Synthesis and Evaluation of 6-Aza-2′-deoxyuridine Monophosphate Analogs as Inhibitors of Thymidylate Synthases, and as Substrates or Inhibitors of Thymidine Monophosphate Kinase in *Mycobacterium tuberculosis*

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A series of 5-substituted analogs of 6-aza-2′-deoxyuridine 5′-monophosphate, 6-aza-dUMP, has been synthesized and evaluated as potential inhibitors of the two mycobacterial thymidylate synthases (*i.e.*, a flavin-dependent thymidylate synthase, ThyX, and a classical thymidylate synthase, ThyA). Replacement of C(6) of the natural substrate dUMP by a N-atom in 6-aza-dUMP **1a** led to a derivative with weak ThyX inhibitory activity (33% inhibition at 50 μM). Introduction of alkyl and aryl groups at C(5) of **1a** resulted in complete loss of inhibitory activity, whereas the attachment of a 3-(octanamido)prop-1-ynyl side chain in derivative **3** retained the weak level of mycobacterial ThyX inhibition (40% inhibition at 50 μM). None of the synthesized derivatives displayed any significant inhibitory activity against mycobacterial ThyA. The compounds have also been evaluated as potential inhibitors of mycobacterial thymidine monophosphate kinase (TMPKmt). None of the derivatives showed any significant TMPKmt inhibition. However, replacement of C(6) of the natural substrate (dTMP) by a N-atom furnished 6-aza-dTMP (**1b**), which still was recognized as a substrate by TMPKmt.

Introduction. – The emergence of multidrug resistant (MDR) and more recently, extremely drug resistant (XDR) strains of *Mycobacterium tuberculosis* has complicated the successful implementation of tuberculosis (TB) control programs [1]. MDR-TB is defined as resistance to the first-line drugs isoniazid and rifampin [2], whereas XDR-TB is defined as resistance to isoniazid and rifampin, to any fluoroquinolone, and to at least one of the three injectable antibiotics (capreomycin, kanamycin, and amikacin), all belonging to the aminoglycoside family [3]. MDR and XDR-TB are particularly significant in developing countries where HIV-prevalence is high [4]. Co-infection with HIV and malaria has exacerbated the spread of the TB epidemic in those countries, due to poor infrastructure and socioeconomic conditions, inadequate regimens, and insufficient resources [5]. Globally, the number of prevalent cases of MDR-TB has increased by 64% from 2000 to 2004, and the number of countries that have observed at least one case of XDR-TB has tripled from 20 in 2007 to 69 by the end of 2010 [6]. Due to the exclusion of the fluoroquinolones, which are the most potent

and least toxic second line antibiotics from the treatment regimens, patients being diagnosed with XDR-TB must be subjected to strict supervision and patient monitoring for an extended period of time, up to 24 months, and must be treated with less efficacious and more expensive second line drugs. In view of these recent developments, the emergence of even *totally* drug-resistant strains might become reality within the next decade [7]. It highlights the urgent demand for new antimycobacterial drugs with novel mode of actions to avoid cross-resistance.

Drug-susceptible TB is treated in a six-month short course (the so-called DOTS program) with first-line drugs. The standard regimen comprises of a two-month initiation period with isoniazid, rifampin, pyrazinamide, and ethambutol, followed by a four-month period of isoniazid and rifampin [8]. Isoniazid and ethambutol target essential enzymes involved in the biosynthesis of components of the mycobacterial cell wall [9]. Rifampin binds to the β-subunit of the RNA-polymerase, resulting in abortive initiation of transcription [10]. Pyrazinamide is enzymatically deaminated to pyrazinoic acid which is the active agent [11]. Its activity is most probably due to its capacity in lowering the membrane potential which alters the bacterial uptake of nutrients.

In the search for novel tuberculosis medication, drug-discovery programs starting from marketed antibacterials have been implemented. Using the licensed drug linezolid (**I**) as starting point, new oxazolidinon derivatives (*e.g.*, PNU-100480; **II**) have been prepared with better antimycobacterial activity [12]. Their antibacterial activity is due to inhibition of protein synthesis by binding to ribosomal RNA and thereby blocking formation of the initiation complex. New targets that are currently being approached are ATP synthase [13] (*e.g.*, TMC207; **III**) and mycobacterial phosphatases [14]. A library-based search for potent and selective inhibitors of mycobacterial PTPB resulted in the discovery of I-A09 (**IV**), a non-competitive inhibitor with an IC_{50} value of 1.26 μM, which was able to restore IFN-γ induced apoptosis in Raw264.7 macrophages expressing mPTPB (*Fig. 1*).

Bacterial thymidylate synthase (TS) is a promising target for antimicrobial agents, since the sequences of the TS enzymes are highly conserved among different bacterial species [15]. The amino acid sequences of TS are, however, also highly conserved across species, particularly among the residues that form the substrate and cofactor-binding pockets [16]. Therefore, the synthesis of selective bacterial TS inhibitors is highly challenging. Recent genomic analysis has revealed that *M. tuberculosis* carries the genes for both the classical thymidylate synthase, ThyA, as well as for an alternative thymidylate synthase, called ThyX [17]. Both enzymes catalyze the reductive methylation of 2′-deoxyuridine 5′-monophosphate (dUMP) to thymidine 5′-monophosphate, which constitutes an essential step in nucleotide metabolism. While ThyA uses N^5,N^{10}-methylenetetrahydrofolic acid (CH_2THF) as both, carbon and hydride donor [18], ThyX uses CH_2THF only as carbon donor, but it depends on the NADPH/FAD redox system to fulfill the role as hydride donor [19]. The biochemical reaction mechanism of ThyX [20] involves the transfer of a hydride from reduced flavin adenine dinucleotide ($FADH_2$, generated *via* reduction of FAD by NADPH) to C(6) of dUMP, thereby generating an enolate anion at C(4)/C(5), which, in turn, nucleophilically attacks the iminium cation of CH_2THF. The resulting CH_2THF–dUMP adduct subsequently undergoes β-elimination of the H-atom at C(5) of dUMP which generates

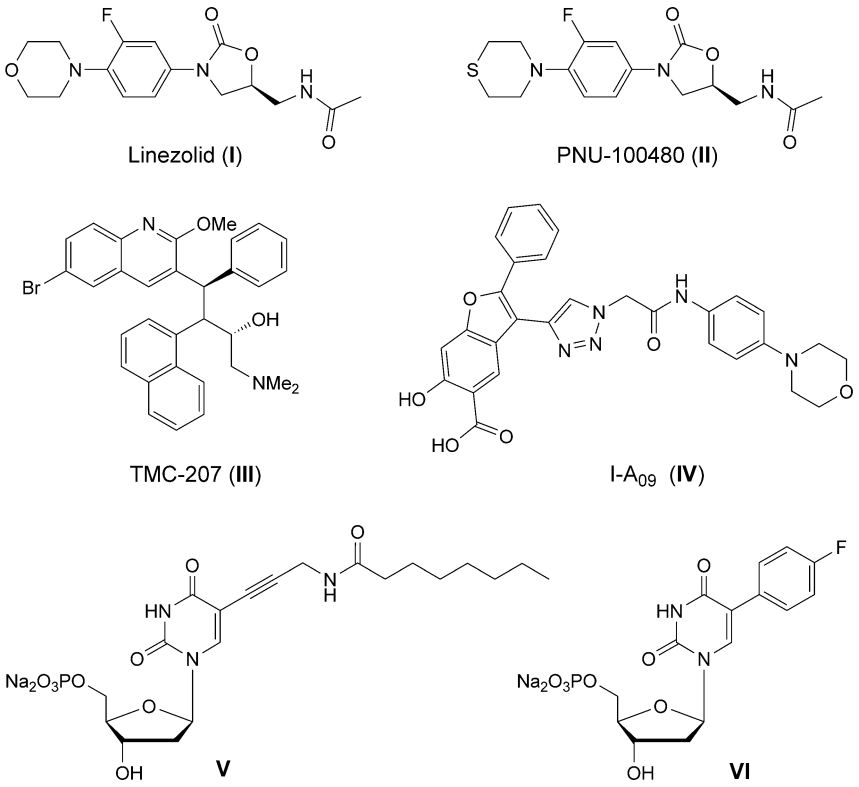


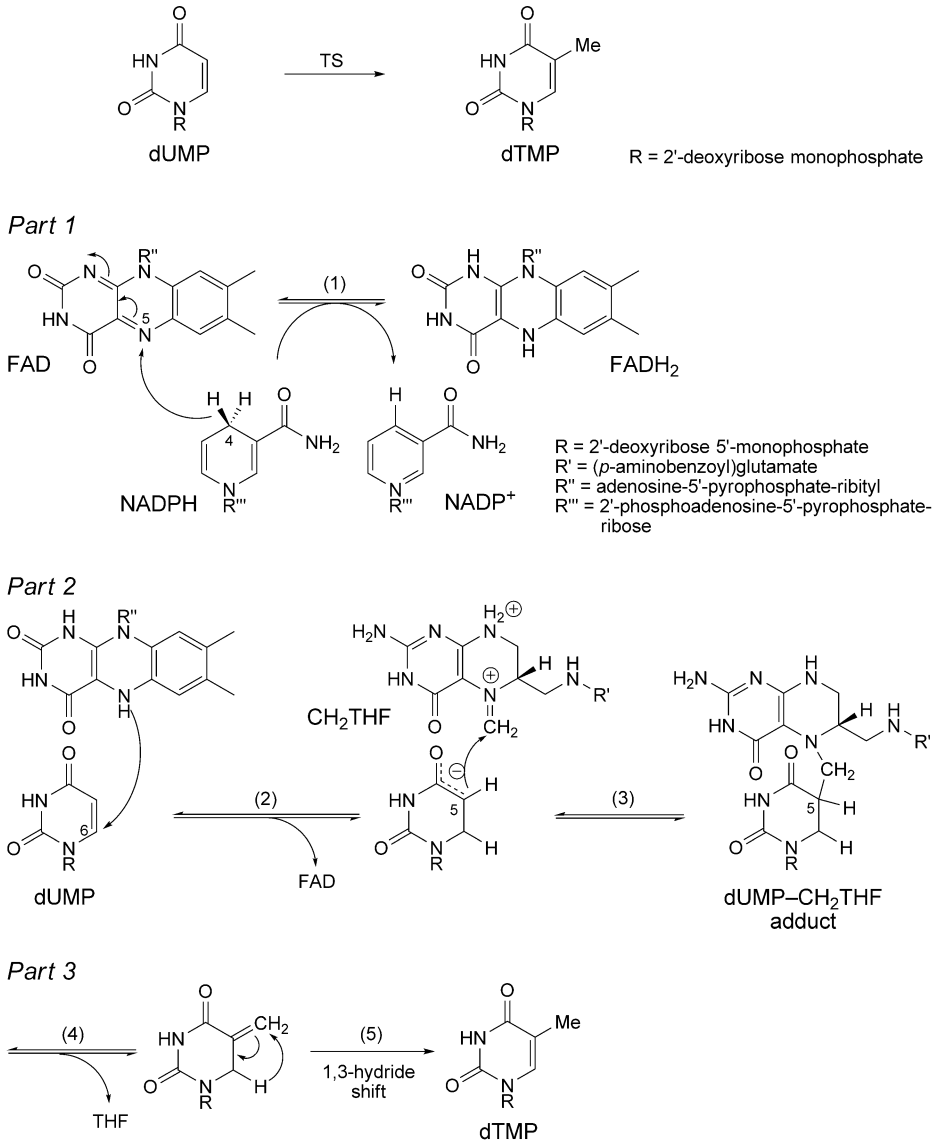
Fig. 1. *Drugs in the clinical pipeline and recently reported selective mycobacterial ThyX inhibitors*

an exocyclic methylidene group at C(5). An intramolecular 1,3-hydride shift, finally, furnishes dTMP (*Scheme 1*).

ThyA and ThyX show neither structural nor sequential similarity, and substantially differ in their biochemical reaction mechanism. Therefore, the design of selective ThyX inhibitors does not have to rely on small structural differences between ThyX and human ThyA proteins. In addition, transposon site hybridization experiments have shown that mycobacterial ThyX is an essential gene for growth of the pathogen [21].

