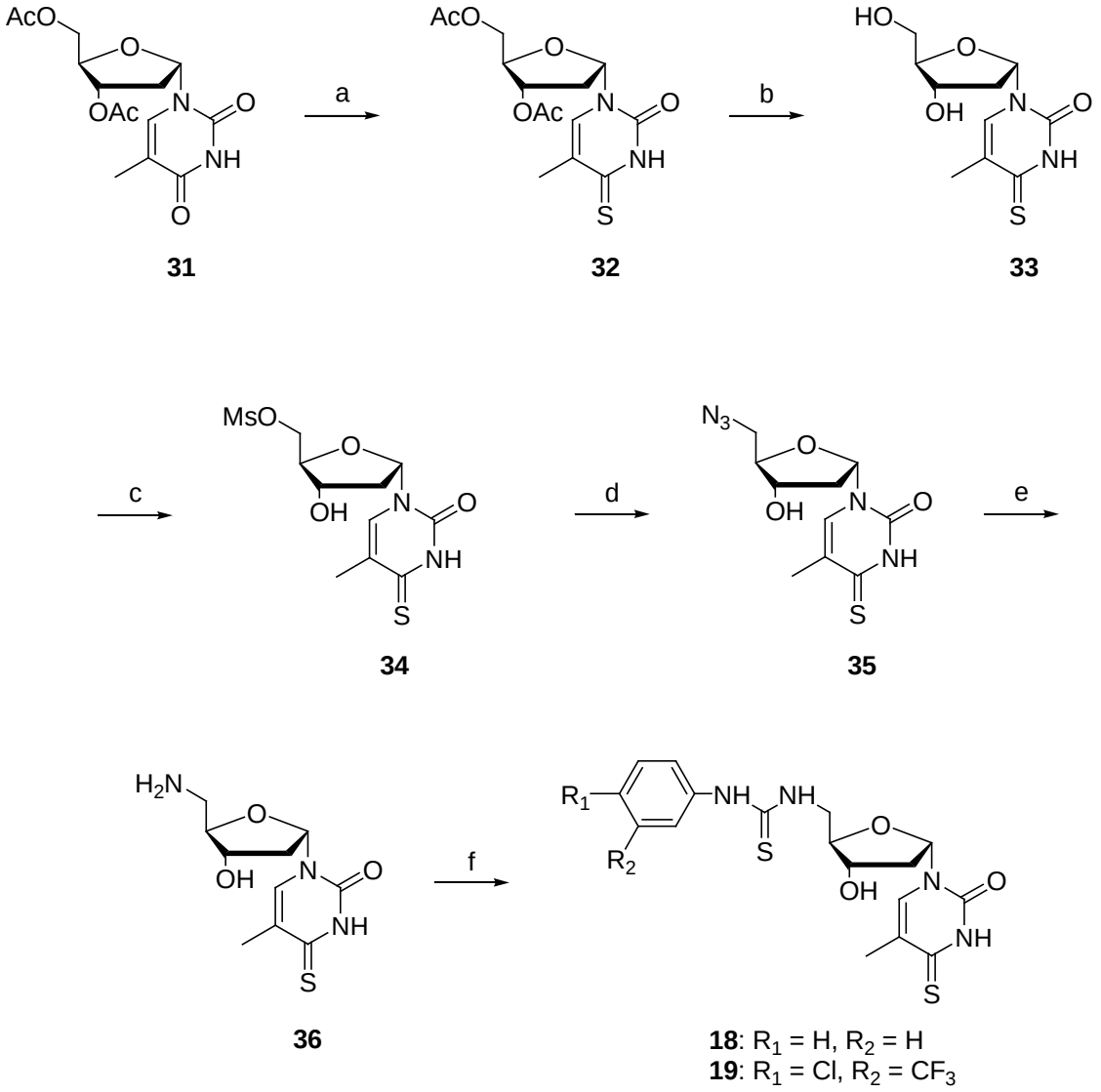
The synthesis of the 5'-substituted β-thymidine analogue **16** started from 5'-azido-5'-deoxy-β-thymidine[17] (Scheme 3). "Click chemistry" followed by HPLC purification allowed to isolate enough pure material of **16** for testing.



30 **16**

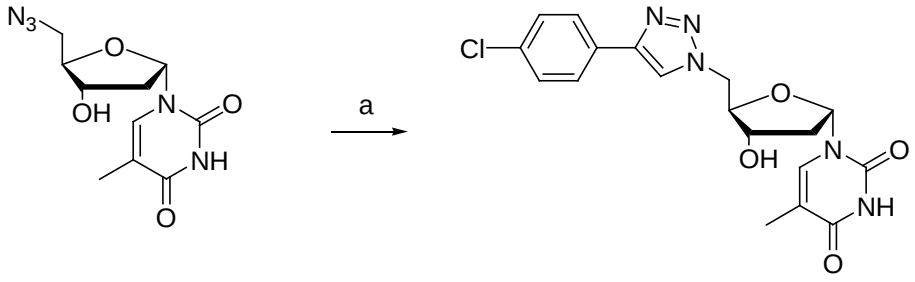
Scheme 3. Reagents and conditions: (a) 1-chloro-4-ethynylbenzene, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*-BuOH 1:2, rt, 7 d, 2%.

For the synthesis of compounds **18** and **19**, 3',5'-*O*-diacetyl-α-D-thymidine **31**[18] was first thionated using Lawesson's reagent followed by deprotection and conversion to the monomesylate ester **34** (Scheme 4). Upon treatment with NaN_3, **34** was converted into azide **35**, which was reduced to afford the 5'-amino-5'-deoxy-4-thio-α-D-thymidine **36**. Final treatment of this amine with the appropriate isothiocyanate analogues afforded derivatives **18** and **19**.



Scheme 4. Reagents and conditions. (a) Lawesson's reagent, anhydrous 1,4-dioxane, reflux, 4 h; (b) 7N NH_3 in MeOH, rt, 4 h, 37% over 2 steps; (c) MsCl, pyridine, -78 °C → 0 °C, 1 h, 70%; (d) NaN_3, DMF, 60 °C, overnight, 89%; (e) PPh_3, THF, H_2O, rt, 1 d, 89%; (f) appropriate isothiocyanate, DMF, 0 °C → rt, 3 h, 42-69%.