In view of these findings, mycobacterial ThyX was selected as a promising target for the discovery of novel tuberculosis medication. Recently, 5-aryl- and 5-alkynyl-dUMP derivatives have been developed in our laboratory as selective ThyX inhibitors (*Fig. 1*) [22]. To further study the structure–activity relationship (SAR) of dUMP analogs as mycobacterial ThyX inhibitors, we envisioned to prepare 6-aza-dUMP analogs in which C(6) of the uracil moiety is replaced by a N-atom. This would preclude the nucleophilic attack of a hydride from $FADH_2$. In this report, the synthesis and biological evaluation of 6-aza-dUMP analogs, whose structures are depicted in *Fig. 2*, are described. These compounds can also be considered as structural analogs of thymidine monophosphate, and, therefore, they also have been evaluated as potential inhibitors of thymidine monophosphate kinase from *M. tuberculosis* (TMPKmt). TMPKmt is responsible for

Scheme 1. *Biochemical Reaction Mechanism Reported by* Koehn et al. [20]



the phosphorylation of thymidine monophosphate to thymidine diphosphate, using ATP as the preferred phosphate donor. It is considered as an attractive target for the development of novel tuberculosis medication for the following reasons: *i*) TMPK acts at the junction of the *de novo* and salvage pathways for thymidine triphosphate metabolism, and is the last specific enzyme for its synthesis [23]; *ii*) it has been demonstrated that TMPKmt is an essential enzyme for mycobacterial DNA synthesis

[24], and *iii*) biochemical [25] and structural [26] characterizations revealed that TMPKmt is sufficiently different from its human counterpart, which allows the synthesis of selective TMPKmt inhibitors. A number of inhibitors of TMPKmt are known [27]. These include sugar- and base-modified nucleosides and nucleotides [28], as well as substituted pyrimidine analogs [29]. To the best of our knowledge, 6-aza-dUMP analogs have never been evaluated as potential inhibitors of TMPKmt.

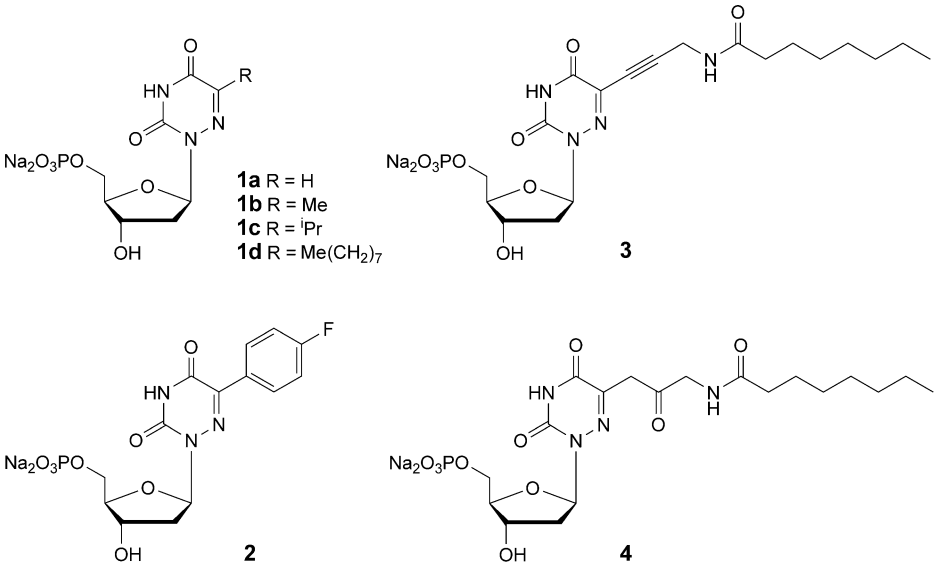
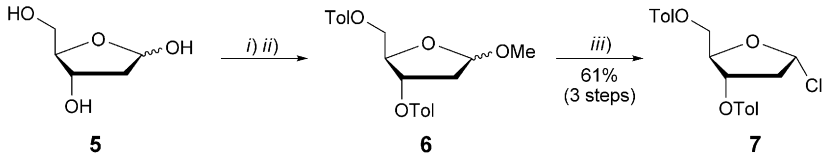


Fig. 2. *Structures of synthesized 6-aza-dUMP analogs*

Results and Discussion. – *Chemistry.* The syntheses of 6-aza-dUMP derivatives **1a**–**1d** and **2**–**4** have been carried out *via Vorbrüggen*-type glycosylation [30] of suitable 5-substituted 6-azauracils with *Hoffer*'s chloro sugar **7** [31]. Chloro sugar **7** was prepared in three steps from 2-deoxy-D-ribose **5** (*Scheme 2*). Upon treatment of intermediate **6** with saturated HCl in AcOH, diastereomerically pure compound **7** precipitated out after a few minutes.

Scheme 2

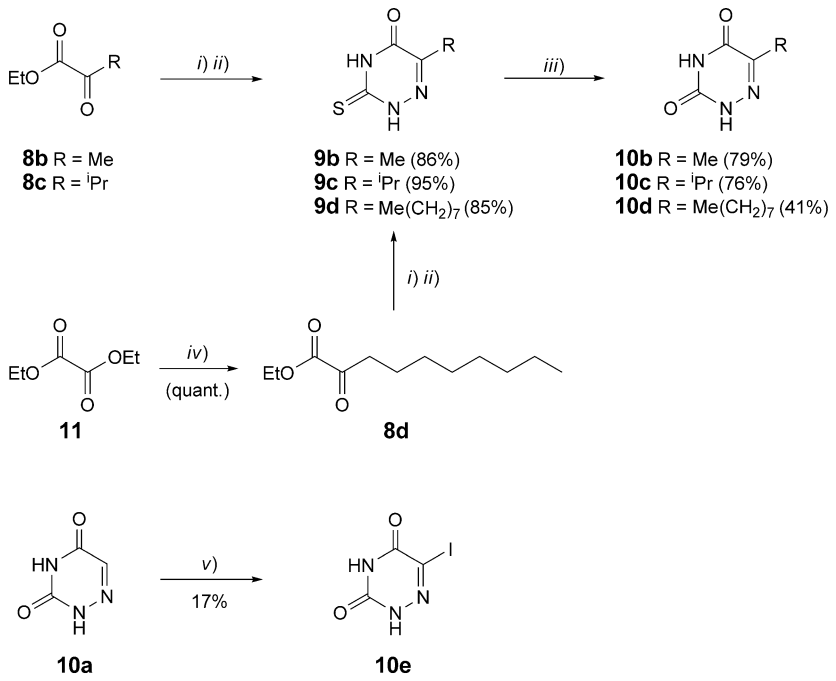


i) HCl/MeOH, r.t. *ii*) *p*-TolCl, Pyr, r.t. *iii*) HCl/AcOH, r.t.

The synthesis of 6-azathymine **10b** and 5-iPr-6-azauracil **10c**, outlined in *Scheme 3*, starts from commercially available keto esters **8b** and **8c**, respectively. Keto ester **8d**, necessary for the preparation of 5-octyl-6-azauracil **10d**, was prepared from diethyl

oxalate **11** in quantitative yield by a *Grignard* reaction. Keto esters **8b**–**8d** were treated with thiosemicarbazide in EtOH, followed by ring closure under aqueous alkaline conditions, to furnish 2-thio-6-azauracil derivatives **9b**–**9d**, respectively [32]. Conversion of **9b**–**9d** to the desired 6-azauracil derivatives **10b**–**10d**, respectively, was performed in alkaline H_2O_2 at room temperature [32]. 5-Iodo-6-azauracil **10e** was prepared according to a literature procedure by iodination of commercially available 6-azauracil, albeit in low yield [33].

Scheme 3

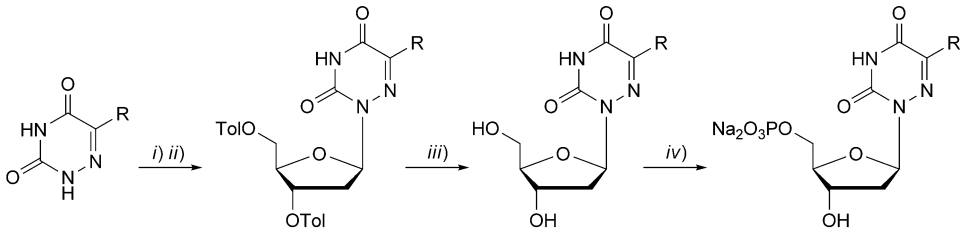


i) Thiosemicarbazide, EtOH, 90°. *ii*) NaOH, H_2O, reflux. *iii*) 35% aq. H_2O_2, 1M NaOH, r.t. *iv*) $Me(CH_2)_7MgBr$, THF/Et_2O, −60°. *v*) I_2, KI, NaOH, H_2O, reflux.

Coupling reactions between 6-azauracil moieties **10a**–**10e** and the chloro sugar **7** were performed in the presence of CuI as catalyst according to the method by *Freskos* (*Scheme 4*) [34]. Yields were usually good, however, in contrast to the literature [34], only a modest β/α-selectivity (usually *ca.* 60%, as determined by ^{1}H-NMR spectroscopy) was observed. The pure β-epimers were obtained either by recrystallization or silica-gel column chromatography. Addition of TMSCl (1 equiv.) led to a slight improvement of that ratio to *ca.* 70% but can generally be omitted. The nucleosides **12a**–**12d** were subsequently subjected to alkaline deprotection of the toluoyl groups [35], followed by phosphorylation of the primary OH groups [36], to yield the nucleoside monophosphate derivatives **1a**–**1d**, respectively.

Toluoyl-protected 5-iodo-6-aza-2′-deoxyuridine derivative **12e** served as a key intermediate for the preparation of 5-(fluorophenyl)-6-aza derivative **2**, as well as the

Scheme 4



10a R = H
10b R = Me
10c R = ^{i}Pr
10d R = Me(CH$_2$)$_7$

12a R = H (58%)
12b R = Me (73%)
12c R = ^{i}Pr (43%)
12d R = Me(CH$_2$)$_7$ (66%)

13a R = H
13b R = Me
13c R = ^{i}Pr
13d R = Me(CH$_2$)$_7$

1a R = H (40%)
1b R = Me (53%)
1c R = ^{i}Pr (17%)
1d R = Me(CH$_2$)$_7$ (47%)

i) Hexamethyldisilazane (HMDS), $(NH_4)_2SO_4$, reflux. *ii*) Chloro sugar **7**, CuI, Me_3SiCl (TMSCl), $CHCl_3$, r.t. *iii*) 0.1M MeONa, MeOH, r.t. *iv*) $POCl_3$, proton sponge, $PO(OMe)_3$, 0°.