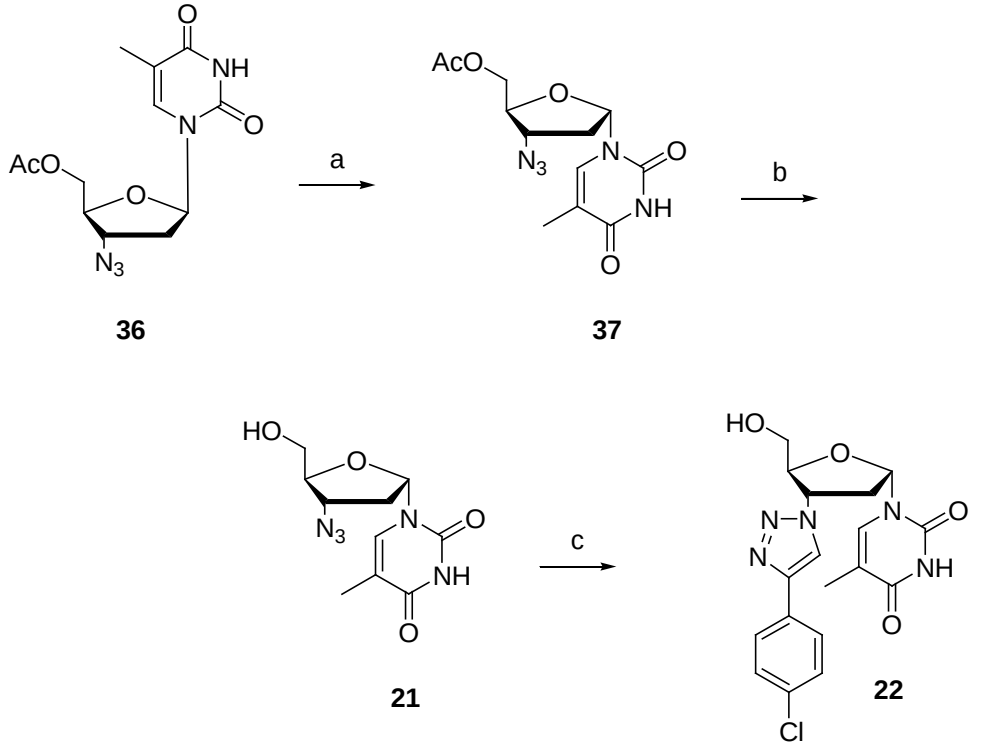
Starting from 5'-azido-5'-deoxy-α-D-thymidine **17**,[8] compound **20** was synthesized using the same method as described for triazole **16** (Scheme 5).



17 **20**

Scheme 5. Reagents and conditions. (a) 1-chloro-4-ethynylbenzene, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*-BuOH 2:1, rt, 4 d, 31%.

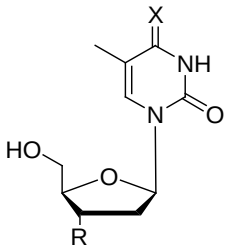
The synthesis of 3'-modified α-thymidine analogue **21** started with the anomerisation of 5'-*O*-acetylated AZT **36**.[13] Deprotection of **37** followed by CuAAC with 1-chloro-4-ethynylbenzene afforded triazole **21** in moderate yield (Scheme 6).



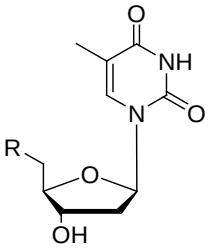
36 **37** **21** **22**

Scheme 6. Reagents and conditions: (a) acetic anhydride, H_2SO_4, CH_2Cl_2, rt, 2 h, 20% (b) 7N NH_3 in MeOH, rt, overnight, 72%, (c) 1-chloro-4-ethynylbenzene, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*-BuOH 1:3, rt, 24 h, 47%.

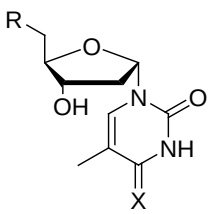
Table 1. Kinetic Parameters of TMPKmt with Compounds **3-22**.



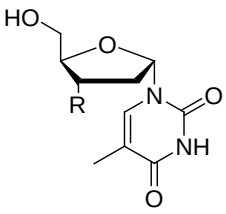
4-13



14-16



3, 17-20



21-22

Compound	X	R	K_i (μM) TMPKmt	K_i (μM) TMPKh	SI (K_i TMPKh/ K_i TMPKmt)	MIC_{99} *M. bovis* (μg/mL)
3	O	3-CF_3-4-Cl-phenylthiourea	0.6			
4	O	3-CF_3-4-Cl-phenylurea	2.8	95	34	
5	O	3-CF_3-4-Cl-phenylthiourea	9.9			
6 (AZT)	O	N_3	28			
7	S	N_3	≥ 100			
8	O	4-(Phenyl)-triazol-1-yl	4.2			
9	O	4-(*p*-Chlorophenyl)-triazol-1-yl	2.1			
10	S	4-(*p*-Chlorophenyl)-triazol-1-yl	15			
11	O	4-(Benzyl)-triazol-1-yl	2.7	N.I.[b]	> 100	
12	O	1-(3-CF_3-4-Cl-phenyl)-tetrazol-5-amine	45			
13	O	5-(Aminobenzyl)-tetrazol-1-yl	2.3	N.I.[b]	> 100	
14	O	3-CF_3-4-Cl-phenylthiourea	14.5			
15	O	1-(3-CF_3-4-Cl-phenyl)-tetrazol-5-amine	73			
16	O	4-(*p*-Chlorophenyl)-triazol-1-yl	201			
17	O	N_3	26.5			
18	S	Phenylthiourea	N. I.[a]			>> 100
19	S	3-CF_3-4-Cl-phenylthiourea	0.17	N.I.[a]	> 100	25
20	O	4-(*p*-Chlorophenyl)-triazol-1-yl	9			
21	O	N_3	6			
22	O	4-(*p*-Chlorophenyl)-triazol-1-yl	35			

N.I.: no inhibition detected at a final concentration of (a) 0.05 mM and (b) 1 mM.

2.1 Biological evaluation

All compounds have been evaluated for TMPKmt inhibition as described in the Experimental Section and results are summarized in Table 1. Replacement of the 3'-azido group of AZT (**6**) by a 3-CF_3-4-Cl-phenylurea substituent (**4**) resulted in a 10-fold increased activity, while this trend was less pronounced with the thiourea analogue **5**. In the 1,4-substituted 1,2,3-triazole series, the anti-TMPKmt activity was clearly influenced by the nature of the substituent at C-4 of the triazole. The click product of AZT and phenylacetylene (**8**) proved to be more potent than AZT itself. *p*-Chloro-substitution of the phenyl ring of **8** or introduction of a methylene between the triazole and the phenyl caused a moderate increase in activity (**11**). In this series of 3'-modified thymidine analogues, replacement of the oxygen at position 4 of the thymine moiety by a sulfur typically led to a significant drop in affinity for the target enzyme (compare couples **6/7** and **9/10**). Compounds **12** and **13**, both containing a 1,5-disubstituted tetrazole, significantly differed in their capacity to inhibit TMPKmt. The aminotetrazole analogue **13**, in which the tetrazole ring is directly attached to the sugar ring, showed a significantly better activity compared to analogue **12** in which the tetrazole ring is connected to C-3' via a NH-bridge. Remarkably, an opposite trend was observed for these tetrazole analogues on mitochondrial thymidine kinase 2.[11]

The inhibitory activity of a series of 5'-modified β-thymidine analogues appeared to be weak. Introduction of a 3-CF_3-4-Cl-phenylthiourea substituent (**14**) gave micromolar inhibition, while replacement of the thiourea by a 1-(3-CF_3-4-Cl-phenyl)-tetrazol-5-amine (**15**) or a 4-(*p*-chlorophenyl)-triazol-1-yl (**16**) caused a 5 and 14-fold drop in K_i value, respectively. Comparison of the anomeric couples **3**/**14** and **16**/**20** demonstrate that the α-anomers, which feature a trans orientation of the nucleobase and the 5'-substituent, exhibit superior TMPKmt inhibition compared to their β-epimers (factor 22-24).