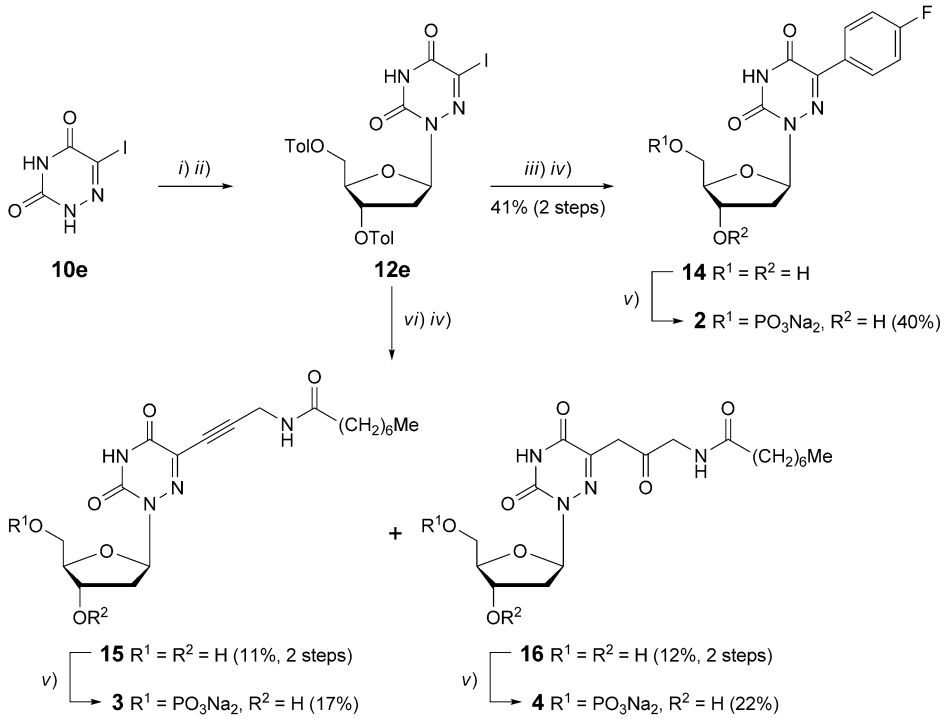
5-(prop-1-ynyl)-6-aza derivative **3** (*Scheme 5*). Coupling of compound **12e** with (4-fluorophenyl)boronic acid under *Suzuki–Liebeskind* reaction conditions [37] led to the corresponding toluoyl protected nucleoside derivative. Alkaline deprotection, followed by phosphorylation of the primary OH group of derivative **14**, yielded the 5-(4-flurophenyl) nucleotide **2**. *Sonogashira* coupling [38] of **12e** with *N*-(prop-2-ynyl)octanamide [22] under standard reaction conditions with $[Pd(PPh_3)_4]$ as catalyst gave unsatisfactory results, due to low conversion and difficult purification of the reaction mixture. Changing to $[Pd_2(dba)_3]$ along with the addition of PPh_3 led to a remarkable increase in reactivity. However, the *Sonogashira* coupling reaction under these conditions afforded substantial amounts of the corresponding hydrated congener. The alkynyl derivative **15** and the hydrated side product **16** were separated by flash chromatography after alkaline deprotection of the toluoyl groups. Standard phosphorylation of derivatives **15** and **16**, finally, gave the desired monophosphate **3** along with **4**, respectively.

The correct structure of derivative **4** was established by 1D- and 2D-NMR spectroscopy. The ^{13}C-NMR spectrum of this derivative in D_2O clearly showed two CD_2 peaks at δ 40.3 and 48.3 ppm, respectively (*Fig. 3*).

These intriguing finding indicated the presence of two enolizable CH_2 H-atoms adjacent to a C=O group. The corresponding regioisomer **17** would give rise essentially to only one CD_2 signal, which is indicative for the correct structure in derivative **4**. When the NMR spectra were run in (D_6)DMSO as solvent, enolization was impossible, and two 'normal' CH_2 peaks were obtained instead of the CD_2 peaks (*Fig. 3*).

Furthermore, the signals of the two CH_2 H-atoms were missing in the ^{1}H-NMR spectrum recorded in D_2O, whereas the ^{1}H-NMR spectrum of regioisomer **17** would still show the signals of the two CH_2 H-atoms β to the C=O group. The correct structure of analog **4** was further confirmed by 2D-COSY-NMR spectroscopy (*Fig. 4*). In (D_6)DMSO (in contrast to the spectrum in D_2O), the ^{1}H-NMR spectrum showed resonances of two additional CH_2 groups at δ 3.65 and 4.0 ppm, respectively. In the 2D-

Scheme 5



i) HMDS, $(NH_4)_2SO_4$, reflux. *ii*) Chloro sugar **7**, CuI, TMSCl, $CHCl_3$, r.t. *iii*) (4-Fluorophenyl)boronic acid, $Pd(PPh_3)_4$, copper(I) thiophene-2-carboxylate (CuTC), THF, 50°. *iv*) 0.1M MeONa, MeOH, r.t. *v*) $POCl_3$, proton sponge, $PO(OMe)_3$, 0°. *vi*) *N*-(Prop-2-ynyl)octanamide, $[Pd_2(dba)_3]$, PPh_3, CuI, Et_3N, DMF, r.t.

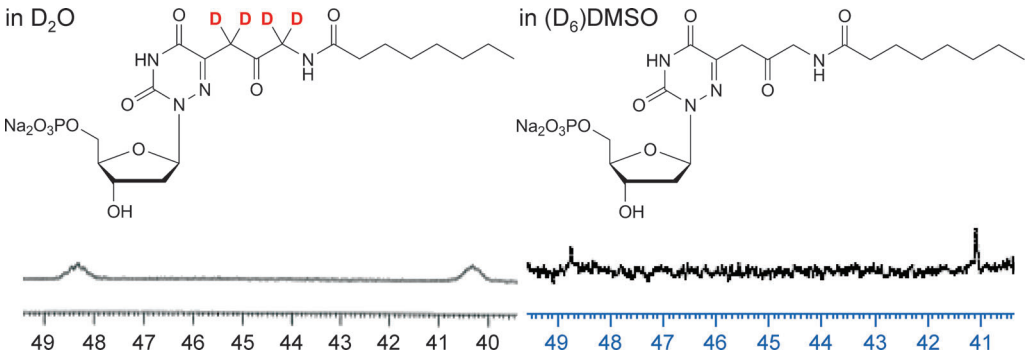


Fig. 3. *^{13}C-NMR Spectrum of compound* **4** *in D_2O (left) and $(D_6)DMSO$ (right)*

COSY spectrum, no cross-peak between these two CH_2 groups was observed, ruling out the correct structure to be that of the regioisomer **17**.

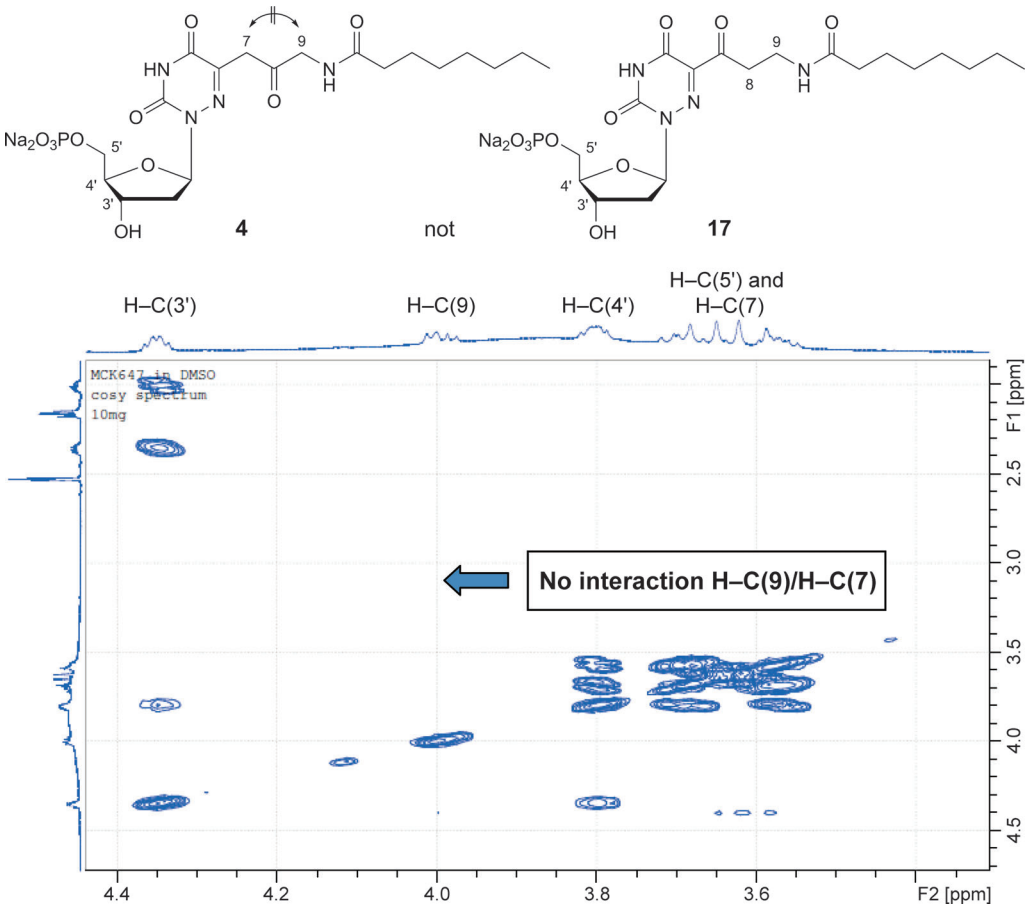


Fig. 4. *COSY Spectrum of compound* **4** *in* (D_6)*DMSO*

The correct connection of the nucleobase to the 2′-deoxyribose moiety was determined by 2D-HMBC-NMR spectroscopy by using compound **1c** as representative example. *Fig. 5* shows H–C(1′) being coupled to C(2) and C(5) of the nucleobase. It indicates that the sugar moiety is connected to N(1) of the 5-^{i}Pr-6-azauracil base. In case of the corresponding N(3)-regioisomer, an additional HMBC correlation with C(4) would be expected. This is absent in the HMBC spectrum and confirms the identity of compound **1c**.

Biological Evaluation. Compounds have been evaluated for their inhibitory activity against mycobacterial ThyX and ThyA. Cloning the ThyX and ThyA gene, protein expression and purification were performed as described in [22]. Similarly, the biochemical assays have been performed as reported in [22][39]. Compounds were evaluated at a concentration of 50 μM in the ThyX as well as in the ThyA assay. In both assays, 5-F-dUMP was included as reference compound and positive control.

The co-crystal structure [40] of the ternary complex ThyX-FAD-5-BrdUMP revealed that the O-atoms of the 5′-monophosphate moiety of 5-Br-dUMP are engaged

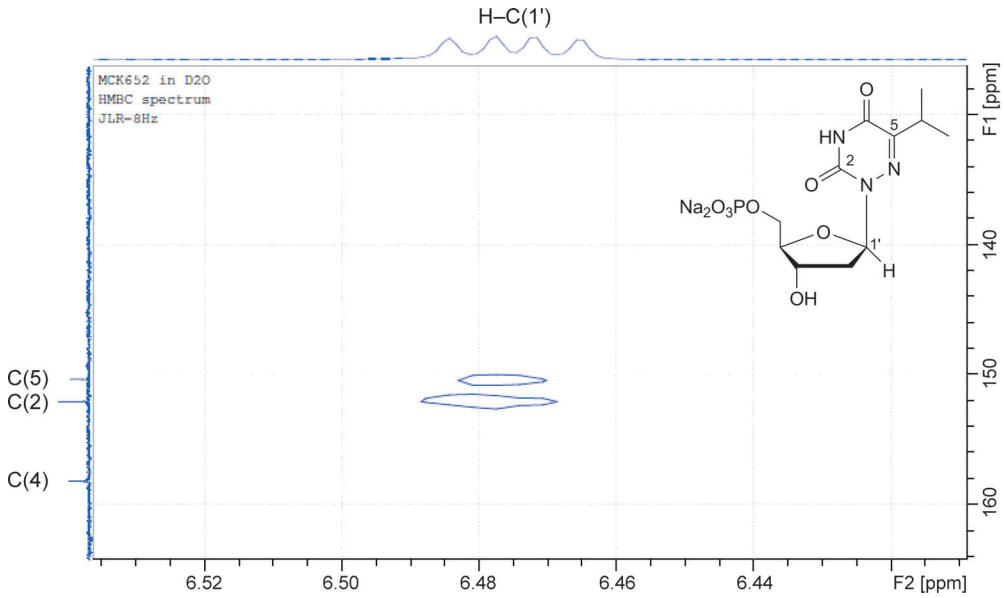


Fig. 5. *HMBC Spectrum of compound* **1c**

in crucial interactions with residues in the active site of the ThyX enzyme. Furthermore, the pyrimidine ring of 5-Br-dUMP and the isoalloxazine ring of FAD are close enough to interact *via* H-bonds and π-stacking. Based on this information from X-ray crystallography studies, the structure–activity relationship (SAR) study at C(5) of the natural dUMP substrate has been studied already quite extensively. The presence of small substituents (F, Br) led to potent ThyX inhibitory activities. Furthermore, it has been demonstrated by our group that introduction of sterically demanding substituents such as a 4-fluorophenyl moiety (compound **VI**) and a 3-(octanamido)prop-1-ynyl side chain (compound **V**) yielded nucleotide derivatives with potent and selective mycobacterial ThyX inhibitory activities, with IC_{50} values of 10 and 0.91 μM, respectively [22]. The finding that some structural variety is tolerated at C(5) of the natural uracil base prompted us to prepare a series of 6-aza-dUMP analogs, in which the substituent at C(5) was varied in a systematic way. Because of the above mentioned crucial interactions of the phosphate group, all derivatives synthesized in this study have been evaluated as their corresponding 5′-monophosphate congeners.