The 5'-modified α-thymidine analogues (**3**, **17-20**) demonstrated moderate to excellent inhibitory activity for TMPKmt. Also in this small series, the activity is influenced by the nature of the substituent at the 5'-position. Derivative **17**, containing an azide function, gave moderate inhibition with a strikingly comparable K_i value (26.5 µM) as its AZT counterpart (28 µM). As observed in the 3'-modified β-series, conversion of the 5'-azide moiety by a 4-(*p*-chlorophenyl)-1,2,3-triazol-1-yl substituent improved the inhibitory activity, although to a lesser extent. Most interestingly and in contrast to what was observed in the 3'-modified β-series, substitution of the 4-*O* of the original hit **3** by a 4-*S*, increased the activity by a factor 3, ranking compound **19** amongst the most potent TMPKmt inhibitors together with the (*Z*)-butenylthymines with a naphtholactam or naphthosultam moiety at position 4 (K_i values of 0.42 and 0.27 µM, respectively).[19]

In addition, α-analogue **22**, which was synthesized to further assess the influence of the relative orientation between the aryl moiety and the nucleobases, showed poor inhibitory activity against TMPKmt, indicating that the preferred trans-orientation of the base and the aromatic substituent also holds for the 3'-modified analogues.

Compound **19** was evaluated for its in vitro inhibitory activity against *Mycobacterium bovis* BCG. It showed 100% inhibition of bacterial growth at a concentration of 25 μg/mL.

In an effort to rationalize the 3-fold increase in affinity upon replacement of the 4-O of the original hit **3** by a 4-S (**19**), both compound were docked into the substrate binding site of the TMPKmt enzyme (Figure 2).[8] The interactions of the base moiety with the surrounding residues is in inhibitor **3** are similar to the ones with the natural substrate[6]: a stacking with Phe70, H-bond of base atom N3 with Asn100 and base atom O4 hydrogen bonding with Arg74. An additional hydrogen bond involving O3' and Tyr39 may explain the better activity of **3** compared to its 3'-deoxygenated analogue.[8] In compound **19** where the C(4)=O at is replaced by a C(4)=S, the hydrogen bonds to Arg74 is lost. However, due to the bigger size of the sulfur atom, a better van der Waals interaction is seen with surrounding residues Phe70, Arg74 and Asn100 which may explain a higher affinity for this inhibitor.

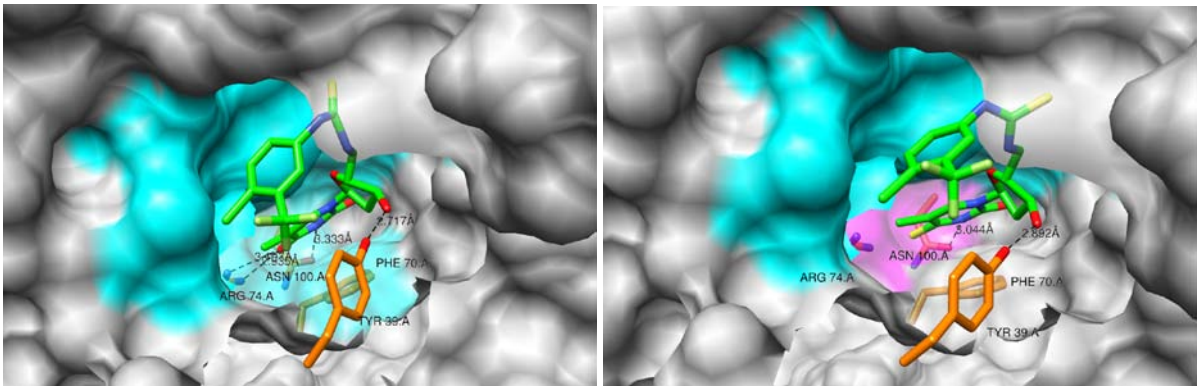


Figure 2. Compound **3** (a) and compound **19** (b) docked in the substrate binding site of TMPKmt. The carbon atoms of both inhibitors are colored green. The enzyme contact surface is colored cyan, and the contact surface with the 4S atom is colored magenta.

3. Conclusions

On the basis of the structures of nucleosides **2** and **3**, which were identified earlier as potent TMPKmt inhibitors, this paper describes the synthesis and biological evaluation of a series of new thymidine analogues, including α- and β-derivatives. In both the 3'- and the 5'-derivatised analogues, the anomer that places the thymine base trans to the aromatic substituent showed the best TMPKmt inhibition. In addition, several analogues were synthesized in which the 4-oxygen was replaced by a more lipophilic sulphur atom to probe the influence of this modification on TMPKmt inhibitory activity. Remarkably, a 4-thio modification of the pyrimidine base was favorable for the 5'-modified α-analogues, while it caused an opposite effect the 3'-modified β-analogues. Several compounds showed an inhibitory

potency in the low micromolar range, with the 5'-arylthiourea 4-thio-α-thymidine analogue **19** being the most active one (K_i = 0.17 μM). This compound is capable of inhibiting *M. bovis a*t a concentration of 25 μg/mL, promoting TMPKmt as an attractive target for further inhibitor design.

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4. Experimental Section

Spectrophotometric Binding Assay. TMPKmt activities were determined using the coupled spectrophotometric assay described by Blondin *et al*.[20] using an Eppendorf ECOM 6122 photometer and a wavelength of 334 nm. The reaction medium (0.5 mL final volume) contained 50 mM Tris-HCl, pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate, and 2 units each of lactate dehydrogenase, pyruvate kinase, and nucleoside diphosphate kinase. The concentrations of ATP and dTMP were kept constant at 0.5 and 0.05 mM, respectively, whereas the concentrations of analogues varied between 0.003 and 1.5 mM. Equation 1 was used to calculate the Ki values using Equations 2 and 3 (classical competitive inhibition model following the Lineweaver-Burk representation):

$$K_i = \frac{K_m\,[I]}{\left(\frac{v}{v_i} - 1\right)\left(K_m + [S]\right)} \quad \text{(Eq.1)}$$

$$v = \frac{V_m\,[S]}{[S] + K_m} \quad \text{(Eq.2)} \qquad v_i = \frac{V_m\,[S]}{[S] + K_m\left(1 + \frac{[I]}{K_i}\right)} \quad \text{(Eq.3)}$$

where v and v_i are the reaction velocities respectively in the absence and in the presence of the analogue at a concentration value [I]; Km is the Km for dTMP (4.5 μM for TMPKmt and 5 μM for TMPKh); [S] is the concentration of dTMP (50 μM). For each compound, v_i determinations were performed at least at two different concentration values [I].