The investigation of 6-aza-dUMP derivatives as potential ThyX-inhibitors started with the synthesis of the parent compound 6-aza-dUMP **1a**. This derivative exhibited weak mycobacterial ThyX inhibitory activity (33% inhibition at 50 μM; *Table 1*). To increase activity, small modifications were introduced at C(5) of the 6-azauracil base moiety, starting with a Me group in the analog **1b**. This resulted in a complete loss of inhibitory activity. Similarly, increasing the size of the substituent to an ^{i}Pr group (compound **1c**) or an octyl chain (compound **1d**) led to complete loss of ThyX-inhibitory activity.

Table 1. *Inhibition of Mtb-ThyX and Mtb-ThyA by Compounds* **1a**–**1d** *and* **2**–**4**[a])

Compound	ThyX Inhibition		ThyA Inhibition	
	% Inhibition (50 μM)	IC_{50} [μM]	% Inhibition (50 μM)	IC_{50} [μM]
5-F-dUMP[b])	95.1	0.29	100	0.57
1a	33.5	>50	0.99	>50
1b	−9.3	>50	8.78	>50
1c	−42.8	>50	20.04	>50
1d	5.7	>50	3.67	>50
2	−14.1	>50	8.65	>50
3	40.9	>50	13.18	>50
4	−12.6	>50	14.71	>50

[a]) Values are means of three independent experiments. [b]) Positive control.

As, within the dUMP series, a 4-fluorophenyl group and a long amidopropargyl side chain at C(5) were found to be optimal for ThyX inhibition, these substituents were also introduced in the current 6-aza-dUMP series. This led to the synthesis of 6-aza-dUMP derivatives **2** (bearing a 5-(4-fluorophenyl) group) and **3** (with a long amidopropargyl side chain). As can be deduced from *Table 1*, compound **2** lacks any inhibitory activity, whereas derivative **3** displays only low levels of ThyX inhibitory activity (40% inhibition at 50 μM). Both analogs are totally devoid of ThyA-inhibitory activity. These data highlight the fact that replacement of C(6) by a N-atom is detrimental for ThyX inhibitory activity.

Compounds have also been evaluated on TMPKmt (*Table 2*), as their structures were closely related to the natural 5′-monophosphate substrate, namely dTMP. No activity was detected in the presence of 0.5 mM ATP and with the different compounds at concentrations from 0.5 up to 1 mM, even with a large excess of TMPKmt (four-times more than with dTMP), except for 6-aza-dTMP **1b**. The latter was found to be a substrate (K_m value 20-fold higher and reaction rate by 21% than that with dTMP). On the other hand, compounds **1a**, **1c**, **1d**, **2**, and **3** were able to inhibit TMPKmt activity, yet with different potencies. As for the thymidine analogs [41], 6-aza-dUMP **1a** was the weakest inhibitor. The presence of an octyl chain at C(5) of the base moiety (compound **1d**) led to the most potent inhibitor in this series, displaying an IC_{50} value of 400 μM.

Table 2. *Inhibitory Activity of Compounds* **1a**–**1d** *and* **2**–**3** *against TMPKmt*

Compound	K_m^{app} [μM][a])	% Inhibition (1.5 mM)	IC_{50} [mM]
dTMP	33		
1a		30	>2
1b	610		
1c		50	1.5
1d		95	0.4
2		50	1.5
3		40	>1.5

[a]) K_m^{app} Values were calculated by fitting the experimental data to the *Michaelis–Menten* equation using the Kaleidagraph software.

Conclusions. – In view of the recent rise in TB incidence, as well as the steady spread of MDR- and XDR-TB, the targets set by the *WHO* to halve TB prevalence by 2015 are fully out of reach. These figures highlight an urgent demand for new drugs acting on novel targets. Due to the structural and sequence dissimilarities of mycobacterial ThyX and human ThyA, as well as the essential role of ThyX in nucleotide metabolism in *M. tuberculosis*, ThyX is a promising target for the discovery of novel antimycobacterial agents. Here, the synthesis of novel 6-aza-dUMP analogs with structural variation at C(5) of the base moiety is described. These compounds were evaluated as potential inhibitors of mycobacterial ThyX and ThyA. Within this series, only the parent nucleotide 6-aza-dUMP **1a** and the 5-substituted 6-aza-dUMP derivative **3**, bearing a amidopropargyl side chain displayed weak ThyX inhibitions (30–40% inhibition at 50 μM) and completely lacked any activities against ThyA. Moreover, this series of compounds were found to interact within the dTMP site of TMPKmt, being either substrate or inhibitor of this enzyme. Therefore, these compounds are very promising, as they would be able to act simultaneously on different targets specific of the mycobacterial metabolism. Further input from structural biology, X-ray crystallographic studies, and modeling are required to improve their current binding properties.

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Experimental Part

General. Abbreviations. AcOH, Acetic acid; CH_2THF, methylenetetrahydrofolic acid; CuTC, copper(I) thiophene-2-carboxylate; dba, dibenzylideneacetone; DMSO, dimethyl sulfoxide; DOTS, directly observed treatment short; dTMP, 2′-deoxythymidine 5′-monophosphate; dUMP, 2′-deoxyuridine 5′-monophosphate; ATP, adenosine 5′-triphosphate; FAD, flavin adenine dinucleotide; HIV, human immunodeficiency virus; HMDS, 1,1,1,3,3,3-hexamethyldisilazane; HR-MS, high-resolution mass spectrometry; IC_{50}, half maximal (50%) inhibitory concentration; MDR-TB, multidrug-resistant TB; mPTPB, mycobacterial protein tyrosine phosphatase B; NADPH, nicotinamide adenine nucleotide phosphate hydride; PE, petroleum ether; RP-HPLC, reversed-phase high-performance liquid chromatography; SAR, structure–activity relationship; TB, tuberculosis; TEAB, triethylammonium bicarbonate; ThyA, thymidylate synthase; ThyX, flavin-dependent thymidylate synthase; TMPKmt, thymidine monophosphate kinase from *M. tuberculosis*; TMSCl, trimethylsilyl chloride; TS, thymidylate synthase; *WHO*, *World Health Organization*; XDR-TB, extensively drug-resistant TB.

For all reactions, anal.-grade solvents were used. Dry MeOH was obtained by distillation over CaH_2. Dry $CHCl_3$, THF, Et_2O, and DMF were purchased from commercial suppliers. All moisture-sensitive reactions were carried out in oven-dried glassware (120°). TLC: Precoated aluminum sheets (*Fluka* silica gel/TLC cards, 254 nm). Column chromatography (CC): *ICN* silica gel (SiO_2) *63–200*, 60 Å. All final compounds possessed a purity of at least 95% as determined by anal. RP-HPLC analysis on an *XBridge* column (*C-18*, 5 μm, 4.6 × 150 mm) in combination with a *Waters 600 HPLC* system, a *Waters 717 plus* autosampler and a *Waters 2996* photodiode array detector from *Waters*, Milford, Massachusetts, USA. Prep. HPLC purification: the same instrument with a prep. *XBridge* column (*C-18*, 5 μm, 19 × 150 mm) from *Waters*, Milford, Massachusetts, USA. ^{1}H- and ^{13}C-NMR spectra: *Bruker Advance 300* (^{1}H: 300, ^{13}C: 75, and ^{31}P: 121 MHz) or *500* (^{1}H: 500 and ^{13}C: 125 MHz) spectrometer, Me_4Si as internal standard for ^{1}H-NMR, and (D_6)DMSO (39.52 ppm) or $CDCl_3$ (77.16 ppm) for ^{13}C-NMR spectra; chemical shifts δ in

ppm; coupling constants *J* in Hz. MS: *Finnigan LCQ advantage Max* (ion trap) mass spectrometer from *Thermo Finnigan*, San Jose, CA, USA; exact mass measurements performed on a quadrupole time-of-flight mass spectrometer (*Q-tof-2*, *Micromass*, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface; samples infused in ^{i}PrOH/H_2O 1:1 at 3 µl/min.

*2-Deoxy-α-*D-erythro-*pentofuranosyl Chloride Bis(4-methylbenzoate)* (**7**). *2-Deoxy-*D-*ribose* (**5**; 4 g, 29.84 mmol) was dissolved in MeOH (48 ml), and a soln. of 1% HCl in MeOH (8 ml) was added. The mixture was stirred at r.t. for 25 min, and then the reaction was quenched through addition of solid $NaHCO_3$ (1.6 g). The resulting suspension was stirred for *ca.* 5 min, then filtered off, washed with MeOH, and evaporated *in vacuo*. The residue was co-evaporated twice with dry pyridine, and subsequently dissolved in dry pyridine (24 ml) under Ar and cooled to 0°. *p*-Toluoyl chloride (8.8 ml, 65.8 mmol) was added drop- to portionwise, the ice bath was removed, and the mixture was stirred at r.t. overnight. The mixture was diluted with H_2O (40 ml) at 0° and extracted with CH_2Cl_2 (3 × 40 ml). The org. layer was washed with sat. $NaHCO_3$ (1 × 100 ml), 2N HCl (2 × 100 ml), and H_2O (1 × 100 ml), dried ($MgSO_4$), and evaporated. The resulting yellow-orange oil was dissolved in AcOH (16 ml) and treated drop- to portionwise with a sat. soln. of HCl in AcOH (25 ml) at r.t. Towards the end of the addition, **7** (7.07 g, 61% over three steps) readily precipitated, thereby forming a thick milky suspension, which was rapidly filtered off, washed with dry Et_2O, and dried *in vacuo*. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.99 (*d*, *J* = 8.2, 2 arom. H); 7.89 (*d*, *J* = 8.2, 2 arom. H); 7.25 (*m*, 4 arom. H); 6.47 (*d*, *J* = 5.1, H–C(1)); 5.56 (*m*, H–C(3)); 4.86 (*m*, H–C(4)); 4.71–4.57 (*ddd*, *J* = 42.6, 12.1, 3.7, CH_2(5)); 2.91–2.82 (*m*, 1 H, CH_2(2)); 2.74 (*d*, *J* = 9.0, 1 H, CH_2(2)); 2.42 (*s*, Me); 2.41 (*s*, Me). ^{13}C-NMR (125 MHz, $CDCl_3$): 166.55; 166.21; 144.44; 144.21; 130.06 (2 C); 129.82 (2 C); 129.39 (2 C); 129.36 (2 C); 126.95; 126.83; 95.47; 84.85; 73.69; 63.64; 44.68; 21.87; 21.83. ESI-MS (pos.): 352.80 ($[M-Cl]^+$, $C_{21}H_{21}O_5^+$; calc. 353.14).

*3,4-Dihydro-6-methyl-3-thioxo-1,2,4-triazin-5(2*H*)-one* (**9b**). To a soln. of *ethyl pyruvate* (**8b**; 1.5 ml, 13.5 mmol) in EtOH (30 ml) was added thiosemicarbazide (1.14 g, 12.45 mmol), and the resulting suspension was stirred at 90° for 30 min. The clear soln. obtained was allowed to cool to r.t. and concentrated *in vacuo*. The residue was suspended in H_2O (30 ml), NaOH (920 mg, 23 mmol) was added, and the mixture was refluxed for 30 min. The resulting clear soln. was allowed to cool to r.t. and acidified to pH 4 with AcOH at 0°. The aq. layer was extracted with AcOEt (3 × 40 ml), and the org. layer was dried ($MgSO_4$) and evaporated to afford pure **9b** (1.53 g, 86%). White solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 6:1): 2.25 (*s*, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 6:1): 173.09; 153.52; 148.41; 15.93. ESI-MS (neg.): 141.80 ($[M-H]^-$, $C_4H_4N_3OS^-$; calc. 142.01).

*3,4-Dihydro-6-(1-methylethyl)-3-thioxo-1,2,4-triazin-5(2*H*)-one* (**9c**). To a soln. of *ethyl dimethylpyruvate* (**8c**; 1.8 ml, 12.35 mmol) in EtOH (28 ml) was added thiosemicarbazide (1.043 g, 11.39 mmol), and the resulting suspension was stirred at 90° for 30 min. The clear soln. obtained was allowed to cool to r.t. and concentrated *in vacuo*. The residue was suspended in H_2O (28 ml), NaOH (842 mg, 21.04 mmol) was added, and the mixture was refluxed for 30 min. The resulting clear soln. was allowed to cool to r.t. and acidified to pH 4 with AcOH at 0°. The aq. layer was extracted with AcOEt (3 × 40 ml), and the org. layer was dried ($MgSO_4$) and evaporated to afford pure **9c** (1.86 g, 95%). White solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 7:1): 3.17 (*m*, Me_2CH); 1.22 (*s*, Me); 1.19 (*s*, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 7:1): 173.44; 155.10; 152.70; 28.66; 19.44 (2 C). ESI-MS (neg.): 169.80 ($[M-H]^-$, $C_6H_8N_3OS^-$; calc. 170.04).

*3,4-Dihydro-6-octyl-3-thioxo-1,2,4-triazin-5(2*H*)-one* (**9d**). *Diethyl oxalate* (**11**; 1 ml, 7.39 mmol) was dissolved in dry THF (8 ml) and dry Et_2O (8 ml) at 0° under Ar, and the soln. was cooled to −60°. Octylmagnesium bromide (2M in Et_2O, 4.4 ml, 8.8 mmol) was added over 10 min, and the stirred mixture was allowed to warm to 0° over 2½ h. The reaction was quenched by addition of 2N H_2SO_4 (7 ml), diluted with H_2O, and the phases were separated. The aq. layer was extracted with Et_2O (1 × 15 ml), and the org. phase was dried ($MgSO_4$) and evaporated. The resulting yellow oil **8d** (1.84 g, quant.) was used in the next step without further purification. This intermediate was dissolved in EtOH (14 ml), and thiosemicarbazide (561 mg, 6.16 mmol) was added in one portion. The mixture was stirred at 90° for 30 min, cooled to r.t., and the solvent was evaporated. The residue was taken up in H_2O (15 ml), and NaOH (460 mg, 11.5 mmol) was added. The mixture was refluxed for 30 min, allowed to cool to r.t., and acidified to pH 4 with AcOH at 0°. The suspension was diluted with H_2O (25 ml), extracted with AcOEt (3 × 40 ml), and the org. layer was dried ($MgSO_4$) and evaporated. The residue was purified by CC (SiO_2;

PE/AcOEt 4:1 containing 1% HCOOH) to yield **9d** (1.262 g, 85%) as a white solid, which contained substantial amounts of impurities and was, therefore, used in the next step without any further purification.

6-Azathymine (=*6-Methyl-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **10b**). Compound **9b** (716 mg, 5 mmol) was dissolved in 1M NaOH (15 ml), and the soln. was cooled to 0°. Aq. H_2O_2 (35%; 1.9 ml, 22.27 mmol) was added dropwise, whereupon the color of the mixture changed from colorless to yellow and finally back to colorless towards the end of the H_2O_2 addition. The mixture was stirred at r.t. for 30 min and subsequently acidified with conc. HCl at 0°. The aq. layer was extracted with AcOEt (6 × 30 ml), the combined org. layer was dried ($MgSO_4$) and evaporated, which afforded pure **10b** (504 mg, 79%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 5:2): 2.20 (*s*, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 5:2): 157.56; 150.20; 143.68; 15.56. ESI-MS (neg.): 125.50 ($[M-H]^-$, $C_4H_4N_3O_2^-$; calc. 126.03)

5-(1-Methylethyl)-6-azauracil (=*6-(1-Methylethyl)-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **10c**). Compound **9c** (1.20 g, 7 mmol) was dissolved in 1M NaOH (21 ml), and the soln. was cooled to 0°. Aq. H_2O_2 (35%; 2.65 ml, 31.06 mmol) was added dropwise, whereupon the color of the mixture changed from colorless to yellow and finally back to colorless towards the end of the H_2O_2 addition. The mixture was stirred at r.t. for 30 min and subsequently acidified with conc. HCl at 0°. The aq. layer was extracted with AcOEt (6 × 30 ml), and the combined org. layer was dried ($MgSO_4$) and evaporated to afford pure **10c** (830 mg, 76%). White solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 6:1): 3.15 (*m*, Me_2CH); 1.21 (*s*, Me); 1.18 (*s*, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 6:1): 156.81; 150.82; 150.22; 28.55; 19.62 (2 C). ESI-MS (neg.): 153.90 ($[M-H]^-$, $C_6H_8N_3O_2^-$; calc. 154.06).

5-Octyl-6-azauracil (=*6-Octyl-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **10d**). Crude **9d** (1.02 g, 4.23 mmol) was suspended in 1M NaOH (13 ml), and 35% aq. H_2O_2 (1.6 ml, 18.75 mmol) was added dropwise at r.t. The mixture was stirred at r.t. for 30 min, then acidified with conc. HCl, and diluted with H_2O (30 ml). The aq. layer was extracted with AcOEt (3 × 40 ml), and the org. layer was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was suspended in petroleum ether, filtered off, and washed with PE to afford **10d** (392 mg, 41%). White solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 6:1): 2.57 (*t*, *J* = 7.7, $CH_2(\alpha)$); 1.61 (*m*, $CH_2(\beta)$); 1.27 (*m*, 5 × CH_2); 0.87 (*t*, *J* = 6.8, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 6:1): 157.24; 150.20; 147.08; 31.76; 29.58; 29.22; 29.14; 29.10; 26.28; 22.56; 13.92. ESI-MS (neg.): 224.16 ($[M-H]^-$, $C_{11}H_{18}N_3O_2^-$; calc. 224.14).

5-Iodo-6-azauracil (=*6-Iodo-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **10e**). To a soln. of **10a** (3.39 g, 30 mmol) in H_2O (105 ml) were added KI (15.9 g, 95.79 mmol), NaOH (4.8 g, 120 mmol), and I_2 (15 g, 59.1 mmol) at r.t. The mixture was refluxed for 40 h, then allowed to cool to r.t., and acidified with conc. HCl at 0°. The dark mixture was decolorized with 5% $NaHSO_3$/$Na_2S_2O_5$ soln. (60 ml) at 0° and subsequently extracted with AcOEt (3 × 150 ml). The org. layer was washed with a soln. containing 150 ml of H_2O and 10 ml of 5% $NaHSO_3$/$Na_2S_2O_5$, dried ($MgSO_4$), and evaporated. The residue was purified by CC (SiO_2; 2% EtOH in CH_2Cl_2 containing 1% HCOOH) to afford **10e** (1.21 g, 17%). Off-white solid. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.60 (br. *s*, NH); 12.24 (br. *s*, NH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 155.08; 148.90; 110.58. ESI-MS (neg.): 237.98 ($[M-H]^-$, $C_3HIN_3O_2^-$; calc. 237.91).

3′,5′-Di-O-p-*toluoyl-2′-deoxy-6-azauridine* (=*2-[2-Deoxy-3,5-bis*-O-*(4-methylbenzoyl)-β*-D-erythro-*pentofuranosyl]-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **12a**). A suspension of **10a** (372 mg, 3.3 mmol) and $(NH_4)_2SO_4$ (39 mg, 0.3 mmol) in HMDS (20 ml) was refluxed overnight under Ar. The clear soln. obtained was allowed to cool to r.t., and the solvent was evaporated under exclusion of air and moisture. To the resulting oil were added **7** (1.17 g, 3 mmol), CuI (573 mg, 3 mmol), and dry $CHCl_3$ (60 ml), and the mixture was stirred at r.t. for 2 h. The reaction was quenched by addition of sat. $NaHCO_3$ (5 ml), and the mixture was stirred vigorously for 5 min and evaporated to near dryness. The residue was taken up in AcOEt (70 ml), and the insoluble material was filtered off. The filtrate was washed with sat. $NaHCO_3$ (1 × 60 ml) and brine (1 × 60 ml), dried ($MgSO_4$), and evaporated. The residue was purified by CC (SiO_2; 1% EtOH in CH_2Cl_2) to yield **12a** as an anomeric mixture. Recrystallization from hot EtOH afforded pure *β*-anomer **12a** (810 mg, 58%). White solid. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.36 (br. *s*, NH); 7.95 (*m*, 4 arom. H); 7.25 (*m*, 4 arom. H); 6.69 (*t*, *J* = 6.4, H–C(1′)); 5.72 (*m*, H–C(3′)); 4.66 (*m*, H–C(4′)); 4.56–4.40 (*m*, CH_2(5′)); 3.04–2.93 (*m*, 1 H, CH_2(2′)); 2.55–2.35 (*m*, 1 H, CH_2(2′)); 2.43 (*s*, Me); 2.41 (*s*, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.36; 166.16; 155.58; 148.05; 144.51; 144.07; 136.19; 129.95 (2 C); 129.91

(2 C); 129.37 (2 C); 129.25 (2 C); 127.15; 126.67; 86.07; 82.72; 75.04; 64.09; 35.18; 21.85; 21.82. ESI-MS (pos.): 488.10 ($[M+Na]^+$, $C_{24}H_{23}N_3NaO_7^+$; calc. 488.14).

3′,5′-Di-O-p-*toluoyl-2′-deoxy-6-azathymidine* (=*2-[2-Deoxy-3,5-bis*-O-*(4-methylbenzoyl)-β*-D-erythro-*pentofuranosyl]-6-methyl-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **12b**). A suspension of **10b** (210 mg, 1.65 mmol) and $(NH_4)_2SO_4$ (20 mg, 0.153 mmol) in HMDS (15 ml) was refluxed overnight under Ar. The clear soln. obtained was allowed to cool to r.t., and the solvent was evaporated under exclusion of air and moisture. Compound **7** (585 mg, 1.5 mmol) was added, and the mixture was dissolved in dry $CHCl_3$ (30 ml) under Ar. TMSCl (192 µl, 1.5 mmol) and CuI (287 mg, 1.5 mmol) were then added, and the mixture was stirred at r.t. for 3 h. The reaction was quenched by addition of sat. $NaHCO_3$ (3 ml), and the mixture was stirred vigorously for 5 min and evaporated to near dryness. The residue was taken up in AcOEt (50 ml), and the insoluble material was filtered off. The filtrate was washed with sat. $NaHCO_3$ (1×40 ml) and brine (1×40 ml), dried ($MgSO_4$), and evaporated. The residue was purified by CC (SiO_2; 3–5% acetone in CH_2Cl_2) to yield **12b** as an anomeric mixture. Recrystallization from hot EtOH afforded pure *β*-anomer **12b** (525 mg, 73%). White solid. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.15 (br. *s*, NH); 7.88 (*m*, 4 arom. H); 7.33 (*m*, 4 arom. H); 6.49 (*t*, *J* = 6.0, H–C(1′)); 5.63 (*m*, H–C(3′)); 4.58–4.37 (*m*, H–C(4′), CH_2(5′)); 2.94–2.77 (*m*, 1 H, CH_2(2′)); 2.60–2.20 (*m*, 1 H, CH_2(2′)); 2.40 (*s*, Me); 2.38 (*s*, Me); 2.03 (*s*, Me). ^{13}C-NMR (75 MHz, (D_6)DMSO): 165.39; 165.26; 156.57; 148.73; 143.99; 143.95; 143.74; 129.38, 129.26, 129.22 (8 C); 126.69; 126.49; 85.00; 81.11; 74.79; 64.13; 34.38; 21.15 (2 C); 16.11. ESI-MS (pos.): 502.10 ($[M+Na]^+$, $C_{25}H_{25}N_3NaO_7^+$; calc. 502.16).