Biological Assays on *Mycobacterium bovis* (BCG). Compounds **18** and **19** were assayed for their inhibitory potency on *Mycobacterium bovis* var. BCG growth *in vitro*.[21] A micro-method of culture was performed in 7H9 Middlebrook broth medium containing 0.2% glycerol and 0.5% Tween-80. Serial 2-fold 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 per mL, were delivered in 100 µL per well. The covered plates were sealed with parafilm and incubated at 37 °C in plastic boxes containing a humidified normal atmosphere. At day 8 of incubation, 30 µL of a resazurin (Sigma) solution at 0.01% (wt/vol) in water was added to each well. After an overnight incubation at 37 °C, the plates were assessed for color development using the optical density difference at 570 and 630 nm on a microplate reader. The change from blue to pink indicates reduction of resazurin and therefore bacterial growth. The lowest compound concentration that prevented the color change determined the MIC for the assayed compound.

Synthesis. General. All reagents were from standard commercial sources and of analytical grade. Precoated Merck silica gel F254 plates were used for TLC, spots were examined under ultraviolet light at 254 nm and further visualized by sulfuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (63-200 µm, 60Å, Biosolve, Valkenswaard, The Netherlands). NMR spectra were determined using a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent peak: in the case of DMSO-d_6, it is 2.54 ppm for ^{1}H and 40.5 ppm for ^{13}C; in the case of $CDCl_3$, it is 7.26 ppm for ^{1}H and 77.4 ppm for ^{13}C. Structural assignment was confirmed with COSY and DEPT. All signals assigned to hydroxyl groups were exchangeable with D_2O. Exact mass measurements were performed on a Waters LCT Premier XETM Time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/water (1:1) mixture at 10 µL/min.

5'-*O*-Acetyl-3'-azido-3'-deoxy-4-thio-β-D-thymidine (24). Lawesson's reagent (154 mg, 0.38 mmol) was added to a solution of compound **23** (111 mg, 0.36 mmol) in 10 mL anhydrous toluene. The mixture was refluxed overnight and the solvent was removed *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2/ MeOH 95:5) to give compound **24** as a brown-yellow solid (23 mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.99 (3H, d, *J*= 0.9 Hz, 5-CH_3), 2.06 (3H, s, OAc), 2.34-2.43 (1H, m, H-2'a), 2.53-2.57 (1H, m, H-2'b), 3.99-4.04 (1H, m, H-4'), 4.21-4.32 (2H,m, H-5'a an H-5'b), 4.44-4.50 (1H, m, H-3'), 6.07 (1H, dd, *J*= 5.7 Hz, *J*= 7.2 Hz, H-1'), 7.58 (1H, s, H-6), 12.75 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.78 (5-CH_3), 20.58 (OAc), 35.96 (C-2'), 59.81 (C-3'), 63.09 (C-5'), 81.10 (C-4'), 84.64 (C-1'), 117.95 (C-5), 133.32 (C-6), 147.67 (C-2), 171.92 (OAc), 190.95 (C-4). Exact mass (ESI-MS) for $C_{12}H_{16}N_5O_4S$ $[M+H]^+$ found, 326.0941; calcd, 326.0918.

3'-Azido-3'-deoxy-4-thio-β-D-thymidine (7). Compound **24** (16 mg, 0.050 mmol) was dissolved in a 7N NH_3 in MeOH solution (1 mL) and stirred at room temperature for 6 hours. The reaction mixture was concentrated *in vacuo* and the residue was purified on a silica gel column using CH_2Cl_2/MeOH (95:5) as the eluent to afford compound **7** as a yellow powder (6.0 mg, 42%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.97 (3H, d, *J*= 0.6 Hz, 5-CH_3), 2.29-2.38 (1H, m, H-2'a), 2.41-2.48 (1H, m, H-2'b), 3.59-3.71 (2H, m, H-5'a and H-5'b), 3.83-3.87 (1H, m, H-4'), 4.40 (1H, app dd, *J*= 6.0 Hz, *J*= 12.6 Hz, H-3'), 5.28 (1H, br s, 5'-OH), 6.04 (1H, app t, *J*= 5.7 Hz, H-1'), 7.86 (1H, s, H-6). ^{13}C NMR (75 MHz, DMSO-

d_6): δ 16.90 (5-CH_3), 36.64 (C-2'), 59.44 (C-3'), 60.35 (C-5'), 84.32 and 84.44 (C-4' and C-1'), 117.63 (C-5), 133.34 (C-6), 147.71 (C-2), 190.76 (C-4). Exact mass (ESI-MS) for $C_{10}H_{14}N_5O_3S$ $[M+H]^+$ found, 326.284.0813; calcd, 284.0812. Spectroscopic data of **7** were in accordance with literature data.[22]

5'-*O*-acetyl-3'-(4-chlorophenyl-1,2,3-triazol-1-yl)-3'-deoxy-β-D-thymidine (25). Compound **23** (146 mg, 0.47 mmol), sodium ascorbate (5 mg, 0.024 mmol) and $CuSO_4$ ·$5H_2O$ (5 mg, 0.019 mmol) were suspended in 9 mL of H_2O/*t*-BuOH (2:1). 1-Chloro-4-ethynylbenzene (129 mg, 0.94 mmol) was added after 15 minutes and the mixture was stirred at room temperature for 24 h. The reaction mixture was extracted with EtOAc and the combined organic phases were dried over anhydrous $MgSO_4$ and evaporated to anhydrousness. The crude product was purified column chromatography (CH_2Cl_2/MeOH 95:5) affording **25** (87 mg, 41 %) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.84 (3H, s, 5-CH_3), 2.04 (3H, s, OAc), 2.73-2.94 (2H, m, H-2'a and H-2'b), 4.27-4.37 (2H, m, H-5'a and H-5'b), 4.43-4.49 (1H, m, H-4'), 5.47-5.54 (1H, m, H-3'), 6.45 (1H, t, *J*= 7.2 Hz, H-1'), 7.52-7.57 (2H, m, subs Ph), 7.63 (1H, d, *J*= 1.2 Hz, H-6), 7.85-7.90 (2H, m, subs Ph), 8.86 (1H, s, H-5"), 11.41 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.14 (5-CH_3), 20.53 (OAc), 36.36 (C-2'), 59.50 and 63.29 (C-5' and C-3'), 80.85 (C-4'), 84.15 (C-1'), 110.00 (C-5), 121.34 (C-5"), 126.86, 129.07, 129.42 and 132.48 (subs Ph), 136.39 (C-6), 145.54 (C-4"), 150.44 (C-2), 163.74 (C-4), 170.08 (OAc). Exact mass (ESI-MS) for $C_{20}H_{21}ClN_5O_5$ $[M+H]^+$ found, 446.1238; calcd, 446.1226.