5-Isopropyl-3′,5′-di-O-p-*toluoyl-2′-deoxy-6-azauridine* (=*2-[2-Deoxy-3,5-bis*-O-*(4-methylbenzoyl)-β*-D-erythro-*pentofuranosyl]-6-(1-methylethyl)-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **12c**). A suspension of **10c** (341 mg, 2.2 mmol) and $(NH_4)_2SO_4$ (25 mg, 0.191 mmol) in HMDS (20 ml) was refluxed overnight under Ar. The clear soln. obtained was allowed to cool to r.t., and the solvent was evaporated under exclusion of air and moisture. Compound **7** (780 mg, 2 mmol) was added, and the mixture was dissolved in dry $CHCl_3$ (40 ml) under Ar. TMSCl (256 µl, 2 mmol) and CuI (383 mg, 2 mmol) were then added, and the mixture was stirred at r.t. for 3 h. The reaction was quenched by addition of sat. $NaHCO_3$ (3 ml), and the mixture was stirred vigorously for 5 min and evaporated to near dryness. The residue was taken up in AcOEt (60 ml), and the insoluble material was filtered off. The filtrate was washed with sat. $NaHCO_3$ (1×50 ml) and brine (1×50 ml), dried ($MgSO_4$), and evaporated. The residue was purified by CC (SiO_2; 2% acetone in CH_2Cl_2) to yield pure *β*-anomer **12c** (434 mg, 43%). White solid. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.15 (br. *s*, NH); 7.86 (*m*, 4 arom. H); 7.30 (*m*, 4 arom. H); 6.52 (*dd*, *J* = 7.0, 4.7, H–C(1′)); 5.70 (*m*, H–C(3′)); 4.56–4.35 (*m*, H–C(4′), CH_2(5′)); 3.09–2.98 (*m*, Me_2CH); 2.91–2.79 (*m*, 1 H, CH_2(2′)); 2.60–2.20 (*m*, 1 H, CH_2(2′)); 2.39 (*s*, Me); 2.35 (*s*, Me); 1.16 (*d*, *J* = 6.9, Me); 1.12 (*d*, *J* = 6.9, Me). ^{13}C-NMR (75 MHz, (D_6)DMSO): 165.42; 165.32; 156.0; 150.37; 148.48; 144.0; 143.77; 129.42, 129.30, 129.23 (8 C); 126.61; 126.49; 85.21; 80.98; 74.87; 64.57; 34.51; 28.25; 21.19; 21.15; 19.75 (2 C). ESI-MS (pos.): 530.0 ($[M+Na]^+$, $C_{27}H_{29}N_3NaO_7^+$; calc. 530.19).

5-Octyl-3′,5′-di-O-p-*toluoyl-2′-deoxy-6-azauridine* (=*2-[2-Deoxy-3,5-bis*-O-*(4-methylbenzoyl)-β*-D-erythro-*pentofuranosyl]-6-octyl-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **12d**). A suspension of **10d** (248 mg, 1.1 mmol) and $(NH_4)_2SO_4$ (13 mg, 0.1 mmol) in HMDS (10 ml) was refluxed overnight under Ar. The clear soln. obtained was allowed to cool to r.t., and the solvent was evaporated under exclusion of air and moisture. To the resulting oil were added **7** (389 g, 1 mmol), CuI (191 mg, 1 mmol), and dry $CHCl_3$ (20 ml), and the mixture was stirred at r.t. for 4 h. The reaction was quenched by addition of sat. $NaHCO_3$ (5 ml), and the mixture was stirred vigorously for 5 min and evaporated to near dryness. The residue was taken up in AcOEt (40 ml), and the insoluble material was filtered off. The filtrate was washed with sat. $NaHCO_3$ (1×40 ml), and the aq. layer was extracted with AcOEt (1×40 ml). The combined org. layer was dried ($MgSO_4$) and evaporated. Upon purification by CC (SiO_2; 3% acetone in CH_2Cl_2), the desired *β*-isomer **12d** (385 mg, 66%, white solid) eluted first, followed by the *α*-isomer of **12d** (25 mg, 4%, white solid). ^{1}H-NMR (300 MHz, $CDCl_3$; *β*-isomer): 8.63 (br. *s*, NH); 7.93 (*m*, 4 arom. H); 7.23 (*m*, 4 arom. H); 6.69 (*t*, *J* = 6.4, H–C(1′)); 5.70 (*m*, H–C(3′)); 4.53 (*m*, H–C(4′), CH_2(5′)); 3.06–2.94 (*m*, 1 H, CH_2(2′)); 2.66–2.45 (*m*, 3 H, CH_2(2′), CH_2(*α*)); 2.43 (*s*, Me); 2.40 (*s*, Me); 1.60 (*m*, CH_2(*β*)); 1.26 (*m*, $5\times CH_2$); 0.86 (*t*, *J* = 6.7, Me). ^{13}C-NMR (75 MHz, $CDCl_3$; *β*-isomer): 166.33; 166.10; 155.60; 148.39; 148.04; 144.45; 143.99; 129.94, 129.36, 129.24 (8 C); 127.12; 126.75; 85.95; 82.28; 75.20; 64.54; 35.07; 31.99; 30.13; 29.39;

29.37; 29.35; 26.45; 22.77; 21.81 (2 C); 14.23. ESI-MS (pos.): 600.10 ($[M+Na]^+$, $C_{32}H_{39}N_3NaO_7^+$; calc. 600.29).

5-Iodo-3′,5′-di-O-p-*toluoyl-2′-deoxy-6-azauridine* (=*2-[2-Deoxy-3,5-bis*-O-*(4-methylbenzoyl)-β*-D-erythro-*pentofuranosyl]-6-iodo-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **12e**). A suspension of **10e** (789 mg, 3.3 mmol) and $(NH_4)_2SO_4$ (39 mg, 0.3 mmol) in HMDS (30 ml) was refluxed overnight under Ar. The clear soln. obtained was allowed to cool to r.t., and the solvent was evaporated under exclusion of air and moisture. To the resulting oil were added **7** (1.17 g, 3 mmol), CuI (573 mg, 3 mmol), and dry $CHCl_3$ (60 ml). TMSCl (383 µl, 3 mmol) was added in one portion, and the mixture was stirred at r.t. for 3 h. The reaction was quenched by addition of sat. $NaHCO_3$ (5 ml), and the mixture was stirred vigorously for 5 min and evaporated to near dryness. The residue was taken up in AcOEt (100 ml), and the insoluble material was filtered off. The filtrate was washed with sat. $NaHCO_3$ (1 × 80 ml) and brine (1 × 80 ml), dried ($MgSO_4$), and evaporated. The residue was purified by CC (SiO_2; 3–5% acetone in CH_2Cl_2) to yield **12e** as an anomeric mixture. Recrystallization from hot EtOH afforded pure *β*-anomer **12e** (798 mg, 45%). White solid. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.43 (br. *s*, NH); 7.91–7.84 (*m*, 4 arom. H); 7.36–7.30 (*m*, 4 arom. H); 6.44 (*dd*, *J* = 6.8, 5.4, H–C(1′)); 5.58 (*m*, H–C(3′)); 4.46 (*m*, H–C(4′), CH_2(5′)); 2.85–2.78 (*m*, 1 H, CH_2(2′)); 2.56–2.49 (*m*, 1 H, CH_2(2′)); 2.39 (*s*, Me); 2.37 (*s*, Me). ^{13}C-NMR (75 MHz, (D_6)DMSO): 165.55; 165.37; 154.58; 147.94; 144.08; 143.83; 129.51, 129.44, 129.37 (8 C); 126.70; 126.54; 112.29; 85.66; 81.41; 74.53; 64.39; 34.92; 21.27 (2 C). ESI-MS (pos.): 614.07 ($[M+Na]^+$, $C_{24}H_{22}IN_3NaO_7^+$; calc. 614.04).

2′-Deoxy-6-azauridine (=*2-(2-Deoxy-β*-D-erythro-*pentofuranosyl)-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **13a**). To a suspension of **12a** (350 mg, 0.752 mmol) in dry MeOH (10 ml) was added MeONa (30% soln. in MeOH, 200 µl, 1.05 mmol), and the mixture was stirred at r.t. for 6 h. The resulting soln. was neutralized with *Dowex 50 WX-8* (H^+ form), and the resin was filtered off and washed with MeOH. After evaporation of the solvent, the residue was purified by CC (SiO_2; 10% EtOH in CH_2Cl_2) to yield **13a** (113 mg, 66%). Off-white solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 5:2): 7.44 (*s*, H–C(5)); 6.51 (*t*, *J* = 6.0, H–C(1′)); 4.48 (*m*, H–C(3′)); 3.94 (*m*, H–C(4′)); 3.75–3.60 (*m*, CH_2(5′)); 2.66–2.50 (*m*, 1 H, CH_2(2′)); 2.40–2.20 (*m*, 1 H, CH_2(2′)). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 5:2): 156.59; 148.22; 135.77; 87.04; 85.73; 70.99; 62.44; 37.37. HR-ESI-MS (pos.): 252.0607 ($[M+Na]^+$, $C_8H_{11}N_3NaO_5^+$; calc. 252.0591).

2′-Deoxy-6-azathymidine (=*2-(2-Deoxy-β*-D-erythro-*pentofuranosyl)-6-methyl-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **13b**). To a suspension of **12b** (400 mg, 0.834 mmol) in dry MeOH (14 ml) was added MeONa (30% soln. in MeOH, 266 µl, 1.4 mmol), and the mixture was stirred at r.t. for 6 h. The resulting soln. was neutralized with *Dowex 50 WX-8* (H^+ form), and the resin was filtered off and washed with MeOH. After evaporation of the solvent, the residue was purified by CC (SiO_2; 7–10% EtOH in CH_2Cl_2) to yield **13b** (188 mg, 92%). Off-white hygroscopic solid. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.08 (br. *s*, NH); 6.31 (*dd*, *J* = 7.0, 5.5, H–C(1′)); 5.15 (br. *s*, OH); 4.60 (br. *s*, OH); 4.27 (*m*, H–C(3′)); 3.70 (*m*, H–C(4′)); 3.49–3.43 (*dd*, *J* = 11.5, 5.4, 1 H, CH_2(5′)); 3.39–3.33 (*dd*, *J* = 11.6, 6.2, 1 H, CH_2(5′)); 2.44–2.37 (*m*, 1 H, CH_2(2′)); 2.08 (*s*, Me); 2.07–2.0 (*m*, 1 H, CH_2(2′)). ^{13}C-NMR (75 MHz, (D_6)DMSO): 156.80; 148.95; 143.54; 87.33; 84.62; 70.76; 62.36; 37.04; 16.39. HR-ESI-MS (pos.): 266.0769 ($[M+Na]^+$, $C_9H_{13}N_3NaO_5^+$; calc. 266.0748).

5-Isopropyl-2′-deoxy-6-azauridine (=*2-(2-Deoxy-β*-D-erythro-*pentofuranosyl)-6-(1-methylethyl)-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **13c**). To a suspension of **12c** (434 mg, 0.855 mmol) in dry MeOH (14 ml) was added MeONa (30% soln. in MeOH, 273 µl, 1.44 mmol), and the mixture was stirred at r.t. for 6 h. The resulting soln. was neutralized with *Dowex 50 WX-8* (H^+ form), and the resin was filtered off and washed with MeOH. After evaporation of the solvent, the residue was purified by CC (SiO_2; 5–7% MeOH in CH_2Cl_2) to yield **13c** (223 mg, 96%). White solid. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.06 (br. *s*, NH); 6.35 (*dd*, *J* = 7.2, 4.8, H–C(1′)); 5.17 (br. *s*, OH); 4.62 (br. *s*, OH); 4.32 (*m*, H–C(3′)); 3.69 (*m*, H–C(4′)); 3.54–3.44 (*m*, 1 H, CH_2(5′)); 3.43–3.33 (*m*, 1 H, CH_2(5′)); 3.13–2.97 (*m*, Me_2CH); 2.48–2.37 (*m*, 1 H, CH_2(2′)); 2.14–2.03 (*m*, 1 H, CH_2(2′)); 1.13 (*d*, *J* = 6.8, Me); 1.12 (*d*, *J* = 6.8, Me). ^{13}C-NMR (75 MHz, (D_6)DMSO): 155.99; 149.72; 148.58; 87.31; 84.48; 70.67; 62.28; 37.0; 28.12; 19.87; 19.78. HR-ESI-MS (pos.): 294.1079 ($[M+Na]^+$, $C_{11}H_{17}N_3NaO_5^+$; calc. 294.1061).