5'-*O*-acetyl-3'-(4-chlorophenyl-1,2,3-triazol-1-yl)-3'-deoxy-4-thio-β-D-thymidine (26). Lawesson's reagent (158 mg, 0.39 mmol) was added to a solution of compound **25** (87 mg, 0.20 mmol) in 10 mL anhydrous toluene. The mixture was refluxed overnight and the solvent was removed *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2/ MeOH 95:5) to give compound **26** as a brown-yellow solid (44 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.00 (3H, s, 5-CH_3), 2.05 (3H, s, OAc), 2.86-2.94 (1H, m, H-2'a), 3.17-3.26 (1H, m, H-2'b), 4.35 (2H, d, *J*= 3.3 Hz, H-5'a and H-5'b), 4.59-4.65 (1H, m, H-4'), 5.49 (1H, app dd, *J*= 7.5 Hz, *J*= 15.3 Hz, H-3'),6.01-6.04 (1H, m, H-1'), 7.24 (1H, s, H-6), 7.32 (2H, d, *J*= 8.4 Hz, subs Ph), 7.68 (2H, d, *J*= 8.4 Hz, subs Ph), 7.93 (1H, s, H-5"), 11.10 (1H, s, 3-NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.41 (5-CH_3), 21.00 (OAc), 38.16 (C-2'), 60.31 and 63.45 (C-5' and C-3'), 82.99 (C-4'), 89.57 (C-1'), 120.21 and 120.71 (C-5 and C-5"), 127.23, 128.77, 129.39, 134.01 and 134.47 (C-6 and subs Ph), 147.20 and 148.49 (C-4" and C-2), 170.66 (OAc), 190.91 (C-4). Exact mass (ESI-MS) for $C_{20}H_{21}ClN_5O_4S$ $[M+H]^+$ found, 462.1042; calcd, 462.0997.

3'-(4-Chlorophenyl-1,2,3-triazol-1-yl)-3'-deoxy-4-thio-β-D-thymidine (10). Compound **26** (42 mg, 0.090 mmol) was dissolved in a 7N NH_3 in MeOH solution (1 mL) and stirred at room temperature for 6 hours. The reaction mixture was concentrated *in vacuo* and the residue was purified on a silica gel column using CH_2Cl_2/MeOH (95:5) as the eluent to afford compound **10** as a yellow powder (25.2 mg, 66%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (3H, d, *J*= 0.9 Hz, 5-CH_3), 1.96 (1H, app dt, *J*= 3.6 Hz, *J*= 14.1 Hz, H-2'a), 2.45-2.55 (1H, m, H-2'b), 4.28 (1H, app s, H-3'), 4.47-4.64 (3H, m, H-4', H-5'a and H-5'b), 5.63 (1H, s, 5'-OH), 6.18 (1H, dd, *J*= 3.9 Hz, *J*= 7.5 Hz, H-1'), 7.49-7.54 (2H, m, subs Ph), 7.72 (1H, d, J= 1.2 Hz, H-6), 7.86-7.91 (2H, m, subs Ph), 8.62 (1H, s, H-5"). ^{13}C NMR (75 MHz, DMSO-d_6):

δ 16.94 (5-CH_3), 37.51 (C-2'), 59.02 (C-3'), 60.41 (C-5'), 84.80 and 84.90 (C-4' and C-1'), 117.81 (C-5), 121.40 (C-5"), 126.87, 129.03, 129.47, 132.44 and 133.50 (C-6 and subs Ph), 145.49 and 147.79 (C-4" and C-2), 190.90 (C-4). Exact mass (ESI-MS) for $C_{18}H_{19}ClN_5O_3S$ $[M+H]^+$ found, 420.0915; calcd, 420.0892.

N-(5'-Deoxy-3'-*O*-tert-butyldimethylsilyl-β-D-thymidin-5'-yl)-N'-(4-chloro-3-trifluoromethyl-phenyl)-thiourea (28). To a solution of compound **27** (403 mg, 1.13 mmol) in DMF (4 mL) was added a solution of 4-chloro-3-(trifluoromethyl)phenylisothiocyanate (0.18 mL, 1.13 mmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred for 1 h. The solvents were evaporated to anhydrousness and the residue was purified by column chromatography (CH_2Cl_2/MeOH 98:2) affording compound **28** as a colorless solid (510 mg, 76%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.098 (6H, s, TBDMS), 0.87-0.88 (9H, m, TBDMS), 1.80 (3H, s, 5-CH_3), 2.02-2.10 (1H, m, H-2'a), 2.24-2.34 (1H, m, H-2'b), 3.55-3.65 (1H, m, H-4'), 3.98-3.99 (2H, m, H-5'a and H-5'b), 4.45-4.47 (1H, m, H-3'), 6.15-6.19 (1H, m, H-1'), 7.52 (1H, s, subs Ph), 7.62-7.72 (2H, m, subs Ph and H-6), 7.96 (1H, subs Ph), 8.12 (1H, s, 5'-NH), 9.93 (1H, s, N'H), 11.33 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ -4.99 and -4.93 (TBDMS), 11.95 (5-CH_3), 17.57 (TBDMS), 25.57 (TBDMS), C-2' (under solvent peak), 45.74 (C-5'), 72.80 (C-3'), 83.98 and 84.19 (C-1' and C-4'), 109.74 (C-5), 124.50-139.00 (subs Ph, CF_3 and C-6), 150.36 (C-2), 163.56 (C-4), 180.77 (C=S). Exact mass (ESI-MS) for $C_{24}H_{33}ClF_3N_4O_4Si$ $[M+H]^+$ found, 593.1643; calcd, 593.1627.

N-(5'-Deoxy-β-D-thymidin-5'-yl)-N'-(4-chloro-3-trifluoromethylphenyl)-thiourea (14). Compound **28** (84 mg, 0.14 mmol) was dissolved in THF (0.9 mL). A solution of 1M tetra-*n*-butylammoniumfluoride in THF (0.31 mL) was added. After 1 h at room temperature the reaction was completed. The solvent was evaporated and the anhydrous residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5) to give pure compound **14** (40 mg, white solid) in 60% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.79 (3H, s, 5-CH_3), 2.05-2.13 (1H, m, H-2'a), 2.17-2.27 (1H, m, H-2'b), 3.59-3.66 (1H, m, H-4'), 3.87-3.96 (2H, m, H-5'a and H-5'b), 4.23-4.24 (1H, m, H-3'), 5.38 (1H, d, *J*= 4.2 Hz, 3'-OH), 6.18-6.22 (1H, m, H-1'), 7.51-7.7.75 (3H, m, subs Ph and H-6), 8.17-8.22 (2H, m, subs Ph and 5'-NH), 9.99 (1H, s, N'H), 11.32 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.73 (5-CH_3), C-2' (under solvent peak), 46.95 (C-5'), 71.97 (C-3'), 84.56 and 84.69 (C-1' and C-4'), 110.54 (C-5), 121.59-139.91 (subs Ph, CF_3 and C-6), 151.18 (C-2), 164.38 (C-4), 181.44 (C=S). Exact mass (ESI-MS) for $C_{18}H_{19}ClF_3N_4O_4$ $[M+H]^+$ found, 479.0778; calcd, 479.0762.

5-(5'-Amino-5'-deoxy-3'-*O*-*tert*-butyldimethylsilyl-β-D-thymidin-5'*N*-yl)-1-(4-chloro-3-trifluoro-methylphenyl)-tetrazole (29). To a suspension of compound **28** (504 mg, 0.85 mmol), sodium azide (166 mg, 2.55 mmol) and $HgCl_2$ (253 mg, 0.93 mmol) in anhydrous DMF (3.3 mL) was added Et_3N (0.36 mL, 2.55 mmol) under N_2 atmosphere. The resulting black suspension was stirred overnight at room temperature. The mixture was filtered through a pad of Celite, washing with CH_2Cl_2. The filtrate was diluted with water and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by silica

gel chromatography (CH_2Cl_2/MeOH 98:2) affording compound **29** (424 mg, 83%) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.029-0.042 (6H, m, TBDMS), 0.84-0.86 (9H, m, TBDMS), 1.78 (3H, s, 5-CH_3), 2.00-2.07 (1H, m, H-2'a), 2.23-2.33 (1H, m, H-2'b), 3.48-3.62 (2H, m, H-5'a and H-5'b), 3.94-3.99 (1H, m, H-4'), 4.42-4.45 (1H, m, H-3'), 6.10-6.14 (1H, m, H-1'), 7.41 (1H, t, *J*= 6.0 Hz, 5'-NH), 7.53 (1H, d, *J*= 1.2 Hz, H-6), 7.88-8.03 (3H, m, subs Ph), 11.31 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ -5.06 and -4.91 (TBDMS), 11.92 (5-CH_3), 17.56 (TBDMS), 25.56 (TBDMS), C-2' (under solvent peak), 45.82 (C-5'), 72.90 (C-3'), 84.07 and 84.58 (C-1' and C-4'), 109.61 (C-5), 128.00-136.27 (subs Ph, CF_3 and C-6), 150.35 (C-2), 155.28 (C=N), 163.62 (C-4). Exact mass (ESI-MS) for $C_{24}H_{32}ClF_3N_7O_4Si$ $[M+H]^+$ found, 602.1910; calcd, 602.1920.

5-(5'-Amino-5'-deoxy-β-D-thymidin-5'*N*-yl)-1-(4-chloro-3-trifluoromethylphenyl)-tetrazole (15). Compound **29** (241 mg, 0.400 mmol) was dissolved in THF (2.5 mL). A solution of 1 M tetra-*n*-butylammoniumfluoride in THF (0.88 mL) was added. After 1 h at room temperature the reaction was completed. The solvent was evaporated and the anhydrous residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5) to give pure compound **15** (104 mg, white solid) in 53% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76 (3H, d, *J*= 0.9 Hz, 5-CH_3), 2.04-2.11 (1H, m, H-2'a), 2.14-2.24 (1H, m, H-2'b), 3.49-3.65 (2H, m, H-5'a and H-5'b), 3.93-3.98 (1H, m, H-4'), 4.22-4.27 (1H, m, H-3'), 5.32 (1H, d, *J*= 4.5 Hz, 3'-OH), 6.12-6.17 (1H, m, H-1'), 7.39 (1H, t, *J*= 5.7 Hz, 5'-NH), 7.50 (1H, d, *J*= 1.5 Hz, H-6), 7.90-8.07 (3H, m, subs Ph), 11.29 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.02 (5-CH_3), C-2' (under solvent peak), 46.16 (C-5'), 71.24 (C-3'), 83.91 (C-1'), 84.28 (C-4'), 109.73 (C-5), 124.95-136.26 (subs Ph, CF_3 and C-6), 150.47 (C-2), 155.42 (C=N), 163.76 (C-4). Exact mass (ESI-MS) for $C_{18}H_{18}ClF_3N_7O_4$ $[M+H]^+$ found, 488.1052; calcd, 488.1055.

5'-(4-Chlorophenyl-1,2,3-triazol-1-yl)-5'-deoxy-β-D-thymidine (16). Compound **30** (56 mg, 0.21 mmol), sodium ascorbate (cat. amount) and $CuSO_4 \cdot 5H_2O$ (cat. amount) were suspended in 3 mL of H_2O/*t*-BuOH (1:2). 1-Chloro-4-ethynylbenzene (57 mg, 0.42 mmol) was added after 15 minutes and the mixture was stirred at room temperature for 7 days. The reaction mixture was extracted with EtOAc and the combined organic phases were dried over anhydrous $MgSO_4$ and evaporated to anhydrousness. Purification of the crude using RP-HPLC (Phenomenex Luna C-18, H_2O/0.1% HCOOH in CH_3CN, 90:10 → 0:100 in 23 min, flow 17.5 mL/min) afforded compound **16** (2.0 mg, 2%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.68 (3H, s, 5-CH_3), 2.02-2.24 (2H, m, H-2'a and H-2'b), 4.09-4.14 (1H, m, H-4'), 4.28-4.31 (1H, m, H-3'), 4.64-4.80 (2H, m, H-5'a and H-5'b), 5.59 (1H, br s, 3'-OH), 6.18 (1H, app t, *J*= 6.9 Hz, H-1'), 7.23 (1H, d, *J*= 1.2 Hz, H-6), 7.49-7.53 (2H, m, subs Ph), 7.85-7.90 (2H, m, subs Ph), 8.62 (1H, s, H-5"), 11.30 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.96 (5-CH_3), 37.84 (C-2'), 51.13 (C-5'), 70.47 (C-3'), 83.66 (C-4'), 83.77 (C-1'), 109.79 (C-5), 122.53-132.25 (subs Ph and C-5"), 135.96 (C-6), 145.26 (C-4"), 150.37 (C-2), 163.56 (C-4). Exact mass (ESI-MS) for $C_{18}H_{19}ClN_5O_4$ $[M+H]^+$ found, 404.1125; calcd, 404.1120.

4-Thio-α-D-thymidine (33). Lawesson's reagent (777 mg, 1.92 mmol) was added to a solution of compound 3'-5'-di-*O*-acetyl-α-D-thymidine (**31**) (519 mg, 1.59 mmol) in 15 mL anhydrous 1,4-dioxane.

The mixture was refluxed for 4 hours. After the reaction mixture had been cooled, the solvent was removed *in vacuo*. The crude product thus obtained was treated with 8 mL of a 7N NH_3 in MeOH solution and stirred at room temperature for 4 hours. The reaction mixture was concentrated *in vacuo* and the residue was purified on a silica gel column using CH_2Cl_2/MeOH (94:6) as the eluent to afford compound **33** as a yellow foam (150 mg, 37%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (3H, s, 5-CH_3), 1.94-1.98 (1H, m, H-2'a), 2.51-2.57 (1H, m, H-2'b), 3.40 (2H, t, *J*= 5.2 Hz, H-5'a and H-5'b), 4.23-4.25 (2H, m, H-3' and H-4'), 4.86 (1H, t, *J*= 5.7 Hz, 5'-OH), 5.26 (1H, d, *J*= 2.7 Hz, 3'-OH), 6.04 (1H, dd, *J*= 2.7 Hz, *J*= 7.5 Hz, H-1'), 7.81 (1H, d, *J*= 0.9 Hz, H-6), 12.65 (1H, s, 3-NH). Exact mass (ESI-MS) for $C_{10}H_{15}N_2O_4S$ $[M+H]^+$ found, 259.0753; calcd, 259.0747.