5-Octyl-2′-deoxy-6-azauridine (=*2-(2-Deoxy-β*-D-erythro-*pentofuranosyl)-6-octyl-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **13d**). To a suspension of **12d** (380 mg, 0.658 mmol) in dry MeOH (12 ml) was added

MeONa (30% soln. in MeOH, 228 µl, 1.2 mmol), and the mixture was stirred at r.t. for 6 h. The resulting soln. was neutralized with *Dowex 50 WX-8* (H^+ form), and the resin was filtered off and washed with MeOH. After evaporation of the solvent, the residue was purified by CC (SiO_2; 5% EtOH in CH_2Cl_2) to yield **13d** (195 mg, 87%). Off-white hygroscopic solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 5:2): 6.53 (*t*, *J* = 6.3, H–C(1′)); 4.50 (*m*, H–C(3′)); 3.96 (*m*, H–C(4′)); 3.77–3.62 (*m*, CH_2(5′)); 2.73–2.50 (*m*, 3 H, CH_2(2′), CH_2(*α*)); 2.32–2.19 (*m*, 1 H, CH_2(2′)); 1.71–1.52 (*m*, CH_2(*β*)); 1.28 (*m*, 5 × CH_2); 0.89 (*t*, *J* = 6.0, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 5:2): 156.54; 148.91; 147.64; 87.26; 85.73; 71.58; 62.92; 37.69; 31.67; 29.72; 29.12; 29.05 (2 C); 26.10; 22.46; 13.80. HR-ESI-MS (pos.): 364.1863 ([*M* + Na]$^+$, $C_{16}H_{27}N_3NaO_5^+$; calc. 364.1843).

5-(4-Fluorophenyl)-2′-deoxy-6-azauridine (= *2-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-6-(4-fluorophenyl)-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **14**). Compound **12e** (473 mg, 0.8 mmol), (4-fluorophenyl)boronic acid (143 mg, 1.02 mmol), $Pd(PPh_3)_4$ (65 mg, 0.056 mmol), and CuTC (195 mg, 1.02 mmol) were flushed with Ar and suspended in dry THF (9 ml). The mixture was stirred at 50° for 24 h, and then the solvent was evaporated *in vacuo*. The residue was taken up in AcOEt (50 ml) and washed with sat. $NaHCO_3$ (50 ml) and brine (50 ml), dried ($MgSO_4$), and evaporated. The crude mixture was purified by CC (SiO_2; 2% acetone in CH_2Cl_2), which afforded 376 mg of impure material, which was used for the deprotection step without further purification. To a suspension of this impure intermediate in dry MeOH (13 ml) was added MeONa (30% soln. in MeOH, 255 µl, 1.34 mmol), and the mixture was stirred at r.t. for 6 h. The resulting soln. was neutralized with *Dowex 50 WX-8* (H^+ form), and the resin was filtered off and washed with MeOH. After evaporation of the solvent, the residue was purified by CC (SiO_2; 5% MeOH in CH_2Cl_2), early and late fractions were combined, evaporated, and subjected to a second column using the same eluent to yield **14** (106 mg, 41% over two steps). White solid. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 6:2): 7.99 (*m*, 2 arom. H); 7.14 (*t*, *J* = 8.7, 2 arom. H); 6.61 (*t*, *J* = 6.0, H–C(1′)); 4.58 (*m*, H–C(3′)); 3.96 (*m*, H–C(4′)); 3.72–3.60 (*ddd*, *J* = 26.2, 11.9, 4.6, CH_2(5′)); 2.76–2.63 (*m*, 1 H, CH_2(2′)); 2.40–2.26 (*m*, 1 H, CH_2(2′)). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 6:2): 163.89 (*d*, *J*(C,F) = 251.2, 1 C); 156.06; 148.64; 141.83; 130.37 (*d*, *J*(C,F) = 8.5, 2 C); 127.62 (*d*, *J*(C,F) = 3.2, 1 C); 115.27 (*d*, *J*(C,F) = 21.7, 2 C); 87.10; 85.92; 71.06; 62.38; 37.68. HR-ESI-MS (pos.): 346.0814 ([*M* + Na]$^+$, $C_{14}H_{14}FN_3NaO_5^+$; calc. 346.0810).

N-*[3-(2′-Deoxy-6-azauridin-5-yl)prop-2-ynyl]octanamide* (= *2-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-6-[3-[(1-oxooctyl)amino]prop-1-yn-1-yl]-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **15**). Compound **12e** (1 g, 1.692 mmol), [Pd_2(dba)$_3$] (142 mg, 0.156 mmol), PPh_3 (82 mg, 0.312 mmol), and CuI (60 mg, 0.316 mmol) were dissolved in anh. DMF (12 ml) under Ar, and Et_3N (432 µl, 3.11 mmol) and *N*-(prop-2-ynyl)octanamide [22] (846 mg, 4.66 mmol) were then added. After stirring at r.t. for 2 h, the mixture was diluted with AcOEt (120 ml), and the insoluble material was filtered off. The filtrate was washed with brine (2 × 120 ml) and H_2O (2 × 120 ml), dried ($MgSO_4$), and evaporated. The residue was purified by CC (SiO_2; 5% acetone in CH_2Cl_2 containing 1% HCOOH), followed by a second silica-gel column with the same eluent. Appropriate fractions were combined, evaporated, and the residue was recrystallized from hot EtOH, which afforded a crude product mixture (331 mg, 2 crops) as a white solid, which was subjected to detoluoylation without further purification. To a suspension of this crude intermediate (331 mg) in dry MeOH (9 ml) was added MeONa (30% soln. in MeOH, 164 µl, 0.863 mmol), and the mixture was stirred at r.t. for 6 h. The resulting soln. was neutralized with *Dowex 50 WX-8* (H^+ form), and the resin was filtered off and washed with MeOH. After evaporation of the solvent, the residue was purified by CC (SiO_2; 5–7% MeOH in CH_2Cl_2) to afford two compounds: **15** (73 mg, 11%, 2 steps) and side-product **16** (87 mg, 12%, two steps) as slightly yellow oils.

Data of **15**. ^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 6:2): 6.50 (*dd*, *J* = 6.8, 5.1, H–C(1′)); 4.51 (*m*, H–C(3′)); 4.25 (*s*, CH_2(*α*)); 3.96 (*m*, H–C(4′)); 3.79–3.70 (*dd*, *J* = 11.9, 4.2, 1 H, CH_2(5′)); 3.70–3.60 (*dd*, *J* = 11.9, 4.2, 1 H, CH_2(5′)); 2.65–2.51 (*m*, 1 H, CH_2(2′)); 2.35–2.20 (*m*, 1 H, CH_2(2′)); 2.23 (*t*, *J* = 7.7, C*H*$_2$CONH); 1.63 (*m*, C*H*$_2$CH$_2$CONH); 1.30 (*s*, 4 × CH_2); 0.88 (*t*, *J* = 6.7, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 6:2): 174.19; 155.70; 147.90; 130.55; 93.20; 87.31; 86.18; 73.76; 70.98; 62.49; 37.70; 35.86; 31.42; 29.30; 28.96; 28.71; 25.38; 22.31; 13.62. HR-ESI-MS (pos.): 431.1925 ([*M* + Na]$^+$, $C_{19}H_{28}N_4NaO_6^+$; calc. 431.1901).

Data of N-*[3-(2′-Deoxy-6-azauridin-5-yl)-2-oxopropyl]octanamide* (= *2-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-6-[2-oxo-3-[(1-oxooctyl)amino]propyl]-1,2,4-triazine-3,5(2*H,*4*H*)-dione*; **16**). ^{1}H-NMR

(300 MHz, $CDCl_3$/MeOD 6:2): 6.49 (*dd*, *J* = 6.8, 4.9, H–C(1′)); 4.48 (*m*, H–C(3′)); 4.22 (*d*, *J* = 1.6, $COCH_2NH$); 3.92 (*m*, H–C(4′)); 3.79–3.55 (*m*, CH_2(5′)); 2.66–2.52 (*m*, 1 H, CH_2(2′)); 2.34–2.19 (*m*, 1 H, CH_2(2′)); 2.27 (*t*, *J* = 7.6, $NHCOCH_2$); 1.63 (*m*, $NHCOCH_2CH_2$); 1.29 (*m*, 4 × CH_2); 0.88 (*t*, *J* = 6.7, Me). ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 6:2): 201.43; 174.85; 156.21; 148.71; 141.26; 86.94; 85.69; 70.88; 62.28; 48.88; 37.48; 35.76; 31.41; 29.82; 28.94; 28.71; 25.40; 22.34; 13.65. HR-ESI-MS (pos.): 427.2186 ($[M+H]^+$, $C_{19}H_{31}N_4O_7^+$; calc. 427.2187).

General Procedure for the Phosphorylation of 5-Substituted Derivatives **13a**–**13d** *and* **14**–**16** (*GP*). To a soln. of an unprotected 5-substituted nucleoside derivative (1 equiv.) and proton sponge (1.5–3 equiv.) in $PO(OMe)_3$ was added $POCl_3$ (1.5–3 equiv.) in one portion at 0°, and the mixture was stirred at 0° for 3 h. The mixture was poured into ice/H_2O and brought to pH 8–9 with NH_4OH or 1M NaOH, then the volatiles were removed *in vacuo*, and the residue was purified by CC (SiO_2; gradient of ^{i}PrOH/H_2O/NH_4OH). The phosphates isolated after lyophilization were further purified by prep. RP-HPLC (*C-18*, 5 μm, 19 × 150 mm; gradient of MeCN and TEAB (30 mM)) to yield anal. pure compounds. Next, the phosphates were subjected to ion exchange (*Dowex 50 WX-8*, Na^+) and lyophilized to afford the corresponding disodium salts.

2′-Deoxy-6-azauridine 5′-Monophosphate (*=2-(2-Deoxy-5-*O*-phosphono-β-*D*-*erythro*-pentofuranosyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione Sodium Salt (1:2)*; **1a**). Prepared according to *GP:* compound **13a** (94 mg, 0.411 mmol), $POCl_3$ (76 μl, 0.82 mmol), and proton sponge (177 mg, 0.82 mmol) in 2.5 ml of $PO(OMe)_3$. The mixture was stirred at 0° for 3 h and worked up as described above, including CC (SiO_2; ^{i}PrOH/NH_4OH/H_2O 75:15:10 → 70:20:5 → 65:25:5) and RP-HPLC (30 mM aq. TEAB/MeCN 99:1 → 98:2, 16 ml/min) to yield **1a** (59 mg, 40%). White solid. ^{1}H-NMR (500 MHz, D_2O): 7.54 (*s*, H–C(5)); 6.51 (*dd*, *J* = 6.9, 5.4, H–C(1′)); 4.60 (*m*, H–C(3′)); 4.09 (*m*, H–C(4′)); 3.93–3.85 (*m*, 1 H, CH_2(5′)); 3.85–3.78 (*m*, 1 H, CH_2(5′)); 2.75–2.66 (*m*, 1 H, CH_2(2′)); 2.33–2.24 (*m*, 1 H, CH_2(2′)). ^{13}C-NMR (125 MHz, D_2O): 162.59; 152.96; 136.22; 85.51; 85.01 (*d*, *J*(C,P) = 8.0, 1 C); 71.0; 64.17 (*d*, *J*(C,P) = 4.7, 1 C); 35.64. ^{31}P-NMR (121 MHz, D_2O): 2.88. HR-ESI-MS (neg.): 308.0280 ($[M-H]^-$, $C_8H_{11}N_3O_8P^-$; calc. 308.0289).

2′-Deoxy-6-azathymidine 5′-Monophosphate (*=2-(2-Deoxy-5-*O*-phosphono-β-*D*-erythro-pentofuranosyl)-6-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione Sodium Salt (1:2)*; **1b**). Prepared according to *GP:* compound **13b** (114.2 mg, 0.469 mmol), $POCl_3$ (88 μl, 0.94 mmol), and proton sponge (205 mg, 0.95 mmol) in 3 ml of $PO(OMe)_3$. The mixture was stirred at 0° for 3 h and worked up as described above, including CC (SiO_2; ^{i}PrOH/NH_4OH/H_2O 85:10:5 → 80:10:10 → 75:15:10) and RP-HPLC (30 mM aq. TEAB/MeCN 97:3 → 80:20, 16 ml/min), to yield **1b** (91 mg, 53%). White solid. ^{1}H-NMR (500 MHz, D_2O): 6.51 (*dd*, *J* = 7.0, 5.8, H–C(1′)); 4.62 (*m*, H–C(3′)); 4.07 (*m*, H–C(4′)); 3.94–3.88 (*m*, 1 H, CH_2(5′)); 3.85–3.79 (*m*, 1 H, CH_2(5′)); 2.77–2.69 (*m*, 1 H, CH_2(2′)); 2.27–2.17 (*m*, 1 H, CH_2(2′)); 2.19 (*s*, Me). ^{13}C-NMR (125 MHz, D_2O): 167.67; 158.16; 145.11; 85.64; 85.18 (*d*, *J*(C,P) = 7.7, 1 C); 71.68; 64.50 (*d*, *J*(C,P) = 4.6, 1 C); 36.01; 16.48. ^{31}P-NMR (121 MHz, D_2O): 3.93. HR-ESI-MS (neg.): 322.0448 ($[M-H]^-$, $C_9H_{13}N_3O_8P^-$; calc. 322.0446).