5'-*O*-Methanesulfonyl-4-thio-α-D-thymidine (34). To a solution of 4-thio-α-D-thymidine **33** (146 mg, 0.57 mmol) in pyridine (5 mL) at -78 °C, methanesulfonylchloride (42 µL,0.54 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C. The reaction was quenched with saturated aqueous $NaHCO_3$-solution and extracted with CH_2Cl_2 three times, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (CH_2Cl_2/ MeOH 95:5) to give mesylated compound **34** as a yellow foam (133 mg, 70%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.97 (3H, d, *J*= 0.6 Hz, 5-CH_3), 1.99-2.04 (1H, m, H-2'a), 2.53-2.30 (1H, m, H-2'b), 3.22 (3H, s, SO_2CH_3), 4.08-4.10 (2H, m, H-5'a and H-5'b), 4.16-4.30 (1H, m, H-3'), 4.40-4.46 (1H, m, H-4'), 5.55 (1H, br s, 3'-OH), 6.09 (1H, dd, *J*= 3.6 Hz, *J*= 7.5 Hz, H-1'), 7.69 (1H, s, H-6). Exact mass (ESI-MS) for $C_{11}H_{17}N_2O_6S_2$ $[M+H]^+$ found, 337.0533; calcd, 337.0523.

5'-Azido-5'-deoxy-4-thio-α-D-thymidine (35). A solution of 5'-mesylated 4-thio-α-D-thymidine **34** (129 mg, 0.38 mmol) and NaN_3 (250 mg, 3.86 mmol) in DMF (7 mL) was heated to 60 °C overnight. The reaction mixture was evaporated *in vacuo*. The residue was resolved in CH_2Cl_2 and washed with brine. The organic layer was dried over $MgSO_4$, evaporated and purified by column chromatography (CH_2Cl_2/MeOH 95:5) to afford compound **35** (97 mg, 89%) as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.98 (3H, d, *J*= 0.9 Hz, 5-CH_3), 2.04 (1H, t, *J*= 3.3 Hz, H-2'a), 2.56-2.65 (1H, m, H-2'b), 3.40-3.44 (2H, m, H-5'a and H-5'b), 4.14-4.17 (1H, m, H-3'), 4.34-4.39 (1H, m, H-4'), 5.47 (1H, br s, 3'-OH), 6.09 (1H, dd, *J*= 3.6 Hz, *J*= 7.5 Hz, H-1'), 7.78 (1H, s, H-6). Exact mass (ESI-MS) for $C_{10}H_{14}N_5O_3S$ $[M+H]^+$ found, 284.0813; calcd, 284.0812.

5'-Amino-5'-deoxy-4-thio-α-D-thymidine (36). Compound **35** (97 mg, 0.34 mmol) and PPh_3 (187 mg, 0.71 mmol) were dissolved in THF (6 mL). After stirring for 10 minutes, H_2O was added (883 µL) and the mixture was stirred for 1 day. The mixture was extracted with CH_2Cl_2 and the water phase lyophilized to give amine **36** (78 mg, 89%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.96 (3H, d, *J*= 0.6 Hz, 5-CH_3), 2.00 (1H, t, *J*= 3.6 Hz, H-2'a), 2.58-2.68 (1H, m, H-2'b), 2.76-2.87 (1H, m, H-5'a), 2.96-3.04 (1H, m, H-5'b), 4.18-4.20 (1H, m, H-3'), 4.32 (1H,dt, *J*= 3.3 Hz, *J*= 9.6 Hz, H-4'), 5.57 (1H, br s, 3'-OH), 6.15 (1H, dd, *J*= 3.3 Hz, *J*= 7.5 Hz, H-1'), 7.77 (1H, s, H-6). Exact mass (ESI-MS) for $C_{10}H_{16}N_3O_3S$ $[M+H]^+$ found, 258.0907; calcd, 258.0907.

N-(5'-Deoxy-4-thio-α-D-thymidin-5'-yl)-N'-phenylthiourea (18). For the synthesis of compound **18**, amine **36** (26 mg, 0.10 mmol) was dissolved in DMF (1 mL). At 0 °C, phenyl isothiocyanate (16 mg, 0.12 mmol) was added and the reaction mixture was allowed to stir at room temperature during 3 h. After completion of the reaction, the reaction mixture was evaporated to anhydrousness and the residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5) to obtain the pure final compound **18** (27.0 mg, 69%) as a yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.97 (3H, d, *J*= 0.9 Hz, 5-CH_3), 2.01-2.03 (1H, m, H-2'a), 2.54-2.63 (1H, m, H-2'b), 3.53-3.70 (2H, m, H-5'a and H-5'b), 4.21-4.24 (1H, m, H-3'), 4.42-4.46 (1H, m, H-4'), 5.43 (1H, d, *J*= 2.7 Hz, 3'-OH), 6.12 (1H, dd, *J*= 2.7 Hz, *J*= 7.5 Hz, H-1'), 7.08-7.13 (1H, m, Ph), 7.29-7.34 (2H, m, Ph), 7.43-7.47 (2H, m, Ph), 7.80 (1H, s, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 17.03 (5-CH_3), under DMSO (C-2'), 45.49 (C-5'), 70.79 (C-3'), 86.08 (C-1'), 86.78 (C-4'), 117.22 (C-5), 123.22, 124.30, 128.63 and 134.25 (Ph), 139.13 (C-6), 147.87 (C-2), 180.74 (C=S), 190.56 (C-4). Exact mass (ESI-MS) for $C_{17}H_{21}N_4O_3S_2$ $[M+H]^+$ found,393.1053; calcd, 393.1050.

N-(5'-Deoxy-4-thio-α-D-thymidin-5'-yl)-N'-(3-trifluoromethyl-4-chlorophenyl)thiourea (19). Compound **19** was synthesized from amine **36** (52 mg, 0.20 mmol) and 4-chloro-3-trifluoromethylphenyl isothiocyanate (57 mg,0.24 mmol) using the same procedure as described for the synthesis of compound **18**. After purification by column chromatography (CH_2Cl_2/MeOH 95:5), compound **19** (41.7mg, 42%) was obtained as a yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.98 (3H, d, *J*= 0.6 Hz, 5-CH_3), 2.06 (1H, t, *J*= 2.1 Hz, H-2'a), 2.57-2.66 (1H, m, H-2'b), 3.56-3.59 (1H, m, H-5'a), 3.67-3.72 (1H, m, H-5'b), 4.25-4.26 (1H, m, H-3'), 4.44-4.48 (1H, m, H-4'), 5.47 (1H, d, *J*= 3.0 Hz, 3'-OH), 6.14 (1H, dd, *J*= 2.7 Hz, *J*= 7.5 Hz, H-1'), 7.64 (2H, d, *J*= 8.7 Hz, subs Ph), 7.74 (2H, dd; *J*= 2.1 Hz, *J*= 8.4 Hz, subs Ph), 7.83 (1H, d, *J*= 0.9 Hz, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 17,11 (5-CH_3), under DMSO (C-2'), 45.62 (C-5'), 70.95 (C-3'), 86.18 and 86.55 (C-4' and C-1'), 117.22 (C-5), 124.61, 127.50, 131.77 and 134.28 (CF_3 and subs Ph), 139.23 (C-6), 148.00 (C-2), 180.95 (C=S), 190.70 (C-4). Exact mass (ESI-MS) for $C_{18}H_{19}ClF_3N_4O_3S_2$ $[M+H]^+$ found,495.0508; calcd, 495.0534.

5'-(4-Chlorophenyl-1,2,3-triazol-1-yl)-5'-deoxy-α-D-thymidine (20). Compound **17** (85 mg, 0.32 mmol), sodium ascorbate (3 mg, 0.016 mmol) and $CuSO_4$ ·$5H_2O$ (3 mg, 0.013 mmol) were suspended in 3 mL of H_2O/*t*-BuOH (2:1). 1-Chloro-4-ethynylbenzene (87 mg, 0.64 mmol) was added after 15 minutes and the mixture was stirred at room temperature for 4 days. Water was added and the triazole product precipitated. Filtration of the mixture afforded pure compound **20** (40.0 mg, 31%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (3H, d, *J*= 1.2 Hz, 5-CH_3), 1.94 (1H, app t, *J*= 3.9 Hz, H-2'a), 1.99 (1H, app t, *J*= 3.9 Hz, H-2'b), 4.22-4.32 (1H, m, H-4'), 4.43-4.66 (3H, m, H-3', H-5'a and H-5'b), 5.65 (1H, br s, 3'-OH), 6.18 (1H, dd, *J*= 4.2 Hz, *J*= 7.5 Hz, H-1'), 7.49-7.54 (2H, m, subs Ph), 7.72 (1H, d, *J*= 1.2 Hz, H-6), 7.86-7.91 (2H, m, subs Ph), 8.62 (1H, s, H-5"), 11.26 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.15 (5-CH_3), 51.20 (C-5'), 70.53 (C-4'), 84.72 (C-1'), 85.37 (C-3'), 108.81 (C-5), 122.41 (C-5"), 126.73, 128.83, 129.45 and 132.15 (subs Ph), 136.64 (C-6), 145.12 (C-4"), 150.29 (C-2), 163.65 (C-4). Exact mass (ESI-MS) for $C_{18}H_{19}ClN_5O_4$ $[M+H]^+$ found, 404.1129; calcd, 404.1120.

5'-*O*-Acetyl-3'-azido-3'-deoxy-α-D-thymidine (38). To a solution of compound **37** (642 mg, 2.08 mmol) in 1 mL anhydrous CH_2Cl_2, was added a freshly prepared solution, containing 34 µL H_2SO_4 and 140 µL acetic acid anhydride in 1 mL anhydrous CH_2Cl_2. After 2 h, the mixture was quenched with saturated $NaHCO_3$-solution and extracted three times with EtOAc. Purification of the crude on a silica gel column (EtOAc/hexane 9:1) yielded compound **38** as a white foam (126 mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.80 (3H, d, *J*= 0.9 Hz, 5-CH_3), 2.06 (3H, s, OAc), 2.15-2.22 (1H, m, H-2'a), 2.70-2.75 (1H, m, H-2'b), 4.11-4.14 (2H, m, H-5'a and H-5'b), 4.34-4.39 (1H, m, H-3'), 4.42-4.45 (1H, m, H-4'), 6.07 (1H, dd, *J*= 6.0 Hz, *J*= 6.9 Hz, H-1'), 7.59 (1H, d, *J*= 1.2 Hz, H-6), 11.32 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.15 (5-CH_3), 20.62 (OAc), 36.23 (C-2'), 60.43 (C-3), 63.68 (C-5'), 81.46 (C-4'), 84.93 (C-1'), 109.38 (C-5), 136.25 (C-6), 150.36 (C-2), 163.80 (C-4), 170.15 (OAc). Exact mass (ESI-MS) for $C_{12}H_{16}N_5O_5$ $[M+H]^+$ found, 310.1164; calcd, 310.1146. Spectroscopic data of **38** were in accordance with literature data.[23]

3'-Azido-3'-deoxy-α-D-thymidine (21). Compound **38** (146 mg, 0.47 mmol) was dissolved in a 7N NH_3 in MeOH solution (2.6 mL) and stirred at room temperature for 6 hours. The reaction mixture was concentrated *in vacuo* and the residue was purified on a silica gel column using EtOAc/hexane (8:2) as the eluent to afford compound **21** as a colorless solid (91 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.95 (3H, d, *J*= 0.9 Hz, 5-CH_3), 2.12-2.17 (1H, m, H-2'a), 2.82-2.91 (1H, m, H-2'b), 3.08 (1H, t, *J*= 5.4 Hz, 5'-OH), 3.65-3.72 (1H, m, H-5'a), 3.81-3.85 (1H, m, H-5'b), 4.27-4.35 (2H, m, H-3' and H-4'), 6.29 (1H, dd, *J*= 4.2 Hz, *J*= 6.9 Hz, H-1'), 7.32 (1H, d, *J*= 1.5 Hz, H-6), 9.48 (1H, s, 3-NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.67 (5-CH_3), 38.22 (C-2'), 60.83 (C-3), 62.62 (C-5'), 85.93 and 86.02 (C-4' and C-1'), 111.24 (C-5), 135.35 (C-6), 150.72 (C-2), 164.03 (C-4). Exact mass (ESI-MS) for $C_{10}H_{14}N_5O_4$ $[M+H]^+$ found, 268.1020; calcd, 268.1040. Spectroscopic data of **39** were in accordance with literature data.[23]

3'-(4-Chlorophenyl-1,2,3-triazol-1-yl)-3'-deoxy-α-D-thymidine (22). Compound **21** (72 mg, 0.27 mmol), sodium ascorbate (3 mg, 0.024 mmol) and $CuSO_4 \cdot 5H_2O$ (3 mg, 0.011 mmol) were suspended in 3 mL of H_2O/*t*-BuOH (1:2). 1-Chloro-4-ethynylbenzene (74 mg, 0.54 mmol) was added after 15 minutes and the mixture was stirred at room temperature for 24 h. The reaction mixture was extracted with EtOAc and the combined organic phases were dried over anhydrous $MgSO_4$ and evaporated to anhydrousness. The crude product was purified column chromatography (EtOAc/hexane 8:2) affording **22** (50.8 mg, 47%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (3H, s, 5-CH_3), 2.71-2.80 (1H, m, H-2'a), 2.98-3.07 (1H, m, H-2'b), 3.54-3.70 (2H, m, H-5'a and H-5'b), 4.65-4.70 (1H, m, H-4'), 5.12 (1H, t, *J*= 5.4 Hz, 5'-OH), 5.29-5.36 (1H, m, H-3'), 6.26 (1H, app t, *J*= 6.6 Hz, H-1'), 7.50-7.54 (2H, m, subs Ph), 7.63 (1H, d, *J*= 1.2 Hz, H-6), 7.84-7.87 (2H, m, subs Ph), 8.82 (1H, s, H-5"), 11.29 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.18 (5-CH_3), 37.12 (C-2'), 59.23 (C-3), 61.12 (C-5'), 83.89 (C-4'), 84.43 (C-1'), 109.58 (C-5), 121.38 (C-5"), 126.88-132.47 (subs Ph), 136.07 (C-6), 145.49 (C-4"), 150.46 (C-2), 163.78 (C-4). Exact mass (ESI-MS) for $C_{18}H_{19}ClN_5O_4$ $[M+H]^+$ found, 404.1156; calcd, 404.1120.

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