5-Isopropyl-2′-deoxy-6-azauridine 5′-Monophosphate (*=2-(2-Deoxy-5-*O*-phosphono-β-*D*-erythro-pentofuranosyl)-6-(1-methylethyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione Sodium Salt (1:2)*; **1c**). Prepared according to *GP:* compound **13c** (108.5 mg, 0.4 mmol), $POCl_3$ (75 μl, 0.8 mmol), and proton sponge (175 mg, 0.81 mmol) in 2.5 ml of $PO(OMe)_3$. The mixture was stirred at 0° for 3 h and worked up as described above, including CC (SiO_2; ^{i}PrOH/NH_4OH/H_2O 85:10:5 → 80:10:10 → 75:15:10) and RP-HPLC (30 mM aq. TEAB/MeCN 95:5 → 90:10 → 85:15, 16 ml/min), to give **1c** (27.1 mg, 17%). White solid. ^{1}H-NMR (500 MHz, D_2O): 6.48 (*dd*, *J* = 7.4, 4.1, H–C(1′)); 4.60 (*m*, H–C(3′)); 4.07 (*m*, H–C(4′)); 3.97–3.91 (*m*, 1 H, CH_2(5′)); 3.84–3.77 (*m*, 1 H, CH_2(5′)); 3.09 (*m*, Me_2CH); 2.74–2.68 (*m*, 1 H, CH_2(2′)); 2.33–2.26 (*m*, 1 H, CH_2(2′)); 1.18 (*d*, *J* = 6.9, Me); 1.17 (*d*, *J* = 6.0, Me). ^{13}C-NMR (125 MHz, D_2O): 158.18; 152.06; 150.35; 85.56; 85.30 (*d*, *J*(C,P) = 7.5, 1 C); 71.45; 65.20 (*d*, *J*(C,P) = 4.5, 1 C); 36.14; 28.66; 19.19; 19.13. ^{31}P-NMR (121 MHz, D_2O): 2.01. HR-ESI-MS (neg.): 350.0742 ($[M-H]^-$, $C_{11}H_{17}N_3O_8P^-$; calc. 350.0759).

5-Octyl-2′-deoxy-6-azauridine 5′-Monophosphate (*=2-(2-Deoxy-5-*O*-phosphono-β-*D*-erythro-pentofuranosyl)-6-octyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione sodium salt (1:2)*; **1d**). Prepared according to *GP:* compound **13d** (154 mg, 0.451 mmol), $POCl_3$ (84 μl, 0.9 mmol), and proton sponge (177 mg, 0.9 mmol) in 3 ml of $PO(OMe)_3$. The mixture was stirred at 0° for 3 h and worked up as described above, including CC

(SiO_2; ^{i}PrOH/NH_4OH/H_2O 80:10:10→75:15:10) and RP-HPLC (30 mM aq. TEAB/MeCN 80:20→75:25, 16 ml/min), to yield **1d** (98 mg, 47%). White solid. ^{1}H-NMR (500 MHz, D_2O): 6.45 (*dd*, *J* = 7.4, 4.0, H–C(1′)); 4.55 (*m*, H–C(3′)); 4.07 (*m*, H–C(4′)); 4.0–3.92 (*m*, 1 H, CH_2(5′)); 3.84–3.76 (*m*, 1 H, CH_2(5′)); 2.70–2.50 (*m*, 3 H, CH_2(2′) and CH_2(*α*)); 2.34–2.26 (*m*, 1 H, CH_2(2′)); 1.69–1.53 (*m*, CH_2(*β*)); 1.35–1.14 (*m*, 5 CH_2); 0.80 (*t*, *J* = 6.9, Me). ^{13}C-NMR (125 MHz, D_2O): 157.91; 149.82; 147.81; 85.31; 84.96 (*d*, *J*(C,P) = 7.5, 1 C); 70.07; 65.09 (*d*, *J*(C,P) = 5.1, 1 C); 35.95; 30.88; 28.71; 28.22; 28.18; 27.88; 24.79; 21.74; 13.12. ^{31}P-NMR (121 MHz, D_2O): 1.37. HR-ESI-MS (neg.): 420.1537 ([*M* − H]$^-$, $C_{16}H_{27}N_3O_8P^-$; calc. 420.1541).

5-(4-Fluorophenyl)-2′-deoxy-6-azauridine 5′-Monophosphate (= *2-(2-Deoxy-5-*O*-phosphono-β-*D*-*erythro*-pentofuranosyl)-6-(4-fluorophenyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione Sodium Salt (1:2)*; **2**). Prepared according to *GP:* compound **14** (88.7 mg, 0.274 mmol), $POCl_3$ (51 μl, 0.55 mmol), and proton sponge (123 mg, 0.56 mmol) in 1.8 ml of $PO(OMe)_3$. The mixture was stirred at 0° for 3 h and worked up as described above, including CC (SiO_2; ^{i}PrOH/NH_4OH/H_2O 85:10:5→80:10:10→75:15:10) and RP-HPLC (30 mM aq. TEAB/MeCN 92:8→80:20, 16 ml/min), to give **2** (49 mg, 40%). White solid. ^{1}H-NMR (500 MHz, D_2O): 7.84 (*m*, 2 arom. H); 7.24 (*m*, 2 arom. H); 6.57 (*dd*, *J* = 7.2, 5.0, H–C(1′)); 4.58 (*m*, H–C(3′)); 4.09 (*m*, H–C(4′)); 3.90–3.83 (*m*, 1 H, CH_2(5′)); 3.82–3.75 (*m*, 1 H, CH_2(5′)); 2.82–2.74 (*m*, 1 H, CH_2(2′)); 2.35–2.27 (*m*, 1 H, CH_2(2′)). ^{13}C-NMR (150 MHz, D_2O): 163.24 (*d*, *J*(C,F) = 247.0, 1 C); 160.19; 156.59; 143.54; 130.71 (*d*, *J*(C,F) = 8.5, 2 C); 129.08; 115.22 (*d*, *J*(C,F) = 21.9, 2 C); 85.78; 85.17 (*d*, *J*(C,P) = 7.1, 1 C); 71.52; 64.60 (*d*, *J*(C,P) = 3.8, 1 C); 35.95. ^{31}P-NMR (121 MHz, D_2O): 1.37. HR-ESI-MS (neg.): 402.0493 ([*M* − H]$^-$, $C_{14}H_{14}FN_3O_8P^-$; calc. 402.0508.

N-*[3-(2′-Deoxy-5′-*O*-phosphono-6-azauridin-5-yl)prop-2-ynyl]octanamide* (= *2-(2-Deoxy-5-*O*-phosphono-β-*D*-*erythro*-pentofuranosyl)-6-[3-[(1-oxooctyl)amino]prop-1-yn-1-yl]-1,2,4-triazine-3,5(2*H*,4*H*)-dione Sodium Salt (1:2)*; **3**). Prepared according *GP:* compound **15** (71.3 mg, 0.175 mmol), $POCl_3$ (37 μl, 0.395 mmol), and proton sponge (89 mg, 0.41 mmol) in 1.5 ml of $PO(OMe)_3$. The mixture was stirred at 0° for 3 h and worked up as described above, including CC (SiO_2; ^{i}PrOH/NH_4OH/H_2O 85:10:5→80:10:10→75:15:10) and RP-HPLC (30 mM aq. TEAB/MeCN 85:15→80:20→65:35, 16 ml/min), to afford **3** (16 mg, 17%). White solid. ^{1}H-NMR (500 MHz, D_2O): 6.51 (*t*, *J* = 6.0, H–C(1′)); 4.59 (*m*, H–C(3′)); 4.23 (*s*, CH_2(*α*)); 4.10 (*m*, H–C(4′)); 3.98–3.91 (*m*, 1 H, CH_2(5′)); 3.88–3.80 (*m*, 1 H, CH_2(5′)); 2.75–2.66 (*m*, 1 H, CH_2(2′)); 2.35–2.25 (*m*, 1 H, CH_2(2′)); 2.30 (*t*, *J* = 7.1, CH_2CO); 1.63 (*m*, C*H*$_2$CH$_2$CO); 1.30 (br. *s*, 2 CH_2); 1.23 (br. *s*, 2 CH_2); 0.81 (*t*, *J* = 6.8, Me). ^{13}C-NMR (150 MHz, D_2O): 177.40; 163.93; 154.79; 131.01; 91.79; 86.25; 85.28 (*d*, *J*(C,P) = 7.7, 1 C); 74.86; 71.34; 65.04 (*d*, *J*(C,P) = 4.6, 1 C); 36.16; 35.56; 31.04; 29.41; 28.05; 27.93; 25.27; 21.88; 13.38. ^{31}P-NMR (121 MHz, D_2O): 2.14. HR-ESI-MS (neg.): 487.1594 ([*M* − H]$^-$, $C_{19}H_{28}N_4O_9P^-$; calc. 487.1599.

N-*[3-(2′-Deoxy-5′-*O*-phoshono-6-azauridine-5-yl)-2-oxopropyl]octanamide* (= *2-(2-Deoxy-5-*O*-phosphono-β-*D*-*erythro*-pentofuranosyl)-6-[2-oxo-3-[(1-oxooctyl)amino]propyl]-1,2,4-triazine-3,5(2*H*,4*H*)-dione Sodium Salt (1:2)*; **4**). Prepared according to *GP:* compound **16** (85.9 mg, 0.201 mmol), $POCl_3$ (38 μl, 0.406 mmol), and proton sponge (89 mg, 0.41 mmol) in 1.5 ml of $PO(OMe)_3$. The mixture was stirred at 0° for 3 h and worked up as described above, including CC (SiO_2; ^{i}PrOH/NH_4OH/H_2O 85:10:5→80:10:10→75:15:10) and RP-HPLC (30 mM aq. TEAB/MeCN 85:15→80:20→75:25, 16 ml/min), to yield **4** (24.6 mg, 22%). White solid. ^{1}H-NMR (600 MHz, D_2O): 6.49 (*dd*, *J* = 7.2, 4.7, H–C(1′)); 4.54 (*m*, H–C(3′)); 4.07 (*m*, H–C(4′)); 3.94–3.97 (*m*, 1 H, CH_2(5′)); 3.83–3.76 (*m*, 1 H, CH_2(5′)); 2.70–2.64 (*m*, 1 H, CH_2(2′)); 2.32–2.23 (*m*, 1 H, CH_2(2′)); 2.28 (*t*, *J* = 7.4, NHCOC*H*$_2$); 1.57 (*m*, NHCOCH$_2$C*H*$_2$); 1.31–1.18 (*m*, 4 × CH_2); 0.81 (*t*, *J* = 6.9, Me). ^{13}C-NMR (150 MHz, D_2O): 204.97; 177.82; 158.31; 150.62; 141.96; 85.89; 85.32 (*d*, *J*(C,P) = 7.9, 1 C); 71.26; 65.0 (*d*, *J*(C,P) = 4.2, 1 C); 48.32 (br. *m*, CD_2); 40.32 (br. *m*, CD_2); 36.19; 35.34; 30.88; 28.05; 27.99; 25.17; 21.87; 13.31. ^{31}P-NMR (121 MHz, D_2O): 1.25. HR-ESI-MS (neg.): 505.1695 ([*M* − H]$^-$, $C_{19}H_{30}N_4O_{10}P^-$; calc. 505.1705.

ThyX and ThyA Assays. Cloning, expression, and purification of mycobacterial ThyX and ThyA, as well as the inhibition assays, were performed according to protocols described in [22].

TMPKmt Assays. Expression and purification of recombinant TMPKmt as well as TMPKmt activity determination were described in [25].

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